GL 23 Teleworking
Approved by Director: Dr. Guy Vallaro

A. PURPOSE:

This temporary telework program provides an option for certain employees to work remotely and for managers to help manage their Section during the COVID-19 pandemic. This policy applies to all teleworking activities of the Division of Scientific Services (DSS). All managers, supervisors, and teleworkers should be familiar with the contents of this policy.

B. DEFINITIONS:

Telework: The concept of working from home on a full-time or part-time basis. Telework is not a formal employee benefit. Rather, it is an alternative method of meeting the needs of the Division of Scientific Services remotely. Employees are not required to telework. Employees have the right to refuse to telework if the option is made available.

Teleworker: someone who works at home, and communicates with their office by phone, email, or internet: Must be a regular employee who has successfully passed their probationary period and has a documented history of job performance that meets or exceeds their supervisor's expectations.

VPN: A virtual private network (VPN) extends a private network across a public network and enables users to send and receive data across shared or public networks as if their computing devices were directly connected to the private network.

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C. RESPONSIBILITIES AND ADMINISTRATIVE POLICIES:

Director: To direct and approve all teleworking.

Teleworkers: Responsible to ensure all organization policies, rules and practices at the Division will apply at the telework site. The teleworker must have a portion of their workload that can be performed away from the office in order to be approved to telework.

Deputy Directors/Assistant Directors: To manage the telework operations and ensure staff is trained in the teleworking policy.

Quality Manager: To ensure that the same level of quality is achieved and to ensure policy aligns with all ANAB accreditation standards and the standards within the FBI DNA QAS.

ADMINISTRATIVE POLICIES:

The telework program is a work flexibility arrangement under which an employee performs the duties and responsibilities of such employee's position, and other authorized activities, from an approved worksite other than the location from which the employee would otherwise work.

1. Participation in the program is voluntary and subject to management approval.

2. The employee must possess the appropriate equipment to allow the job to be performed away from the

central worksite. Equipment will be provided to teleworking employees in order to fulfill their job duties.

3. The employee will be in compliance with all applicable DSS, DESPP, and DESPP IT policies and procedures, including security, privacy, and confidentiality.
4. The manager's/supervisor's will assess performance by monitoring the quality of results and productivity. Established objectives measures such as analyst error rates, turn-around-time, case reports volume, case review (technical and administrative) volume will be utilized to ensure employees are meeting expectations.
5. A telework arrangement can be terminated by management at any time. The manager/supervisor will assess telework performance by monitoring the quality of results and productivity. Established objective measures such as analyst turn-around-times, case report volume, and case review (technical and administrative) volume will be utilized to ensure employees are meeting expectations.
6. All property owned by the organization used at home by the teleworker is to be returned to the organization when the teleworker ceases to telework.
7. Duration and schedule of teleworking will be dependent on the individual's manager and the need for teleworking.
8. Identification of the eligible individuals for telework are based on a review of the characteristics and duties of their position.
9. Management may require teleworkers to report to the laboratory on scheduled telework days when necessary to meet operational needs.
10. A teleworker must maintain a daily log of activity on days working from home.

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D. CHEMICAL ANALYSIS SECTION

1. Current practices regarding Quality Assurance/Quality Control (QA/QC) protocols will be followed for all evidence submissions, handling, accessioning, storage, and analyses - with the exception of certain situations wherein initials or signatures are needed (e.g. physically initialing documents not-contemporaneous with remote review of batches or reports).
2. Upon inventorying evidence analysts will ensure that evidence corresponds to the items listed within the Request for Analysis (RFA) forms. If there are any discrepancies then they will be noted and the assigned analyst(s) (or designee) will follow-up accordingly. If an evidence discrepancy occurs within the Controlled Substance Unit then a supervisor (or higher) may be contacted and they can possibly assist with the correction process. When not under current examination, all evidence will be stored under proper seal and in proper evidence storage areas according to protocols for each unit and/or the section.
3. Analysis of evidence will occur according to each current standard operating procedure (SOP). The processing of data from evidence analysis can occur via Remote Connection and/or Virtual Private Networking (VPN). All instrumentation within the section will have secure connectivity to remote connections.

4. Data may need to be transferred from instrument computers to a networked location where such data will be processed remotely. .
5. Teleworking will usually commence after instrumental data collection has occurred. Analysts will remotely connect to computers/servers which house analytical data and process such data as if they were normally at work in the laboratory setting.
6. By remotely logging-in the analysts will use the same instrumental software and parameters as if they were physically at the laboratory.
7. Documentation of data may be in the form of printing to networked laboratory printers. When analysts return to work they shall log into applicable print jobs on the networked printers and obtain hardcopies of previously processed data.
8. Alternatively, processed data may be saved as pdf-files and sent to shared drives so co-workers could perform technical reviews. Such reviews will be done using pdf files that will be accessible to teleworking employees.
9. Each step may have to be documented within LIMS-Plus software in order to ensure efficiency and casework completeness. Given no remediation after reviews, analysts will be able to send their reports to shared drives so co-workers can perform further [administrative] reviews. Teleworking analysts will then be able to release reports remotely.
10. The physical mailing of toxicology reports will not be performed. Reports will be transmitted electronically and documented accordingly within LIMS-Plus.
11. Once fully reviewed and no remedial steps, technical and administrative reviews can occur as within non-batch casework.
12. Once physically back in the laboratory, staff will compile all relevant data and paperwork in order to ensure everything went into the respective places (i.e., batch folders or casefile folders).
13. All communications (e.g., receipt of paperwork/case documents, issued reports) will be done electronically and captured within LIMS-Plus. .
14. Evidence will be placed under proper seal and returned via normal in-house laboratory operations when staff are physical in the laboratory.

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E. Forensic Biology & DNA

I. Telework SOP – Using Adobe Acrobat 2017

Adobe Acrobat 2017 will be used to make edits on paperwork, such as additions, cross-outs, initials and dates. All edits made in this manner should be made in colored font to easily distinguish (i.e. anything that would have been handwritten can be done in blue font). When working electronically using VPN, typed initials are an acceptable form of identification.

1. Adding initials/dates/comments

   a. Under Tools select 'Fill & Sign'.

   b. Checkmarks can be added using the 'Checkmark' option from the top header toolbar. This option is helpful for the batch and case review checklists and injection sheets.

   c. Initials, dates or any corrections/notes can be added by choosing the 'Ab Text' option.
      i. An alternative to this is to select 'Comments' in the main Tools option. Choose the plain 'T' option called 'Add Text Comment'. This option is sometimes easier to move around; colors can also be changed using this feature to help distinguish markings.

   d. Initials/date must also be added to the end of comments to remain in compliance with current GLs and SOPs.

2. Making cross-outs
   a. In Tools, select "Fill & Sign". The "Line" option allows a line be added. Depending on the length of this line, it may be too thick to view what is being crossed out.

   b. An alternative is to select "Comments" in the Tools option. Choose the pencil called "Draw Free Form" to add a cross out.

   c. Any cross out added must be accompanied by an initial/date text comment.

3. Organizing scanned documents
   a. The "Organize Pages" option in Tools allows for numerous actions such as changing page order, inserting new pages and replacing pages.

      "Extract" option: remove pages to add to a different file. This is helpful for moving e-grams and various QRs to case folders. This option can also replace the need for photocopies.

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II. Telework SOP – Forensic Biology

Report writing will be conducted in accordance with current applicable GLs, FB SOPs and WIs in addition to below remote instructions. Any corrections or discussion conducted via email will be printed and included in case paperwork. Case jackets may not be removed from laboratory for the purposes of teleworking.

1. Cases to be processed remotely will be scanned to S:\FB\Telework.

2. Reports/notifications will be drafted as usual. The draft will be saved in Word format in the Final Reports drive.
3. The draft along with FB-QR 21 and the appropriate checklist (FB QR-12, -12a, -12b) will be saved in PDF format from Qualtrax to S:\FB\Telework.
4. Corrections and reviews will be documented by reviewers in Adobe Acrobat 2017.
5. For cases processed remotely, LIMS milestone completions will stand in place of case jacket labels.
6. The analyst or any personnel available onsite can print the completed casefile paperwork and add to case jacket.

II. Telework SOP – Male Screen Batch Processing

Male screen processing will be conducted in accordance with current applicable GLs, DNA SOPs and WIs in addition to below remote instructions. Any corrections or discussion conducted via email will be printed and included in batch paperwork.

1. Case jackets and batch paperwork will be scanned by the FB examiner processing the batch. Scanned paperwork will include a blank DNA QR-4E. Scanned documents will be kept in their respective folders on the U:Drive (U:\Telework\Cases and U:\Telework\BatchPaperwork).

   FB examiner to notify a DNA analyst competent in the male screen procedure that batch is ready to be analyzed.

2. Remote Analysis

   a. DNA analyst will electronically record male screen results and assign extraction requests using the Male Screen Results QR-346 (QR-346-A); This QR will be saved to U:\Telework\BatchPaperwork.

   b. DNA analyst will enter male screen results and create requests in LIMS as usual.

   c. DNA QR-4E will be completed electronically using Adobe Acrobat 2017.

3. Remote Review

   Batch review will be conducted as usual by a qualified analyst by accessing the U:Drive folders. The review and any noted corrections will be recorded using Adobe Acrobat 2017.

4. Final Scans/Copies

   a. Once corrections are finalized, the analyst will ensure a completed version of the batch is added to U:\Batch Paperwork Files and reviewer will verify.

   b. The analyst or any personnel available onsite can print the final scanned batch and file in appropriate location. They will make copies of necessary QRs to add to case files. The case filed will be forwarded to Case Management.

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c. Analysts or technicians assigned negative sample and/or cases will move those samples virtually (in LIMS) and physically to the "Freezer Storage" storage location.

IV. Telework SOP – Batch Processing and Analysis Workflow

Batch processing and analysis will be conducted in accordance with current applicable GLs, DNA SOPs and WIs in addition to below remote instructions. Any corrections or discussion conducted via email will be printed and included in batch paperwork. Necessary TL sign offs may be accomplished via scanning and email. These approvals will be printed and included in the batch paperwork.

1. Case Scanning

   a. Cases to be processed remotely will be scanned onto the U:Drive (U:\Telework\Cases) by the batch processor or any available onsite personnel.

   b. Scanning shall include all paperwork included in case jacket. Transfer sheets will be scanned after batch processor has made handwritten notes pertaining to sample verification/consumption, etc.

2. Batch Processing

   a. Remote worksheet creation
      i. Batch processor (technician) will scan paperwork to analyst (this scan does not need to be saved long-term; it is for worksheet creation purposes only).

      ii. Qualified DNA analyst will create halt/dilution/amplification sheets as needed and save to technician's amplification folder per usual. The 'prepared by' line will be initial/dated using Adobe Acrobat 2017 at the time of batch ownership. (See below).

      iii. Save each staff search worksheet to the appropriate case folder to U:\Telework\Cases.

   b. Once injection(s) are complete, batch processor will scan entire batch to analyst for ownership. A blank QR-4A will be included. The scanned batch will be saved to folder on U:Drive (U:\Telework\BatchPaperwork).

   c. Analyst will review batch, initial pages and document necessary corrections using Adobe Acrobat 2017.

3. Remote Analysis

   a. Any 'prepared by' lines will be filled in via Adobe at this time.

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b. Analysis and contributor estimations will be conducted as usual. The injection sheet(s) (QR-325, QR-328) will be completed and initialed using Adobe Acrobat 2017.

c. E-grams and associated QRs (NOC, project comparison, concordance checker, etc.) will be printed, initialed and scanned to U:\Telework\BatchPaperwork. Alternatively, applicable documents may be converted to PDF format and processed via Adobe.

4. Remote Technical/Administrative Reviews

a. Reviews will be conducted per usual by accessing necessary paperwork on U:drive and documented using Adobe.

b. Electropherograms may be viewed by opening the associated GeneMarker/GeneMapper ID-X projects.

5. Scanning

a. Fully completed paperwork with all necessary initials will be scanned to currently used location (U:\Batch Paperwork Files) and filed. Alternatively, the PDFs may be moved to the appropriate folder(s) using the 'extract' option of Adobe. The reviewer will check contents of U:\Batch Paperwork Files and complete the Batch Paperwork Review Spreadsheet.

b. The analyst or any personnel available onsite can print the completed batch and file in appropriate storage location. Final completed paperwork will supersede any other versions generated in the process.

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6. **Telework SOP – DNA Reports and STRmix™**

Report writing will be conducted in accordance with current applicable GLs, DNA SOPs and WIs in addition to below remote instructions. Any corrections or discussion conducted via email will be printed and included in case paperwork. Necessary TL sign offs may be accomplished via scanning and email. These approvals will be printed and included in the batch paperwork.
Case jackets may not be removed from laboratory for the purposes of teleworking.

1. STRmix™

a. The STRmix™ server and F:drive will only be accessed by using the remote desktop function.

b. STRmix™ runs will be conducted as usual. Pages 1-3 of the report and QR-303 will be printed, initialed and scanned to U:\Telework\Cases.

2. Reports

   a. Reports will be drafted as usual. The draft will be saved in Word format in U:\CASEREPORTS.

   b. The draft along with QR-4 will be converted to PDF format by using the ACROBAT tab in Microsoft Word and saved to U:\Telework\Cases.

   c. Any batch paperwork normally photocopied into case jacket will be copied to U:\Telework\Cases. This included e-grams and NOC QRs. These steps can be done via the 'Organize Pages' feature of Adobe.

   d. Corrections and reviews will be documented by reviewers in Adobe Acrobat 2017.
   e. For cases processed remotely, LIMS milestone completions will stand in place of case jacket labels.

   f. The analyst or any personnel available onsite can print the completed casefile paperwork and add to case jacket.

F. Identification Section

The Identification Section is comprised of the Firearms, Latent Print, Computer Crimes, Multimedia, Questioned Documents and Imprint Units of the DSS. Each Unit performs "hands on" work on evidence, but also has the ability to conduct analysis in an electronic environment. It is the electronic environment of these Units that can be conducted remotely in teleworking situations. The examiners assigned to each unit will have a laptop off site that will use a VPN to remote into the examiner's desktop computer or other computer as indicated below. Each Unit is unique in the type of analysis conducted and this SOP will address the areas that can be conducted remotely for each Unit.

The examiners should plan ahead and be prepared to have multiple cases available in queue that can be worked on in a remote telework situation. The SOPs below describe the work that may be conducted in a remote telework environment. At the completion of the Administrative Review, the analyst may send the document to the DSS printer and select a delayed print option. Once back on-site at DSS, the analyst will print the final report, prepare the evidence and work product if applicable for transfer to the Evidence Receiving Unit.

Firearms Unit

1. The Firearms Unit of DSS receives evidence submitted as physical evidence that must be processed on-site. The following processes are conducted on-site: NIBIN acquisition/correlation, test firing of weapons, serial number restorations and microscopic comparisons.
2. This Unit's worksheets are able to be completed digitally and uploaded to the examiner's FAH Drive. Paperwork generated from the NIBIN Brass Trax and Matchpoint are converted to PDF and uploaded to the examiner's FAH Drive.

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3. The examiner will assemble their final conclusions on worksheets by remoting in to the FAH drive via their desktop computers.
4. Reports will be generated through the Justice Trax LIMS.
5. The technical review will be assigned through Justice Trax. The technical reviewer will receive the email from Justice Trax and will conduct the remote review of the digital file located in analyst's FAH drive.
6. Review of these documents is done on the standard technical review/administrative review Quality Record (QR FA-9) and also using the Remote Review Form to address corrections needed.
7. Once the technical review has been completed, the Remote Review Form will be added to the FAH drive and the Administrative Review will be assigned through Justice Trax.
8. At the conclusion of the Administrative Review, the milestone will be updated and the final report will be printed out and added to the final paper case file on-site.

Latent Print Unit

1. The Latent Print Unit of DSS receives evidence in both a digital format and also as physical evidence. When evidence is submitted as physical evidence in nature, there are scanning and processing techniques that must be conducted on-site prior to converting impressions of value to a digital file.
2. Once the latent evidence is in a digital format, the remainder of the comparison and verification work can be done in a remote environment.
3. Requests for the release of fingerprint cards will be done via an electronic request to SPBI for the comparison part of the remote analysis.
4. The examiner will conduct enhancements, sufficiency charts and comparisons by remoting into their desktop computers. Their desktop computer contains the necessary enhancement and processing software to conduct their comparative casework. NGI searches will be done in a remote telework environment using a LEO email account. AFIS searches must be conducted on-site due to CJIS security requirements.
5. The examiner will place their verification requests in their coworker's electronic folder, where the co-worker can conduct the verification in a remote setting. Once all casework is completed and the worksheets are saved, the examiner can also generate the report through Justice Trax.
6. The technical review and administrative review is assigned through Justice Trax and the review is conducted using the standard technical review/administrative review Quality Records (QR-LP-4) and also using the Remote Review Form to address corrections needed.

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Computer Crime Unit

1. The Computer Crimes Unit of the DSS receives physical evidence which must be processed upon receipt at the laboratory for the acquisition of data. The acquisition process of this evidence must be performed on site and then the imaged files or the data files are uploaded to the Computer Crimes Unit server and are accessible to the examiner on this physical workstation/computer.
2. These computers are not connected to the DESPP-BEST network. Remote teleworking connectivity to these workstation are done through the Cox Cable internet using Cisco Meraki VPN software. the examiner will use this VPN on the laptop to access their desktop workstation. This connection will allow the examiner to use their analysis software such as (Axiom, Encase, FTK, Cellebrite 4PC) to analyze the extracted data.
3. The Computer Crimes Unit worksheets, report and work product are all in electronic format and will be completed at the time of their analysis and uploaded to a centralized location for review by the technical reviewer and administrative reviewer.
4. Review of these documents is done on the standard technical review/administrative review Quality Records (QR CC-15, QR CC-29 or QR CC-53) and also using the Remote Review Form to address corrections needed.
5. No cases that are submitted to the DSS and are labeled as Child Pornography will be analyzed or reviewed in a remote teleworking environment. It is understood that sometimes images suspected of being child pornography are encountered in the data analysis of other types of offenses. In circumstances in which these types of images are encountered, the examiner should stop analyzing this case in a remote environment and continue on this case once back at the DSS.

Multimedia and Image Enhancement Unit

1. The Multimedia and Image Enhancement Unit of the DSS receives evidence in a physical form and digital format. This Unit also responds off-site to assist in the retrieval of video evidence at crime scenes or locations related to a crime scene.
2. Depending on the format of the evidence submitted, some of the analysis work must be conducted completely on-site. This evidence includes tapes submitted in analog format that must be converted. Digital Video Recorders that need video exported are also worked on-site up to the point in which the digital images are imported into the examiner's working folder.
3. If the evidence submitted is digital in nature or has been exported/converted into a digital format, the files are transferred into the examiner's working folder for enhancement. The enhancements and accompanying worksheets may be conducted in a remote telework environment by the analyst remoting into that computer.
4. The analysis will be completed remotely and all worksheets associated with the analysis are completed at the time of analysis. Upon completion of the analysis, the examiner will upload and work products

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created to the working folder for technical review. The examiner will update Justice Trax and assign the technical review to the other examiner. Review of these documents is done on the standard technical review/administrative review Quality Records (MMIE Review Checklist QR) and also using the Remote Review Form to address corrections needed.

5. Upon completion of the AR, the final report will be printed out once on-site and the final product created for return to the submitting agency.

Imprint Unit

1. The Imprint Unit of the DSS received evidence in both physical form and also as digital files that may be on disk. In the event that the evidence is in a physical nature, the enhancements and production of test impressions must be done on-site. These test impressions can then be digitized and uploaded to a working folder on the computer for enhancements using Photoshop or for searching in the Solemate database.
2. Comparisons may be conducted using the digitized images and the worksheets are in an electronic format and are updated contemporaneously as the examination is conducted.
3. Once the analysis is completed, the examiner will generate a report through Justice Trax and assign the technical review to the other examiner.
4. Review of these documents is done on the standard technical review/administrative review Quality Records (QD-IM QR1 Check Sheet) and also using the Remote Review Form to address corrections needed. Upon completion of the AR, the final report will be printed out once on-site and the final product created for return to the submitting agency.

Questioned Documents Unit

1. The Questioned Document Unit of the DSS conducts analysis of documents for handwriting analysis, indented writing and determination of alteration of a document. This Unit also conducts security testing for the Connecticut Lottery Corporation. Lottery security tests are conducted on lots of tickets prior to release to public consumer purchase. This testing is involved and it conducted on-site.
2. The forensic analysis of documents is divided into stages that can be conducted on-site and then off-site. The initial examination of a document, testing for indented writing, analysis using the Video Spectral Comparator and scanning of the document to digital format are all conducted on-site. Once the document is in a digital format, comparison of handwriting and the corresponding worksheets may be completed in a remote teleworking environment.
3. The completed analysis worksheets, notes and supporting images are saved to a virtual folder that may be accessed by the technical reviewer for review. The report is generated through Justice Trax in the remote telework setting and the assignments for technical and administrative reviews are assigned and tracked through the LIMS system.
4. Review of these documents is done on the standard technical review/administrative review. Quality Records (QD-IM QR1 Check Sheet) and also using the Remote Review Form to address corrections needed. Upon completion of the AR, the final report will be printed out once on-site and the final product created for return to the submitting agency.

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Appendix A
The following documents are the policies and procedures of the technical work that will be utilized during Telework

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CASE RECORDS AND REPORTS

5.1 PURPOSE

To complete case paperwork and write a report of examination results.

5.2 RESPONSIBILITY

Forensic Science Examiners (however titled) from the Division of Scientific Services who have been trained in case completion and writing of reports according to FB SOP-26 (Training Manual and Checklist) and GL-4 (LIMS).

5.3 DEFINITIONS

A. LIMS: Laboratory Information Management System
B. SAECK: Sexual Assault Evidence Collection Kit

5.4 PROCEDURE

A. A secure and password protected LIMS computer system is used in accordance with GL-3 (Security) and GL-4 (LIMS).

B. The case number and analyst's initials must be on every page in the case jacket. The case number (when necessary to write) and analyst's initials are typically written in the upper right hand corner.

C. Place all completed Quality Record Worksheets in the labeled case jacket.

D. Place the completed General Reagent Sheet (FBQR-09) in the case jacket if reagents are used and the Quality Record Worksheets utilized don't provide a space to record this information.

E. If further examination in another Unit is required, print the appropriate LIMS Evidence Transfer Receipt(s) when necessary. Additional LIMS Evidence Transfer Receipt(s) will be printed for verification purposes when necessary. Include a copy of the print out(s) in the FB case jacket.

F. For cases in which a Forensic Biology report is issued, follow the instructions below. (Forensic Biology reports may not be issued for cases examined according to Case Management Work Instruction-01 (DNA Outsourcing and Sample Prep) or those with only a Biology Knowns request, an Autopsy Sample request, a Forensic Biology Sample Prep request or a 'CT 100' Male Screen Sample Prep request.

G. Place any additional paperwork in the case jacket. Examples may include but are not limited to:
   1. Applicable Casework Checklist Review Sheet (FBQR-12) or Amended Report Checklist Review Sheet (FBQR-12A).
   2. Review Notes Worksheet (FBQR-21)
   3. Evidence Receipt/Request for Analysis Form

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4. LIMS case notes/Emails as needed
5. Conversation/Message Forms

H. Generate a report (including Supplemental and Amended reports) through the LIMS computer system according to GL-4 (LIMS).

Note: Notifications will be generated for closed cases according to section 5.6 (Guidelines for Closed Case Notification Writing).

1. Include the following:
   a. List of evidence with Laboratory submission numbers, include submitting agency item numbers if available. Note: Include only the initials of the victim's name in a sexual assault case report.
   b. Examination method
   c. Evidence description
   d. Results of examination/test(s)
   e. Date and location of off-site examination when applicable
   f. Disposition of sample(s) collected/sub-itemized and preserved for future testing
   g. Disposition of evidence
   h. Report statements (see below)
   i. The signatures of the Analyst and Technical Reviewer
   j. The Analyst's and Technical Reviewer's names and titles
   k. Glossary of Terms and Concepts

2. Maintain the draft report(s) and a copy of the final report in the case jacket.

3. If samples are being forwarded to another Unit for further examination, forward the appropriate paperwork.

4. Supplemental Reports will be identified within the report header.
   a. Include a statement referencing a previously generated report(s).
   b. Maintain the draft report(s) and a copy of the final report in the case jacket.

5. Amended Reports will be identified within the report header.
   a. A cover letter will accompany the Amended Report referencing the original report and what was amended.
   b. The amendment will be identified in bold font or similar, according to GL-1 (Quality Manual).
   c. Maintain the draft report(s) and a copy of the letter and final report in the case jacket.

6. Reports will be sub-itemized in LIMS for return to the submitting agency according to GL-4 (LIMS) and GL-11 (Control of Records).

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I. All paperwork in the case jacket and the report are reviewed by the Technical Reviewer and the Administrative Reviewer (a Lead of the Forensic Biology Unit or designee).

J. The Technical and Administrative Reviewers will check off, date and initial the applicable Casework Checklist Review Sheet (FBQR-12)/Amended Report Checklist Review Sheet (FBQR-12A) and Review Notes Worksheet (FBQR-21). The duties of the Technical and Administrative Reviewers are specified on quality records FBQR-12 and FBQR-12A.

K. The Analyst and the Technical Reviewer will complete the LIMS computer system milestones according to GL-4 (LIMS) when their case review responsibilities have been completed.

L. For additional information, please refer to GL-18 (Case Reviews).

5.5 *SUGGESTED REPORT STATEMENTS*

Serological test result statements, Trace material and Physical match report statements are included in the specific FB SOP.

[ ] = appropriate description

A. Supplemental Report
See Forensic Biology Report(s) dated [ ] for additional information.

B. Examination method
All examinations were conducted macroscopically unless otherwise noted.
Serological results reflect the analysis of only the portion of the sample tested.

May be otherwise noted: *Stereoscopic/microscopic examination was conducted on this item.*

C. General Description
1. *Submission/item # [ ] consisted of one (1)/a Sexual Assault Evidence Collection Kit containing the above-/previously listed items.*
2. *Submission/item # [ ] consisted of one (1)/a [ ].*

D. Tissue-like Material
1. *Tissue-like material(s) was/were located on [ ].*
2. *Tissue-like material(s) was/were located upon/during microscopical examination of [ ].*
3. *No tissue-like material(s) was/were noted on [ ].*
4. *No tissue-like material(s) was/were noted upon/during microscopical examination of [ ].*

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E. No further/serological testing/examination
   1. *No further testing was conducted on this/these [ ] at this time.*
   2. *No further serological testing was conducted on this/these [ ] at this time.*
   3. *No serological testing was conducted on this/these [ ] at this time.*
   4. *No further examination was conducted on this/these [ ] at this time*

F. Not Examined At This Time
   Submission(s)/item(s) # [ ] was/were not examined at this time.

G. Unsuitable for testing/examination
   This [ ] was determined to be unsuitable for further serological testing/further examination due to [ ].

H. Disposition of evidence
   1. Retained
      a. *A sample(s) from submission(s)/item(s) # [ ] was/were retained at the Laboratory.*
      b. *Submission(s)/item(s) # [ ] was/were retained at the Laboratory.*
      c. *Any remaining extracts from the above-tested item(s) are retained at the Laboratory.*
      d. SAECKs and other evidence related to sexual assault cases:
         i. *Submission [ ] will be retained at the Laboratory per Public Act No. 15-207.*
         ii. *Item [ ], as previously listed, was not examined at this time and was retained at the Laboratory. Further examination may be conducted upon request.*

   2. Forwarded/Transferred
      a. *A sample(s) from submission(s)/item(s) # [ ] was/were forwarded/transferred to the [ ]Unit for further analysis/examination.*
      b. *Submission(s)/item(s) #[ ] was/were forwarded/transferred to the [ ] Unit for further analysis/examination.*

   3. Returned
      Evidence not related to sexual assault cases: *Submission(s)/Item(s) # [ ] will be returned to the (appropriate) Submitting Agency.*

I. No suspect
   If a suspect is developed in this case, please notify the Case Management Unit as soon as possible.

J. Request for a known sample from victim/suspect
   A known biological sample is requested from the victim/suspect for comparison purposes.

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K. The following statements are to be included at the end of each report:
   1. ***Further analysis upon request***
   2. *This report reflects the test results, conclusions, interpretations and/or findings of the Analyst and Technical Reviewer as indicated by their signatures below. Results relate only to the items tested.*

5.6 GUIDELINES FOR CLOSED CASE NOTIFICATION WRITING

A. Notifications will be generated for closed cases through LIMS requiring only an administrative review and the author's signature.

B. Notifications cannot be written if serological testing was performed on any item in the case. A REPORT must be written to include the results of the testing.

5.6.1 Author

A. Evidentiary Samples
   1. FB prepped samples
      a. Transfer to 'Freezer Storage' - physically and in LIMS.
      b. Write on the original transfer sheet (from examiner to 'Freezer Storage – DNA Sample' sheet) that case has been closed and the date of closure notification. As always, date and initial.
      c. If the transfer was not previously verified (i.e. prior to 3/5/18) then you must print out the transfer receipt (from you to 'Freezer storage'). This transfer must be verified by someone other than yourself and the original examiner.
   2. DNA Knowns – Not processed by DNA
      a. Physically transfer from storage to self. (If you do not have access, contact Case Management (CM) to complete the next 2 steps.)
      b. Photocopy packaging and place in case jacket.
      c. Transfer Knowns to Evidence Receiving (ER) for return to Agency.
      d. Write on the photocopy that case has been closed, evidence returned to ER/Agency, date and initial.

B. Worksheets
   1. Cross-off the "to DNA" for each sample.
   2. Document: Retained at laboratory for each sample.
   3. Initial and date each entry appropriately.
   4. Add FB QR-12b and FB QR-21 to case jacket.

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5. Initial all pages in case jacket.
6. If additional paperwork was added after the original examiner completed the work, there is no need for the original examiner to initial the additional paperwork.

C. LIMS – General
1. If not already done:
    a. Un-assign and cancel appropriate FB/DNA requests.
    b. Choose "FB Case Closed" request (not "FB-other") and relate submissions and sub-items to the request.
        i. This list will appear on the notification. Remember to include Known Samples in this list.
        ii. Check/edit the request date, as necessary, to reflect when it was requested to be closed. This date will appear on the notification.
    c. Assign "FB Closed Case" request to yourself.
2. Sub-itemize a barcode for the Notification.
    a. Make sure this Notification sub-item is in your name.
    b. Affix barcode and Notification sticker to envelope.

D. LIMS – Creating Notification Document:
1. Highlight the "FB case closed" request line and right click.
2. Click on "Edit findings".
3. In the menu: right click on FB Case Closed.
4. In 'Results Menu' click on "Add Result" Click "Apply".
5. You will see a window temporarily pop us saying "generating word final report document" while the report is being created. Click "Close".
6. The milestone will change to "Findings Entered" in the request line.
7. Highlight the "FB case closed" request line and right click.
8. Choose "Print Final Report" – a window may appear that says "your PC printer is not configured to your machine…" click OK to remove this window.
9. "Print report" window appears, choose screen, click OK and the *Report* template will appear (may need to minimize/move JusticeTrax window).

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10. Click the red "Close Print Preview" button located on the top bar and the document will open in Word (with 'FB Report Tools' tab located on the top bar) – If you do not have the tab, contact your supervisor.
11. Click on "FB Reports Tools" tab.
12. Click "FB Notification" button (located on far right). This will transform the document from the Report Template to the Notification template.

E. Enter Data into DRAFT Notification

1. Use the Notification shortcut buttons to enter the final disposition of each item of evidence.
    a. Place the cursor at the end of each listed item. Click on the appropriate disposition button to enter the disposition next to the item.
    b. If necessary, edit request date to the date that the case was requested to be closed.
    c. Remove "Analyst" from the signature line (Author's name and title only should be beneath the signature line!).
2. SAVE your changes before exiting as usual.
3. REMEMBER: Every time you need to go back to the notification for editing you must choose "Print Final Report" or risk losing the previous edits.
4. If an FB Report was previously written, use the shortcut button to include "See Forensic Biology report(s) dated xx/xx/xx for additional information".
5. All pages need "DRAFT xx/xx/xx", where xx/xx/xx is the draft date. Watermarks are useful for this.
6. Print and initial all pages of Draft Notification.
7. Give to AR for review.

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5.6.2 Administrative Reviewer (AR)

A. Ensures Draft Notification is accurate. Edits hard copy as needed.

B. Reviews Case Jacket and enters comments onto FB QR-21 for Author and Examiner as appropriate.

C. Completes appropriate portions of FB QR-12b.

1. If necessary, gives case jacket to examiner to address FB QR-21 comments.

2. It is NOT necessary to have examiner initial paperwork that was placed in case jacket after the work was completed. (Those placing the paperwork into the case jacket need to initial those pages.) If the examiner's initials are missing from a page that should have been initialed at or around the time of examination, then those pages need to be flagged for the examiner to initial.
3. When necessary, Examiner addresses issues noted by AR on FB QR-12b and returns case jacket to AR.
4. AR completes FB QR-12b and gives case jacket back to author.

5.6.3 Author

A. Write FINAL Notification
   1. Addresses issues noted by AR on FB QR-12b.
   2. Make all corrections to Draft Notification.
   3. Print Final Notification with date.
   4. Sign Notification.
   5. "Draft complete" in LIMS.
   6. Completed Milestone sticker – End Date is same as the date on Final Notification.
   7. Make two (2) copies of Notification:
      a. Copy of Notification (no initials) placed in barcoded envelope.
      b. Copy of Notification (with original) initials placed in case jacket.
   8. Bring to ER:
      a. Original Notification
      b. Barcoded envelope containing copy of Notification.
   9. File case jacket appropriately.

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5.7 REFERENCES

A. GL-1 (Quality Manual).
B. GL-3 (Security)
C. GL-4 (LIMS)
D. GL-11 (Control of Records)
E. GL-18 (Case Reviews)
F. Case Management Work Instruction-01

1.1 General Procedures:

Evidence and samples from evidence, must be collected, received, handled, sampled and stored so as to preserve the identity, integrity, condition, and security of the item.

1.1.1 Persons handling physical evidence will wear gloves, lab coats, facemasks, and other appropriate attire for safety considerations and to prevent contamination. White lab coats are worn in "Pre-Amplification" areas and blue lab coats are worn in "Amplification" and "Post Amplification" areas. Only one piece of evidence is examined at a time.

1.1.2 Change bench covering between cases, or between items if needed.

1.1.3 Single use consumable supplies will be used (when appropriate) for each item. Forceps, scissors, scalpels, and other non-consumables will be cleansed appropriately between items.

1.1.4 Polymerase Chain Reaction (PCR) product and genomic DNA are separated by space.

1.1.5 Physical evidence in the DNA Unit will be handled and documented according to standard DESPP Division of Scientific Services procedures (GL 4 and GL 13) and the FBI Quality Assurance Standards (QAS). All case results are documented on the appropriate DNA records. Evidence sampled for DNA extraction will not be consumed unless testing is expected to require the entire sample. Disposition of evidence and extracts will be documented on reports and in LIMS.

Casework knowns/reference samples submitted for processing are considered evidence and are handled as such according to GL4, GL13, and the FBI QAS.

1.1.6 Amplification products (amplification tubes or plates) are considered to be work product; material that is generated as a function of analysis. Work product is not evidence and therefore not sub-itemized in LIMS or given a barcode. They are not preserved for future testing.

1.1.7 Extracts containing genomic DNA are considered evidence and treated as such, are uniquely identified and are maintained in locked freezers within the DNA Unit. The boxes containing the DNA extracts are sealed with tape and initialled. Evidence extracts have a unique identifier by being labelled with case number and item number. Control extracts have a unique identifier by being labelled with the batch number (initials/date in XXXMMDDYY format). Reagent blanks, extraction positive controls, and evidence extracts are tracked in LIMS with a chain of custody when the samples are preserved for future testing. The extracts will be clearly designated in LIMS; for example, "#001-001-01 - DNA Extract of #001-001". When the extract is exhausted; the extract will be virtually transferred to "sample consumed in testing". For controls (reagent blank/extraction positive control), a "DNA controls" case covering a time period specified in the case name will be made in LIMS where the controls assigned to specific batches will be tracked.

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For example: CONTROLS-2019-2 for the second quarter of 2019. In each case, month names are entered as submissions, and control extracts are added as sub-items of the corresponding month. If the controls are exhausted; they will be virtually transferred to "sample consumed in testing". The extracts and controls will be maintained in storage boxes, and virtually in the "DNA Extract- Long Term Freezer" location in JusticeTrax. In the notes field of each extract and control, the storage box number will be noted. While the DNA extracts are in progress and not in the analyst's custody, they may be virtually placed in the "DNA Extract – Temporary Refrigerator" location, and kept where each analyst has a slot for their extract racks. This is for overnight refrigerator storage if not all steps in the DNA process are completed in one day.

Casework known/reference sample and database extracts are not preserved for future testing, and therefore not tracked. Work product stored in 96-well plates (used for known and database samples) will have a unique identifier to the plate itself, see DNA SOP-12. There will be no separate identifier for controls on these plates.

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1.1.8 Consumption of Evidence (exhaustive testing only)

Per the Connecticut Practice Book (40-9), the defense must be notified in the event of exhaustive testing (if the suspect has been arrested) to determine whether the defense wishes to observe the testing. See CM WI-04: Consumption Letters, for procedures about notification. If the defense wants a representative to be present during the exhaustive testing, the Case Management section or designee will notify the TL/supervisor and the analyst assigned to the case (if already assigned). The Case Management section, designee or analyst assigned to the case will schedule the testing date with the expert. If no arrest has been made, the analyst/technician may proceed with the testing without a defense expert being present/notified. If there is no documentation within the case jacket, return the case jacket to Case Management or designee for confirmation. Consult with the TL or your supervisor with any questions or concerns.

If no suspect is listed on the evidence receipt, the analyst/technician may proceed with the testing. Case Management will determine arrest status prior to an analyst/technician receiving a case. There is no need for any further confirmation by the analyst or technician.

1.1.9 dH_2O is defined as de-ionized water. The source of this water can be from the laboratory's in-house purification system or Molecular Biology Grade Water from an approved vendor. Molecular Biology Grade Water is manufactured and certified to be de-ionized, purified and DNase free. dH_2O used for sample preparation through DNA amplification is lot tracked and quality tested before use in case work.

All casework analysis is conducted using the appropriate lot-tested reagents as defined in DNA SOP-6 and DNA SOP-8. Reagents are lot tested before their use in casework.
Reagents are not used for casework analysis or validations past their control date (expiration date). Reagents may be used for research purposes past their control date where appropriate and

are labelled as such.

1.1.10 When examining evidentiary samples, DNA analysts (qualified in collection of evidence samples) will use Forensic Biology protocols found in Qualtrax. Refer to: FB SOP-01: Evidence Exam, FB SOP-03: Guidelines-Collection & Forwarding, and GL 4 LIMS. Physical Evidence Worksheets DNA QR-1 and DNA-QR-1A may also be used to document evidence and examination. Please see GL 4 LIMS for details on itemizing in JusticeTrax.

1.1.11 A complete list of all applicable DNA Unit abbreviations can be found below in APPENDIX II.

Note: The words submission and item are interchangeable unless describing a sub-item taken at the laboratory. (i.e. An evidentiary sample may be called a submission in a Forensic Biology report but called an item in a DNA report).

1.1.12 DNA analysts can perform Forensic Biology screening and confirmatory tests for which they are authorized. Qualified DNA analysts will follow Forensic Biology protocols. These protocols are found in Qualtrax. Refer to FB SOPs 1 through 27 and FB Appendices 1 and 2.

1.1.13 Prior to beginning an extraction, all samples retrieved for DNA testing will be verified for correct labeling and contents by a second individual (however titled).

The first individual will review the samples retrieved and verify that the labeling and contents agree with the LIMS evidence transfer sheet. They will initial and date the evidence transfer sheet to document their review.

The second individual will review the samples retrieved and verify that the labeling and contents agree with the LIMS evidence transfer sheet.

a. If in agreement, the second individual will initial and date the LIMS evidence transfer sheet.
b. If the second individual discovers a discrepancy, the Technical Leader, Assistant Director and/or Deputy Director will be informed and the root cause will be determined and corrected.

Further appropriate action may be taken by the Technical Leader, Assistant Director and/or Deputy Director.

The initialed and dated LIMS evidence transfer sheet(s) (or copy) will be retained in the appropriate case jacket(s).

Once verified, the samples can proceed to DNA extraction.

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1.2 Preparation of Discovery Materials (in conjunction with GL-11, Control of Records):

1.2.1 It is preferred that discovery requests are made in writing at least 1 month prior to the anticipated court date. Discovery materials (official laboratory receipts and records) will be made available to authorized individuals according to DESPP policy. Any sample identifying information not pertaining to the case in question will be obliterated. No additional information is to be added to the discovery copy of the original case material. The requested information will be duplicated, scanned, and maintained on the "dps domain" shared drive (S:). Once on the S: Drive, the duplicated hard copy will be shredded. In addition, all requested electronic data will be maintained on the S: Drive. The case jacket records and electronic data (if requested) maintained on the S: Drive will be the retained copy of the discovery materials at the laboratory. A designee will send the discovery materials to the requesting agency. Release of case material will be noted in the case file. A fee may be charged to cover administrative costs. Requests for discovery that are non-case specific will be performed on site at the laboratory. Please refer to CM WI-06 Discovery and FOIA Requests for further details.

1.2.2 Preparation of GeneMarker electronic data sample files and project files for discovery: The samples applicable to the discovery request are selected by opening the saved project with GeneMarker, and then selecting the applicable samples, controls, and ladders by double clicking on them. Next, select 'Save Selected Samples' making sure to save the project for discovery with a name that is different from the original file name (e.g. original project name_case#discovery; save on S: Drive in Case Management's designated folder).

1.2.3 Preparation of GeneMapper (ID and IDX) electronic data sample files and project files for discovery: The project containing samples for discovery is opened, and all files not associated with the case in question are deleted from the project. "Save Project As" should then be selected, and given a name different from original project name. Using the GeneMapper Manager, the newly created project shall be exported to the appropriate folder on the S: Drive in Case Management's designated folder.

1.3 DNA Analysis Workflow:

Upon receipt and documentation of any item for DNA analysis, the first step is to extract the DNA and estimate the quantity of human DNA recovered. The quantity of human DNA is estimated using a human qPCR kit or equivalent for question samples. The Nuclear DNA typing system is fluorescent STR or Y-STR analysis.

Personnel not qualified in DNA analysis (i.e. "technicians") may not make decisions about sample processing, such as which samples to halt or move forward to amp, or reinject. This is in accordance with the QAS. Qualified analysts will review DNA quantitation to create halt at

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quantitation and amplification QRs, and inform technicians of any samples in need of re-work, including re-injections or re-extractions.
The starting date of DNA testing for a request is documented on the DNA Extraction Worksheets for the particular request. Testing is completed on the date of the draft report for that particular request.

1.4 Report Writing:

DNA results are reported according to standard DESPP Division of Scientific Services guidelines, ANAB ISO 17025:2017, and the current FBI DNA QAS. Please refer to GL-18, Case Reviews. Reports are signed by two individuals. The signature on the left is the analyst responsible for the case. The signature on the right is the technical reviewer of the case.

1.4.1 Final reports will include DNA typing results and a qualitative interpretative statement. A quantitative statement is given where appropriate. Please refer to DNA SOP-6 and the DNA Report Template file for general report statements.

Note: Casework forensic samples and knowns, processed using different STR kits, may be compared and interpreted at overlapping loci.

1.4.2 The results from each item tested are included in the DNA report. Conclusions are required in the DNA report. At a minimum, a qualitative statement will be made for every comparison. A statistical statement will also be made where appropriate. A DNA report is not issued until the work on a set of samples tested is completed and reviewed.

1.4.3 Evidence examined by a DNA analyst authorized in Forensic Biology methods:

If the evidence was examined and forensic biology tests were performed by a DNA analyst authorized in those testing methods, the results may be incorporated into a DNA report. Refer to FB SOP-05 for Forensic Biology result statements or DNA SOP-6.

1.4.4 All DNA Reports will clearly communicate all items tested and those not tested, all evidence and extract dispositions, all eliminations (e.g., elimination of a victim or other known source) when comparisons are made, and all profiles which were entered into CODIS or which were not suitable for CODIS entry. Any updates to the level of a CODIS entry (e.g. SDIS, NDIS, deleted from NDIS and/or SDIS) will be communicated, please refer to DNA SOP-13 CODIS Profile Entry and Data Bank.

1.4.5 Draft reports, while being worked and/or in the review process, should be located on the U: Drive in the case reports folder in the subfolder for the proper year of the case.

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1.4.6 Draft reports that have not been issued, will have a date (may be handwritten) to reflect the "end of testing" until the final report is released.

1.4.7 Final Report

1.4.7.1 The draft report is edited, and then changed from a draft report to the final report to include the report date. The report is considered finalized when the primary analyst makes all necessary edits to include adding a date to the report. This is saved as the final report (without signatures) on the U: Drive.

1.4.7.2 The final report (with signatures) will be scanned as a PDF, and saved to the U: Drive in the case reports folder and the subfolder for the proper year of the case. This PDF will also be uploaded to LIMS.

1.4.7.3 The original final report with signatures will reside in the case file in evidence receiving.

1.4.7.4 A copy of the final report with signatures will reside in the case jacket.

1.4.7.5 After a case is "draft complete" in JusticeTrax, the analyst will assign a technical review request to a Technical Reviewer. When the Technical Reviewer has completed their technical review of the case, they will assign an administrative review request to the Administrative Reviewer. Upon completion of the administrative review, the request will be released in JusticeTrax. This release will be confirmed by the analyst and documented on the appropriate QR.

1.5 General Considerations for PCR Amplification of Evidentiary Genomic DNA:

1.5.1 All amplification reactions for evidence samples and controls must be prepared in a PCR hood.

1.5.2 Gloves, masks, and lab coats must be worn at all times when working in PCR set-up.

1.5.3 All reagents and microfuge tubes must be opened with caution. At no time should open tubes of reagents be removed from the laminar flow hood.

1.5.4 For all mixing and pipetting pertaining to PCR set-up: only use designated pipettes from the PCR set-up hood. At no time should these pipettes be removed from the set-up area, except for calibration checks. Pipettes from other areas of the lab should not be brought into PCR set-up.

1.5.5 Aerosol blocking pipette tips shall be used at all times when preparing PCR reactions.

1.5.6 At no time should any amplified product be brought into the PCR set-up area.

1.5.7 All PCR reactions must be prepared using designated "PCR set-up only" microfuge tube racks.

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1.5.8 Prior to setting up any PCR amplifications, the working area (laminar flow hood) must be cleaned with a bleach solution made in-house or 10% stabilized bleach followed by ethanol and subjected to UV irradiation for at least 15 minutes. See DNA WI-21 for 20% Bleach formulation.

1.5.9 A PCR master mix is prepared and dispensed to each tube prior to the addition of DNA. Limit the time PCR reagents are out of storage for long exposure of light and incorrect storage temperatures could negatively affect the reagents.

1.5.10 After PCR reactions are set-up; the work area must be cleaned as described in 1.5.8.

1.6 Controls for DNA analysis:

Four types of controls are processed with each case:

1.6.1 Reagent Blank (RB). A reagent blank (RB) is processed with each set of extractions. The extract volume of a RB must be at or below the extract volume of all samples for which it controls. During a manual extraction, after measuring the volume with a pipette, if a sample elutes at a lower volume than its RB, dH_2O is added to the sample to raise it to the volume of the RB. Extractions performed on automation instruments where elution volumes are set, such as the EZ1 or Biomek instrument, are an exception to this. After the extraction and elution, visual inspections of all elution volumes are made. If any visual discrepancies are noted, a manual measurement of all the extracts in the set is conducted. If it is determined that an adjustment is needed, dH_2O will be added to the sample to bring the final volume to 50µL (as intended by the program setting). Any adjustments made to the samples will be recorded on the corresponding DNA QR sheet. The volume of the RB used for the amplification step must be the same as (or greater than) the maximum volume used to amplify the evidentiary samples. Re-amplification of the RB may be omitted when re-amplifying the same volume (or less) of the evidentiary sample(s). When re-amplifying a greater volume of the evidentiary sample(s), the RB must be re-amplified with that volume. With differential extractions, the RB is subjected to the same processing steps as the evidence.

If multiple amplification test kits are to be used and the reagent blank associated with the extraction set or sample being amplified has been depleted, continuation on to a different amplification test kit shall not be performed. This is specifically for DNA extracted after July 1, 2009. Multiple RBs may be processed in a single extraction set to avoid this situation; if this is done, each RB may control for any sample in the extraction set. RBs for an extraction set should be used for testing in order of highest signal to lowest.

The reagent blank shall be extracted in parallel and concurrently with its respective samples.

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1.6.2 Lab Positive Control. A positive control (EP1) is processed for each set of extractions. This positive control is carried through all STR analysis steps. The EP1 is also considered an amplification positive control that is used to determine if the PCR performed properly. The EP1 may be omitted for re-amplifications if expected results were previously generated for the extraction set for that kit.

1.6.3 Negative Control. An amplification blank (NEG) is processed with each set of amplifications. The maximum volume (dH_2O) possible for each kit is used for the amplification step. When practicable the same lot of dH_2O used in extraction/dilution is used for amplification.

1.6.4 Kit Positive Control (POS). A kit amplification positive control is processed with each set of amplifications.

1.6.5 Expected results of controls (correct genotype) will be verified by the analyst and technical reviewer and documented on DNA QR-4A, QR-4b, or QR4c which is maintained in the case file. Amplification and extraction positive controls are verified using DNA-QR-37. The corresponding QR worksheet is maintained in the batch paperwork. Instructions to use the worksheet is within the excel workbook. When using a positive extraction control besides EP1 (i.e. KJL, RKO, TMP), the control may be checked against the known genotype of those controls and documented in the batch paperwork and on the review worksheets in the case file.

1.7 Contamination:

Contamination is defined as the introduction of a secondary source of DNA (genomic or amplified) into a sample at the DESPP Division of Scientific Services. This is to be distinguished from sample mixtures or contamination at the time of collection, which may require different action.

See below for examples of contamination prevention methods during DNA analysis of forensic unknown samples:

1. Kimwipe method: Use a fresh Kimwipe or a portion of a Kimwipe to open and close flip-top tubes.

2. Tube Opener method: Use a plastic tube opener to open tubes. Bleach tube openers between uses.

All laboratory personnel are to supply a known sample for elimination purposes prior to working in the lab. If a known sample is not submitted by an individual, that person cannot work on evidence involving DNA requests.

If contamination is detected, review the results to determine the source of the contamination. Perform appropriate corrective measures as warranted by the nature/source of the

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contamination. Each incident of contamination (peaks ≥ AT for casework items and knowns) will be documented and maintained by the Quality Section as workflow instances in Qualtrax. The TL, CODIS Admin, Assistant Director and Deputy Director will be notified of all incidents of contamination through the workflow in Qualtrax. If a QAR is required, it will be approved by the TL. Please see DNA-SOP-39 and DNA SOP-40 for more details. A quarterly review of contamination will be performed and shared with the FB/DNA Section.

All applicable profile results are checked against the staff index (see DNA SOP-13)

If systemic contamination is detected:

1.7.1 Discard any reagents that are contaminated.

1.7.2 Thoroughly clean the affected lab area.

1.7.3 On occasion, when objects/equipment are repeatedly identified as contaminated, after a thorough cleaning, set up blank reactions to verify that the contamination has been eliminated. Do not conduct DNA testing of case samples until the situation is rectified.

1.7.4 Review the data and the analytical techniques of the analyst(s) in question and take remedial action as required.

1.8 Monitoring, cleaning, and decontaminating facilities and equipment:

1.8.1 DNA testing facilities and equipment are monitored, cleaned, and decontaminated (when applicable) per FBI Quality Assurance Standards (Standard 6) and laboratory SOPs.

1.8.2 The EZ1extraction robots have maintenance worksheets with each instrument. Cleaning before and after use is required and is documented on the respective worksheets.

1.8.3 For each set of amplifications, document the batch number, the results of the controls and cleaning performed on worksheet DNA QR-8.

1.9 Case documentation:

Only official case receipts, technical and administrative records shall be maintained in the DNA case file. Where appropriate, case results and records shall be documented on controlled worksheets. See SOP-23 “Case Documentation and Review” for specific procedures. In addition, phone calls and email correspondence can be maintained in the DNA case file and notes are made in LIMS. For STR analysis, the electropherograms are included in the case folder with the exception of convicted offender samples. All electronic files regarding STR analysis (Complete run folder, GeneMarker HID, GeneMapper ID, GeneMapper IDX, sample

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and project files: .sgf, .fsa, .hid, and .ser files) are archived on optical disks. In addition, the U: Drive is backed up on a regular basis. See APPENDIX I below for more detail in regards to Archiving Data.

1.10 Case review:

Definitions as per the current FBI QAS:

Technical Review: is an evaluation of reports, notes, data, and other documents to ensure that there is appropriate and sufficient basis for the scientific conclusions.

Technical Reviewer: is an employee who is a current or previously qualified/competent analyst in the methodology being reviewed and is not an author of the applicable report. The Technical Reviewer is required to have been competency tested and authorized for the process they are reviewing and has had casework experience prior to performing any technical reviews. The Technical Reviewer must be qualified in the method, technology, typing test kit, platform and interpretation software that the review encompasses.

Administrative Review: is an evaluation of the report and supporting documentation for consistency with laboratory policies and editorial correctness.

Administrative Reviewer: is an employee who is not an author of the applicable report.

See DNA SOP-23: General Procedures for Case Documentation and Review

1.10.1 All DNA cases and reports are technically (100%) and administratively (100%) reviewed. The technical review function is performed by the technical reviewer and documented on DNA QR-4, QR-4b, or QR-4c. For batch paperwork, the technical review is documented on DNA QR-4A or QR-4E.

Technical reviews are performed on all case files prior to reports being issued. Technical reviews are performed by any qualified analyst to verify that the conclusions and data are scientifically supported through the documentation in the case file.

Administrative reviews are performed on all case files prior to reports being issued. Administrative reviews are performed by any laboratory personnel who have been trained to review DNA cases and have access to LIMS. These reviews are used to ensure that the case demographics are correct, there are not typographical errors, and the documentation is consistent with laboratory policies.

In addition, a DNA analyst trainee who has been approved to be an administrative reviewer may AR cases where interpretations are reported. These reviews are used to ensure that the

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case demographics are correct, there are not typographical errors, and the documentation is consistent with laboratory policies. For outsourced cases where there will be no ownership in the data, QR-4d is used. Please see DNA SOP-21 for further outsourcing guidance.
Note: No analyst may technically review their own work.

1.10.2 For male-screen batch paperwork, the technical review and administrative review are completed simultaneously by one qualified analyst.

1.10.3 In the event of discrepant conclusions, the examiners will discuss/review the results. Should a discrepancy persist, the data will be reviewed and conclusions approved by the Technical Leader (TL).

1.10.4 A DNA case file review (independent of QAS Standard 12 and Standard 15) will be conducted by the TL on a quarterly basis. The scope of each quarterly review will be defined and approved by the TL. The scope may change based on factors such as corrective actions or interpretational issues as needed. Where practicable, one case per analyst will be reviewed quarterly by the TL. In general, a representative sampling of cases will be selected for TL review that cover the types of cases and range of tasks that are typically encountered by the DNA Unit. Typically, cases will be pulled by Case Management based on the types of cases submitted (property crimes, sexual assaults, homicides, etc.) during the quarter. The list of cases for review will be emailed to the TL for pre-approval. The TL will email the Quality Manager/Deputy Director/Assistant Director to document when the review has been completed and note if any problems/issues were identified. That email will be printed and maintained in a designated binder. Further investigation/remediation will occur as appropriate.

A representative sampling of DNA database sample processing records will be reviewed by the TL on a quarterly basis (independent of QAS Standard 12 and Standard 15). The scope of each quarterly review will be defined and approved by the TL (documented via email). In general, DNA processing records for 1 plate (~ 86 samples) per quarter will be reviewed by the TL. The TL will email the Quality Manager/Deputy Director/Assistant Director when the review has been completed and note if any problems/issues were identified. That email will be printed and maintained in a designated binder. Further investigation/remediation will occur as appropriate.

1.10.5 If you are taking ownership of a case that had a previous report in which you were not the signatory or the Technical Reviewer, a review must be completed of the relevant examination and supplemental records, as well as previous conclusions drawn that are necessary to review in order to write the additional and/or associated reports for that case. In addition to appropriate initials on supporting paperwork, this additional review will be documented on DNA QR-315 and maintained in the case jacket.

1.10.6 If you are to testify to a report that references known samples not processed/analyzed by you, a review of the case record(s) pertaining to those known/reference samples processed, including

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electropherograms and data projects, must be reviewed. Your agreement with the results reported for the known/reference sample is to be documented on DNA-QR-316, and maintained in the case jacket. In addition, initials will be added to all paperwork reviewed to support this, if not already added during the initial case review.

1.10.7 In the circumstance where the original analyst and Technical Reviewer are not able to testify to their report, the analyst to testify must complete a full technical review of the case record. This includes all electropherograms, data projects, and conclusions reported out. Your agreement with the results reported is to be documented by initialling all reviewed paperwork, and on DNA-QR-317, and maintained in the case jacket.

1.11 Corrective Action:

Where warranted, corrective action will be taken as outlined in the Quality Manual GL-9. Any corrective action in the DNA Unit shall be approved by the TL (documented in Qualtrax through a DNA- Corrective Action Workflow) prior to implementation. The TL has the authority to initiate, suspend, and resume DNA analytical operations for the DNA Unit or an individual.

1.12 Laboratory Safety protocols:

Laboratory safety protocols are outlined in the Division of Scientific Services Quality Manual GL-2. The DNA health and safety program is reviewed annually by the TL and documented on QR-258. The program includes (1) a blood borne pathogen and chemical hygiene plan and (2) documented training on the blood borne pathogen and chemical hygiene plan.

1.13 Quality Manual:

General DESPP, Division of Scientific Services protocols are outlined in the Quality Manual (GL 1-22 and subcomponents). The DNA Unit follows accordingly to these SOPs.

GL 1 (Quality Manual)
GL 2 (Safety)
GL 3 (Security)
GL 4 (LIMS)
GL 5 (Ethics)
GL 6 (Purchasing)
GL 7 (Audits)
GL 8 (Management System)
GL 9 (Quality Action Requests)
GL 10 (Customer Inquiries)
GL 11 (Control of Records)
GL 12 (Evidence Receiving)

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GL 13 (General Evidence Handling)
GL 14 (General Training)
GL 15 (Professional Development)
GL 16 (Proficiency Tests)
GL 17 (Court Monitoring)
GL 18 (Case Reviews)
GL 19 (Document Control)
GL 20 (Review of Requests and Tenders)
GL 21 (General Lab Equipment)
GL 22 (Policy on Validation and Performance Checks)

1.14 DNA Analyst Training and Continuing Education:

1.14.1 Transcripts and educational qualifications of all analysts are documented and maintained in each analyst's professional development file by the Quality section which are approved by the Technical Leader and documented by memo. Such documentation may also be found in Qualtrax in the analyst's electronic professional development file.

1.14.2 All DNA analysts are trained prior to assuming casework duties as outlined in the Quality Manual, the DNA Training Manual, and the FBI QAS. If appropriate and necessary, the training period may be extended, or an analyst may undergo retraining following guidance in GL-14 and DNA SOP-7.

The TL will assess and document any adjustments to the established training program with aid of the Training Coordinator. DNA analysts will successfully complete a qualifying test prior to independent casework analysis. The TL is responsible for the oversight and approval of training in the DNA Unit and will document the approval by memo. The current DNA Training Manual is found in DNA SOP-7.

1.14.3 Proficiency Tests are samples submitted to the laboratory by an external source, usually a qualified commercial vendor with ISO 17043 accreditation. Individuals taking the test do not know the results.

Competency Tests are samples that have either been prepared internally or received from an outside source. The Training Coordinator or designee knows the results for the samples but the analyst conducting the test does not. Each analyst must successfully complete a competency test prior to being assigned casework that covers the spectrum of anticipated activities related to the work the analyst will perform. Per the Quality Manual, competency tests include the following:

1. The examination of sufficient practical samples to cover the anticipated spectrum of assigned duties.

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2. A written report (if applicable) to demonstrate the individual's ability to convey results and the significance of the results.

3. A written or oral examination, which assess the individual's knowledge of the discipline.

4. Court Testimony.

Competency memos recommending authorization for individuals to conduct independent forensic DNA testing/analysis or databasing using the applicable methods, technology, typing test kits and platforms shall be approved by the TL. Personnel who review and authorize results, an opinion or an interpretation or technical review of results or testimony shall be competency tested as above. The authorization for technical review may be concurrent with authorization as an analyst or as a separate authorization but will be clearly addressed. Authorizations are approved by the Director through a Qualtrax workflow.

1.14.4 Proficiency testing is used to demonstrate the quality of the scientific service offered by the laboratory and it serves as a mechanism for critical self-evaluation. The test due date is the date used to indicate when a DNA proficiency test is performed.

The TL is responsible for the oversight and approval of the proficiency testing program in the DNA Unit. A Qualtrax workflow "DNA Proficiency Testing" is used to capture the proficiency results, TL notification of results, and analysts' notification of their results.

All DNA analysts will undergo external proficiency tests semi-annually (twice a year) as outlined in the Quality Manual (GL-16) and the FBI DNA QAS. A report will be written for the proficiency test and kept in the case file. Semi-annual requires testing to take place two times during one calendar year, with the first event taking place in the first six months of that year and the second event taking place in the second six months of that year, and where the interval between events is at least four months and not more than eight months.

If the analyst is qualified in only one technology then the analyst will take both semi-annual tests in that technology. All applicable samples in a single proficiency test shall be worked for each technology. It is permissible for multiple technologies to be reported on a single proficiency test. Alternatively, an analyst qualified in multiple technologies may be separately tested in each technology. For example, the laboratory may administer one test in the first half of the year with their YSTR technology and one test in the second half of the year with their autosomal STR technology.

1.14.5 DNA analysts will complete at least 8 hours of continuing education each year as outlined in the FBI DNA QAS. Continuing education documentation and necessary approvals per the FBI DNA QAS are maintained in Qualtrax through the "DNA Continuing Education" workflow. The

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analysts may self-enter their CE and the workflow will automatically send the information to the DNA TL and their lead. Pre-approval for webinars and other various items (i.e. sign in sheets for internal training) are kept in a binder in the Training Coordinator's office. CV's of outside lecturers are kept on the U: drive in a specific folder.

Please note: As electronic record keeping is phased in and paper format phased out, there may be some information still maintained in paper format. Eventually, all Professional Development files and future Continuing Education documentation are to be kept electronically.

Analysts will maintain familiarity with the scientific literature of their field by reading and/or reviewing pertinent articles. In general, analysts should try to read 4-6 articles a year (minimum is 1 article per year by FBI QAS). This program will be reviewed and approved by the TL, in association with the Training Coordinator, and documented using DNA QR-30, kept in a binder by the Training Coordinator.

Continuing education in topics relevant to the field of forensic or databasing DNA analysis may include seminars on new methods and techniques for obtaining DNA profiles, lectures on troubleshooting current methods or techniques, courses on providing testimony on DNA results and conclusions, as well as the QAS auditor training or relevant CODIS training.

Reading of scientific literature and subsequent lab-sponsored discussions (e.g., journal club, article presentation) do not count toward the continuing education hours. Activities required as part of the laboratory's training program and/or that are required for establishing an individual's competency do not count toward the continuing education hours.

Regional, national, or international conferences related to forensic or biological sciences that include presentations relevant to forensic or databasing DNA typically provide sufficient content to satisfy the continuing education requirement. The program agenda, record of presentations, or curriculum vitae of presenters is not required for regional, national, or international conferences.

The technical leader must approve the use of multimedia or internet delivered programs to satisfy continuing education hours. The approval of multimedia or internet delivered continuing education, to include the QAS auditor training or relevant CODIS training, may be documented for the specific course or may be documented for each individual completing a course. Completion is documented through Qualtrax to include the time required to complete the program. For multimedia training that is internally generated (e.g., video recording of an internal lecture), technical leader approval and the time needed to complete the training may be documented prior to or with the dissemination of such training.

1.14.6 The Training Coordinator will work with Quality Section, the Technical Leader and CODIS State Administrator to ensure appropriate levels of training and competence are maintained for DNA Unit personnel. The training program found in DNA SOP-7, to the extent necessary based

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on job function, shall include criteria for acceptable performance. Retraining will occur if acceptable performance is not found. The Training Coordinator will maintain documentation of education and training processes in the laboratory.

1.15 Method Validation:

The DNA Unit shall only use validated methodologies for DNA analyses and computer software programs that affect the results of testing. Any software program developed in the laboratory will also have a planned validation.

Before any new/updated method is implemented for casework, the DNA Unit must perform an internal validation to demonstrate the reliability of the procedure in-house.

Validation is the process used by the scientific community to acquire information to assess the ability of a procedure to obtain a desired result, determine the conditions under which such results can be obtained and to determine the limitations of the procedure. The validation process identifies the critical aspects of a procedure and must address the quality assurance parameters and interpretation guidelines for the procedure.

Prior to the start of a validation, a validation plan must be written and approved by the Technical Leader. Internal validations (according to the FBI QAS and the Quality Manual), if applicable, must include the following criteria:

1. Reproducibility/Concordance
2. Precision
3. Sensitivity and stochastic studies
4. Mixture studies
5. Knowns and Non-probative casework or Mock Evidence.
6. Contamination assessment
7. Associated data interpretation
8. Data required reporting a result, opinion or interpretation
9. Identify the limitations of a method, reported results, opinions and interpretations

During the validation process, additional testing may be necessary which can alter the validation plan. Please refer to GL-22 “Policy on Validation and Performance Checks” for more details and other necessary requirements for validations.

Validation Summaries should capture what was tested and the results of the testing. The validation results will be reviewed and approved by the TL. In addition, all methods, work instructions, and SOPs are approved by the TL. The Assistant Director and/or Deputy Director will also review validations and SOP changes. The Director will be the final approver of the validation of a new method as described in GL-22.

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DNA validations will support that all DNA test methods that involve the comparison of an unknown to a known shall require the evaluation of the unknown to identify characteristics suitable for comparison and, if applicable, characteristics suitable for statistics prior to comparison to one or more known items.

1.16 References: DNA Unit:

All DNA protocols are based on standard techniques of molecular biology and forensic science, and have been extensively peer reviewed and conform to the general practices of forensic and molecular biology. Please refer to the controlled DNA Manual list found in Qualtrax.

NOTE: Various other scientific articles and references are kept by the Training Coordinator. Developmental Validations of methods used in the DNA unit are kept on the dps-dnasrv01 (DNA Server).

1.17 Equipment Maintenance and Calibration Checks:

All equipment used for casework analysis is maintained in proper working condition. Any equipment in need of repair or out of calibration is tagged and will not be used for casework until repairs/calibrations are completed. If necessary and depending on the situation, an incident report may be needed.

Performance checks are performed on critical equipment annually. A performance check is typically done following the annual (service contract) preventative maintenance (if applicable) or after a repair of an instrument to ensure the instrument is operating within normal parameters and gives the expected results, prior to the instrument being put back online for casework analysis. (Refer to DNA SOP-9).

Instrument Performance Checks will be documented on the respective DNA Quality Record referenced in DNA SOP-9 and kept in its respective "Performance Check" binder by the DNA Unit.

1.18 Audits:

In accordance with the FBI QAS standards, the DNA Unit of the laboratory will be audited using the most current standards annually. Every other year, the audit must be performed by personnel external to the Division of Scientific Services in accordance with Standard 15 of the FBI QAS documents. Per the FBI QAS, "the required annual audit shall, at a minimum, occur once every calendar year and shall be at least 6 months but no more than 18 months apart."

Internal audits will be conducted by the CT Division of Scientific Services DNA audit team using the current FBI QAS audit document in accordance with guidelines established in the

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Laboratory Quality Manual (GL 7). The DNA audit team will consist of at least one person that is, or has previously been, a qualified DNA analyst for each specific DNA technology performed (STR and Y-STR) and platform used and at least one person who is a qualified auditor who has successfully completed the FBI QAS auditor training course. The qualifications of each audit team member are documented on Appendix C of the QAS. The internal audit review/team will be documented on DNA QR-257A and B. Internal Audits shall include direct observation of DNA personnel performing a sampling of the spectrum of services provided.

The audit documents (internal and external) are maintained for a minimum of ten years by the Quality Section.

If any difficulty arises in scheduling an external audit during the required year, the laboratory will immediately notify the NDIS Custodian and the NIJ Grants Program Manager of the nature of the problem scheduling the external audit.

The TL will annually review the DNA Quality System (independent of the annual audit) and document the approval on DNA QR-258. This document will be kept indefinitely by the Quality Section.

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1.19 Outsourcing:

The Connecticut Department of Emergency Services and Public Protection Division of Scientific Services (CT DSS) may contract or subcontract forensic DNA casework samples to a Vendor Laboratory (e.g. Bode, DNA Labs International). If samples are outsourced to a Vendor Laboratory, this Vendor Laboratory and CT DSS shall comply with the *FBI Quality Assurance Standards for Forensic DNA Testing Laboratories* and the *FBI Quality Assurance Standards for DNA Databasing Laboratories (QAS)* and the accreditation requirements of federal law. The vendor laboratory shall provide documentation of this compliance to the CTDSS. Documentation of this compliance will be maintained by the Quality Section (Refer to DNA SOP-21 and GL 1).

1.19.1 DNA laboratories outsourcing DNA sample(s) to a vendor laboratory or accepting ownership of DNA data from a vendor laboratory shall have and follow a procedure to perform an onsite visit(s).

The CTDSS procedure includes, at a minimum, one of the following elements:

1.19.1.1 A documented initial on-site visit prior to the vendor laboratory's beginning of casework analysis for the CTDSS. This on-site visit is performed by the DNA TL or a designated employee of the CTDSS who is a qualified or previously qualified DNA analyst in the technology, platform and typing amplification test kit, used to generate the DNA data. If the on-site visit is performed by the DNA TL or designated employee of the CTDSS, at a

minimum, Standard 17 of the FBI QAS will be printed out and completed by the analyst performing the visit. Any discrepancies or issues found will be documented and reviewed.

1.19.1.2 Instead of a CTDSS employee performing the initial on-site visit as stated in above, the CTDSS Technical Leader may accept an on-site visit conducted by another NDIS participating laboratory (within 12 months) using the same technology, platform and typing amplification test kit, for the generation of the DNA data. If the on-site visit is performed by another NDIS laboratory, the DNA TL will document the review and approval of the on-site visit. The date the onsite visit was performed, a summary of the visit and the personnel who performed the on-site visit will all be maintained.

1.19.2 If the outsourcing agreement extends beyond one year, an annual on-site visit shall be required. Each annual on-site visit shall occur every calendar year and shall be at least 6 months and no more than 18 months apart. Either element described above may be followed. All reviews and approvals by the DNA TL will be documented.

1.20 Work Instructions:

Work Instructions are defined as documents containing detailed instructions that specify exactly what steps to follow to carry out an activity. A work instruction is more detailed than a SOP and is only created if more detailed instructions are necessary. Work Instructions may be referenced in specific SOPs or may be appended to the end of an SOP. Work Instructions will be treated as a controlled document.

1.21 Facility/Security:

The laboratory security protocol is outlined in GL 3 Security. The laboratory's approach for maintaining the integrity of evidence is outlined in GL13 General Evidence Handling.

1.22 Technical Records:

Please see GL11 Control of Records for more details on technical records. Below are some of the specific requirements found in GL 11.

1.22.1 All records of observations, data or calculations made while performing specific tests shall be documented at the time they are made, within the constraints of reasonable and accepted scientific practice.

1.22.2 If an observation, data, or a test result is rejected, the reason, the identity of the individual(s) taking the action and the date shall be recorded in the technical record.

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1.22.3 Testing dates shall be clearly document in the case records. Testing dates may be reflected as a range of dates or the date of individual test performance.

1.22.4 All pertinent records generated during the course of laboratory analysis shall be maintained in the case file or within a batch file.

1.22.5 It is incumbent upon case analysts to enable the test to be reproduced under conditions as close as possible to the original and to enable a competent analyst to come to the same conclusion and/or results. In addition, all personnel responsible for the sampling, performance, and review processes of these tests shall be readily identifiable in case documentation within the casefile. For units that perform batch analyses, casefiles will have documentation that shows where records can be located when not based in casefiles. Case documentation shall be clear as to which samples are associated with which batches. The batch documentation will be maintained per unit guidance and in a manner similar to case files.

1.23 Reinterpretation of Legacy Data

1.23.1 Reinterpretation of legacy data is defined as re-evaluating allele/base calls, genotype calls (to include potential allelic drop-out), changing the assumptions used, or removing alleles (or entire loci) from statistics estimates. Generating a report for the comparison of two profiles as a result of a CODIS high stringency match is not considered reinterpretation of legacy data. Likewise, if the interpretation of the DNA profile has been previously documented regarding the genotypes of possible contributors, that interpretation is not considered reinterpretation.

1.23.2 DNA analysts qualified (or previously qualified) in STR, Y-STR and/or mtDNA technology, may be authorized to reinterpret data from a legacy STR, Y-STR and/or mtDNA amplification kit(s). The elements of the analyst's training (listed below) for reinterpretation of legacy data are documented on DNA QR-305. TL approval is documented on DNA QR-305, which is emailed to the Quality Manager and Assistant Director/Deputy Director. The approval to reinterpret legacy data is good for a period of two years.

1. The analyst will review the applicable portions of DNA SOP-33 (Interpretation of STR profiles from Legacy Protocols) and/or DNA SOP-43 (mtDNA Legacy) and the applicable portions of DNA SOP-29 (Stutters and Controls for Legacy STR kits).

2. Legacy kit validation data covering non-probative casework, mixture interpretation, kit sensitivity, reproducibility, thresholds, and stutter will be reviewed by the analyst.

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APPENDIX I

Data Archived

All 3500 data files (.hid) and GeneMapper projects (.ser), GeneMarker projects (.sgf), GeneMapper IDX projects (.ser), and STRmix data containing case data are periodically (2x a year) archived on permanent storage disks. Other data may also be archived.

Security of Archived Data

Archived data is stored on non-rewriteable disks. These are stored in a location which may only be accessed by authorized personnel. Only designated analysts should have write-access to the network folder containing archived data, to prevent accidental changes.

Considerations When Generating Data that will be Archived

1. Semicolons should not be used in Collection Sample File Names, as they prevent their file from burning properly to DVDs.

2. Run file names generated by 3500s should not be altered, except by adding to the end if necessary.

3. Run folders, GeneMapper, GeneMapper IDX, and GeneMarker projects should be moved into the "Completed" folder as soon as they are done being analyzed.

4. Never place anything into a subfolder containing the word "Archived" in its name, which may be present within the "Completed" folder.

Procedure for Archiving Data

1. Leave at least one month between the latest data archived and the date of archiving. The data from this month(s) should remain on the network in the "Completed" folder until the next data burn. Use the injection date in the 3500 run folder name as the "date" of everything in that folder.

2. Convicted Offender data is archived separately from case data. Follow the same procedure, using the Convicted Offender plate number for sorting.

3. Create a new folder within the "Completed" folder. Call this folder "Archived [date]".

4. Move all data to be archived into the "Archived [date]" folder. Check carefully to be sure that only data from the proper date range has been moved, and that no data from the date range to be burned remains in the "Completed" folder.

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5. Follow the instructions in a data-burning program to burn the "Archived [date]" folder to a disk. Indicate the type of data (Nuclear/CO 3500 Data) and the date of burning in the disk title.

6. When the burn is finished, insert the disk into a computer, open it, and right-click the "Archived [date]" folder and choose "Properties". Do the same for the "Archived [date]" folder which is still on the network. Compare the Size and the Contains fields. The Size of each folder and the numbers of Files and Folders Contained should be identical. If not, find what is causing the difference and fix it before continuing. Note: the Size-on-disk may be slightly different between the two folders; this is ok.

7. Label the disk with the disk title (as in step 5) and the range of dates substantially covered.

8. Import 1 project from the disk into GeneMarker.

9. Assure that they open properly and that all data appears to display correctly.

10. The currently archived data is stored in the DNA Unit Section storage room (206).

11. Do one final check immediately before moving data to be sure that the Size and numbers of Files and Folders Contained in the "Archived [date]" folder on the network have not changed, then move this folder to the designated Archived Data folder on the DNA network. (If any of this information *has* changed, the folder has been altered since it was burned – resolve the discrepancy before moving and be sure that what you are moving is exactly what was burned to the disks.)

12. Any disk generated that contains an error and is therefore not being used for the final archive should be disposed of. It must be rendered unreadable first (i.e. by breaking in pieces or shredding).

13. In addition to disk archival, all data residing on the U: Drive is archived on a weekly basis.

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APPENDIX II

List of Abbreviations

a = allele

@ = at or about

-A = minus A peak

ABI = Life Technologies (formerly Applied Biosystems Incorporated)

ABC = Anode Buffer Container

A(L)L = Average log likelihood

AP = Acid Phosphatase

* Peak = peak ≥ 50 RFU & <75 RFU (potential allele below threshold)

AT = analytical threshold

AV = Allele variance

BLS = Blood-like stain

bp = base pair

Č = contained

CBC = Cathode Buffer Container

CBE = cannot be eliminated

CDMCS = Central district major crime squad

CIDI = case, item, date, initials

CO = Click Off

CODIS = Combined DNA Index System

CM or Conv Match = Conviction Match

da = dye artifact

dH_20 = de-ionized water

DOI = date of incident

DNA = deoxyribonucleic acid

DNR = Data Not Reported

DTT = Dithiothreitol

E = evidence

EDMCS = Eastern district major crime squad

EF = epithelial rich fraction

EP1 = Extraction Positive Control

EP-MS = Extraction Positive – Male Screen

ESS = effective sample size

F6C = PowerPlex Fusion 6C System

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FH or For Hit = Forensic Hit

GF = GlobalFiler

GFE = GlobalFiler Express

GM-HID = GeneMarker HID Software

GMID = GeneMapper ID Software

GR = Gelman-Rubin Convergence

H (circled) = high stringency match

H_1 = Hypothesis consistent with inclusion of POI

H_2 = Hypothesis consistent with a random person matching

HBAP = High Background Artifact Peak

H_p = Prosecutor's Hypothesis

H_d = Defense's Hypothesis

HPB = Heterozygote peak balance

HPD = Highest Posterior Density

ID = Identifiler

ID + = Identifiler Plus

IDP = Identifiler Plus

IFC = Insufficient For Comparison

ILS = Internal Size Standard

II or Inv Info = Investigative Information

Inc. = Inconclusive

KM = Kastle-Meyer

KJL = Laboratory Positive Control (used previously)

LR = Likelihood ratio

MajorMix = major mixture

MCMC = Markov Chain Monte Carlo

MF = Minifiler

MM = master mix

mt = mitochondrial

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mito = mitochondrial

mtDNA = mitochondrial DNA

N = No

n = additional allele potentially dropped out

NAA = no alleles assigned

N/A = not applicable

ND = not deduced

NE = No Edits

NEATT = not examined at this time

NEG = Negative amplification control

NFT = No further testing

NR = No Results

nt = nucleotides

NTATT = not tested at this time

OB = out of bin

OCME = Office of the Chief Medical Examiner

OH or Off hit = Offender Hit

OLa = Off Ladder allele

OL = Off Ladder

ORI = Originating Agency Identifier

pd = Pull down peak

PD = Police Department or Penta D

PE = Penta E

Pks = peaks

PHR = Peak Height Ratio

POI = person of interest

POS = Positive amplification control

PPY = PowerPlex Y

Pr = Probability

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Prot K = Proteinase K

pu = pull up peak

Q samples = question samples

QC samples = quality control samples

rb = raised baseline

RAL = retained at the laboratory

RB = extraction negative control (reagent blank)

R/B = Reddish Brown

RBS = Reddish Brown Stain

R-CPI = restricted CPI

Re-amp = re-amplification

re-inj. = re-inject

RET = red evidence tape

RFU = relative fluorescent units

RKO = Laboratory Positive Control (used previously)

RMP = random match probability

rxns = reactions

SA = sexual assault

SAK = sex assault kit

SAO = State's Attorney's Office

SF = sperm rich fraction

Sld. = sealed

sm. amt. = small amount

sp = spike

SR = stutter ratio

ss = single source

ST = stochastic threshold

st = stutter peak

std = standard

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sus = suspect

SV = Stutter variance

TL = Technical Leader

TMP = Laboratory Positive Control (used previously)

U-CPI = unrestricted CPI

V (circled) or vic = victim

WDMCS = Western district major crime squad

Y = yes

YF = Y-Filer

YFP = Y-Filer Plus

YHRD = Y-Chromosome STR Haplotype Reference Database

Y-Mix = Y-Mix Database Filter

CSF = CSF1PO

D1 = D1S1656

D2B = D2S441

D2G = D2S1338

D3 = D3S1358

D5 = D5S818

D7 = D7S820

D8 = D8S1179

D10 = D10S1248

D12 = D12S391

D13 = D13S317

D16 = D16S539

D18 = D18S51

D19 = D19S433

D21 = D21S11

D22 = D22S1045

TH0 = TH01

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Amel = Amelogenin

| = given

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6.1 Responsibility:

DNA Section Personnel.

6.2 DNA Report Templates:

Note: The DNA Report Template is located in the controlled Current NUC Report Templates folder on the S: Drive.

6.2.1 Nuclear DNA Reports: Formerly, there were multiple types of DNA reports. Currently, all DNA reports are called DNA Reports. "Supplemental" and "Amended" may be added to this title as appropriate.

6.2.2 Mitochondrial DNA (mtDNA) Reports: mtDNA report statements are incorporated in the DNA Report Template. See mtDNA SOP-43 for guidelines on mtDNA report writing.

6.2.3 Supplemental Report Numbering: Supplemental reports for each report heading are numbered independently of each other. For example:

DNA Report
Supplemental DNA Report
Supplemental DNA Report II
Amended DNA Report

6.2.4 Statistics Templates

Note: See DNA SOPs 21, 25, 39, and 40 for single source and mixture profile interpretations and statistic details.

6.2.4.1 Statistics are calculated and documented for case files using STRmix (single source and mixture STR profiles), DNA QR-44 (match probability of parentage), Y-Mix and YHRD, (Y-STR profiles), PopStats with DNA QR-46 (probability of kinship), and DNA QRs-307 and 318 (for deconvolution). When applicable, the Excel workbooks containing these listed quality records are found on the S: Drive.

6.2.4.2 Frequency data to calculate the statistical weight of DNA matches may be derived from the following databases:

CODIS Popstats Database
Y-Chromosome STR Haplotype Reference Database (yhrd.org)

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Per specific requests, with TL and DD/AD approval, other peer reviewed databases may be used.

6.2.5 GlobalFiler, Fusion 6C and ID/IDP Conclusion Statements:

Note: If multiple amplification kits are reported out together, specify kit name in conclusion statements.

6.2.5.1 Elimination through STRmix: The following statements will be used when the LR calculated through STRmix is less than 1:

6.2.5.1.1 Single source profiles: "The results are consistent with the DNA profile from item #xx originating from a single (add if can determine: male/female) individual. The results do not support the hypothesis that John Doe is the source of this profile. Assuming one individual, John Doe is eliminated as the source of the DNA profile from item #xx."

6.2.5.1.2 Mixture profiles: "The results are consistent with the DNA profile from item #xx being a mixture of xx contributors (add if can determine: with (at least) xx of them being male). The results do not support the hypothesis that John Doe is a contributor to this profile. Assuming xx contributors, John Doe is eliminated as a contributor to the DNA profile from item #xx."

6.2.5.2 Elimination without using STRmix: The following statements will be used when an individual is eliminated visually by an analyst, without running STRmix software:

6.2.5.2.1 Single Source: "The results are consistent with the DNA profile from item #xx originating from a single (add if can determine: male/female) individual. Jane Doe is eliminated as the source of the DNA profile from item #xx."

6.2.5.2.2 Mixture profiles (profile not too complex to run through STRmix): "The results are consistent with the DNA profile from item #xx being a mixture of xx contributors (add if can determine: with (at least) xx of them being male). Jane Doe is eliminated as a contributor to the DNA profile from item #xx."

6.2.5.3 Inconclusive through STRmix: The following statements will be used when the LR calculated through STRmix is ≥ 1 but less than 10,000 for Fusion 6C, and ≥ 1 but less than 1,000 for GlobalFiler and ID/IDP:

6.2.5.3.1 Single source profiles: "The results are consistent with the DNA profile from item #xx originating from a single (add if can determine: male/female) individual. Assuming one individual, given the low likelihood ratio(s) calculated, the results are inconclusive as to whether John Doe could be the source of the DNA profile from item #xx."

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6.2.5.3.2 Mixture profiles: "The results are consistent with the DNA profile from item #xx being a mixture of xx contributors (add if can determine: with (at least) xx of them being male). Assuming xx contributors, given the low likelihood ratio(s) calculated, the results are inconclusive as to whether John Doe could be a contributor to the DNA profile from item #xx."

6.2.5.4 Other conclusions without running STRmix:

6.2.5.4.1 The following statement will be used when the DNA profile is a mixture too complex for STRmix deconvolution: "The results indicate that the DNA profile from item #xx is a mixture that is too complex for STRmix interpretation. Due to the complexity of the DNA profile from item #xx, no comparisons will be made."

6.2.5.4.2 The following statement will be used when the profile has limited data detected to run STRmix software, and the profile does not warrant an elimination conclusion: "Due to limited data detected from item #xx, the comparison to John Doe is inconclusive."

6.2.5.5 Positive associations (i.e. LR's higher than the laboratory's inconclusive zone) as determined through STRmix:

Note: The lowest calculated likelihood ratio HPD lower bound on the STRmix report, ceilinged at 100 billion, will be reported out. The following statement will be used when LR ≥ 10,000 for Fusion 6C and LR ≥ 1,000 for GlobalFiler and ID/IDP:

6.2.5.5.1 Single source profiles: "The results are consistent with the DNA profile from item #xx originating from a single (add if can determine: male/female) individual. Assuming one individual, the DNA profile from item #xx is at least xx times more likely to occur if it originated from John Doe than if it originated from an unknown individual."

6.2.5.5.1.1 For samples that are identical (same data all above the stochastic threshold regardless of injection) to one another, there is no need to run STRmix again. The same LR may be reported as follows:

"The results from items #xx, #yy, and #zz are identical to the results from item #aa. Therefore, assuming one individual, the DNA profile(s) from item(s) #xx, #yy, and #zz is/are at least xx times more likely to occur if it/they originated from Joe Smith than if it/they originated from an unknown individual."

6.2.5.5.2 Mixture profiles (without conditioning): "The results are consistent with the DNA profile from item #xx being a mixture of xx contributors (add if can determine: with (at least) xx of them being male). Assuming xx contributors, the DNA profile obtained from item #xx is at

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least xx times more likely to occur if it originated from John Doe and xx unknown individual(s) than if it originated from xx unknown individuals."

6.2.5.5.3 Mixture profiles (with conditioning): "The results are consistent with the DNA profile from item #xx being a mixture of xx contributors (add if can determine: with (at least) xx of them being male). Jane Doe is assumed to be a contributor to the DNA profile from item #xx." Assuming xx contributors, where Jane Doe is one of the contributors, the DNA profile obtained from item #xx is at least xx times more likely to occur if it originated from John Smith, (and) Jane Doe (and xx unknown individual(s)) than if it originated from Jane Doe and xx unknown individual(s)."

6.2.5.6 The following statement will be used when assuming a known to a single source profile "The results are consistent with the DNA profile from item #xx originating from a single (male/female) individual. J. Doe is assumed to be the source of the DNA profile from item #xx."

6.2.5.7 Statement when conditioning a laboratory staff member:

"The results are consistent with the DNA profile from item #xx being a mixture of xx contributors (add if can determine: with (at least) xx of them being male). The results are consistent with a laboratory staff member being a contributor to the DNA profile from item #xx. Assuming xx contributors, where the laboratory staff member is one of the contributors, the DNA profile obtained from item #xx is at least xx times more likely to occur if it originated from John Smith, (and) the laboratory staff member (and xx unknown individual(s)) than if it originated from the laboratory staff member and xx unknown individual(s)."

6.2.6 Report wording for clear major in 5+ person mixtures.

6.2.6.1 Consistent with source wording: "The results are consistent with the DNA profile from item #xx being a mixture. A major (male/female) profile was deduced from item #xx. The results are consistent with J. Doe being the source of the major profile deduced from item #xx. The major profile deduced from item #xx is at least xx times more likely to occur if it originated from J. Doe than if it originated from an unknown individual.

6.2.6.2 Eliminated as source wording: "The results are consistent with the DNA profile from item #xx being a mixture. A major (male/female) profile was deduced from item #xx. J. Smith is eliminated as the source of the major profile deduced from item #xx."

6.2.6.3 Wording for full profile with major component deduced: "The results are consistent with the DNA profile from item #xx being a mixture of (at least) five contributors (with at least xx of them being male). This profile is too complex for STRmix interpretation. Due to the

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complexity of the DNA profile from item #xx, no comparisons to the minor alleles present in the profile will be made."

6.2.7 Y-STR Conclusion Statements:

Note: The statistic being reported is the highest figure (most common) from the African American, Caucasian, or Hispanic population with 95% confidence interval already applied. Loci not used in statistical calculations will be noted through a footnote designation (Locus not used for statistics) in the allele table, located in the appendix of the report.

Note: If multiple amplification kits are reported out together, specify kit name in conclusion statements.

Note: Wherever a mixture statement specifies "xx" contributors, know that for Yfiler profiles, "xx" cannot exceed 2 contributors, based upon our validations.

6.2.7.1 Single-source profiles with full detection at all loci with results: "The results are consistent with the DNA profile from item #xx originating from a single male lineage. The results are consistent with John Doe (or another member of the same male lineage) being the source of the DNA profile from item #xx. The expected frequency of individuals who could be the source of the DNA profile from item #xx is less than 1 in xx in the general male population."

6.2.7.2 Single-source profiles with partial detection at all loci with results: "The results are consistent with the DNA profile from item #xx originating from a single male lineage. John Doe (or another member of the same male lineage) cannot be eliminated as the source of the DNA profile from item #xx. The expected frequency of individuals who could be the source of the DNA profile from item #xx (at loci…/ at all loci tested except…) is less than 1 in xx general male population."

6.2.7.3 Mixture profiles with full detection at all loci with results: "The results are consistent with item #xx being a mixture of xx male lineages. John Doe (or another member of the same male lineage) is included as a potential contributor to the DNA profile from item #xx. The expected frequency of individuals who could be a contributor to the DNA profile item #xx is less than 1 in xx in the general male population."

6.2.7.4 Mixture profiles with partial detection at loci with results: "The results are consistent with item #xx being a mixture of xx male lineages. John Doe (or another member of the same male lineage) cannot be eliminated as a potential contributor to the DNA profile from item #xx. The expected frequency of individuals who could be a contributor to the DNA profile from item #xx (at loci…/ at all loci tested except…) is less than 1 in xx in the general male population."

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6.2.7.4.1 Deconvolution of major contributor: "The results are consistent with item #xx being a mixture of xx male lineages. A major profile was deduced from item #xx. The results are consistent with John Doe (or another member of the same male lineage) being the source of the major DNA profile deduced from item #xx. The expected frequency of individuals who could be the source the major DNA profile from item #xx is less than 1 in xx in the general male population."

6.2.7.5 Elimination from Y–STR profiles:

6.2.7.5.1 Single Source Profiles: "The results are consistent with the DNA profile from item #xx originating from a single male lineage. J. Doe is eliminated as the source of the DNA profile from item #xx."

6.2.7.5.2 Mixture Profiles: "The results are consistent with item #xx being a mixture of (at least) xx male lineages. J. Doe is eliminated as a contributor to the DNA profile from item #xx." If deconvolution: John Doe is eliminated as the source of the major DNA profile deduced from item #xx.

6.2.7.6 If limited data is detected from an item for a comparison, report as follows: "Due to limited data detected from item #xx, the comparison to J. Doe is inconclusive."

6.2.7.7 Mixture too complex (more than 3 lineages for Yfiler Plus, and more than 2 lineages for Yfiler): "Due to the complexity of the DNA profile from item #xx, the comparison to J. Smith is inconclusive."

6.2.8 Statistics statement for paternity (RMNE): "The expected frequency of males who could be the father of NAME is less than 1 in xx in the general population."

6.2.8.1 Statistics statement for Kinship analysis: see DNA SOP-25 Kinship Analysis.

6.2.9 Conclusion Templates:

6.2.9.1 The following footnote will be used when STRmix was used to calculate LR: "Profile analyzed and comparison made using STRmix™ analysis software."

6.2.9.2 The following statement shall be used when a profile is a 5 person mixture, too complex for STRmix interpretation, but case information indicates assuming individual(s) will be appropriate and could therefore make the mixture interpretable: "Item #xx could potentially be interpreted with STRmix if elimination known(s) are submitted."

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Note: This statement is not warranted with every 5 person mixture. An example of an exception: A weapon possession case with evidence not being touched by a victim, etc. Case scenario should determine utilization of this wording.

6.2.9.3 Statement stating that a known is assumed as a contributor to a profile shall be stated prior to any statistical statements involving that assumption.

6.2.9.4 Analysts should combine like conclusions and/or separate those conclusions from the number of contributor's statement as warranted, making statements grammatically correct while increasing ease of interpretation for our clients.

6.2.10 Additional Report Phrasing: The following are commonly used in DNA Reports; however, this is not an exhaustive list of all possible remarks or report wording allowed by the DNA Section. All buttons in the DNA Report Buttons tab or More DNA Report Buttons tab in the DNA Report Document located on the S: Drive are approved wording. Any variation or additions to these phrases are allowed with TL approval.

6.2.10.1 Evidence disposition: The following statements will be used, depending on how the evidence is disposed:

6.2.10.1.1 For evidence consumed in testing: "Item #xx was consumed in testing." For DNA extracts consumed in testing: "The DNA extract from item #xx was consumed in testing."

6.2.10.1.2 Item #xx (or a sample from item #xx) was retained at the laboratory. For reports containing new questioned sample testing, with those extracts being retained at the laboratory, an additional statement will be added to the remarks section: "Any remaining DNA extracts are retained at the laboratory." There is no need to list the item numbers of these extracts, nor a need to list them in the Evidence Description section.

6.2.10.1.3 Item #xx was returned to the submitting agency.

6.2.10.1.4 Item #xx was forwarded to the xx Unit of the laboratory.

6.2.10.1.5 If only empty packaging for a known sample is being returned, with the testable portion of that known sample being retained at the laboratory, analysts shall use the following statement: "The testable portion from item #xx was retained at the laboratory. Item #xx was returned to the submitting agency."

6.2.10.2 If trace materials (that could be tested) were noted in a physical examination worksheet, and not reported on in a separate Forensic Biology Report, those findings must be noted in the "Remarks" section of the DNA report.

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6.2.10.2.1 Trace materials only noted, as appropriate: “Hair-like fibers and other trace materials were noted on item #xx but not collected.”

6.2.10.2.2 Trace materials collected and returned to original packaging: “Hair-like fibers and other trace material were collected from item #xx and returned to the submission packaging.”

6.2.10.2.3 There is no need to report out trace material on cigarette/tobacco material.

6.2.10.2.4 When an evidence description is used, it is not necessary to carry forward the “container”. For example, Submission #001: Box “with stained knife”; item #001-001: stain on knife. The box that the knife was submitted in does not have to continue in the description.

6.2.10.3 For cases in which a sample was entered into CODIS, the “Remarks” section will state that the submitting agency will be notified of any positive CODIS associations.

6.2.10.4 Items listed in the under “Evidence Description”, but not tested, shall be noted as such in the “Remarks” section.

6.2.10.5 Any request for appropriate known samples shall be noted in the “Remarks” section.

6.2.10.6 If the Quantifiler-Trio results dictate that additional male-specific testing may be beneficial to the client if a known sample is submitted, this shall be noted in the “Remarks” section, so long as controls are still present to perform this testing.

6.2.10.7 Any testing results from Kastle-Meyer (KM) (whether sent to DNA or not sent to DNA), are reported out. For blood screening testing not reported by FB: “A screening test for the presence of blood was performed on (additional) (stains located on) item #1. …

6.2.10.7.1 Positive: Blood was indicated (on the (additional) stains) with this test.”

6.2.10.7.2 Negative: Blood was not indicated (on the (additional) stains) with this test.”

6.2.10.7.3 Inconclusive: Due to indeterminate results and/or substrate interference, this testing was determined to be inconclusive.”

6.2.10.8 Notes of non-blood like stains, dirt, grass, etc. are not reported out, but noted on worksheets.

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6.2.10.9 KM Testing Reporting Summary:

In Testing Summary Table AND in Conclusions Section	Stain KM tested, collected, and tested for DNA or collected, tested, and retained for possible DNA testing
In Conclusions Section	Stain KM tested, but not collected for DNA
In Remarks Section	Reddish-brown stains noted on worksheets, but not tested

6.2.10.10 Stop at Quantitation statement (property crimes): When no DNA has been detected in quantitation, report in Remarks section: "Since no human DNA was detected from item #xx using the Quantifiler Trio DNA quantitation method, no further testing has been performed." This will be noted in the "Testing Summary" table, under a "Halted after Quantitation" column.

6.2.10.11 Reporting out items that underwent the Male Screen procedure:

6.2.10.11.1 In the "Conclusions" section, the actual male screen procedure will be reported out as follows: All to start with "A screening test for the presence of male DNA was performed on item #xx. …

6.2.10.11.1.1 Positive screen: Male DNA was detected with this test."

6.2.10.11.1.2 Negative screen: Male DNA was not detected with this test."

6.2.10.11.1.3 Inconclusive result (insufficient human DNA detected): Due to the low quantity of total human DNA, this testing was determined to be inconclusive."

6.2.10.11.1.4 Inconclusive result (Microcon failure): Due to indeterminate results, this testing was determined to be inconclusive."

6.2.10.11.2 All male screen procedure results will also be summarized in the "Testing Summary" table as follows:

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TESTING SUMMARY

Item #	Screening	Profile Obtained[1]	Type	CODIS Entry
		GF		
1-1	Male DNA (-)			
1-2	Male DNA (+)	Yes	Male	Yes – CT and National
1-3	Male DNA (inc)	Yes	Female	No - Not Appropriate

[1]GF = GlobalFiler STR DNA amplification kit

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6.2.10.11.3 Halt at quant statements for casework questioned samples that previously tested positive or inconclusive through the male screen procedure, and not being amplified after EZ1 extraction (to be reported in remarks section):

6.2.10.11.3.1 No male DNA detected: "Since no (male) DNA was detected from item #xx using the Quantifiler Trio DNA Quantitation method, no further testing has been performed."

6.2.10.11.3.2 Sufficient male DNA detected for male-specific testing only, known from suspect is not in-house: "Male DNA was indicated in item #xx with high levels of total human DNA compared to male DNA, therefore no further testing has been performed. Additional male-specific testing may be performed if a known sample from a/the suspect is submitted."

6.2.10.11.3.3 Sufficient male DNA detected for testing, but more probative/positive samples in case amplified: "Sufficient male DNA on item #xx was detected, but no further testing was performed at this time."

6.2.10.11.4 Additional statement to be added to "Remarks" section when sexual assault kits have items not tested: "Further examination on item #xx may be conducted upon request."

6.2.11 Additional DNA Report Sections

6.2.11.1 Evidence Description: Exhaustive list of all items of evidence pertaining to report.

6.2.11.1.1 Multiple cuttings of a singular item taken for different purposes may be listed under parent item, so long as all different purposes are listed on report. For example vaginal swabs from a sexual assault kit (#001-002-02) may have a Y-screen cutting, a cutting for DNA testing, and a cutting for additional serological testing. Notation for and fractions of a differential extraction would be off of this item. The Y-screen result will be listed for #001-002-02, and

the "Remarks" section note will that item #001-002-02 was retained at the laboratory for further serological testing. For example:

EVIDENCE DESCRIPTION

#001-002-02 Vaginal swabs[1]
#001-002-02A Vaginal swabs, EF
#001-002-02B Vaginal swabs, SF

[1]A differential extraction was performed, separating the DNA extracted from this item into an epithelial-rich fraction (EF) and a sperm-rich fraction (SF). Detection of some DNA from one fraction in the other may occur due to incomplete separation.

TESTING SUMMARY

Item #	Screening	Profile Obtained[1]	CODIS Entry
		GF	
001-002-02	Male DNA(+)		
001-002-02A		Yes	No - Not Appropriate
001-002-02B		Yes	Yes - CT and National

[1]GF = GlobalFiler STR DNA amplification kit

REMARKS

Item #001-002-02 was retained at the laboratory. Additional serological testing may be conducted upon request.

6.2.11.2 Testing Summary: Contains table documenting any items tested, which, if any, DNA profiles were obtained for that item (or if the sample was halted at quantitation), information regarding CODIS entries for appropriate samples, and when applicable, details regarding previously tested samples and screening test results (including blood and male DNA). If the DNA Report does not have a conclusions summary table, the type of DNA profile (single source or mixture), will also be documented in the testing summary. For example:

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TESTING SUMMARY

Item #	Screening	Profile Obtained[1]	Type	CODIS Entry
		GF		
1S1		Yes	Mixture	Yes - CT and National
1S3	Blood(+)	Yes	Male	No - Not Appropriate

[1]GF = GlobalFiler STR DNA amplification kit

6.2.11.3 Conclusions Summary: Contains table documenting items that had profile results, the description of that evidence, the type of DNA profile (single source or mixture), and conclusions to all knowns associated in the case. For example:

CONCLUSIONS SUMMARY

Item #	Description	Type	J. Doe (#2)	J. Smith (#3)
1S1	Swabbing – Steering Wheel	Mixture	Assumed	Included
1S3	Cutting – Cigarette Butt	Female	Consistent with Source	Eliminated

6.2.11.4 Appendix: Contains all DNA profiles in appropriate allele tables.

6.2.12 Report templates for CT DSS reporting results generated by outsourcing labs:

6.2.12.1 Report statements for single-source profiles (if STRmix is not being run by outsourcing vendor):

a. "The results are consistent with NAME being the source of the DNA profile from item #xx. The expected frequency of individuals who could be the source of the DNA profile from item #xx is less than 1 in xx in the general population." Note: The statistic being reported is the highest figure (most common point estimate) from the African American, Caucasian, or Hispanic populations.

b. "NAME cannot be eliminated as the source of the DNA profile from item #xx. The expected frequency of individuals who could be the source of the DNA profile from item #xx (at loci…/ at all loci tested except…) is less than 1 in xx in the general population."

6.2.12.2 Report statements for mixture profiles (if STRmix is not being run by outsourcing vendor):

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a. "The results are consistent with item #xx being a mixture. NAME is included as a potential contributor to the DNA profile from item #xx. The expected frequency of individuals who could be a contributor to the DNA profile (at loci…/ at all loci tested except…) from item #xx is less than 1 in xx in the general population."

b. "The results are consistent with item #xx being a mixture. NAME cannot be eliminated as a potential contributor to the DNA profile from item #xx. The expected frequency of individuals who could be a contributor to the DNA profile from item #xx (at loci…/ at all loci tested except…) is less than 1 in xx in the general population."

c. Assumed 2-person mixture (major): "The results are consistent with item #xx being a mixture. Assuming two contributors to the mixture, a major profile was deduced. The results are consistent with NAME being the source of the major profile deduced from item #xx. The expected frequency of individuals who could be the source of the major DNA profile deduced from item #xx is less than 1 in xx in the general population."

d. Assumed 2-person mixture (minor): "The results are consistent with item #xx being a mixture. Assuming two contributors to the mixture, a minor profile was deduced. The results are consistent with NAME being the source of the minor profile deduced from item #xx. The expected frequency of individuals who could be the source of the minor DNA profile deduced from item #xx is less than 1 in xx in the general population."

e. 3 & 4 person mixtures with deduced major: "The results are consistent with item #xx being a mixture. A major profile was deduced. The results are consistent with NAME being the source of the major DNA profile from item #xx. The expected frequency of individuals who could be the source of the major DNA profile deduced from item #xx is less than 1 in xx in the general population."

6.2.12.3 F6C report statement for mixture profiles when using the restricted CPI calculation: "The results are consistent with item #xx being a mixture. A major DNA mixture profile was detected. NAME is included as a potential contributor to the major DNA mixture profile from item #xx. The expected frequency of individuals who could be a contributor to the major DNA mixture profile deduced from item #xx is less than 1 in xx in the general population."

6.2.12.4 Report wording for 5+ person mixtures:

6.2.12.4.1 No clear major: "The results are consistent with item #xx being a mixture of 5 contributors (with at least xx of them being male). This profile is too complex for STRmix interpretation. Due to the complexity of the DNA profile from item #xx, no comparisons will be made."

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6.2.12.4.2 >5 person mixtures: "The results are consistent with item #xx being a mixture of at least 5 contributors (with at least xx of them being male). This profile is too complex for STRmix interpretation. Due to the complexity of the DNA profile from item #xx, no comparisons will be made."

6.2.12.4.3 Deduced major portion of profile: "The results are consistent with the DNA profile from item #xx being a mixture. A major (male/female) profile was deduced from item #xx. J. Smith is eliminated as the source of the major profile deduced from item #xx."

6.2.12.4.4 Full profile with deduced major: "The results are consistent with the DNA profile from item #xx being a mixture of five contributors (with at least xx of them being male). This profile is too complex for STRmix interpretation. Due to the complexity of the DNA profile from item #xx, no comparisons to the minor alleles present in the profile will be made."

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6.2.13 Amended report letter (sample):

Date

Asst. State's Attorney
Hartford State's Attorney's Office
101 Lafayette St.
Hartford, CT 06106

RE: Connecticut DESPP, Division of Scientific Services, Case ID, Police Department Case #.

Dear Ms. Attorney:

During a review of the supplemental DNA report dated xx-xx-xx for the above case a typographical error was noted.

In section 3 the second to last sentence should read:
Item #1 was returned to the appropriate submitting agency.

Enclosed is an amended copy of the report. Please forward this information to the appropriate individual(s).

Thank you for your attention in this matter.

Sincerely,

_____
Analyst
Forensic Science Examiner

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6.2.14 No Ownership Notifications

STATE OF CONNECTICUT
DEPARTMENT OF
EMERGENCY SERVICES AND PUBLIC PROTECTION
DIVISION OF SCIENTIFIC SERVICES

Guy M. Vallaro, Ph.D.
Director

DNA NOTIFICATION

Laboratory Case	**Submitting Agency**	**Date of Request**
DSS-19-00TEST	Ansonia Police Department 2 Elm St Ansonia, CT 06401	1/1/2019
Agency Case		**Date of Notification**
12345		Report Date

The above mentioned case was outsourced to DNA Labs International (260 SW Natura Ave, Deerfield Beach, FL 33441). No CODIS entry was able to be made. Please refer to the attached DNA Labs International report dated 4/xx/2019 for DNA testing results and conclusions. Evidence will be returned to the submitting agency. Any remaining extract from the tested items will be retained at the laboratory. Results apply only to the evidence as received by the DSS.

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Melanie G. Russell(Analyst)
Forensic Science Examiner 1

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278 Colony Street, Meriden, Connecticut 06451
Phone (203) 639-6400 Fax (203) 639-6485
An Affirmative Action / Equal Opportunity Employer

6.3 List of Critical Reagents and Equipment

All critical reagents will be appropriately tested to ensure proper performance prior to their use in casework samples. See SOP-8 for QC testing.

6.3.1 Critical reagent chemicals and solutions:

Anode Buffer Container
Cathode Buffer Container
dH_2O (used for sample prep through amplification)
Dithiothreitol (DTT)
Extraction Buffer
Formamide
PCIA (Phenol/Chloroform/Isoamyl Alcohol 25:24:1 v/v)
Prep-N-Go solution
TE Buffer

6.3.2 Critical Enzymes/Biologicals/Kits:

Proteinase K
Quantifiler Trio Kits
Primer Sets (if not as part of an assembled kit)
Allelic Ladders (if not as part of an assembled kit)
EZ1 Extraction Kits
Yfiler Plus Kits
GeneScan™ -600 LIZ™
POP-4
GlobalFiler Amplification Kits
GlobalFiler Express Amplification Kits
cRNA

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6.3.3 Evaluation and testing of critical reagents and solutions Refer to DNA SOP-8.

6.3.4 Critical Instruments: those requiring an annual performance check prior to use. Below is a list of what is included as a critical instrument.

Note: Refer to DNA SOP-9 for performance check information on the below listed equipment.

Thermometers that are traceable to national or international standard(s)
Balance/Scales
Incubators/heat blocks used in analytical procedures

Thermal cyclers
Thermal cycler temperature-verification system
Note: this instrument is not currently in use due to an outside vendor conducting performance checks of thermal cyclers (see DNA SOP-9)
Real-Time PCR Instruments
Robotic Systems (EZ1, etc.)
Electrophoresis detection systems (Genetic Analyzers)
Pipettes
Centrifuges

6.4 General Chemical Inventory

6.4.1 Dry Chemicals:

Dithiothreitol (DTT)
Dryrite
Ethylenediaminetetraacetic acid disodium, dihydrate (EDTA)
Potassium Chloride
Sodium Chloride
Sodium Dodecyl Sulfate (lauryl sulfate)
Sodium Hydroxide
TRIS, ULtraPure Grade
TRIS Hydrochloride
TRIS-base (hydroxymethyl amino methane)

6.4.2 Liquid Chemicals:

Ethanol, Reagent
Hydrogen Peroxide
pH Buffer (4.00, 7.00 & 10.00)
Proprietary Solvent, anhydrous
Sodium Hypochlorite (bleach)
Hydrochloric acid
Conditioning Reagent

6.4.3 Enzymes/Biologicals:

DS-36 Matrix Standard (Dye Set J6)

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21.1 INTRODUCTION:

The Connecticut Department of Emergency Services and Public Protection Division of Scientific Services (CTDSS) may contract or subcontract forensic DNA casework samples to a Vendor Laboratory (e.g. Bode Cellmark, DNA Labs International). If samples are outsourced to a Vendor Laboratory, this Vendor Laboratory shall comply with the *FBI Quality Assurance Standards for Forensic DNA Testing Laboratories* and the *FBI Quality Assurance Standards for DNA Databasing Laboratories (QAS)* and the accreditation requirements of federal law. The Vendor Laboratory shall provide documentation of this compliance to the CTDSS. Documentation of this compliance will be maintained by the Quality Section.

21.2 RESPONSIBILITY:

It is the responsibility of the DNA Section Technical Leader (TL) to document and maintain the approval of the technical specification of the outsourcing agreement before it is awarded. The DNA Section TL is responsible for documenting the approval of the technical specifications of the outsourcing agreement and/or documenting the approval of acceptance of ownership of the DNA data prior to any upload of data generated by the Vendor Laboratory to CODIS.

If the pre-approved Vendor Laboratory is performing forensic DNA analysis for a law enforcement agency or entity other than for the CTDSS, documented approval from the DNA Section TL accepting ownership of the DNA data generated shall be obtained by the Vendor Laboratory prior to the initiation of analysis.

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21.3 Procedure for Outsourced Casework:

With the approval of the CTDSS, the vendor laboratory may perform evidence screening, DNA data analysis, and issue a DNA report. DNA analysis typing systems used and data analysis parameters are to be approved by the CTDSS TL.If necessary, the CTDSS may prep the evidence in-house including serological screening according to Forensic Biology Section (SOP-FB-01) and/or DNA Section (SOP-1) protocols and procedures.

21.3.1 The CTDSS will receive evidence from submitting agencies.

21.3.2 Samples will be collected and packaged for outsourcing (according to the Memorandum of Understanding (MOU/contract), Technical Specifications, and Case Management WI-01).

21.3.3 Samples, along with paperwork (specific) and a copy of the electronic spreadsheet will be shipped via Mail Transport to the outsourcing vendor.

21.3.4 The vendor will maintain the chain of custody.

21.3.5 The vendor will process the sample (according to the Memorandum of Understanding (MOU/contract) and Technical Specifications).

21.3.6 The vendor will ship all samples (if any are left unconsumed), genomic DNA, electronic data, case files, and DNA reports to the CTDSS.

21.3.7 The CTDSS will receive the returned data, samples, genomic DNA, and DNA reports via Mail Transport from the Vendor Laboratory.

21.3.7.1 An optical disk containing the casework data and DNA processing worksheets generated by the vendor laboratory will be held in an electronic media storage binder and kept in case file storage areas. An additional copy of this material will be archived on the DNA Section U-drive.

21.3.7.2 Another option of data transfer/result delivery from the vendor lab to CTDSS is through restricted access to a secure FTP (file transfer protocol) site. A unique user name and password will be given to CTDSS. The FTP site serves as a portal that grants access to upload and/or download the results. The FTP site will provide the necessary encryption to protect such data transfers through the web interface.

The data/results downloaded from the FTP site will be kept on the DNA U drive. For long-term storage, this data will be burned to discs and kept in the data archive binders.

21.3.8 A DNA analyst will receive a set of cases grouped into a batch that were processed together at the vendor laboratory or a subset thereof. The vendor laboratory may have a batch identification number. If not, then the DNA analyst will assign a unique batch identification number. The analyst will print out the pertinent case specific records for the cases they are assigned and those records will be maintained in the case file. The batch paperwork for the set will be referenced in the case file and will be stored on the U drive.
A copy of the vendor's DNA report is added to the CTDSS case jacket for each case in the batch. The analyst initials all DNA worksheets and the vendor's report and also ensures that the DSS case number is on each page. Add the DSS case number if it is not identified on the page.

21.3.9 **Standard 17 Review**
From the FBI QAS:
The Standard 17 Review is the technical review required by the FBI QAS standards for DNA data that has been outsourced and the lab takes or retains ownership (see current QAS audit document for ownership definition). This Standard 17 review is to be distinguished from the administrative and technical reviews required by Standard 12. For outsourced

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DNA data, the vendor laboratory is responsible for conducting the administrative and technical reviews required by Standard 12.

21.3.9.1 Once being assigned a set of cases, the DNA analyst will review the vendor's report and submitting agency's case documentation to evaluate the following to see if a "Standard 17 Review" is necessary:

1. The NDIS participating laboratory will use any samples, extracts, or materials from the vendor laboratory for the purposes of forensic testing (e.g., a vendor laboratory prepares an extract that will be analyzed by the NDIS laboratory);

2. The NDIS participating laboratory will interpret the data generated by the vendor laboratory;

3. The NDIS participating laboratory will issue a report describing or drawing conclusions on the results of the DNA analysis performed by the vendor laboratory; or

4. The NDIS participating laboratory will enter or search a DNA profile in CODIS from data generated by the vendor laboratory.

If yes, please follow steps starting at 21.3.9.1.1 to 21.3.9.3 below.

RETIRED

OWNERSHIP CASES

21.3.9.1.1 A "Standard 17 Review" is documented on DNA QR-4c; Section 1 through 4. This review will be performed by a DNA Analyst who is qualified or previously qualified in the technology, platform, and typing amplification test kit used to generate the data. The analyst will also be proficiency tested to the full extent in which he or she participates in casework. The Analyst will draft a DNA Report and perform a Standard 17 "Review". DNA QR-4c will list the aspects of this review per QAS Standard 17. The analyst will then have a reviewer complete an additional review of elements that are outlined on DNA QR-4c. Between the analyst and the reviewer, the administrative review aspects will be completed. The "outsource review" as described in GL-18, will be covered on DNA QR-4c.

21.3.9.2 The DNA Report sent to the submitting agency for "ownership" cases will at a minimum, document the entry of any CODIS-eligible profiles and refer to the vendor laboratory report.

Known reference samples processed in-house and data sent to the vendor laboratory will be documented on a separate report. This will be a standard DNA report coming from the CT DSS to apprise the submitting agency that the known they submitted was processed at CT DSS and the data will be sent to the vendor lab for comparisons and conclusions.

If for some reason, the known reference sample processed needs to be included in an "ownership" report, then Section 4 of DNA QR-4c will be filled out by an analyst and a combined technical/administrative reviewer. The known processed will be analyzed, technically and administratively reviewed following all requirements of the FBI QAS (Standard 12) and CT DSS protocol prior to the DNA Report being sent out.

The "reviewer" for Section 4 on DNA QR-4c, is a technical reviewer. This technical reviewer is used when a known was processed by CT DSS and data sent to the vendor laboratory, or, the CT DSS determines that CT DSS will "own" the data; see Section 21.7 of this SOP.

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21.3.9.3 A Standard 17 Review for ownership of data shall include at a minimum:

1. Review of all DNA types to verify that they are supported by the analyzed data (electropherograms or images). For cases with Fusion 6C and/or GF, this will be a review of all required printed, pdf, or images of electropherograms.

2. A review of all associated controls, internal lane size standards, and allelic ladders to verify that the expected results were obtained. For cases with Fusion 6C and/or GF, this will be a review of all required printed, pdf, or images of electropherograms.

3. A review of the final report to verify that the results/conclusions are supported by the data and that the report addresses each tested item (or its probative fraction) that was submitted to the vendor laboratory.

4. Verification of the DNA types, eligibility, and the correct specimen category for entry into CODIS. This verification is reviewed by a second qualified analyst and this entire process is documented on DNA QR-13. The specimen detail report once verified, will be printed out and also kept in the case file. All procedures outlined in DNA SOP-13 "Profile Entry and Data Bank" shall be followed.

5. The "outsource review" for outsourced cases as outlined in GL-18 will be performed as part of the Standard 17 review. This can be the same individual as the reviewer as described above. This "outsource review" includes the following:

    1. Verify evidence (if any) and/or results returned from the vendor laboratory represents what was sent.
    2. Verify that the signed report matches the DSS case number and represents what was submitted to the contract laboratory.
    3. Verify that the requested testing was performed.
    4. Update LIMs to the appropriate request.
    5. Check chain of custody.
    6. Review for clerical errors and review final report with signatures.

NOTE: For cases with multiple samples; if one sample in a case is CODIS eligible or "owned" and one sample is not, Standard 17 Review (DNA QR-4c) will be used for the entire case.

NO OWNERSHIP CASES

21.3.9.4 Vendor laboratory cases that CT DSS deems "no ownership" do not require a Standard 17 Review. The review necessary for these cases will be documented on QR-4d.

21.3.9.4.1 A letter/notification will be written to the submitting agency documenting that their case was outsourced, there was no entry into CODIS, and that evidence was returned. Please see Appendix 1 below for example of this letter/notification. The vendor lab report will be referred to and sent out with this notification. CT DSS does not perform any testing for these cases and only uses approved and competent vendors. All ANAB requirements for reporting results of testing will reside on the vendor laboratory report.

An "Outsourcing Review" of these cases will be performed as outlined in GL-18 Case Review. The assigned analyst will complete the following and document on QR-4d.

1. Verify evidence (if any) and/or results returned from the vendor laboratory represents what was sent.
2. Verify that the signed report matches the DSS case number and represents what was submitted to the contract laboratory.

RETIRED

3. Verify that the requested testing was performed.
4. Verify that there are no CODIS eligible profiles (requiring a standard 17 review)
5. Update LIMs to the appropriate request.
6. Check chain of custody
7. Review for clerical errors and review final report with signatures.

21.3.10 If DNA analysis (such as a known reference sample) was performed at the CTDSS, a DSS DNA Report will be issued (separately from the above letter) outlining what was done, and that the data was sent to the vendor lab for further comparison to the question samples. All testing performed at CT DSS will be completed in compliance with FBI QAS and accreditation standards.

Knowns processed (extraction through analysis) at CT DSS **will not be** incorporated into the "no ownership" letters. A standard CT DSS report will be issued for the known sample.

A known sample which is examined merely for retention purposes and is not processed for DNA can be incorporated into the "no ownership" letter.

RETIRED

21.4 Hit Notification

21.4.1 Hit notifications from a Vendor Laboratory case, will follow the normal CT DSS protocol DNA SOP-15 "Hit Notifications". Confirmed matches will also follow the same protocol as described in DNA SOP-15. STRmix will not be used to evaluate any matches with forensic profiles processed with a kit not validated for use with STRmix.

Send to the Vendor laboratory for confirmation if a confirmatory known is submitted for OR re-amp the question sample and re-enter the profile to CODIS and process the confirmation in house if necessary.

21.5 Storage and LIMS

21.5.1 The long term storage of the electronic data on optical disk will be kept in a binder in a case file storage area and on the DNA U-drive.

21.5.2 Ensure that the optical disk has a label with the Vendor name and a unique identifier. A tracking method will be in place to associate that optical disk with specific cases.

21.5.3 The final case file will be stored in the case file storage area according to the CTDSS case number.

21.5.4 The original copy of the final report will be stored in Evidence Receiving.

21.5.5 The milestones in LIMS for outsourcing will be slightly different than standard protocol. No ownership cases will be released at "draft complete" by the assigned analyst. Ownership cases will have findings entered and draft competed entered by the analyst and the "technical review" and "administrative review" will be completed by the reviewer of the case.

21.5.6 For the known requests in LIMS, the report generated will follow normal milestone procedure. The analyst, technical reviewer and administrative reviewer will have their distinct roles as per GL-4 LIMS and DNA unit SOPs.

21.6 On-Site Visit and QAS Standard 17

21.6.1 The CTDSS will follow all standards defined in the *FBI Quality Assurance Standards for Forensic DNA Testing Laboratories* and *FBI Quality Assurance Standards for DNA Databasing Laboratories* Standard 17.

21.6.2 An on-site visit of the Vendor Laboratory will be performed prior to the initiation of analysis. The on-site visit will be conducted by the TL or a designated employee of an NDIS laboratory that uses the same technology, platform, and typing amplification test kit. The TL shall document approval of this on-site visit. The on-site visit may also be performed by a designated FBI employee accepted by the TL.

Site Visits may include, but are not limited to a review of the following:

1. Accreditation Certificates
2. Recent Audits (internal and external)
3. SOPs
4. Quality Manual

21.6.3 If the outsourcing agreement extends beyond one year, an annual on-site visit shall be conducted. The TL shall document the approval of the annual on-site visit.

21.6.4 The CTDSS shall maintain documentation of the Vendor Laboratory's external QAS audit document report, the Vendor Laboratory's responses and/or follow-up actions to any findings detailed in the report.

21.6.5 The CTDSS may elect to accept audit documentation generated from another NDIS laboratories' external audit conducted on the Vendor Laboratory following FBI QAS Standard 15. Such documentation shall be retained at the laboratory by the CTDSS Quality Section.

RETIRED

21.6.6 Documentation shall be retained demonstrating the date on which the TL provided approval to the Vendor Laboratory for the technical specifications of the outsourcing agreement to be used prior to the Vendor Laboratory initiating analysis. Approval may be in the form of an e-mail, fax or documented phone call, etc.

21.6.7 Documentation shall be retained demonstrating the TL had prior approval of the acceptance of ownership of the DNA data from the vendor lab as well as the date that the CTDSS first uploaded DNA data to CODIS.

Per the QAS document, ownership occurs when any of the following criteria are applicable:

1. The originating laboratory will use any samples, extracts, or materials for the vendor laboratory for the purposes of forensic testing (i.e, a vendor lab prepares an extract that will be analysed by the originating laboratory).
2. The originating laboratory will interpret the data generated by the vendor laboratory.
3. The originating laboratory will issue a report on the results of the analysis.
4. The originating laboratory will enter or search a DNA profile in CODIS from data generated by the vendor laboratory.

21.6.8 If a Vendor Laboratory is performing testing for a law enforcement agency or entity other that the CTDSS, it is the responsibility of the Vendor Laboratory to maintain the dated, documented approval obtained from the TL agreeing to accept ownership of the DNA data, as well as the date that the Vendor Laboratory first initiated analysis for a specific case or set of cases. All approval documents will be kept by the Quality Section.

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21.7 Evaluation of Outsource DNA Results by DSS DNA Analysts-Special Circumstances

The following applies to the deconvolution and statistical analysis of Fusion 6C (F6C) profiles generated at outsourcing laboratories when STRmix analysis is not performed by the vendor laboratory and/or when the protocols of the outsourcing lab do not permit statistics/comparisons and CT DSS believes a CODIS entry is available.

Please refer to DNA SOP-6 for the applicable general report statements and DNA SOP-33 & WI-37 (Analysis of Vendor Generated F6C Profiles).

The vendor generated profiles which have gone through all QAS requirements of analysis and Standard 12 review (technical and administrative review) will be used to make any comparisons or CODIS entries that the CT DSS accepts and "owns". A DNA report will be sent out for the ownership cases as stated above in 21.3.9.1. The CT DSS will evaluate these vendor-generated profiles as discussed below:

21.7.1 Mixture Deconvolution for F6C Outsource Results (Bode Cellmark/DNA Labs International)

Definitions:

Mixture Ratio: The ratio between an apparent minor and apparent major allele to see if the results fits within the laboratory set threshold.

Peak Height Ratio (PHR): The peak balance between two sister alleles in a heterozygous pair.

21.7.1.1 Assumed Two-Person Mixture:

A mixture profile in which the number of detected peaks (≥ AT, see WI-37) per locus does not exceed four, barring other scientific reasons, can be assumed to be a two person mixture. This assumption will be clearly indicated on DNA reports.

If there are ≥ 10 loci (where the mixture is detected) in which all the detected peaks/alleles are above the stochastic threshold (Bode Cellmark = 350 RFU using the ABI 3500XL; DNA Labs International (DLI) = 325 RFU using the 3130XL), the DSS analyst may perform the unrestricted combined probability of inclusion (CPI) calculation at those loci for inclusionary/CBE conclusions instead of deconvolution as per SOP 21.8.2. The stochastic thresholds are based on the vendor lab's validation data (mean + 3 SD).

If there are < 10 loci (where the mixture is detected) where all the detected peaks/alleles are above the stochastic threshold, an attempt to deconvolute the major and minor contributor will be made.

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21.7.1.1.1 A deconvoluted 2 person mixture with a major and minor contributor may be able to be determined following the procedure below. If deconvolution is not possible, the CPI calculation may be performed for inclusions on loci with all peaks above the stochastic threshold and after "certifying" what loci are to be used for the calculation. It is important to note that all inclusionary/CBE statements will have the same statistical weight with a CPI calculation regardless of the known profile being compared.

21.7.1.2 For genotype assignment of a heterozygous contributor above the stochastic threshold, the peak balance must be ≥ 65%. For the minor contributor, (if at or below the stochastic threshold) the heterozygous peak balance must be ≥ 50%.

21.7.1.3 Determining a major and minor contributor for assumed two person mixtures

When determining the major or minor, calculate according to the number of alleles present at each locus (see below). For all mixture ratio calculations, perform the calculations as described using macro QR-307. Each locus will be calculated independently.

21.7.1.3.1 Loci with 4 Alleles

If the Mixture Ratio is:

$$\frac{\text{Height of the highest allele from apparent minor}}{\text{Height of the lowest allele from apparent major}} \leq 50\%$$

The two highest alleles may be called as the major contributor and the remaining alleles may be called the minor contributor. If the above calculation is > 50%, the locus is deemed indistinguishable and cannot be deconvoluted for statistical purposes. A CPI calculation would be made where appropriate.

21.7.1.3.2 Loci with 3 Alleles

If there is a heterozygous major, and the peak height ratio of the two highest alleles is ≥ 65% (if above the stochastic threshold, ≥50% if below) and the mixture ratio between the smallest allele and the 2nd highest allele is ≤ 40%, then the two largest peaks are reported as the major contributor and the remaining allele as the minor contributor. However, for inclusionary/CBE comparisons, for the statistical weight of the minor, the homozygous minor genotype is considered ambiguous and will be calculated by random match probability using 2p-p2. If these conditions are not met, the locus is deemed indistinguishable and cannot be used in statistical calculations.

If there is a homozygous major, and the mixture ratio of the larger minor allele to the largest allele is ≤ 30%, then the largest allele is reported as the major contributor and the remaining alleles as the minor. If this condition is not met, the locus is deemed indistinguishable and cannot be used in statistical calculations. For the heterozygous minor; if the alleles are above the stochastic threshold, the PHR must be ≥ 65% and if the alleles are below the stochastic threshold, the PHR must be ≥ 50%.

21.7.1.3.3 Loci with 2 Alleles

If there is a heterozygous major and the peak height ratio of those two alleles is ≥ 65% above the stochastic threshold, or ≥ 50% at or below the stochastic threshold, then report the two alleles as the major contributor. No minor contributor is reported for this locus. (If the heterozygous major alleles are below the stochastic threshold, the locus may be deemed indistinguishable and not used for statistics with TL approval).

If there is a homozygous major and peak height ratio of the two alleles is ≤ 20%, then the highest allele is reported as the major contributor and the lower allele is assigned as the minor. For the minor genotype, use $2p\text{-}p^2$ for the RMP calculation. If the

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peak height ratio between the two alleles is between <65% and >20% (above stochastic threshold) and <50% and >20% (at or below stochastic threshold), the locus is deemed indistinguishable and cannot be used in statistical calculations.

21.7.1.3.4 Loci with 1 Allele

Evaluate the mixture profile overall. The allele is reported out as the major. No minor contributor is reported for this locus. For statistical purposes, if the allele is above the stochastic threshold, $p^2+p(1-p)\theta$ will be used and if the allele is at or below the stochastic threshold, $2p-p^2$ will be used.

21.7.1.4 Three and 4 person mixtures

Deconvolution is only possible for the major contributor. There needs to be 10 or more loci that meet the below criteria to deduce a major contributor.

If there is an apparent heterozygous major (above ST) and the peak height ratio between those two alleles is ≥ 65% and the mixture ratio between the highest minor allele and lowest of the major alleles is ≤ 33%, the two highest alleles are reported as the major contributor.

If there is an apparent homozygous major (above ST) and the mixture ratio between the homozygous allele and the next highest allele is ≤ 25%, the highest peak is reported as the major contributor.

21.7.1.5 Deducing a Clear Major Contributor in Profiles with > 5 Contributors

A clear major contributor may be deduced from profiles containing ≥ 5 contributors as outlined below (requires TL & Assistant Director approval). The clear major contributor must be deduced without consideration of any known profile.

1. Where the allele(s) of the major contributor are at least 500 RFU at ≥10 autosomal loci, the heterozygous peak balance of the major profile is ≥ 65% at ≥10 autosomal loci, and the following conditions are met:

A. **Apparent heterozygous major**: the height of the highest minor allele is ≤ 33% of the height of the lowest major allele. If not, the locus does not qualify for statistics.

B. **Apparent homozygous major**: the height of the highest minor allele is ≤ 25% of the height of the major allele. If not, the locus does not qualify for statistics.

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2. The allele(s) of the major profile should be called at the remaining loci that do not qualify for statistics.

   Notes: The impact of stutter on the height of a minor peak should be addressed by evaluating how much of the peak height would be the result of average stutter from the major allele. Given that the PCR biological model shows continuous peak height variation, exceptions to the binary criteria listed above may be made with TL & Assistant Director approval as warranted.

3. If conditions listed above are met, CODIS entry of the deduced major contributor is permitted with CODIS Administrator/designee approval.

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21.8 Statistics

A macro is used to assist analysts in determining what loci meet the deconvolution rules. The loci that qualify and do not qualify for statistical purposes will be documented on QR-307 and will be kept in the case file.

21.8.1 Random Match Probabilities (RMP)

Note: For standard reporting purposes, statistics are calculated for the African American, Caucasian, and Southeast Hispanic populations (PopStats allele frequencies)

21.8.1.1 Autosomal STR Statistics:

Match probabilities are calculated according to the Hardy-Weinberg equilibrium and the product rule with the exceptions listed below. A statistical evaluation of any scientifically relevant result is included on the case report where comparisons to known profiles are possible except for where the results are inconclusive, IFC, exclusionary or if there is a non- probative comparison of a known to their own intimate sample, such as a vaginal swab, epithelial rich sample. An intimate sample is defined as any sample collected directly from the body, e.g., body cavity, skin swabs, nail clippings/swabbings/scrapings, etc.

21.8.1.2 Autosomal RMP Ceiling:

21.8.1.2.1 For results generated by vendor laboratories, when required the CT Division of Scientific Services will report a RMP ceiling of 1.43×10^{-10}. Any match probability (or CPI) less than 1.43×10^{-10} (1 in 7 billion) will be reported as follows: the expected frequency of individuals who could be the source (contributor) of the observed DNA profile is less than 1 in 7 billion. This does not imply that the product rule calculation (without a ceiling) is scientifically unwarranted. In the event that the ceiling is not reached, match probabilities are reported according to FBI DNA Quality Assurance Standards and as described in NRC II:

Heterozygotes	$2pq$
Homozygotes	$p^2 + p(1-p)\theta$; where $\theta = 0.01$

(Note: for homozygotes below stochastic threshold, $(2p-p^2)$ will be used since the genetoype is ambiguous at that locus.)

21.8.1.2.2 The calculation of RMP (random match probability) is performed on the following for inclusionary comparisons:

a. The profile detected has been determined to come from a single source. Note again that the RMP calculation does not need to be calculated for known profiles compared to their own intimate sample as stated above in 21.8.1.1.
b. A major contributor has been separated out of a mixture following procedure in section 21.7.1.
c. A minor contributor has been separated out from an assumed 2-person mixture as described in section 21.7.1.3.
d. For an assumed 2-person mixture produced from an intimate sample, the RMP can be calculated on a deduced "foreign" profile generated from "subtracting out" the expected known contributor compared to their own intimate sample. Where the "foreign" profile genotype is unambiguous, the standard RMP is used. If the locus is ambiguous, regardless of stochastic threshold, the $2p-p^2$ is used. The locus is not used in the statistical calculation if the expected known profile would completely mask the foreign profile.

21.8.2 Autosomal Mixtures:

21.8.2.1 The unrestricted or restricted combined probability of inclusion (CPI) is calculated for unresolved mixtures (see F6C mixture discussion in SOP 33).

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When single source profiles cannot be resolved from a DNA mixture, the unrestricted Combined Probability of Inclusion (CPI) calculation may be used to signify the weight of the match. The CPI requires no assumptions about the number of contributors and is used only on loci where all alleles are above the stochastic threshold. The probability of dropout increases at or below the stochastic threshold as demonstrated by the laboratory's validation data (Bode Cellmark--350 RFU; DLI--325 RFU). Loci which contain alleles/peaks below the defined stochastic threshold are not used in the CPI calculation. The loci that can and cannot be used for statistics will be documented in the case notes and will be determined prior to comparisons to any known reference samples. There needs to be at least one locus in an evidentiary profile that is deemed suitable for statistics to make any inclusionary/CBE association.

21.8.2.2 The loci that the unrestricted CPI is calculated from will be stated in the report. For example:

Known A is included as a potential contributor to the DNA profile from item #xxx. The expected frequency of individuals who could be a contributor to the DNA profile (at loci: D8S1179, vWA, and TPOX) from item #xxx is approximately 1 in xxx in the African American population, approximately 1 in xxx in the Caucasian population, and approximately 1 in xxx in the Hispanic population.

Known B cannot be eliminated as a potential contributor to the DNA profile from item #xxx. The expected frequency of individuals who could be a contributor to the DNA profile (at loci: D8S1179, vWA, and TPOX) from item #xxx is approximately 1 in xxx in the African American population, approximately 1 in xxx in the Caucasian population, and approximately 1 in xxx in the Hispanic population.

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CPI calculation:

$PI = P^2$, where P is the sum of the observed allelic frequencies. PI is the probability of inclusion at one locus.

$CPI = (PI_i)(PI_j)(PI_k)\ldots(PI_n)$ = probability of inclusion at multiple loci.

21.8.2.3 A locus is used in the unrestricted CPI calculation only when all the peaks detected are above the stochastic threshold and the known source(s) have been detected in the evidentiary profile(s).

21.8.2.4 The restricted combined probability of inclusion (CPI) may be calculated for 3-4 person mixtures where there is a clear "major" mixture component above the stochastic threshold. The subset of "major" allele peak heights must be at least 500 RFU and the distinct group must be ≥ 250

RFU from any minor alleles/peaks detected at the locus. Additionally, the mixture ratio of the highest non restricted allele to the lowest restricted allele must be < 50%. For restricted CPI, the calculation is performed only using the restricted set of alleles, determined prior to any comparison, and as described in.21.8.2.1 All alleles ≥ 500 RFU must qualify by the above rules and must be used in the restricted set. If these conditions are not met, then the locus is deemed indistinguishable and is not used for statistical calculations.

21.8.2.5 A macro is used to assist analysts in determining what loci can be used for the CPI calculations. The loci that qualify and do not qualify will be documented on QR-307 and will be kept in the case file.

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APPENDIX 1

RETIRED

STATE OF CONNECTICUT

DEPARTMENT OF
EMERGENCY SERVICES and PUBLIC PROTECTION
DIVISION OF SCIENTIFIC SERVICES

Guy M. Vallaro, Ph.D.
Director

DNA NOTIFICATION

Laboratory Case	**Submitting Agency**	**Date of Request**
DSS-19-00TEST	Ansonia Police Department 2 Elm St Ansonia, CT 06401	1/1/2019
Agency Case		**Date of Report**
12345		Report Date

The above mentioned case was outsourced to DNA Labs International (260 SW Natura Ave, Deerfield Beach, FL 33441). No CODIS entry was able to be made. Please refer to the attached DNA Labs International report dated 04/xx/2019 for DNA testing results and conclusions. Evidence will be returned to the submitting agency.

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Melanie G. Russell (Analyst)
Forensic Science Examiner 1

RETIRED

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An Affirmative Action / Equal Opportunity Employer

23.1 **PURPOSE**

Technical reviews are performed on all case files prior to reports being issued. Technical reviews are performed by any qualified analyst to verify that the conclusions and data are scientifically supported through the documentation in the case file.

Administrative reviews are performed on all case files prior to reports being issued. Administrative reviews are performed by any laboratory personnel who have been trained to review DNA cases and have access to LIMS. These reviews are used to ensure that the case demographics are correct, there are not typographical errors, and the documentation is consistent with laboratory policies.

In addition, a DNA analyst trainee who has been approved to be an administrative reviewer may AR cases where interpretations are reported. These reviews are used to ensure that the case demographics are correct, there are not typographical errors, and the documentation is consistent with laboratory policies.

Please refer to GL-18: Case Reviews (Technical &Administrative Reviews) for general guidelines.

Note: Technical and Administrative reviews for outsourced case work will be addressed in DNA SOP 21 and GL 18.

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23.2 **RESPONSIBILITY**

DNA section personnel.

23.3 **DEFINITIONS**

Definitions as per the FBI QAS:

DNA Technician: is an employee or contract employee who performs analytical procedures on forensic samples and/or database, known or casework reference samples under the direction of qualified analyst. This individual has successfully completed the laboratory's training requirements for casework and/or database sample processing, passed a competency test, and has entered into a proficiency testing program. Technicians do not interpret data to reach conclusions on typing results or prepare final reports.

DNA Analyst: is an employee or contract employee, that has successfully completed the laboratory's training requirements for casework sample analysis and/or database analysis, passed a competency test, and has entered into a proficiency testing program. This

individual conducts and/or directs the analysis of forensic samples and/or database, known or casework reference samples, interprets data and reaches conclusions.

Technical Review: is an evaluation of reports, notes, data, and other documents to ensure that there is appropriate and sufficient basis for the scientific conclusions. Elements are documented on DNA QR-4A "Batch Paperwork Review Checklist", DNA QR-4 "Case Review Checklist", and DNA QR-4E "Male Screen Batch paperwork Review Checklist".

Technical Reviewer (TR): is an employee or contract employee who is a current or previously qualified DNA analyst in the methodology being reviewed and is not an author of the applicable report. The Technical Reviewer is authorized to conduct technical reviews and has had casework experience prior to performing any technical reviews. The Technical Reviewer must be qualified in the method, technology, typing test kit, platform and interpretation software that the review encompasses. A DNA Technical Reviewer must be proficiency tested semi-annually to the extent to which he or she performs casework.

Administrative review: is an evaluation of the report and supporting documentation for consistency with laboratory policies and for editorial correctness. Elements are documented on DNA QR-4A "Batch Paperwork Review Checklist", DNA QR-4 "Case Review Checklist", and DNA QR-4E "Male Screen Batch Paperwork Review Checklist". AR and TR for male screen batch paperwork completed by one qualified analyst.

Administrative reviewer (AR): is an employee who is not an author of the applicable report.

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23.4 **PROCEDURE**

23.4.1 Case documentation:

A DNA analyst/technician receives a set of cases for processing and assigns a unique batch identifier to all cases to be processed together. Batch identifiers typically include the analyst's initials, date batch is started, and the batch number for that date (i.e. XXX010101).

The Batch ID is a unique identifier that acts as an address for traceability. Each batch shall be assigned a Batch ID and the Batch ID shall be documented on every page of the batch paperwork.

The batch paperwork for a case is considered part of the entire case record. Inside the physical case file folder will be additional technical and administrative records for the specific case, such as, but not limited to TR/AR checklists, evidence submission receipts,

communications, evidence exam worksheets, electropherograms for question samples and known samples (if applicable), CODIS documentation (if applicable), and draft reports.

When sample processing and analysis is completed, the Analyst prints electropherograms to be included in case jackets (for case samples) and relevant control electropherograms (RB, NEG, EP1, and POS) for the batch and initials all benchwork worksheets (from extraction through injection). It is not necessary to print ladders.

All case jackets, electropherograms, and paperwork for one batch are transferred to the Technical Reviewer (TR). The TR technically reviews the electronic data and all bench paperwork. The TR then transfers the batch paperwork and case jackets to the Administrative Reviewer (AR). The AR administratively reviews the batch paperwork and returns it to the Analyst. All reviews of the batch paperwork are documented on DNA QR-4A "Batch Paperwork Review Checklist".

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The Analyst separates electropherograms by case, and photocopies the injection worksheet(s) and Quantifiler Trio reports associated with Q sample(s) for each case in the set. All original worksheets (extraction, quant, amp, injection) and control electropherograms for each batch are scanned to the DNA network. The TR checks that scanning was done appropriately. The batch papers are retained together in the appropriate folder located in the Casework Processing Filing Cabinets. As needed, batch paperwork may be archived in room III-9. Batch paperwork will be stored in file folders by year in alphabetical order using the first initial of the analyst's first name in appropriately labelled bankers boxes.

Electropherograms for casework known samples, with their corresponding injection worksheets and batch paperwork review worksheets, are distributed to the assigned case Analyst. If a case has not been assigned to an Analyst the paperwork is given to Case Management.

The Analyst writes reports for each case in the batch, adds additional worksheets, as needed, and adds case # and initials to all pages in each case jacket. A copy of DNA QR-4A (Batch Paperwork Review Checklist) is placed in each case jacket.

If a sample fails during processing and it will be processed with a different batch, place a copy of the failed worksheet in the appropriate case jacket (i.e. if it fails Quant, put the failed Quant worksheet in the appropriate case jacket and note which sample failed; If you

determine based on injection that it needs to be re-amplified, put the failed injection worksheet in the appropriate case jacket with the reason why it will be reprocessed).

If a sample(s) within one previously processed batch is amped later, either as a re-amp or using a different amplification kit, label the batch paperwork for the re-amp with the original Batch ID for the sample. After first and second analysis, add the new batch paperwork to the original batch papers and refile them in the filing cabinet.

If pooling samples from several different previously processed batches to be amped later either as re-amps or using a different amplification kit (i.e Y-STR, etc.), a new unique Batch ID shall be assigned and documented on all pages in that set.

Note: Only official case receipts and records shall be maintained in the DNA case file. All case results/records shall be documented on the appropriate, controlled QR worksheets. For STR analysis, the electropherograms are included in the case folder with the exception of convicted offender samples. All electronic files regarding STR analysis (ie. complete run folders of .fsa sample files together with .ser or .sgf project files) are archived appropriately.

RETIRED

23.4.2 Case review:

23.4.2.1 Analysts are expected to review their own case work prior to submitting it for technical review. DNA QA/QC Case Work Checklist Review (QR-4) should be used to confirm all appropriate case information resides in the case jacket. In addition, the case jacket should be reviewed for transcriptional and typographical errors.

23.4.2.2 If the evidence was examined and forensic biology tests were performed by a DNA analyst authorized in those testing methods, the results may be incorporated into a DNA report. Refer to FB-SOP-05 for Forensic Biology result statements.

23.4.2.3 The technical review function is performed by the TR and documented on QR-4. In general, cases assigned to FSE 1s and FSE 2s, the TR is done by the direct Lead or designee. In general, cases assigned to FSE 3s, the TR is done by a FSE 2, or designee.

The elements of a Technical Review include but are not limited to the following as per the FBI QAS.

A review of all case notes, worksheets and electronic data (or printed electropherograms/images) that support the conclusions.

A review of all DNA types to verify that they are supported by the raw or analyzed data (electropherograms/images).

A review of all profiles to verify correct inclusions and exclusions (if applicable) as well as a review of any inconclusive result for compliance with laboratory guidelines.

A review of all controls, internal lane standards, and allelic ladders to verify that the expected results were obtained.

A review of statistical analysis (if applicable).

A review of the final report to verify that the results/conclusions are supported by the data and that the report has addressed each tested item.

A verification that all profiles entered into CODIS are eligible, have the correct DNA types, and correct specimen category.QR-13 "CODIS Profile Entry" will document the verification of CODIS eligibility and that the profiles entered have the correct DNA types and correct specimen category (Refer to DNA SOP-13 for more detailed guidance on CODIS Entry).

RETIRED

23.4.2.3.1 If the TR of the case file was not the person who reviewed the electronic data for the evidentiary (forensic unknown) samples, they must go back and technically review the data for all casework results that have not already been reported. The TR may choose not to technically review the data if the results were previously reported. Any non-concordance of analysis will be addressed between the Analyst, the TR, and the TL (if necessary).

23.4.2.3.2 The TR will confirm that all appropriate case information resides in the case jacket.

23.4.2.3.3 The TR will verify that the conclusions made in the case are supported through the documentation in the case file.

23.4.2.3.4 In addition, the case jacket should be reviewed for transcriptional and typographical errors.

23.4.2.3.5 No analyst may technically review their own work.

23.4.2.3.6 If the evidence was examined by a forensic biologist, no forensic biology tests were performed, and the forensic biology worksheets reside in the DNA case jacket: a DNA analyst can TR these worksheets. Essentially, the forensic biologist is being used as a laboratory assistant.

23.4.2.3.7 Cases containing forensic biology tests must be Technically Reviewed by a DNA analyst who is competent and at one time proficient for the tests performed.

23.4.3 The administrative review function is performed by the AR and documented on DNA QR-4 "Case Review Checklist". The elements of an Administrative Review include but are not limited to the following as per the FBI QAS:

A review of the case file and final report for clerical errors.

A review of the chain of custody and disposition of evidence.

23.4.4 In the event of discrepant conclusions, the examiners will discuss/review the results. Should a discrepancy persist, the data will be reviewed and conclusions approved by the Technical Leader (TL).

23.4.5 Where practicable, one case per analyst will be reviewed quarterly by the TL. See DNA SOP-01 for guidance.

RETIRED

25.1 PURPOSE:

25.1.1 Kinship Analysis assesses alternate or competing hypotheses of relatedness. In the forensic context, it is useful for determining familial relationships, the identification of unknown bodies, and the identification of the donor of biological evidence when the donor/body is missing or unavailable.

25.2 RESPONSIBILITY:

DNA Section Personnel trained in Kinship Analysis and CODIS 9.0 Popstats.

25.3 Kinship Evaluations:

25.3.1 The DNA profile from the subject/stain in question is compared to the DNA profile of close biological relatives. For parent(s)/child comparisons, the loci are first evaluated to determine whether the individual in question can be excluded as a biological child of the other individual(s). An individual must be excluded at more than two loci (when 13-23 STRs are typed) in order to be eliminated as a potential parent.

25.3.1.1 Regarding apparent mutations using STR systems, the repeat # difference of the putative mutation may be relevant to the final conclusion.

25.3.1.2 In the event of apparent mutations, additional testing may be conducted as appropriate.

25.3.1.3 If one or two loci have an apparent mutation, they are included in the kinship index since a mutation rate is incorporated in the calculation.

RETIRED

25.3.1.4 If an alleged parent and child are both homozygous at a locus (but for different alleles), a null allele could be a possibility. On a case by case basis and with TL approval, this locus will not be used in the statistical calculation.

25.3.2 If the individual cannot be excluded, or for comparisons not involving a parent(s)/child relationship (i.e. siblings), a KI (kinship index) is calculated for each locus using CODIS 9.0 Popstats.

25.3.3 Popstats then calculates an overall CKI (combined kinship index) by multiplying all of the individual KIs. A probability of relatedness is then calculated using Bayes' theorem and assuming a prior probability of 50% (for non-criminal applications only). In criminal cases with MP/UHR results, the CKI will be reported without the prior probability calculation per State v. Skipper, 228 Conn. 610 (1994). A report is printed out from Popstats and is included in the case file as the statistics sheet.

25.3.4 The FBI Population Databases (African American, Caucasian, and Hispanic) have been validated and are selected within Popstats for the Kinship calculations.

RETIRED

25.4 Relationship Indexes Evaluated (non-criminal):

25.4.1 Single Parentage- (a) Mother or Father, Alleged Child is the MP/UHR
(b) Child, Alleged Mother or Father is the MP/UHR

25.4.2 Reverse Parentage - Mother and Father, Alleged Child is MP/UHR

25.4.3 Parentage Trio - One Parent and One Child, Other Alleged Parent is MP/UHR

25.4.4 Full sibling - Brother or Sister, Alleged Full Sibling is MP/UHR

25.5 Using Popstats:

25.5.1 For Reverse Parentage or Parentage Trio scenarios:

Launch CODIS 9.0 Analyst Workbench. Click on "Popstats" followed by "Parentage". Click on "Reverse" for the Subtype if you have STR profiles from both parents and the alleged child is the MP/UHR sample. Click on "Trio" for the Subtype if you have STR profiles from one parent and one child and the other alleged parent is the MP/UHR sample. Enter the Specimen ID.

The specimen ID # is the laboratory case # followed by a space then the submission/item # (e.g. 14-xxxx 1G1). For specimens already entered into CODIS, the DNA profile can be retrieved by clicking on the "Retrieve" button, after the specimen ID # has been entered. Enter the STR results for all applicable loci. In the instance of genetic inconsistencies, allelic information will not be entered for that locus for all samples in the comparison. Once the STR profiles have been entered, click "Calculate". Calculations for each population group will be computed. Print the results by selecting "Print→ Reverse Parentage Statistics" or "Print→ Parentage Trio Calculations" depending on the type of calculation conducted.

Autosomal CKI's can be combined with mitochondrial DNA and/or Y-STR statistics whenever applicable.

RETIRED

25.5.2 For Single Parentage or Full Sibling scenarios:

Launch CODIS 9.0 Analyst Workbench. Click on "Popstats" followed by "Kinship". Enter the Specimen ID. The specimen ID # is the laboratory case # followed by a space then the submission/item # (e.g. 14-xxxx 1G1). For specimens already entered into CODIS, the DNA profile can be retrieved by clicking on the "Retrieve" button, after the specimen ID # has been entered. Enter the STR results for all applicable loci. The sample in question (i.e. the DNA profile obtained from the UHR/MP) shall be entered as the "Evidence" and the known sample shall be entered as the "Reference". Select the relevant Kinship Abbreviation based upon the case scenario. Parent-Offspring is abbreviated PO and Full Siblings is abbreviated FS. De-select all other abbreviations so only one relationship is being evaluated. In the

instance of genetic inconsistencies (PO only), allelic information will not be entered for that locus for both samples in the comparison (see section 25.7.7 for further guidance). Once the STR profiles have been entered and the relationship being compared has been selected, click "Calculate". Calculations for each population group will be computed. Print the results by selecting "Print→ Popstats Single Parentage Statistics" for PO scenarios or "Print→ Popstats Kinship Locus Report" for FS scenarios.

Autosomal CKI's can be combined with mitochondrial DNA and/or Y-STR statistics whenever applicable.

RETIRED

25.6 Reporting Results

25.6.1 All comparisons will be reported. The DNA report should state the results for one race, preferably the race of the individual in question. If the race is unknown, then all three major population groups (African American, Caucasian, and Hispanic) will be reported. Instances where the race provided is not one of the three major population groups will be handled on a case-by-case basis, with approval by the TL.

25.6.2 The DNA report must list the race(s) used for the calculation(s), the CKI, the assumed prior probability, the probability of relatedness expressed as a percentage (rounded to four decimal places), and a statement that calculations compare the tested individual to either an unrelated or related individual. Any loci not used in the calculation(s) will also be listed in the report.

25.6.3 The following sibling CKI's are used as a guide for report wording:

<u>Full Sibling CKI</u>

<1- does not support sib-ship

1-9- limited support of sib-ship

10-99- moderate support of sib-ship

100-999- strong support of sib-ship

1000+- very strong support of sib-ship

If the race is unknown, the lowest CKI will be used for qualitative purposes.

25.7 Report Examples

25.7.1 The following examples are intended to provide a general guideline for the preparation of DNA reports as it pertains to Kinship Analysis. However, it is not an exhaustive list of all possible casework scenarios. Other reporting strategies may be used with approval of the TL.

25.7.2 Single Parentage:

The DNA results are consistent with Karen Smith being a parental contributor to item #1. It is XX times more likely (assuming a 50% prior probability) that Karen Smith is the biological mother of the source of item #1 as opposed to a random, untested individual in the X population who is unrelated to Karen Smith. The relative probability of maternity is XX.

25.7.3 Reverse Parentage:

The DNA results are consistent with Karen Smith and Mark Smith being parental contributors to item #1. It is XX times more likely (assuming a 50% prior probability) that Karen Smith and Mark Smith are the biological parents of the source of item #1 as opposed to random, untested individuals in the X population who are unrelated to Karen Smith and Mark Smith. The relative probability of parentage is XX.

25.7.4 Parentage Trio:

RETIRED

The DNA results are consistent with Karen Smith and the source of item #1 being parental contributors to the DNA profile obtained from Jason Smith. It is XX times more likely (assuming a 50% prior probability) that Karen Smith and an individual with a DNA profile matching item #1 are the parents of Jason Smith rather than if the DNA profile from item #1 is from a random, untested individual in the X population who is unrelated to Karen Smith and Jason Smith. The relative probability of paternity is XX.

25.7.5 Full Siblings:

It is XX times more likely (assuming a 50% prior probability) that Karen Smith and the source of item #1 are related as full siblings rather than being unrelated individuals in the X population. This is xx support of sib-ship. The relative probability of full sib-ship is XX.

RETIRED

25.7.6 Unknown Race:

The DNA results are consistent with Karen Smith being a parental contributor to item #1. It is XX times more likely (assuming a 50% prior probability) in the African American population group, XX times more likely (assuming a 50% prior probability) in the Caucasian population group, and XX times more likely (assuming a 50% prior probability) in the Hispanic population group that Karen Smith is the biological mother of the source of item #1 as opposed to a random, untested individual in the African American, Caucasian, and Hispanic populations who is unrelated to Karen Smith. The relative probability of maternity is XX in the African American population group, XX in the Caucasian population group, and XX in the Hispanic population group.

25.7.7 Mutation Event:

Note: This statement will be used only if an alleged parent and child are both homozygous at a locus (but for different alleles), and a null allele could be a possibility. It will be used on a case by case basis, with TL approval.

The DNA results are consistent with Mark Smith being a parental contributor to item #1. It is XX times more likely (assuming a 50% prior probability) that Mark Smith is the biological father of the source of item #1 as opposed to a random, untested individual in the X population who is unrelated to Mark Smith. The relative probability of paternity is XX.

A genetic inconsistency between Mark Smith and item #1 was observed at (locus name). This inconsistency could be due to a mutation event; therefore, this locus was not included in the above calculations.

RETIRED

25.8 Probability of Kinship Worksheet

25.8.1 To calculate the relative probabilities of kinship for the appropriate population group(s), the Probability of Kinship Worksheet QR-46 is utilized. The worksheet uses the combined kinship index number calculated by Popstats and the below statistical formula to calculate the overall relative probability:

Prior Probability: 50%

$$\textit{Probability of Kinship} = \frac{CKI}{CKI+1} \times 100$$

Note: This calculation is already listed on the Popstats printout for Parentage Trio Calculations, therefore, it does not need to be repeated.

25.9 Mutation Rates

The below equation is used for the Kinship Index at any locus where an apparent mutation has occurred. Popstats automatically incorporates the mutation rate into the calculation.

$$KI = \frac{\mu}{\bar{A}}$$

Where: μ = mutation rate at the locus

$\bar{A}$ = Average Probability of Exclusion for the locus

Mutation Rates per Locus

Locus	Mutation Rate (%)
CSF1PO	0.16
FGA	0.28
TH01	0.01
TPOX	0.01
vWA	0.17
D3S1358	0.12
D5S818	0.11
D7S820	0.10
D8S1179	0.14
D13S317	0.14
D16S539	0.11
D18S51	0.22
D21S11	0.19
Penta D	0.14
Penta E	0.16
D2S1338	0.12
D19S433	0.11
SE33	0.64
D1S1656	0.002
D2S441	0.002
D10S1248	0.002
D12S391	0.002
D22S1045	0.002

RETIRED

25.10 References

Evett, I.W. and Weir, B.S. (1998) *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists*. Sunderland, MA: Sinauer Associates.

Scientific Working Group on DNA Analysis Methods: Guidelines for Missing Persons Casework. Approved 01/09/14.

STRBase: http://strbase.nist.gov/mutation.htm

25.11 Appendix

Single Parentage Equations

Paternity calculations in cases where only the child's and alleged father's (or alleged mother's) genotypes are known.

Prob (E|U, C, F) is the probability that the evidence would be observed given that the alleged father was a biological parent of the child.

Prob (E|U, C, U) is the probability that the evidence would be observed given that the alleged father was not the biological father of the child.

LR is the ratio of the two possibilities (likelihood ratio) =

$$\frac{\text{Prob (E|U, C, F)}}{\text{Prob (E|U, C, U)}}$$

P_A, etc., is the estimated frequency of the "A" allele in the population

	Child	Alleged Father	Prob (E|U, C, F)	Prob (E|U, C, U)	LR
1	AA	AA	$P_A * (P_A)^2$	$(P_A)^2 * (P_A)^2$	$1/ P_A$
2	AA	AB	$P_A * 2P_AP_B * ½$	$(P_A)^2 * 2P_AP_B$	$1/(2P_A)$

RETIRED

3	AB	AA	$P_B * (P_A)^2$	$2P_AP_B * (P_A)^2$	$1/(2P_A)$
4	AB	AB	$(P_A + P_B) * 2P_AP_B * ½$	$2P_AP_B * 2P_AP_B$	$(P_A + P_B)/(4P_AP_B)$
5	AB	AC	$P_B * 2P_AP_C * ½$	$2P_AP_B * 2P_AP_C$	$1/(4P_A)$

Reverse Parentage Equations

Paternity calculations in cases where the mother's and father's genotypes are known.

Prob ((E|M, F, Q) is the probability that the evidence would be observed given that the mother and the father were the parents of the evidence sample (Q)

Prob (E|M, F, U) is the probability that the evidence would be observed given that a random member of the population was the was the questioned sample

LR is the ratio of the two possibilities (likelihood ratio) =

$$\frac{\text{Prob ((E|M, F, Q)}}{\text{Prob (E|M, F, U)}}$$

P_A, etc., is the estimated frequency of the "A" allele in the population

	Mother	Q	Father	Prob ((E|M, F, Q)	Prob (E|M, F, U)	LR
1	AA	AA	AA	$(P_A)^2 * (P_A)^2$	$(P_A)^2 * (P_A)^2 * (P_A)^2$	$1/(P_A)^2$
2	AA	AA	AB	$(P_A)^2 * 2P_AP_B * ½$	$(P_A)^2 * 2P_AP_B * (P_A)^2$	$1/(2(P_A)^2)$
3	AA	AB	BB	$(P_A)^2 * (P_B)^2$	$(P_A)^2 * (P_B)^2 * 2P_AP_B$	$1/(2P_AP_B)$
4	AA	AB	AB	$(P_A)^2 * 2P_AP_B * ½$	$(P_A)^2 * 2P_AP_B * 2P_AP_B$	$1/(4P_AP_B)$
5	AA	AB	BC	$(P_A)^2 * 2P_BP_C * ½$	$(P_A)^2 * 2P_BP_C * 2P_AP_B$	$1/(4P_AP_B)$
6	AB	AB	BB	$2P_AP_B * (P_B)^2 * ½$	$2P_AP_B * (P_B)^2 * 2P_AP_B$	$1/(4P_AP_B)$

RETIRED

7	AB	AB	AB	$2P_AP_B * 2P_AP_B * (¼ + ¼)$	$2P_AP_B * 2P_AP_B * 2P_AP_B$	$1/(4P_AP_B)$
8	AB	AB	AC	$2P_AP_B * 2P_AP_C * ½ * ½$	$2P_AP_B * 2P_AP_C * 2P_AP_B$	$1/(8P_AP_B)$
9	AB	AA	AA	$2P_AP_B * (P_A)^2 * ½$	$2P_AP_B * (P_A)^2 * (P_A)^2$	$1/(2(P_A)^2)$
10	AB	AA	AB	$2P_AP_B * 2P_AP_B * ½ * ½$	$2P_AP_B * 2P_AP_B * (P_A)^2$	$1/(4(P_A)^2)$
11	AB	AA	AC	$2P_AP_B * 2P_AP_C * ½ * ½$	$2P_AP_B * 2P_AP_C * (P_A)^2$	$1/(4(P_A)^2)$
12	AB	AC	CC	$2P_AP_B * (P_C)^2 * ½$	$2P_AP_B * (P_C)^2 * 2P_AP_C$	$1/(4P_AP_C)$
13	AB	AC	BC	$2P_AP_B * 2P_BP_C * ½ * ½$	$2P_AP_B * 2P_BP_C * 2P_AP_C$	$1/(8P_AP_C)$
14	AB	AC	AC	$2P_AP_B * 2P_AP_C * ½ * ½$	$2P_AP_B * 2P_AP_C * 2P_AP_C$	$1/(8P_AP_C)$
15	AB	AC	CD	$2P_AP_B * 2P_CP_D * ½ * ½$	$2P_AP_B * 2P_CP_D * 2P_AP_C$	$1/(8P_AP_C)$

Parentage Trio Equation

Paternity calculation in cases where mother's, child's and alleged father's genotypes are known.

Prob (E|M, C, F) is the probability that the evidence would be observed given the mother and alleged father were the biological parents of the child.

Prob (E|M, C) is the probability that the evidence would be observed given that a random member of the population was the biological father.

LR is the ratio of the two possibilities (likelihood ratio) =

$$\frac{\text{Prob ((E|M, C, F)}}{\text{Prob (E|M, C)}}$$

P_A, etc., is the estimated frequency of the "A" allele in the population

	Mother	Child	Alleged Father	Prob (E|M, C, F)	Prob (E|M, C)	LR

RETIRED

1	AA	AA	AA	$(P_A)^2 * (P_A)^2$	$(P_A)^2 * (P_A)^2 * P_A$	$1/P_A$
2	AA	AA	AB	$(P_A)^2 * 2P_AP_B * ½$	$(P_A)^2 * 2P_AP_B * P_A$	$1/(2P_A)$
3	AA	AB	BB	$(P_A)^2 * (P_B)^2$	$(P_A)^2 * (P_B)^2 * P_B$	$1/P_B$
4	AA	AB	AB	$(P_A)^2 * 2P_AP_B * ½$	$(P_A)^2 * 2P_AP_B * P_B$	$1/(2P_B)$
5	AA	AB	BC	$(P_A)^2 * 2P_BP_C * ½$	$(P_A)^2 * 2P_BP_C * P_B$	$1/(2P_B)$
6	AB	AB	BB	$2P_AP_B * (P_B)^2 * ½$	$2P_AP_B * (P_B)^2 * ½ * (P_A + P_B)$	$1/(P_A + P_B)$
7	AB	AB	AB	$2P_AP_B * 2P_AP_B * (¼ + ¼)$	$2P_AP_B * 2P_AP_B * ½ * (P_A + P_B)$	$1/(P_A + P_B)$
8	AB	AB	AC	$2P_AP_B * 2P_AP_C * ½ * ½$	$2P_AP_B * 2P_AP_C * ½ * (P_A + P_B)$	$1/(2P_A + 2P_B)$
9	AB	AA	AA	$2P_AP_B * (P_A)^2 * ½$	$2P_AP_B * (P_A)^2 * ½ * P_A$	$1/P_A$
10	AB	AA	AB	$2P_AP_B * 2P_AP_B * ½ * ½$	$2P_AP_B * 2P_AP_B * ½ * P_A$	$1/(2P_A)$
11	AB	AA	AC	$2P_AP_B * 2P_AP_C * ½ * ½$	$2P_AP_B * 2P_AP_C * ½ * P_A$	$1/(2P_A)$
12	AB	AC	CC	$2P_AP_B * (P_C)^2 * ½$	$2P_AP_B * (P_C)^2 * ½ * P_C$	$1/P_C$
13	AB	AC	BC	$2P_AP_B * 2P_BP_C * ½ * ½$	$2P_AP_B * 2P_BP_C * ½ * P_C$	$1/(2P_C)$
14	AB	AC	AC	$2P_AP_B * 2P_AP_C * ½ * ½$	$2P_AP_B * 2P_AP_C * ½ * P_C$	$1/(2P_C)$
15	AB	AC	CD	$2P_AP_B * 2P_CP_D * ½ * ½$	$2P_AP_B * 2P_CP_D * ½ * P_C$	$1/(2P_C)$

RETIRED

Full-Sibling Equations

Note: p and q are the frequencies of the alleles shared between genotypes #1 and #2

	#1	#2	Full-Sib	Unrelated
1	AB	AB	1 + p + q + 2pq	8pq
2	AA	AA	$(1 + p)^2$	$4p^2$
3	AA	AB	1 + p	4p
4	AB	AC	1 + 2p	8p
5	AB	CD	1	4
6	AA	BB	1	4

29.1 Legacy Kit Stutter Thresholds

29.1.1 Identifiler Stutter (N-1):

Locus	Allele	Threshold %
D8S1179	to 11.3	10
	12 +	12
D21S11	to 27.3	10
	28 – 31.3	12
	32 +	13
D7S820	all	10
CSF1PO	all	10
D3S1358	to 14.3	10
	15 +	12
THO1	all	10
D13S317	to 13.3	10
	14 +	12
D16S539	to 11.3	10
	12 +	12
D2S1338	all	12
D19S433	to 13.3	10
	14-15.1	12
	15.2 +	15
vWA	to 15.3	10
	16 +	12
TPOX	all	10

RETIRED

D18S51	to 14.3	10
	15 – 16.3	12
	17 +	14
D5S818	to 12.3	10
	13 +	12
FGA	to 22.3	10
	23 +	12
FGA (Extended)	30.2+	18

29.1.2 Identifiler Plus Stutter:

Locus	Allele	Threshold %	Allele	Threshold %
D8S1179	to 9.3	8	all	2.0
	10 – 12.3	10		
	13 - 16	12		
	16.1+	14		
D21S11	to 27.3	10	all	2.0
	28 – 32.3	12		
	33 – 35.3	13		
	36	14		
D7S820	to 9.3	8	all	2.0
	10 – 13.3	10		
	14+	12		
CSF1PO	to 9.3	8	all	2.0
	10 – 12.3	10		
	13+	12		
D3S1358	to 14.3	10	all	3.5
	15 – 17.3	12		
	18+	14		
TH01	to 7.3	5	all	2.0
	8+	7		
D13S317	to 9.3	7	all	3.0
	10 – 11.3	10		

RETIRED

		12+		12				
D16S539		to 9.3		8		all		2.0
		10 – 11.3		10				
		12 – 12.3		12				
		13+		14				
D2S1338		to 16.3		10		all		2.0
		17 – 23.3		12				
		24+		15				
D19S433		to 13		10		all		2.0
D19S433		13.1 – 14.3		12				
		15+		15				
vWA		to 14.3		10		all		2.0
		15 – 17.3		12				
		18+		15				
TPOX		to 10.3		8		all		2.0
		11+		10				
D18S51		to 10.3		8		all		3.0
		11 - 13		10				
		13.1 - 15		12				
		15.1 - 18		15				
		18.1+		18				
D5S818		to 9.3		10		all		3.0
		10 – 13.3		12				
		14		14				
FGA		to 18.3		10		all		2.0
		19 – 23.3		12				
		24 – 32.2		15				
		32.3+		18				

RETIRED

29.1.3 Yfiler Stutter:

Locus		Allele		Threshold %
DYS456		to 14		10
		14.1 - 17		13
		17.1 +		14

DYS389I		to 13.3		10
		14+		12
DYS390		to 23		10
		23.1 +		12
DYS389II		to 26.3		12
		27 - 29		14
		29.1 +		16
DYS458		to 15.3		10
		16 - 18		12
		18.1 +		14
DYS19		to 15.3		10
		16 - 18		12
		18.1 +		14
n-2 bases		all		14
n+2 bases		all		2.5
DYS385		to 13		10
		13.1 - 15		12
		15.1 - 19		15
		19.1 +		18
DYS393		to 10		10
		10.1 - 13		12
		13.1 - 15		14
		15.1 +		15
DYS391		to 11		10
		11.1 +		12
DYS439		to 10.3		10
		11 +		12
DYS635		to 20		10
		20.1 +		12
DYS392		to 12		12
		12.1 - 13		14
		13.1 +		18
n+3 bases		all		10

RETIRED

GATA_H4		to 11		10
		11.1 - 12		12
		12.1 +		14
DYS437		to 14.3		9
		15 +		11
DYS438		to 12		5
		12.1 +		6
DYS448		all		7

29.1.4 Fusion 6C Stutter (≤ 29 cycles):

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D3S1358	8	9.3	2.7	0	D3S1358	16	12.7	2.7	0
	8.1	9.3	2.7	0		16.1	12.7	2.7	0
	8.2	9.3	2.7	0		16.2	12.7	2.7	0
	8.3	9.3	2.7	0		16.3	12.7	2.7	0
	9	9.3	2.7	0		17	13.3	2.7	0
	9.1	9.3	2.7	0		17.1	13.3	2.7	0
	9.2	9.3	2.7	0		17.2	13.3	2.7	0
	9.3	9.3	2.7	0		17.3	13.3	2.7	0
	10	9.3	2.7	0		18	14	2.7	0
	10.1	9.3	2.7	0		18.1	14	2.7	0
	10.2	9.3	2.7	0		18.2	14	2.7	0
	10.3	9.3	2.7	0		18.3	14	2.7	0
	11	9.3	2.7	0		19	14.7	2.7	0
	11.1	9.3	2.7	0		19.1	14.7	2.7	0
	11.2	9.3	2.7	0		19.2	14.7	2.7	0
	11.3	9.3	2.7	0		19.3	14.7	2.7	0
	12	10	2.7	0		20	15.3	2.7	0
	12.1	10	2.7	0		20.1	15.3	2.7	0
	12.2	10	2.7	0		20.2	15.3	2.7	0
	12.3	10	2.7	0		20.3	15.3	2.7	0
	13	10.7	2.7	0		21	16	2.7	0
	13.1	10.7	2.7	0					
	13.2	10.7	2.7	0					
	13.3	10.7	2.7	0					
	14	11.3	2.7	0					

RETIRED

14.1	11.3	2.7	0
14.2	11.3	2.7	0
14.3	11.3	2.7	0
15	12	2.7	0
15.1	12	2.7	0
15.2	12	2.7	0
15.3	12	2.7	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D1S1656	8	8.7	2.7	3.8
	8.1	8.7	2.7	3.8
	8.2	8.7	2.7	3.8
	8.3	9.9	2.7	3.8
	9	8.7	2.7	3.8
	9.1	8.7	2.7	3.8
	9.2	8.7	2.7	3.8
	9.3	9.9	2.7	3.8
	10	8.7	2.7	3.8
	10.1	8.7	2.7	3.8
	10.2	8.7	2.7	3.8
	10.3	9.9	2.7	3.8
	11	8.7	2.7	3.8
	11.1	8.7	2.7	3.8
	11.2	8.7	2.7	3.8
	11.3	9.9	2.7	3.8
	12	9.8	2.7	3.8
	12.1	9.8	2.7	3.8
	12.2	9.8	2.7	3.8
	12.3	9.9	2.7	3.8
	13	10.8	2.7	3.8
	13.1	10.8	2.7	3.8
	13.2	10.8	2.7	3.8
	13.3	9.9	2.7	3.8
	14	11.8	2.7	3.8
	14.1	11.8	2.7	3.8
	14.2	11.8	2.7	3.8
	14.3	9.9	2.7	3.8

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D1S1656	17	14.9	2.7	3.8
	17.1	14.9	2.7	3.8
	17.2	14.9	2.7	3.8
	17.3	11.7	2.7	3.8
	18	15.9	2.7	3.8
	18.1	15.9	2.7	3.8
	18.2	15.9	2.7	3.8
	18.3	12.6	2.7	3.8
	19	17	2.7	3.8
	19.1	17	2.7	3.8
	19.2	17	2.7	3.8
	19.3	13.5	2.7	3.8
	20	18	2.7	3.8
	20.1	18	2.7	3.8
	20.2	18	2.7	3.8
	20.3	14.4	2.7	3.8
	21	19	2.7	3.8

RETIRED

15	12.9	2.7	3.8
15.1	12.9	2.7	3.8
15.2	12.9	2.7	3.8
15.3	9.9	2.7	3.8
16	13.9	2.7	3.8
16.1	13.9	2.7	3.8
16.2	13.9	2.7	3.8
16.3	10.8	2.7	3.8

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D2S441	8	7.7	2.1	0
	8.1	5.2	2.1	0
	8.2	7.7	2.1	0
	8.3	5.2	2.1	0
	9	7.7	2.1	0
	9.1	5.2	2.1	0
	9.2	7.7	2.1	0
	9.3	5.2	2.1	0
	10	7.7	2.1	0
	10.1	5.2	2.1	0
	10.2	7.7	2.1	0
	10.3	5.2	2.1	0
	11	9.3	2.1	0
	11.1	5.2	2.1	0
	11.2	9.3	2.1	0
	11.3	5.2	2.1	0
	12	10.9	2.1	0
	12.1	5.7	2.1	0
	12.2	10.9	2.1	0
	12.3	6.1	2.1	0
	13	5.5	2.1	0
	13.1	6.5	2.1	0
	13.2	5.5	2.1	0
	13.3	6.9	2.1	0
	14	6.4	2.1	0
	14.1	7.4	2.1	0
	14.2	6.4	2.1	0
	14.3	7.8	2.1	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D2S441	16	8.2	2.1	0
	16.1	9.1	2.1	0
	16.2	8.2	2.1	0
	16.3	9.5	2.1	0
	17	9.1	2.1	0

RETIRED

15	7.3	2.1	0
15.1	8.2	2.1	0
15.2	7.3	2.1	0
15.3	8.6	2.1	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D10S1248	8	7.1	1.3	0
	8.1	7.1	1.3	0
	8.2	7.1	1.3	0
	8.3	7.1	1.3	0
	9	7.1	1.3	0
	9.1	7.1	1.3	0
	9.2	7.1	1.3	0
	9.3	7.1	1.3	0
	10	7.1	1.3	0
	10.1	7.1	1.3	0
	10.2	7.1	1.3	0
	10.3	7.1	1.3	0
	11	8.2	1.3	0
	11.1	8.2	1.3	0
	11.2	8.2	1.3	0
	11.3	8.2	1.3	0
	12	9.2	1.3	0
	12.1	9.2	1.3	0
	12.2	9.2	1.3	0
	12.3	9.2	1.3	0
	13	10.3	1.3	0
	13.1	10.3	1.3	0
	13.2	10.3	1.3	0
	13.3	10.3	1.3	0
	14	11.3	1.3	0
	14.1	11.3	1.3	0
	14.2	11.3	1.3	0
	14.3	11.3	1.3	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D10S1248	17	14.5	1.3	0
	17.1	14.5	1.3	0
	17.2	14.5	1.3	0
	17.3	14.5	1.3	0
	18	15.5	1.3	0
	18.1	15.5	1.3	0
	18.2	15.5	1.3	0
	18.3	15.5	1.3	0
	19	16.6	1.3	0

RETIRED

15	12.4	1.3	0
15.1	12.4	1.3	0
15.2	12.4	1.3	0
15.3	12.4	1.3	0
16	13.4	1.3	0
16.1	13.4	1.3	0
16.2	13.4	1.3	0
16.3	13.4	1.3	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D13S317	5	6.3	2.3	0
	5.1	6.3	2.3	0
	5.2	6.3	2.3	0
	5.3	6.3	2.3	0
	6	6.3	2.3	0
	6.1	6.3	2.3	0
	6.2	6.3	2.3	0
	6.3	6.3	2.3	0
	7	6.3	2.3	0
	7.1	6.3	2.3	0
	7.2	6.3	2.3	0
	7.3	6.3	2.3	0
	8	6.3	2.3	0
	8.1	6.3	2.3	0
	8.2	6.3	2.3	0
	8.3	6.3	2.3	0
	9	7.2	2.3	0
	9.1	7.2	2.3	0
	9.2	7.2	2.3	0
	9.3	7.2	2.3	0
	10	8.1	2.3	0
	10.1	8.1	2.3	0
	10.2	8.1	2.3	0
	10.3	8.1	2.3	0
	11	9	2.3	0
	11.1	9	2.3	0
	11.2	9	2.3	0
	11.3	9	2.3	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D13S317	14	11.8	2.3	0
	14.1	11.8	2.3	0
	14.2	11.8	2.3	0
	14.3	11.8	2.3	0
	15	12.7	2.3	0
	15.1	12.7	2.3	0
	15.2	12.7	2.3	0
	15.3	12.7	2.3	0
	16	13.6	2.3	0
	16.1	13.6	2.3	0
	16.2	13.6	2.3	0
	16.3	13.6	2.3	0
	17	14.6	2.3	0

RETIRED

	12	10	2.3	0
	12.1	10	2.3	0
	12.2	10	2.3	0
	12.3	10	2.3	0
	13	10.9	2.3	0
	13.1	10.9	2.3	0
	13.2	10.9	2.3	0
	13.3	10.9	2.3	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
Penta E	4	3.1	1.9	0	Penta E	15	6.8	1.9	0
	4.1	3.1	1.9	0		15.1	6.8	1.9	0
	4.2	3.1	1.9	0		15.2	6.8	1.9	0
	4.3	3.1	1.9	0		15.3	6.8	1.9	0
	4.4	3.1	1.9	0		15.4	6.8	1.9	0
	5	3.1	1.9	0		16	7.3	1.9	0
	5.1	3.1	1.9	0		16.1	7.3	1.9	0
	5.2	3.1	1.9	0		16.2	7.3	1.9	0
	5.3	3.1	1.9	0		16.3	7.3	1.9	0
	5.4	3.1	1.9	0		16.4	7.3	1.9	0
	6	3.1	1.9	0		17	7.7	1.9	0
	6.1	3.1	1.9	0		17.1	7.7	1.9	0
	6.2	3.1	1.9	0		17.2	7.7	1.9	0
	6.3	3.1	1.9	0		17.3	7.7	1.9	0
	6.4	3.1	1.9	0		17.4	7.7	1.9	0
	7	3.1	1.9	0		18	8.2	1.9	0
	7.1	3.1	1.9	0		18.1	8.2	1.9	0
	7.2	3.1	1.9	0		18.2	8.2	1.9	0
	7.3	3.1	1.9	0		18.3	8.2	1.9	0
	7.4	3.1	1.9	0		18.4	8.2	1.9	0
	8	3.6	1.9	0		19	8.7	1.9	0
	8.1	3.6	1.9	0		19.1	8.7	1.9	0
	8.2	3.6	1.9	0		19.2	8.7	1.9	0
	8.3	3.6	1.9	0		19.3	8.7	1.9	0
	8.4	3.6	1.9	0		19.4	8.7	1.9	0
	9	4.1	1.9	0		20	9.1	1.9	0
	9.1	4.1	1.9	0		20.1	9.1	1.9	0
	9.2	4.1	1.9	0		20.2	9.1	1.9	0

RETIRED

	9.3	4.1	1.9	0	20.3	9.1	1.9	0
	9.4	4.1	1.9	0	20.4	9.1	1.9	0
	10	4.5	1.9	0	21	9.6	1.9	0
	10.1	4.5	1.9	0	21.1	9.6	1.9	0
	10.2	4.5	1.9	0	21.2	9.6	1.9	0
	10.3	4.5	1.9	0	21.3	9.6	1.9	0
	10.4	4.5	1.9	0	21.4	9.6	1.9	0
	11	5	1.9	0	22	10	1.9	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
Penta E	11.1	5	1.9	0	Penta E	22.1	10	1.9	0
	11.2	5	1.9	0		22.2	10	1.9	0
	11.3	5	1.9	0		22.3	10	1.9	0
	11.4	5	1.9	0		22.4	10	1.9	0
	12	5.4	1.9	0		23	10.5	1.9	0
	12.1	5.4	1.9	0		23.1	10.5	1.9	0
	12.2	5.4	1.9	0		23.2	10.5	1.9	0
	12.3	5.4	1.9	0		23.3	10.5	1.9	0
	12.4	5.4	1.9	0		23.4	10.5	1.9	0
	13	5.9	1.9	0		24	11	1.9	0
	13.1	5.9	1.9	0		24.1	11	1.9	0
	13.2	5.9	1.9	0		24.2	11	1.9	0
	13.3	5.9	1.9	0		24.3	11	1.9	0
	13.4	5.9	1.9	0		24.4	11	1.9	0
	14	6.4	1.9	0		25	11.4	1.9	0
	14.1	6.4	1.9	0		25.1	11.4	1.9	0
	14.2	6.4	1.9	0		25.2	11.4	1.9	0
	14.3	6.4	1.9	0		25.3	11.4	1.9	0
	14.4	6.4	1.9	0		25.4	11.4	1.9	0
						26	11.9	1.9	0

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D16S539	4	5.9	3.3	0	D16S539	13	11.6	3.3	0
	4.1	5.9	3.3	0		13.1	11.6	3.3	0
	4.2	5.9	3.3	0		13.2	11.6	3.3	0
	4.3	5.9	3.3	0		13.3	11.6	3.3	0
	5	5.9	3.3	0		14	12.7	3.3	0
	5.1	5.9	3.3	0		14.1	12.7	3.3	0
	5.2	5.9	3.3	0		14.2	12.7	3.3	0
	5.3	5.9	3.3	0		14.3	12.7	3.3	0
	6	5.9	3.3	0		15	13.8	3.3	0
	6.1	5.9	3.3	0		15.1	13.8	3.3	0
	6.2	5.9	3.3	0		15.2	13.8	3.3	0
	6.3	5.9	3.3	0		15.3	13.8	3.3	0
	7	5.9	3.3	0		16	15	3.3	0
	7.1	5.9	3.3	0					
	7.2	5.9	3.3	0					
	7.3	5.9	3.3	0					
	8	5.9	3.3	0					
	8.1	5.9	3.3	0					
	8.2	5.9	3.3	0					
	8.3	5.9	3.3	0					
	9	7	3.3	0					
	9.1	7	3.3	0					
	9.2	7	3.3	0					
	9.3	7	3.3	0					
	10	8.2	3.3	0					
	10.1	8.2	3.3	0					
	10.2	8.2	3.3	0					
	10.3	8.2	3.3	0					
	11	9.3	3.3	0					
	11.1	9.3	3.3	0					
	11.2	9.3	3.3	0					
	11.3	9.3	3.3	0					
	12	10.4	3.3	0					
	12.1	10.4	3.3	0					
	12.2	10.4	3.3	0					

RETIRED

	12.3	10.4	3.3	0					
Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D18S51	7	6.9	3.7	0	D18S51	17	12.2	3.7	0
	7.1	6.9	3.7	0		17.1	12.2	3.7	0
	7.2	6.9	3.7	0		17.2	12.2	3.7	0
	7.3	6.9	3.7	0		17.3	12.2	3.7	0
	8	6.9	3.7	0		18	12.9	3.7	0
	8.1	6.9	3.7	0		18.1	12.9	3.7	0
	8.2	6.9	3.7	0		18.2	12.9	3.7	0
	8.3	6.9	3.7	0		18.3	12.9	3.7	0
	9	6.9	3.7	0		19	13.7	3.7	0
	9.1	6.9	3.7	0		19.1	13.7	3.7	0
	9.2	6.9	3.7	0		19.2	13.7	3.7	0
	9.3	6.9	3.7	0		19.3	13.7	3.7	0
	10	6.9	3.7	0		20	14.5	3.7	0
	10.1	6.9	3.7	0		20.1	14.5	3.7	0
	10.2	6.9	3.7	0		20.2	14.5	3.7	0
	10.3	6.9	3.7	0		20.3	14.5	3.7	0
	11	7.6	3.7	0		21	15.2	3.7	0
	11.1	7.6	3.7	0		21.1	15.2	3.7	0
	11.2	7.6	3.7	0		21.2	15.2	3.7	0
	11.3	7.6	3.7	0		21.3	15.2	3.7	0
	12	8.4	3.7	0		22	16	3.7	0
	12.1	8.4	3.7	0		22.1	16	3.7	0
	12.2	8.4	3.7	0		22.2	16	3.7	0
	12.3	8.4	3.7	0		22.3	16	3.7	0
	13	9.1	3.7	0		23	16.7	3.7	0
	13.1	9.1	3.7	0		23.1	16.7	3.7	0
	13.2	9.1	3.7	0		23.2	16.7	3.7	0
	13.3	9.1	3.7	0		23.3	16.7	3.7	0
	14	9.9	3.7	0		24	17.5	3.7	0
	14.1	9.9	3.7	0		24.1	17.5	3.7	0
	14.2	9.9	3.7	0		24.2	17.5	3.7	0
	14.3	9.9	3.7	0		24.3	17.5	3.7	0
	15	10.7	3.7	0		25	18.3	3.7	0
	15.1	10.7	3.7	0		25.1	18.3	3.7	0
	15.2	10.7	3.7	0		25.2	18.3	3.7	0

RETIRED

	15.3	10.7	3.7	0		25.3	18.3	3.7	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D18S51	16	11.4	3.7	0	D18S51	26	19	3.7	0
	16.1	11.4	3.7	0		26.1	19	3.7	0
	16.2	11.4	3.7	0		26.2	19	3.7	0
	16.3	11.4	3.7	0		26.3	19	3.7	0
						27	19.8	3.7	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D2S1338	10	9.4	2.2	0	D2S1338	20	12.3	2.2	0
	10.1	9.4	2.2	0		20.1	12.3	2.2	0
	10.2	9.4	2.2	0		20.2	12.3	2.2	0
	10.3	9.4	2.2	0		20.3	12.3	2.2	0
	11	9.4	2.2	0		21	13	2.2	0
	11.1	9.4	2.2	0		21.1	13	2.2	0
	11.2	9.4	2.2	0		21.2	13	2.2	0
	11.3	9.4	2.2	0		21.3	13	2.2	0
	12	9.4	2.2	0		22	13.7	2.2	0
	12.1	9.4	2.2	0		22.1	13.7	2.2	0
	12.2	9.4	2.2	0		22.2	13.7	2.2	0
	12.3	9.4	2.2	0		22.3	13.7	2.2	0
	13	9.4	2.2	0		23	14.4	2.2	0
	13.1	9.4	2.2	0		23.1	14.4	2.2	0
	13.2	9.4	2.2	0		23.2	14.4	2.2	0
	13.3	9.4	2.2	0		23.3	14.4	2.2	0
	14	9.4	2.2	0		24	15.1	2.2	0
	14.1	9.4	2.2	0		24.1	15.1	2.2	0
	14.2	9.4	2.2	0		24.2	15.1	2.2	0
	14.3	9.4	2.2	0		24.3	15.1	2.2	0
	15	9.4	2.2	0		25	15.9	2.2	0
	15.1	9.4	2.2	0		25.1	15.9	2.2	0
	15.2	9.4	2.2	0		25.2	15.9	2.2	0
	15.3	9.4	2.2	0		25.3	15.9	2.2	0
	16	9.4	2.2	0		26	16.6	2.2	0
	16.1	9.4	2.2	0		26.1	16.6	2.2	0
	16.2	9.4	2.2	0		26.2	16.6	2.2	0

RETIRED

	16.3	9.4	2.2	0	26.3	16.6	2.2	0
	17	10.1	2.2	0	27	17.3	2.2	0
	17.1	10.1	2.2	0	27.1	17.3	2.2	0
	17.2	10.1	2.2	0	27.2	17.3	2.2	0
	17.3	10.1	2.2	0	27.3	17.3	2.2	0
	18	10.8	2.2	0	28	18	2.2	0
	18.1	10.8	2.2	0	28.1	18	2.2	0
	18.2	10.8	2.2	0	28.2	18	2.2	0
	18.3	10.8	2.2	0	28.3	18	2.2	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D2S1338	19	11.5	2.2	0	D2S1338	29	18.7	2.2	0
	19.1	11.5	2.2	0					
	19.2	11.5	2.2	0					
	19.3	11.5	2.2	0					

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
CSF1PO	5	3.7	4.3	0	CSF1PO	14	13.1	4.3	0
	5.1	3.7	4.3	0		14.1	13.1	4.3	0
	5.2	3.7	4.3	0		14.2	13.1	4.3	0
	5.3	3.7	4.3	0		14.3	13.1	4.3	0
	6	3.7	4.3	0		15	14.5	4.3	0
	6.1	3.7	4.3	0		15.1	14.5	4.3	0
	6.2	3.7	4.3	0		15.2	14.5	4.3	0
	6.3	3.7	4.3	0		15.3	14.5	4.3	0
	7	3.7	4.3	0		16	15.8	4.3	0
	7.1	3.7	4.3	0					
	7.2	3.7	4.3	0					
	7.3	3.7	4.3	0					
	8	5	4.3	0					
	8.1	5	4.3	0					
	8.2	5	4.3	0					
	8.3	5	4.3	0					
	9	6.4	4.3	0					
	9.1	6.4	4.3	0					
	9.2	6.4	4.3	0					
	9.3	6.4	4.3	0					

RETIRED

10	7.7	4.3	0
10.1	7.7	4.3	0
10.2	7.7	4.3	0
10.3	7.7	4.3	0
11	9.1	4.3	0
11.1	9.1	4.3	0
11.2	9.1	4.3	0
11.3	9.1	4.3	0
12	10.4	4.3	0
12.1	10.4	4.3	0
12.2	10.4	4.3	0
12.3	10.4	4.3	0
13	11.8	4.3	0
13.1	11.8	4.3	0
13.2	11.8	4.3	0
13.3	11.8	4.3	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
Penta D	2.2	3.9	3.7	0
	2.3	3.9	3.7	0
	2.4	3.9	3.7	0
	3	3.9	3.7	0
	3.1	3.9	3.7	0
	3.2	3.9	3.7	0
	3.3	3.9	3.7	0
	3.4	3.9	3.7	0
	4	3.9	3.7	0
	4.1	3.9	3.7	0
	4.2	3.9	3.7	0
	4.3	3.9	3.7	0
	4.4	3.9	3.7	0
	5	3.9	3.7	0
	5.1	3.9	3.7	0
	5.2	3.9	3.7	0
	5.3	3.9	3.7	0
	5.4	3.9	3.7	0
	6	3.9	3.7	0
	6.1	3.9	3.7	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
Penta D	11	4.5	3.7	0
	11.1	4.5	3.7	0
	11.2	4.5	3.7	0
	11.3	4.5	3.7	0
	11.4	4.5	3.7	0
	12	4.8	3.7	0
	12.1	4.8	3.7	0
	12.2	4.8	3.7	0
	12.3	4.8	3.7	0
	12.4	4.8	3.7	0
	13	5.1	3.7	0
	13.1	5.1	3.7	0
	13.2	5.1	3.7	0
	13.3	5.1	3.7	0
	13.4	5.1	3.7	0
	14	5.4	3.7	0
	14.1	5.4	3.7	0
	14.2	5.4	3.7	0
	14.3	5.4	3.7	0
	14.4	5.4	3.7	0

RETIRED

	6.2	3.9	3.7	0	15	5.8	3.7	0
	6.3	3.9	3.7	0	15.1	5.8	3.7	0
	6.4	3.9	3.7	0	15.2	5.8	3.7	0
	7	3.9	3.7	0	15.3	5.8	3.7	0
	7.1	3.9	3.7	0	15.4	5.8	3.7	0
	7.2	3.9	3.7	0	16	6.1	3.7	0
	7.3	3.9	3.7	0	16.1	6.1	3.7	0
	7.4	3.9	3.7	0	16.2	6.1	3.7	0
	8	3.9	3.7	0	16.3	6.1	3.7	0
	8.1	3.9	3.7	0	16.4	6.1	3.7	0
	8.2	3.9	3.7	0	17	6.4	3.7	0
	8.3	3.9	3.7	0	17.1	6.4	3.7	0
	8.4	3.9	3.7	0	17.2	6.4	3.7	0
	9	3.9	3.7	0	17.3	6.4	3.7	0
	9.1	3.9	3.7	0	17.4	6.4	3.7	0
	9.2	3.9	3.7	0	18	6.7	3.7	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
Penta D	9.3	3.9	3.7	0					
	9.4	3.9	3.7	0					
	10	4.2	3.7	0					
	10.1	4.2	3.7	0					
	10.2	4.2	3.7	0					
	10.3	4.2	3.7	0					
	10.4	4.2	3.7	0					

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
TH01	3	5.3	1.5	0	TH01	12	8.6	1.5	0
	3.1	5.3	1.5	0		12.1	8.6	1.5	0
	3.2	5.3	1.5	0		12.2	8.6	1.5	0
	3.3	5.3	1.5	0		12.3	7	1.5	0
	4	5.3	1.5	0		13	9.1	1.5	0
	4.1	5.3	1.5	0		13.1	9.1	1.5	0
	4.2	5.3	1.5	0		13.2	9.1	1.5	0
	4.3	5.3	1.5	0		13.3	7.5	1.5	0
	5	5.3	1.5	0					
	5.1	5.3	1.5	0					

RETIRED

5.2	5.3	1.5	0
5.3	5.3	1.5	0
6	5.3	1.5	0
6.1	5.3	1.5	0
6.2	5.3	1.5	0
6.3	5.3	1.5	0
7	5.9	1.5	0
7.1	5.9	1.5	0
7.2	5.9	1.5	0
7.3	5.3	1.5	0
8	6.4	1.5	0
8.1	6.4	1.5	0
8.2	6.4	1.5	0
8.3	5.3	1.5	0
9	7	1.5	0
9.1	7	1.5	0
9.2	7	1.5	0
9.3	5.3	1.5	0
10	7.5	1.5	0
10.1	7.5	1.5	0
10.2	7.5	1.5	0
10.3	5.9	1.5	0
11	8	1.5	0
11.1	8	1.5	0
11.2	8	1.5	0
11.3	6.4	1.5	0

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
vWA	10	9.1	2.7	0	vWA	19	14.6	2.7	0
	10.1	9.1	2.7	0		19.1	14.6	2.7	0
	10.2	9.1	2.7	0		19.2	14.6	2.7	0
	10.3	9.1	2.7	0		19.3	14.6	2.7	0
	11	9.1	2.7	0		20	15.7	2.7	0
	11.1	9.1	2.7	0		20.1	15.7	2.7	0
	11.2	9.1	2.7	0		20.2	15.7	2.7	0
	11.3	9.1	2.7	0		20.3	15.7	2.7	0
	12	9.1	2.7	0		21	16.8	2.7	0
	12.1	9.1	2.7	0		21.1	16.8	2.7	0

	12.2	9.1	2.7	0	21.2	16.8	2.7	0
	12.3	9.1	2.7	0	21.3	16.8	2.7	0
	13	9.1	2.7	0	22	17.8	2.7	0
	13.1	9.1	2.7	0	22.1	17.8	2.7	0
	13.2	9.1	2.7	0	22.2	17.8	2.7	0
	13.3	9.1	2.7	0	22.3	17.8	2.7	0
	14	9.1	2.7	0	23	18.9	2.7	0
	14.1	9.1	2.7	0	23.1	18.9	2.7	0
	14.2	9.1	2.7	0	23.2	18.9	2.7	0
	14.3	9.1	2.7	0	23.3	18.9	2.7	0
	15	10.2	2.7	0	24	20	2.7	0
	15.1	10.2	2.7	0	24.1	20	2.7	0
	15.2	10.2	2.7	0	24.2	20	2.7	0
	15.3	10.2	2.7	0	24.3	20	2.7	0
	16	11.3	2.7	0	25	21.1	2.7	0
	16.1	11.3	2.7	0				
	16.2	11.3	2.7	0				
	16.3	11.3	2.7	0				
	17	12.4	2.7	0				
	17.1	12.4	2.7	0				
	17.2	12.4	2.7	0				
	17.3	12.4	2.7	0				
	18	13.5	2.7	0				
	18.1	13.5	2.7	0				
	18.2	13.5	2.7	0				
	18.3	13.5	2.7	0				

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D21S11	23.2	9.4	3.5	0	D21S11	32	14.3	3.5	0
	23.3	9.7	3.5	0		32.1	14.3	3.5	0
	24	9.7	3.5	0		32.2	12.2	3.5	0
	24.1	9.7	3.5	0		32.3	14.3	3.5	0
	24.2	9.4	3.5	0		33	15.3	3.5	0
	24.3	9.7	3.5	0		33.1	15.3	3.5	0
	25	9.7	3.5	0		33.2	13.1	3.5	0
	25.1	9.7	3.5	0		33.3	15.3	3.5	0
	25.2	9.4	3.5	0		34	16.2	3.5	0
	25.3	9.7	3.5	0		34.1	16.2	3.5	0

26	9.7	3.5	0	34.2	14.1	3.5	0
26.1	9.7	3.5	0	34.3	16.2	3.5	0
26.2	9.4	3.5	0	35	17.1	3.5	0
26.3	9.7	3.5	0	35.1	17.1	3.5	0
27	9.7	3.5	0	35.2	15	3.5	0
27.1	9.7	3.5	0	35.3	17.1	3.5	0
27.2	9.4	3.5	0	36	18.1	3.5	0
27.3	9.7	3.5	0	36.1	18.1	3.5	0
28	10.6	3.5	0	36.2	16	3.5	0
28.1	10.6	3.5	0	36.3	18.1	3.5	0
28.2	9.4	3.5	0	37	19	3.5	0
28.3	10.6	3.5	0	37.1	19	3.5	0
29	11.6	3.5	0	37.2	16.9	3.5	0
29.1	11.6	3.5	0	37.3	19	3.5	0
29.2	9.4	3.5	0	38	19.9	3.5	0
29.3	11.6	3.5	0	38.1	19.9	3.5	0
30	12.5	3.5	0	38.2	17.8	3.5	0
30.1	12.5	3.5	0	38.3	19.9	3.5	0
30.2	10.3	3.5	0	39	20.9	3.5	0
30.3	12.5	3.5	0				
31	13.4	3.5	0				
31.1	13.4	3.5	0				
31.2	11.3	3.5	0				
31.3	13.4	3.5	0				

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D7S820	5	4.3	1.8	0	D7S820	14	11.6	1.8	0
	5.1	4.3	1.8	0		14.1	11.6	1.8	0
	5.2	4.3	1.8	0		14.2	11.6	1.8	0
	5.3	4.3	1.8	0		14.3	11.6	1.8	0
	6	4.3	1.8	0		15	12.7	1.8	0
	6.1	4.3	1.8	0		15.1	12.7	1.8	0
	6.2	4.3	1.8	0		15.2	12.7	1.8	0
	6.3	4.3	1.8	0		15.3	12.7	1.8	0
	7	4.3	1.8	0		16	13.7	1.8	0
	7.1	4.3	1.8	0					

7.2	4.3	1.8	0
7.3	4.3	1.8	0
8	5.3	1.8	0
8.1	5.3	1.8	0
8.2	5.3	1.8	0
8.3	5.3	1.8	0
9	6.4	1.8	0
9.1	6.4	1.8	0
9.2	6.4	1.8	0
9.3	6.4	1.8	0
10	7.4	1.8	0
10.1	7.4	1.8	0
10.2	7.4	1.8	0
10.3	7.4	1.8	0
11	8.5	1.8	0
11.1	8.5	1.8	0
11.2	8.5	1.8	0
11.3	8.5	1.8	0
12	9.5	1.8	0
12.1	9.5	1.8	0
12.2	9.5	1.8	0
12.3	9.5	1.8	0
13	10.6	1.8	0
13.1	10.6	1.8	0
13.2	10.6	1.8	0
13.3	10.6	1.8	0

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D5S818	5	5	2.4	0	D5S818	14	12.5	2.4	0
	5.1	5	2.4	0		14.1	12.5	2.4	0
	5.2	5	2.4	0		14.2	12.5	2.4	0
	5.3	5	2.4	0		14.3	12.5	2.4	0
	6	5	2.4	0		15	13.5	2.4	0
	6.1	5	2.4	0		15.1	13.5	2.4	0
	6.2	5	2.4	0		15.2	13.5	2.4	0
	6.3	5	2.4	0		15.3	13.5	2.4	0
	7	5	2.4	0		16	14.6	2.4	0
	7.1	5	2.4	0		16.1	14.6	2.4	0

7.2	5	2.4	0	16.2	14.6	2.4	0
7.3	5	2.4	0	16.3	14.6	2.4	0
8	6	2.4	0	17	15.7	2.4	0
8.1	6	2.4	0	17.1	15.7	2.4	0
8.2	6	2.4	0	17.2	15.7	2.4	0
8.3	6	2.4	0	17.3	15.7	2.4	0
9	7.1	2.4	0	18	16.7	2.4	0
9.1	7.1	2.4	0	18.1	16.7	2.4	0
9.2	7.1	2.4	0	18.2	16.7	2.4	0
9.3	7.1	2.4	0	18.3	16.7	2.4	0
10	8.2	2.4	0	19	17.8	2.4	0
10.1	8.2	2.4	0				
10.2	8.2	2.4	0				
10.3	8.2	2.4	0				
11	9.3	2.4	0				
11.1	9.3	2.4	0				
11.2	9.3	2.4	0				
11.3	9.3	2.4	0				
12	10.3	2.4	0				
12.1	10.3	2.4	0				
12.2	10.3	2.4	0				
12.3	10.3	2.4	0				
13	11.4	2.4	0				
13.1	11.4	2.4	0				
13.2	11.4	2.4	0				
13.3	11.4	2.4	0				

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
TPOX	4	4.1	1.1	0	TPOX	13	7.8	1.1	0
	4.1	4.1	1.1	0		13.1	7.8	1.1	0
	4.2	4.1	1.1	0		13.2	7.8	1.1	0
	4.3	4.1	1.1	0		13.3	7.8	1.1	0
	5	4.1	1.1	0		14	8.6	1.1	0
	5.1	4.1	1.1	0		14.1	8.6	1.1	0
	5.2	4.1	1.1	0		14.2	8.6	1.1	0
	5.3	4.1	1.1	0		14.3	8.6	1.1	0
	6	4.1	1.1	0		15	9.3	1.1	0
	6.1	4.1	1.1	0		15.1	9.3	1.1	0

6.2	4.1	1.1	0	15.2	9.3	1.1	0
6.3	4.1	1.1	0	15.3	9.3	1.1	0
7	4.1	1.1	0	16	10.1	1.1	0
7.1	4.1	1.1	0				
7.2	4.1	1.1	0				
7.3	4.1	1.1	0				
8	4.1	1.1	0				
8.1	4.1	1.1	0				
8.2	4.1	1.1	0				
8.3	4.1	1.1	0				
9	4.8	1.1	0				
9.1	4.8	1.1	0				
9.2	4.8	1.1	0				
9.3	4.8	1.1	0				
10	5.6	1.1	0				
10.1	5.6	1.1	0				
10.2	5.6	1.1	0				
10.3	5.6	1.1	0				
11	6.3	1.1	0				
11.1	6.3	1.1	0				
11.2	6.3	1.1	0				
11.3	6.3	1.1	0				
12	7.1	1.1	0				
12.1	7.1	1.1	0				
12.2	7.1	1.1	0				
12.3	7.1	1.1	0				

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D8S1179	6	10.6	3.4	0	D8S1179	15	13	3.4	0
	6.1	10.6	3.4	0		15.1	13	3.4	0
	6.2	10.6	3.4	0		15.2	13	3.4	0
	6.3	10.6	3.4	0		15.3	13	3.4	0
	7	10.6	3.4	0		16	13.3	3.4	0
	7.1	10.6	3.4	0		16.1	13.3	3.4	0
	7.2	10.6	3.4	0		16.2	13.3	3.4	0
	7.3	10.6	3.4	0		16.3	13.3	3.4	0
	8	10.6	3.4	0		17	13.7	3.4	0
	8.1	10.6	3.4	0		17.1	13.7	3.4	0

8.2	10.6	3.4	0	17.2	13.7	3.4	0
8.3	10.6	3.4	0	17.3	13.7	3.4	0
9	11	3.4	0	18	14	3.4	0
9.1	11	3.4	0	18.1	14	3.4	0
9.2	11	3.4	0	18.2	14	3.4	0
9.3	11	3.4	0	18.3	14	3.4	0
10	11.3	3.4	0	19	14.4	3.4	0
10.1	11.3	3.4	0	19.1	14.4	3.4	0
10.2	11.3	3.4	0	19.2	14.4	3.4	0
10.3	11.3	3.4	0	19.3	14.4	3.4	0
11	11.6	3.4	0	20	14.7	3.4	0
11.1	11.6	3.4	0				
11.2	11.6	3.4	0				
11.3	11.6	3.4	0				
12	12	3.4	0				
12.1	12	3.4	0				
12.2	12	3.4	0				
12.3	12	3.4	0				
13	12.3	3.4	0				
13.1	12.3	3.4	0				
13.2	12.3	3.4	0				
13.3	12.3	3.4	0				
14	12.7	3.4	0				
14.1	12.7	3.4	0				
14.2	12.7	3.4	0				
14.3	12.7	3.4	0				

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D12S391	13	8.8	2.7	0	D12S391	22	15.9	2.7	0
	13.1	8.8	2.7	0		22.1	15.9	2.7	0
	13.2	8.8	2.7	0		22.2	15.9	2.7	0
	13.3	8.8	2.7	0		22.3	15.9	2.7	0
	14	8.8	2.7	0		23	17	2.7	0
	14.1	8.8	2.7	0		23.1	17	2.7	0
	14.2	8.8	2.7	0		23.2	17	2.7	0
	14.3	8.8	2.7	0		23.3	17	2.7	0
	15	8.8	2.7	0		24	18	2.7	0
	15.1	8.8	2.7	0		24.1	18	2.7	0

15.2	8.8	2.7	0	24.2	18	2.7	0
15.3	8.8	2.7	0	24.3	18	2.7	0
16	9.8	2.7	0	25	19	2.7	0
16.1	9.8	2.7	0	25.1	19	2.7	0
16.2	9.8	2.7	0	25.2	19	2.7	0
16.3	9.8	2.7	0	25.3	19	2.7	0
17	10.8	2.7	0	26	20	2.7	0
17.1	10.8	2.7	0	26.1	20	2.7	0
17.2	10.8	2.7	0	26.2	20	2.7	0
17.3	10.8	2.7	0	26.3	20	2.7	0
18	11.9	2.7	0	27	21	2.7	0
18.1	11.9	2.7	0				
18.2	11.9	2.7	0				
18.3	11.9	2.7	0				
19	12.9	2.7	0				
19.1	12.9	2.7	0				
19.2	12.9	2.7	0				
19.3	12.9	2.7	0				
20	13.9	2.7	0				
20.1	13.9	2.7	0				
20.2	13.9	2.7	0				
20.3	13.9	2.7	0				
21	14.9	2.7	0				
21.1	14.9	2.7	0				
21.2	14.9	2.7	0				
21.3	14.9	2.7	0				

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D19S433	5.2	7.8	2.6	0	D19S433	14	10.7	2.6	0
	5.3	7.8	2.6	0		14.1	10.7	2.6	0
	6	7.8	2.6	0		14.2	11.1	2.6	0
	6.1	7.8	2.6	0		14.3	10.7	2.6	0
	6.2	7.8	2.6	0		15	11.6	2.6	0
	6.3	7.8	2.6	0		15.1	11.6	2.6	0
	7	7.8	2.6	0		15.2	12.1	2.6	0
	7.1	7.8	2.6	0		15.3	11.6	2.6	0
	7.2	7.8	2.6	0		16	12.5	2.6	0
	7.3	7.8	2.6	0		16.1	12.5	2.6	0

8	7.8	2.6	0	16.2	13	2.6	0
8.1	7.8	2.6	0	16.3	12.5	2.6	0
8.2	7.8	2.6	0	17	13.5	2.6	0
8.3	7.8	2.6	0	17.1	13.5	2.6	0
9	7.8	2.6	0	17.2	14	2.6	0
9.1	7.8	2.6	0	17.3	13.5	2.6	0
9.2	7.8	2.6	0	18	14.4	2.6	0
9.3	7.8	2.6	0	18.1	14.4	2.6	0
10	7.8	2.6	0	18.2	14.9	2.6	0
10.1	7.8	2.6	0	18.3	14.4	2.6	0
10.2	7.8	2.6	0	19	15.4	2.6	0
10.3	7.8	2.6	0	19.1	15.4	2.6	0
11	7.8	2.6	0	19.2	15.8	2.6	0
11.1	7.8	2.6	0				
11.2	8.3	2.6	0				
11.3	7.8	2.6	0				
12	8.8	2.6	0				
12.1	8.8	2.6	0				
12.2	9.3	2.6	0				
12.3	8.8	2.6	0				
13	9.7	2.6	0				
13.1	9.7	2.6	0				
13.2	10.2	2.6	0				
13.3	9.7	2.6	0				

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
SE33	4.2	11.6	3.3	8.7	SE33	22	17.5	3.3	8.7
	4.3	10.8	3.3	8.7		22.1	17.5	3.3	8.7
	5	10.8	3.3	8.7		22.2	13.5	3.3	8.7
	5.1	10.8	3.3	8.7		22.3	17.5	3.3	8.7
	5.2	11.6	3.3	8.7		23	18.3	3.3	8.7
	5.3	10.8	3.3	8.7		23.1	18.3	3.3	8.7
	6	10.8	3.3	8.7		23.2	14.1	3.3	8.7
	6.1	10.8	3.3	8.7		23.3	18.3	3.3	8.7
	6.2	11.6	3.3	8.7		24	19	3.3	8.7
	6.3	10.8	3.3	8.7		24.1	19	3.3	8.7

	7	10.8	3.3	8.7		24.2	14.7	3.3	8.7
	7.1	10.8	3.3	8.7		24.3	19	3.3	8.7
	7.2	11.6	3.3	8.7		25	19.8	3.3	8.7
	7.3	10.8	3.3	8.7		25.1	19.8	3.3	8.7
	8	10.8	3.3	8.7		25.2	15.3	3.3	8.7
	8.1	10.8	3.3	8.7		25.3	19.8	3.3	8.7
	8.2	11.6	3.3	8.7		26	20.5	3.3	8.7
	8.3	10.8	3.3	8.7		26.1	20.5	3.3	8.7
	9	10.8	3.3	8.7		26.2	15.9	3.3	8.7
	9.1	10.8	3.3	8.7		26.3	20.5	3.3	8.7
	9.2	11.6	3.3	8.7		27	21.3	3.3	8.7
	9.3	10.8	3.3	8.7		27.1	21.3	3.3	8.7
	10	10.8	3.3	8.7		27.2	16.5	3.3	8.7
	10.1	10.8	3.3	8.7		27.3	21.3	3.3	8.7
	10.2	11.6	3.3	8.7		28	22	3.3	8.7
	10.3	10.8	3.3	8.7		28.1	22	3.3	8.7
	11	10.8	3.3	8.7		28.2	17.1	3.3	8.7
	11.1	10.8	3.3	8.7		28.3	22	3.3	8.7
	11.2	11.6	3.3	8.7		29	22.8	3.3	8.7
	11.3	10.8	3.3	8.7		29.1	22.8	3.3	8.7
	12	10.8	3.3	8.7		29.2	17.7	3.3	8.7
	12.1	10.8	3.3	8.7		29.3	22.8	3.3	8.7
	12.2	11.6	3.3	8.7		30	23.5	3.3	8.7
	12.3	10.8	3.3	8.7		30.1	23.5	3.3	8.7
	13	10.8	3.3	8.7		30.2	18.4	3.3	8.7
	13.1	10.8	3.3	8.7		30.3	23.5	3.3	8.7

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
SE33	13.2	11.6	3.3	8.7	SE33	31	24.3	3.3	8.7
	13.3	10.8	3.3	8.7		31.1	24.3	3.3	8.7
	14	11.5	3.3	8.7		31.2	19	3.3	8.7
	14.1	11.5	3.3	8.7		31.3	24.3	3.3	8.7
	14.2	11.6	3.3	8.7		32	25	3.3	8.7
	14.3	11.5	3.3	8.7		32.1	25	3.3	8.7
	15	12.3	3.3	8.7		32.2	19.6	3.3	8.7
	15.1	12.3	3.3	8.7		32.3	25	3.3	8.7
	15.2	11.6	3.3	8.7		33	25.8	3.3	8.7
	15.3	12.3	3.3	8.7		33.1	25.8	3.3	8.7

RETIRED

16	13	3.3	8.7	33.2	20.2	3.3	8.7
16.1	13	3.3	8.7	33.3	25.8	3.3	8.7
16.2	11.6	3.3	8.7	34	26.5	3.3	8.7
16.3	13	3.3	8.7	34.1	26.5	3.3	8.7
17	13.8	3.3	8.7	34.2	20.8	3.3	8.7
17.1	13.8	3.3	8.7	34.3	26.5	3.3	8.7
17.2	11.6	3.3	8.7	35	27.3	3.3	8.7
17.3	13.8	3.3	8.7	35.1	27.3	3.3	8.7
18	14.5	3.3	8.7	35.2	21.4	3.3	8.7
18.1	14.5	3.3	8.7	35.3	27.3	3.3	8.7
18.2	11.6	3.3	8.7	36	28	3.3	8.7
18.3	14.5	3.3	8.7	36.1	28	3.3	8.7
19	15.3	3.3	8.7	36.2	22	3.3	8.7
19.1	15.3	3.3	8.7	36.3	28	3.3	8.7
19.2	11.6	3.3	8.7	37	28.8	3.3	8.7
19.3	15.3	3.3	8.7	37.1	28.8	3.3	8.7
20	16	3.3	8.7	37.2	22.6	3.3	8.7
20.1	16	3.3	8.7	37.3	28.8	3.3	8.7
20.2	12.3	3.3	8.7	38	29.5	3.3	8.7
20.3	16	3.3	8.7	38.1	29.5	3.3	8.7
21	16.8	3.3	8.7	38.2	23.2	3.3	8.7
21.1	16.8	3.3	8.7	38.3	29.5	3.3	8.7
21.2	12.9	3.3	8.7	39	30.3	3.3	8.7
21.3	16.8	3.3	8.7	39.1	30.3	3.3	8.7
				39.2	23.8	3.3	8.7

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
D22S1045	6	6.9	5.2	0	D22S1045	17	15.6	10.2	0
	6.1	6.9	5.2	0		17.1	15.6	10.2	0
	6.2	6.9	5.2	0		17.2	15.6	10.2	0
	7	6.9	5.2	0		18	17	11	0
	7.1	6.9	5.2	0		18.1	17	11	0
	7.2	6.9	5.2	0		18.2	17	11	0
	8	6.9	5.2	0		19	18.5	11.9	0
	8.1	6.9	5.2	0		19.1	18.5	11.9	0
	8.2	6.9	5.2	0		19.2	18.5	11.9	0
	9	6.9	5.2	0		20	19.9	12.7	0

9.1	6.9	5.2	0
9.2	6.9	5.2	0
10	6.9	5.2	0
10.1	6.9	5.2	0
10.2	6.9	5.2	0
11	6.9	5.2	0
11.1	6.9	5.2	0
11.2	6.9	5.2	0
12	8.3	6	0
12.1	8.3	6	0
12.2	8.3	6	0
13	9.8	6.8	0
13.1	9.8	6.8	0
13.2	9.8	6.8	0
14	11.2	7.7	0
14.1	11.2	7.7	0
14.2	11.2	7.7	0
15	12.7	8.5	0
15.1	12.7	8.5	0
15.2	12.7	8.5	0
16	14.1	9.4	0
16.1	14.1	9.4	0
16.2	14.1	9.4	0

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
DYS391	5	11	2	0	DYS391	14	14.9	2	0
	5.1	11	2	0		14.1	14.9	2	0
	5.2	11	2	0		14.2	14.9	2	0
	5.3	11	2	0		14.3	14.9	2	0
	6	11	2	0		15	15.7	2	0
	6.1	11	2	0		15.1	15.7	2	0
	6.2	11	2	0		15.2	15.7	2	0
	6.3	11	2	0		15.3	15.7	2	0
	7	11	2	0		16	16.5	2	0
	7.1	11	2	0					
	7.2	11	2	0					

	7.3	11	2	0
	8	11	2	0
	8.1	11	2	0
	8.2	11	2	0
	8.3	11	2	0
	9	11	2	0
	9.1	11	2	0
	9.2	11	2	0
	9.3	11	2	0
	10	11.8	2	0
	10.1	11.8	2	0
	10.2	11.8	2	0
	10.3	11.8	2	0
	11	12.6	2	0
	11.1	12.6	2	0
	11.2	12.6	2	0
	11.3	12.6	2	0
	12	13.4	2	0
	12.1	13.4	2	0
	12.2	13.4	2	0
	12.3	13.4	2	0
	13	14.2	2	0
	13.1	14.2	2	0
	13.2	14.2	2	0
	13.3	14.2	2	0

RETIRED

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
FGA	12.2	8.2	2.9	0	FGA	29	15.9	2.9	0
	12.3	8.2	2.9	0		29.1	15.9	2.9	0
	13	8.2	2.9	0		29.2	15.9	2.9	0
	13.1	8.2	2.9	0		29.3	15.9	2.9	0
	13.2	8.2	2.9	0		30	16.6	2.9	0
	13.3	8.2	2.9	0		30.1	16.6	2.9	0
	14	8.2	2.9	0		30.2	16.6	2.9	0
	14.1	8.2	2.9	0		30.3	16.6	2.9	0
	14.2	8.2	2.9	0		31	17.3	2.9	0
	14.3	8.2	2.9	0		31.1	17.3	2.9	0
	15	8.2	2.9	0		31.2	17.3	2.9	0

	15.1	8.2	2.9	0	31.3	17.3	2.9	0
	15.2	8.2	2.9	0	32	18	2.9	0
	15.3	8.2	2.9	0	32.1	18	2.9	0
	16	8.2	2.9	0	32.2	18	2.9	0
	16.1	8.2	2.9	0	32.3	18	2.9	0
	16.2	8.2	2.9	0	33	18	2.9	0
	16.3	8.2	2.9	0	33.1	18	2.9	0
	17	8.2	2.9	0	33.2	18	2.9	0
	17.1	8.2	2.9	0	33.3	18	2.9	0
	17.2	8.2	2.9	0	34	18	2.9	0
	17.3	8.2	2.9	0	34.1	18	2.9	0
	18	8.2	2.9	0	34.2	18	2.9	0
	18.1	8.2	2.9	0	41.2	18	2.9	0
	18.2	8.2	2.9	0	41.3	18	2.9	0
	18.3	8.2	2.9	0	42	18	2.9	0
	19	8.9	2.9	0	42.1	18	2.9	0
	19.1	8.9	2.9	0	42.2	18	2.9	0
	19.2	8.9	2.9	0	42.3	18	2.9	0
	19.3	8.9	2.9	0	43	18	2.9	0
	20	9.6	2.9	0	43.1	18	2.9	0
	20.1	9.6	2.9	0	43.2	18	2.9	0
	20.2	9.6	2.9	0	43.3	18	2.9	0
	20.3	9.6	2.9	0	44	18	2.9	0
	21	10.3	2.9	0	44.1	18	2.9	0
	21.1	10.3	2.9	0	44.2	18	2.9	0

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
FGA	21.2	10.3	2.9	0	FGA	44.3	18	2.9	0
	21.3	10.3	2.9	0		45	18	2.9	0
	22	11	2.9	0		45.1	18	2.9	0
	22.1	11	2.9	0		45.2	18	2.9	0
	22.2	11	2.9	0		45.3	18	2.9	0
	22.3	11	2.9	0		46	18	2.9	0
	23	11.7	2.9	0		46.1	18	2.9	0
	23.1	11.7	2.9	0		46.2	18	2.9	0
	23.2	11.7	2.9	0		46.3	18	2.9	0
	23.3	11.7	2.9	0		47	18	2.9	0
	24	12.4	2.9	0		47.1	18	2.9	0

RETIRED

24.1	12.4	2.9	0	47.2	18	2.9	0
24.2	12.4	2.9	0	47.3	18	2.9	0
24.3	12.4	2.9	0	48	18	2.9	0
25	13.1	2.9	0	48.1	18	2.9	0
25.1	13.1	2.9	0	48.2	18	2.9	0
25.2	13.1	2.9	0	48.3	18	2.9	0
25.3	13.1	2.9	0	49	18	2.9	0
26	13.8	2.9	0	49.1	18	2.9	0
26.1	13.8	2.9	0	49.2	18	2.9	0
26.2	13.8	2.9	0	49.3	18	2.9	0
26.3	13.8	2.9	0	50	18	2.9	0
27	14.5	2.9	0	50.1	18	2.9	0
27.1	14.5	2.9	0	50.2	18	2.9	0
27.2	14.5	2.9	0	50.3	18	2.9	0
27.3	14.5	2.9	0	51	18	2.9	0
28	15.2	2.9	0	51.1	18	2.9	0
28.1	15.2	2.9	0	51.2	18	2.9	0
28.2	15.2	2.9	0				
28.3	15.2	2.9	0				

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
DYS576	10	10.4	5.1	0	DYS576	19	13.2	5.1	0
	10.1	10.4	5.1	0		19.1	13.2	5.1	0
	10.2	10.4	5.1	0		19.2	13.2	5.1	0
	10.3	10.4	5.1	0		19.3	13.2	5.1	0
	11	10.4	5.1	0		20	14.1	5.1	0
	11.1	10.4	5.1	0		20.1	14.1	5.1	0
	11.2	10.4	5.1	0		20.2	14.1	5.1	0
	11.3	10.4	5.1	0		20.3	14.1	5.1	0
	12	10.4	5.1	0		21	15	5.1	0
	12.1	10.4	5.1	0		21.1	15	5.1	0
	12.2	10.4	5.1	0		21.2	15	5.1	0
	12.3	10.4	5.1	0		21.3	15	5.1	0
	13	10.4	5.1	0		22	15.9	5.1	0
	13.1	10.4	5.1	0		22.1	15.9	5.1	0
	13.2	10.4	5.1	0		22.2	15.9	5.1	0

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13.3	10.4	5.1	0	22.3	15.9	5.1	0
14	10.4	5.1	0	23	16.9	5.1	0
14.1	10.4	5.1	0	23.1	16.9	5.1	0
14.2	10.4	5.1	0	23.2	16.9	5.1	0
14.3	10.4	5.1	0	23.3	16.9	5.1	0
15	10.4	5.1	0	24	17.8	5.1	0
15.1	10.4	5.1	0				
15.2	10.4	5.1	0				
15.3	10.4	5.1	0				
16	10.4	5.1	0				
16.1	10.4	5.1	0				
16.2	10.4	5.1	0				
16.3	10.4	5.1	0				
17	11.3	5.1	0				
17.1	11.3	5.1	0				
17.2	11.3	5.1	0				
17.3	11.3	5.1	0				
18	12.3	5.1	0				
18.1	12.3	5.1	0				
18.2	12.3	5.1	0				
18.3	12.3	5.1	0				

Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat	Locus	Allele	N-1 repeat	N+1 repeat	N-0.5 repeat
DYS570	9	12.2	2.4	0	DYS570	18	13.5	2.4	0
	9.1	12.2	2.4	0		18.1	13.5	2.4	0
	9.2	12.2	2.4	0		18.2	13.5	2.4	0
	9.3	12.2	2.4	0		18.3	13.5	2.4	0
	10	12.2	2.4	0		19	14.2	2.4	0
	10.1	12.2	2.4	0		19.1	14.2	2.4	0
	10.2	12.2	2.4	0		19.2	14.2	2.4	0
	10.3	12.2	2.4	0		19.3	14.2	2.4	0
	11	12.2	2.4	0		20	14.9	2.4	0
	11.1	12.2	2.4	0		20.1	14.9	2.4	0
	11.2	12.2	2.4	0		20.2	14.9	2.4	0
	11.3	12.2	2.4	0		20.3	14.9	2.4	0
	12	12.2	2.4	0		21	15.6	2.4	0
	12.1	12.2	2.4	0		21.1	15.6	2.4	0
	12.2	12.2	2.4	0		21.2	15.6	2.4	0

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12.3	12.2	2.4	0	21.3	15.6	2.4	0
13	12.2	2.4	0	22	16.3	2.4	0
13.1	12.2	2.4	0	22.1	16.3	2.4	0
13.2	12.2	2.4	0	22.2	16.3	2.4	0
13.3	12.2	2.4	0	22.3	16.3	2.4	0
14	12.2	2.4	0	23	16.9	2.4	0
14.1	12.2	2.4	0	23.1	16.9	2.4	0
14.2	12.2	2.4	0	23.2	16.9	2.4	0
14.3	12.2	2.4	0	23.3	16.9	2.4	0
15	12.2	2.4	0	24	17.6	2.4	0
15.1	12.2	2.4	0	24.1	17.6	2.4	0
15.2	12.2	2.4	0	24.2	17.6	2.4	0
15.3	12.2	2.4	0	24.3	17.6	2.4	0
16	12.2	2.4	0	25	18.3	2.4	0
16.1	12.2	2.4	0	25.1	18.3	2.4	0
16.2	12.2	2.4	0	25.2	18.3	2.4	0
16.3	12.2	2.4	0	25.3	18.3	2.4	0
17	12.9	2.4	0	26	19	2.4	0
17.1	12.9	2.4	0				
17.2	12.9	2.4	0				
17.3	12.9	2.4	0				

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29.2 Legacy Kit Control Profiles

29.2.1 Fusion 6C Control Profiles:

29.2.1.1 EP1

Dye	**Loci**						
FL-6C	**AMEL**	**D3S1358**	**D1S1656**	**D2S441**	**D10S1248**	**D13S317**	**Penta E**
	X,Y	14,18	15,15.3	14	14,16	10,11	7,15
JOE-6C	**D16S539**	**D18S51**	**D2S1338**	**CSF1PO**	**Penta D**		
	12,13	14,16	17,23	10,11	9		
TMR-6C	**TH01**	**vWA**	**D21S11**	**D7S820**	**D5S818**	**TPOX**	
	7,9.3	16,18	30	8,9	9,11	8,10	
CXR-6C	**D8S1179**	**D12S391**	**D19S433**	**SE33**	**D22S1045**		
	10,14	17,22	14,15	17,28.2	16		
TOM-6C	**DYS391**	**FGA**	**DYS576**	**DYS570**			
	11	20,22	18	18			

29.2.1.2 POS (2800M)

Dye	**Loci**						
FL-6C	**AMEL**	**D3S1358**	**D1S1656**	**D2S441**	**D10S1248**	**D13S317**	**Penta E**
	X,Y	17,18	12,13	10,14	13,15	9,11	7,14
JOE-6C	**D16S539**	**D18S51**	**D2S1338**	**CSF1PO**	**Penta D**		
	9,13	16,18	22,25	12	12,13		
TMR-6C	**TH01**	**vWA**	**D21S11**	**D7S820**	**D5S818**	**TPOX**	
	6,9.3	16,19	29,31.2	8,11	12	11	
CXR-6C	**D8S1179**	**D12S391**	**D19S433**	**SE33**	**D22S1045**		
	14,15	18,23	13,14	15,16	16		
TOM-6C	**DYS391**	**FGA**	**DYS576**	**DYS570**			
	10	20,23	18	17			

29.2.2 Yfiler Control Profiles:

6-FAM (blue)

Control	DYS456	DYS389I	DYS390	DYS389II
EP1	18	13	26	29
RKO	15	13	24	29
POS 007	15	13	24	29

VIC (green)

Control	DYS458	DYS19	DYS385
EP1	16	15	12,13
RKO	17	14	11,15
POS 007	17	15	11,14

NED (yellow)

Control	DYS393	DYS391	DYS439	DYS635	DYS392
EP1	13	11	12	23	14
RKO	13	11	11	24	13
POS 007	13	11	12	24	13

PET (red)

Control	Y GATA H4	DYS437	DYS438	DYS448
EP1	12	15	12	18
RKO	12	15	12	19
POS 007	13	15	12	19

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29.2.3 Profiler Plus and COfiler Controls:

Profiler Plus

Control	D3S1358	vWa	FGA	AMEL	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
POS	14,15	17,18	23,24	X	13	30	15,19	11	11	10,11
KJL	16,18	16,18	23,24	X	13,14	28,29	14,16	11,13	12,14	10,12
TMP	14,16	17,19	25	X,Y	13,14	30,31	14,16	11,12	8,12	10,11

COfiler

Control	D3S1358	D16S539	AMEL	THO1	TPOX	CSF1PO	D7S820
POS	14,15	11,12	X	8,9.3	8	10,12	10,11
KJL	16,18	11,12	X	6,9.3	8,11	9,12	10,12
TMP	14,16	11	X,Y	6,9.3	8,11	10,12	10,11

29.2.4 Minifiler Controls:

6-FAM (blue)

Control	D13S317	D7S820
RKO	9	8,11
mPOS	11	7,12

VIC (green)

Control	Amelogenin	D2S1338	D21S11
RKO	X,Y	17	28,29
mPOS	X,Y	20,23	28,31

NED (yellow)

Control	D16S539	D18S51
RKO	11,12	14,15
mPOS	9,10	12,15

PET (red)

Control	CSF1PO	FGA
RKO	9,11	22,24
mPOS	11,12	24,26

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29.2.5 ID/IDP Controls:

6-FAM (blue)

Control	D8S1179	D21S11	D7S820	CSF1PO
RKO	12,13	28,29	8,11	9,11
EP1	10,14	30	8,9	10,11
POS	13	30	10,11	10,12

VIC (green)

Control	D3S1358	TH01	D13S317	D16S539	D2S1338
RKO	15,18	6,9.3	9	11,12	17
EPI	14,18	7,9.3	10,11	12,13	17,23
POS	14,15	8,9.3	11	11,12	19,23

NED (yellow)

Control	D19S433	vWA	TPOX	D18S51
RKO	13,15.2	14,18	8,11	14,15
EP1	14,15	16,18	8,10	14,16
POS	14,15	17,18	8	15,19

PET (red)

Control	Amelogenin	D5S818	FGA
RKO	X,Y	10,12	22,24
EP1	X,Y	9,11	20,22
POS	X	11	23,24

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29.3 Legacy Kit Common Dye Artifacts

29.3.1 Fusion 6C (from Fusion 6C System Technical Manual)

29.3.1.1 DNA-Dependent Artifacts Observed with Fusion 6C

Locus	**Artifact Size (bp)**
Amelogenin	n-1
D1S1656	n-1, n-2
D13S317	n-2
D18S51	n-2
vWA	n-2, elevated baseline in locus
D7S820	n-2
D5S818	n-2
D19S433	n-2
SE33	n-2
DYS391	n-1
FGA	n-1, n-2

29.3.1.2 DNA-Independent Artifacts Observed with Fusion 6C

Dye Label	**Artifact Size[1]**
FL-6C	~65-75bp ~113-120bp ~137-145bp
JOE-6C	~60-66bp
TMR-6C	~57-62bp

[1]Artifact sizes may vary depending on CE instrumentation and environmental conditions in the laboratory.

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DNA SOP-32 Using STRmix™ Software

32.1 Purpose:

STRmix™ is used to deconvolute an evidentiary profile to obtain probabilistic weights of genotype combinations for all contributors to either a single source or mixture samples with, or without conditioning known profiles. Those weights are used to calculate likelihood ratios which will be reported out on all appropriate samples deconvoluted through STRmix™. The association may be, based on the likelihood ratio, included, cannot be eliminated, inconclusive, or an elimination. STRmix™ may also be used as a tool to determine possible CODIS entries, though this is not currently validated for casework. For further guidance on running STRmix™ software, please refer to the current STRmix™ Operations Manual.

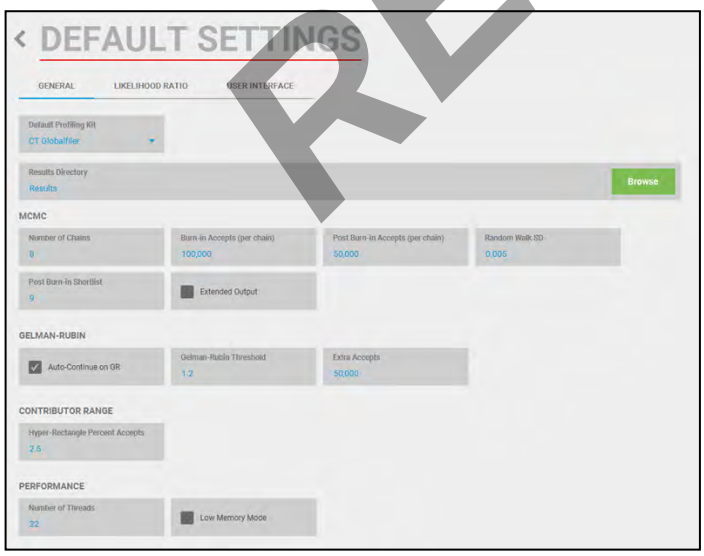
32.2 Responsibility:

DNA Unit personnel

32.3 Set-up of STRmix™

32.3.1 STRmix™ Default Settings

32.3.1.1 Default settings can be edited by clicking on "Administration" in the STRmix™ main menu, then clicking on "STRmix Defaults". See below for how your "Default Settings" should appear.



32.3.1.2 The number of MCMC chains, burn-in accepts, and post-burn-in accepts can be adjusted when scientifically valid (such as for a high Gelman-Rubin score or an expected genotype not being modeled) with TL approval.

32.3.2 Kits

32.3.2.1 Kits can be edited by clicking on "Administration" in the STRmix™ main menu, then clicking on "Profiling Kits".

32.3.2.2 Located in server desktop's local disk ProgramData/STRmix/Kits folder will be 3 different kit files for Fusion 6C, for a maximum, standard, and low injection, 2 different kit files for Identifiler Plus (to be used for

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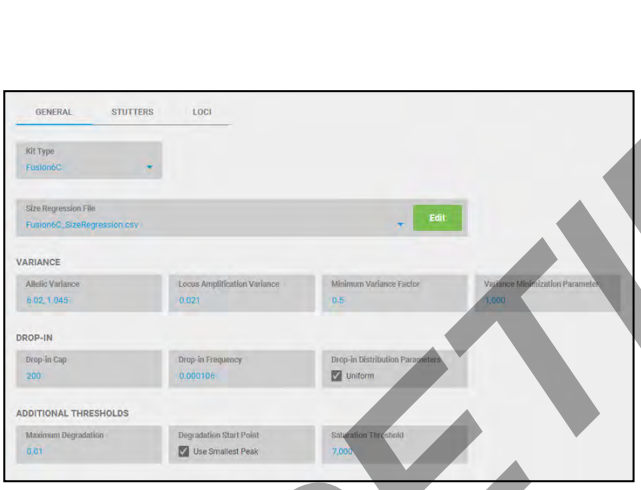
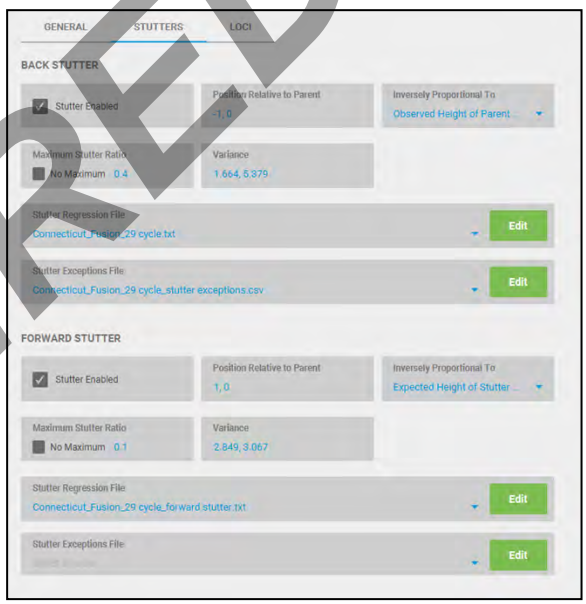
Identifiler as well), for a maximum (15 seconds or more) and standard (less than 15 seconds) injection, and one kit file for GlobalFiler.

32.3.2.3 These are the settings for the F6C – Standard Injection kit, with the loci listed in order being AMEL (gender), D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, SE33, D22S1045, DYS391 (ignore), FGA, DYS576 (ignore), and DYS570 (ignore). Repeat length is 4 for all loci except D22S1045 (3), Penta E (5), and Penta D (5), with a detection threshold of 50 rfu for all loci, and back and forward stutter being modeled for all loci:





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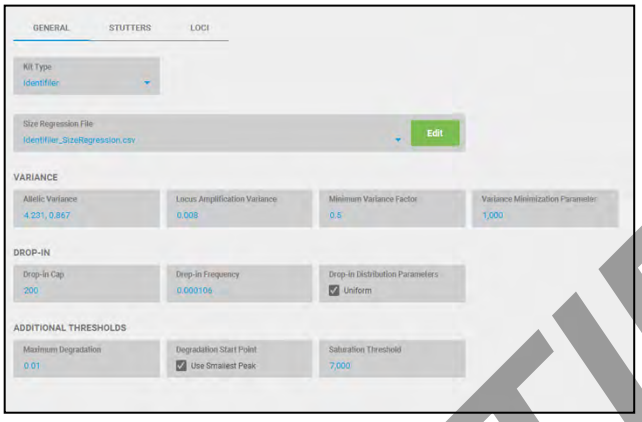
32.3.2.4 The F6C - Maximum Injection kit will have the same standard settings as the F6C – Standard Injection kit with the following exceptions:

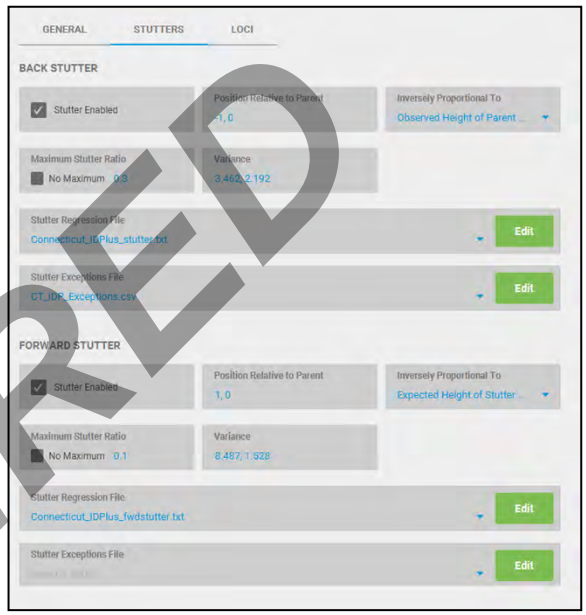
Allelic Variance: 6.725, 1.786
Stutter Variance: 2.666, 5.052
Locus Amp Variance: 0.026

32.3.2.5 The F6C – Low Injection kit will have the same standard settings as the F6C – Standard Injection kit with the following exceptions:

Allelic Variance: 4.129, 1.017
Stutter Variance: 1.75, 3.655
Locus Amp Variance: 0.019

32.3.2.6 These are the settings for the IDP – Standard Injection kit with the loci listed in order being D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, AMEL (gender), D5S818, and FGA. Repeat length is 4 for all loci, with a detection threshold of 50 rfu for all loci, and back and forward stutter being modeled for all loci:





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32.3.2.7 The IDP – Max Injection kit will have the same settings as the IDP – Standard Injection with the following exceptions:

Allelic Variance: 6.262, 1.196
Stutter Variance: 4.218, 2.221
Locus Amp Variance: 0.01

32.3.2.8 These are the settings for the GlobalFiler injection kit (X is being modeled):

	Detection Threshold	Back Stutter	Forward Stutter	Half-Back Stutter	Double Back Stutter	Half-Forward Stutter
D3S1358	40	X	X	X	X	
vWA	40	X	X	X	X	
D16S539	40	X	X	X	X	
CSF1PO	40	X	X		X	
TPOX	40	X	X			
Yindel (Ignore)	55					

	Detection Threshold	Back Stutter	Forward Stutter	Half-Back Stutter	Double Back Stutter	Half-Forward Stutter
AMEL (Gender)						
D8S1179	55	X	X	X	X	
D21S11	55	X	X	X	X	
D18S51	55	X	X		X	
DYS391 (Ignore)	55					
D2S441	35	X	X	X	X	
D19S433	35	X	X	X	X	X
TH01	35	X	X	X		
FGA	35	X	X	X	X	X
D22S1045*	50	X	X		X	
D5S818	50	X	X		X	
D13S317	50	X	X		X	
D7S820	50	X	X		X	
SE33	50	X	X	X	X	X
D10S1248	55	X	X		X	
D1S1656	55	X	X	X	X	
D12S391	55	X	X		X	
D2S1338	55	X	X		X	

*Only 3 repeat locus. All others 4.

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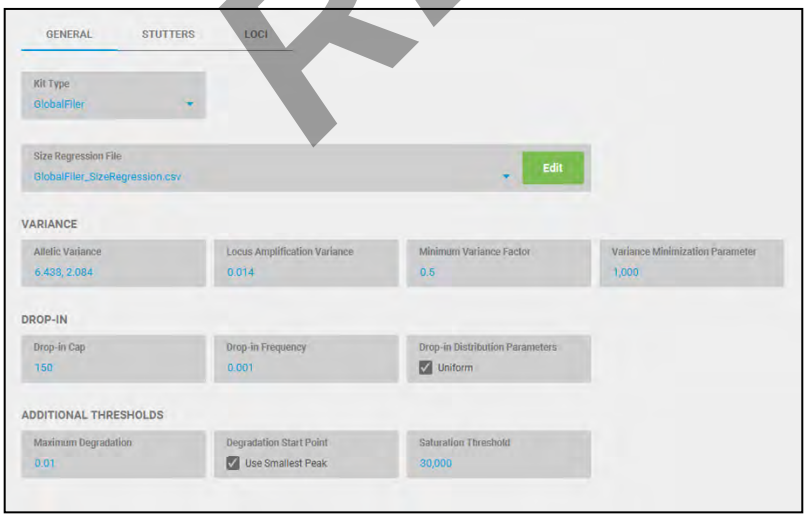

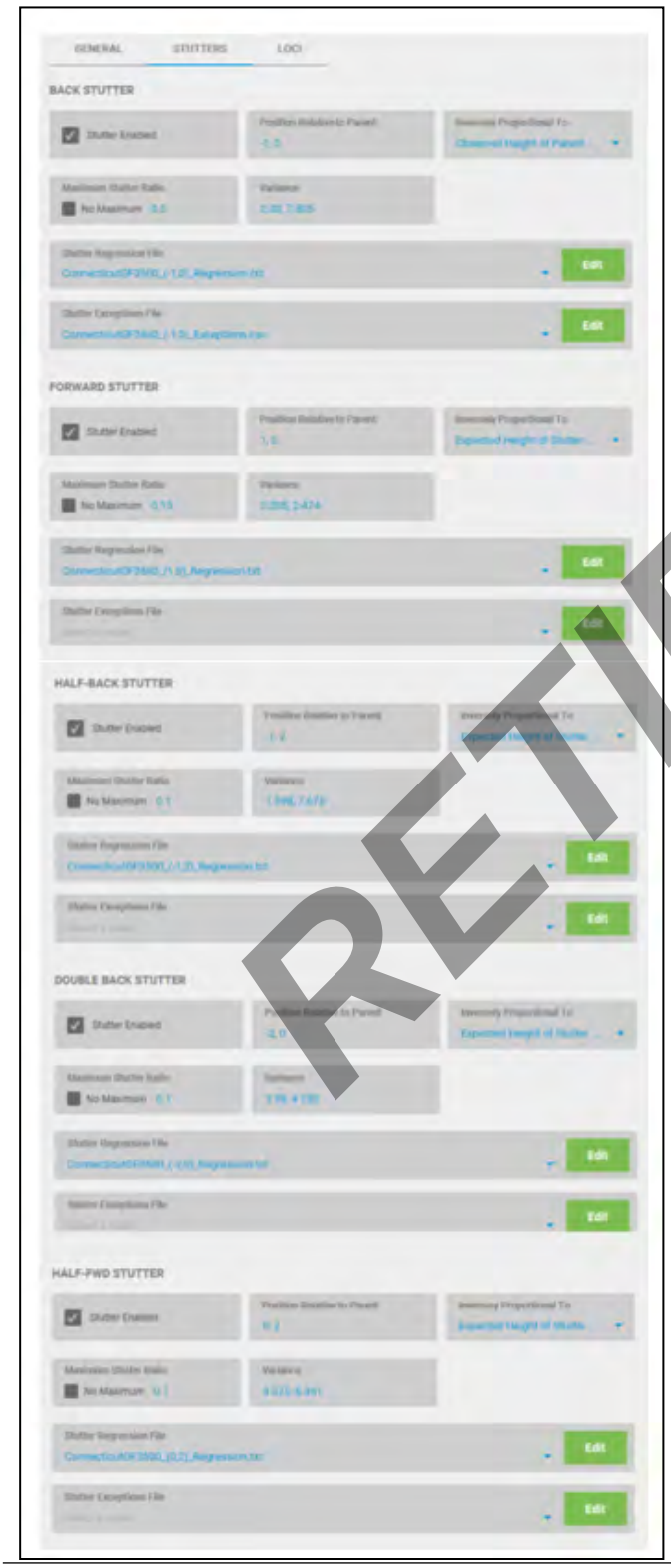

32.3.2.9 Certain situations (such as tri-alleles, discrepant results at one locus as compared to the rest of the profile, or the possibility for stutter exceeding a stutter max threshold) might warrant changing the "Ignore?" setting for autosomal loci during a deconvolution, with TL approval.

32.3.2.10 Please see the Appendix for screen shots of the stutter text files, and charts for the stutter exception files.

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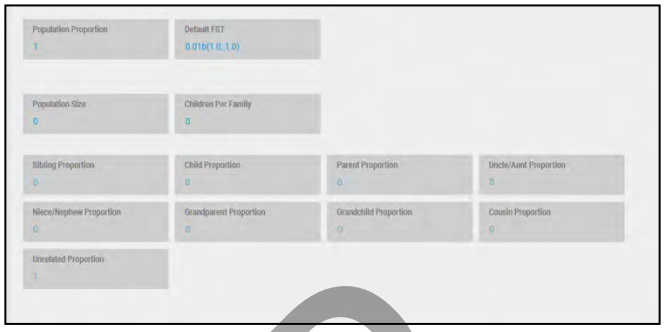
32.3.3 Populations/Allele Frequencies



32.3.3.1 Populations can be edited by clicking on “Administration” in the STRmix™ main menu, then clicking on “Populations” (see figure to right)

32.3.3.2 Each population file could only be assigned to one kit in STRmix™ v2.4, therefore, for backward compatibility with previous deconvolutions each F6C population file is in triplicate in the ProgramData/STRmix/Populations folder, and named to reflect the kit that it is assigned to. Population files for STRmix™ v2.5 and v2.6 are not kit-specific. Each file has an allele frequency file linked to it.

32.3.3.2.1 The allele frequency file that the African American population is linked to is FBI_extended_AfAmBahJam.csv

32.3.3.2.2 The allele frequency file that the Caucasian population is linked to is FBI_extended_Cauc.csf

32.3.3.2.3 The allele frequency file that the Hispanic population is linked to is FBI_extended_SE_Hisp.csv

32.3.3.3 Allele frequencies are from the FBI’s allele frequencies in PopStats.

32.3.3.4 The allele frequencies listed in the CSV files do not have the posterior means equation applied to them.

32.3.3.5 Besides “Default FST” and the allele frequency file, most settings in “Populations” do not currently apply to State of Connecticut SOPs.

32.4 Initial Setup of Reports

32.4.1 Report Default Settings: From the Main Menu click “Administration”, then “Report Defaults”.

32.4.2 Only “Summary Report” should be clicked from the list on the left and set to contain the following options: Post Burn-In Summary, Contributor Summary, LR Summary, Component Information, Locus Efficiencies, Variance Charts, Settings, Interpretation Details, Probability of N Given the Profile, Evidence Files, and Reference Files. (The order of items in the “Include?” list can be changed by dragging.)

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32.4.3 If viewing additional components for a run is necessary, reports can be re-created retroactively with different options selected. To do this click "Reports" from the main menu, and supply the appropriate run folder by browsing or drag-and-drop. Select the desired options, change the "Output File" name at the bottom of the window (as to not overwrite original report) and click "Run". This will not change the default report settings. Please note that the Genotype Probability Distribution is not included in the default report, so if there is a need to look at individual locus/contributor weights, a new report will need to be created. A report including all components shall be created for discovery/FOIA purposes, regardless if electronic data was requested.

32.4.4 With the settings above, the Interpretation Report is generated automatically for every deconvolution and LR from Previous run.

32.5 Using STRmix™ to deconvolute a profile

After analysis of a DNA profile, a determination of the most reasonable number of contributors is chosen and documented on DNA QR-302. Analysts manually compare all knowns associated with a case to all questioned samples associated with that case. If a positive association or inconclusive conclusion (except in cases of insufficient data or complexity of a mixture, where STRmix cannot be run) is made to a questioned DNA profile that questioned sample shall be deconvoluted using STRmix™. Identical profiles (regardless of injection time) compared to the same known, as such will give identical conclusions, need only be run once through STRmix. This will be properly documented in the DNA Report.

32.5.1 Launch STRmix™ software via remote desktop server: Whenever prompted, usernames, passwords, and settings can be stored.

32.5.1.1 Open the following website using your web browser: (Can be saved to favorites) https://10.51.107.36/RDWeb/Pages/en-US/login.aspx. This initially may come up as an unsecure website, but continue to the website when prompted by your browser. This link will bring up your remote application and desktop. If prompted to log in, Domain\username shall be entered as: dps\employee#. Password shall be the same password used to log onto your DPS desktop.

32.5.1.2 Click on "explorer". This will bring up the server desktop, or a prompt to save/open an application. Click "ok", "continue" or "confirm" whenever prompted. Two drives will be accessed through this desktop:

32.5.1.2.1 The F-drive is the STRmix™ server. It is the results folder on this drive to which your results will be automatically saved. Located in F:\results are individual analyst folders. Any deconvolutions an analyst performs are to be saved temporarily in these individual analyst folders. If these are not automatically mapped to save in that location, the analyst shall move

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their results folders into their folder. Analysts further will create case specific folders in their results folders. This can be done by right-clicking in their folder → new → folder. The folder names will be DSS-YY-XXXXXX, for consistency and ease in archiving. Do not create any further sub-folders, as file name sizes are restricted. Upon issuance of a DNA report, this folder shall be moved to the F:\Results\ Completed folder. A record of this move shall be recorded on QR-4, and confirmed by your technical reviewer. There shall be no sub-folders in "Completed" to assist with archiving data.

32.5.1.2.2 The U: Drive that you see on the server desktop is the U: Drive that your GeneMarker/ GeneMapper text files have been previously exported and saved to. You will not be saving anything to the U: Drive while using STRmix™. However, you must access those files from the server desktop, or you will not be allowed by STRmix™ to drag and drop those text files.

32.5.1.3 Click on "STRmix". This will launch the STRmix™ software or a prompt to save/open an application. Click "ok", "continue" or "confirm" whenever prompted. Only 12 analysts can concurrently run the STRmix™ software.

32.5.2 Click "Interpretation" from the start menu.

32.5.3 Enter the following information:

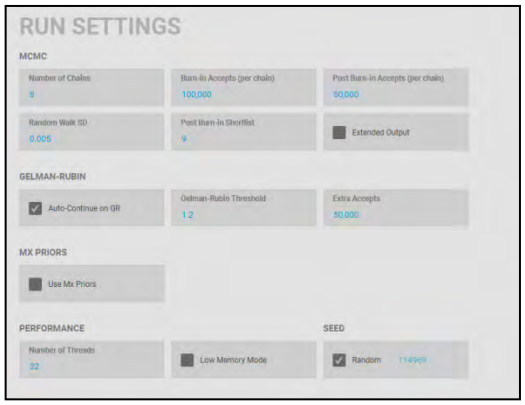
Case number: Lab ID# (ex. DSS-XX-XXXX)

Sample ID: Item #(s) (ex. 1-1 LR to 2)

Case Notes: Add any information that would be important to your technical reviewer. This can include, but not be limited to description of the item, knowns, conditioning information, reason (if applicable) deconvolution is being repeated, etc.

Number of contributors: This should have been pre-determined, and recorded on QR-302 during analysis. ("Contributor Range" should never be selected, as this feature is not currently validated CT DSS.)

32.5.3.1 A click on "Run Settings" will bring up the window to the right, Nothing in here need be changed in routine casework.



32.5.3.2 After everything has been properly added, click "Next". (Note: At any time you can return to earlier steps using the "Back" button. All entries will be saved.)

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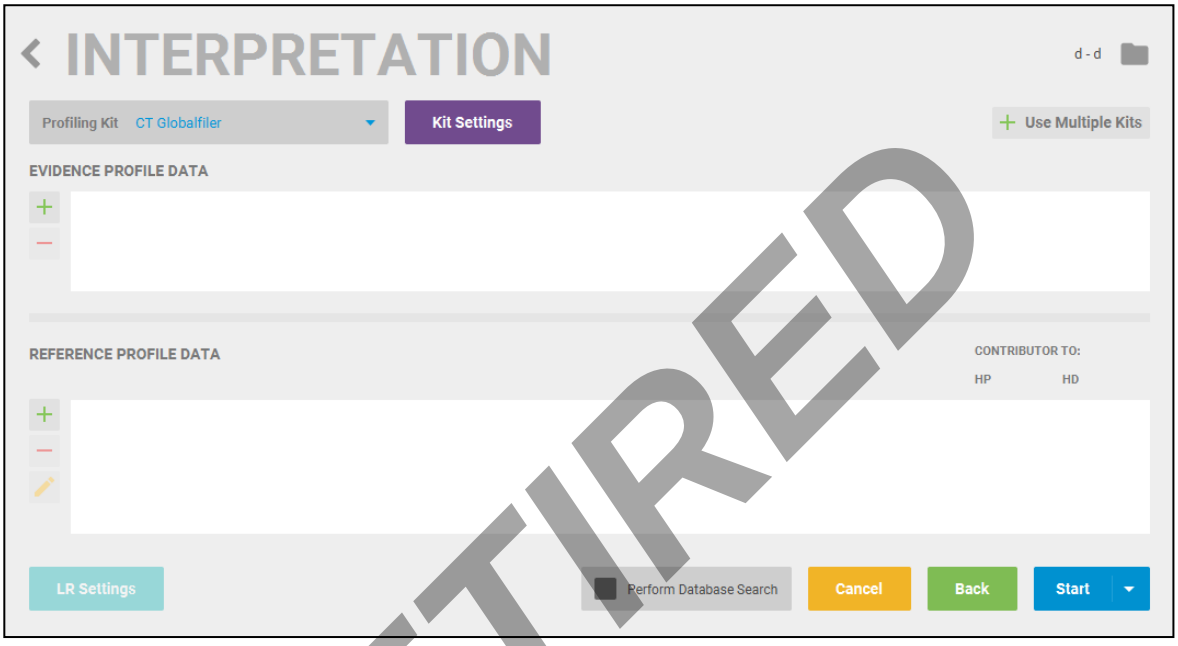
32.5.4 In window shown below, first select the kit appropriate to the amplification system and injection time for the evidentiary sample from the "Profiling Kit" pull-down menu. The "CT GlobalFiler" kit will be there as a default.



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32.5.4.1 Drag text file with evidentiary file results (the text file exported from GeneMarker/ GeneMapper) to be deconvoluted into "Evidence Profile Data" box (or conversely select green plus sign and browse to file). If necessary, browse through the list of samples in that file, select the one you are deconvoluting by checking the box to the left of it, and click "Confirm". The evidentiary input file HAS stutter peaks (these stutter peaks HAVE NOT been filtered out).

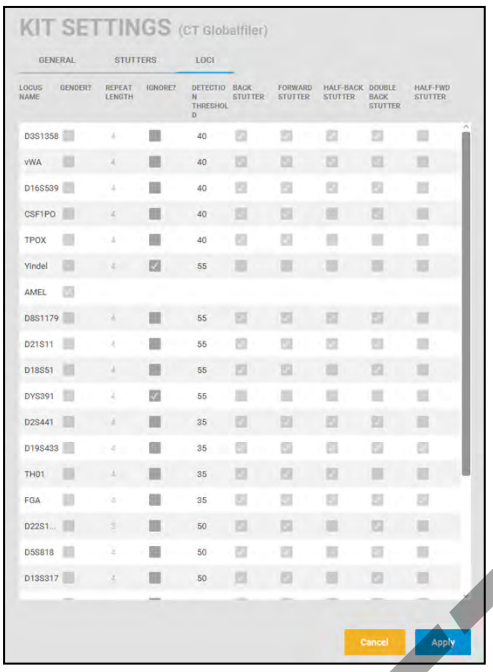
32.5.4.2 Add reference profiles to the "Reference Profile Data" box in the same way as above. The known input file does NOT have stutter peaks (all stutter peaks have been filtered out).

32.5.4.3 For any .csv input files that were generated for STRmix v2.4, STRmix v2.6 will prompt you for the kit used to type the sample being added. Providing any STRmix kit name that uses sample's amplification system will suffice for this; there is no need to look up the injection time used. This step is needed because these input files indicate marker only by reference numbers based on the order of loci in the kit, rather than containing locus names. The kit selected in step 32.5.4 will still be the kit used for the deconvolution.

32.5.4.4 If you are conditioning (a) known(s) to H_d, click on the H_d box that corresponds to that known.

32.5.5 Ignoring Loci (with TL approval)

32.5.5.1 Click the "Kit Settings" button next to the profiling kit pull-down menu, then click on "Loci", to show the window to the left.



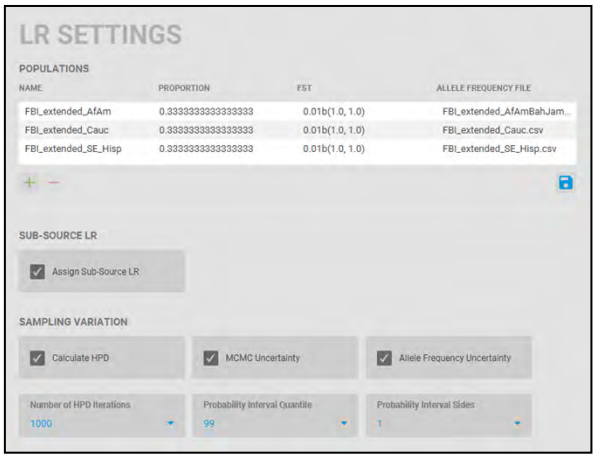
32.5.5.2 Check the box under "Ignore" for the locus you wish to ignore. Click "Apply" to change for this run only. Default settings will not be changed.

32.5.5.3 The stutter max thresholds are expected to be conservative for all loci in all kits.

32.5.6 To see the defaulted populations, click on "LR settings". The window below will appear. When compared knowns are applied to any hypothesis, 3 populations are added to "Population Settings": African American, Caucasian, and Hispanic. This setting is completed and saved the first time STRmix™ is run, and need not be completed on subsequent deconvolutions. Click "Start", making sure that "Perform Database Search" is not checked off.

32.5.7 The report PDF file for the deconvolution opens automatically when the run completes. Print page 1 for a deconvolution-only run, and print pages 1-3 for a run with a likelihood ratio calculation, for the case jacket.

32.5.8 The qualitative comparisons for various kits are as follows:



Profiling Kit	Exclusion	Inconclusive	Positive Association
Identifiler/Identifiler Plus	<1	≥ 1 <1,000	≥1,000
Fusion 6C	<1	≥ 1 <10,000	≥10,000
GlobalFiler	<1	≥ 1 <1,000	≥1,000

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32.6 Using STRmix™ to Create a Likelihood Ratio to a Reference Sample from previously deconvoluted sample

32.6.1 After launching STRmix™, click on "Investigation" then "LR from Previous". Into the "Choose Previous Interpretation for LR" box, you can either drag and drop the outer run file folder of the deconvolution that was previously completed, or browse to select the "config" .xml file within that folder, then click "select".

32.6.2 Next the sample ID may be edited to reflect the current calculation. (i.e. 1-1 LR 2 changes to 1-1 LR 3). This can be further described in the case notes. Since this is to a previous deconvolution, the MCMC settings are unable to be changed. Click "Next".

32.6.3 You will be unable to add or remove evidence profiles, or change kits in analysis. Add reference profiles to the bottom half of the "add profile data" window, as in 32.5.4.2. The known can only be added to H_p. Knowns cannot be added to or removed from H_d for conditioning purposes in "LR from previous analysis" settings, since conditioning a known affects the deconvolution, and not just the likelihood ratio from previously obtained weights.

32.6.3.1 If rework on this sample is being completed because a victim, consensual partner, or other known has been submitted that is to be conditioned to the profile, the deconvolution itself must be repeated with the conditioned known.

32.6.4 Print pages 1-3 of each report for the case jacket.

32.7 Running samples in Batch Mode

32.7.1 On the STRmix™ startup window, click "Batch Mode".

32.7.2 Click the "Add to Batch" pull-down menu, and select either "Add Interpretation" or "Add LR From Previous", depending on the desired calculation, and add samples as you would in 32.5 or 32.6. After a sample has been added to the batch, you will not be able to see your settings. Pay close attention to your settings. Entering something incorrectly could stop the batch from running.

32.7.3 If a sample needs to be run in low memory mode (a complex mixture, for example), please adjust in "Run Settings" for that particular sample.

32.7.4 If all samples have been added, but you do not wish to start the batch at this time, you can click the back arrow next to "Batch Mode" to return to the main menu. The samples that have been added will be there when you return to "Batch Mode".

32.7.5 If you wish to start the batch, click "Start".

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32.7.6 If you wish to end the batch, click "Stop". All samples that have yet to be deconvoluted will remain in the queue, until removed.

32.7.7 To remove a sample from the queue, highlight the sample in the "Calculations In Batch" window, then click "Remove".

32.8 **Review of Run Diagnostics:** In STRmix™, there are a number of diagnostics that may indicate that MCMC analysis did not perform as expected. These are found in the Post Burn-In Summary section of the Report.

For every deconvolution performed in STRmix, DNA-QR-303 must be completed to allow for an overall assessment of how the deconvolution was performed in STRmix. The ideal ranges for the quality parameters based on the internal validation are documented on this QR. No single diagnostic can determine whether a STRmix deconvolution was or was not successful. However, multiple values outside of their ideal ranges, or values falling far from their ideal ranges, are potential indicators of a problem with a deconvolution, and may warrant manual scrutiny of the genotypic weights assigned and possibly a re-run with TL approval.

In the event that any of the secondary diagnostic values are out of range, the analyst will document a brief justification in the "Comments" section of QR-303 for why the deconvolution is (or is not) valid.

32.8.1 Total iterations

The value indicates the total number of post burn-in iterations that the MCMC ran for during its analysis. This value, along with the number of accepts chosen for the analysis informs the analyst as to how often a new proposed set of parameters was accepted. This is referred to as the acceptance rate. For example, the analysis that lead to the above results was carried out with 100,000 burn-in accepts and 500,000 total accepts. This leaves 400,000 post burn-in accepts spread across 800,000 total iterations giving an acceptance rate of 1 in 2.

A very low acceptance rate (e.g. 1 in hundreds or thousands) may, in combination with the other diagnostics, indicate that the analysis needs to be run for additional iterations. On its own, a low acceptance rate is not an indication that reanalysis is required.

As a note, if a deconvolution auto-continues past the standard number of post burn-in accepts (due to an above-threshold Gelman-Rubin diagnostic score), STRmix v2.5.11 factors the total number of post burn-in iterations but only the standard number of accepts into the acceptance rate on the STRmix Report. The macro in QR-303 uses the total post burn-in counts for both iterations and accepts when calculating acceptance rate. The value appearing on the worksheet is the appropriate one to compare to the threshold range. This has been corrected in STRmix v 2.6.2.

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32.8.2 Effective Sample Size

Effective sample size (ESS) is the number of independent genotypic/mass parameter combinations that the MCMC has evaluated. A low ESS in relation to the total number of iterations suggests that the MCMC has not moved very far with each step or has had a low acceptance rate. A low ESS (e.g. 10s or 100s) value means that there is potential for a large difference in weights if the analysis was run again. A low ESS on its own is not an indication that reanalysis is required. In general, an ESS of less than 750 warrants additional scrutiny.

32.8.3 Average (log_{10}) likelihood

This value is the average (log_{10}) likelihood for the entire post burn-in MCMC. It is the log of the average likelihood (or probability) value created at each of the post burn-in MCMC iterations. The larger this value, the better STRmix™ has been able to describe the observed data. A negative value suggests that STRmix™ has not been able to describe the data very well given the information it has been provided. Some possible reasons for this value being low or negative are:

a) The profile is simply very low level and there is very little data making up the likelihood.

b) The number of contributors is wrong which can cause STRmix™ to consider incorrect genotypic combinations (e.g. large heterozygote peak imbalances or variation in mixture proportions across the profile).

c) Data has been removed that was real, particularly stutter peaks, and must now be described in STRmix™ by dropout.

d) Artifact peaks were not removed and must now be accounted for in STRmix™ by drop-in.

A low or negative value for the average (log_{10}) likelihood may indicate that the analysis requires additional scrutiny.

Good quality (adequate template, high molecular weight) mixed DNA profiles often give higher average (log_{10}) likelihood values than comparable single source profiles. So low average (log_{10}) likelihood values alone are not necessarily an indicator of an issue especially if the profile is single source.

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32.8.4 Gelman-Rubin convergence diagnostic

This diagnostic informs the analyst whether the MCMC analysis has likely converged. STRmix™ uses 8 chains to carry out the MCMC analysis and ideally each chain will be sampling in the same space after burn-in. If the chains spend their time in different spaces then it is likely that the analysis has not run for long enough. Whether or not the chains have spent time in the same space can be gauged by the within-chain and between-chain variances. This is known as the Gelman-Rubin convergence diagnostic (GR). If the chains fully converge, the GR is 1.

If the GR is above 1.2 (or 1.35 for 4 or 5 person mixtures), then there exists the possibility that the analysis hasn't converged. If the GR value is above 1.2 (or above 1.35 for 4 or 5 person mixtures), the results of the analysis should be scrutinised. Running the analysis for a larger number of iterations may reduce the GR in these instances. With the default settings a run will continue automatically for an additional 50,000 accepts per chain if the GR score is above 1.2

after the standard accept count; this does not preclude analysts from increasing the iteration count further if appropriate.

32.8.5 Allele Variance and Stutter Variance constants

Both of these values are the average value for variance and stutter variance constants across the entire post burn-in MCMC analysis. These values can be used as a guide as to the level of stochastic variation in peak heights that is present in the profile. A visual representation of how these values compare with the data seen in the laboratory's model maker data can be seen in "Variance Charts" section in the STRmix Report. The dot on the curve represents the value in the current run.

If variance is significantly above the mode value, additional scrutiny is warranted. It may indicate that the DNA profile is sub-optimal or that the number of contributors is incorrect. A higher than average Stutter Variance constant may be indicative of stutter peaks not being called in the STRmix GeneMarker project. A further look into the STRmix GeneMarker project may be necessary to see if all stutter peaks have been properly called. Pay close attention to those peaks adjacent to another peak with only a 1 base pair difference, since those are the more likely to be unresolved than those with greater than a 1 base pair difference.

Used in conjunction with the average (log_{10}) likelihood, a large allele variance or stutter variance may indicate that the PCR did not perform as expected.

If the sample is simply low level, this often results in a low average (log_{10}) likelihood and an average variance constant.

If some data has been omitted or mistakenly left in the STRmix™ input file, this often results in a low average (log_{10}) likelihood and high variances.

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Glossary of Terms

Accept: An iteration that is accepted. Default settings in STRmix™ require 500,000 accepts in the MCMC process. The burn-in, the first 100,000 accepts, allows the MCMC chains to proceed into spaces where "good" genotypes are proposed. The Metropolis-Hastings algorithm determines if a new genotype combination will be accepted or rejected.

Allele Variance: A secondary diagnostic tool, that when compared to the kit's mode, can determine how variable peak heights are in a particular sample, as well as to those samples most commonly seen in the laboratory's model maker.

Average (log) likelihood: A secondary diagnostic tool that calculates the average of the common logarithm of all likelihoods obtained for the post burn-in MCMC process.

Effective Sample Size (ESS) – A secondary diagnostic tool that evaluates the total number of independent genotypic/mass parameter combinations that the MCMC has evaluated.

Gelman-Rubin convergence diagnostic: A secondary diagnostic tool that compares variances within and between MCMC chains, to determine if they have or haven't converged.

Iteration: a proposed genotype combination.

Likelihood Ratio: A statistical test for comparing two competing hypotheses.

Markov chain Monte Carlo (MCMC): An algorithm based on standard mathematical principles that assigns a likelihood for each random genotype combination, in order to provide the best explanation for an observed data set.

Mass Parameters: 4 variable parameters used in the MCMC process to generate expected peak heights given a proposed genotype combination. These are: contributor specific **D**egradation rate, locus specific **A**mplification efficiencies, PCR **R**eplicate (not utilized by CT DSS SOPs), and contributor specific **T**emplate amount. A mnemonic phase to easily remember these parameters is **DART**.

Model Maker: A tool within the software that uses laboratory specific empirical data to create kit specific STRmix™ parameters.

Probabilistic Genotyping: A tool that combines the use of biological modeling, statistical theory, computer algorithms, and probability distributions to calculate likelihood ratios for DNA typing results of forensic samples, providing statistical weighting to different genotype combinations

STRmix™: Forensic analysis software that utilizes a fully continuous (taking peak heights into account) approach to probabilistic genotyping to deconvolute DNA profiles into their most probable components,

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then compare reference samples to that deconvolution, calculating a likelihood ratio to give that comparison a statistical weight.

Stutter Variance: A secondary diagnostic tool, that when compared to the kit's mode, can determine how variable stutter peak heights are in a particular sample, as well as to those samples most commonly seen in the laboratory's model maker.

Total Iterations: A secondary diagnostic tool that tells how many genotype combinations were proposed during the post burn-in MCMC process. Directly related to the acceptance rate, which divides the total number of post burn-in accepts (defaulted to 400,000) by the total number of iterations. Therefore, if the total iterations were 7 million, the acceptance rate would be 1 in 17.5.

Weight: A probability given to a particular genotype combination that correlates to how well that combination explains the observed profile.

Note: In creating the above glossary, the following references were utilized: STRmix Training Workshop handouts, NYC OCME STRmix™ Glossary, the STRmix Support Website, the STRmix Operations Manual, and the STRmix™ User's Manual.

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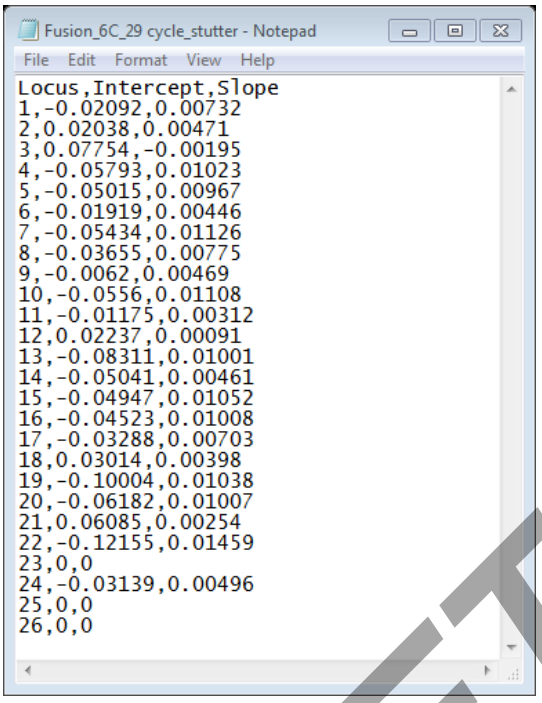
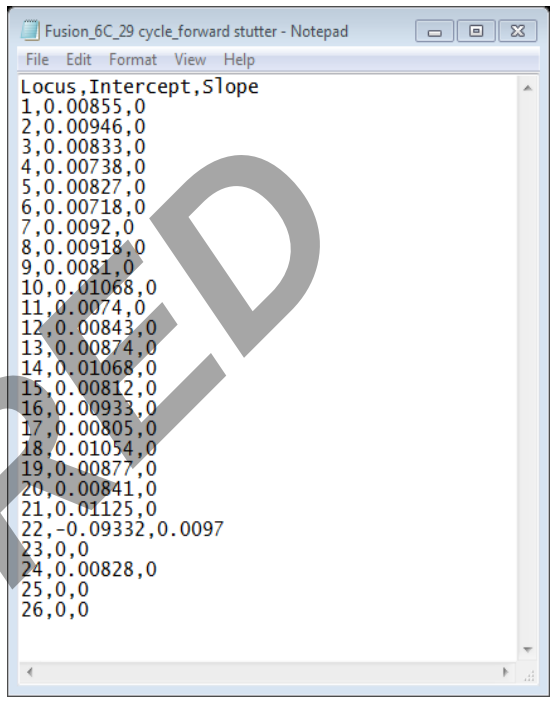
Appendices

Appendix 1: F6C Stutter File



Appendix 2: F6C Forward Stutter File



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Appendix 3: F6C Stutter Exceptions File:

What is shown in this appendix is a truncated version of the stutter exceptions file, listing only all of the stutter exceptions. The full version of this file lists all loci and all repeats in those loci.

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Repeat	D3S1358
13	0.06355
14	0.07844
15	0.09291
16	0.0933
17	0.10154
18	0.11101
19	0.1177

Repeat	D1S1656
11	0.06336
12	0.0734
13	0.08337
14	0.09366
15	0.10519
15.3	0.06924
16	0.11508
16.3	0.07731
17	0.12828
17.3	0.08733
18.3	0.0956
19.3	0.11022

Repeat	D2S441
10	0.04354
11	0.06581
11.3	0.0307
12	0.07349
14	0.04604
15	0.05485

Repeat	D2S1338
16	0.07082
17	0.07211
18	0.07885
19	0.08786
20	0.09115
21	0.0908
22	0.08697
23	0.09679
24	0.10597
25	0.12193
26	0.11848

Repeat	CSF1PO
7	0.02889
8	0.0446
9	0.03888
10	0.05278
11	0.06379
12	0.08068
13	0.09312

Repeat	Penta D
9	0.02391
10	0.02139
11	0.02226
12	0.023
13	0.02947
14	0.03312
15	0.03318

Repeat	TH01
6	0.02126
7	0.03261
8	0.03284
9	0.03904
9.3	0.01725

Repeat	D21S11
27	0.06409
28	0.077798
29	0.083498
30	0.094359
30.2	0.082745
31	0.10219
31.2	0.086892
32	0.110109
32.2	0.096907
33.2	0.10599
35	0.088913

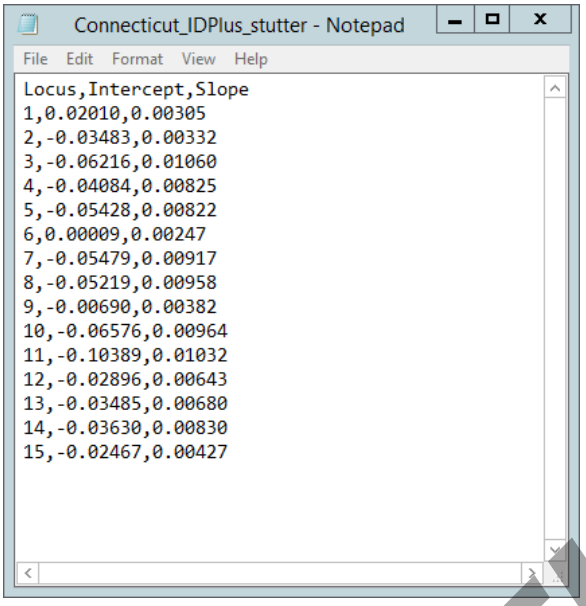
Repeat	D8S1179
9	0.05481
10	0.06995
11	0.08066
12	0.08394
13	0.07828
14	0.08691
15	0.0869
16	0.09698

Repeat	D19S433
11	0.04463
12	0.0616
13	0.06884
13.2	0.07345
14	0.07851
14.2	0.09034
15	0.08811
15.2	0.09411
16	0.09051
16.2	0.10265

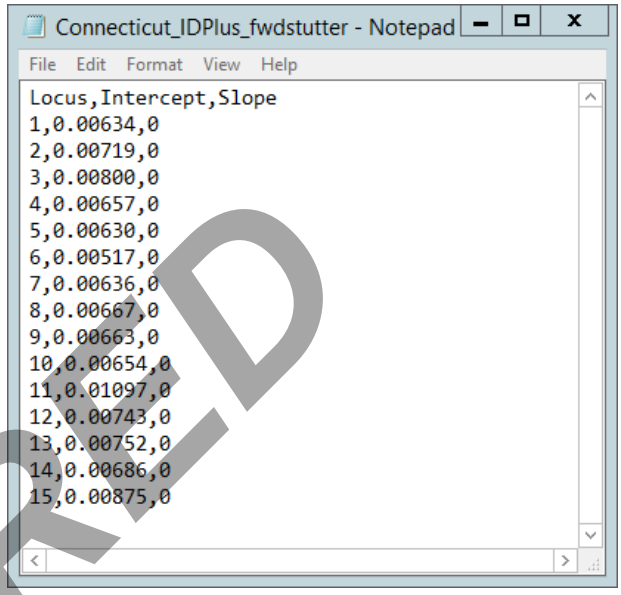
Repeat	SE33
13	0.07679
14	0.08063
15	0.08763
16	0.10257
17	0.10101
18	0.11568
19	0.12278
20	0.12455
21	0.1356
21.2	0.08847
22	0.13586
22.2	0.09782
23.2	0.10263
24.2	0.1108
25.2	0.10887
26.2	0.12206
27.2	0.12681
28.2	0.13212
29.2	0.13372
30.2	0.14036
31.2	0.15647
32.2	0.15267

Repeat	FGA
19	0.05522
20	0.0641
21	0.0703
22	0.08002
23	0.08644
24	0.09284
25	0.09859
26	0.10413
27	0.09188

Appendix 4: ID/IDP Stutter File



Appendix 5: ID/IDP Forward Stutter File



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Appendix 6: ID/IDP Stutter Exceptions File

What is shown in this appendix is a truncated version of the stutter exceptions file, listing only all of the stutter exceptions. The full version of this file lists all loci and all repeats in those loci.

Allele	D21S11
27	0.047908
28	0.054311
29	0.062966
30	0.070931
30.2	0.052559
31	0.077371
31.2	0.062083
32	0.08521
32.2	0.071276
33.2	0.080434
35	0.045518

Allele	TH01
4	0.00245
5	0.00759
6	0.01273
7	0.01787
8	0.02301
8.3	0.00759
9	0.02815
9.3	0.01273
10	0.03329
10.3	0.01273
11	0.03843
12	0.04357
13.3	0.02301

Allele	D2S1338
16	0.049954
17	0.057781
18	0.065838
19	0.06834
20	0.07168
21	0.074861
22	0.070758
23	0.074588
24	0.085853
25	0.091494
26	0.09872

Allele	D19S433
6.2	0.00402
8	0.01308
9	0.01308
10	0.0312
11	0.04026
11.1	0.0312
11.2	0.04932
12	0.04932
12.1	0.00402
12.2	0.05838
13	0.05838
13.2	0.06744
14	0.06744
14.2	0.0765
15	0.0765
15.2	0.08556
16	0.08556
16.2	0.09462
17	0.09462
17.2	0.09915
18	0.10368
18.2	0.11274
19.2	0.1218

Allele	FGA
18.2	0.050704
19	0.046956
20	0.052723
21	0.06027
22	0.074733
23	0.077721
24	0.082077
25	0.09072
26	0.090288
27	0.079836

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Appendix 7: **GlobalFiler Stutter Regression Files (0,2), (1,0), (-1,0), (-1,2) & (-2,0)**

ConnecticutGF3500_(0,2)_Regression

File Edit Format View Help

```
Locus,Intercept,Slope
D3S1358,0,0
vWA,0,0
D16S539,0,0
CSF1PO,0,0
TPOX,0,0
Yindel,0,0
D8S1179,0,0
D21S11,0,0
D18S51,0,0
DYS391,0,0
D2S441,0,0
D19S433,0.00793,0
TH01,0,0
FGA,0.00749,0
D22S1045,0,0
D5S818,0,0
D13S317,0,0
D7S820,0,0
SE33,0.00795,0
D10S1248,0,0
D1S1656,0,0
D12S391,0,0
D2S1338,0,0
```

ConnecticutGF3500_(1,0)_Regression

File Edit Format View Help

```
Locus,Intercept,Slope
D3S1358,0.00777,0
vWA,0.00621,0
D16S539,0.00807,0
CSF1PO,0.00787,0
TPOX,0.00425,0
Yindel,0,0
D8S1179,0.00829,0
D21S11,0.00984,0
D18S51,0.00870,0
DYS391,0,0
D2S441,0.00799,0
D19S433,0.00703,0
TH01,0.00341,0
FGA,0.00839,0
D22S1045,-0.05928,0.00651
D5S818,0.01011,0
D13S317,0.00871,0
D7S820,0.00677,0
SE33,0.01102,0
D10S1248,0.00735,0
D1S1656,0.00995,0
D12S391,0.00916,0
D2S1338,0.00987,0
```

ConnecticutGF3500_(-1,0)_Regression

File Edit Format View Help

```
Locus,Intercept,Slope
D3S1358,-0.04101,0.00725
vWA,-0.07068,0.00816
D16S539,-0.04508,0.00872
CSF1PO,-0.04372,0.00881
TPOX,-0.02447,0.00526
Yindel,0,0
D8S1179,0.01232,0.00370
D21S11,-0.05012,0.00396
D18S51,-0.03297,0.00655
DYS391,0,0
D2S441,0.05263,-0.00064
D19S433,-0.06695,0.00959
TH01,0.01153,0.00106
FGA,-0.06418,0.00599
D22S1045,-0.11235,0.01267
D5S818,-0.03519,0.00804
D13S317,-0.05057,0.00872
D7S820,-0.04024,0.00816
SE33,0.03781,0.00236
D10S1248,-0.02509,0.00689
D1S1656,0.02003,0.00343
D12S391,-0.08615,0.00846
D2S1338,-0.01485,0.00439
```

ConnecticutGF3500_(-1,2)_Regression

File Edit Format View Help

```
Locus,Intercept,Slope
D3S1358,0.00405,0
vWA,0.00449,0
D16S539,0.00453,0
CSF1PO,0,0
TPOX,0,0
Yindel,0,0
D8S1179,0.01331,0
D21S11,0.00537,0
D18S51,0,0
DYS391,0,0
D2S441,0.00757,0
D19S433,0.00671,0
TH01,0.00405,0
FGA,0,0
D22S1045,0,0
D5S818,0,0
D13S317,0,0
D7S820,0,0
SE33,0.04165,0
D10S1248,0,0
D1S1656,0.01731,0
D12S391,0,0
D2S1338,0,0
```

ConnecticutGF3500_(-2,0)_Regression

File Edit Format View Help

```
Locus,Intercept,Slope
D3S1358,0.00517,0
vWA,0.00593,0
D16S539,0.00403,0
CSF1PO,0.00321,0
TPOX,0,0
Yindel,0,0
D8S1179,0.00526,0
D21S11,0.00719,0
D18S51,0.00637,0.00073
DYS391,0,0
D2S441,0.00355,0
D19S433,0.00519,0
TH01,0,0
FGA,0.00553,0
D22S1045,0.00760,0
D5S818,0.00565,0
D13S317,0.00516,0
D7S820,0.00566,0
SE33,0.00717,0
D10S1248,0.00620,0
D1S1656,0.00651,0
D12S391,0.00752,0
D2S1338,0.00653,0
```

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Appendix 8: **GlobalFiler Stutter Exceptions File**

What is shown in this appendix is a truncated version of the stutter exceptions file, listing only all of the stutter exceptions. The full version of this file lists all loci and all repeats in those loci.

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Allele	D3S1358
14	0.057157213
15	0.070214002
16	0.075747885
17	0.083579096
18	0.086703111
19	0.09515721

Allele	vWA
11	0.021647446
14	0.039794758
15	0.05419452
16	0.061073697
17	0.067741678
18	0.076002233
19	0.084110913
20	0.094287847

Allele	D16S539
8	0.027102144
9	0.033088544
10	0.043033654
11	0.04957558
12	0.060711435
13	0.068121727
14	0.075502413

Allele	D8S1179
8	0.030275779
9	0.041166635
10	0.048186229
11	0.05877945
12	0.056821559
13	0.060381419
14	0.064776565
15	0.066719985
16	0.068078219
17	0.076700175

Allele	D21S11
26	0.045255334
27	0.050348048
28	0.058834134
29	0.066059096
30	0.075774293
30.2	0.059425255
31	0.079463683
31.2	0.069985026
32	0.090087629
32.2	0.077065249
33.2	0.083942629
35	0.067970436

Allele	D2S441
10	0.035079766
11	0.054400091
11.3	0.024948462
12	0.059030209
13	0.035869284
14	0.041421602
15	0.04730129

Allele	D19S433
6.2	0.00497
8	0.0136
9	0.0136
10	0.03086
11	0.03949
11.1	0.03086
11.2	0.04812
12	0.04812
12.1	0.00497
12.2	0.05675
13	0.05675
13.2	0.06538
14	0.06538
14.2	0.07401
15	0.07401
15.2	0.08264
16	0.08264
16.2	0.09127
17	0.09127
17.2	0.095585
18	0.0999
18.2	0.10853
19.2	0.11716

Allele	D5S818
8	0.02678807
9	0.035359618
10	0.047376719
11	0.053247853
12	0.061253483
13	0.067554112
14	0.079974379

Allele	TH01
4	0.00356
5	0.00903
6	0.0145
7	0.01997
8	0.02544
8.3	0.00903
9	0.03091
9.3	0.0145
10	0.03638
10.3	0.0145
11	0.04185
12	0.04732
13.3	0.02544

Allele	FGA
18	0.047377555
19	0.045966818
20	0.052071688
21	0.0627042
21.2	0.065717445
22	0.067640009
23	0.07592171
24	0.08225654
25	0.087313067
26	0.092385336
27	0.073225804

Allele	D13S317
8	0.018639181
9	0.027636656
10	0.036289016
11	0.045703807
12	0.053198383
13	0.064382035
14	0.068873716

Allele	D15S659
10	0.038091039
11	0.052122684
12	0.058509489
13	0.066715776
14	0.076017969
14.3	0.044753523
15	0.079769254
15.3	0.055455096
16	0.096188462
16.3	0.061246782
17	0.105950187
17.3	0.068641764
18.3	0.07755221
19.3	0.084337418
20.3	0.097746847

Allele	D10S1248
11	0.045478762
12	0.057250355
13	0.065590485
14	0.070354463
15	0.082042273
16	0.081661818
17	0.096379288

Allele	SE33
12	0.051066981
13	0.057925719
14	0.060510302
15	0.069474101
16	0.074128367
17	0.081578331
18	0.089001609
19	0.089724768
19.2	0.057838049
20	0.099225077
20.2	0.065143047
21	0.112380789
21.2	0.060111285
22	0.113095663
22.2	0.0695048
23.2	0.074836329
24.2	0.081108059
25.2	0.085765998
26.2	0.089076674
27.2	0.099475408
28.2	0.103651021
29.2	0.111906187
30.2	0.114894038
31.2	0.121249021
32	0.100129656
32.2	0.12989792
33	0.090853457
33.2	0.131442883
34	0.09625741

Allele	D12S391
15	0.039509506
16	0.051744278
17	0.058346473
17.3	0.052592996
18	0.068585161
18.3	0.060679173
19	0.07982839
20	0.082668327
21	0.088835583
22	0.098274453
23	0.108756947
24	0.119927917
25	0.130966451

Allele	D2S1338
16	0.050174213
17	0.058245042
18	0.063006326
19	0.072160424
20	0.080432849
21	0.075459958
22	0.07820601
23	0.078338562
24	0.090973177
25	0.093499216
26	0.108471302
27	0.110911362

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DNA SOP-32 Using STRmix™ Software

32.1 Purpose:

STRmix™ is used to deconvolute an evidentiary profile to obtain probabilistic weights of genotype combinations for all contributors to either a single source or mixture samples with, or without conditioning known profiles. Those weights are used to calculate likelihood ratios which will be reported out on all appropriate samples deconvoluted through STRmix™. The association may be, based on the likelihood ratio, included, cannot be eliminated, inconclusive, or an elimination. STRmix™ may also be used as a tool to determine possible CODIS entries, though this is not currently validated for casework. For further guidance on running STRmix™ software, please refer to the current STRmix™ Operations Manual.

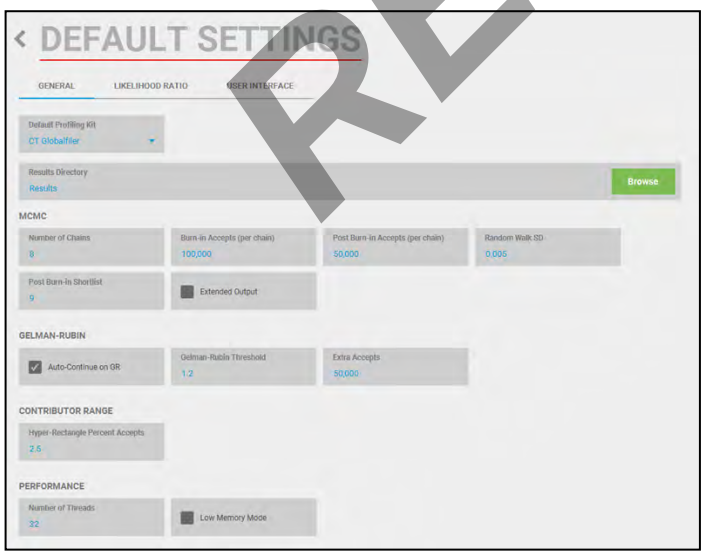
32.2 Responsibility:

DNA Unit personnel

32.3 Set-up of STRmix™

32.3.1 STRmix™ Default Settings

32.3.1.1 Default settings can be edited by clicking on "Administration" in the STRmix™ main menu, then clicking on "STRmix Defaults". See below for how your "Default Settings" should appear.



32.3.1.2 The number of MCMC chains, burn-in accepts, and post-burn-in accepts can be adjusted when scientifically valid (such as for a high Gelman-Rubin score or an expected genotype not being modeled) with TL approval.

32.3.2 Kits

32.3.2.1 Kits can be edited by clicking on "Administration" in the STRmix™ main menu, then clicking on "Profiling Kits".

32.3.2.2 Located in server desktop's local disk ProgramData/STRmix/Kits folder will be 3 different kit files for Fusion 6C, for a maximum, standard, and low injection, 2 different kit files for Identifiler Plus (to be used for

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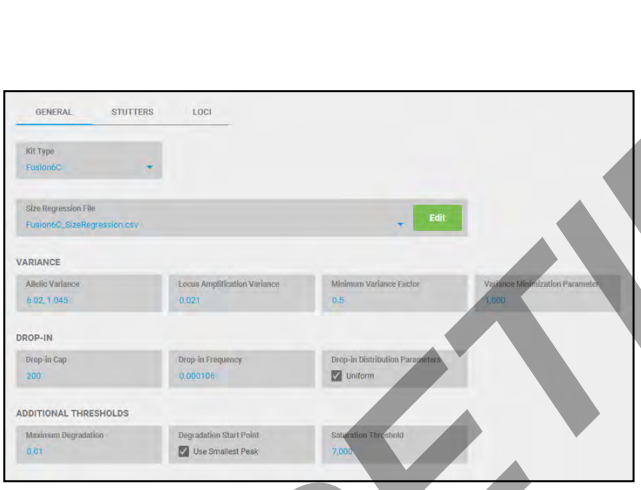
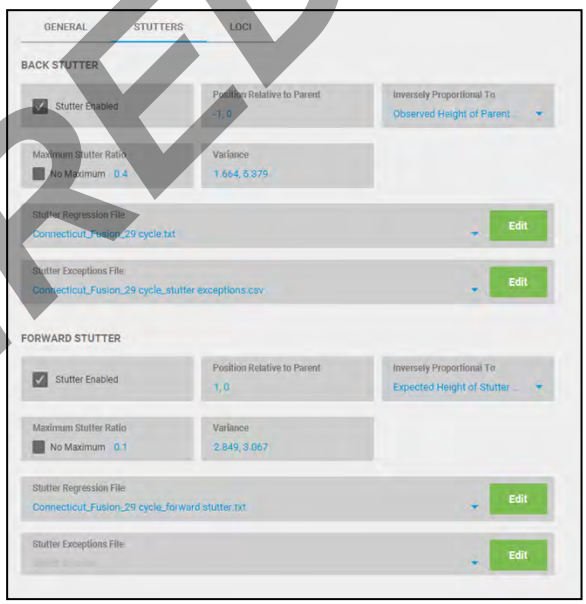
Identifiler as well), for a maximum (15 seconds or more) and standard (less than 15 seconds) injection, and one kit file for GlobalFiler.

32.3.2.3 These are the settings for the F6C – Standard Injection kit, with the loci listed in order being AMEL (gender), D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, SE33, D22S1045, DYS391 (ignore), FGA, DYS576 (ignore), and DYS570 (ignore). Repeat length is 4 for all loci except D22S1045 (3), Penta E (5), and Penta D (5), with a detection threshold of 50 rfu for all loci, and back and forward stutter being modeled for all loci:





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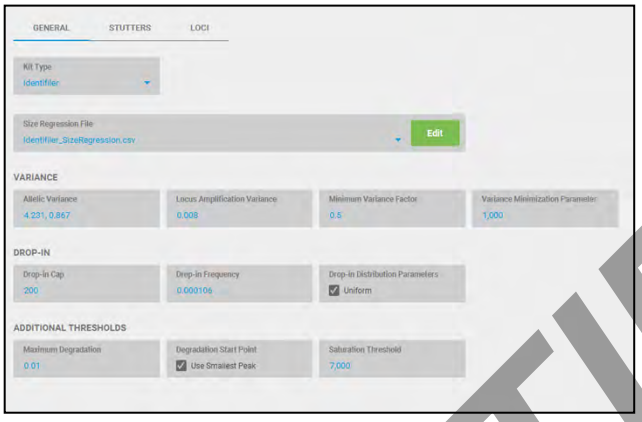
32.3.2.4 The F6C - Maximum Injection kit will have the same standard settings as the F6C – Standard Injection kit with the following exceptions:

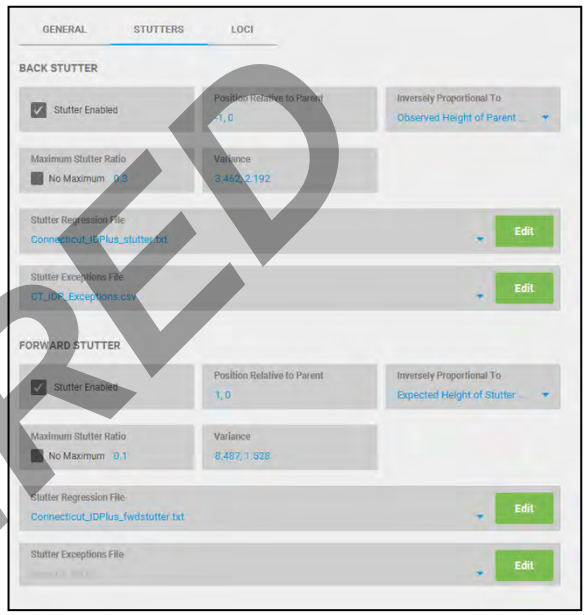
Allelic Variance: 6.725, 1.786
Stutter Variance: 2.666, 5.052
Locus Amp Variance: 0.026

32.3.2.5 The F6C – Low Injection kit will have the same standard settings as the F6C – Standard Injection kit with the following exceptions:

Allelic Variance: 4.129, 1.017
Stutter Variance: 1.75, 3.655
Locus Amp Variance: 0.019

32.3.2.6 These are the settings for the IDP – Standard Injection kit with the loci listed in order being D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, AMEL (gender), D5S818, and FGA. Repeat length is 4 for all loci, with a detection threshold of 50 rfu for all loci, and back and forward stutter being modeled for all loci:





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32.3.2.7 The IDP – Max Injection kit will have the same settings as the IDP – Standard Injection with the following exceptions:

Allelic Variance: 6.262, 1.196
Stutter Variance: 4.218, 2.221
Locus Amp Variance: 0.01

32.3.2.8 These are the settings for the GlobalFiler injection kit (X is being modeled):

	Detection Threshold	Back Stutter	Forward Stutter	Half-Back Stutter	Double Back Stutter	Half-Forward Stutter
D3S1358	40	X	X	X	X	
vWA	40	X	X	X	X	
D16S539	40	X	X	X	X	
CSF1PO	40	X	X		X	
TPOX	40	X	X			
Yindel (Ignore)	55					

	Detection Threshold	Back Stutter	Forward Stutter	Half-Back Stutter	Double Back Stutter	Half-Forward Stutter
AMEL (Gender)						
D8S1179	55	X	X	X	X	
D21S11	55	X	X	X	X	
D18S51	55	X	X		X	
DYS391 (Ignore)	55					
D2S441	35	X	X	X	X	
D19S433	35	X	X	X	X	X
TH01	35	X	X	X		
FGA	35	X	X	X	X	X
D22S1045*	50	X	X		X	
D5S818	50	X	X		X	
D13S317	50	X	X		X	
D7S820	50	X	X		X	
SE33	50	X	X	X	X	X
D10S1248	55	X	X		X	
D1S1656	55	X	X	X	X	
D12S391	55	X	X		X	
D2S1338	55	X	X		X	

*Only 3 repeat locus. All others 4.

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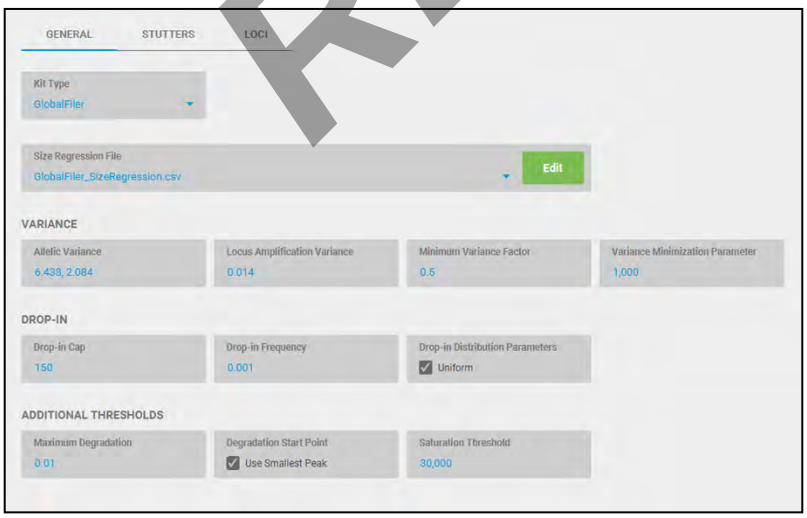

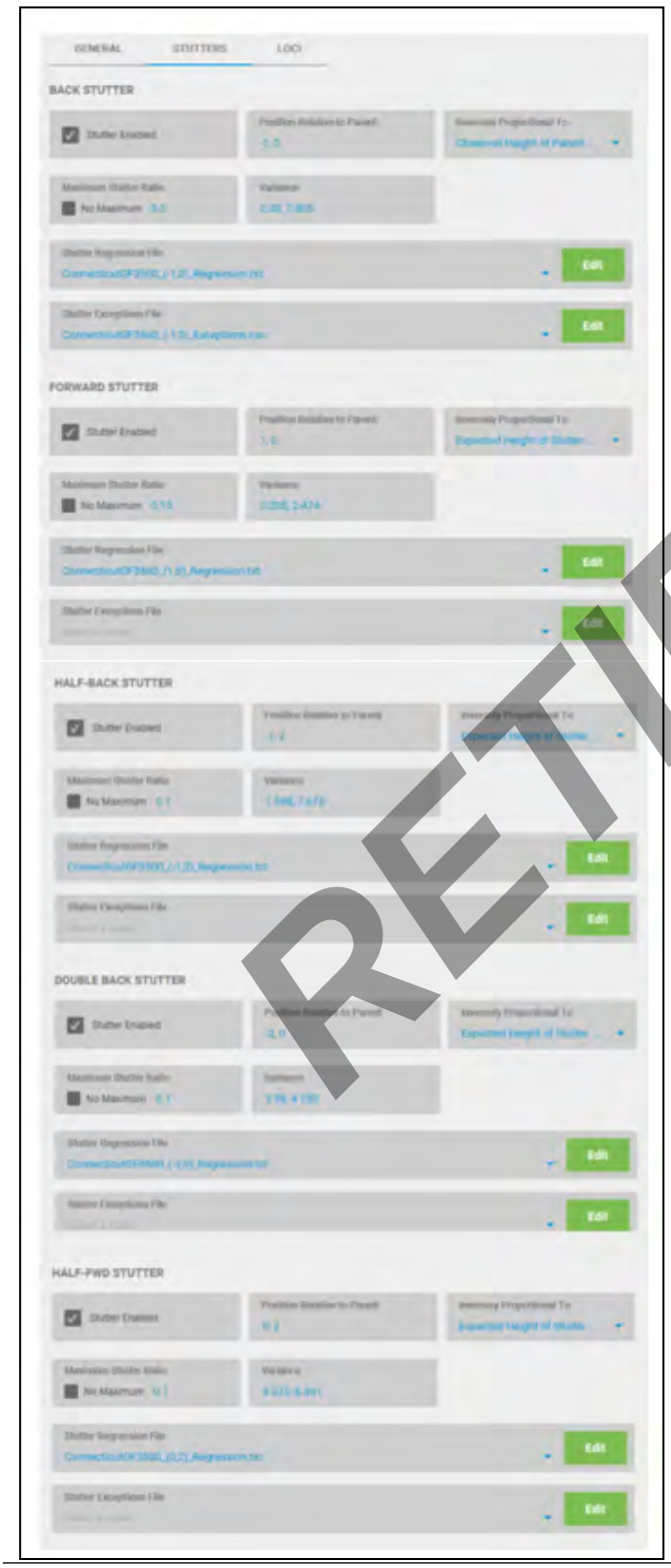

32.3.2.9 Certain situations (such as tri-alleles, discrepant results at one locus as compared to the rest of the profile, or the possibility for stutter exceeding a stutter max threshold) might warrant changing the "Ignore?" setting for autosomal loci during a deconvolution, with TL approval.

32.3.2.10 Please see the Appendix for screen shots of the stutter text files, and charts for the stutter exception files.

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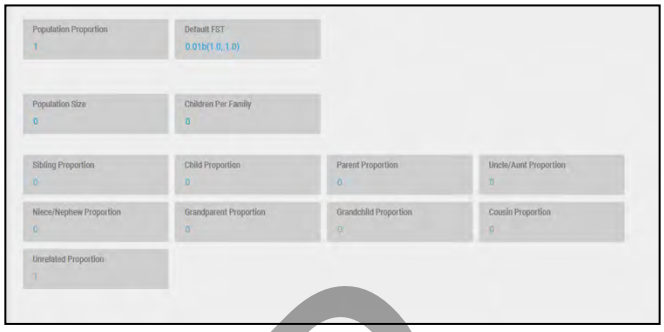
32.3.3 Populations/Allele Frequencies



32.3.3.1 Populations can be edited by clicking on “Administration” in the STRmix™ main menu, then clicking on “Populations” (see figure to right)

32.3.3.2 Each population file could only be assigned to one kit in STRmix™ v2.4, therefore, for backward compatibility with previous deconvolutions each F6C population file is in triplicate in the ProgramData/STRmix/Populations folder, and named to reflect the kit that it is assigned to. Population files for STRmix™ v2.5 and v2.6 are not kit-specific. Each file has an allele frequency file linked to it.

32.3.3.2.1 The allele frequency file that the African American population is linked to is FBI_extended_AfAmBahJam.csv

32.3.3.2.2 The allele frequency file that the Caucasian population is linked to is FBI_extended_Cauc.csf

32.3.3.2.3 The allele frequency file that the Hispanic population is linked to is FBI_extended_SE_Hisp.csv

32.3.3.3 Allele frequencies are from the FBI’s allele frequencies in PopStats.

32.3.3.4 The allele frequencies listed in the CSV files do not have the posterior means equation applied to them.

32.3.3.5 Besides “Default FST” and the allele frequency file, most settings in “Populations” do not currently apply to State of Connecticut SOPs.

32.4 Initial Setup of Reports

32.4.1 Report Default Settings: From the Main Menu click “Administration”, then “Report Defaults”.

32.4.2 Only “Summary Report” should be clicked from the list on the left and set to contain the following options: Post Burn-In Summary, Contributor Summary, LR Summary, Component Information, Locus Efficiencies, Variance Charts, Settings, Interpretation Details, Probability of N Given the Profile, Evidence Files, and Reference Files. (The order of items in the “Include?” list can be changed by dragging.)

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32.4.3 If viewing additional components for a run is necessary, reports can be re-created retroactively with different options selected. To do this click "Reports" from the main menu, and supply the appropriate run folder by browsing or drag-and-drop. Select the desired options, change the "Output File" name at the bottom of the window (as to not overwrite original report) and click "Run". This will not change the default report settings. Please note that the Genotype Probability Distribution is not included in the default report, so if there is a need to look at individual locus/contributor weights, a new report will need to be created. A report including all components shall be created for discovery/FOIA purposes, regardless if electronic data was requested.

32.4.4 With the settings above, the Interpretation Report is generated automatically for every deconvolution and LR from Previous run.

32.5 Using STRmix™ to deconvolute a profile

After analysis of a DNA profile, a determination of the most reasonable number of contributors is chosen and documented on DNA QR-302. Analysts manually compare all knowns associated with a case to all questioned samples associated with that case. If a positive association or inconclusive conclusion (except in cases of insufficient data or complexity of a mixture, where STRmix cannot be run) is made to a questioned DNA profile that questioned sample shall be deconvoluted using STRmix™. Identical profiles (regardless of injection time) compared to the same known, as such will give identical conclusions, need only be run once through STRmix. This will be properly documented in the DNA Report.

32.5.1 Launch STRmix™ software via remote desktop server: Whenever prompted, usernames, passwords, and settings can be stored.

32.5.1.1 Open the following website using your web browser: (Can be saved to favorites) https://10.51.107.36/RDWeb/Pages/en-US/login.aspx. This initially may come up as an unsecure website, but continue to the website when prompted by your browser. This link will bring up your remote application and desktop. If prompted to log in, Domain\username shall be entered as: dps\employee#. Password shall be the same password used to log onto your DPS desktop.

32.5.1.2 Click on "explorer". This will bring up the server desktop, or a prompt to save/open an application. Click "ok", "continue" or "confirm" whenever prompted. Two drives will be accessed through this desktop:

32.5.1.2.1 The F-drive is the STRmix™ server. It is the results folder on this drive to which your results will be automatically saved. Located in F:\results are individual analyst folders. Any deconvolutions an analyst performs are to be saved temporarily in these individual analyst folders. If these are not automatically mapped to save in that location, the analyst shall move

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their results folders into their folder. Analysts further will create case specific folders in their results folders. This can be done by right-clicking in their folder → new → folder. The folder names will be DSS-YY-XXXXXX, for consistency and ease in archiving. Do not create any further sub-folders, as file name sizes are restricted. Upon issuance of a DNA report, this folder shall be moved to the F:\Results\ Completed folder. A record of this move shall be recorded on QR-4, and confirmed by your technical reviewer. There shall be no sub-folders in "Completed" to assist with archiving data.

32.5.1.2.2 The U: Drive that you see on the server desktop is the U: Drive that your GeneMarker/ GeneMapper text files have been previously exported and saved to. You will not be saving anything to the U: Drive while using STRmix™. However, you must access those files from the server desktop, or you will not be allowed by STRmix™ to drag and drop those text files.

32.5.1.3 Click on "STRmix". This will launch the STRmix™ software or a prompt to save/open an application. Click "ok", "continue" or "confirm" whenever prompted. Only 12 analysts can concurrently run the STRmix™ software.

32.5.2 Click "Interpretation" from the start menu.

32.5.3 Enter the following information:

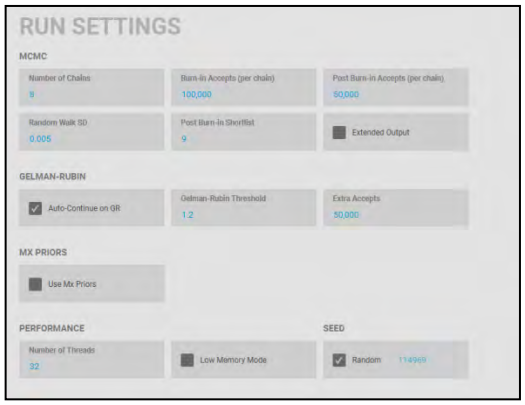
Case number: Lab ID# (ex. DSS-XX-XXXX)

Sample ID: Item #(s) (ex. 1-1 LR to 2)

Case Notes: Add any information that would be important to your technical reviewer. This can include, but not be limited to description of the item, knowns, conditioning information, reason (if applicable) deconvolution is being repeated, etc.

Number of contributors: This should have been pre-determined, and recorded on QR-302 during analysis. ("Contributor Range" should never be selected, as this feature is not currently validated CT DSS.)

32.5.3.1 A click on "Run Settings" will bring up the window to the right, Nothing in here need be changed in routine casework.



32.5.3.2 After everything has been properly added, click "Next". (Note: At any time you can return to earlier steps using the "Back" button. All entries will be saved.)

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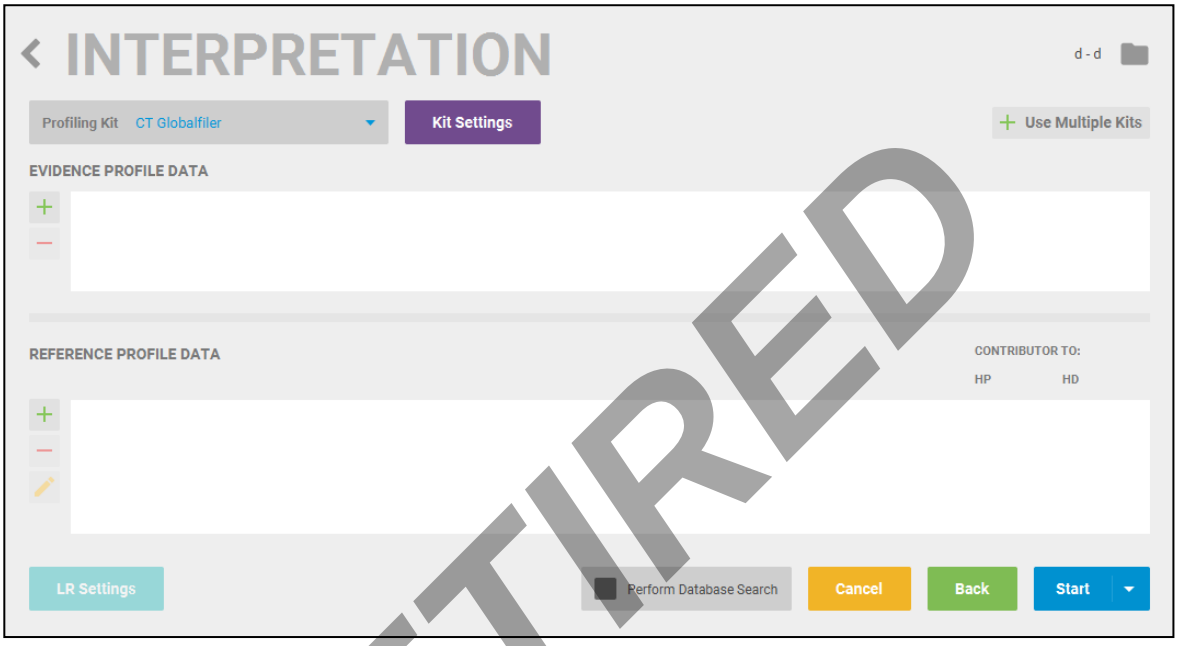
32.5.4 In window shown below, first select the kit appropriate to the amplification system and injection time for the evidentiary sample from the "Profiling Kit" pull-down menu. The "CT GlobalFiler" kit will be there as a default.



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32.5.4.1 Drag text file with evidentiary file results (the text file exported from GeneMarker/ GeneMapper) to be deconvoluted into "Evidence Profile Data" box (or conversely select green plus sign and browse to file). If necessary, browse through the list of samples in that file, select the one you are deconvoluting by checking the box to the left of it, and click "Confirm". The evidentiary input file HAS stutter peaks (these stutter peaks HAVE NOT been filtered out).

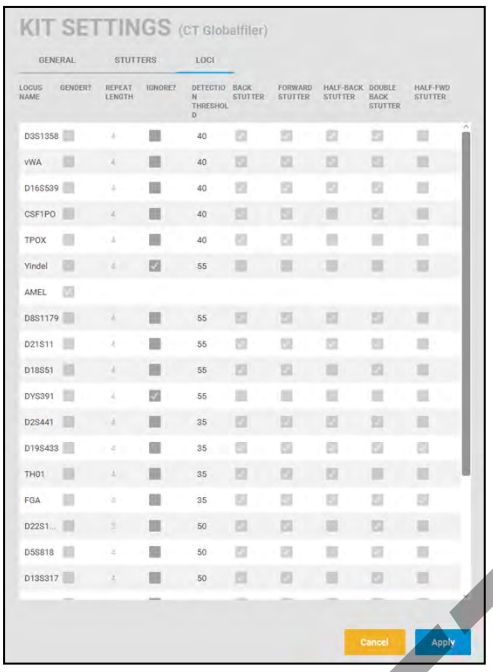
32.5.4.2 Add reference profiles to the "Reference Profile Data" box in the same way as above. The known input file does NOT have stutter peaks (all stutter peaks have been filtered out).

32.5.4.3 For any .csv input files that were generated for STRmix v2.4, STRmix v2.6 will prompt you for the kit used to type the sample being added. Providing any STRmix kit name that uses sample's amplification system will suffice for this; there is no need to look up the injection time used. This step is needed because these input files indicate marker only by reference numbers based on the order of loci in the kit, rather than containing locus names. The kit selected in step 32.5.4 will still be the kit used for the deconvolution.

32.5.4.4 If you are conditioning (a) known(s) to H_d, click on the H_d box that corresponds to that known.

32.5.5 Ignoring Loci (with TL approval)

32.5.5.1 Click the "Kit Settings" button next to the profiling kit pull-down menu, then click on "Loci", to show the window to the left.



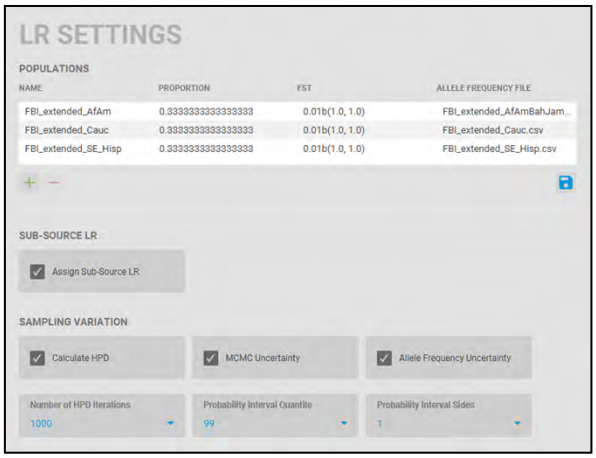
32.5.5.2 Check the box under "Ignore" for the locus you wish to ignore. Click "Apply" to change for this run only. Default settings will not be changed.

32.5.5.3 The stutter max thresholds are expected to be conservative for all loci in all kits.

32.5.6 To see the defaulted populations, click on "LR settings". The window below will appear. When compared knowns are applied to any hypothesis, 3 populations are added to "Population Settings": African American, Caucasian, and Hispanic. This setting is completed and saved the first time STRmix™ is run, and need not be completed on subsequent deconvolutions. Click "Start", making sure that "Perform Database Search" is not checked off.

32.5.7 The report PDF file for the deconvolution opens automatically when the run completes. Print page 1 for a deconvolution-only run, and print pages 1-3 for a run with a likelihood ratio calculation, for the case jacket.

32.5.8 The qualitative comparisons for various kits are as follows:



Profiling Kit	Exclusion	Inconclusive	Positive Association
Identifiler/Identifiler Plus	<1	≥ 1 <1,000	≥1,000
Fusion 6C	<1	≥ 1 <10,000	≥10,000
GlobalFiler	<1	≥ 1 <1,000	≥1,000

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32.6 Using STRmix™ to Create a Likelihood Ratio to a Reference Sample from previously deconvoluted sample

32.6.1 After launching STRmix™, click on "Investigation" then "LR from Previous". Into the "Choose Previous Interpretation for LR" box, you can either drag and drop the outer run file folder of the deconvolution that was previously completed, or browse to select the "config" .xml file within that folder, then click "select".

32.6.2 Next the sample ID may be edited to reflect the current calculation. (i.e. 1-1 LR 2 changes to 1-1 LR 3). This can be further described in the case notes. Since this is to a previous deconvolution, the MCMC settings are unable to be changed. Click "Next".

32.6.3 You will be unable to add or remove evidence profiles, or change kits in analysis. Add reference profiles to the bottom half of the "add profile data" window, as in 32.5.4.2. The known can only be added to H_p. Knowns cannot be added to or removed from H_d for conditioning purposes in "LR from previous analysis" settings, since conditioning a known affects the deconvolution, and not just the likelihood ratio from previously obtained weights.

32.6.3.1 If rework on this sample is being completed because a victim, consensual partner, or other known has been submitted that is to be conditioned to the profile, the deconvolution itself must be repeated with the conditioned known.

32.6.4 Print pages 1-3 of each report for the case jacket.

32.7 Running samples in Batch Mode

32.7.1 On the STRmix™ startup window, click "Batch Mode".

32.7.2 Click the "Add to Batch" pull-down menu, and select either "Add Interpretation" or "Add LR From Previous", depending on the desired calculation, and add samples as you would in 32.5 or 32.6. After a sample has been added to the batch, you will not be able to see your settings. Pay close attention to your settings. Entering something incorrectly could stop the batch from running.

32.7.3 If a sample needs to be run in low memory mode (a complex mixture, for example), please adjust in "Run Settings" for that particular sample.

32.7.4 If all samples have been added, but you do not wish to start the batch at this time, you can click the back arrow next to "Batch Mode" to return to the main menu. The samples that have been added will be there when you return to "Batch Mode".

32.7.5 If you wish to start the batch, click "Start".

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32.7.6 If you wish to end the batch, click "Stop". All samples that have yet to be deconvoluted will remain in the queue, until removed.

32.7.7 To remove a sample from the queue, highlight the sample in the "Calculations In Batch" window, then click "Remove".

32.8 **Review of Run Diagnostics:** In STRmix™, there are a number of diagnostics that may indicate that MCMC analysis did not perform as expected. These are found in the Post Burn-In Summary section of the Report.

For every deconvolution performed in STRmix, DNA-QR-303 must be completed to allow for an overall assessment of how the deconvolution was performed in STRmix. The ideal ranges for the quality parameters based on the internal validation are documented on this QR. No single diagnostic can determine whether a STRmix deconvolution was or was not successful. However, multiple values outside of their ideal ranges, or values falling far from their ideal ranges, are potential indicators of a problem with a deconvolution, and may warrant manual scrutiny of the genotypic weights assigned and possibly a re-run with TL approval.

In the event that any of the secondary diagnostic values are out of range, the analyst will document a brief justification in the "Comments" section of QR-303 for why the deconvolution is (or is not) valid.

32.8.1 Total iterations

The value indicates the total number of post burn-in iterations that the MCMC ran for during its analysis. This value, along with the number of accepts chosen for the analysis informs the analyst as to how often a new proposed set of parameters was accepted. This is referred to as the acceptance rate. For example, the analysis that lead to the above results was carried out with 100,000 burn-in accepts and 500,000 total accepts. This leaves 400,000 post burn-in accepts spread across 800,000 total iterations giving an acceptance rate of 1 in 2.

A very low acceptance rate (e.g. 1 in hundreds or thousands) may, in combination with the other diagnostics, indicate that the analysis needs to be run for additional iterations. On its own, a low acceptance rate is not an indication that reanalysis is required.

As a note, if a deconvolution auto-continues past the standard number of post burn-in accepts (due to an above-threshold Gelman-Rubin diagnostic score), STRmix v2.5.11 factors the total number of post burn-in iterations but only the standard number of accepts into the acceptance rate on the STRmix Report. The macro in QR-303 uses the total post burn-in counts for both iterations and accepts when calculating acceptance rate. The value appearing on the worksheet is the appropriate one to compare to the threshold range. This has been corrected in STRmix v 2.6.2.

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32.8.2 Effective Sample Size

Effective sample size (ESS) is the number of independent genotypic/mass parameter combinations that the MCMC has evaluated. A low ESS in relation to the total number of iterations suggests that the MCMC has not moved very far with each step or has had a low acceptance rate. A low ESS (e.g. 10s or 100s) value means that there is potential for a large difference in weights if the analysis was run again. A low ESS on its own is not an indication that reanalysis is required. In general, an ESS of less than 750 warrants additional scrutiny.

32.8.3 Average (log_{10}) likelihood

This value is the average (log_{10}) likelihood for the entire post burn-in MCMC. It is the log of the average likelihood (or probability) value created at each of the post burn-in MCMC iterations. The larger this value, the better STRmix™ has been able to describe the observed data. A negative value suggests that STRmix™ has not been able to describe the data very well given the information it has been provided. Some possible reasons for this value being low or negative are:

a) The profile is simply very low level and there is very little data making up the likelihood.

b) The number of contributors is wrong which can cause STRmix™ to consider incorrect genotypic combinations (e.g. large heterozygote peak imbalances or variation in mixture proportions across the profile).

c) Data has been removed that was real, particularly stutter peaks, and must now be described in STRmix™ by dropout.

d) Artifact peaks were not removed and must now be accounted for in STRmix™ by drop-in.

A low or negative value for the average (log_{10}) likelihood may indicate that the analysis requires additional scrutiny.

Good quality (adequate template, high molecular weight) mixed DNA profiles often give higher average (log_{10}) likelihood values than comparable single source profiles. So low average (log_{10}) likelihood values alone are not necessarily an indicator of an issue especially if the profile is single source.

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32.8.4 Gelman-Rubin convergence diagnostic

This diagnostic informs the analyst whether the MCMC analysis has likely converged. STRmix™ uses 8 chains to carry out the MCMC analysis and ideally each chain will be sampling in the same space after burn-in. If the chains spend their time in different spaces then it is likely that the analysis has not run for long enough. Whether or not the chains have spent time in the same space can be gauged by the within-chain and between-chain variances. This is known as the Gelman-Rubin convergence diagnostic (GR). If the chains fully converge, the GR is 1.

If the GR is above 1.2 (or 1.35 for 4 or 5 person mixtures), then there exists the possibility that the analysis hasn't converged. If the GR value is above 1.2 (or above 1.35 for 4 or 5 person mixtures), the results of the analysis should be scrutinised. Running the analysis for a larger number of iterations may reduce the GR in these instances. With the default settings a run will continue automatically for an additional 50,000 accepts per chain if the GR score is above 1.2

after the standard accept count; this does not preclude analysts from increasing the iteration count further if appropriate.

32.8.5 Allele Variance and Stutter Variance constants

Both of these values are the average value for variance and stutter variance constants across the entire post burn-in MCMC analysis. These values can be used as a guide as to the level of stochastic variation in peak heights that is present in the profile. A visual representation of how these values compare with the data seen in the laboratory's model maker data can be seen in "Variance Charts" section in the STRmix Report. The dot on the curve represents the value in the current run.

If variance is significantly above the mode value, additional scrutiny is warranted. It may indicate that the DNA profile is sub-optimal or that the number of contributors is incorrect. A higher than average Stutter Variance constant may be indicative of stutter peaks not being called in the STRmix GeneMarker project. A further look into the STRmix GeneMarker project may be necessary to see if all stutter peaks have been properly called. Pay close attention to those peaks adjacent to another peak with only a 1 base pair difference, since those are the more likely to be unresolved than those with greater than a 1 base pair difference.

Used in conjunction with the average (log_{10}) likelihood, a large allele variance or stutter variance may indicate that the PCR did not perform as expected.

If the sample is simply low level, this often results in a low average (log_{10}) likelihood and an average variance constant.

If some data has been omitted or mistakenly left in the STRmix™ input file, this often results in a low average (log_{10}) likelihood and high variances.

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Glossary of Terms

Accept: An iteration that is accepted. Default settings in STRmix™ require 500,000 accepts in the MCMC process. The burn-in, the first 100,000 accepts, allows the MCMC chains to proceed into spaces where "good" genotypes are proposed. The Metropolis-Hastings algorithm determines if a new genotype combination will be accepted or rejected.

Allele Variance: A secondary diagnostic tool, that when compared to the kit's mode, can determine how variable peak heights are in a particular sample, as well as to those samples most commonly seen in the laboratory's model maker.

Average (log) likelihood: A secondary diagnostic tool that calculates the average of the common logarithm of all likelihoods obtained for the post burn-in MCMC process.

Effective Sample Size (ESS) – A secondary diagnostic tool that evaluates the total number of independent genotypic/mass parameter combinations that the MCMC has evaluated.

Gelman-Rubin convergence diagnostic: A secondary diagnostic tool that compares variances within and between MCMC chains, to determine if they have or haven't converged.

Iteration: a proposed genotype combination.

Likelihood Ratio: A statistical test for comparing two competing hypotheses.

Markov chain Monte Carlo (MCMC): An algorithm based on standard mathematical principles that assigns a likelihood for each random genotype combination, in order to provide the best explanation for an observed data set.

Mass Parameters: 4 variable parameters used in the MCMC process to generate expected peak heights given a proposed genotype combination. These are: contributor specific **D**egradation rate, locus specific **A**mplification efficiencies, PCR **R**eplicate (not utilized by CT DSS SOPs), and contributor specific **T**emplate amount. A mnemonic phase to easily remember these parameters is **DART**.

Model Maker: A tool within the software that uses laboratory specific empirical data to create kit specific STRmix™ parameters.

Probabilistic Genotyping: A tool that combines the use of biological modeling, statistical theory, computer algorithms, and probability distributions to calculate likelihood ratios for DNA typing results of forensic samples, providing statistical weighting to different genotype combinations

STRmix™: Forensic analysis software that utilizes a fully continuous (taking peak heights into account) approach to probabilistic genotyping to deconvolute DNA profiles into their most probable components,

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then compare reference samples to that deconvolution, calculating a likelihood ratio to give that comparison a statistical weight.

Stutter Variance: A secondary diagnostic tool, that when compared to the kit's mode, can determine how variable stutter peak heights are in a particular sample, as well as to those samples most commonly seen in the laboratory's model maker.

Total Iterations: A secondary diagnostic tool that tells how many genotype combinations were proposed during the post burn-in MCMC process. Directly related to the acceptance rate, which divides the total number of post burn-in accepts (defaulted to 400,000) by the total number of iterations. Therefore, if the total iterations were 7 million, the acceptance rate would be 1 in 17.5.

Weight: A probability given to a particular genotype combination that correlates to how well that combination explains the observed profile.

Note: In creating the above glossary, the following references were utilized: STRmix Training Workshop handouts, NYC OCME STRmix™ Glossary, the STRmix Support Website, the STRmix Operations Manual, and the STRmix™ User's Manual.

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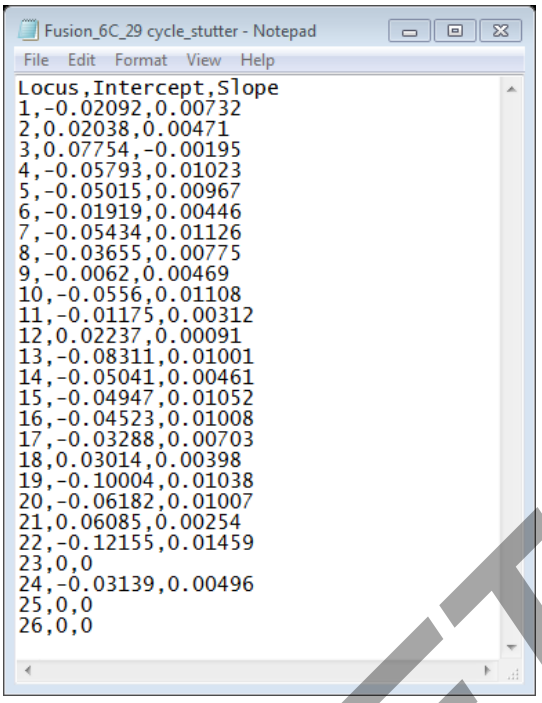
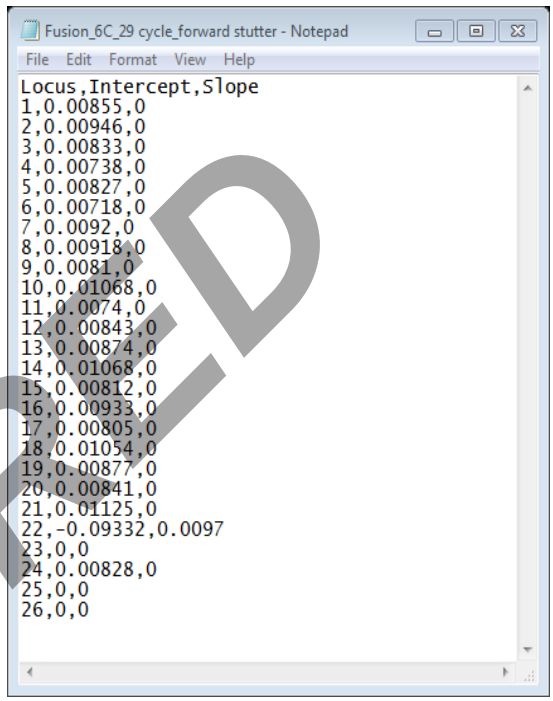
Appendices

Appendix 1: F6C Stutter File



Appendix 2: F6C Forward Stutter File



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Appendix 3: F6C Stutter Exceptions File:

What is shown in this appendix is a truncated version of the stutter exceptions file, listing only all of the stutter exceptions. The full version of this file lists all loci and all repeats in those loci.

RETIRED

Repeat	D3S1358
13	0.06355
14	0.07844
15	0.09291
16	0.0933
17	0.10154
18	0.11101
19	0.1177

Repeat	D1S1656
11	0.06336
12	0.0734
13	0.08337
14	0.09366
15	0.10519
15.3	0.06924
16	0.11508
16.3	0.07731
17	0.12828
17.3	0.08733
18.3	0.0956
19.3	0.11022

Repeat	D2S441
10	0.04354
11	0.06581
11.3	0.0307
12	0.07349
14	0.04604
15	0.05485

Repeat	D2S1338
16	0.07082
17	0.07211
18	0.07885
19	0.08786
20	0.09115
21	0.0908
22	0.08697
23	0.09679
24	0.10597
25	0.12193
26	0.11848

Repeat	CSF1PO
7	0.02889
8	0.0446
9	0.03888
10	0.05278
11	0.06379
12	0.08068
13	0.09312

Repeat	Penta D
9	0.02391
10	0.02139
11	0.02226
12	0.023
13	0.02947
14	0.03312
15	0.03318

Repeat	TH01
6	0.02126
7	0.03261
8	0.03284
9	0.03904
9.3	0.01725

Repeat	D21S11
27	0.06409
28	0.077798
29	0.083498
30	0.094359
30.2	0.082745
31	0.10219
31.2	0.086892
32	0.110109
32.2	0.096907
33.2	0.10599
35	0.088913

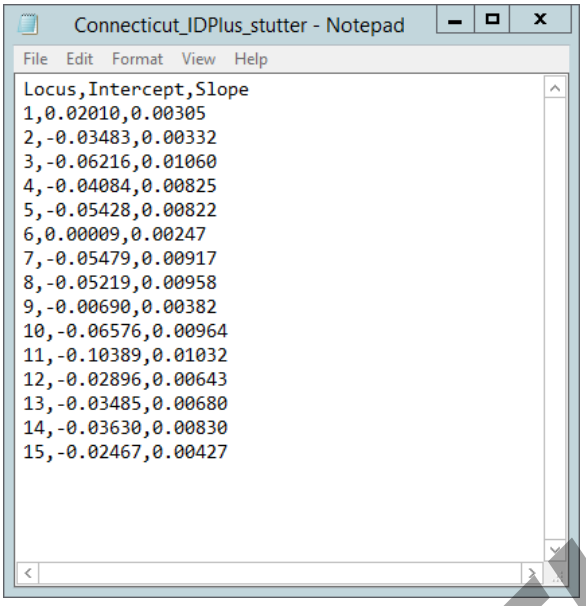
Repeat	D8S1179
9	0.05481
10	0.06995
11	0.08066
12	0.08394
13	0.07828
14	0.08691
15	0.0869
16	0.09698

Repeat	D19S433
11	0.04463
12	0.0616
13	0.06884
13.2	0.07345
14	0.07851
14.2	0.09034
15	0.08811
15.2	0.09411
16	0.09051
16.2	0.10265

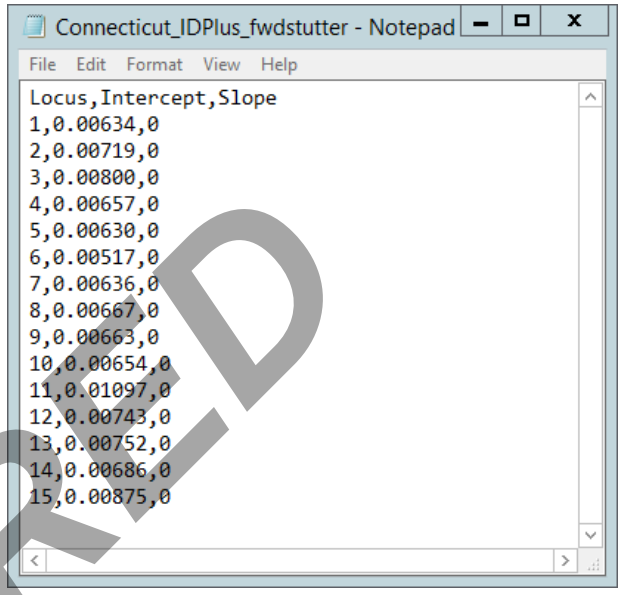
Repeat	SE33
13	0.07679
14	0.08063
15	0.08763
16	0.10257
17	0.10101
18	0.11568
19	0.12278
20	0.12455
21	0.1356
21.2	0.08847
22	0.13586
22.2	0.09782
23.2	0.10263
24.2	0.1108
25.2	0.10887
26.2	0.12206
27.2	0.12681
28.2	0.13212
29.2	0.13372
30.2	0.14036
31.2	0.15647
32.2	0.15267

Repeat	FGA
19	0.05522
20	0.0641
21	0.0703
22	0.08002
23	0.08644
24	0.09284
25	0.09859
26	0.10413
27	0.09188

Appendix 4: ID/IDP Stutter File



Appendix 5: ID/IDP Forward Stutter File



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Appendix 6: ID/IDP Stutter Exceptions File

What is shown in this appendix is a truncated version of the stutter exceptions file, listing only all of the stutter exceptions. The full version of this file lists all loci and all repeats in those loci.

Allele	D21S11
27	0.047908
28	0.054311
29	0.062966
30	0.070931
30.2	0.052559
31	0.077371
31.2	0.062083
32	0.08521
32.2	0.071276
33.2	0.080434
35	0.045518

Allele	TH01
4	0.00245
5	0.00759
6	0.01273
7	0.01787
8	0.02301
8.3	0.00759
9	0.02815
9.3	0.01273
10	0.03329
10.3	0.01273
11	0.03843
12	0.04357
13.3	0.02301

Allele	D2S1338
16	0.049954
17	0.057781
18	0.065838
19	0.06834
20	0.07168
21	0.074861
22	0.070758
23	0.074588
24	0.085853
25	0.091494
26	0.09872

Allele	D19S433
6.2	0.00402
8	0.01308
9	0.01308
10	0.0312
11	0.04026
11.1	0.0312
11.2	0.04932
12	0.04932
12.1	0.00402
12.2	0.05838
13	0.05838
13.2	0.06744
14	0.06744
14.2	0.0765
15	0.0765
15.2	0.08556
16	0.08556
16.2	0.09462
17	0.09462
17.2	0.09915
18	0.10368
18.2	0.11274
19.2	0.1218

Allele	FGA
18.2	0.050704
19	0.046956
20	0.052723
21	0.06027
22	0.074733
23	0.077721
24	0.082077
25	0.09072
26	0.090288
27	0.079836

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Appendix 7: **GlobalFiler Stutter Regression Files (0,2), (1,0), (-1,0), (-1,2) & (-2,0)**

ConnecticutGF3500_(0,2)_Regression

File Edit Format View Help

```
Locus,Intercept,Slope
D3S1358,0,0
vWA,0,0
D16S539,0,0
CSF1PO,0,0
TPOX,0,0
Yindel,0,0
D8S1179,0,0
D21S11,0,0
D18S51,0,0
DYS391,0,0
D2S441,0,0
D19S433,0.00793,0
TH01,0,0
FGA,0.00749,0
D22S1045,0,0
D5S818,0,0
D13S317,0,0
D7S820,0,0
SE33,0.00795,0
D10S1248,0,0
D1S1656,0,0
D12S391,0,0
D2S1338,0,0
```

ConnecticutGF3500_(1,0)_Regression

File Edit Format View Help

```
Locus,Intercept,Slope
D3S1358,0.00777,0
vWA,0.00621,0
D16S539,0.00807,0
CSF1PO,0.00787,0
TPOX,0.00425,0
Yindel,0,0
D8S1179,0.00829,0
D21S11,0.00984,0
D18S51,0.00870,0
DYS391,0,0
D2S441,0.00799,0
D19S433,0.00703,0
TH01,0.00341,0
FGA,0.00839,0
D22S1045,-0.05928,0.00651
D5S818,0.01011,0
D13S317,0.00871,0
D7S820,0.00677,0
SE33,0.01102,0
D10S1248,0.00735,0
D1S1656,0.00995,0
D12S391,0.00916,0
D2S1338,0.00987,0
```

ConnecticutGF3500_(-1,0)_Regression

File Edit Format View Help

```
Locus,Intercept,Slope
D3S1358,-0.04101,0.00725
vWA,-0.07068,0.00816
D16S539,-0.04508,0.00872
CSF1PO,-0.04372,0.00881
TPOX,-0.02447,0.00526
Yindel,0,0
D8S1179,0.01232,0.00370
D21S11,-0.05012,0.00396
D18S51,-0.03297,0.00655
DYS391,0,0
D2S441,0.05263,-0.00064
D19S433,-0.06695,0.00959
TH01,0.01153,0.00106
FGA,-0.06418,0.00599
D22S1045,-0.11235,0.01267
D5S818,-0.03519,0.00804
D13S317,-0.05057,0.00872
D7S820,-0.04024,0.00816
SE33,0.03781,0.00236
D10S1248,-0.02509,0.00689
D1S1656,0.02003,0.00343
D12S391,-0.08615,0.00846
D2S1338,-0.01485,0.00439
```

ConnecticutGF3500_(-1,2)_Regression

File Edit Format View Help

```
Locus,Intercept,Slope
D3S1358,0.00405,0
vWA,0.00449,0
D16S539,0.00453,0
CSF1PO,0,0
TPOX,0,0
Yindel,0,0
D8S1179,0.01331,0
D21S11,0.00537,0
D18S51,0,0
DYS391,0,0
D2S441,0.00757,0
D19S433,0.00671,0
TH01,0.00405,0
FGA,0,0
D22S1045,0,0
D5S818,0,0
D13S317,0,0
D7S820,0,0
SE33,0.04165,0
D10S1248,0,0
D1S1656,0.01731,0
D12S391,0,0
D2S1338,0,0
```

ConnecticutGF3500_(-2,0)_Regression

File Edit Format View Help

```
Locus,Intercept,Slope
D3S1358,0.00517,0
vWA,0.00593,0
D16S539,0.00403,0
CSF1PO,0.00321,0
TPOX,0,0
Yindel,0,0
D8S1179,0.00526,0
D21S11,0.00719,0
D18S51,0.00637,0.00073
DYS391,0,0
D2S441,0.00355,0
D19S433,0.00519,0
TH01,0,0
FGA,0.00553,0
D22S1045,0.00760,0
D5S818,0.00565,0
D13S317,0.00516,0
D7S820,0.00566,0
SE33,0.00717,0
D10S1248,0.00620,0
D1S1656,0.00651,0
D12S391,0.00752,0
D2S1338,0.00653,0
```

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Appendix 8: **GlobalFiler Stutter Exceptions File**

What is shown in this appendix is a truncated version of the stutter exceptions file, listing only all of the stutter exceptions. The full version of this file lists all loci and all repeats in those loci.

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Allele	D3S1358
14	0.057157213
15	0.070214002
16	0.075747885
17	0.083579096
18	0.086703111
19	0.09515721

Allele	vWA
11	0.021647446
14	0.039794758
15	0.05419452
16	0.061073697
17	0.067741678
18	0.076002233
19	0.084110913
20	0.094287847

Allele	D16S539
8	0.027102144
9	0.033088544
10	0.043033654
11	0.04957558
12	0.060711435
13	0.068121727
14	0.075502413

Allele	D8S1179
8	0.030275779
9	0.041166635
10	0.048186229
11	0.05877945
12	0.056821559
13	0.060381419
14	0.064776565
15	0.066719985
16	0.068078219
17	0.076700175

Allele	D21S11
26	0.045255334
27	0.050348048
28	0.058834134
29	0.066059096
30	0.075774293
30.2	0.059425255
31	0.079463683
31.2	0.069985026
32	0.090087629
32.2	0.077065249
33.2	0.083942629
35	0.067970436

Allele	D2S441
10	0.035079766
11	0.054400091
11.3	0.024948462
12	0.059030209
13	0.035869284
14	0.041421602
15	0.04730129

Allele	D19S433
6.2	0.00497
8	0.0136
9	0.0136
10	0.03086
11	0.03949
11.1	0.03086
11.2	0.04812
12	0.04812
12.1	0.00497
12.2	0.05675
13	0.05675
13.2	0.06538
14	0.06538
14.2	0.07401
15	0.07401
15.2	0.08264
16	0.08264
16.2	0.09127
17	0.09127
17.2	0.095585
18	0.0999
18.2	0.10853
19.2	0.11716

Allele	D5S818
8	0.02678807
9	0.035359618
10	0.047376719
11	0.053247853
12	0.061253483
13	0.067554112
14	0.079974379

Allele	TH01
4	0.00356
5	0.00903
6	0.0145
7	0.01997
8	0.02544
8.3	0.00903
9	0.03091
9.3	0.0145
10	0.03638
10.3	0.0145
11	0.04185
12	0.04732
13.3	0.02544

Allele	FGA
18	0.047377555
19	0.045966818
20	0.052071688
21	0.0627042
21.2	0.065717445
22	0.067640009
23	0.07592171
24	0.08225654
25	0.087313067
26	0.092385336
27	0.073225804

Allele	D13S317
8	0.018639181
9	0.027636656
10	0.036289016
11	0.045703807
12	0.053198383
13	0.064382035
14	0.068873716

Allele	D15S659
10	0.038091039
11	0.052122684
12	0.058509489
13	0.066715776
14	0.076017969
14.3	0.044753523
15	0.079769254
15.3	0.055455096
16	0.096188462
16.3	0.061246782
17	0.105950187
17.3	0.068641764
18.3	0.07755221
19.3	0.084337418
20.3	0.097746847

Allele	D10S1248
11	0.045478762
12	0.057250355
13	0.065590485
14	0.070354463
15	0.082042273
16	0.081661818
17	0.096379288

Allele	SE33
12	0.051066981
13	0.057925719
14	0.060510302
15	0.069474101
16	0.074128367
17	0.081578331
18	0.089001609
19	0.089724768
19.2	0.057838049
20	0.099225077
20.2	0.065143047
21	0.112380789
21.2	0.060111285
22	0.113095663
22.2	0.0695048
23.2	0.074836329
24.2	0.081108059
25.2	0.085765998
26.2	0.089076674
27.2	0.099475408
28.2	0.103651021
29.2	0.111906187
30.2	0.114894038
31.2	0.121249021
32	0.100129656
32.2	0.12989792
33	0.090853457
33.2	0.131442883
34	0.09625741

Allele	D12S391
15	0.039509506
16	0.051744278
17	0.058346473
17.3	0.052592996
18	0.068585161
18.3	0.060679173
19	0.07982839
20	0.082668327
21	0.088835583
22	0.098274453
23	0.108756947
24	0.119927917
25	0.130966451

Allele	D2S1338
16	0.050174213
17	0.058245042
18	0.063006326
19	0.072160424
20	0.080432849
21	0.075459958
22	0.07820601
23	0.078338562
24	0.090973177
25	0.093499216
26	0.108471302
27	0.110911362

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33.1 Purpose:

Occasionally, a DNA typing result that was developed using a protocol that is no longer in use may need to be further evaluated for comparison to newly submitted samples or to assess a candidate match. Please see DNA SOP-15 CODIS Hit Confirmations for information on the need for reinterpretation.

33.2 Responsibility:

DNA Unit Personnel authorized for Legacy Data Interpretation

33.3 Procedure:

For Profiler Plus® and COfiler®, the analyst will refer to Section 33.4 below.

For autosomal data generated using AmpFℓSTR Identifiler®, the analyst will refer to Section 33.9 below.

For autosomal data generated using AmpFℓSTR Identifiler® Plus, the analyst will refer to Section 33.9 below.

For autosomal data generated using AmpFℓSTR Minifiler, the analyst will refer to Section 33.16 below.

For autosomal data generated using PowerPlex® Fusion 6C, the analyst will refer to Section 33.17 below.

For Y-STR data generated using Yfiler, the analyst will refer to Section 33.18 below.

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33.4 Profiler Plus® and COfiler®:

This procedure pertains to both Perkin Elmer AmpF*l*STR Profiler Plus and AmpF*l*STR CO*f*iler PCR amplification kits. When Profiler Plus and CO*f*iler are employed, the original thirteen core CODIS STR loci are typed in two amplifications (two overlapping STRs + Amelogenin) according to manufacturer's protocols. These STR loci are D3S1358, vWA, FGA, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, CSF1PO, D7S820.

Amplification product from these kits were analyzed using an ABI 377 DNA Sequencer and the supplied GeneScan and Genotyper software.

33.5 GeneScan Analysis:

Following the electrophoretic run, all data is automatically sent to GeneScan (data is saved in a run folder with the date and time of the run) and all software programs are closed. Analysis of the data is outlined below. The gel image is opened. An approved matrix is installed. (Note: A new matrix is generated approximately once a month or when a major reagent is changed, the new matrix is logged into the matrix notebook). Gel lanes are auto-tracked. The top of the gel is cut off above the 400 bp MW standard (Profiler Plus) or the 350 bp MW standard (CO*f*iler). The gel bottom is cut off between the 75 bp standard and the primers. The tracking is inspected and any changes necessary are made. Extract data from the tracked lanes. A GeneScan project is created and stored in the run folder.

33.5.1 GeneScan gel preference defaults are as follows:

Auto-track gel	on
Scan range	0-10,000
Image generation defaults	0-12,000
Multi-component gel image	on
Estimated maximum peak height	3500
3 channel averaging	

33.5.2 GeneScan analysis parameters are as follows:

Analysis parameters (Q sample):
Analysis Range: full range
Data Processing: baseline
Multi-component on
Light smoothing
Peak Detection:
Minimum peak amplitude thresholds B: 50 Y: 50 G: 50 R: 50
Size call range: full
Size calling method: Local Southern
Split Peak correction: none

33.5.3 DNA samples are first analyzed using the standard parameters listed above. To address issues such as shoulder effects, the samples may be analyzed using different parameters provided that the change (e.g. to sample 100) does not remove true alleles. Both the primary and the second analyst must confirm that the new parameters do not adversely affect the results.

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33.5.4 When the cases on a gel are analyzed individually, the negative controls for the case in question (on a particular gel) are analyzed at the same parameters as the evidentiary samples for that case. When multiple cases are analyzed on one gel (at the same time) using the same set of controls, the negative controls are analyzed using the parameters of the most stringent case, i.e, the lowest sample parameters of the evidence.

33.5.5 Check the analysis log to ascertain that size calling was successful in the selected range. If standard ROX peaks were missed, check the tracking to make sure they were included. Re-analyze at lower threshold as needed.

33.5.6 Look at the negative control and the reagent blank.

33.5.7 Look at the ladder. Ensure that one ladder has all allele peaks above the threshold. If not, the gel must be rerun.

33.5.8 Look at the positive controls.

33.5.9 Look at the size standards--aligned by size. Verify that all size standards are called within the appropriate size range for the STR system.

33.5.10 Look at each sample. Check for the number of peaks at each locus, poorly formed peaks, or any other feature that may be important in the interpretation of the data. Samples that show background noise >50 RFU should be noted. In Results Control, look for pull-up peaks and raised baselines. Look at maximum peak intensities and level of sample degradation.

33.5.11 Save the project in the run folder.

33.6 Genotyper:

33.6.1 Launch the appropriate template alias (Profiler Plus or CO*f*iler) for the amplifications being analyzed.

33.6.2 Import the appropriate GeneScan sample files including controls (RB, NEG, KJL/TMP, POS) and at least one ladder. At least one set of controls (for each amplification set) must give the expected results*.

*RB and NEG samples should not have any callable profiles. If minor peaks are detected and the evidentiary samples are not affected, the set of extracted samples may be interpreted with caution.

33.6.3 Control Profiles: Look at each color in the controls individually. Ensure that all alleles were included and that the positive controls give the expected genotypes.

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See DNA SOP-29 for profiles for the kit positive control (POS) and the laboratory positive control (KJL)

33.6.4 Allelic Ladder: Look at each color individually. Ensure that all alleles that are in the allelic ladder are called.

33.7 Interpretation of DNA Profiles in Genotyper:

The results are evaluated for each locus. However, considering the data from all loci tested will assist in the composite interpretation of the results.

33.7.1 Off-Scale Data: If too much DNA is added to the amplification reaction or is loaded onto the gel the fluorescent intensity for the PCR products may exceed the linear dynamic range for detection by the instrument. Samples with off-scale peaks may exhibit raised baselines, split peaks, increased –A peaks, excessive pull-up and/or higher than expected stutter levels in one or more of the colors. Examine the peak heights in GeneScan software. Any peaks greater than 3500 RFU may be off-scale. These samples should be interpreted with care.

33.7.2 Peak identification: Only allele peaks with a height >50 RFU are reported. The identity of all peaks called by Genotyper, is assigned to one of the following categories:

a) Allele Peak: A called allele has a peak height greater than 50 RFU, a fragment size that falls within the base pair range, and has the appropriate dye color for the locus. However, not all peaks greater than 50 RFU are typed as alleles. Stutter peaks, shoulders, pull-up peaks, and background noise could fall above 50 RFU, especially if the allele peaks are high. Given factory macro parameters, peaks above 50 RFU that are called by GeneScan but removed by Genotyper may be called by the analysts on a case-by-case basis. Such peaks are interpreted with caution. Peaks between 30 and 50 RFU are not reported, but should be noted when appropriate. For these reasons, scientist experience is critical in reporting STR results.

b) Detection of variant alleles: Variant alleles have been identified from many STR loci. These alleles may contain full or partial repeats. Variant alleles are labeled as Off Ladder Alleles (OLA) in Genotyper. If a particular variant has been observed before by the CT DSS, Life Technologies, STRbase, NIST, or in published literature, the sample does not need to be repeated. In Genotyper change the OLA designation to the proper allele call.

c) Stutter peaks (generally N-4): A stutter peak has a fragment length typically four base pairs smaller than the true allele. If the peak height in the stutter

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position is less than the PE stutter threshold, the peak is considered a stutter peak and is filtered out by Kazam. CT Forensic Laboratory stutter thresholds are determined by evaluating the PE and CT Forensic Laboratory empirical stutter data on an allele-by-allele basis (see Appendix 8.4). If the height of the N-4 peak is greater than the CT stutter threshold, this indicates a possible mixture, an overloaded sample, or a higher than normal stutter percentage. Additional information provided by other loci may be of assistance in these cases. For offender database samples, Kazam 20% will filter out all N-4 peaks that are less than 20% of the N peak.

d) -A Peaks: AmpF*l*STR kits are optimized to add an extra nucleotide to the 3' end of the PCR product. The nucleotide is predominantly adenosine (A). Lack of a full A addition may be observed especially when the amount of input DNA is greater than approximately 2.5 ng. However, sample degradation can increase the effective concentration of a small amplicon relative to the larger amplicons, leading to -A product at the smaller loci. Data from other loci may be of assistance in these cases. The level of –A product may be reduced by incubating the sample at 60 ^{o}C for an additional 30-90'. When appropriate, a smaller quantity of sample may be re-amplified.

e) Pull-up Peaks: Pull-up peaks are generally seen when excess amplification product is loaded onto the gel or when the matrix is sub-optimal for the gel. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-up peaks are often displayed in several colors. Pull-up peaks should have approximately the same fragment size in each of the colors as the true allele peak. Additional information provided by other loci may be of assistance in these cases.

f) Peaks < 50 RFU: Low-level peaks are those less than 50 RFU but > 30 RFU that are clearly distinguishable from background noise. These peaks cannot be called but their presence is noted when appropriate. This information may be used in overall sample interpretation when appropriate.

33.8 Interpretation of Mixed Samples for Profiler Plus and CO*f*iler**:**

Generally, the detection of more than two alleles per locus indicates a mixed sample. Variation in peak height between alleles in a single locus may assist in the interpretation of such results. Peaks below the 50 RFU threshold may assist in the interpretation of mixed DNA samples. The following guidelines pertain to the interpretation of mixtures:

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33.8.1 A DNA mixture is conclusively identified by (a) the detection of three or more alleles at two or more loci or (b) 4 or more alleles detected at one or more loci. Other results are consistent with (but not proof of) a mixture as discussed below. Three peak patterns (at a single locus) have been observed from single sources, but they are rare.

33.8.2 Peak imbalance involving the stutter position, e.g., where the N-4 peak is 15-25% of the N peak, may indicate a mixture. With the possibility of allele sharing among individuals, 3 alleles may not be detected especially with degraded samples or partial profiles.

33.8.3 General peak imbalance: Heterozygous peak imbalance greater than 40% may indicate a mixture, i.e., the smaller peak is less than 60% of the larger peak. However, microvariants in the primer-binding domain may cause either PCR enhancement or suppression.

33.8.4 Highly Degraded/Low Template Samples:

Highly degraded/low template samples are interpreted with caution. Degraded samples may "appear" to exhibit high stutter and greater –A at the smaller amplicons with off-scale data. In addition, degraded/low template samples may display unbalanced heterozygous alleles and locus/allele dropout. Partial profiles may be called as determined by the data present for each locus. Given the possibility of stochastic allele sampling, especially with low quantity and/or degraded samples, single peaks less than approximately 150 RFU are interpreted with caution (pertains to putative genotype assignment at that locus). To maximize the number of loci with peaks above the threshold, different amount of amplification product from one sample may be run on analytical gels. However, any off-scale data at the smaller amplicons are interpreted with caution.

33.8.5 Inhibited Samples:

Inhibited samples may exhibit allele or locus drop out and unbalanced heterozygous alleles.

33.9 Identifiler/Identifiler Plus:

33.9.1 This procedure is a general guideline for the analysis and interpretation of STR profiles when using the Identifiler/Identifiler Plus Kits (ID/IDP). These procedures generally follow those outlined in ABI Prism GeneMapper ID-X Software v1.5 Reference Guide.

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33.9.2 When the Identifiler/Identifiler Plus Kit is employed, the non-expanded, original thirteen core CODIS STR loci (prior to January 1, 2017), two of the expanded core CODIS STR loci (D2S1338 and D19S433), and Amelogenin are typed in a single PCR amplification reaction according to the manufacturer's and laboratory's protocols.

33.9.3 Identifiler/Identifiler Plus amplification products are separated and detected using an ABI 3130xl Genetic Analyzer and the supplied Run 3130xl Data Collection v4.0 and GeneMapper ID-X v1.6 (GMID-X) software.

33.9.4 For the GMID-X software, the Analysis Method is as outlined in the user's manual except for the following: Under the Allele Tab, confirm that the Bin set is for the appropriate kit and that Marker Specific Stutter Ratio is checked for Allele projects and unchecked for STRmix projects. Under the Peak Detector Tab, the Analysis Range is Partial Range but shall include the full range of required size standard peaks, the Sizing Range is Partial Sizes with the Start Size set to 75 for Identifiler/Identifiler Plus and the Stop Size set to at least 450 for ID/IDP. Smoothing is set to Light for all three kits and the Size Calling Method is set to Local Southern Method for ID/IDP. For the Panel, use Identifiler_Plus_Panels_v1 for IDP and Identifiler_CODIS_v1X_Panels for ID. For the Size Standard, use CE_G5_Identifiler_Plus_GS500 for Identifiler Plus.

33.9.5 The analysis and interpretation of STR results are listed below.

33.9.5.1 ID/IDP Analysis:

33.9.5.2 A GMID-X project is created by importing all of the pertinent data files. The standard analysis parameters for forensic unknowns, knowns, ladders, and controls are B, G, Y, R = 50 RFU, O = 150 RFU (IDP-50). Samples originally analyzed with a prior analysis method, (i.e. IDP-75, where B, G, Y, R = 75), will need reanalysis if the comparison shows a positive association to a newly submitted known sample, as well as those comparisons thought to possibly be inconclusive. Please refer to WI-36 for a more detailed explanation on how to reanalyze unknown samples for use with STRmix. Evidentiary profiles (forensic unknowns) with peaks >5000 RFU are interpreted with caution with approval from the DNA TL. With known samples and controls, any peaks > 6500 are interpreted with caution. For known samples, including positive controls, the thresholds for B, G, Y, & R may be raised where appropriate, and the RFU threshold for O may be lowered to 50 RFU. See below:

33.9.5.3 Ladders and any samples with ILS peaks lower than the standard values are interpreted with caution, with the restriction noted in #2.

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33.9.5.4 The minimum analysis parameters for ladder peaks and the internal lane standards are: Ladder: B, G, Y, R, and O > 49 RFU (including 250 peak (and 340 peak for IDP)).

33.9.5.5 With all samples and controls, the ILS peaks must be >49 RFU (including 250 peak (and 340 peak for IDP)).

33.9.5.6 Ensure that all required internal lane size standard peaks are called in all samples (75 – 450 nt, GS500-LIZ; 60 – 400 nt for IDP). If a sample's internal size standard fails due to a low sizing quality (e.g. pull-up peaks into the size standard), the option to override the sample's size quality is available and shall be noted on the analysis worksheet. Both the primary and the second analyst must confirm that the size quality failure was not due to the loss of internal lane size standard peaks. The sample may then be analyzed as normal.

33.9.5.7 Ensure that each ladder in each injection has all allele peaks detected above the minimum threshold. If a ladder does not meet these requirements, change the sample type from allelic ladder to sample and reanalyze set. One ladder in each injection must meet the requirements.

33.9.5.8 GMID-X Analysis-- Control Requirements:

33.9.5.8.1 Import the appropriate GMID-X sample data files including controls (RB, NEG, EP1/RKO, POS) and at least one ladder. Controls for each extraction/amplification set must give the expected results.

33.9.5.8.2 Expected results of controls will be verified and documented on DNA QR-4a which is maintained in the case file.

33.9.5.8.3 Potential contamination is assessed by evaluating the evidentiary samples, RB, NEG, and positive controls. All samples within a batch will be cross-checked against each other to detect potential contamination. This is not necessary for samples reanalyzed for STRmix analysis. Analysts should use their experience and judgment during this process. An excel macro is available to assist with batch cross-comparisons. The appropriate response for a contamination event (including how the sample(s) are reported) is determined on a case-by-case basis with approval of the TL. RB and NEG samples should not have any callable peaks If callable peaks are detected and the evidentiary samples are not affected, the set of extracted samples may be interpreted with caution with TL approval.

33.9.5.8.4 For those samples reanalyzed for STRmix, negative controls associated with those samples must be reassessed for peaks below the analytical threshold, down to 25

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RFU. If peaks in this range are present, and the evidentiary samples are not affected, those samples may be interpreted with caution with TL approval.

33.9.5.8.5 Depending on factors such as the type and extent of contamination, and whether the source of the contamination can be identified, the contaminated sample(s) and/or amplification sets may be interpreted with caution in the report or deemed unsuitable for comparison following TL review and approval.

33.9.5.8.6 See SOP-29 for ID/IDP profiles for the Kit Positive Control DNA 9947A (POS) and the laboratory Extraction Positive Control (EP1/RKO):

33.10 Interpretation of DNA Profiles in GMID-X:

The results are evaluated for each locus. However, considering the data from all loci tested will assist in the overall interpretation of the results for each sample.

33.10.1 Off-Scale Data: If too much DNA is added to the amplification reaction or is loaded onto the 3130, the fluorescent intensity for the PCR products may exceed the linear dynamic range for detection by the instrument. Samples with off-scale peaks may exhibit raised baselines, split peaks, increased minus A peaks, excessive pull-up and/or higher than expected stutter levels, and/or amplification artifacts in one or more of the colors. Examine the peak heights in GMID-X software. Any peaks greater than 5000 RFU may be off-scale.

33.10.2 Peak identification: Only allele peaks with a height of ≥ 50 RFU in GMID-X are reported. The identity of peaks called by GMID-X is generally assigned to one of the following categories:

33.10.3 Allele Peak: A called allele has a peak height (≥50 RFU for those samples reanalyzed for STRmix analysis), a fragment size that falls within the base pair range, and has the appropriate dye color for the locus. However, not all peaks are typed as alleles. Stutter peaks, pull-up/pull-down peaks, background noise, and amplification or injection artifacts could fall above the RFU threshold, especially if the allele peaks are high. Peaks that do not meet the Laboratory's calling criteria/thresholds (< 50 RFU) are not analyzed by STRmix. Peaks that are not called by GMID-X may be called by the analysts on a case-by-case basis with TL approval. Such peaks are interpreted with caution.

33.10.4 Peaks (GMID-X Peaks): Sub analytical threshold (AT) peaks are those less than 50 RFU (25-49 RFU) that have good peak morphology and are clearly distinguishable from background noise and other artifacts. Any sub AT peaks observed are considered only to evaluate the number of contributors to the profile if STRmix analysis is required. Sub AT peaks in the evidentiary samples or

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controls are noted on the appropriate worksheets.. With those samples reanalyzed for use with STRmix analysis software, there are no * peaks, as the calling threshold is 50 RFU. Also, peaks 25-49 RFU are evaluated in the negative controls, and in the evidentiary samples to determine number of contributors.

33.10.5 Detection of variant alleles: Variant alleles have been identified from many STR loci. These alleles may contain full or partial repeats. Variant alleles are labeled as Off Ladder (OL) alleles in GMID-X. If a particular variant has been observed before by the DESPP CT Division of Scientific Services (CTDSS), Applied Biosystems, NIST, or in published literature, the sample does not need to be repeated. In GMID-X, change the OL to the proper allele call.

33.10.6 Stutter peaks (generally N-4 bases, but ID/IDP also has N+4 bases for all STR loci): A stutter peak has a fragment length typically four base pairs smaller than the true allele. If the peak height in the stutter position is less than the user-defined stutter threshold, the peak is considered a stutter peak and is filtered out by the GMID-X program. Any peaks < the CT stutter threshold are manually edited as required (edits recorded on DNA QR-330). CT DSS stutter thresholds are listed in DNA SOP-29. Peaks in the stutter position that are below the Laboratory's stutter thresholds are not listed on the report and are not used for statistical purposes.

33.10.7 Minus A Peaks: AmpF*l*STR kits are optimized to add an extra nucleotide to the 3' end of the PCR product. The nucleotide is predominantly adenosine (A). Lack of a full A addition may be observed especially when the amount of input DNA is greater than approximately 2.5 ng. However, sample degradation can increase the effective concentration of a small amplicon relative to the larger amplicons, leading to minus A product at the smaller loci. Data from other loci may be of assistance in these cases. When appropriate, for known samples, a smaller quantity of sample may be re-amplified.

33.10.8 Pull-up Peaks: Pull-up peaks are generally seen when excess amplification product is injected on the CE or when the spectral calibration is sub-optimal. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-up peaks are often displayed in several colors. Pull-up peaks should have approximately the same fragment size in each of the colors as the true allele peak. Additional information provided by other loci may be of assistance in these cases. When evaluating allelic peaks that may contain a pull-up (PU) contribution, an estimate of the PU contribution is subtracted from the height of the potential allelic peak to determine if the remaining peak height is sufficient for allele or asterisk designation. The estimated pull-up contribution is determined by evaluating the

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height of unambiguous pull-up peaks (pull-up with e.g., no stutter or allelic contribution) at each locus where pull-up is detected for a particular color.

33.10.8.1 Example: The percentage of pull-up peaks as compared to the height of the peaks causing the pull-up is calculated. (PU percentage = PU peak height/height of the peak causing the PU). This percentage is converted to an RFU value (multiply percent by height of peak causing the pull-up) which is then subtracted from the potential allelic peak in question.

33.10.8.2 The resulting "subtracted" peak is called where DNA Section allele calling criteria are met. The same methodology would be applied to potential allelic peaks with a pull-down component (see below).

33.10.9 Pull-down Peaks: Pull-down peaks are generally seen when excess amplification product is run on the CE or when the spectral calibration is sub-optimal for the run. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-down peaks can be displayed in several colors. Pull-down peaks can have approximately the same fragment size, but often the pull-down peak will be slightly a larger or smaller size as compared to the true allele. Additional information provided by other loci may be of assistance in these cases.

33.10.10 Spikes: fluorescent spikes can be seen within GMID-X data. Spikes are typically sharp peaks caused by air bubbles or polymer crystals in a capillary, which can be displayed in different colors, and have approximately the same "fragment size". Additional inspection of the raw data or re-injection may assist in cases where spikes are believed to be present.

33.10.11 Dye Artifacts/Dye Blobs: Broad peaks may be the result of dissociated dyes or fluorescent particles that are trapped within the polymer matrix and then fluorescently excited by the laser. Additional inspection of other samples within the module may assist in interpretation in cases where dye blobs are believed to be present.

33.10.12 Amplification Artifact Peaks: Nonspecific amplification product peaks, generally with good peak morphology, may result when excessive template DNA is amplified. When appropriate, a smaller quantity of sample may be re-amplified.

33.10.13 High Background: Background fluorescence may reach an RFU level above the calling threshold. Generally such high background peaks exhibit atypical peak morphology. When appropriate, re-injection (only of known samples) may assist with interpretation.

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33.10.14 Raised Baseline: Elevated baseline of one color over a broader (multiple bin) scan range may occur. Note: pull-down from two adjacent peaks may cause raised baseline between the pull-down peaks; the "pull-down" designation should be used for the editing notation when raised baseline is caused by pull-down.

33.11 Mixed Samples in Identifiler/Identifiler Plus:

Generally, the detection of more than two alleles per locus indicates a mixed sample. Variation in peak height between alleles in a single locus may assist in the interpretation of such results. Peaks below the 50 RFU threshold for GMID-X (25-49) may assist in the interpretation of mixed DNA samples. The following guidelines pertain to the interpretation of mixtures.

33.11.1 A DNA profile is consistent with being a mixture by the following; (a) the detection of three or more alleles at one or more loci or (b) or as discussed below in 33.11.2 and 33.11.3.

33.11.2 Allelic peak imbalance involving the stutter position, e.g., where the N-4 peak is 15-25% of the N peak, may indicate a mixture. With the possibility of allele sharing among individuals, 3 alleles may not be detected especially with degraded samples or partial profiles.

33.11.3 General allelic peak imbalance: Heterozygous peak imbalance greater than 40% may indicate a mixture, i.e., the smaller peak is less than 60% of the larger peak. However, microvariants in the primer-binding domain may cause either PCR enhancement or suppression.

33.11.4 Per the "SWGDAM Guidelines on STR Interpretation" A Stochastic Threshold is defined as the "value above which it is reasonable to assume that allelic dropout has not occurred within a single-source sample". The below table lists the applicable stochastic thresholds for CT DSS based on 3130xl instruments and injection times.

Injections Time Ranges

Stochastic Threshold	3130-1	3130-2	3130-3	3130-4	3130-6
150 RFU	2 – 18 sec	2 – 10 sec	2 – 10 sec	2 – 10 sec	2 – 10 sec
200 RFU	19 – 28 sec	11 – 16 sec	11 – 16 sec	11 – 16 sec	11 – 16 sec
250 RFU	29 – 35 sec	17 – 20 sec	17 – 20 sec	17 – 20 sec	17 – 20 sec

33.12 CODIS Entries

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Profiles are entered into CODIS as warranted by the results. See DNA SOP-13 CODIS Profile Entry.

33.13 General Interpretation of STR Results: (without STRmix interpretational software)

When a known is submitted to the laboratory for comparison to samples previously amplified with Identifiler/Identifiler Plus, the analyst can visually eliminate that person as the source of, or a contributor to, the questioned DNA sample(s). The following is a general description of the standard DNA report conclusions. Other conclusions may be reported as warranted by the results with approval of the technical leader. (See DNA SOP-6)

33.13.1 Elimination: used when the known sample is not the source of, or a contributor to, the DNA profile detected from item xx. The known sample has alleles that are not detected in the evidentiary sample and the lack of detection is not reasonably explainable by degradation/inhibition/allele dropout (stochastic effects) within the evidentiary sample.

33.13.2 If the previously amplified and analyzed sample only had reported * peaks (peaks between 50 & 74 RFU analyzed when the laboratory's calling and analytical thresholds were 75 RFU) present in a profile, the result can be used for elimination. If those results suggest an inclusion, the sample must be reanalyzed with a calling threshold of 50 RFU.

33.13.3 If the previously amplified and analyzed sample only has results (whether it be * or called) at one locus, the sample can be reported out as an elimination, or an inconclusive result. If unable to eliminate, the comparison to J. Smith is inconclusive.

If the sample is not being reanalyzed for STRmix analysis, a conclusion as to the number of contributors is not given.

33.13.4 Interpretation of single source DNA profiles: The use of "undetermined" in the Report Conclusions Summary table (in the "Type" column): Applies to the detection of male vs female DNA. Used when the analyst is unable to determine whether a profile that appears to be single source is male or female. In this situation, follow the single source interpretation protocol.

33.14 Interpretation of DNA mixtures (without STRmix interpretational software)

These general approaches do not account for all possible mixture scenarios and no two mixtures are exactly the same.

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33.14.1 The analyst will consider the minimum number of persons in the mixture; the minimum number of persons in a mixture is determined by assessing the maximum number of peaks detected at any locus (total number of peaks above the AT). For example, if the maximum number is 5 or 6 peaks, it is indicative of a 3 or more person mixture; 7 or 8 is indicative of a 4 or more person mixture.

33.14.2 The analyst will also consider the quantity of DNA amplified, the peak heights of the alleles, the extent of degradation/inhibition, the impact of stutter (particularly if there is above average stutter [mean + ~1.5 SD for that locus/allele (see Appendix 1)]), potential carryover between fractions in a differential extraction, and if a known profile compared to the mixture can be objectively determined to be a contributor (e.g. intimate samples).

33.14.3 Factors taken into consideration when interpreting mixtures:

33.14.3.1 If one person can be objectively determined to be included in the mixture given an intimate sample origin, the mixture is evaluated by a consideration of what loci have DNA profile results (above the AT) that could not be from the known source.

33.14.3.2 Incomplete separation. In mixtures from samples that were subjected to the differential extraction protocol, the mixture is evaluated by a consideration of whether the results are consistent with incomplete separation from the other fraction (e.g., A vs B fractions).

33.14.3.3 The ratio of male: female DNA and the extent of degradation in sample as determined by Quantifiler Trio and the STR results.

33.14.3.4 Peak height consistency, i.e. the peak heights in the evidentiary profile potentially attributable to the known DNA profile should generally be consistent at all loci applicable.

33.14.3.5 The "zygosity" of the known: homozygous peaks are generally higher than heterozygous peaks of the same contributor. Therefore, homozygous peaks are less likely to dropout as compared to one allele from a heterozygote.

33.14.3.6 While allele dropout (below the stochastic threshold), can occur at any locus, extensive validation and case experience has shown that smaller amplicons amplify more efficiently and are less affected by degradation than are larger amplicons. Therefore, the results at the smaller amplicons can be particularly diagnostic.

33.14.3.7 Mixture DNA profiles that involve relatives are interpreted with caution.

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33.15 Deducing a Clear Major Contributor in Profiles with ≥ 5 Contributors

33.15.1 A clear major contributor may be deduced from Identifiler/Identifiler Plus profiles containing ≥ 5 contributors as outlined below (requires TL & Assistant Director approval). The clear major contributor must be deduced without consideration of any known profile.

1. Where the allele(s) of the major contributor are at least 500 RFU at ≥10 autosomal loci, the heterozygous peak balance of the major profile is ≥ 65% at ≥10 autosomal loci, and the following conditions are met:

A. **Apparent heterozygous major**: the height of the highest minor allele is ≤ 33% of the height of the lowest major allele. If not, the locus does not qualify for statistics.

B. **Apparent homozygous major**: the height of the highest minor allele is ≤ 25% of the height of the major allele. If not, the locus does not qualify for statistics.

2. The allele(s) of the major profile should be called at the remaining loci that do not qualify for statistics.

Notes: The impact of stutter on the height of a minor peak should be addressed by evaluating how much of the peak height would be the result of average stutter from the major allele. Given that the PCR biological model shows continuous peak height variation, exceptions to the binary criteria listed above may be made with TL & Assistant Director approval as warranted.

3. If conditions listed above are met, CODIS entry of the deduced major contributor is permitted with CODIS Administrator/designee approval.

33.15.2 The exported allele table text file will be imported into the Deconvolution Workbook, "CT DSS" is selected as the laboratory, the sample selected, and "5+" selected for contributors. Click "Calculate". If a clear major contributor is present at 10 or more loci, the "Major from 5P+ mixture" button becomes active. Clicking on that button will bring you to DNA-QR-307d Five or More Person Mixture Major Deconvolution.

33.15.3 Print DNA-QR-307d Five or More Person Mixture Major deconvolution, and keep as part of the case record. TL and AD approval will be recorded on this QR. The presence of a clear major profile will be noted on DNA-QR-302 Contributor Estimation Worksheet.

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33.15.4 Statistics for Clear Major Profiles

31.15.4.1 If a known sample is positively associated with a clear major profile, a binary LR (1/RMP) for the major contributor (using the Popstats database with posterior means distribution, NRC 4.2, and a factor of 10 lower bound confidence interval) is calculated. The population with the lowest LR is reported.

31.15.4.2 Repeat 33.14.4.1, and click on "LR for Major". DNA-QR-307h, Likelihood Ratio, is printed out for the case jacket. "Min LR/10" will be reported.

33.16 Minifiler:

Minifiler DNA profiles are analyzed and interpreted as described in Section 33.13 above. See SOP-29 for the Minifiler Kit Positive Control DNA 007 (mPOS) and the laboratory Extraction Positive Control (RKO).

33.17 Fusion 6C Analysis and Interpretation:

33.17.1 PowerPlex Fusion F6C System:

33.17.2 When the F6C Kit is employed, the twenty core CODIS STR loci, plus three additional loci (Penta D, Penta E, SE33), 3 Y-STRs (DYS391, DYS576, DYS570) and Amelogenin are typed in a single PCR amplification reaction according to the manufacturer's and laboratory's protocols.

33.17.3 PowerPlex® Fusion 6C System amplification products are separated, detected, and analyzed using an ABI 3130xl Genetic Analyzer, the supplied 3130xl Data Collection v4.0 and GeneMarker HID STR Software (GM-HID) v.2.9.0.

33.17.4 These procedures generally follow those outlined in the GeneMarker HID (GM-HID) Software User Manual for the version in use.

33.17.5 PowerPlex® Fusion 6C Analysis:

33.17.5.1 Analysis of DNA Profiles in GM-HID

33.17.5.2 The standard analysis parameters for forensic unknowns, knowns, ladders, and controls are B,G,Y,R,P = 50 RFU. Evidentiary profiles (forensic unknowns) with peaks > 4000 RFU, and known samples with peaks > 6000 RFU, at loci other than Amelogenin, are not used unless necessary with TL or Lead approval.

33.17.5.3 The results are evaluated for each locus. However, considering the data from all loci tested will assist in the overall interpretation of the results for each profile.

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33.17.5.4 Overloaded Data: If too much DNA is added to the amplification reaction or is injected into the 3130, the fluorescent intensity for the PCR products may result in various interpretational challenges. Samples with off-scale peaks may exhibit raised baselines, split peaks, increased minus A peaks, excessive pull-up and/or higher than expected stutter levels, and/or amplification artifacts in one or more of the colors. Examine the peak heights in GM-HID software. Peaks greater than 4000 RFU (6000 RFU for knowns and database samples) but not off-scale, may also lead to artifacts peaks being detected ≥ 50 RFU.

33.17.5.5 Peak identification: Only allele peaks with a height of ≥50 RFU in GM-HID are called/reported and evaluated using STRmix™. This is also the laboratory's analytical threshold (50 RFU). Peaks < 50 RFU may be evaluated to address issues such as whether a sample has been contaminated or whether a sample should be re-amped/re-injected, and also in contributor number assessment. The identity of peaks called by GM-HID is generally assigned to one of the following categories:

33.17.5.6 Allele Peak: A called allele has a peak height ≥50 RFU, a fragment size that falls within the base pair range, good peak morphology, and has the appropriate dye color for the locus. However, not all peaks ≥50 RFU are typed as alleles. Stutter peaks, pull-up/pull-down peaks, background noise, and amplification or injection artifacts could fall above the RFU threshold, especially if the allele peaks are high. Peaks that do not meet the Laboratory's calling criteria are not used by STRmix™ with the exception of the N-1 and N+1 stutter peaks that are modeled. Peaks ≥ 50 RFU that are not called by GM-HID may be called by the analysts on a case-by-case basis with TL approval. Such peaks are interpreted with caution.

33.17.5.7 Detection of variant alleles: Variant alleles have been identified for many STR loci. These alleles may contain full or partial repeats. Variant alleles are labeled as Out of Bin (OB) or Off Ladder (OL) alleles in GM-HID.

33.17.5.8 Stutter peaks (N-1, N-0.5, N+0.5, and N+1 repeats, but N-2 is also possible for Fusion 6C data if over-amplified/injected): A stutter peak has a fragment length typically 1 repeat smaller than the true allele (see exceptions above). If the peak height in the stutter position is less than the CT_DSS allele-specific stutter thresholds, the peak is filtered out by the GM-HID program. CT_DSS stutter thresholds are listed in DNA SOP-29. Peaks in the stutter position that are below the Laboratory's stutter thresholds are not listed on the electropherograms or the case report. Note: N-1 and N+1 peaks are evaluated by STRmix™

33.17.5.9 Minus A Peaks: F6C kits are optimized to add an extra nucleotide to the 3' end of the PCR product. The nucleotide is predominantly adenosine (A). Lack of a full A addition may be observed especially when the amount of input DNA generates peaks ≥ 4000 RFU. However, sample degradation can increase the effective

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concentration of a small amplicon relative to the larger amplicons, leading to minus A product at the smaller loci. Data from other loci may be of assistance in these cases. When appropriate, a smaller quantity of sample may be re-amplified.

33.17.5.10 Pull-up Peaks: Pull-up peaks are generally seen when excess amplification product is injected on the CE or when the spectral calibration is sub-optimal. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-up peaks can be displayed in several colors. Pull-up peaks should have approximately the same fragment size in each of the colors as the true allele peak. Additional information provided by other loci may be of assistance in these cases. When evaluating allelic peaks that may contain a pull-up (PU) contribution, an estimate of the PU contribution is subtracted from the height of the potential allelic peak to determine if the remaining peak height is sufficient for allele designation. The estimated pull-up contribution is determined by evaluating the height of unambiguous pull-up peaks (pull-up with e.g., no stutter or allelic contribution) at each locus where pull-up is detected for a particular color.

Example: The percentage of pull-up peaks as compared to the height of the peaks causing the pull-up is calculated. (PU percentage = PU peak height/height of the peak causing the PU). This percentage is converted to an RFU value (multiply percent by height of peak causing the pull-up) which is then subtracted from the potential allelic peak in question. The resulting “subtracted” peak is called where DNA Section allele calling criteria are met. The same methodology would be applied to potential allelic peaks with a pull-down component (see below).

33.17.5.11 Pull-down Peaks: Pull-down peaks are generally seen when excess amplification product is run on the CE or when the spectral calibration is sub-optimal for the run. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-down peaks can be displayed in several colors. Pull-down peaks can have approximately the same fragment size, but often the pull-down peak will be a slightly larger or smaller size as compared to the true allele. Additional information provided by other loci may be of assistance in these cases.

33.17.5.12 Spikes: fluorescent spikes can be seen within GM-HID data. Spikes are typically sharp peaks caused by air bubbles or polymer crystals in a capillary, which can be displayed in different colors, and have approximately the same “fragment size”. Additional inspection of the raw data or re-injection may assist in cases where spikes are believed to be present.

33.17.5.13 Dye Artifacts/Dye Blobs: Broad peaks may be the result of dissociated dyes or fluorescent particles that are trapped within the polymer matrix and then fluorescently excited by the laser. Additional inspection of other samples (especially negative controls) within the module may assist in interpretation in cases where dye blobs are believed to be present (see SOP-29.

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33.17.5.14 Amplification Artifact Peaks: Nonspecific amplification product peaks, generally with good peak morphology, may result when excessive template DNA is amplified.

33.17.5.15 High Background: Background fluorescence may reach an RFU level above the analytical threshold. Generally such high background peaks exhibit atypical peak morphology.

33.17.5.16 Raised Baseline: Elevated baseline of one color over a broader (multiple bin) scan range may occur.

33.17.5.17 Non-specific amplification peaks: With some samples, primers may bind to non-human DNA or human DNA other than the target region, and amplification may occur. Resulting peaks do not exhibit stutter but typically have good morphology otherwise. Such peaks may be observed at characteristic base pair sizes. Most often they fall outside of bins or at fractional repeat numbers. More than one non-specific amplification peak may be seen in the same sample.

33.17.6 Control Requirements

33.17.6.1 For evidentiary sample (forensic unknown) amplifications, all controls (RB, NEG, EP1, POS) for each extraction/amplification set must give the expected results. For known or database samples, only one positive control (EP1 or POS) is required to give the expected results; all other controls must give the expected results.

33.17.6.2 If either the NEG or the RB for a database plate does not inject properly (ex. size standard fails during multiple re-preps/re-injections), results from the plate may be used with TL approval.

33.17.6.3 Expected results of controls will be verified and documented on DNA-QR-4a DNA QA/QC Case Review Checklist which is maintained in the case file.

33.17.6.4 The F6C profiles for the Kit Positive Control DNA 2800M (POS) and the laboratory Extraction Positive Control (EP1) are listed in SOP-29.

33.17.7 Contamination Assessment

33.17.7.1 Potential contamination is assessed by evaluating the evidentiary samples, RB, NEG, and positive controls.

33.17.7.2 RB and NEG samples should not have any callable peaks. If callable peaks are detected, the profile may be used if the evidentiary sample is not affected.

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33.17.7.3 Since peaks between 25 and 49 RFU may be used in contributor number assessment for any evidentiary samples in the set, the potential for contamination at this level that could impact contributor number determination should be assessed using the negative controls.

33.17.7.4 All samples within a batch will be cross-checked against each other to detect potential contamination. Analysts should use their experience and judgment during this process. An excel macro is available to assist with batch cross-comparisons.

33.17.7.5 The appropriate response for a contamination event (including how the sample(s) are reported) is determined on a case-by-case basis with approval of the TL.

33.17.7.6 Depending on factors such as the type and extent of contamination, and whether the source of the contamination can be identified, the contaminated sample(s) and/or amplification sets may be interpreted with caution or deemed unsuitable for comparison following TL review and approval (see Alternate Report Template Statements located within the DNA Report Template).

33.17.8 Documentation of controls and standards on DNA QR-300:

The following key should be used as a guideline to determine how controls and standards should be documented on DNA-QR-300. This list is not all encompassing, and other situations may arise not fully documented here. Any questions on how to document controls and standards on DNA QR-300 should be brought to the TL.

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Did the ILS perform as expected?

Sizing quality score less than 88	No
Not all 21 peaks called*	No
Score 88 or more and all 21 peaks called	Yes
Broad peaks, but score is 88 or more and all 21 peaks are called	Yes

* The 500 peak does need a green arrow in the “Calibration Chart” window. However, it does not need a call in the “All Color Browser”, as the settings only call peaks up to 500 BP in size, and this peak may size at say, 500.2.

Did the negative controls perform as expected?

Called peaks in Neg or RB*	No
Peaks below AT in Neg or RB*	Yes

Broad peaks in WEN, but no called peaks or peaks below AT	Yes

* Need TL initials in comments section showing that no affect to samples

Did the positive controls perform as expected?

Broad peaks, but genotype still correctly called	Yes
Broad peaks causing peaks not to be called	No
Large peak height imbalance, but genotype still correctly called	Yes
Large peak height imbalance causing dropout	No
High peaks, needing reinjection at low, but genotype still correctly called	Yes
Extremely high peaks (i.e. overamplified sample), sample needs re-amplification	No
Low peaks, needing reinjection at MAX, peaks not called	No

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33.17.9 Mixed Samples in F6C:

33.17.9.1 Generally, the detection of more than two alleles per locus indicates a mixed sample. Variation in peak height between alleles in a single locus may assist in the interpretation of such results. Peaks below the 50 RFU threshold for HID may assist in the determining the number of contributors. (see section 33.17.10 for detailed guidance on determining the number of contributors to a DNA profile).

33.17.9.2 A DNA profile is generally consistent with being a mixture when three or more alleles are detected at any locus and as discussed below. Note that three peak patterns (at a single locus) have been observed from single sources, but they are rare.

33.17.9.3 Allelic peak imbalance involving the stutter position, e.g., where the N-1 repeat peak is outside the expected range, may be indicative of a mixture. With the possibility of allele sharing among individuals, 3 alleles may not be detected especially with degraded samples or partial profiles.

33.17.9.4 General allelic peak imbalance: Heterozygous peak imbalance greater than expected for a set of parameters may indicate a mixture. However, microvariants in the primer-binding domain may cause either PCR enhancement or suppression.

33.17.9.5 CT DSS Stochastic thresholds are 150 RFU for Standard and Low injection times and 300 RFU for Maximum injection times. (see below for Low, Standard, and Maximum injection times on each 3130 instrument for DNA samples.)

33.17.9.6 The following injection times were validated for use on specific 3130 instruments that are online for casework:

3130-1	2sec – 35sec (CO/knowns only)
3130-2	5sec, 11sec, 22sec
3130-3	5sec, 10sec, 20sec
3130-4	5sec, 11sec, 22sec
3130-6	5sec, 8sec, 16sec

33.17.9.7 Known and database samples are injected at 3kV for 2-35 seconds. For the 29 cycle Fusion 6C amplification of knowns and database samples using extract from EZ1 normalization, the stochastic threshold is 150 RFU for 3kV5sec or less and 300 RFU for anything greater than 3kV5sec. For the 26 cycle Fusion 6C amplification of knowns and database samples using extract from DNA IQ or Fusion 6C Direct Amplification, the stochastic threshold is 85 RFU for 3kV5sec or less and 125 RFU for anything greater than 3kV5sec. The CT-DSS Knowns panel is used for analysis.

33.17.9.8 Mixtures are deconvoluted for CODIS entry purposes as warranted by the results. See DNA SOP-13.

33.17.10 Determination of Single Source or Mixture Profile & Number of Contributors

Single Source Profile Attributes: A single source profile will generally have no more than 2 alleles at any locus. Expected heterozygote peak balance (HPB) decreases as a function of peak height (see Fig. 1). In rare instances, an individual may exhibit a tri-allelic pattern at a locus. When a suspected tri-allelic pattern is observed but not confirmed by multiple observations in a case, the sample would typically be re-amplified to confirm its presence.

Note: If an electropherogram has only an X at Amelogenin then it is considered a No Result.

When a tri-allelic pattern is noted, the results must be documented in the case file, but the locus is not included in the statistical calculation (see STRmix™ DNA SOP-32).

General Mixture Profile Attributes: Use ‘consistent with a mixture’ for profiles with multiple contributors. A profile with no more than 2 alleles can be best explained as a mixture if there is significant peak height imbalance at one or more loci (see Fig. 1 re: expected heterozygous peak balance vs peak height).

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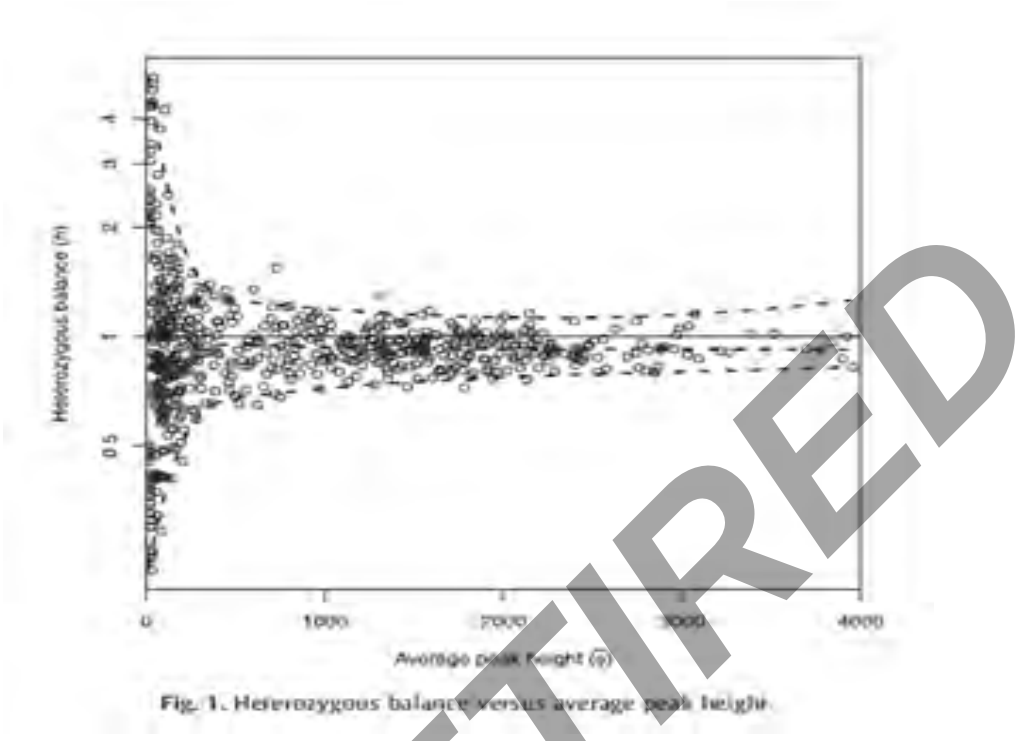

Fig. 1. Heterozygous balance versus average peak height

"Modelling heterozygote balance in forensic DNA profiles"
Kelly et al, FSI Genetics 6 (2012) 729-734

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33.17.10.1 Determination of the number of contributors to the DNA profile

Determination of the number of contributors to a profile begins with assessing the minimum number of contributors from the locus that exhibits the greatest number of allelic peaks. For example, if at most five alleles are observed at a locus, then the DNA results are consistent with having arisen from at least three individuals. Proceed with caution when only one additional allele at a locus would lead to an increased number of possible contributors, as peaks in stutter positions, and the potential for a tri-allele can complicate mixture interpretation. While counting allele peaks is useful in determining a minimum number of contributors, the analyst must also consider that allele sharing between individuals may result in an underestimation of the actual number of contributors. The potential of peaks in stutter positions should be considered. The number of contributors chosen for STRmix™ analysis reflects the analyst's assessment of the most likely number of contributors required to reasonably explain the observed profile.

A determination of the number of contributors to a profile is generally made by the DNA analyst prior to STRmix™ deconvolution and prior to the comparison of

the profile to any reference samples in STRmix™. However, when scientifically warranted, the comparison of an evidentiary profile to a conditioned elimination known might lead the analyst to alter the original number of contributor determination. This change needs to be reviewed by the technical reviewer, and technical lead approval must be obtained prior to STRmix deconvolutions or report writing. A detailed explanation of the reasoning for the change must be documented on DNA QR-308 Changed and/or Not Reported Interpretations. The original DNA QR-302 Contributor Estimate for the sample shall be DNR'd with initials and date of the reassessment.

Sometimes the number of contributors is ambiguous. This could be because the profile is too complex and may contain putative indications of additional contributors. DNA analysts will use their professional judgement to assess the number of contributors, and may evaluate peaks less than 50 RFU that are above the 3130 limit of detection (non-artifact peaks 25-49 RFU with no raised baseline issue) and high stutter as appropriate.

If a profile is generated that contains one called allele but there are peaks between 25-49 RFU that would show evidence of a mixture; the profile will be reported as consistent with being a mixture. The report would state insufficient for STRmix analysis but may be used for comparison (inconclusive or elimination). The peaks between 25-49 RFU shall be used to determine the assumption of the number of contributors.

Note: the limit of detection is the average amount of machine noise plus 3 standard deviations.

In circumstances when the number of contributors cannot be adequately assessed, or when there are greater than 4 unknown/ unconditioned contributors without a clear major contributor being present, the profile is reported as too complex to interpret.

After analysis of the STRmix™ MCMC (Markov Chain Monte Carlo) output, it may be determined that the deconvolution does not conform to scientific expectations and may be re-run under a different number of contributors/iterations. In this event and with TL approval, the analyst's assumptions are documented on DNA QR-308 Changed and/or Not Reported Interpretations, the original STRmix™ printout is DNR'd and kept in the case jacket, and the electronic file of both deconvolutions is retained in the STRmix™ server's casework folder.

33.17.10.2 Method for assigning the number of contributors for a profile

The following steps are followed to assign the likely number of contributors to a profile:

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1. Review the profile as a whole, assessing the level of degradation, presence of low level peaks, noisy or clean baseline and general quality (template) of the profile.
2. Identify likely stutter peaks (both forward and back) by reference to DSS allele stutter ratio (SR) expectations (plots of SR per each Allele).
3. Find the locus with the highest number of unambiguous allelic peaks, A. If A is an odd number, add 1. A/2 gives the initial assessment of the number of contributors to the profile.
4. Review peak height imbalances at the most informative locus (greatest number of alleles). Taking into account potential allele sharing among contributors, visually attempt to 'pair' alleles and assign them to contributors. If there is too much imbalance between alleles, this may mean the presence of an additional contributor(s) above that indicated by allele count alone. Refer to figure 1 for an indication of expected heterozygote balance at varying peak heights.
5. If one or more contributors at this locus are either minor or a clear major, check that this pattern is represented at other loci.
6. Apply the general pattern of contributors (number and proportion) to other loci in the profile. If it holds, assign this number of contributors to the profile; otherwise consider the addition or subtraction of one.
7. Analysts may utilize the Contributor Estimator Macro to assist in determining the number of contributors to a profile. (See WI-34 Step 16 under "Analyst".)

Notes

1. Discriminating loci such as FGA and SE33 are particularly informative for determining the number of likely contributors to a profile; however important information can be gleaned at any locus.
2. The presence of one or two minor peaks can sometimes be indicative of drop-in and not a true additional contributor.
3. Non-artifact peaks below 50 RFU, but above the limit of detection, may be useful to identify the presence of low level/minor additional contributors where genotype assignment of contributor(s) by STRmix would be impacted. Peaks < 50 RFU are not used for NOC determination where there is a reasonable expectation that all genotypes associated with the contributor(s) of the reportable alleles can be identified by STRmix, i.e., reported alleles for contributors are all above the ST and genotype assignment is unambiguous. When peaks <50 RFU are used to infer an extra contributor there should be data elsewhere in the profile to support this. The effects of the mis-

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assignment of the number of contributors are typically restricted to false exclusions of true contributors and false inconclusive LRs.

33.17.11 Highly Degraded/Low Template Samples:

33.17.11.1 Highly degraded/low template samples are interpreted with caution. Degraded samples may "appear" to exhibit high stutter and greater –A at the smaller amplicons with off-scale data. In addition, degraded/low template samples may exhibit unbalanced heterozygous alleles and locus/allele dropout. Partial profiles are interpreted as determined by the data present for each locus. Given the possibility of stochastic effects, especially with low quantity and/or degraded samples, results from low template amplifications are interpreted with caution. Note that stochastics effects can be different for each contributor to a mixture based on the quantity/quality of the template.

33.17.12 Inhibited Samples:

33.17.12.1 Inhibited samples may exhibit allele, locus, or complete profile dropout, unbalanced heterozygous alleles, and inter-locus peak imbalance. To possibly overcome an inhibitor, smaller quantities of sample may be re-amplified. Quantitation data may aid in determining whether re-amplification is warranted as well as the amount of sample to be re-amplified. Before the genotyping amplification is performed, an evaluation of the Quantifiler Trio IPC for each sample is useful. The Quantifiler Trio IPC result for a sample being undetected or having an elevated C_T may indicate that a sample has an inhibitor present.

33.17.13 Qualitative Interpretation of STR Results: The following is a general description of the standard DNA report conclusions. Other conclusions may be reported as warranted by the results with approval of the technical leader (see DNA SOP-6.

33.17.13.1 Elimination: Conclusion reported in two situations: (1) When it has been determined by manual comparison that the known sample is not the source of, or a contributor to, the DNA profile detected from item #xx. The known sample has alleles that are not detected in the evidentiary sample and the lack of detection is not reasonably explainable by degradation/inhibition/allele dropout (stochastic effects) within the evidentiary profile. (2) When the LR is less than 1 by STRmix™ analysis.

33.17.13.2 Inconclusive: No conclusion can be drawn from the comparison between the known sample and the evidentiary sample due to uncertainty. There are 2 reasons for inconclusive results:

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33.17.13.2.1 The likelihood ratio obtained to a STRmix deconvolution is greater than or equal to 1 but less than 10,000.

33.17.13.2.2 MCMC secondary diagnostics did not perform as expected: "the MCMC deconvolution process did not meet scientific expectations; therefore the comparison to Known A is inconclusive."

Note: If a known individual can be manually eliminated as a source of/contributor to a profile that has sub-par MCMC secondary diagnostics, this elimination is reported.

33.17.13.3 Positive Associations

33.17.13.3.1 Cannot be eliminated (CBE): An individual cannot be eliminated (CBE) as the source of (or a contributor to) a DNA profile if there is a strong positive association between the known sample and the evidentiary sample (see calculation of likelihood ratios) but some of the alleles present in the known sample are not detected in the evidentiary sample above the analytical threshold. In this instance, STRmix considers the probability of dropout taking into consideration peak height data, overall quantity of template DNA, information regarding the extent of degradation, inhibition, stochastic effects, and potential masking by stutter or contributor ratio. An LR ≥ 10,000 is obtained using STRmix.

33.17.13.3.2 Consistent with Source: An individual is consistent with being the source of a single-source DNA profile if all alleles consistent with the known sample are detected (≥50 RFU) in the evidentiary profile at all loci where results are generated (see report templates). An LR ≥ 10,000 is obtained using STRmix.

33.17.13.3.3 Inclusion: An individual is included as a potential contributor to a mixture DNA profile if all alleles consistent with the known sample are detected (≥ 50 RFU) in the evidentiary profile at all loci where results are generated (see SOP-6. An LR ≥ 10,000 is obtained using STRmix.

33.17.13.4 No STRmix Interpretation: STRmix is unable to be run to obtain a likelihood ratio to a deconvolution from a questioned sample. There are 2 reasons for this:

33.17.13.4.1 The mixture too complex. With > 4 unknown (unconditioned) contributors, or if the analyst is unable to determine the number of contributors with confidence. An exception to this is when a mixture has a clear major contributor (SOP-33.17.18).

33.17.13.4.2 Insufficient data is obtained for the questioned DNA Profile. This determination is made for low-level, partial DNA profiles when there is insufficient data from

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the evidentiary DNA profile for comparison to a particular known using STRmix™. DNA profiles with only one resulting allele are unable to be run through STRmix. This includes a low level contributor(s) associated with a clear major contributor. With TL approval, other low level profiles can be deemed insufficient for comparison to a reference sample without being run through STRmix. If this determination is made after a STRmix likelihood ratio has already been calculated, document the non-reporting of the likelihood ratio on DNA QR-308 Changed and/or Not Reported Interpretations. The STRmix electronic files shall be maintained in the case folder, and the STRmix printouts shall be DNR'd, and remain in the case jacket.

Note: If a known individual can be manually eliminated as a source of/contributor to a profile insufficient for STRmix™ interpretation, this elimination is reported.

33.17.14 Interpretation of single source DNA profiles

33.17.14.1 For single source partial profiles with peaks ≥ 50 RFU, report a positive association with potential dropout when the observed allele(s) are consistent with the known source and the LR ≥ 10,000.

33.17.14.2 Note: for low-level profiles in situations where no loci have more than 2 peaks above the AT [e.g. such as if there is unexpected "heterozygous" peak imbalance], it is important to consider if the profile could be a partial composite of 2 or more individuals.

33.17.14.3 Note: Report "undetermined" in the "Type" column of the Testing or Conclusions Summary table when the analyst is unable to determine whether a single source profile is male or female if the Amelogenin X peak is below the stochastic threshold and no Y peaks are detected. For single source profiles where the individual is assumed, the "Type" would be reported as "Male" or "Female" in the Conclusions Summary table as appropriate (peaks above or below the stochastic threshold).

33.17.15 Interpretation of DNA mixtures

33.17.15.1 The analyst considers the quantity of DNA amplified, the peak heights of the alleles, the extent of degradation/inhibition, the impact of stutter, potential carryover between fractions in a differential extraction, and if a known profile compared to the mixture can be objectively determined to be a contributor such as for intimate samples.

33.17.15.2 If one person can be objectively determined to be included in the mixture given an intimate sample origin, the mixture may be evaluated by a consideration of what

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loci have DNA profile results (above the AT) that could not be from the known source.

33.17.15.3 Incomplete separation: In mixtures from samples that were subjected to the differential extraction protocol, the mixture is evaluated by a consideration of whether the results are consistent with incomplete separation from the other fraction (e.g., A vs B fractions).

33.17.15.4 The ratio of male:female DNA and the extent of degradation in a sample as determined by Quantifiler Trio and the STR results.

33.17.15.5 Peak height consistency, i.e. the peak heights in the evidentiary profile potentially attributable to the known DNA profile should generally be consistent at all loci applicable. If not, this may not optimally fit the CBE conclusion (note: this may not apply to degraded profiles).

33.17.15.6 The "zygosity" of the known: homozygous peaks are generally higher than heterozygous peaks of the same contributor. Therefore, homozygous peaks are less likely to dropout as compared to one allele from a heterozygote.

33.17.15.7 The total number of dropouts (overall and at each locus) that would be required to still be consistent with a CBE conclusion: While allele dropout (below the stochastic threshold), can occur at any locus, extensive validation and case experience has shown that smaller amplicons amplify more efficiently and are less affected by degradation than are larger amplicons. Therefore, the results at the smaller amplicons can be particularly diagnostic.

33.17.15.8 To what degree (at how many loci) would a putative low-level contributor coincidently be included by a high-level contributor due to overlap? This is particularly important to consider with mixtures involving relatives.

33.17.15.9 Mixture DNA profiles that involve relatives are interpreted with caution.

33.17.16 Statistics – Likelihood ratios

Determining hypotheses for calculating the likelihood ratio (LR)

An LR is calculated using STRmix™ Analysis software for forensic unknown DNA profiles as discussed in SOP-39 (see also STRmix™ SOP-32).

33.17.17 Reporting

See DNA SOP-6 for a general discussion regarding reporting profile results.

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33.17.17.1 Likelihood Ratio

The 99% 1-sided lower HPD (highest posterior density) LR value is reported for F6C DNA profiles using STRmix™ for the population with the lowest LR. Exceptions can be made if the scenario indicates an alternate LR is more appropriate. Likelihood ratios are rounded down when reported as listed in the table below:

<1	Elimination, no LR reported
1-9,999	Comparison inconclusive, no LR reported
10,000 – 100 billion	LR rounded down—use up to two significant figures
>100 billion	Ceiling—report LR is at least 100 billion

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33.17.17.2 Reporting Comparisons using STRmix™ (see DNA SOP-6 for Report Templates)

33.17.17.2.1 LR obtained is less than 1:

The likelihood of obtaining the profile is greater under H_2 than H_1); reported as an exclusion of the individual considered in H_1.

Note that reference samples may also be eliminated through manual comparison to the questioned sample.

33.17.17.2.2 LR obtained is ≥ 1 but less than 10,000; the comparison is inconclusive.

33.17.17.2.3 LR obtained is ≥ 10,000; the comparison is a positive association.

33.17.17.2.4 For samples that are identical to one another, there is no need to run STRmix again.

33.17.18 **5+ Person Mixtures with a Clear Major Profile**

33.17.18.1 During analysis and number of contributor estimation, an analyst can determine (with TL and AD approval) that a 5 or more person mixture has a clear major contributor. The following criteria for this must be met at at least 10 loci. If any of these criteria are not met, the locus does not qualify for statistics:

33.17.18.1.1 All of the alleles of the major contributor are at least 500 RFU.

33.17.18.1.2 The heterozygous peak balance of the major profile ≥ 65%

33.17.18.1.3 For an apparent heterozygous major, the height of the highest minor allele is ≤ 33% of the height of the lowest major allele

33.17.18.1.4 For an apparent homozygous major, the height of the highest minor allele is ≤ 25% of the height of the major allele

33.17.18.2 After determining the number of contributors to be 5 or greater, the analyst will determine if a clear major profile is present.

33.17.18.3 The exported allele table text file will be imported into the Fusion 6C Deconvolution Workbook, "CT DSS" is selected as the laboratory, the sample selected, and "5+" selected for contributors. Click "Calculate". If a clear major contributor is present at 10 or more loci, the "Major from 5P+ mixture" button becomes active. Clicking on that button will bring you to DNA-QR-307d Five or More Person Mixture Major Deconvolution.

33.17.18.4 Print DNA-QR-307d Five or More Person Mixture Major deconvolution, and keep a part of the case record. TL and DD approval will be recorded on this QR. The presence of a clear major profile will be noted on DNA-QR-302 Contributor Estimation Worksheet.

33.17.18.5 Qualitative comparisons to known profiles: Known profiles are compared to the clear major profile as described in 33.17.13.

33.17.18.5.1 The alleles of the major profile should typically be called at the remaining loci that do not qualify for statistics. Note: this is not checked by the deconvolution macro.

33.17.18.6 Statistics for Clear Major Profiles:

33.17.18.6.1 If a known sample is positively associated with a clear major profile, a binary LR (1/RMP) for the major contributor (using the Popstats database with posterior means distribution, NRC 4.2, and a factor of 10 lower bound confidence interval) is calculated. The population with the lowest LR is reported.

33.17.18.6.2 Repeat 33.15.2.1, and click on "LR for Major". DNA-QR-307h, Likelihood Ratio, is printed out for the case jacket. "Min LR/10" will be reported.

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33.17.18.6.3 Since statistics are able to be calculated, profiles that have a clear major are eligible for CODIS, with CODIS Administrator approval.

33.17.18.7 Reporting Out Complex Mixtures with Clear Majors

33.17.18.7.1 Reports without comparisons to knowns: Major profile will not be included in the Testing Summary table, or the Appendix. Remarks section will not discuss complexity of this mixture.

33.17.18.7.2 Reports with comparisons to knowns: Both profile and major profile will be included in Testing Summary table. If there is a known consistent with the source of the major profile, the comparison to the full profile is N/A. If there is a known eliminated as the source of the major profile, the Testing Summary table will note "No Comparison" to the full profile.

33.17.18.7.3 5+ person mixture wording: see DNA SOP-6.

33.17.19 Criminal Parentage Testing:

For criminal parentage testing, standard statistical methods for autosomal STR results will be used as described below:

33.17.19.1 The statistic calculated (for inclusions) is the expected frequency (parentage inclusion probability) of individuals who could contribute the paternally (or maternally) transmitted alleles. The formula used is: $p^2 + 2p(1-p) = 2p-p^2$, where p is the frequency of the obligatory allele.

33.17.19.2 No statistics are required for non-matches. Due to the occurrence of mutations between generations, an individual must be excluded at more than two loci in order to be eliminated as a potential parent. Regarding apparent mutations using STR systems, the repeat # difference of the putative mutation (child's obligatory allele vs. alleged parent's alleles typically varies by +/- 1 repeat) may be relevant to the final conclusion. In the event of apparent mutations, additional testing may be conducted as appropriate.

33.17.19.3 Statistics statement for paternity (RMNE): "The expected frequency of individuals who could be the father of NAME is less than 1 in xx in the general male population" (using the population with the most common match probability). The RMNE ceiling that is reported for Fusion 6C criminal parentage testing is 1 in 100 billion.

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33.17.9.4 In the event that the evidentiary sample is a maternal/child mixture, the fetal/child component is deconvoluted as described in DNA SOP-21using the CT DSS analytical and stochastic thresholds.

33.18 Yfiler Analysis:

33.18.1 This procedure is a general guideline for the analysis and interpretation of Y-STR profiles when using the Yfiler (YF) STR kits. These procedures generally follow those outlined in ABI Prism GeneMapper ID-X Software v1.5 Reference Guide.

33.18.2 When the Yfiler kit is employed, seventeen Y-STR loci; DYS391, DYS389I, DYS439, DYS389II, DYS438 (5 base repeat), DYS437, DYS19, DYS392 (3 base repeat), DYS393, DYS390, DYS385 a/b (2 loci amped with one set of primers), DYS456, DYS458, DYS635, Y-GATA H4, and DYS448 (6 base repeat) are typed in a single PCR amplification reaction according to manufacturer's and laboratory protocols.

33.18.3 Yfiler amplification products are separated and detected using an ABI 3130xl Genetic Analyzer and the supplied Run 3130xl Data Collection v4.0 and GeneMapper ID-X v1.6 (GMID-X) softwares.

33.18.4 For the GMID-X software for YF, the Analysis Method is as outlined in the user's manual except for the following: Under the Allele Tab, confirm that the Bin set is for the appropriate kit and that Marker Specific Stutter Ratio is used. Note: The Yfiler Kit contains one locus that is a 3 base repeat, one locus that is a 5 base repeat, and one locus that is a 6 base repeat, so there are some extra parameter values needed for the Analysis Method under the Allele Tab, Marker Repeat Type. Under the Peak Detector Tab, the Peak Detection Algorithm is Advanced, the Analysis Range is Partial Range but shall include the full range of required size standard peaks, the Sizing Range is Partial Sizes with the Start Size set to 75 and the Stop Size set to at least 400. Smoothing is set to Light and the Size Calling Method is set to Local Southern Method. For the Panel, use Yfiler_v2. For the Size Standard, use CE_G5_HID_GS500. In panel manager, confirm that the marker specific stutter thresholds match those listed in DNA SOP-29 for the Yfiler amplification kit.

33.18.5 Analysis of Y-STR DNA Profiles using GMID-X:

33.18.5.1 The results are evaluated for each locus. However, considering the data from all loci tested will assist in the overall interpretation of the results for each sample. See DNA SOP-6 for standard report conclusions.

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33.18.5.2 Off-Scale Data: If too much DNA is added to the amplification reaction or is loaded onto the 3130, the fluorescent intensity for the PCR products may exceed the linear dynamic range for detection by the instrument. Samples with off-scale peaks may exhibit raised baselines, split peaks, increased minus A peaks, excessive pull-up and/or higher than expected stutter levels, and/or amplification artifacts in one or more of the colors. Examine the peak heights in GMID-X software. Peaks greater than 5000 RFU may be off-scale.

33.18.5.3 Peak identification: The laboratory's analytical threshold (AT) is 50 RFU. The identity of peaks called by GMID-X is generally assigned to one of the following categories:

33.18.5.4 Allele Peak: A called allele has a peak height ≥ 50 RFU, a fragment size that falls within the base pair range, and has the appropriate dye color for the locus. However, not all peaks ≥ 50 RFU are typed as alleles. Stutter peaks, pull-up/pull-down peaks, background noise, and amplification or injection artifacts could fall above the RFU threshold, especially if the allele peaks are high. Peaks ≥ 50 RFU that are not called by GMID-X may be called by the analysts on a case-by-case basis. Such peaks are interpreted with caution.

33.18.5.5 Detection of variant alleles: Variant alleles have been identified from many STR loci. These alleles may contain full or partial repeats. Variant alleles are labeled as Off Ladder (OL) alleles in GMID-X. If a particular variant has been observed before by the DESPP CT Division of Scientific Services (CTDSS), ThermoFisher (Life Technologies), NIST, or in published literature, the sample does not need to be repeated. In GMID-X, change the OL to the proper allele call.

33.18.5.6 Stutter peaks (N-2, N-3, N-4 N-5, N-6, N+2, and N+3—depending on the locus): A stutter peak has a fragment length typically four base pairs smaller than the true allele (see exceptions above). If the peak height in the stutter position is less than the user-defined stutter threshold, the peak is considered a stutter peak and is filtered out using the GMID-X program. Any peaks < the CT stutter threshold are manually edited (edits recorded on DNA QR-330) as required. (see DNA SOP-29. Peaks in the stutter position that are below the Laboratory's stutter thresholds are not listed on the report and are not used for statistical purposes. If the height of the peak in the stutter position is greater than the CT stutter thresholds, this indicates a possible mixture, an overloaded sample, or a higher than normal stutter ratio. Additional information provided by other loci may be of assistance in these cases.

33.18.5.7 Minus A Peaks: AmpF*l*STR kits are optimized to add an extra nucleotide to the 3' end of the PCR product. The nucleotide is predominantly adenosine (A). Lack of a full A addition may be observed especially when the amount of input DNA is

greater than approximately 2.5 ng. However, sample degradation can increase the effective concentration of a small amplicon relative to the larger amplicons, leading to minus A product at the smaller loci. Data from other loci may be of assistance in these cases.

33.18.5.8 Pull-up Peaks: Pull-up peaks are generally seen when excess amplification product is injected on the CE or when the spectral calibration is sub-optimal. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-up peaks are often displayed in several colors. Pull-up peaks should have approximately the same fragment size in each of the colors as the true allele peak. Additional information provided by other loci may be of assistance in these cases. When evaluating allelic peaks that may contain a pull-up (PU) contribution, an estimate of the PU contribution is subtracted from the height of the potential allelic peak to determine if the remaining peak height is sufficient for allele or asterisk designation. The estimated pull-up contribution is determined by evaluating the height of unambiguous pull-up peaks (pull-up with e.g., no stutter or allelic contribution) at each locus where pull-up is detected for a particular color.

Example: The percentage of pull-up peaks as compared to the height of the peaks causing the pull-up is calculated. (PU percentage = PU peak height/height of the peak causing the PU). This percentage is converted to an RFU value (multiply percent by height of peak causing the pull-up) which is then subtracted from the potential allelic peak in question. The resulting "subtracted" peak is called where DNA Section allele calling criteria are met. The same methodology would be applied to potential allelic peaks with a pull-down component (see below).

33.18.5.9 Pull-down Peaks: Pull-down peaks are generally seen when excess amplification product is run on the CE or when the spectral calibration is sub-optimal for the run. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-down peaks can be displayed in several colors. Pull-down peaks can have approximately the same fragment size, but often the pull-down peak will be slightly a larger or smaller size as compared to the true allele. Additional information provided by other loci may be of assistance in these cases.

33.18.5.10 Spikes: fluorescent spikes can be seen within GMID-X data. Spikes are typically sharp peaks caused by air bubbles or polymer crystals in a capillary, which can be displayed in different colors, and have approximately the same "fragment size". Additional inspection of the raw data or re-injection may assist in cases where spikes are believed to be present.

33.18.5.11 Dye Artifacts/Dye Blobs: Broad peaks may be the result of dissociated dyes or fluorescent particles that are trapped within the polymer matrix and then fluorescently excited by the laser. Additional inspection of other samples within

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the module may assist in interpretation in cases where dye blobs are believed to be present.

33.18.5.12 Amplification Artifact Peaks: Nonspecific amplification product peaks, generally with good peak morphology, may result when excessive template DNA is amplified.

33.18.5.13 High Background: Background fluorescence may reach an RFU level above the calling threshold. Generally such high background peaks exhibit atypical peak morphology.

33.18.5.14 Raised Baseline: Elevated baseline of one color over a broader (multiple bin) scan range may occur. Note: pull-down from two adjacent peaks may cause raised baseline between the pull-down peaks; the "pull-down" designation should be used for the editing notation when raised baseline is caused by pull-down.

33.18.6 Highly Degraded/Low Template Samples:

Highly degraded/low template samples are interpreted with caution. Degraded samples may "appear" to exhibit high stutter and greater –A at the smaller amplicons with off-scale data. In addition, degraded/low template samples may display unbalanced heterozygous alleles and locus/allele dropout. Partial profiles may be interpreted as determined by the data present for each locus. Given the possibility of stochastic allele sampling, especially with low quantity and/or degraded samples, single peaks (at DYS385) less than the stochastic threshold are interpreted with caution (pertains to putative haplotype assignment at that locus).

33.18.7 Inhibited Samples:

33.18.7.1 Inhibited samples may exhibit allele, locus, or complete profile dropout, unbalanced alleles at DYS385, and interlocus peak imbalance.

33.18.7.2 Input of excessive female DNA may result in inhibition of Y-STR amplification.

33.18.8 X-homologous peaks:

Rarely, (especially with large quantities of female DNA) the Y primers may generate X-homologous or non-specific peaks. If the known female sample is amplified, any X-homologous peaks may be removed from the evidentiary DNA profile.

33.18.9 General Analysis of Yfiler Results

33.18.9.1 The standard analysis parameters for samples and controls are B,G,Y,R, O = 50 RFU. Profiles (forensic unknowns) with peaks >3000 RFU are interpreted with

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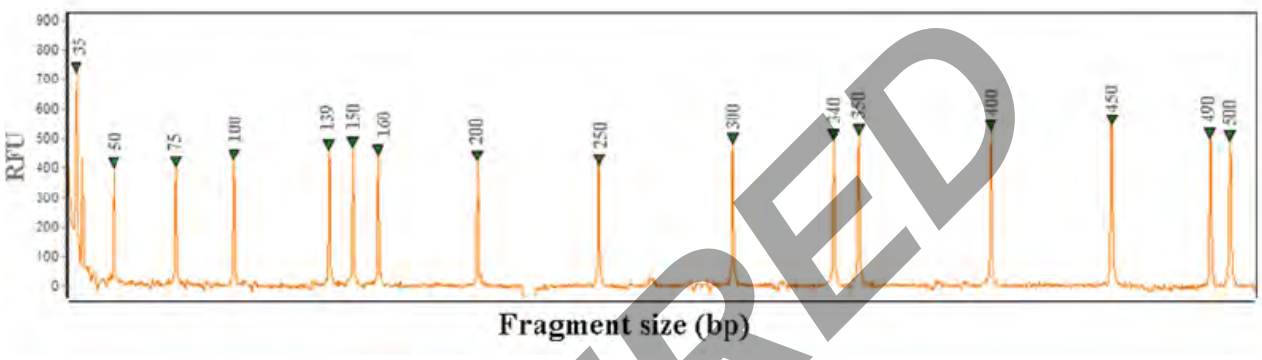
caution with approval of the TL. Known profiles > 5000 RFU are interpreted with caution with approval of the TL.

33.18.9.2 Ensure that all required internal lane size standard (75-400) peaks are called in all samples.



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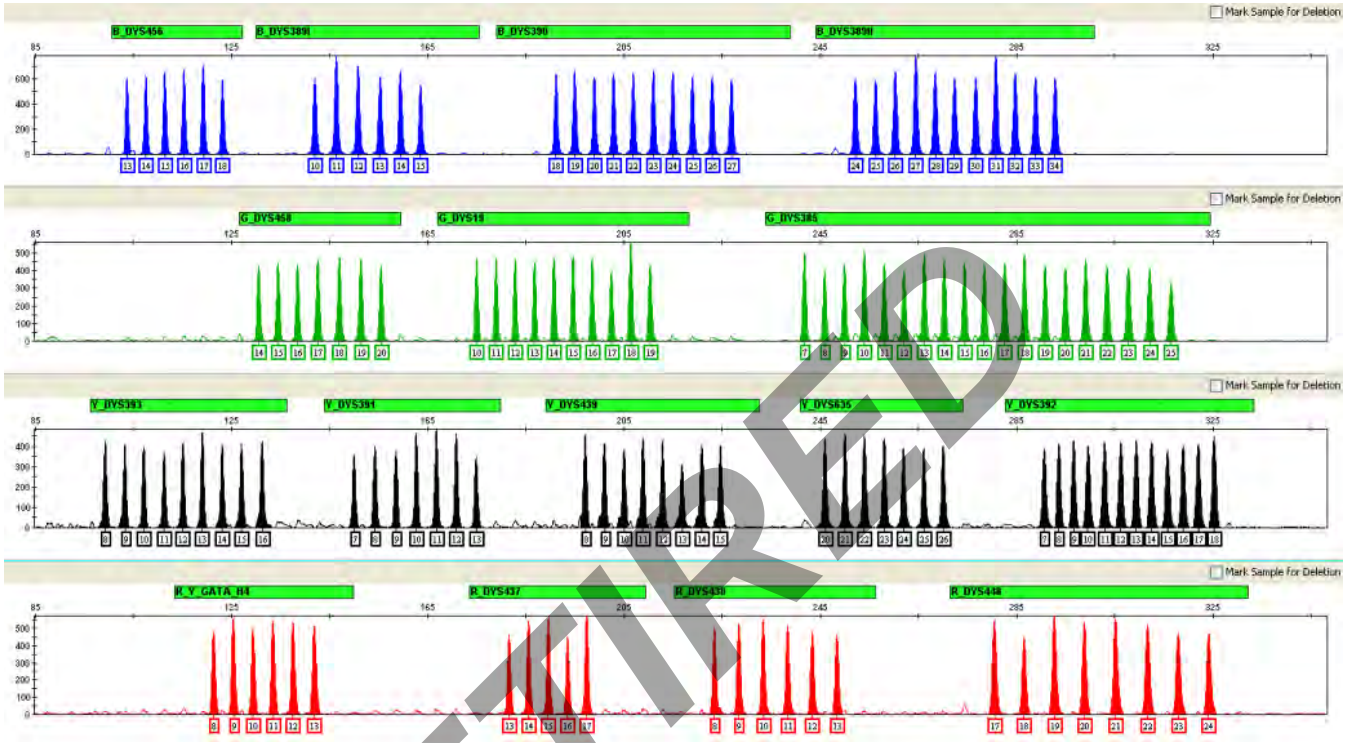
33.18.9.3 If a sample's internal size standard fails due to a low sizing quality (e.g. pull-up peaks into the size standard), the option to override the sample's size quality is available and shall be noted on the analysis worksheet. Both the primary and the second analyst must confirm that the size quality failure was not due to the loss of internal lane size standard peaks. The sample may then be analyzed as normal.

33.18.9.4 Ensure that each ladder in each injection has all allele peaks detected above the minimum threshold. If a ladder does not meet these requirements, change the sample type from allelic ladder to sample. One ladder in each injection must meet the requirements.

33.18.9.5 The Yfiler Allelic Ladder should appear as follows:



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33.18.10 Y-STR Control Requirements:

33.18.10.1 Import the appropriate sample files including controls (RB, NEG, RKO/EP1*, POS control DNA 007 for YF) and at least one ladder. At least one set of controls (for each amplification set) must give the expected results (see DNA SOP-29).

*With extraction sets where a male laboratory control (RKO/EP1) is not available, using the YF kit positive control is sufficient.

33.18.10.2 Potential contamination is assessed by evaluating the evidentiary samples, RB, NEG, and positive controls. All samples within a batch will be cross-checked against each other to detect potential contamination. An excel macro is available to assist with batch cross-comparisons.

RB and NEG samples should not have any callable peaks. If callable peaks are detected and the evidentiary samples are not affected, the set of extracted samples may be interpreted with caution with TL approval. If sub AT peaks are detected and the evidentiary samples are not affected, the set of extracted samples may be interpreted with caution.

If contamination is detected, depending on factors such as the type and extent of contamination and whether the source of the contamination can be identified, the contaminated sample(s) and/or amplification sets may be interpreted with caution in the report or deemed unsuitable for comparison following TL review and approval. Contaminated profiles that are deemed unsuitable for comparisons are reported out as such.

33.18.10.3 Positive Control Profiles: Ensure that all alleles were included.

33.18.10.4 If a peak is labelled as off ladder (OL) or is outside the ladder region and therefore not labelled by the GMID-X software, review the data to determine that it is a true microvariant or off-ladder allele.

33.18.10.5 The analytical threshold is 50 RFU and the stochastic threshold (ST) is 150-250 RFU for DYS385a/b (see table below).

33.18.10.6 For Yfiler, the stochastic threshold is the peak height above which one can expect that both peaks at DYS385a/b would be observed.

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Injections Time Ranges

Stochastic Threshold	3130-1	3130-2	3130-3	3130-4	3130-6
150 RFU	2 – 18 sec	2 – 11 sec	2 – 10 sec	2 – 11 sec	2 – 8 sec
200 RFU	19 – 29 sec	12 – 18 sec	11 – 16 sec	12 – 18 sec	9 – 13 sec
250 RFU	30 – 35 sec	19 – 22 sec	17 – 20 sec	19 – 22 sec	14 – 16 sec

33.18.11 Determination of Single Source or Mixture Profile & Number of Contributors

Single Source Profile Attributes: A single source profile will generally have no more than 1 allele at any locus except DYS385a/b (typically 2 alleles). Expected peak balance at DYS385a/b decreases as a function of peak height similar to that seen with autosomal loci. In rare instances, an individual may exhibit a bi/tri-allelic pattern at a locus.

When an extra-allelic pattern is identified, the results must be documented in the case file. The locus is able to be searched in the YHRD database.

33.18.11.1 Determination of the number of contributors (different Y lineages) to the DNA profile.

33.18.11.1.1 Evidence profiles are assessed for the number of possible contributors. Data above/below the Stochastic Threshold at the DYS385a/b locus, the amount of visible drop out (peaks observed below analytical threshold but clearly discernable from noise (25-49 RFU), peak heights of called alleles, etc., are evaluated when making this determination as discussed below:

The following steps are followed to assign the likely number of lineages in the profile:

1. Review the profile as a whole, assessing the level of degradation, presence of low level peaks, noisy or clean baseline and general quality (template) of the profile.
2. Identify likely stutter peaks by reference to DSS allele stutter ratio (SR) expectations (plots of SR per each allele).
3. Find the locus with the highest number of unambiguous allelic peaks, A. A gives the initial assessment of the likely number of contributors to the profile.
4. Review peak height balance at DYS385.

The detection of more than one allele per locus (other than the DYS385 locus) is generally indicative of a mixed sample. Variation in peak heights between alleles between loci and at DYS385 may assist in the interpretation of such results. Typically, if a maximum of 2 alleles are observed at a locus (except for DYS385), the DNA results are consistent with a mixture of two male lineages. Two peak patterns (at a single locus other than DYS385) have been observed from single source samples, but they are rare. The analyst should consider the possibility of high stutter peaks, which can complicate interpretation. While counting allele peaks is critical in determining the number of contributors, the analyst must also consider that allele sharing between different haplotype lineages may result in an underestimation of the actual number of contributors. The number of lineages identified reflects the analyst's assessment of the most likely number of different haplotypes required to reasonably explain the observed profile.

A Y-STR mixture simulation tool has been validated by the Division of Scientific Services to assist in the determination of the number of haplotypes that are present in Yfiler profiles. This tool may be used by analysts to demonstrate the expected amount of allele sharing at each locus per contributor number in the absence of dropout.

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Note: Non-artifact peaks below 50 RFU (25-49 RFU) are evaluated when assessing the number of contributors to the profile.

33.18.12 Qualitative Assessment of Yfiler Profiles—Comparisons to Knowns

33.18.12.1 Single source haplotypes. If 1 peak was called ≥ 50 RFU) (except at DYS385) with nothing else detected above 25 RFU at any locus), and "Known A" has that allele, the results would be consistent with "Known A" being the source. One would consider the issue of "heterozygous" allelic peak imbalance at DYS385. Therefore, with good peak balance: the results would be consistent with "Known A" being the source of the DNA profile for item #xx. Significant peak imbalance (> 50%) at DYS385, may be indicative of a mixture.

33.18.12.2 For single source partial profiles with peaks ≥ 50 RFU, report a CBE interpretation when partial results are detected at DYS385, as long as the observed alleles are consistent with the known source.

Note: in situations where no loci have more than 1 peak above the AT (except 2 peaks at DYS385 [e.g. such as if there is significant peak imbalance (> 50%)], it is important to consider if the profile could be a mixture (partial composite of 2 or more individuals).

33.18.13 Interpretation of DNA mixtures

33.18.13.1 The analyst considers the quantity of DNA amplified, the peak heights of the alleles, the extent of degradation/inhibition, the impact of stutter, potential carryover between fractions in a differential extraction, and if a known profile compared to the mixture can be objectively determined to be a contributor (e.g. intimate samples).

33.18.13.2 Factors taken into consideration when interpreting mixtures:

33.18.13.2.1 If one person can be objectively determined to be included in the mixture (e.g. consensual partner), the mixture is evaluated by a consideration of what loci have DNA profile results (above the AT) that could not be from the known source.

33.18.13.2.2 The total number of dropouts (overall and at each locus) that would be required to still be consistent with a CBE conclusion. While allele dropout (including peaks below the stochastic threshold at DYS385), can occur at any locus, extensive validation and case experience has shown that smaller amplicons amplify more

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efficiently and are less affected by degradation than are larger amplicons. Therefore, the results at the smaller amplicons can be particularly diagnostic.

33.18.13.2.3 To what degree would a putative low-level contributor coincidently be included by a high-level contributor due to overlap.

33.18.13.2.4 If the analyst and reviewers feel that the above rules do not adequately apply to a specific mixture comparison, consult the TL.

33.18.13.2.5 Y-STR profiles will be searched against the Staff Index and documented on DNA QR-20 Staff Search Worksheet.

33.18.14 General Report Conclusions for Y-STR Results:

33.18.14.1 Y-STR report templates are described in DNA SOP-6.

33.18.14.2 Elimination: used when the known sample is not the source of, or a contributor to, the DNA profile detected from item xx. The known sample has alleles that are not detected in the evidentiary sample and the lack of detection is not reasonably explainable by degradation/inhibition/allele dropout (stochastic effects) within the evidentiary sample.

33.18.14.3 Inconclusive: No conclusion can be drawn from the comparison between the known sample and the evidentiary sample due to uncertainty. There are two reasons for inconclusive results. See 33.18.14.3.1 and 33.18.14.3.2 below:

33.18.14.3.1 Based on limited data: This statement is made when the data does not provide sufficient support for any conclusion regarding a comparison of a mixture to a particular known.

33.18.14.3.2 Mixture too complex: This statement is made for mixtures of 3 or more male lineages where the known is not eliminated.

33.18.14.4 Cannot be eliminated (CBE) as the source of, or a contributor to a DNA profile: This conclusion is used when there is a strong correlation between the known sample and the evidentiary sample, but some of the alleles present in the known sample are not detected in the evidentiary sample above the analytical threshold. In this instance, dropout is a reasonable explanation taking into consideration peak height data, overall quantity of template DNA, information regarding the extent of degradation, inhibition, stochastic effects, and masking by stutter or contributor ratio.

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33.18.14.5 Inclusion: All alleles consistent with the known sample are detected (≥ 50 RFU) in the evidentiary profile at all loci where results are generated (See 33.18.15 for statistics).

33.18.15 Y-STR Statistics: (see 33.18.16 - YF Workflow and qualification of loci for statistics)

33.18.15.1 For single source Y-STR profiles statistics are calculated at all loci where DNA results are obtained above the analytical threshold (except for DYS385). The Y-STR random match probability is calculated using the counting method. A haplotype frequency is generated by counting the number of times a haplotype has been observed in the population database divided by the total number of haplotypes in the Database. Haplotype frequencies are given an upper bound 95% confidence interval (See DNA SOP-6. The Y-Chromosome STR Haplotype Reference Database (YHRD) Y-STR population database (available at https://yhrd.org) is used.

33.18.15.2 On the YHRD website homepage, click "Search the Database" on the upper left of the site. From there, click on "Manually enter the haplotype/haplotypes to search for". "Yfiler" is the default for the order the loci are listed in, which can be adjusted if needed. Enter the haplotype, with a comma separating the two alleles (even if a homozygote) at DYS385, then click "Search". When search is complete, click "Add feature to this report" and select "National Database (with Subpopulations, current SWGDAM-compliant version). Click the "X" to close the "worldwide" results before printing page for the case jacket.

33.18.15.3 An exact upper bound 95% confidence interval (Clopper-Pearson method) is calculated from the results of a database search in order to estimate the population frequency of a haplotype using the population (United States data set: African American, Caucasian, or Hispanic) with the most common haplotype frequency. The upper bound estimate is dependent on the size of the database, and these estimates may change as the database size changes.

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33.18.15.4 The formula used to calculate the 95% upper bound frequency estimate is:

$$\sum_{k=0}^{x}\binom{n}{k}p_0^k(1-p_0)^{n-k}=0.05$$

https://strbase.nist.gov/pub_pres/SWGDAM-Jan2012-YSTRs.pdf

Clopper, C. and Pearson, E. *The use of confidence or fiducial limits illustrated in the case of binomial,* Biometrika (1934) 26:404-413.

33.18.15.5 Y-STR haplotypes (and mtDNA haplotypes) are inherited independently from autosomal loci. Therefore, Y haplotype (and cumulative haplotype) frequencies may be combined with autosomal STR RMPs or CPIs using the product rule to generate an overall profile frequency estimate (applies to outsourced cases only with no STRmix analysis).

33.18.15.6 Y-STR Mixtures: (see 33.18.16--YF Workflow and qualification of loci for statistics)

33.18.15.6.1 The expected frequency of haplotypes that could contribute to a Y-STR mixture is calculated using a combined counting method (CCM). A cumulative haplotype frequency is generated by counting how many times each of the possible haplotypes are observed in the population Database, summing them and dividing by the total number of haplotypes in the database (i.e. cumulative haplotype frequency = x/N, where x is the number of potentially contributing haplotypes observed in a population and N is the total number of haplotypes searched in that population). An exact upper bound 95% confidence interval is then applied using the population (African American, Caucasian, or Hispanic) with the most common CCM frequency. Note: The frequency and confidence interval for a mixture profile are calculated using the software tool Y-Mix, which contains the U.S. portion of the YHRD database.

The cumulative haplotype frequency (with an upper bound 95% confidence interval) is calculated for samples with an additional allele at any locus, i.e., 2 alleles at DYS393, DYS19, DYS389II, DYS390, DYS391, or 3 alleles at DYS385.

33.18.15.6.2 The File for the Y-Mix software tool is located on the S drive. The loci in the Excel folder are in alphanumeric order. Enter the mixture profile under the appropriate loci, with one row per allele. Defaults (Limit database to samples with all the loci entered above?: Yes, Treat this profile as a single source sample?: No, Desired UCI: 95%, Use (x+1)/(N+1)?: No) are to be unchanged.

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Click "compare the profile to the database" when profile entry is complete. Print page when results are obtained.

33.18.16 **YF Workflow and Qualification of Loci for Statistics**

33.18.16.1 Evaluate data at all loci (≥ 25 RFU) to determine if profile is a single haplotype or a mixture (see 33.18.11).

33.18.16.2 Qualify results for statistics at DYS385 using a ST of 150-250 RFU (based on injection time) and at other loci as discussed below. Statistics are calculated as described in 33.18.15 for single lineage YF profiles and 2 lineage mixtures only.

33.18.16.3 Compare known(s) to evidentiary profiles as in 33.18.12-15.

33.18.16.4 Single Haplotype Profile:

33.18.16.4.1 2 alleles detected at DYS385: use data (above & below ST) at DYS385 and other loci where included.

33.18.16.4.2 1 allele detected at DYS385: (A) Qualify locus if allele is above ST and use data for statistics at other loci where included. (B) Disqualify locus for statistics if allele is below ST and use data at remaining loci where included. Note: the known would be eliminated as the source if the known does not have the allele found in the evidence profile.

33.18.16.5 Mixture of 2 Haplotypes (CCM): Evaluate results at all loci as described in 33.18.16.1-3.

33.18.16.5.1 Mixture evident at DYS385 with < 4 alleles and all alleles above ST at DYS385: Qualify locus for stats and use data for statistics at other loci where the mixture is detected.

33.18.16.5.2 Mixture evident at DYS385 with < 4 alleles and some allele(s) below ST at DYS385: Disqualify locus for stats and use data at other loci where the mixture is detected. Note: the known (2 alleles at DYS385) would be eliminated as a contributor if the known does not have at least 1 allele found in the evidence profile. If the known has only l allele at DYS385, the known may not be eliminated depending on the results at other loci.

33.18.16.5.3 Mixture with 4 alleles at DYS385 and allele(s) above or below ST at DYS385: qualify locus for statistics and use data for statistics at other loci where the mixture is detected.

33.18.16.5.4 Mixture not evident at DYS385, e.g., 2 alleles detected at DYS385 and no significant peak imbalance at DYS385 (where the minor allele is > 20% of the major allele): Disqualify locus for statistics. Use data for statistics at other loci where the mixture is detected.

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33.18.17 Deconvolution of 2 Male Lineage Mixtures

33.18.17.1 A major contributor to mixtures of 2 male lineages may be deconvoluted in lieu of the combined counting method (CCM) discussed in 33.18.16.5 with TL/DD approval as follows:

33.18.17.2 Deconvolution of mixtures at all loci except DYS385: A major contributor may be deconvoluted where the height of the major allele is at least 5X greater than the minor allele (minor is ≤ 20% of major). Example: if the minor allele is 100 RFU, the major allele must be ≥ 500 RFU.

33.18.17.3 Where only 1 allele is detected at any locus except DYS385, the "major contributor allele" must be greater than 150-250 RFU depending on the injection time (see 33.18.10.7). If not, the locus is not eligible for deconvolution.

33.18.17.4 To qualify the results at DYS385 for deconvolution: where 1 allele is present at DYS385, the major contributor may be deduced if the peak height is above the stochastic threshold and the mixture ratio at all loci where the mixture is detected is at least 5:1.

33.18.17.5 To qualify the results at DYS385 for deconvolution: where 2 alleles are present at DYS385, the "heterozygous" major contributor may be deduced if the peaks are above the stochastic threshold, the mixture ratio at all loci where the mixture is detected is at least 5:1, and "heterozygous" peak balance is at least 50%.

33.18.17.6 To qualify the results at DYS385 for deconvolution: where 2 alleles are present at DYS385 ("homozygous" major with significant peak imbalance), the major contributor may be deduced if the major peak is above the stochastic threshold and the mixture ratio at DYS385 is at least 5:1 (and at all other loci where the mixture is detected).

33.18.17.7 To qualify the results at DYS385 for deconvolution: where 3 alleles are present at DYS385 ("heterozygous" major): the major contributor may be deduced if both peaks are above the stochastic threshold, the mixture ratio of the lowest major allele to the minor allele is at least 5:1 (and at all other loci where the mixture is detected), and "heterozygous" peak balance is at least 50%.

33.18.17.8 To qualify the results at DYS385 for deconvolution: where 3 alleles are present at DYS385 ("homozygous" major): the major contributor may be deduced if the major allele is above the stochastic threshold and the mixture ratio of the major

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allele to the highest minor allele is at least 5:1 (and at all other loci where the mixture is detected).

33.18.17.9 To qualify the results at DYS385 for deconvolution: where 4 alleles are present at DYS385: the major contributor may be deduced if the two highest peaks are above the stochastic threshold, the mixture ratio is at least 5:1 (lowest major allele must be at least 5X greater than the highest minor allele), and "heterozygous" peak balance is at least 50%.

33.18.17.10 Once deconvolution is completed, the known(s) are compared to the profile of the deconvoluted major contributor (see 33.18.13 & 33.18.14).

33.18.17.11 If an individual is eliminated as a potential source of the major contributor but cannot be eliminated from the overall mixture (i.e. a potential minor contributor), the CCM statistic is calculated as discussed in 33.18.16.5.

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34.1 PURPOSE

To determine if sufficient male DNA is present in a sample to warrant if subsequent EZ1 extraction and STR/Y-STR amplifications are required.

34.2 RESPONSIBILITY

DNA and FB section personnel, as assigned.

34.3 EXTRACTION

34.3.1 (If cuttings previously collected, skip to 34.3.3) Per FB SOP-02 (SAECK Exam) – Multiple swabs collected from the same orifice/area and submitted as one item will be considered one sample. For each item, test a portion of each swab as one sample (i.e. 4 swabs from a vaginal swab submitted; ~1/10 of each of the 4 swabs will be placed in 1 tube). A sample (1/4 of swab) will be taken for any future request of serology testing. The remaining sample will be used for DNA testing if applicable based on the Male Screen Results. Samples taken will be documented on FB QR-05b SAEK Worksheet for Male Screen. Please note: depending on the case scenario (i.e possibly non-semen male DNA), a differential or a non-differential request may be made in LIMS if the sample results lead to a DNA EZ1 extraction.

34.3.2 Take a small cutting (~1/10) of each swab of the item and place all cuttings of that item into one Spinease tube (Note on tube how many ~1/10 cuttings in the tube).

34.3.3 Repeat 34.3.2 for any other items to be tested to generate a set of swab cutting samples for extraction.

34.3.4 Document samples and number of swab cuttings on DNA QR-314 Male Screen Extraction Worksheet.

34.3.5 Alongside the cutting samples, process a male screen positive control (EP-MS), consisting of 10 microliters (µl) of diluted semen pre-aliquoted in a Spinease tube, and a reagent blank (RB), consisting of reagents added to an empty Spinease tube.

34.3.6 Add 50 µl of 1N Sodium Hydroxide for each ~1/10 cutting in the tube. Add the 1N Sodium Hydroxide directly onto the swab cutting(s) in each tube. Add 100 µl of 1N

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Sodium Hydroxide to the male screen positive control (EP-MS). Maximum volume used for any sample is the volume used for the RB (i.e. 2 cuttings = 100 µL).

34.3.7 Incubate the Spinease tubes in a thermomixer at 80°C and shaking at 750 rpm for 10 minutes. Verify that the thermometer reads the correct temperature and document on DNA QR-204 Incubation Temperature Log.

34.3.8 Remove the tubes from the thermomixer and pulse spin to maximum speed (15,000 rpm) in centrifuge by holding the "short" button for ~15 seconds.

34.3.9 Add 2 µl of Glacial Acetic Acid for every 50 µl of 1N Sodium Hydroxide to each tube and vortex for 1-2 seconds to mix.

34.3.10 Add 200 µl of Milli-Q purified water for every 50 µl of 1 N Sodium Hydroxide to each tube and vortex ~3 seconds to mix. Pulse spin the set of samples to maximum speed (15,000 rpm) in a centrifuge by holding the "short" button for ~15 seconds.

34.3.11 Use 2 µl of each extract in a Quantifiler Trio reaction (see DNA SOP-3, DNA WI-07). If not quantifying for several hours or more, store the extracts at 4°C.

34.3.12 Review the Quantifiler Trio results. The positive control Y quant value should be within 5 standard deviations of the aliquot set average (see DNA QR-312 Male Screen Extraction Positive Control (EP-MS) QC). The RB and Quant NEG should be 'Undet.' for all quant values. If DNA (≥1pg/µl in SA or LA value, and not undetermined for the Y value) is detected in the Trio negative control or reagent blanks, bring it to the attention of the TL. Depending on the amount detected, the Trio results may be interpreted with caution with TL approval. Detection of less than 1pg/uL in the SA or LA target does not need TL notification.

34.3.12.1 Any samples that produce a Y quant value that isn't undetermined will be forwarded to DNA for extraction. This is reported as a positive male screen result.

34.3.12.2 Any samples that produce a Y quant value that is undetermined **AND** an SA value of < 0.001 ng/µl will be forwarded to DNA for extraction. This is reported as an inconclusive male screen result, due to the low quantity of total human DNA.

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34.3.11.3 For any samples that produce an undetermined Y quant value **AND** an SA value of ≥ 0.001 ng/µl; perform the following purifying/concentration procedure with a Microcon DNA Fast Flow centrifugal filter (including the RB).

34.4 MICROCON (Document this step on DNA QR-313 Male Screen Concentration Worksheet.)

34.4.1 Assemble a Microcon DNA Fastflow filter cup and tube. Add 400 µl of sterile Milli-Q purified water to the Microcon DNA Fastflow filter cup. Add 100 µl of the Male Screen extract (Sodium Hydroxide and Glacial Acetic Acid diluted with H20) to the cup. Reminder: purify/concentrate the RB associated with the samples.

34.4.2 Spin the Microcon device in a centrifuge at 2900 rpm (790 rcf) for ~15 minutes to reduce the 500 µl starting volume to <20 µl, but do not spin the filter cup dry. A second 400 µl Milli-Q water wash may be performed as needed.

34.4.3 Invert the filter cup into a clean 1.5 ml tube and spin the remaining volume from the cup into the tube in a centrifuge at 2900 rpm for 2 minutes.

34.4.4 Measure the volume with a micro-pipette and bring the sample to a 20 µl total volume by adding sterile Milli-Q purified water.

34.4.5 Use 2 µl of each extract in a Quantifiler Trio reaction (see DNA SOP-3, DNA WI-07).

34.4.6 Review the Quantifiler Trio results. The RB and Quant NEG should be 'Undet.' for all quant values. If DNA (≥1pg/ul in SA or LA value, and not undetermined for the Y value) is detected in the Trio negative control or reagent blanks, bring it to the attention of the TL. Depending on the amount detected, the Trio results may be interpreted with caution with TL approval. Detection of less than 1pg/uL in the SA or LA target does not need TL notification.

34.4.6.1 Any samples that produce a Y quant value that isn't undetermined will be forwarded to DNA for extraction. This is reported as a positive male screen result.

34.4.6.2 Any samples that produce an undetermined Y quant value **AND** an SA value that did **not** increase by ≥ 1.5 fold will be forwarded to DNA for extraction. This is reported

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as an inconclusive male screen result, due to the lack of sufficient concentration by the Microcon device. The statement will contain the reason as "due to indeterminate results."

34.4.6.3 Any samples that produce an undetermined Y quant value **AND** an SA value that **did** increase by ≥ 1.5 fold will be stopped for testing. This is reported as a negative male screen result. DNA extraction will not be performed.

34.4.6.4 DNA-QR-313 Male Screen Concentration Worksheet is additionally used to flag samples that would need to go through a Microcon step. This worksheet has been validated to follow all guidance in this SOP outlining when a sample is to go through the Microcon step.

34.5 REVIEW

After all the samples have been processed, a DNA analyst competent in the male screen procedure will be given the batch to review, determine male screen results, and assign appropriate LIMS requests (differential, non-differential, male negative, knowns, consumption). In addition, the batch will be technically/administratively reviewed by a qualified analyst. This review will be documented on DNA-QR-4E. After review, only QR, and worksheets containing results will be photocopied and placed in each case jacket. The original batch paperwork will be scanned to the U: Drive, and filed with benchwork batch paperwork. After LIMS requests are made, the case files will be given to Case Management. Analysts or technicians assigned negative samples and/or cases will move those samples virtually (in LIMS) and physically to the "Freezer Storage" storage location.

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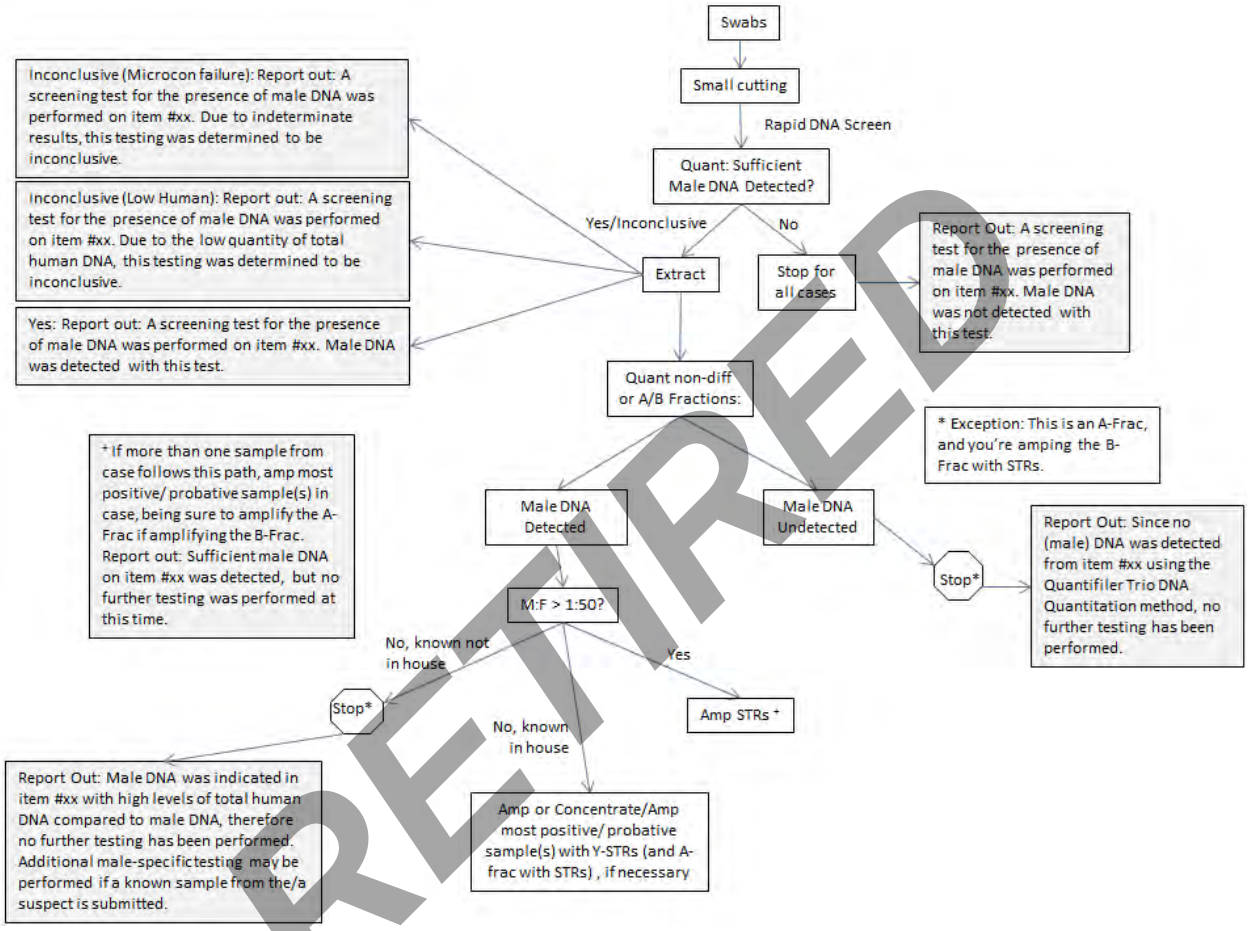
34.6 WORKFLOW



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34.6.1 Please note: In General, if multiple samples were sent for DNA extraction, one sample (the most positive/probative) will be forwarded to STR amplification. However, based on the case scenario (number of perpetrators, consensual partners, different orifices, etc.) two or more samples may be forwarded to STR amplification. Keep in mind offense, and the potential for CODIS entries when making this determination. If the case scenario is unknown, all samples that qualify for amplification will be amplified.

34.6.2 If a suspect known is available and Y-STR testing is performed, the epithelial-rich fraction will be amplified for autosomal STRs for a quality assurance check.

34.6.3 Only perform Y-STR testing when necessary. For example, if the flow chart indicates y-testing on an A-frac, but the B-frac has a GF male profile, or if the flow chart indicates y-testing on the genital swabs, but you've already obtained a male GF profile from the vaginal swabs, etc.

34.7 **REFERENCES**

34.7.1 DNA Quality Records

34.7.1.1 DNA QR-311 QC Male Screen Extraction Reagents QC: Used to capture QC results of the Male Screen Extraction Reagent Aliquots.

34.7.1.2 DNA QR-312 Male Screen Extraction Positive Control (EP-MS): Used to capture QC results of a lot of Male Screen Positive Control samples. The Bulk Male Semen Samples will be tracked on a document "BioChemed Semen Samples" that is found on the DNA server in the Male Screen Folder.

34.7.1.3 DNA-QR-313 Male Screen Concentration Worksheet: Used to capture purification and concentration step of the process when needed.

34.7.1.4 DNA-QR-314 Male Screen Extraction Worksheet**:** Used to capture samples and controls run in a Male Screen Batch. QR will have reagent lot numbers and thermal mixer used.

34.7.2 LIMS Requests

34.7.2.1 Forensic Biology Request = "CT 100 – Male screen sample prep" (released at draft complete)

34.7.2.2 DNA Request = "Male Screen" (released at draft complete)

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39.1 Purpose:

The primary objective reflected in this section is for the analyst to render the best interpretation of the data.

39.2 Responsibility:

DNA Unit personnel.

39.3 STR Analysis and Interpretation:

This procedure is a general guideline for the analysis and interpretation of STR profiles when using the GlobalFiler® PCR Amplification kit (GF) and STRmix™ (see WI-34 for GF/STRmix™ work flow overview and WI-35 for GeneMarker overview). However, it is not an exhaustive list of all possible casework scenarios. Analyst training, experience, and judgment must be considered when reporting STR profiles. STR data are interpreted by evaluating the results at all loci.

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39.4 GlobalFiler® System:

39.4.1 When the GF Kit is employed, the twenty core CODIS STR loci, plus one additional STR locus (SE33), 1 Y-STRs (DYS391), a Y-Indel and Amelogenin are typed in a single PCR amplification reaction according to the manufacturer's and laboratory's protocols.

39.4.2 GlobalFiler® PCR amplification products are separated, detected, and analyzed using an ABI 3500xL Genetic Analyzer, the supplied 3500 Series Data Collection Software 4 and GeneMarker HID (GM-HID) v.2.9.7 software.

39.4.3 These procedures generally follow those outlined in the GeneMarker HID Software User Manual for the version in use.

39.5 GlobalFiler® Analysis:

39.5.1 Analysis of DNA Profiles in GM-HID

39.5.1.1 Standard analysis parameters: The analytical thresholds for 3500xl data (AT) for forensic unknowns, knowns, ladders, and controls are B-40 RFU, G-55 RFU, Y-35 RFU, R-50 RFU, P-55 RFU. Evidentiary profiles (forensic unknowns) with peaks > 10,000 RFU, and known samples with peaks > 30,000 RFU, at loci other than Amelogenin, may be used where appropriate with TL or Lead approval.

39.5.1.2 The results are evaluated for each locus. However, considering the data from all loci tested will assist in the overall interpretation of the results for each profile.

39.5.1.3 Overloaded Data: If too much DNA is added to the amplification reaction or is injected into the 3500xl, the fluorescent intensity for the PCR products may result in various interpretational challenges. Samples with off-scale peaks may exhibit raised baselines, split peaks, increased minus A peaks, excessive pull-up and/or higher than expected stutter levels including uncommon stutter types, and/or amplification artifacts in one or more of the colors. Examine the peak heights in GM-HID software. Peaks greater than 10,000 RFU (30,000 RFU for knowns and database samples) but not off-scale, may also lead to artifacts peaks being detected ≥ AT. These samples may need to be reinjected for a shorter time or diluted and re-amplified.

39.5.1.4 Peak identification: Only allele peaks with a height of ≥ AT in GM-HID are called/reported and evaluated using STRmix™. Peaks < AT may be evaluated to address issues such as whether a sample should be re-amped/re-injected, but are not used for contributor number or contamination assessment. The identity of peaks called by GM-HID is generally assigned to one of the following categories:

39.5.1.5 Allele Peak: A called allele has a peak height ≥ AT, a fragment size that falls within the range of 60-460 base pairs, good peak morphology, and has the appropriate dye color for the locus. However, not all peaks ≥ AT are typed as alleles. Stutter peaks, pull-up/pull-down peaks, background noise, and amplification or injection artifacts could fall above the RFU threshold, especially if the allele peaks are high. Peaks that do not meet the Laboratory's calling criteria are not used by STRmix™ with the exception of the stutter peaks that are modeled. Peaks ≥ AT that are not called by GM-HID may be called by the analysts on a case-by-case basis with TL approval. Such peaks are interpreted with caution.

39.5.1.6 Detection of variant alleles: Variant alleles have been identified for many STR loci. These alleles may contain full or partial repeats. Variant alleles may be labeled as Out of Bin (OB) or Off Ladder (OL) alleles in GM-HID. If a particular variant has been observed before by the DESPP CT Division of Scientific Services (CT_DSS), Thermo Fisher, NIST, or in published literature, the sample does not need to be repeated. In GM-HID, change the OB/OL to the proper allele call if needed. However, if the repeat has not been observed, the sample may be re-amplified and/or reinjected to confirm the variant allele as warranted.

39.5.1.7 Stutter peaks (N-1, N-2, N-0.5, N+0.5, and N+1 repeats; N-2 is particularly prevalent if sample is over-amplified/injected): A stutter peak has a fragment length typically 1 repeat smaller than the true allele (see exceptions above). If the peak height in the stutter

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position is less than the CT_DSS allele-specific stutter thresholds/combined stutter thresholds, the peak is filtered out by the GM-HID program. CT_DSS stutter thresholds were determined by evaluating the CT_DSS empirical stutter data on an allele-by-allele basis (see DNA SOP-41). Peaks in the stutter position that are below the Laboratory's stutter thresholds are not listed on the electropherograms or the case report. Note: see DNA SOP-32 for stutter peak types that are evaluated by STRmix™.

39.5.1.8 Minus A Peaks: GF kits are optimized to add an extra nucleotide to the 3' end of the PCR product. The nucleotide is predominantly adenosine (A). Lack of a full A addition may be observed especially when the amount of input DNA generates peaks ≥ 10,000 RFU. However, sample degradation can increase the effective concentration of a small amplicon relative to the larger amplicons, leading to minus A product at the smaller loci. Data from other loci may be of assistance in these cases. When appropriate, a smaller quantity of sample may be re-amplified.

39.5.1.9 Pull-up Peaks: Pull-up peaks are generally seen when an elevated quantity of amplification product is injected on the CE or when the spectral calibration is sub-optimal. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-up peaks can be displayed in several colors. Pull-up peaks should have approximately the same fragment size in each of the colors as the true allele peak. Additional information provided by other loci may be of assistance in these cases. When evaluating allelic peaks that may contain a pull-up (PU) contribution, an estimate of the PU contribution is subtracted from the height of the potential allelic peak to determine if the remaining peak height is sufficient for allele designation. The estimated pull-up contribution is determined by evaluating the height of unambiguous pull-up peaks (pull-up with e.g., no stutter or allelic contribution) at each locus where pull-up is detected for a particular color.

Note: the 3500 Series Data Collection Software 4 has a pull-up correction function applied to the raw data. This function is not necessarily consistent across the entire base-pair range for the detection of peaks from amplified DNA, therefore care must be taken when identifying the percentage of signal for unambiguous pull-up peaks. Using unambiguous pull-up peak(s) in the same locus will most often be optimal. In the case where there are no unambiguous pull-up peaks for the dye channel, consider that the median percentage of pull-up peaks observed during the validation was 0.7% with 95.1% of the pull-up peaks being < 3% and 99.4% being < 5%.

Example: The percentage of pull-up peaks as compared to the height of the peaks causing the pull-up is calculated. (PU percentage = PU peak height/height of the peak causing the PU). This percentage is converted to an RFU value (multiply percent by height of peak causing the pull-up) which is then subtracted from the potential allelic peak in question.

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The resulting "subtracted" peak is called where DNA section allele calling criteria are met. The same methodology would be applied to potential allelic peaks with a pull-down component (see below).

39.5.1.10 Pull-down Peaks: Pull-down peaks are generally seen when an elevated quantity of amplification product is run on the CE or when the spectral calibration is sub-optimal for the run. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-down peaks can be displayed in several colors. Pull-down peaks can have approximately the same fragment size, but often the pull-down peak will be a slightly larger or smaller size as compared to the true allele. Additional information provided by other loci may be of assistance in these cases.

39.5.1.11 Spikes: fluorescent spikes can be seen within GM-HID data. Spikes are typically sharp peaks caused by air bubbles or polymer crystals in a capillary, which can be displayed in different colors, and have approximately the same "fragment size". Additional inspection of the raw data or re-injection may assist in cases where spikes are believed to be present.

39.5.1.12 Dye Artifacts/Dye Blobs: Broad peaks may be the result of dissociated dyes or fluorescent particles that are migrating within the polymer matrix and then fluorescently excited by the laser. Additional inspection of other samples (especially negative controls) within the module may assist in interpretation in cases where dye blobs are believed to be present (see DNA SOP-41).

39.5.1.13 Amplification Artifact Peaks: Nonspecific amplification product peaks, generally with good peak morphology, may result when a higher quantity of template is amplified. When appropriate, a smaller quantity of sample may be re-amplified.

39.5.1.14 High Background: Background fluorescence may reach an RFU level above the analytical threshold. Generally such high background peaks exhibit atypical peak morphology. When appropriate, re-injection may assist with interpretation.

39.5.1.15 Raised Baseline: Elevated baseline of one color over a broader (multiple bin) scan range may occur.

39.5.1.16 Non-specific amplification peaks: With some samples, primers may bind to non-human DNA or human DNA other than the target region, and amplification may occur. Resulting peaks do not exhibit stutter but typically have good morphology otherwise. Such peaks may be observed at characteristic base pair sizes. Most often they fall outside of bins or at fractional repeat numbers. More than one non-specific amplification peak may be seen in the same sample. Whenever a non-specific amplification peak is

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suspected the TL should be consulted for interpretational input and for tracking of common artifact peaks to watch for.

39.5.1.17 Analysis of known samples amplified with GlobalFiler Express or GlobalFiler

Routine click-offs do not need to be noted on the QR-325 injection worksheet (i.e. pull-up, spikes, dye artifacts, stutter peaks below the CT_DSS allele-specific stutter thresholds/combined stutter thresholds, etc.). Allele changes and sizes of peaks out of ladder ranges, called stutter peaks above the CT_DSS allele-specific stutter thresholds/combined stutter thresholds, possible tri-alleles, non-human artifact peaks, non-routine click-offs, and any low peak height ratios (<60%), should be noted on the appropriate QR-325 injection worksheet. Analysts should be aware that since the GFE_CTDSS_20% knowns panel filters out any peaks < 20% in height of the parent peaks at each locus, there could be a stutter peak present above the allele-specific stutter threshold/combined stutter threshold that does not receive an allele call due to its height being < 20% of the parent peak. The Known PHR Filter Macro is used to assist analysts in identifying any results warranting further review or documentation.

39.5.2 Control Requirements

39.5.2.1 For evidentiary sample (forensic unknown) amplifications, all controls (RB, NEG, EP1, POS) for each extraction/amplification set must give the expected results. For known or database samples, only one positive control (EP1 or POS) is required to give the expected results; all other controls must give the expected results. At times, when the amplification positive control does not give expected results, the TL can approve the passing extraction positive control to serve as the amplification control.

39.5.2.1.1 If either the NEG or the RB for a database plate does not inject properly (ex. size standard fails during multiple re-preps/re-injections), results from the plate may be used with TL approval.

39.5.2.2 If the required control results are not obtained, the amplification set may be re-injected, re-amplified, or re-extracted as warranted with input from the TL.

39.5.2.3 Note that the RB may be omitted for re-amplifications using the same volume (or less) of template, provided the RB is not the control with the unexpected result. The EP1 may be omitted for re-amplifications if expected results were previously generated for the extraction set. All re-amplifications must include amplification negative and positive controls. POS/EP1 may be omitted for re-injections. The RB and NEG may be omitted for re-injections of equal or lesser time/sensitivity.

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39.5.2.4 Expected results of controls will be verified and documented on DNA-QR-4a DNA QA/QC Case Review Checklist which is maintained in the case file.

39.5.2.5 The GF profiles for the Kit Positive Control DNA 007 (POS) and the laboratory Extraction Positive Control (EP1) are listed in DNA SOP-41.

39.5.3 Contamination Assessment

39.5.3.1 Potential contamination is assessed by evaluating the evidentiary samples, RB, NEG, and positive controls.

39.5.3.2 RB and NEG samples should not have any callable peaks. If callable peaks are detected, the set of samples may be re-run, re-amplified, or re-extracted as warranted with input from the TL. If callable peaks are detected and the evidentiary samples are not affected, bring to the TL for further guidance and notify the Quality Manager for tracking. If a neg/RB is re-amplified (with TL approval) to assess possible contamination (allelic peaks ≥ AT), all evidentiary samples associated with the impacted controls must also be re-amplified.

39.5.3.3 All samples within a batch will be cross-checked against each other to detect potential contamination. Analysts should use their experience and judgment during this process. An excel macro is available to assist with batch cross-comparisons.

39.5.3.4 The appropriate response for a contamination event (including how the sample(s) are reported) is determined on a case-by-case basis with approval of the TL.

39.5.3.5 Depending on factors such as the type and extent of contamination, and whether the source of the contamination can be identified, the contaminated sample(s) and/or amplification sets may be interpreted with caution or deemed unsuitable for comparison following TL review and approval (see Alternate Report Template Statements located within the DNA Report Template).

39.5.4 Documentation of controls and standards on DNA QR-325. The following key should be used as a guideline to determine how controls and standards should be documented on DNA-QR-325. This list is not all encompassing, and other situations may arise not fully documented here. Any questions on how to document controls and standards should be brought to the technical leader.

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Did the ILS perform as expected?

Sizing quality score less than 88	No
Not all LIZ peaks between 60 and 500 bp are called	No
Score 88 or more and all peaks between 60 and 500 bp are called	Yes
Broad peaks, but score is 88 or more and all peaks between 60 and 500 bp are called	Yes

Did the negative controls perform as expected?

Called peaks in Neg or RB*	No
Broad peaks in LIZ, but no called peaks +	Yes

* Need TL initials in comments section showing that no affect to samples

+ Still needs reinjection

Did the positive controls perform as expected?

Broad peaks, but genotype still correctly called	Yes
Broad peaks causing peaks not to be called	No
Large peak height imbalance, but genotype still correctly called	Yes
Large peak height imbalance causing dropout	No
High peaks, needing reinjection at low, but genotype still correctly called	Yes
Extremely high peaks (i.e. overamplified sample), sample needs re-amplification	No
Low peaks, needing reinjection at MAX, peaks not called	No

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39.6 Mixed Samples in GF:

39.6.1 Generally, the detection of more than two alleles per locus indicates a mixed sample. Variation in peak height between alleles in a single locus may assist in the interpretation of such results. Peaks below the AT thresholds for GM-HID are not used in the determining the number of contributors. (see section 39.7 for detailed guidance on determining the number of contributors to a DNA profile).

39.6.2 A DNA profile is generally consistent with being a mixture when three or more alleles are detected at any locus and as discussed below. Note that three peak patterns (at a single locus) have been observed from single sources, but they are rare.

39.6.3 Allelic peak imbalance involving the stutter position, e.g., where the N-1 repeat peak is above the stutter threshold, may be indicative of a mixture. With the possibility of allele sharing among individuals, 3 alleles may not be detected especially with degraded samples or partial profiles.

39.6.4 General allelic peak imbalance: Heterozygous peak imbalance greater than expected for a set of parameters may indicate a mixture. However, stochastic effects and microvariants in the primer-binding domain may cause either PCR enhancement or suppression.

39.6.5 Per the "SWGDAM Guidelines on STR Interpretation", a Stochastic Threshold is defined as the "value above which it is reasonable to assume that allelic dropout has not occurred within a single-source sample". CT DSS Stochastic thresholds are 500 RFU for Standard and Low injection times and 700 RFU for Maximum injection times. Injection time ranges listed on next page.

Injection	**3500-1, 2, 3, 5, & 6**	**3500-4**
Low	1.2 kV, 16 sec	1.2 kV, 12 sec
Standard	1.2 kV, 24 sec	1.2 kV, 18 sec
High	1.6 kV, 32 sec	1.6 kV, 24 sec

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39.6.6 For the 25 cycle GlobalFiler Express Amplification, the stochastic threshold will be 115 RFU for Low injection and 150 RFU for Max. If samples contain homozygote peaks below these thresholds, inject the sample longer or re-process the sample, as warranted. The CT-DSS Knowns panel (GFE_CTDSS_20%) will be used for analysis.

Note: Since the GFE_CTDSS_20% knowns panel filters out any peaks < 20% in height of the parent peaks at each locus, it is imperative to look carefully at the baseline of knowns to check for contamination. Using this filter could result in peaks greater than the analytical threshold not receiving an allele call due to their peak height being < 20% of the parent peak at that particular locus. In this instance, contamination is still present and needs to be addressed and documented accordingly.

True alleles of a heterozygous pair may not receive an allele call if the peak height ratio of the pair is < 20%. True tri-alleles may also not receive an allele call if one of the peaks is < 20% of either of the other two peaks. In both of these instances, analysts would need to manually add the allele call of the smaller peak onto the electropherogram. These instances should be noted on the corresponding QR-325 injection worksheet.

39.6.7 Mixtures are deconvoluted for CODIS entry purposes as warranted by the results. See DNA SOP-13.

39.7 Determination of Single Source or Mixture Profile & Number of Contributors

Single Source Profile Attributes: A single source profile will generally have no more than 2 alleles at any locus. Expected heterozygote peak balance (HPB) decreases as a function of peak height (see Fig. 1). In rare instances, an individual may exhibit a tri-allelic pattern at a locus. When a suspected tri-allelic pattern is observed but not confirmed by multiple observations in a case, the sample would typically be re-amplified to confirm its presence.

Note: If an electropherogram has only an X at Amelogenin then it is considered a No Result.

When a tri-allelic pattern is noted, the results must be documented in the case file, but the locus is not included in the statistical calculation (see STRmix™ DNA SOP-32).

General Mixture Profile Attributes: Use ‘consistent with a mixture’ for profiles with multiple contributors. A profile with no more than 2 alleles can be best explained as a mixture if there is significant peak height imbalance at one or more loci (see Fig. 1 re: expected heterozygous peak balance vs peak height. Note: Fig. 1 represents 3130xl data, and x-axis scale would be considerably different considering 3500 data, but general trends would remain similar.)

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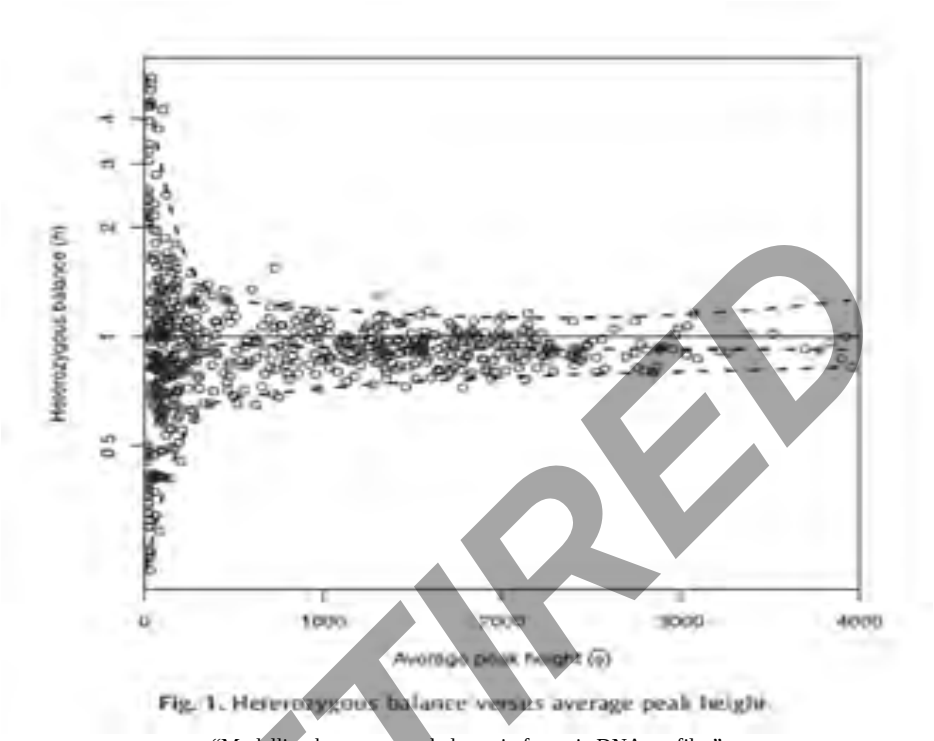

Fig. 1. Heterozygous balance versus average peak height.

"Modelling heterozygote balance in forensic DNA profiles"
Kelly et al, FSI Genetics 6 (2012) 729-734

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39.7.1 Determination of the number of contributors to the DNA profile

Determination of the number of contributors to a profile begins with assessing the minimum number of contributors from the locus that exhibits the greatest number of allelic peaks. For example, if at most five alleles are observed at a locus, then the DNA results are consistent with having arisen from at least three individuals. Proceed with caution when only one additional allele at a locus would lead to an increased number of possible contributors, as peaks in stutter positions, and the potential for a tri-allele can complicate mixture interpretation. While counting allele peaks is useful in determining a minimum number of contributors, the analyst must also consider that allele sharing between individuals may result in an underestimation of the actual number of contributors. The potential of peaks in stutter positions should be considered. The number of contributors chosen for STRmix™ analysis reflects the analyst's assessment of the most likely number of contributors required to reasonably explain the observed profile.

A determination of the number of contributors to a profile is generally made by the DNA analyst prior to STRmix™ deconvolution and prior to the comparison of the profile to any reference samples in STRmix™. However, when scientifically warranted, the

comparison of an evidentiary profile to a conditioned elimination known might lead the analyst to alter the original number of contributor determination. This change needs to be reviewed by the Technical Reviewer, and TL approval must be obtained prior to STRmix deconvolutions or report writing. A detailed explanation of the reasoning for the change must be documented on DNA QR-308 Changed and/or Not Reported Interpretations. The original DNA QR-302 Contributor Estimate for the sample shall be DNR'd with initials and date of the reassessment.

Sometimes the number of contributors is ambiguous. This could be because the profile is too complex and may contain putative indications of additional contributors. DNA analysts will use their professional judgement to assess the number of contributors, and will evaluate if any called peaks should be removed as high stutter where appropriate.

In circumstances when the number of contributors cannot be adequately assessed, or when there are greater than 5 unknown/ unconditioned contributors without a clear major contributor being present (see 39.15), the profile is reported as too complex to interpret.

After analysis of the STRmix™ MCMC (Markov Chain Monte Carlo) output, it may be determined that the deconvolution does not conform to scientific expectations and may be re-run under a different number of contributors/iterations. In this event and with TL approval, the analyst's assumptions are documented on DNA QR-308 Changed and/or Not Reported Interpretations, the original STRmix™ printout is DNR'd and kept in the case jacket, and the electronic file of both deconvolutions is retained in the STRmix™ server's casework folder.

39.7.2 Method for assigning the number of contributors for a profile

The following steps are followed to assign the likely number of contributors to a profile:

1. Review the profile as a whole, assessing the level of degradation, presence of low level peaks, noisy or clean baseline and general quality (template) of the profile.
2. Identify likely stutter peaks (both forward and back) by reference to DSS allele stutter ratio (SR) expectations (plots of SR per each allele).
3. Find the locus with the highest number of unambiguous allelic peaks, A. If A is an odd number, add 1. A/2 gives the initial assessment of the number of contributors to the profile.
4. Review peak height imbalances at the most informative locus (greatest number of alleles). Taking into account potential allele sharing among contributors, visually attempt to 'pair' alleles and assign them to contributors. If there is too much imbalance between alleles, this may mean the presence of an additional contributor(s) above that indicated by allele count alone. Refer to figure 1 for an indication of expected heterozygote balance at varying peak heights.

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5. If one or more contributors at this locus are either minor or a clear major, check that this pattern is represented at other loci.
6. Apply the general pattern of contributors (number and proportion) to other loci in the profile. If it holds, assign this number of contributors to the profile; otherwise consider the addition or subtraction of one.
7. Analysts may utilize the Contributor Estimator Macro to assist in determining the number of contributors to a profile.

Notes:

1. Discriminating loci such as D1S1656, FGA, and SE33 are particularly informative for determining the number of likely contributors to a profile; however important information can be gleaned at any locus.
2. The presence of one or two minor peaks can sometimes be indicative of drop-in and not a true additional contributor.
3. Amplification results from low-template quantities (particularly less than 100 pg) may exhibit increased stutter and peak variance. Based on the GF/3500xl validation data, it was noted that profiles from low-template contributors may exhibit multiple high stutter peak that can be considerably above the standard stutter thresholds. A similar impact may occur with respect to peak imbalance. Therefore, when these low-template peaks pertaining to stutter or peak imbalance are used to infer an extra contributor, there should be data elsewhere in the profile to support this. The effects of the misassignment of the number of contributors are typically restricted to false exclusions of true contributors and false inconclusive LRs.
4. The low-template stutter thresholds are not included in the GeneMarker panel (GF_CTDSS). For each stutter type being evaluated by CT DSS, the "low" data thresholds are included in DNA SOP-41 to aid in determining the number of contributors and to highlight the increased expected stutter variance associated with low-template single source profiles and/or profiles with low-template contributors.

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39.8 Highly Degraded/Low Template Samples:

39.8.1 Highly degraded/low template samples are interpreted with caution. Degraded samples may "appear" to exhibit high stutter and greater –A at the smaller amplicons with off-scale data. In addition, degraded/low template samples may exhibit unbalanced heterozygous alleles and locus/allele dropout. Partial profiles are interpreted as determined by the data present for each locus. Given the possibility of stochastic effects, especially with low quantity and/or degraded samples, results from low template amplifications are interpreted with caution. Note that stochastics effects can be different for each contributor to a mixture based on the quantity/quality of the template. To

maximize data for a profile, the sample may be injected as appropriate at the standard or maximum time on the 3500xl. Maximum injection requires TL approval.

39.9 Inhibited Samples:

39.9.1 Inhibited samples may exhibit allele, locus, or complete profile dropout, unbalanced heterozygous alleles, and inter-locus peak imbalance. To possibly overcome an inhibitor, smaller quantities of sample may be re-amplified. Quantitation data may aid in determining whether re-amplification is warranted as well as the amount of sample to be re-amplified. Before the genotyping amplification is performed, an evaluation of the Quantifiler Trio IPC for each sample is useful. The Quantifiler Trio IPC result for a sample being undetected or having an elevated C_T may indicate that a sample has an inhibitor present. If this is the case, multiple quantities or dilutions of a sample may be amplified for the original amplification. If necessary, a microcon purification can be performed to reduce the amount of inhibitors in a sample.

39.10 Qualitative Interpretation of STR Results: The following is a general description of the standard DNA report conclusions. Other conclusions may be reported as warranted by the results with approval of the technical leader. (See DNA SOP-6)

39.10.1 Elimination: Conclusion reported in two situations: (1) When it has been determined by manual comparison that the known sample is not the source of, or a contributor to, the DNA profile detected from item #xx. The known sample has alleles that are not detected in the evidentiary sample and the lack of detection is not reasonably explainable by degradation/inhibition/allele dropout (stochastic effects) within the evidentiary profile. (2) When the LR is less than 1 by STRmix™ analysis.

39.10.2 Inconclusive: No conclusion can be drawn from the comparison between the known sample and the evidentiary sample due to uncertainty. There are 3 reasons for inconclusive results:

39.10.2.1 The likelihood ratio obtained for a STRmix deconvolution is greater than or equal to 1 but less than 1,000.

39.10.2.2 MCMC secondary diagnostics did not perform as expected: “the MCMC deconvolution process did not meet scientific expectations; therefore the comparison to Known A is inconclusive.”

Note: If a known individual can be manually eliminated as a source of/contributor to a profile that has sub-par MCMC secondary diagnostics, this elimination is reported.

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39.10.2.3 Profile results only include 1 allele (see 39.10.4.2 below).

39.10.3 Positive Associations

39.10.3.1 Cannot be eliminated (CBE): An individual cannot be eliminated (CBE) as the source of (or a contributor to) a DNA profile if there is a strong positive association between the known sample and the evidentiary sample (see calculation of likelihood ratios) but some of the alleles present in the known sample are not detected in the evidentiary sample above the analytical threshold. In this instance, STRmix considers the probability of dropout taking into consideration peak height data, overall quantity of template DNA, information regarding the extent of degradation, inhibition, stochastic effects, and potential masking by stutter or contributor ratio. An LR ≥ 1,000 is obtained using STRmix.

39.10.3.2 Consistent with Source: An individual is consistent with being the source of a single-source DNA profile if all alleles consistent with the known sample are detected in the evidentiary profile at all loci where results are generated (see report templates). An LR ≥ 1,000 is obtained using STRmix.

39.10.3.3 Inclusion: An individual is included as a potential contributor to a mixture DNA profile if all alleles consistent with the known sample are detected (≥ AT) in the evidentiary profile at all loci where results are generated (see report templates). An LR ≥ 1,000 is obtained using STRmix.

39.10.4 No STRmix Interpretation: STRmix is unable to be run to obtain a likelihood ratio to a deconvolution from a questioned sample. There are 2 reasons for this:

39.10.4.1 The mixture too complex. With > 4 unknown (unconditioned) contributors, or when the analyst is unable to determine the number of contributors with confidence. An exception to this is when a mixture has a clear major contributor (see 39.15).

39.10.4.2 Insufficient data is obtained for the questioned DNA Profile. This is for low-level, partial DNA profiles when there is insufficient data from the evidentiary DNA profile for comparison to a particular known using STRmix™. DNA profiles with only one resulting allele are unable to be run through STRmix. This includes a low level contributor(s) associated with a clear major contributor. With TL approval, other low level profiles can be deemed insufficient for comparison to a reference sample without being run through STRmix. If this determination is made after a STRmix likelihood ratio has already been calculated, document the non-reporting of the likelihood ratio on DNA QR-308 Changed

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and/or Not Reported Interpretations. The STRmix electronic files shall be maintained in the case folder, and the STRmix printouts shall be DNR'd, and remain in the case jacket.

Note: If a known individual can be manually eliminated as a source of/contributor to a profile insufficient for STRmix™ interpretation, this elimination is reported.

39.11 Interpretation of single source DNA profiles

39.11.1 For single source partial profiles with peaks ≥ AT, report a positive association with potential dropout when the observed allele(s) are consistent with the known source and the LR ≥ 1000.

39.11.2 Note: for low-level profiles in situations where no loci have more than 2 peaks above the AT [e.g. such as if there is unexpected "heterozygous" peak imbalance], it is important to consider if the profile could be a partial composite of 2 or more individuals.

39.11.3 Note: Report "undetermined" in the "Type" column of the Testing or Conclusions Summary table when the analyst is unable to determine whether a single source profile is male or female if the Amelogenin X peak is below the stochastic threshold and no Y peaks are detected. For single source profiles where the individual is assumed, the "Type" would be reported as "Male" or "Female" in the Conclusions Summary table as appropriate (regardless of X peak above or below the stochastic threshold).

39.12 Interpretation of DNA mixtures

39.12.1 These general approaches do not account for all possible mixture scenarios and no two mixtures are exactly the same.

The analyst will also consider the quantity of DNA amplified, the peak heights of the alleles, the extent of degradation/inhibition, the impact of stutter, potential carryover between fractions in a differential extraction, and if a known profile compared to the mixture can be objectively determined to be a contributor such as for intimate samples.

39.12.2 If one person can be objectively determined to be included in the mixture given an intimate sample origin, the mixture may be evaluated by a consideration of what loci have DNA profile results (above the AT) that could not be from the known source.

39.12.3 Incomplete separation: In mixtures from samples that were subjected to the differential extraction protocol, the mixture is evaluated by a consideration of whether the results are consistent with incomplete separation from the other fraction (e.g., A vs B fractions).

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39.12.4 The ratio of male:female DNA and the extent of degradation in a sample as determined by Quantifiler Trio and the STR results.

39.12.5 Peak height consistency, i.e. the peak heights in the evidentiary profile potentially attributable to the known DNA profile should generally be consistent at all loci applicable. If not, this may not optimally fit the CBE conclusion (note: this may not apply to degraded profiles).

39.12.6 The "zygosity" of the known: homozygous peaks are generally higher than heterozygous peaks of the same contributor. Therefore, homozygous peaks are less likely to dropout as compared to one allele from a heterozygote.

39.12.7 The total number of dropouts (overall and at each locus) that would be required to still be consistent with a CBE conclusion: While allele dropout (below the stochastic threshold), can occur at any locus, extensive validation and case experience has shown that smaller amplicons amplify more efficiently and are less affected by degradation than are larger amplicons. Therefore, the results at the smaller amplicons can be particularly diagnostic.

39.12.8 To what degree (at how many loci) would a putative low-level contributor coincidently be included by a high-level contributor due to overlap? This is particularly important to consider with mixtures involving relatives.

39.12.9 Mixture DNA profiles that involve relatives are interpreted with caution.

39.13 Statistics – Likelihood ratios

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Determining hypotheses for calculating the likelihood ratio (LR)

An LR is calculated using STRmix™ Analysis software for forensic unknown DNA profiles when the known individual(s) cannot be eliminated as the source of or a contributor to the evidence (or is consistent with being the source or included as a contributor) by a manual comparison. (See DNA SOP-32 STRmix™) LRs are calculated according to the following equation:

$$LR = \frac{\Pr\left(E \mid H_1\right)}{\Pr\left(E \mid H_2\right)} \text{ where}$$

Pr = Probability
E = Evidence
| = Given

The likelihood ratio assesses the probability of the evidentiary profile given two alternate (mutually exclusive) hypotheses; (H_1 and H_2). H_1 is typically where the data is explained by an inclusion of the person of interest (POI). H_2 is typically where the data is explained by a person selected at random from the general population.

In general, an LR is calculated for 1 set of hypotheses (see examples below) based on the nature of the DNA profile (single source, mixture, # of contributors, etc.), the scenario of the crime, and any relevant information supplied to the DSS Laboratory. LRs may be calculated based on new information where appropriate and/or upon request by the prosecution or the defense. At times this new information may lead to a repeat of the deconvolution itself (i.e. submission of elimination knowns after a LR to the POI has already been calculated).

Examples:

1. Scenario: a single source profile from evidence at scene matching J. Smith.

H_1: DNA profile originates from J. Smith.

H_2: DNA profile originates from an unknown person selected at random from the general population.

2. Scenario: a two person mixture from an intimate swab collected from Person A. The DNA profile can be explained by a mixture of J. Smith and A.

H_1: DNA profile originates from A and J. Smith.

H_2: DNA profile originates from A and an unknown person selected at random from the general population.

3. Scenario: a three person mixture from evidence recovered from the scene. One contributor corresponds to J. Smith.

H_1: DNA profile originates from J. Smith and two unknowns.

H_2: DNA profile originates from three unknowns selected at random from the general population.

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39.14 Reporting

The following information is described when reporting profile results:

1. A general description of the DNA profile, such as mixed, single source, etc.
2. The hypotheses/assumptions used when the LR has been calculated.

General description: Use the standard terminology regarding single source samples and mixtures per current SOPs.

39.14.2 Assumptions: All assumptions that are made as part of the interpretation will be clearly stated within the report. This includes an assigned number of contributors and any assumed/conditioned contributors.

39.14.3 Conditioning the hypotheses: an assumption that a person can be included in a DNA profile (such as for intimate samples) regarding the person swabbed.

39.14.3.1 To condition an individual in the MCMC, the assumption must be consistent with the DNA profile results (i.e., the known is not eliminated) and there must be objective support for the assumption i.e., there must be a reasonable scientific expectation that an individual's DNA is in the evidentiary profile. In general, this would apply to non-probative comparisons for intimate samples and other items that have been regularly handled or worn by the person conditioned. The conditioned individual appears in both the numerator and the denominator.

The LR threshold to condition ambiguous (where there is uncertainty as to whether the known source is contributing) profiles generated from non-intimate samples is the same as our threshold for reporting positive associations. The known profile in question is first evaluated using STRmix. The known is assumed to the profile and will be used for conditioning purposes when the resulting LR is ≥ 1000. Since this likelihood ratio will not be reported, proper documentation on DNA QR-308 Changed and/or Not Reported Interpretations must be maintained in the case jacket. If the resulting LR is less than 1000, the known profile is not assumed/conditioned to the profile, and the comparisons is reported out either as inconclusive or eliminated, based on the likelihood ratio.

39.14.3.2 If a staff search indicates a putative non-random positive association between a staff member and an evidentiary profile, the staff member may be conditioned in the MCMC on a case-by-case basis with TL/DD approval when the LR to the individual is at least 1000 (conditioning threshold, non-intimate samples). It has been shown in validations studies that conditioning true contributors to a profile lowers LRs for non-contributors and increases LRs for other true contributors. In general, the staff contributor would be conditioned for comparison to submitted known samples and/or for CODIS entry purposes when reprocessing the evidence is not a viable option or when reprocessing has not resolved the issue. The staff contributor may also be conditioned in scenarios where the contamination is

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trace (e.g., the presence of the contaminant is not expected to significantly impact the LR to the overall profile) or where there is a clear major contributor that is not the staff member.

Notes:

1) Unambiguous contributors to non-intimate profiles do not require STRmix evaluation for conditioning. Both analyst and Technical Reviewer must be in agreement. If any uncertainty arises, STRmix will be run and handled as above. Any comparison where the reference sample's DNA profile is fully present (i.e if known is a heterozygote, both alleles must be called) at less than half the loci with results is considered ambiguous, and therefore must be run through STRmix.

2) When there are two or more elimination knowns for consideration, it is appropriate to condition the unambiguous known when evaluating the other. In situations with multiple ambiguous elimination samples, consult TL for further guidance and approval.

3) Likelihood ratios to ambiguous potentially assumed contributors in 5 person mixtures are calculated using STRmix. If the resulting LR is <1000, the comparison shall be reported as inconclusive, or an elimination, based on the likelihood ratio calculated (an LR of 1-999 is inconclusive, while an LR of <1 is an elimination). No probative comparisons will be made to this profile, and reported out as "No additional comparisons to item #XX will be made".

4) Mixtures containing 6 persons or more may be considered too complex for determination of conditioning depending on the number of unambiguous and ambiguous profiles associated with the sample. These comparisons should be reported out as uninterpretable for STRmix, as in 39.10.4.1.

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39.14.4 Likelihood Ratio

A conservative 1-sided lower bound HPD (highest posterior density) LR value is reported for GF DNA profiles using STRmix™ for the population with the lowest LR. Exceptions can be made if the scenario indicates an alternate LR is more appropriate.

Likelihood ratios are rounded down when reported as listed in the table below:

<1	Elimination, no LR reported
1-999	Comparison inconclusive, no LR reported
1000 – 100 billion	LR rounded down—use up to two significant figures
>100 billion	Ceiling—report LR is at least 100 billion

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39.14.5 Reporting Comparisons using STRmix™ - See DNA SOP 6.

39.15 **5+ Person Mixtures with a Clear Major Profile**

39.15.1 During analysis and number of contributor estimation, an analyst can determine (with TL and DD approval) that a 5 or more person mixture has a clear major contributor. The following criteria for this must be met at ≥ 10 loci. If any of the following criteria are not met, the locus does not qualify for statistics:

39.15.1.1 All of the alleles of the major contributor are ≥ ST (500 RFU, 700 RFU).

39.15.1.2 The heterozygous peak balance of the major profile ≥ 65%

39.15.1.3 For an apparent heterozygous major, the height of the highest minor allele is ≤ 33% of the height of the lowest major allele

39.15.1.4 For an apparent homozygous major, the height of the highest minor allele is ≤ 25% of the height of the major allele

39.15.2 After determining the number of contributors to be 5 or greater, the analyst will determine if a clear major profile is present. A macro has been created to assist in this determination. The exported allele table text file will be imported into the GlobalFiler Deconvolution Workbook. If a clear major is present at 10 or more loci, print out DNA QR-318, and keep as a part of the case record. Required TL and DD approval will be recorded on this QR. The presence of a clear major profile will be noted on DNA-QR-302 Contributor Estimation Worksheet.

39.15.3 Qualitative comparisons to known profiles. Known profiles are compared to the clear major profile as described in 39.10.

39.15.3.1 The alleles of the major profile should typically be called at the remaining loci that do not qualify for statistics. Note: this is not checked by the deconvolution macro.

39.15.4 Statistics for Clear Major Profiles

39.15.4.1 If a known sample is positively associated with a clear major profile, a binary LR (1/RMP) for the major contributor (using the Popstats database with posterior means distribution, NRC 4.2, and a factor of 10 lower bound confidence interval) is calculated. The population with the lowest LR is reported.

39.15.4.2 Repeat the steps in 39.15.2, when you determined a clear major was present, then click on "LR for Major". DNA-QR-318a, Likelihood Ratio, is printed out for the case jacket. "Min LR/10" will be reported.

39.15.4.3 Since statistics are able to be calculated, profiles that have a clear major are eligible for CODIS, with CODIS Administrator approval.

39.15.5 Reporting Out Complex Mixtures with Clear Majors

39.15.5.1 Reports without comparisons to knowns: Major profile will not be included in the Testing Summary table, or the Appendix. Remarks section will not discuss complexity of this mixture.

39.15.5.2 Reports with comparisons to knowns: Both profile and major profile will be included in Conclusions Summary table. If there is a known consistent to the source of the major profile, the comparison to the full profile is N/A. If there is a known eliminated as the source of the major profile, the Conclusions Summary table will note "No Comparison" to the full profile.

39.15.5.3 For report wording, see DNA SOP-6.

39.16 Criminal Parentage Testing:

For criminal parentage testing, standard statistical methods for GlobalFiler results will be used as described below:

39.16.1 The statistic calculated (for inclusions) is the expected frequency (parentage inclusion probability) of individuals who could contribute the paternally (or maternally) transmitted alleles. The formula used is: $p^2 + 2p(1-p) = 2p-p^2$, where p is the frequency of the obligatory allele.

RETIRED

39.16.2 No statistics are required for non-matches. Due to the occurrence of mutations between generations, an individual must be excluded at more than two loci in order to be eliminated as a potential parent. Regarding apparent mutations using STR systems, the repeat # difference of the putative mutation (child's obligatory allele vs. alleged parent's alleles typically varies by +/- 1 repeat) may be relevant to the final conclusion. In the event of apparent mutations, additional testing may be conducted as appropriate.

39.16.3 The RMNE ceiling that is reported for GlobalFiler criminal parentage testing is 1 in 100 billion.

39.16.4 In the event that the evidentiary sample is a maternal/child mixture, the fetal/child component is deconvoluted as described in DNA SOP-21using the CT DSS analytical and stochastic thresholds.

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41.1 Stutter Thresholds

41.1.1 GlobalFiler

GlobalFiler Standard (STD) and Low Amp Data Stutter Thresholds (≤29 cycles)

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
D3S1358	8	6.9	9.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	8.1	6.9	9.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	8.2	6.9	9.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	8.3	6.9	9.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	9	6.9	9.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	9.1	6.9	9.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	9.2	6.9	9.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	9.3	6.9	9.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	10	7.6	10.4	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	10.1	7.6	10.4	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	10.2	7.6	10.4	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	10.3	7.6	10.4	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	11	8.3	11.1	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	11.1	8.3	11.1	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	11.2	8.3	11.1	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	11.3	8.3	11.1	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	12	9	11.8	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	12.1	9	11.8	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	12.2	9	11.8	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	12.3	9	11.8	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	13	9.8	12.6	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	13.1	9.8	12.6	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	13.2	9.8	12.6	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	13.3	9.8	12.6	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	14	10.5	13.3	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	14.1	10.5	13.3	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	14.2	10.5	13.3	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	14.3	10.5	13.3	4.6	6.4	1.3	2.3	1.6	1.6	0	0
D3S1358	15	11.2	14	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	15.1	11.2	14	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	15.2	11.2	14	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	15.3	11.2	14	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	16	11.9	14.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	16.1	11.9	14.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	16.2	11.9	14.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	16.3	11.9	14.7	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	17	12.6	15.4	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	17.1	12.6	15.4	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	17.2	12.6	15.4	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	17.3	12.6	15.4	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	18	13.3	16.1	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	18.1	13.3	16.1	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	18.2	13.3	16.1	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	18.3	13.3	16.1	4.6	6.4	1.3	2.3	1.6	1.6	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	19	14	16.8	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	19.1	14	16.8	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	19.2	14	16.8	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	19.3	14	16.8	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	20	14.7	17.5	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	20.1	14.7	17.5	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	20.2	14.7	17.5	4.6	6.4	1.3	2.3	1.6	1.6	0	0
	20.3	14.7	17.5	4.6	6.4	1.3	2.3	1.6	1.6	0	0
D3S1358	21	15.4	18.2	4.6	6.4	1.3	2.3	1.6	1.6	0	0
vWA	10	7.9	12.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	10.1	7.9	12.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	10.2	7.9	12.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	10.3	7.9	12.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	11	7.9	12.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	11.1	7.9	12.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	11.2	7.9	12.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	11.3	7.9	12.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	12	8.7	13.6	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	12.1	8.7	13.6	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	12.2	8.7	13.6	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	12.3	8.7	13.6	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	13	9.5	14.4	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	13.1	9.5	14.4	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	13.2	9.5	14.4	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	13.3	9.5	14.4	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	14	10.4	15.3	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	14.1	10.4	15.3	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	14.2	10.4	15.3	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	14.3	10.4	15.3	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	15	11.2	16.1	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	15.1	11.2	16.1	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	15.2	11.2	16.1	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	15.3	11.2	16.1	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	16	12.1	17	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	16.1	12.1	17	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	16.2	12.1	17	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	16.3	12.1	17	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	17	12.9	17.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	17.1	12.9	17.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
vWA	17.2	12.9	17.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	17.3	12.9	17.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	18	13.7	18.6	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	18.1	13.7	18.6	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	18.2	13.7	18.6	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	18.3	13.7	18.6	2.7	2.7	2.1	2.1	1.7	1.7	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	19	14.6	19.5	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	19.1	14.6	19.5	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	19.2	14.6	19.5	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	19.3	14.6	19.5	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	20	15.4	20.3	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	20.1	15.4	20.3	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	20.2	15.4	20.3	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	20.3	15.4	20.3	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	21	16.3	21.2	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	21.1	16.3	21.2	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	21.2	16.3	21.2	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	21.3	16.3	21.2	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	22	17.1	22	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	22.1	17.1	22	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	22.2	17.1	22	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	22.3	17.1	22	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	23	17.9	22.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	23.1	17.9	22.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	23.2	17.9	22.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	23.3	17.9	22.8	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	24	18.8	23.7	2.7	2.7	2.1	2.1	1.7	1.7	0	0
vWA	24.1	18.8	23.7	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	24.2	18.8	23.7	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	24.3	18.8	23.7	2.7	2.7	2.1	2.1	1.7	1.7	0	0
	25	19.6	24.5	2.7	2.7	2.1	2.1	1.7	1.7	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
D16S539	4	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	4.1	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	4.2	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	4.3	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	5	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	5.1	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	5.2	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	5.3	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	6	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	6.1	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	6.2	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	6.3	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	7	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	7.1	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	7.2	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	7.3	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	8	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	8.1	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	8.2	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	8.3	5.8	10.9	2.3	2.3	1	1	1	1	0	0
	9	6.7	11.8	2.3	2.3	1	1	1	1	0	0
	9.1	6.7	11.8	2.3	2.3	1	1	1	1	0	0
	9.2	6.7	11.8	2.3	2.3	1	1	1	1	0	0
	9.3	6.7	11.8	2.3	2.3	1	1	1	1	0	0
D16S539	10	7.5	12.6	2.3	2.3	1	1	1	1	0	0
	10.1	7.5	12.6	2.3	2.3	1	1	1	1	0	0
	10.2	7.5	12.6	2.3	2.3	1	1	1	1	0	0
	10.3	7.5	12.6	2.3	2.3	1	1	1	1	0	0
	11	8.4	13.5	2.3	2.3	1	1	1	1	0	0
	11.1	8.4	13.5	2.3	2.3	1	1	1	1	0	0
	11.2	8.4	13.5	2.3	2.3	1	1	1	1	0	0
	11.3	8.4	13.5	2.3	2.3	1	1	1	1	0	0
	12	9.3	14.4	2.3	2.3	1	1	1	1	0	0
	12.1	9.3	14.4	2.3	2.3	1	1	1	1	0	0
	12.2	9.3	14.4	2.3	2.3	1	1	1	1	0	0
	12.3	9.3	14.4	2.3	2.3	1	1	1	1	0	0
	13	10.1	15.2	2.3	2.3	1	1	1	1	0	0
	13.1	10.1	15.2	2.3	2.3	1	1	1	1	0	0
	13.2	10.1	15.2	2.3	2.3	1	1	1	1	0	0
	13.3	10.1	15.2	2.3	2.3	1	1	1	1	0	0
	14	11	16.1	2.3	2.3	1	1	1	1	0	0
	14.1	11	16.1	2.3	2.3	1	1	1	1	0	0
	14.2	11	16.1	2.3	2.3	1	1	1	1	0	0
	14.3	11	16.1	2.3	2.3	1	1	1	1	0	0
	15	11.9	17	2.3	2.3	1	1	1	1	0	0
	15.1	11.9	17	2.3	2.3	1	1	1	1	0	0
	15.2	11.9	17	2.3	2.3	1	1	1	1	0	0
	15.3	11.9	17	2.3	2.3	1	1	1	1	0	0
D16S539	16	12.8	17.9	2.3	2.3	1	1	1	1	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
CSF1PO	5	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	5.1	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	5.2	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	5.3	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	6	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	6.1	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	6.2	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	6.3	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	7	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	7.1	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	7.2	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	7.3	4.1	8.7	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	8	4.9	9.5	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	8.1	4.9	9.5	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	8.2	4.9	9.5	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	8.3	4.9	9.5	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	9	5.8	10.4	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	9.1	5.8	10.4	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	9.2	5.8	10.4	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	9.3	5.8	10.4	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	10	6.7	11.3	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	10.1	6.7	11.3	2.2	2.2	0.9	0.9	0	0	1.6	1.6
CSF1PO	10.2	6.7	11.3	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	10.3	6.7	11.3	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	11	7.6	12.2	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	11.1	7.6	12.2	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	11.2	7.6	12.2	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	11.3	7.6	12.2	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	12	8.4	13	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	12.1	8.4	13	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	12.2	8.4	13	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	12.3	8.4	13	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	13	9.3	13.9	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	13.1	9.3	13.9	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	13.2	9.3	13.9	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	13.3	9.3	13.9	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	14	10.2	14.8	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	14.1	10.2	14.8	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	14.2	10.2	14.8	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	14.3	10.2	14.8	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	15	11	15.6	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	15.1	11	15.6	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	15.2	11	15.6	2.2	2.2	0.9	0.9	0	0	1.6	1.6
	15.3	11	15.6	2.2	2.2	0.9	0.9	0	0	1.6	1.6
CSF1PO	16	11.9	16.5	2.2	2.2	0.9	0.9	0	0	1.6	1.6

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
TPOX	4	2	4.5	1.4	1.4	0	0	0	0	0	0
	4.1	2	4.5	1.4	1.4	0	0	0	0	0	0
	4.2	2	4.5	1.4	1.4	0	0	0	0	0	0
	4.3	2	4.5	1.4	1.4	0	0	0	0	0	0
	5	2	4.5	1.4	1.4	0	0	0	0	0	0
	5.1	2	4.5	1.4	1.4	0	0	0	0	0	0
	5.2	2	4.5	1.4	1.4	0	0	0	0	0	0
	5.3	2	4.5	1.4	1.4	0	0	0	0	0	0
	6	2	4.5	1.4	1.4	0	0	0	0	0	0
TPOX	6.1	2	4.5	1.4	1.4	0	0	0	0	0	0
	6.2	2	4.5	1.4	1.4	0	0	0	0	0	0
	6.3	2	4.5	1.4	1.4	0	0	0	0	0	0
	7	2.5	5	1.4	1.4	0	0	0	0	0	0
	7.1	2.5	5	1.4	1.4	0	0	0	0	0	0
	7.2	2.5	5	1.4	1.4	0	0	0	0	0	0
	7.3	2.5	5	1.4	1.4	0	0	0	0	0	0
	8	3	5.5	1.4	1.4	0	0	0	0	0	0
	8.1	3	5.5	1.4	1.4	0	0	0	0	0	0
	8.2	3	5.5	1.4	1.4	0	0	0	0	0	0
	8.3	3	5.5	1.4	1.4	0	0	0	0	0	0
	9	3.5	6	1.4	1.4	0	0	0	0	0	0
	9.1	3.5	6	1.4	1.4	0	0	0	0	0	0
	9.2	3.5	6	1.4	1.4	0	0	0	0	0	0
	9.3	3.5	6	1.4	1.4	0	0	0	0	0	0
TPOX	10	4	6.5	1.4	1.4	0	0	0	0	0	0
	10.1	4	6.5	1.4	1.4	0	0	0	0	0	0
	10.2	4	6.5	1.4	1.4	0	0	0	0	0	0
	10.3	4	6.5	1.4	1.4	0	0	0	0	0	0
	11	4.6	7.1	1.4	1.4	0	0	0	0	0	0
	11.1	4.6	7.1	1.4	1.4	0	0	0	0	0	0
	11.2	4.6	7.1	1.4	1.4	0	0	0	0	0	0
	11.3	4.6	7.1	1.4	1.4	0	0	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	12	5.1	7.6	1.4	1.4	0	0	0	0	0	0
	12.1	5.1	7.6	1.4	1.4	0	0	0	0	0	0
	12.2	5.1	7.6	1.4	1.4	0	0	0	0	0	0
	12.3	5.1	7.6	1.4	1.4	0	0	0	0	0	0
	13	5.6	8.1	1.4	1.4	0	0	0	0	0	0
	13.1	5.6	8.1	1.4	1.4	0	0	0	0	0	0
	13.2	5.6	8.1	1.4	1.4	0	0	0	0	0	0
	13.3	5.6	8.1	1.4	1.4	0	0	0	0	0	0
	14	6.1	8.6	1.4	1.4	0	0	0	0	0	0
	14.1	6.1	8.6	1.4	1.4	0	0	0	0	0	0
	14.2	6.1	8.6	1.4	1.4	0	0	0	0	0	0
	14.3	6.1	8.6	1.4	1.4	0	0	0	0	0	0
	15	6.6	9.1	1.4	1.4	0	0	0	0	0	0
	15.1	6.6	9.1	1.4	1.4	0	0	0	0	0	0
	15.2	6.6	9.1	1.4	1.4	0	0	0	0	0	0
	15.3	6.6	9.1	1.4	1.4	0	0	0	0	0	0
TPOX	16	7.1	9.6	1.4	1.4	0	0	0	0	0	0
D8S1179	4	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	4.1	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	4.2	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	4.3	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	5	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	5.1	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	5.2	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	5.3	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	6	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	6.1	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	6.2	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	6.3	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	7	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	7.1	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	7.2	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	7.3	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	8	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	8.1	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	8.2	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	8.3	10.4	14.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	9	10.7	14.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	9.1	10.7	14.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	9.2	10.7	14.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	9.3	10.7	14.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	10	11.1	15.2	2.8	3.6	1.4	3	4.3	4.9	4	4
	10.1	11.1	15.2	2.8	3.6	1.4	3	4.3	4.9	4	4
	10.2	11.1	15.2	2.8	3.6	1.4	3	4.3	4.9	4	4
	10.3	11.1	15.2	2.8	3.6	1.4	3	4.3	4.9	4	4
	11	11.4	15.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	11.1	11.4	15.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	11.2	11.4	15.5	2.8	3.6	1.4	3	4.3	4.9	4	4
	11.3	11.4	15.5	2.8	3.6	1.4	3	4.3	4.9	4	4

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
D8S1179	12	11.7	15.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	12.1	11.7	15.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	12.2	11.7	15.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	12.3	11.7	15.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	13	12	16.1	2.8	3.6	1.4	3	4.3	4.9	4	4
	13.1	12	16.1	2.8	3.6	1.4	3	4.3	4.9	4	4
	13.2	12	16.1	2.8	3.6	1.4	3	4.3	4.9	4	4
	13.3	12	16.1	2.8	3.6	1.4	3	4.3	4.9	4	4
	14	12.3	16.4	2.8	3.6	1.4	3	4.3	4.9	4	4
	14.1	12.3	16.4	2.8	3.6	1.4	3	4.3	4.9	4	4
	14.2	12.3	16.4	2.8	3.6	1.4	3	4.3	4.9	4	4
	14.3	12.3	16.4	2.8	3.6	1.4	3	4.3	4.9	4	4
	15	12.7	16.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	15.1	12.7	16.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	15.2	12.7	16.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	15.3	12.7	16.8	2.8	3.6	1.4	3	4.3	4.9	4	4
	16	13	17.1	2.8	3.6	1.4	3	4.3	4.9	4	4
	16.1	13	17.1	2.8	3.6	1.4	3	4.3	4.9	4	4
	16.2	13	17.1	2.8	3.6	1.4	3	4.3	4.9	4	4
	16.3	13	17.1	2.8	3.6	1.4	3	4.3	4.9	4	4
	17	13.3	17.4	2.8	3.6	1.4	3	4.3	4.9	4	4
	17.1	13.3	17.4	2.8	3.6	1.4	3	4.3	4.9	4	4
	17.2	13.3	17.4	2.8	3.6	1.4	3	4.3	4.9	4	4
	17.3	13.3	17.4	2.8	3.6	1.4	3	4.3	4.9	4	4
	18	13.6	17.7	2.8	3.6	1.4	3	4.3	4.9	4	4
D8S1179	18.1	13.6	17.7	2.8	3.6	1.4	3	4.3	4.9	4	4
	18.2	13.6	17.7	2.8	3.6	1.4	3	4.3	4.9	4	4
	18.3	13.6	17.7	2.8	3.6	1.4	3	4.3	4.9	4	4
	19	13.9	18	2.8	3.6	1.4	3	4.3	4.9	4	4
	19.1	13.9	18	2.8	3.6	1.4	3	4.3	4.9	4	4
	19.2	13.9	18	2.8	3.6	1.4	3	4.3	4.9	4	4
	19.3	13.9	18	2.8	3.6	1.4	3	4.3	4.9	4	4
	20	14.3	18.4	2.8	3.6	1.4	3	4.3	4.9	4	4
D21S11	23.2	10.6	15.4	2.7	4.4	3.4	3.4	0	0	0	0
	23.3	8	12.8	2.7	4.4	3.4	3.4	0	0	0	0
	24	8	12.8	2.7	4.4	3.4	3.4	0	0	0	0
	24.1	8	12.8	2.7	4.4	3.4	3.4	0	0	0	0
	24.2	10.6	15.4	2.7	4.4	3.4	3.4	0	0	0	0
	24.3	8	12.8	2.7	4.4	3.4	3.4	0	0	0	0
	25	8	12.8	2.7	4.4	3.4	3.4	0	0	0	0
	25.1	8	12.8	2.7	4.4	3.4	3.4	0	0	0	0
	25.2	10.6	15.4	2.7	4.4	3.4	3.4	0	0	0	0
	25.3	8	12.8	2.7	4.4	3.4	3.4	0	0	0	0
	26	8	12.8	2.7	4.4	3.4	3.4	0	0	0	0
	26.1	8	12.8	2.7	4.4	3.4	3.4	0	0	0	0
	26.2	10.6	15.4	2.7	4.4	3.4	3.4	0	0	0	0
	26.3	8	12.8	2.7	4.4	3.4	3.4	0	0	0	0
	27	8.7	13.5	2.7	4.4	3.4	3.4	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	27.1	8.7	13.5	2.7	4.4	3.4	3.4	0	0	0	0
	27.2	10.6	15.4	2.7	4.4	3.4	3.4	0	0	0	0
	27.3	8.7	13.5	2.7	4.4	3.4	3.4	0	0	0	0
	28	9.5	14.3	2.7	4.4	3.4	3.4	0	0	0	0
	28.1	9.5	14.3	2.7	4.4	3.4	3.4	0	0	0	0
	28.2	10.6	15.4	2.7	4.4	3.4	3.4	0	0	0	0
	28.3	9.5	14.3	2.7	4.4	3.4	3.4	0	0	0	0
	29	10.2	15	2.7	4.4	3.4	3.4	0	0	0	0
	29.1	10.2	15	2.7	4.4	3.4	3.4	0	0	0	0
	29.2	10.6	15.4	2.7	4.4	3.4	3.4	0	0	0	0
	29.3	10.2	15	2.7	4.4	3.4	3.4	0	0	0	0
	30	11	15.8	2.7	4.4	3.4	3.4	0	0	0	0
	30.1	11	15.8	2.7	4.4	3.4	3.4	0	0	0	0
	30.2	10.6	15.4	2.7	4.4	3.4	3.4	0	0	0	0
D21S11	30.3	11	15.8	2.7	4.4	3.4	3.4	0	0	0	0
	31	11.7	16.5	2.7	4.4	3.4	3.4	0	0	0	0
	31.1	11.7	16.5	2.7	4.4	3.4	3.4	0	0	0	0
	31.2	11.4	16.2	2.7	4.4	3.4	3.4	0	0	0	0
	31.3	11.7	16.5	2.7	4.4	3.4	3.4	0	0	0	0
	32	12.5	17.3	2.7	4.4	3.4	3.4	0	0	0	0
	32.1	12.5	17.3	2.7	4.4	3.4	3.4	0	0	0	0
	32.2	12.3	17.1	2.7	4.4	3.4	3.4	0	0	0	0
	32.3	12.5	17.3	2.7	4.4	3.4	3.4	0	0	0	0
	33	13.2	18	2.7	4.4	3.4	3.4	0	0	0	0
	33.1	13.2	18	2.7	4.4	3.4	3.4	0	0	0	0
	33.2	13.1	17.9	2.7	4.4	3.4	3.4	0	0	0	0
	33.3	13.2	18	2.7	4.4	3.4	3.4	0	0	0	0
	34	14	18.8	2.7	4.4	3.4	3.4	0	0	0	0
	34.1	14	18.8	2.7	4.4	3.4	3.4	0	0	0	0
	34.2	13.9	18.7	2.7	4.4	3.4	3.4	0	0	0	0
	34.3	14	18.8	2.7	4.4	3.4	3.4	0	0	0	0
D21S11	35	14.7	19.5	2.7	4.4	3.4	3.4	0	0	0	0
	35.1	14.7	19.5	2.7	4.4	3.4	3.4	0	0	0	0
	35.2	14.7	19.5	2.7	4.4	3.4	3.4	0	0	0	0
	35.3	14.7	19.5	2.7	4.4	3.4	3.4	0	0	0	0
	36	15.5	20.3	2.7	4.4	3.4	3.4	0	0	0	0
	36.1	15.5	20.3	2.7	4.4	3.4	3.4	0	0	0	0
	36.2	15.5	20.3	2.7	4.4	3.4	3.4	0	0	0	0
	36.3	15.5	20.3	2.7	4.4	3.4	3.4	0	0	0	0
	37	16.2	21	2.7	4.4	3.4	3.4	0	0	0	0
	37.1	16.2	21	2.7	4.4	3.4	3.4	0	0	0	0
	37.2	16.3	21.1	2.7	4.4	3.4	3.4	0	0	0	0
	37.3	16.2	21	2.7	4.4	3.4	3.4	0	0	0	0
	38	17	21.8	2.7	4.4	3.4	3.4	0	0	0	0
	38.1	17	21.8	2.7	4.4	3.4	3.4	0	0	0	0
	38.2	17.1	21.9	2.7	4.4	3.4	3.4	0	0	0	0
	38.3	17	21.8	2.7	4.4	3.4	3.4	0	0	0	0
D21S11	39	17.7	22.5	2.7	4.4	3.4	3.4	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
D18S51	6	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	6.1	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	6.2	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	6.3	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	7	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	7.1	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	7.2	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	7.3	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	8	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	8.1	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	8.2	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	8.3	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	9	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	9.1	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	9.2	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	9.3	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	10	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	10.1	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	10.2	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	10.3	6.2	10.8	3.4	6	0.7	0.7	0	0	0	0
	11	6.9	11.5	3.4	6	0.7	0.7	0	0	0	0
	11.1	6.9	11.5	3.4	6	0.7	0.7	0	0	0	0
	11.2	6.9	11.5	3.4	6	0.7	0.7	0	0	0	0
	11.3	6.9	11.5	3.4	6	0.7	0.7	0	0	0	0
	12	7.6	12.2	3.4	6	0.7	0.7	0	0	0	0
	12.1	7.6	12.2	3.4	6	0.7	0.7	0	0	0	0
	12.2	7.6	12.2	3.4	6	0.7	0.7	0	0	0	0
	12.3	7.6	12.2	3.4	6	0.7	0.7	0	0	0	0
	13	8.3	12.9	3.4	6	0.8	0.8	0	0	0	0
	13.1	8.3	12.9	3.4	6	0.8	0.8	0	0	0	0
D18S51	13.2	8.3	12.9	3.4	6	0.8	0.8	0	0	0	0
	13.3	8.3	12.9	3.4	6	0.8	0.8	0	0	0	0
	14	8.9	13.5	3.4	6	0.9	0.9	0	0	0	0
	14.1	8.9	13.5	3.4	6	0.9	0.9	0	0	0	0
	14.2	8.9	13.5	3.4	6	0.9	0.9	0	0	0	0
	14.3	8.9	13.5	3.4	6	0.9	0.9	0	0	0	0
	15	9.6	14.2	3.4	6	1	1	0	0	0	0
D18S51	15.1	9.6	14.2	3.4	6	1	1	0	0	0	0
	15.2	9.6	14.2	3.4	6	1	1	0	0	0	0
	15.3	9.6	14.2	3.4	6	1	1	0	0	0	0
	16	10.3	14.9	3.4	6	1	1	0	0	0	0
	16.1	10.3	14.9	3.4	6	1	1	0	0	0	0
	16.2	10.3	14.9	3.4	6	1	1	0	0	0	0
	16.3	10.3	14.9	3.4	6	1	1	0	0	0	0
	17	11	15.6	3.4	6	1.1	1.1	0	0	0	0
	17.1	11	15.6	3.4	6	1.1	1.1	0	0	0	0
	17.2	11	15.6	3.4	6	1.1	1.1	0	0	0	0
	17.3	11	15.6	3.4	6	1.1	1.1	0	0	0	0
	18	11.7	16.3	3.4	6	1.2	1.2	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	18.1	11.7	16.3	3.4	6	1.2	1.2	0	0	0	0
	18.2	11.7	16.3	3.4	6	1.2	1.2	0	0	0	0
	18.3	11.7	16.3	3.4	6	1.2	1.2	0	0	0	0
	19	12.3	16.9	3.4	6	1.3	1.3	0	0	0	0
	19.1	12.3	16.9	3.4	6	1.3	1.3	0	0	0	0
	19.2	12.3	16.9	3.4	6	1.3	1.3	0	0	0	0
	19.3	12.3	16.9	3.4	6	1.3	1.3	0	0	0	0
	20	13	17.6	3.4	6	1.4	1.4	0	0	0	0
	20.1	13	17.6	3.4	6	1.4	1.4	0	0	0	0
D18S51	20.2	13	17.6	3.4	6	1.4	1.4	0	0	0	0
	20.3	13	17.6	3.4	6	1.4	1.4	0	0	0	0
	21	13.7	18.3	3.4	6	1.4	1.4	0	0	0	0
	21.1	13.7	18.3	3.4	6	1.4	1.4	0	0	0	0
	21.2	13.7	18.3	3.4	6	1.4	1.4	0	0	0	0
	21.3	13.7	18.3	3.4	6	1.4	1.4	0	0	0	0
	22	14.4	19	3.4	6	1.5	1.5	0	0	0	0
	22.1	14.4	19	3.4	6	1.5	1.5	0	0	0	0
	22.2	14.4	19	3.4	6	1.5	1.5	0	0	0	0
	22.3	14.4	19	3.4	6	1.5	1.5	0	0	0	0
	23	15.1	19.7	3.4	6	1.6	1.6	0	0	0	0
	23.1	15.1	19.7	3.4	6	1.6	1.6	0	0	0	0
	23.2	15.1	19.7	3.4	6	1.6	1.6	0	0	0	0
	23.3	15.1	19.7	3.4	6	1.6	1.6	0	0	0	0
	24	15.7	20.3	3.4	6	1.7	1.7	0	0	0	0
	24.1	15.7	20.3	3.4	6	1.7	1.7	0	0	0	0
	24.2	15.7	20.3	3.4	6	1.7	1.7	0	0	0	0
	24.3	15.7	20.3	3.4	6	1.7	1.7	0	0	0	0
	25	16.4	21	3.4	6	1.8	1.8	0	0	0	0
	25.1	16.4	21	3.4	6	1.8	1.8	0	0	0	0
	25.2	16.4	21	3.4	6	1.8	1.8	0	0	0	0
	25.3	16.4	21	3.4	6	1.8	1.8	0	0	0	0
	26	17.1	21.7	3.4	6	1.8	1.8	0	0	0	0
	26.1	17.1	21.7	3.4	6	1.8	1.8	0	0	0	0
	26.2	17.1	21.7	3.4	6	1.8	1.8	0	0	0	0
	26.3	17.1	21.7	3.4	6	1.8	1.8	0	0	0	0
	27	17.8	22.4	3.4	6	1.9	1.9	0	0	0	0
	27.1	17.8	22.4	3.4	6	1.9	1.9	0	0	0	0
	27.2	17.8	22.4	3.4	6	1.9	1.9	0	0	0	0
	27.3	17.8	22.4	3.4	6	1.9	1.9	0	0	0	0
D18S51	28	18.5	23.1	3.4	6	2	2	0	0	0	0
DYS391	6	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	6.1	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	6.2	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	6.3	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	7	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	7.1	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	7.2	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	7.3	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	8	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	8.1	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	8.2	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	8.3	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	9	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	9.1	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	9.2	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	9.3	7.4	11.8	3.9	3.9	1.4	1.4	0	0	0	0
	10	8.3	12.7	3.9	3.9	1.4	1.4	0	0	0	0
	10.1	8.3	12.7	3.9	3.9	1.4	1.4	0	0	0	0
	10.2	8.3	12.7	3.9	3.9	1.4	1.4	0	0	0	0
	10.3	8.3	12.7	3.9	3.9	1.4	1.4	0	0	0	0
	11	9.2	13.6	3.9	3.9	1.4	1.4	0	0	0	0
	11.1	9.2	13.6	3.9	3.9	1.4	1.4	0	0	0	0
	11.2	9.2	13.6	3.9	3.9	1.4	1.4	0	0	0	0
	11.3	9.2	13.6	3.9	3.9	1.4	1.4	0	0	0	0
	12	10.1	14.5	3.9	3.9	1.4	1.4	0	0	0	0
	12.1	10.1	14.5	3.9	3.9	1.4	1.4	0	0	0	0
	12.2	10.1	14.5	3.9	3.9	1.4	1.4	0	0	0	0
	12.3	10.1	14.5	3.9	3.9	1.4	1.4	0	0	0	0
	13	11	15.4	3.9	3.9	1.4	1.4	0	0	0	0
	13.1	11	15.4	3.9	3.9	1.4	1.4	0	0	0	0
	13.2	11	15.4	3.9	3.9	1.4	1.4	0	0	0	0
	13.3	11	15.4	3.9	3.9	1.4	1.4	0	0	0	0
DYS391	14	11.9	16.3	3.9	3.9	1.4	1.4	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
D2S441	8	7	11.7	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	8.1	7	11.7	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	8.2	7	11.7	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	8.3	5.2	9.9	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	9	7	11.7	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	9.1	7	11.7	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	9.2	7	11.7	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	9.3	5.2	9.9	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	10	8.3	13	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	10.1	8.3	13	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	10.2	8.3	13	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	10.3	5.2	9.9	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	11	9.5	14.2	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	11.1	9.5	14.2	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	11.2	9.5	14.2	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	11.3	5.2	9.9	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	12	10.8	15.5	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	12.1	10.8	15.5	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	12.2	10.8	15.5	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	12.3	6	10.7	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	13	6.2	10.9	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	13.1	6.2	10.9	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	13.2	6.2	10.9	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	13.3	6.8	11.5	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	14	7	11.7	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	14.1	7	11.7	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	14.2	7	11.7	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	14.3	7.6	12.3	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	15	7.8	12.5	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	15.1	7.8	12.5	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	15.2	7.8	12.5	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	15.3	8.3	13	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	16	8.5	13.2	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	16.1	8.5	13.2	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	16.2	8.5	13.2	2.7	4.2	1.7	1.7	2.3	3.1	0	0
	16.3	9.1	13.8	2.7	4.2	1.7	1.7	2.3	3.1	0	0
D2S441	17	9.3	14	2.7	4.2	1.7	1.7	2.3	3.1	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
D19S433	5.2	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	5.3	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	6	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	6.1	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	6.2	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	6.3	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	7	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	7.1	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	7.2	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	7.3	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	8	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	8.1	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	8.2	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	8.3	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	9	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	9.1	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	9.2	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
D19S433	9.3	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	10	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	10.1	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	10.2	6.8	10	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	10.3	6.4	9.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	11	7.2	10.4	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	11.1	7.2	10.4	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	11.2	7.6	10.8	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	11.3	7.2	10.4	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	12	8	11.2	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	12.1	8	11.2	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	12.2	8.3	11.5	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	12.3	8	11.2	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	13	8.7	11.9	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	13.1	8.7	11.9	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	13.2	9.1	12.3	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	13.3	8.7	11.9	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	14	9.5	12.7	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	14.1	9.5	12.7	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	14.2	9.9	13.1	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	14.3	9.5	12.7	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	15	10.3	13.5	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	15.1	10.3	13.5	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	15.2	10.7	13.9	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	15.3	10.3	13.5	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	16	11	14.2	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	16.1	11	14.2	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	16.2	11.4	14.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	16.3	11	14.2	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	17	11.8	15	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	17.1	11.8	15	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	17.2	12.2	15.4	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	17.3	11.8	15	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	18	12.6	15.8	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	18.1	12.6	15.8	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	18.2	13	16.2	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	18.3	12.6	15.8	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	19	13.4	16.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
	19.1	13.4	16.6	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
D19S433	19.2	13.7	16.9	2.9	2.9	2.4	3.3	4.5	4.5	1.4	1.4
TH01	3	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	3.1	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	3.2	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	3.3	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	4	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	4.1	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	4.2	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	4.3	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	5	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	5.1	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	5.2	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	5.3	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	6	3.6	6.4	0	0	0	0	1.2	1.2	1.2	1.2
	6.1	3.6	6.4	0	0	0	0	1.2	1.2	1.2	1.2
TH01	6.2	3.6	6.4	0	0	0	0	1.2	1.2	1.2	1.2
	6.3	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	7	4	6.8	0	0	0	0	1.2	1.2	1.2	1.2
	7.1	4	6.8	0	0	0	0	1.2	1.2	1.2	1.2
	7.2	4	6.8	0	0	0	0	1.2	1.2	1.2	1.2
	7.3	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	8	4.5	7.3	0	0	0	0	1.2	1.2	1.2	1.2
	8.1	4.5	7.3	0	0	0	0	1.2	1.2	1.2	1.2
	8.2	4.5	7.3	0	0	0	0	1.2	1.2	1.2	1.2
	8.3	3.1	5.9	0	0	0	0	1.2	1.2	1.2	1.2
	9	5	7.8	0	0	0	0	1.2	1.2	1.2	1.2
	9.1	5	7.8	0	0	0	0	1.2	1.2	1.2	1.2
	9.2	5	7.8	0	0	0	0	1.2	1.2	1.2	1.2
	9.3	3.6	6.4	0	0	0	0	1.2	1.2	1.2	1.2
	10	5.5	8.3	0	0	0	0	1.2	1.2	1.2	1.2
	10.1	5.5	8.3	0	0	0	0	1.2	1.2	1.2	1.2
	10.2	5.5	8.3	0	0	0	0	1.2	1.2	1.2	1.2
	10.3	4	6.8	0	0	0	0	1.2	1.2	1.2	1.2
	11	6	8.8	0	0	0	0	1.2	1.2	1.2	1.2
	11.1	6	8.8	0	0	0	0	1.2	1.2	1.2	1.2
	11.2	6	8.8	0	0	0	0	1.2	1.2	1.2	1.2
	11.3	4.5	7.3	0	0	0	0	1.2	1.2	1.2	1.2
	12	6.4	9.2	0	0	0	0	1.2	1.2	1.2	1.2
	12.1	6.4	9.2	0	0	0	0	1.2	1.2	1.2	1.2
	12.2	6.4	9.2	0	0	0	0	1.2	1.2	1.2	1.2
	12.3	5	7.8	0	0	0	0	1.2	1.2	1.2	1.2
	13	6.9	9.7	0	0	0	0	1.2	1.2	1.2	1.2
	13.1	6.9	9.7	0	0	0	0	1.2	1.2	1.2	1.2
	13.2	6.9	9.7	0	0	0	0	1.2	1.2	1.2	1.2
TH01	13.3	5.5	8.3	0	0	0	0	1.2	1.2	1.2	1.2

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
FGA	12.2	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	12.3	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	13	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	13.1	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	13.2	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	13.3	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	14	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	14.1	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	14.2	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	14.3	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	15	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	15.1	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	15.2	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	15.3	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	16	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	16.1	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	16.2	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	16.3	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	17	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	17.1	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	17.2	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	17.3	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	18	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	18.1	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
FGA	18.2	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	18.3	7	10	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	19	7.7	10.7	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	19.1	7.7	10.7	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	19.2	7.7	10.7	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	19.3	7.7	10.7	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	20	8.4	11.4	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	20.1	8.4	11.4	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	20.2	8.4	11.4	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	20.3	8.4	11.4	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	21	9.1	12.1	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	21.1	9.1	12.1	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	21.2	9.1	12.1	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	21.3	9.1	12.1	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	22	9.8	12.8	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	22.1	9.8	12.8	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	22.2	9.8	12.8	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	22.3	9.8	12.8	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	23	10.5	13.5	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	23.1	10.5	13.5	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	23.2	10.5	13.5	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	23.3	10.5	13.5	3.2	4.8	1.6	1.6	0	0	1.3	1.3

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	24	11.2	14.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	24.1	11.2	14.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	24.2	11.2	14.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	24.3	11.2	14.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	25	11.9	14.9	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	25.1	11.9	14.9	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	25.2	11.9	14.9	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	25.3	11.9	14.9	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	26	12.6	15.6	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	26.1	12.6	15.6	3.2	4.8	1.6	1.6	0	0	1.3	1.3
FGA	26.2	12.6	15.6	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	26.3	12.6	15.6	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	27	13.3	16.3	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	27.1	13.3	16.3	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	27.2	13.3	16.3	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	27.3	13.3	16.3	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	28	14	17	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	28.1	14	17	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	28.2	14	17	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	28.3	14	17	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	29	14.7	17.7	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	29.1	14.7	17.7	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	29.2	14.7	17.7	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	29.3	14.7	17.7	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	30	15.4	18.4	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	30.1	15.4	18.4	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	30.2	15.4	18.4	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	30.3	15.4	18.4	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	31	16.1	19.1	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	31.1	16.1	19.1	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	31.2	16.1	19.1	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	31.3	16.1	19.1	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	32	16.8	19.8	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	32.1	16.8	19.8	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	32.2	16.8	19.8	3.2	4.8	1.6	1.6	0	0	1.3	1.3
FGA	32.3	16.8	19.8	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	33	17.5	20.5	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	33.1	17.5	20.5	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	33.2	17.5	20.5	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	33.3	17.5	20.5	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	34	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	34.1	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	34.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
FGA	41.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	41.3	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	42	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	42.1	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	42.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	42.3	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	43	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	43.1	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	43.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	43.3	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	44	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	44.1	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	44.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	44.3	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	45	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	45.1	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	45.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	45.3	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	46	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	46.1	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	46.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	46.3	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	47	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	47.1	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	47.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	47.3	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	48	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	48.1	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	48.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	48.3	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	49	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	49.1	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	49.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	49.3	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	50	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	50.1	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	50.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	50.3	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	51	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
	51.1	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
FGA	51.2	18.2	21.2	3.2	4.8	1.6	1.6	0	0	1.3	1.3
D22S1045	7	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0
	7.1	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0
	7.2	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0
	8	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0
	8.1	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0
	8.2	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0
	9	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0
	9.1	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0
	9.2	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0
	10	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0
	10.1	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0
	10.2	4.9	9.5	3.7	5.8	2.1	2.1	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	11	6.2	10.8	4.3	6.4	2.1	2.1	0	0	0	0
	11.1	6.2	10.8	4.3	6.4	2.1	2.1	0	0	0	0
	11.2	6.2	10.8	4.3	6.4	2.1	2.1	0	0	0	0
	12	7.5	12.1	4.9	7	2.1	2.1	0	0	0	0
	12.1	7.5	12.1	4.9	7	2.1	2.1	0	0	0	0
	12.2	7.5	12.1	4.9	7	2.1	2.1	0	0	0	0
D22S1045	13	8.8	13.4	5.5	7.6	2.1	2.1	0	0	0	0
	13.1	8.8	13.4	5.5	7.6	2.1	2.1	0	0	0	0
	13.2	8.8	13.4	5.5	7.6	2.1	2.1	0	0	0	0
	14	10.1	14.7	6.2	8.3	2.1	2.1	0	0	0	0
	14.1	10.1	14.7	6.2	8.3	2.1	2.1	0	0	0	0
	14.2	10.1	14.7	6.2	8.3	2.1	2.1	0	0	0	0
	15	11.4	16	6.8	8.9	2.1	2.1	0	0	0	0
	15.1	11.4	16	6.8	8.9	2.1	2.1	0	0	0	0
	15.2	11.4	16	6.8	8.9	2.1	2.1	0	0	0	0
	16	12.6	17.2	7.4	9.5	2.1	2.1	0	0	0	0
	16.1	12.6	17.2	7.4	9.5	2.1	2.1	0	0	0	0
	16.2	12.6	17.2	7.4	9.5	2.1	2.1	0	0	0	0
	17	13.9	18.5	8.1	10.2	2.1	2.1	0	0	0	0
	17.1	13.9	18.5	8.1	10.2	2.1	2.1	0	0	0	0
	17.2	13.9	18.5	8.1	10.2	2.1	2.1	0	0	0	0
	18	15.2	19.8	8.7	10.8	2.1	2.1	0	0	0	0
	18.1	15.2	19.8	8.7	10.8	2.1	2.1	0	0	0	0
	18.2	15.2	19.8	8.7	10.8	2.1	2.1	0	0	0	0
	19	16.5	21.1	9.3	11.4	2.1	2.1	0	0	0	0
	19.1	16.5	21.1	9.3	11.4	2.1	2.1	0	0	0	0
	19.2	16.5	21.1	9.3	11.4	2.1	2.1	0	0	0	0
D22S1045	20	17.8	22.4	10	12.1	2.1	2.1	0	0	0	0
D5S818	6	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	6.1	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	6.2	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	6.3	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	7	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	7.1	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	7.2	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	7.3	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	8	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	8.1	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	8.2	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	8.3	6.3	10.3	3.5	6.8	2.4	2.4	0	0	0	0
	9	6.9	10.9	3.5	6.8	2.4	2.4	0	0	0	0
	9.1	6.9	10.9	3.5	6.8	2.4	2.4	0	0	0	0
D5S818	9.2	6.9	10.9	3.5	6.8	2.4	2.4	0	0	0	0
	9.3	6.9	10.9	3.5	6.8	2.4	2.4	0	0	0	0
	10	7.6	11.6	3.5	6.8	2.4	2.4	0	0	0	0
	10.1	7.6	11.6	3.5	6.8	2.4	2.4	0	0	0	0
	10.2	7.6	11.6	3.5	6.8	2.4	2.4	0	0	0	0
	10.3	7.6	11.6	3.5	6.8	2.4	2.4	0	0	0	0
	11	8.3	12.3	3.5	6.8	2.4	2.4	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	11.1	8.3	12.3	3.5	6.8	2.4	2.4	0	0	0	0
	11.2	8.3	12.3	3.5	6.8	2.4	2.4	0	0	0	0
	11.3	8.3	12.3	3.5	6.8	2.4	2.4	0	0	0	0
	12	9	13	3.5	6.8	2.4	2.4	0	0	0	0
	12.1	9	13	3.5	6.8	2.4	2.4	0	0	0	0
	12.2	9	13	3.5	6.8	2.4	2.4	0	0	0	0
	12.3	9	13	3.5	6.8	2.4	2.4	0	0	0	0
	13	9.7	13.7	3.5	6.8	2.4	2.4	0	0	0	0
	13.1	9.7	13.7	3.5	6.8	2.4	2.4	0	0	0	0
	13.2	9.7	13.7	3.5	6.8	2.4	2.4	0	0	0	0
	13.3	9.7	13.7	3.5	6.8	2.4	2.4	0	0	0	0
	14	10.4	14.4	3.5	6.8	2.4	2.4	0	0	0	0
	14.1	10.4	14.4	3.5	6.8	2.4	2.4	0	0	0	0
	14.2	10.4	14.4	3.5	6.8	2.4	2.4	0	0	0	0
	14.3	10.4	14.4	3.5	6.8	2.4	2.4	0	0	0	0
	15	11.1	15.1	3.5	6.8	2.4	2.4	0	0	0	0
	15.1	11.1	15.1	3.5	6.8	2.4	2.4	0	0	0	0
	15.2	11.1	15.1	3.5	6.8	2.4	2.4	0	0	0	0
	15.3	11.1	15.1	3.5	6.8	2.4	2.4	0	0	0	0
	16	11.8	15.8	3.5	6.8	2.4	2.4	0	0	0	0
	16.1	11.8	15.8	3.5	6.8	2.4	2.4	0	0	0	0
	16.2	11.8	15.8	3.5	6.8	2.4	2.4	0	0	0	0
	16.3	11.8	15.8	3.5	6.8	2.4	2.4	0	0	0	0
	17	12.5	16.5	3.5	6.8	2.4	2.4	0	0	0	0
	17.1	12.5	16.5	3.5	6.8	2.4	2.4	0	0	0	0
	17.2	12.5	16.5	3.5	6.8	2.4	2.4	0	0	0	0
	17.3	12.5	16.5	3.5	6.8	2.4	2.4	0	0	0	0
	18	13.2	17.2	3.5	6.8	2.4	2.4	0	0	0	0
	18.1	13.2	17.2	3.5	6.8	2.4	2.4	0	0	0	0
	18.2	13.2	17.2	3.5	6.8	2.4	2.4	0	0	0	0
	18.3	13.2	17.2	3.5	6.8	2.4	2.4	0	0	0	0
D5S818	19	13.8	17.8	3.5	6.8	2.4	2.4	0	0	0	0
D13S317	5	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	5.1	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	5.2	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	5.3	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	6	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	6.1	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	6.2	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	6.3	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	7	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	7.1	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	7.2	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	7.3	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	8	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	8.1	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	8.2	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
D13S317	8.3	5.3	9.4	2.9	4.3	2.1	2.1	0	0	0	0
	9	6.1	10.2	2.9	4.3	2.1	2.1	0	0	0	0
	9.1	6.1	10.2	2.9	4.3	2.1	2.1	0	0	0	0
	9.2	6.1	10.2	2.9	4.3	2.1	2.1	0	0	0	0
	9.3	6.1	10.2	2.9	4.3	2.1	2.1	0	0	0	0
	10	7	11.1	2.9	4.3	2.1	2.1	0	0	0	0
	10.1	7	11.1	2.9	4.3	2.1	2.1	0	0	0	0
	10.2	7	11.1	2.9	4.3	2.1	2.1	0	0	0	0
	10.3	7	11.1	2.9	4.3	2.1	2.1	0	0	0	0
	11	7.8	11.9	2.9	4.3	2.1	2.1	0	0	0	0
	11.1	7.8	11.9	2.9	4.3	2.1	2.1	0	0	0	0
	11.2	7.8	11.9	2.9	4.3	2.1	2.1	0	0	0	0
	11.3	7.8	11.9	2.9	4.3	2.1	2.1	0	0	0	0
	12	8.7	12.8	2.9	4.3	2.1	2.1	0	0	0	0
	12.1	8.7	12.8	2.9	4.3	2.1	2.1	0	0	0	0
	12.2	8.7	12.8	2.9	4.3	2.1	2.1	0	0	0	0
	12.3	8.7	12.8	2.9	4.3	2.1	2.1	0	0	0	0
	13	9.6	13.7	2.9	4.3	2.1	2.1	0	0	0	0
	13.1	9.6	13.7	2.9	4.3	2.1	2.1	0	0	0	0
	13.2	9.6	13.7	2.9	4.3	2.1	2.1	0	0	0	0
	13.3	9.6	13.7	2.9	4.3	2.1	2.1	0	0	0	0
	14	10.4	14.5	2.9	4.3	2.1	2.1	0	0	0	0
	14.1	10.4	14.5	2.9	4.3	2.1	2.1	0	0	0	0
	14.2	10.4	14.5	2.9	4.3	2.1	2.1	0	0	0	0
	14.3	10.4	14.5	2.9	4.3	2.1	2.1	0	0	0	0
	15	11.3	15.4	2.9	4.3	2.1	2.1	0	0	0	0
	15.1	11.3	15.4	2.9	4.3	2.1	2.1	0	0	0	0
	15.2	11.3	15.4	2.9	4.3	2.1	2.1	0	0	0	0
	15.3	11.3	15.4	2.9	4.3	2.1	2.1	0	0	0	0
	16	12.1	16.2	2.9	4.3	2.1	2.1	0	0	0	0
	16.1	12.1	16.2	2.9	4.3	2.1	2.1	0	0	0	0
	16.2	12.1	16.2	2.9	4.3	2.1	2.1	0	0	0	0
	16.3	12.1	16.2	2.9	4.3	2.1	2.1	0	0	0	0
D13S317	17	13	17.1	2.9	4.3	2.1	2.1	0	0	0	0
D7S820	5	5	7.3	2.1	6.5	2.5	2.5	0	0	0	0
	5.1	5	7.3	2.1	6.5	2.5	2.5	0	0	0	0
	5.2	5	7.3	2.1	6.5	2.5	2.5	0	0	0	0
	5.3	5	7.3	2.1	6.5	2.5	2.5	0	0	0	0
	6	5	7.3	2.1	6.5	2.5	2.5	0	0	0	0
	6.1	5	7.3	2.1	6.5	2.5	2.5	0	0	0	0
	6.2	5	7.3	2.1	6.5	2.5	2.5	0	0	0	0
	6.3	5	7.3	2.1	6.5	2.5	2.5	0	0	0	0
	7	5.8	8.1	2.1	6.5	2.5	2.5	0	0	0	0
	7.1	5.8	8.1	2.1	6.5	2.5	2.5	0	0	0	0
	7.2	5.8	8.1	2.1	6.5	2.5	2.5	0	0	0	0
	7.3	5.8	8.1	2.1	6.5	2.5	2.5	0	0	0	0
	8	6.6	8.9	2.1	6.5	2.5	2.5	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	8.1	6.6	8.9	2.1	6.5	2.5	2.5	0	0	0	0
	8.2	6.6	8.9	2.1	6.5	2.5	2.5	0	0	0	0
	8.3	6.6	8.9	2.1	6.5	2.5	2.5	0	0	0	0
	9	7.5	9.8	2.1	6.5	2.5	2.5	0	0	0	0
	9.1	7.5	9.8	2.1	6.5	2.5	2.5	0	0	0	0
	9.2	7.5	9.8	2.1	6.5	2.5	2.5	0	0	0	0
	9.3	7.5	9.8	2.1	6.5	2.5	2.5	0	0	0	0
	13.1	10.8	13.1	2.1	6.5	2.5	2.5	0	0	0	0
	13.2	10.8	13.1	2.1	6.5	2.5	2.5	0	0	0	0
	13.3	10.8	13.1	2.1	6.5	2.5	2.5	0	0	0	0
	14	11.6	13.9	2.1	6.5	2.5	2.5	0	0	0	0
	14.1	11.6	13.9	2.1	6.5	2.5	2.5	0	0	0	0
	14.2	11.6	13.9	2.1	6.5	2.5	2.5	0	0	0	0
	14.3	11.6	13.9	2.1	6.5	2.5	2.5	0	0	0	0
	15	12.5	14.8	2.1	6.5	2.5	2.5	0	0	0	0
	15.1	12.5	14.8	2.1	6.5	2.5	2.5	0	0	0	0
	15.2	12.5	14.8	2.1	6.5	2.5	2.5	0	0	0	0
	15.3	12.5	14.8	2.1	6.5	2.5	2.5	0	0	0	0
D7S820	16	13.3	15.6	2.1	6.5	2.5	2.5	0	0	0	0
SE33	4.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	4.3	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	5	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	5.1	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	5.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	5.3	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	6	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	6.1	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	6.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	6.3	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	7	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	7.1	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	7.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	7.3	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	8	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	8.1	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	8.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	8.3	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	9	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	9.1	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	9.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	9.3	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	10	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	10.1	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	10.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	10.3	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	11	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	11.1	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	11.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
SE33	11.3	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	12	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	12.1	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	12.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	12.3	9.4	14.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	13	10	15	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	13.1	10	15	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	13.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	13.3	10	15	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	14	10.6	15.6	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	14.1	10.6	15.6	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	14.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	14.3	10.6	15.6	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	15	11.2	16.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	15.1	11.2	16.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	15.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	15.3	11.2	16.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	16	11.9	16.9	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	16.1	11.9	16.9	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	16.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	16.3	11.9	16.9	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	17	12.5	17.5	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	17.1	12.5	17.5	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	17.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	17.3	12.5	17.5	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	18	13.1	18.1	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	18.1	13.1	18.1	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	18.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	18.3	13.1	18.1	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	19	13.7	18.7	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	19.1	13.7	18.7	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	19.2	10.8	15.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	19.3	13.7	18.7	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
SE33	20	14.3	19.3	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	20.1	14.3	19.3	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	20.2	11.4	16.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	20.3	14.3	19.3	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	21	14.9	19.9	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	21.1	14.9	19.9	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	21.2	12	17	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	21.3	14.9	19.9	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	22	15.5	20.5	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	22.1	15.5	20.5	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	22.2	12.5	17.5	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	22.3	15.5	20.5	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	23	16.1	21.1	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	23.1	16.1	21.1	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	23.2	13.1	18.1	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	23.3	16.1	21.1	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	24	16.7	21.7	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	24.1	16.7	21.7	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	24.2	13.7	18.7	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	24.3	16.7	21.7	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	25	17.3	22.3	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	25.1	17.3	22.3	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	25.2	14.3	19.3	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	25.3	17.3	22.3	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
SE33	26	18	23	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	26.1	18	23	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	26.2	14.9	19.9	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	26.3	18	23	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	27	18.6	23.6	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	27.1	18.6	23.6	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	27.2	15.4	20.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	27.3	18.6	23.6	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	28	19.2	24.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	28.1	19.2	24.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	28.2	16	21	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	28.3	19.2	24.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	29	19.8	24.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	29.1	19.8	24.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	29.2	16.6	21.6	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	29.3	19.8	24.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	30	20.4	25.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	30.1	20.4	25.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	30.2	17.2	22.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	30.3	20.4	25.4	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	31	21	26	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	31.1	21	26	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	31.2	17.8	22.8	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	31.3	21	26	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	32	21.6	26.6	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	32.1	21.6	26.6	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	32.2	18.3	23.3	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	32.3	21.6	26.6	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	33	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	33.1	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	33.2	18.9	23.9	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	33.3	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	34	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	34.1	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	34.2	19.5	24.5	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	34.3	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	35	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	35.1	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	35.2	20.1	25.1	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	35.3	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	36	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	36.1	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	36.2	20.7	25.7	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	36.3	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	37	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	37.1	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	37.2	21.2	26.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
	37.3	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
SE33	38	22.2	27.2	5.6	11.7	3.1	3.1	7	11.9	2.3	5.3
D10S1248	7	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	7.1	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	7.2	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	7.3	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	8	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	8.1	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	8.2	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	8.3	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	9	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	9.1	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	9.2	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	9.3	7.7	11.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	10	8.4	12.6	2.7	2.7	2	2.9	1.1	2.5	0	0
	10.1	8.4	12.6	2.7	2.7	2	2.9	1.1	2.5	0	0
	10.2	8.4	12.6	2.7	2.7	2	2.9	1.1	2.5	0	0
	10.3	8.4	12.6	2.7	2.7	2	2.9	1.1	2.5	0	0
	11	9	13.2	2.7	2.7	2	2.9	1.1	2.5	0	0
	11.1	9	13.2	2.7	2.7	2	2.9	1.1	2.5	0	0
	11.2	9	13.2	2.7	2.7	2	2.9	1.1	2.5	0	0
	11.3	9	13.2	2.7	2.7	2	2.9	1.1	2.5	0	0
	12	9.7	13.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	12.1	9.7	13.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	12.2	9.7	13.9	2.7	2.7	2	2.9	1.1	2.5	0	0
	12.3	9.7	13.9	2.7	2.7	2	2.9	1.1	2.5	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
D10S1248	13	10.3	14.5	2.7	2.7	2	2.9	1.1	2.5	0	0
	13.1	10.3	14.5	2.7	2.7	2	2.9	1.1	2.5	0	0
	13.2	10.3	14.5	2.7	2.7	2	2.9	1.1	2.5	0	0
	13.3	10.3	14.5	2.7	2.7	2	2.9	1.1	2.5	0	0
	14	11	15.2	2.7	2.7	2	2.9	1.1	2.5	0	0
	14.1	11	15.2	2.7	2.7	2	2.9	1.1	2.5	0	0
	14.2	11	15.2	2.7	2.7	2	2.9	1.1	2.5	0	0
	14.3	11	15.2	2.7	2.7	2	2.9	1.1	2.5	0	0
	15	11.6	15.8	2.7	2.7	2	2.9	1.1	2.5	0	0
	15.1	11.6	15.8	2.7	2.7	2	2.9	1.1	2.5	0	0
	15.2	11.6	15.8	2.7	2.7	2	2.9	1.1	2.5	0	0
	15.3	11.6	15.8	2.7	2.7	2	2.9	1.1	2.5	0	0
	16	12.3	16.5	2.7	2.7	2	2.9	1.1	2.5	0	0
	16.1	12.3	16.5	2.7	2.7	2	2.9	1.1	2.5	0	0
	16.2	12.3	16.5	2.7	2.7	2	2.9	1.1	2.5	0	0
	16.3	12.3	16.5	2.7	2.7	2	2.9	1.1	2.5	0	0
	17	12.9	17.1	2.7	2.7	2	2.9	1.1	2.5	0	0
	17.1	12.9	17.1	2.7	2.7	2	2.9	1.1	2.5	0	0
	17.2	12.9	17.1	2.7	2.7	2	2.9	1.1	2.5	0	0
	17.3	12.9	17.1	2.7	2.7	2	2.9	1.1	2.5	0	0
	18	13.6	17.8	2.7	2.7	2	2.9	1.1	2.5	0	0
	18.1	13.6	17.8	2.7	2.7	2	2.9	1.1	2.5	0	0
	18.2	13.6	17.8	2.7	2.7	2	2.9	1.1	2.5	0	0
	18.3	13.6	17.8	2.7	2.7	2	2.9	1.1	2.5	0	0
D10S1248	19	14.2	18.4	2.7	2.7	2	2.9	1.1	2.5	0	0
D1S1656	8	8.3	11.5	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	8.1	8.3	11.5	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	8.2	8.3	11.5	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	8.3	8.2	11.4	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	9	8.3	11.5	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	9.1	8.3	11.5	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	9.2	8.3	11.5	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	9.3	8.2	11.4	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	10	9.1	12.3	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	10.1	9.1	12.3	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	10.2	9.1	12.3	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	10.3	8.2	11.4	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	11	10	13.2	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	11.1	10	13.2	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	11.2	10	13.2	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	11.3	8.2	11.4	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	12	10.8	14	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	12.1	10.8	14	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	12.2	10.8	14	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	12.3	8.2	11.4	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	13	11.7	14.9	2.9	4.6	2.2	4.5	4.1	9.3	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	13.1	11.7	14.9	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	13.2	11.7	14.9	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	13.3	8.2	11.4	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	14	12.5	15.7	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	14.1	12.5	15.7	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	14.2	12.5	15.7	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	14.3	8.2	11.4	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	15	13.3	16.5	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	15.1	13.3	16.5	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	15.2	13.3	16.5	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	15.3	9	12.2	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	16	14.2	17.4	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	16.1	14.2	17.4	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	16.2	14.2	17.4	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	16.3	9.7	12.9	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	17	15	18.2	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	17.1	15	18.2	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	17.2	15	18.2	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	17.3	10.5	13.7	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	18	15.9	19.1	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	18.1	15.9	19.1	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	18.2	15.9	19.1	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	18.3	11.2	14.4	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	19	16.7	19.9	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	19.1	16.7	19.9	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	19.2	16.7	19.9	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	19.3	11.9	15.1	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	20	17.5	20.7	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	20.1	17.5	20.7	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	20.2	17.5	20.7	2.9	4.6	2.2	4.5	4.1	9.3	0	0
	20.3	12.7	15.9	2.9	4.6	2.2	4.5	4.1	9.3	0	0
D1S1656	21	18.4	21.6	2.9	4.6	2.2	4.5	4.1	9.3	0	0
D12S391	13	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	13.1	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	13.2	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	13.3	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	14	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	14.1	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	14.2	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	14.3	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	15	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	15.1	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	15.2	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	15.3	9.2	14.4	3.7	3.7	2	2	0	0	0	0
	16	10	15.2	3.7	3.7	2	2	0	0	0	0
	16.1	10	15.2	3.7	3.7	2	2	0	0	0	0
	16.2	10	15.2	3.7	3.7	2	2	0	0	0	0
	16.3	10	15.2	3.7	3.7	2	2	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	17	10.8	16	3.7	3.7	2	2	0	0	0	0
	17.1	10.8	16	3.7	3.7	2	2	0	0	0	0
	17.2	10.8	16	3.7	3.7	2	2	0	0	0	0
	17.3	10.8	16	3.7	3.7	2	2	0	0	0	0
	18	11.6	16.8	3.7	3.7	2	2	0	0	0	0
	18.1	11.6	16.8	3.7	3.7	2	2	0	0	0	0
	18.2	11.6	16.8	3.7	3.7	2	2	0	0	0	0
	18.3	11.6	16.8	3.7	3.7	2	2	0	0	0	0
	19	12.4	17.6	3.7	3.7	2	2	0	0	0	0
	19.1	12.4	17.6	3.7	3.7	2	2	0	0	0	0
	19.2	12.4	17.6	3.7	3.7	2	2	0	0	0	0
	19.3	12.4	17.6	3.7	3.7	2	2	0	0	0	0
	20	13.2	18.4	3.7	3.7	2	2	0	0	0	0
	20.1	13.2	18.4	3.7	3.7	2	2	0	0	0	0
	20.2	13.2	18.4	3.7	3.7	2	2	0	0	0	0
	20.3	13.2	18.4	3.7	3.7	2	2	0	0	0	0
	21	14	19.2	3.7	3.7	2	2	0	0	0	0
	21.1	14	19.2	3.7	3.7	2	2	0	0	0	0
	21.2	14	19.2	3.7	3.7	2	2	0	0	0	0
	21.3	14	19.2	3.7	3.7	2	2	0	0	0	0
	22	14.8	20	3.7	3.7	2	2	0	0	0	0
	22.1	14.8	20	3.7	3.7	2	2	0	0	0	0
	22.2	14.8	20	3.7	3.7	2	2	0	0	0	0
	22.3	14.8	20	3.7	3.7	2	2	0	0	0	0
	23	15.7	20.9	3.7	3.7	2	2	0	0	0	0
	23.1	15.7	20.9	3.7	3.7	2	2	0	0	0	0
	23.2	15.7	20.9	3.7	3.7	2	2	0	0	0	0
	23.3	15.7	20.9	3.7	3.7	2	2	0	0	0	0
	24	16.5	21.7	3.7	3.7	2	2	0	0	0	0
	24.1	16.5	21.7	3.7	3.7	2	2	0	0	0	0
	24.2	16.5	21.7	3.7	3.7	2	2	0	0	0	0
	24.3	16.5	21.7	3.7	3.7	2	2	0	0	0	0
	25	17.3	22.5	3.7	3.7	2	2	0	0	0	0
	25.1	17.3	22.5	3.7	3.7	2	2	0	0	0	0
	25.2	17.3	22.5	3.7	3.7	2	2	0	0	0	0
	25.3	17.3	22.5	3.7	3.7	2	2	0	0	0	0
	26	18.1	23.3	3.7	3.7	2	2	0	0	0	0
	26.1	18.1	23.3	3.7	3.7	2	2	0	0	0	0
	26.2	18.1	23.3	3.7	3.7	2	2	0	0	0	0
	26.3	18.1	23.3	3.7	3.7	2	2	0	0	0	0
D12S391	27	18.9	24.1	3.7	3.7	2	2	0	0	0	0
D2S1338	10	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	10.1	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	10.2	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	10.3	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	11	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	11.1	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	11.2	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	11.3	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	12	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	12.1	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	12.2	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	12.3	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	13	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	13.1	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	13.2	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	13.3	6.4	10.6	3.4	3.4	1.7	3.6	0	0	0	0
	14	7.1	11.3	3.4	3.4	1.7	3.6	0	0	0	0
	14.1	7.1	11.3	3.4	3.4	1.7	3.6	0	0	0	0
	14.2	7.1	11.3	3.4	3.4	1.7	3.6	0	0	0	0
	14.3	7.1	11.3	3.4	3.4	1.7	3.6	0	0	0	0
	15	7.9	12.1	3.4	3.4	1.7	3.6	0	0	0	0
	15.1	7.9	12.1	3.4	3.4	1.7	3.6	0	0	0	0
	15.2	7.9	12.1	3.4	3.4	1.7	3.6	0	0	0	0
	15.3	7.9	12.1	3.4	3.4	1.7	3.6	0	0	0	0
	16	8.6	12.8	3.4	3.4	1.7	3.6	0	0	0	0
	16.1	8.6	12.8	3.4	3.4	1.7	3.6	0	0	0	0
	16.2	8.6	12.8	3.4	3.4	1.7	3.6	0	0	0	0
	16.3	8.6	12.8	3.4	3.4	1.7	3.6	0	0	0	0
	17	9.3	13.5	3.4	3.4	1.7	3.6	0	0	0	0
	17.1	9.3	13.5	3.4	3.4	1.7	3.6	0	0	0	0
	17.2	9.3	13.5	3.4	3.4	1.7	3.6	0	0	0	0
	17.3	9.3	13.5	3.4	3.4	1.7	3.6	0	0	0	0
	18	10	14.2	3.4	3.4	1.7	3.6	0	0	0	0
	18.1	10	14.2	3.4	3.4	1.7	3.6	0	0	0	0
	18.2	10	14.2	3.4	3.4	1.7	3.6	0	0	0	0
	18.3	10	14.2	3.4	3.4	1.7	3.6	0	0	0	0
	19	10.8	15	3.4	3.4	1.7	3.6	0	0	0	0
	19.1	10.8	15	3.4	3.4	1.7	3.6	0	0	0	0
D2S1338	19.2	10.8	15	3.4	3.4	1.7	3.6	0	0	0	0
	19.3	10.8	15	3.4	3.4	1.7	3.6	0	0	0	0
	20	11.5	15.7	3.4	3.4	1.7	3.6	0	0	0	0
	20.1	11.5	15.7	3.4	3.4	1.7	3.6	0	0	0	0
	20.2	11.5	15.7	3.4	3.4	1.7	3.6	0	0	0	0
	20.3	11.5	15.7	3.4	3.4	1.7	3.6	0	0	0	0
	21	12.2	16.4	3.4	3.4	1.7	3.6	0	0	0	0
	21.1	12.2	16.4	3.4	3.4	1.7	3.6	0	0	0	0
	21.2	12.2	16.4	3.4	3.4	1.7	3.6	0	0	0	0
	21.3	12.2	16.4	3.4	3.4	1.7	3.6	0	0	0	0
	22	13	17.2	3.4	3.4	1.7	3.6	0	0	0	0
	22.1	13	17.2	3.4	3.4	1.7	3.6	0	0	0	0
	22.2	13	17.2	3.4	3.4	1.7	3.6	0	0	0	0
	22.3	13	17.2	3.4	3.4	1.7	3.6	0	0	0	0
	23	13.7	17.9	3.4	3.4	1.7	3.6	0	0	0	0
	23.1	13.7	17.9	3.4	3.4	1.7	3.6	0	0	0	0
	23.2	13.7	17.9	3.4	3.4	1.7	3.6	0	0	0	0
	23.3	13.7	17.9	3.4	3.4	1.7	3.6	0	0	0	0
	24	14.4	18.6	3.4	3.4	1.7	3.6	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
D2S1338	24.1	14.4	18.6	3.4	3.4	1.7	3.6	0	0	0	0
	24.2	14.4	18.6	3.4	3.4	1.7	3.6	0	0	0	0
	24.3	14.4	18.6	3.4	3.4	1.7	3.6	0	0	0	0
	25	15.2	19.4	3.4	3.4	1.7	3.6	0	0	0	0
	25.1	15.2	19.4	3.4	3.4	1.7	3.6	0	0	0	0
	25.2	15.2	19.4	3.4	3.4	1.7	3.6	0	0	0	0
	25.3	15.2	19.4	3.4	3.4	1.7	3.6	0	0	0	0
	26	15.9	20.1	3.4	3.4	1.7	3.6	0	0	0	0
	26.1	15.9	20.1	3.4	3.4	1.7	3.6	0	0	0	0
	26.2	15.9	20.1	3.4	3.4	1.7	3.6	0	0	0	0
	26.3	15.9	20.1	3.4	3.4	1.7	3.6	0	0	0	0
	27	16.6	20.8	3.4	3.4	1.7	3.6	0	0	0	0
	27.1	16.6	20.8	3.4	3.4	1.7	3.6	0	0	0	0
	27.2	16.6	20.8	3.4	3.4	1.7	3.6	0	0	0	0
	27.3	16.6	20.8	3.4	3.4	1.7	3.6	0	0	0	0
	28	17.3	21.5	3.4	3.4	1.7	3.6	0	0	0	0
	28.1	17.3	21.5	3.4	3.4	1.7	3.6	0	0	0	0
	28.2	17.3	21.5	3.4	3.4	1.7	3.6	0	0	0	0
	28.3	17.3	21.5	3.4	3.4	1.7	3.6	0	0	0	0
D2S1338	29	18.1	22.3	3.4	3.4	1.7	3.6	0	0	0	0

RETIRED

41.1.2 Yfiler Plus

Yfiler Plus Standard (STD) and Low Amp Data Stutter Thresholds (≤30 cycles)

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
DYS576	9	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	9.1	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	9.2	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	9.3	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	10	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	10.1	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	10.2	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	10.3	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	11	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	11.1	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	11.2	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	11.3	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	12	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	12.1	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	12.2	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	12.3	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	13	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	13.1	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	13.2	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0
	13.3	9.4	12	3.3	3.7	1.2	2.1	0	0	0	0

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	14	10.3	12.9	3.3	3.7	1.2	2.1	0	0	0	0
	14.1	10.3	12.9	3.3	3.7	1.2	2.1	0	0	0	0
	14.2	10.3	12.9	3.3	3.7	1.2	2.1	0	0	0	0
	14.3	10.3	12.9	3.3	3.7	1.2	2.1	0	0	0	0
	15	11.2	13.8	3.3	3.7	1.3	2.2	0	0	0	0
	15.1	11.2	13.8	3.3	3.7	1.3	2.2	0	0	0	0
	15.2	11.2	13.8	3.3	3.7	1.3	2.2	0	0	0	0
	15.3	11.2	13.8	3.3	3.7	1.3	2.2	0	0	0	0
	16	12.2	14.7	3.3	3.7	1.5	2.3	0	0	0	0
	16.1	12.2	14.7	3.3	3.7	1.5	2.3	0	0	0	0
	16.2	12.2	14.7	3.3	3.7	1.5	2.3	0	0	0	0
	16.3	12.2	14.7	3.3	3.7	1.5	2.3	0	0	0	0
	17	13.1	15.6	3.3	3.7	1.6	2.4	0	0	0	0
	17.1	13.1	15.6	3.3	3.7	1.6	2.4	0	0	0	0
	17.2	13.1	15.6	3.3	3.7	1.6	2.4	0	0	0	0
	17.3	13.1	15.6	3.3	3.7	1.6	2.4	0	0	0	0
DYS576	18	14	16.6	3.3	3.7	1.7	2.6	0	0	0	0
	18.1	14	16.6	3.3	3.7	1.7	2.6	0	0	0	0
	18.2	14	16.6	3.3	3.7	1.7	2.6	0	0	0	0
	18.3	14	16.6	3.3	3.7	1.7	2.6	0	0	0	0
	19	14.9	17.5	3.3	3.7	1.9	2.7	0	0	0	0
	19.1	14.9	17.5	3.3	3.7	1.9	2.7	0	0	0	0
	19.2	14.9	17.5	3.3	3.7	1.9	2.7	0	0	0	0
	19.3	14.9	17.5	3.3	3.7	1.9	2.7	0	0	0	0
	20	15.8	18.4	3.3	3.7	2	2.8	0	0	0	0
	20.1	15.8	18.4	3.3	3.7	2	2.8	0	0	0	0
	20.2	15.8	18.4	3.3	3.7	2	2.8	0	0	0	0
	20.3	15.8	18.4	3.3	3.7	2	2.8	0	0	0	0
	21	16.8	19.3	3.3	3.7	2.1	3	0	0	0	0
	21.1	16.8	19.3	3.3	3.7	2.1	3	0	0	0	0
	21.2	16.8	19.3	3.3	3.7	2.1	3	0	0	0	0
	21.3	16.8	19.3	3.3	3.7	2.1	3	0	0	0	0
	22	17.7	20.2	3.3	3.7	2.3	3.1	0	0	0	0
	22.1	17.7	20.2	3.3	3.7	2.3	3.1	0	0	0	0
	22.2	17.7	20.2	3.3	3.7	2.3	3.1	0	0	0	0
	22.3	17.7	20.2	3.3	3.7	2.3	3.1	0	0	0	0
	23	18.6	21.2	3.3	3.7	2.4	3.2	0	0	0	0
DYS576	23.1	18.6	21.2	3.3	3.7	2.4	3.2	0	0	0	0
	23.2	18.6	21.2	3.3	3.7	2.4	3.2	0	0	0	0
	23.3	18.6	21.2	3.3	3.7	2.4	3.2	0	0	0	0
	24	19.5	22.1	3.3	3.7	2.5	3.4	0	0	0	0
	24.1	19.5	22.1	3.3	3.7	2.5	3.4	0	0	0	0
	24.2	19.5	22.1	3.3	3.7	2.5	3.4	0	0	0	0
	24.3	19.5	22.1	3.3	3.7	2.5	3.4	0	0	0	0
	25	20.4	23	3.3	3.7	2.6	3.5	0	0	0	0
	25.1	20.4	23	3.3	3.7	2.6	3.5	0	0	0	0
	25.2	20.4	23	3.3	3.7	2.6	3.5	0	0	0	0
	25.3	20.4	23	3.3	3.7	2.6	3.5	0	0	0	0
DYS576	26	21.4	23.9	3.3	3.7	2.8	3.6	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
DYS389I	8	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	8.1	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	8.2	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	8.3	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	9	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	9.1	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	9.2	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	9.3	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	10	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	10.1	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	10.2	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	10.3	4.2	7.1	2.3	2.3	1.1	1.1	0	0	0	0
	11	5.5	8.4	2.3	2.3	1.1	1.1	0	0	0	0
	11.1	5.5	8.4	2.3	2.3	1.1	1.1	0	0	0	0
	11.2	5.5	8.4	2.3	2.3	1.1	1.1	0	0	0	0
	11.3	5.5	8.4	2.3	2.3	1.1	1.1	0	0	0	0
	12	6.8	9.7	2.3	2.3	1.1	1.1	0	0	0	0
	12.1	6.8	9.7	2.3	2.3	1.1	1.1	0	0	0	0
	12.2	6.8	9.7	2.3	2.3	1.1	1.1	0	0	0	0
	12.3	6.8	9.7	2.3	2.3	1.1	1.1	0	0	0	0
	13	8.1	10.9	2.3	2.3	1.1	1.1	0	0	0	0
	13.1	8.1	10.9	2.3	2.3	1.1	1.1	0	0	0	0
	13.2	8.1	10.9	2.3	2.3	1.1	1.1	0	0	0	0
	13.3	8.1	10.9	2.3	2.3	1.1	1.1	0	0	0	0
	14	9.4	12.2	2.3	2.3	1.1	1.1	0	0	0	0
	14.1	9.4	12.2	2.3	2.3	1.1	1.1	0	0	0	0
	14.2	9.4	12.2	2.3	2.3	1.1	1.1	0	0	0	0
	14.3	9.4	12.2	2.3	2.3	1.1	1.1	0	0	0	0
	15	10.7	13.5	2.3	2.3	1.1	1.1	0	0	0	0
	15.1	10.7	13.5	2.3	2.3	1.1	1.1	0	0	0	0
	15.2	10.7	13.5	2.3	2.3	1.1	1.1	0	0	0	0
	15.3	10.7	13.5	2.3	2.3	1.1	1.1	0	0	0	0
	16	11.9	14.8	2.3	2.3	1.1	1.1	0	0	0	0
	16.1	11.9	14.8	2.3	2.3	1.1	1.1	0	0	0	0
	16.2	11.9	14.8	2.3	2.3	1.1	1.1	0	0	0	0
	16.3	11.9	14.8	2.3	2.3	1.1	1.1	0	0	0	0
	17	13.2	16.1	2.3	2.3	1.1	1.1	0	0	0	0
	17.1	13.2	16.1	2.3	2.3	1.1	1.1	0	0	0	0
	17.2	13.2	16.1	2.3	2.3	1.1	1.1	0	0	0	0
	17.3	13.2	16.1	2.3	2.3	1.1	1.1	0	0	0	0
DYS389I	18	14.5	17.4	2.3	2.3	1.1	1.1	0	0	0	0

DYS635	14	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	14.1	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	14.2	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	14.3	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	15	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW

RETIRED

	15.1	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	15.2	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	15.3	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	16	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	16.1	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	16.2	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	16.3	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	17	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	17.1	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	17.2	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	17.3	5.8	12	2.3	11.4	1.7	1.7	0	0	0	0
	18	7	13.1	2.3	11.4	1.7	1.7	0	0	0	0
	18.1	7	13.1	2.3	11.4	1.7	1.7	0	0	0	0
	18.2	7	13.1	2.3	11.4	1.7	1.7	0	0	0	0
	18.3	7	13.1	2.3	11.4	1.7	1.7	0	0	0	0
	19	8.1	14.2	2.3	11.4	1.7	1.7	0	0	0	0
	19.1	8.1	14.2	2.3	11.4	1.7	1.7	0	0	0	0
	19.2	8.1	14.2	2.3	11.4	1.7	1.7	0	0	0	0
	19.3	8.1	14.2	2.3	11.4	1.7	1.7	0	0	0	0
	20	9.2	15.4	2.3	11.4	1.7	1.7	0	0	0	0
	20.1	9.2	15.4	2.3	11.4	1.7	1.7	0	0	0	0
	20.2	9.2	15.4	2.3	11.4	1.7	1.7	0	0	0	0
	20.3	9.2	15.4	2.3	11.4	1.7	1.7	0	0	0	0
DYS635	21	10.4	16.5	2.3	11.4	1.7	1.7	0	0	0	0
	21.1	10.4	16.5	2.3	11.4	1.7	1.7	0	0	0	0
	21.2	10.4	16.5	2.3	11.4	1.7	1.7	0	0	0	0
	21.3	10.4	16.5	2.3	11.4	1.7	1.7	0	0	0	0
	22	11.5	17.7	2.3	11.4	1.7	1.7	0	0	0	0
	22.1	11.5	17.7	2.3	11.4	1.7	1.7	0	0	0	0
	22.2	11.5	17.7	2.3	11.4	1.7	1.7	0	0	0	0
	22.3	11.5	17.7	2.3	11.4	1.7	1.7	0	0	0	0
	23	12.7	18.8	2.3	11.4	1.7	1.7	0	0	0	0
	23.1	12.7	18.8	2.3	11.4	1.7	1.7	0	0	0	0
	23.2	12.7	18.8	2.3	11.4	1.7	1.7	0	0	0	0
	23.3	12.7	18.8	2.3	11.4	1.7	1.7	0	0	0	0
	24	13.8	19.9	2.3	11.4	1.7	1.7	0	0	0	0
	24.1	13.8	19.9	2.3	11.4	1.7	1.7	0	0	0	0
	24.2	13.8	19.9	2.3	11.4	1.7	1.7	0	0	0	0
	24.3	13.8	19.9	2.3	11.4	1.7	1.7	0	0	0	0
	25	14.9	21.1	2.3	11.4	1.7	1.7	0	0	0	0
	25.1	14.9	21.1	2.3	11.4	1.7	1.7	0	0	0	0
	25.2	14.9	21.1	2.3	11.4	1.7	1.7	0	0	0	0
	25.3	14.9	21.1	2.3	11.4	1.7	1.7	0	0	0	0
	26	16.1	22.2	2.3	11.4	1.7	1.7	0	0	0	0
	26.1	16.1	22.2	2.3	11.4	1.7	1.7	0	0	0	0
	26.2	16.1	22.2	2.3	11.4	1.7	1.7	0	0	0	0
	26.3	16.1	22.2	2.3	11.4	1.7	1.7	0	0	0	0
	27	17.2	23.4	2.3	11.4	1.7	1.7	0	0	0	0
	27.1	17.2	23.4	2.3	11.4	1.7	1.7	0	0	0	0
	27.2	17.2	23.4	2.3	11.4	1.7	1.7	0	0	0	0
	27.3	17.2	23.4	2.3	11.4	1.7	1.7	0	0	0	0
	28	18.4	24.5	2.3	11.4	1.7	1.7	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
DYS635	28.1	18.4	24.5	2.3	11.4	1.7	1.7	0	0	0	0
	28.2	18.4	24.5	2.3	11.4	1.7	1.7	0	0	0	0
	28.3	18.4	24.5	2.3	11.4	1.7	1.7	0	0	0	0
	29	19.5	25.6	2.3	11.4	1.7	1.7	0	0	0	0
	29.1	19.5	25.6	2.3	11.4	1.7	1.7	0	0	0	0
	29.2	19.5	25.6	2.3	11.4	1.7	1.7	0	0	0	0
	29.3	19.5	25.6	2.3	11.4	1.7	1.7	0	0	0	0
	30	20.6	26.8	2.3	11.4	1.7	1.7	0	0	0	0
	30.1	20.6	26.8	2.3	11.4	1.7	1.7	0	0	0	0
	30.2	20.6	26.8	2.3	11.4	1.7	1.7	0	0	0	0
	30.3	20.6	26.8	2.3	11.4	1.7	1.7	0	0	0	0
DYS635	31	21.8	27.9	2.3	11.4	1.7	1.7	0	0	0	0
DYS389II	23	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	23.1	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	23.2	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	23.3	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	24	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	24.1	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	24.2	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	24.3	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	25	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	25.1	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	25.2	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	25.3	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	26	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	26.1	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	26.2	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	26.3	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	27	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	27.1	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	27.2	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	27.3	14.6	23.4	3.6	7.9	1.8	3.6	0	0	0	0
	28	15.6	24.4	3.6	7.9	2	3.8	0	0	0	0
	28.1	15.6	24.4	3.6	7.9	2	3.8	0	0	0	0
	28.2	15.6	24.4	3.6	7.9	2	3.8	0	0	0	0
	28.3	15.6	24.4	3.6	7.9	2	3.8	0	0	0	0
	29	16.7	25.5	3.6	7.9	2.2	4	0	0	0	0
	29.1	16.7	25.5	3.6	7.9	2.2	4	0	0	0	0
	29.2	16.7	25.5	3.6	7.9	2.2	4	0	0	0	0
	29.3	16.7	25.5	3.6	7.9	2.2	4	0	0	0	0
DYS389II	30	17.7	26.5	3.6	7.9	2.4	4.2	0	0	0	0
	30.1	17.7	26.5	3.6	7.9	2.4	4.2	0	0	0	0
	30.2	17.7	26.5	3.6	7.9	2.4	4.2	0	0	0	0
	30.3	17.7	26.5	3.6	7.9	2.4	4.2	0	0	0	0
	31	18.8	27.6	3.6	7.9	2.6	4.3	0	0	0	0
	31.1	18.8	27.6	3.6	7.9	2.6	4.3	0	0	0	0
	31.2	18.8	27.6	3.6	7.9	2.6	4.3	0	0	0	0
	31.3	18.8	27.6	3.6	7.9	2.6	4.3	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	32	19.8	28.6	3.6	7.9	2.7	4.5	0	0	0	0
	32.1	19.8	28.6	3.6	7.9	2.7	4.5	0	0	0	0
	32.2	19.8	28.6	3.6	7.9	2.7	4.5	0	0	0	0
	32.3	19.8	28.6	3.6	7.9	2.7	4.5	0	0	0	0
	33	20.9	29.7	3.6	7.9	2.9	4.7	0	0	0	0
	33.1	20.9	29.7	3.6	7.9	2.9	4.7	0	0	0	0
DYS389II	33.2	20.9	29.7	3.6	7.9	2.9	4.7	0	0	0	0
	33.3	20.9	29.7	3.6	7.9	2.9	4.7	0	0	0	0
	34	21.9	30.7	3.6	7.9	3.1	4.9	0	0	0	0
	34.1	21.9	30.7	3.6	7.9	3.1	4.9	0	0	0	0
	34.2	21.9	30.7	3.6	7.9	3.1	4.9	0	0	0	0
	34.3	21.9	30.7	3.6	7.9	3.1	4.9	0	0	0	0
	35	23	31.8	3.6	7.9	3.3	5.1	0	0	0	0
	35.1	23	31.8	3.6	7.9	3.3	5.1	0	0	0	0
	35.2	23	31.8	3.6	7.9	3.3	5.1	0	0	0	0
	35.3	23	31.8	3.6	7.9	3.3	5.1	0	0	0	0
DYS389II	36	24	32.8	3.6	7.9	3.5	5.2	0	0	0	0
DYS627	10	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	10.1	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	10.2	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	10.3	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	11	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	11.1	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	11.2	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	11.3	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	12	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	12.1	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	12.2	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	12.3	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	13	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	13.1	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	13.2	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	13.3	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	14	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	14.1	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	14.2	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	14.3	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	15	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	15.1	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	15.2	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	15.3	9.8	12.1	2.8	4.4	1	1.9	2.5	3.7	0	0
	16	10.6	12.9	2.8	4.4	1	1.9	2.5	3.7	0	0
	16.1	10.6	12.9	2.8	4.4	1	1.9	2.5	3.7	0	0
	16.2	10.6	12.9	2.8	4.4	1	1.9	2.5	3.7	0	0
	16.3	10.6	12.9	2.8	4.4	1	1.9	2.5	3.7	0	0
	17	11.3	13.6	2.8	4.4	1.1	2	2.5	3.7	0	0
	17.1	11.3	13.6	2.8	4.4	1.1	2	2.5	3.7	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	17.2	11.3	13.6	2.8	4.4	1.1	2	2.5	3.7	0	0
	17.3	11.3	13.6	2.8	4.4	1.1	2	2.5	3.7	0	0
	18	12.1	14.4	2.8	4.4	1.2	2.1	2.5	3.7	0	0
	18.1	12.1	14.4	2.8	4.4	1.2	2.1	2.5	3.7	0	0
	18.2	12.1	14.4	2.8	4.4	1.2	2.1	2.5	3.7	0	0
	18.3	12.1	14.4	2.8	4.4	1.2	2.1	2.5	3.7	0	0
DYS627	19	12.8	15.1	2.8	4.4	1.3	2.2	2.5	3.7	0	0
	19.1	12.8	15.1	2.8	4.4	1.3	2.2	2.5	3.7	0	0
	19.2	12.8	15.1	2.8	4.4	1.3	2.2	2.5	3.7	0	0
	19.3	12.8	15.1	2.8	4.4	1.3	2.2	2.5	3.7	0	0
	20	13.6	15.9	2.8	4.4	1.4	2.3	2.5	3.7	0	0
	20.1	13.6	15.9	2.8	4.4	1.4	2.3	2.5	3.7	0	0
	20.2	13.6	15.9	2.8	4.4	1.4	2.3	2.5	3.7	0	0
DYS627	20.3	13.6	15.9	2.8	4.4	1.4	2.3	2.5	3.7	0	0
	21	14.4	16.7	2.8	4.4	1.5	2.4	2.5	3.7	0	0
	21.1	14.4	16.7	2.8	4.4	1.5	2.4	2.5	3.7	0	0
	21.2	14.4	16.7	2.8	4.4	1.5	2.4	2.5	3.7	0	0
	21.3	14.4	16.7	2.8	4.4	1.5	2.4	2.5	3.7	0	0
	22	15.1	17.4	2.8	4.4	1.6	2.5	2.5	3.7	0	0
	22.1	15.1	17.4	2.8	4.4	1.6	2.5	2.5	3.7	0	0
	22.2	15.1	17.4	2.8	4.4	1.6	2.5	2.5	3.7	0	0
	22.3	15.1	17.4	2.8	4.4	1.6	2.5	2.5	3.7	0	0
	23	15.9	18.2	2.8	4.4	1.7	2.6	2.5	3.7	0	0
	23.1	15.9	18.2	2.8	4.4	1.7	2.6	2.5	3.7	0	0
	23.2	15.9	18.2	2.8	4.4	1.7	2.6	2.5	3.7	0	0
	23.3	15.9	18.2	2.8	4.4	1.7	2.6	2.5	3.7	0	0
	24	16.6	18.9	2.8	4.4	1.8	2.7	2.5	3.7	0	0
	24.1	16.6	18.9	2.8	4.4	1.8	2.7	2.5	3.7	0	0
	24.2	16.6	18.9	2.8	4.4	1.8	2.7	2.5	3.7	0	0
	24.3	16.6	18.9	2.8	4.4	1.8	2.7	2.5	3.7	0	0
	25	17.4	19.7	2.8	4.4	1.9	2.8	2.5	3.7	0	0
	25.1	17.4	19.7	2.8	4.4	1.9	2.8	2.5	3.7	0	0
	25.2	17.4	19.7	2.8	4.4	1.9	2.8	2.5	3.7	0	0
	25.3	17.4	19.7	2.8	4.4	1.9	2.8	2.5	3.7	0	0
	26	18.2	20.5	2.8	4.4	2	2.9	2.5	3.7	0	0
	26.1	18.2	20.5	2.8	4.4	2	2.9	2.5	3.7	0	0
	26.2	18.2	20.5	2.8	4.4	2	2.9	2.5	3.7	0	0
	26.3	18.2	20.5	2.8	4.4	2	2.9	2.5	3.7	0	0
	27	18.9	21.2	2.8	4.4	2.1	3	2.5	3.7	0	0
	27.1	18.9	21.2	2.8	4.4	2.1	3	2.5	3.7	0	0
	27.2	18.9	21.2	2.8	4.4	2.1	3	2.5	3.7	0	0
	27.3	18.9	21.2	2.8	4.4	2.1	3	2.5	3.7	0	0
DYS627	28	19.7	22	2.8	4.4	2.2	3.1	2.5	3.7	0	0
DYS460	6	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	6.1	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	6.2	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	6.3	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	7	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	7.1	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	7.2	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	7.3	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	8	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	8.1	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	8.2	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	8.3	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	9	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	9.1	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	9.2	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	9.3	7.4	13	3.1	6.5	1.7	1.7	0	0	0	0
	10	8.8	14.5	3.1	6.5	1.9	1.9	0	0	0	0
	10.1	8.8	14.5	3.1	6.5	1.9	1.9	0	0	0	0
	10.2	8.8	14.5	3.1	6.5	1.9	1.9	0	0	0	0
	10.3	8.8	14.5	3.1	6.5	1.9	1.9	0	0	0	0
	11	10.3	15.9	3.1	6.5	2.1	2.1	0	0	0	0
	11.1	10.3	15.9	3.1	6.5	2.1	2.1	0	0	0	0
	11.2	10.3	15.9	3.1	6.5	2.1	2.1	0	0	0	0
	11.3	10.3	15.9	3.1	6.5	2.1	2.1	0	0	0	0
	12	11.7	17.3	3.1	6.5	2.3	2.3	0	0	0	0
	12.1	11.7	17.3	3.1	6.5	2.3	2.3	0	0	0	0
	12.2	11.7	17.3	3.1	6.5	2.3	2.3	0	0	0	0
	12.3	11.7	17.3	3.1	6.5	2.3	2.3	0	0	0	0
	13	13.1	18.7	3.1	6.5	2.5	2.5	0	0	0	0
	13.1	13.1	18.7	3.1	6.5	2.5	2.5	0	0	0	0
	13.2	13.1	18.7	3.1	6.5	2.5	2.5	0	0	0	0
	13.3	13.1	18.7	3.1	6.5	2.5	2.5	0	0	0	0
	14	14.5	20.2	3.1	6.5	2.7	2.7	0	0	0	0
	14.1	14.5	20.2	3.1	6.5	2.7	2.7	0	0	0	0
	14.2	14.5	20.2	3.1	6.5	2.7	2.7	0	0	0	0
	14.3	14.5	20.2	3.1	6.5	2.7	2.7	0	0	0	0
DYS460	15	16	21.6	3.1	6.5	2.9	2.9	0	0	0	0
DYS458	10	8.2	14.9	2.6	5	1.7	3.1	0	0	0	0
	10.1	8.2	14.9	2.6	5	1.7	3.1	0	0	0	0
	10.2	8.2	14.9	2.6	5	1.7	3.1	0	0	0	0
	10.3	8.2	14.9	2.6	5	1.7	3.1	0	0	0	0
	11	8.2	14.9	2.6	5	1.7	3.1	0	0	0	0
	11.1	8.2	14.9	2.6	5	1.7	3.1	0	0	0	0
	11.2	8.2	14.9	2.6	5	1.7	3.1	0	0	0	0
	11.3	8.2	14.9	2.6	5	1.7	3.1	0	0	0	0
	12	9.2	15.8	2.6	5	1.7	3.1	0	0	0	0
	12.1	9.2	15.8	2.6	5	1.7	3.1	0	0	0	0
	12.2	9.2	15.8	2.6	5	1.7	3.1	0	0	0	0
	12.3	9.2	15.8	2.6	5	1.7	3.1	0	0	0	0
	13	10.1	16.7	2.6	5	1.7	3.1	0	0	0	0
	13.1	10.1	16.7	2.6	5	1.7	3.1	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	13.2	10.1	16.7	2.6	5	1.7	3.1	0	0	0	0
	13.3	10.1	16.7	2.6	5	1.7	3.1	0	0	0	0
	14	11	17.7	2.6	5	1.7	3.1	0	0	0	0
DYS458	14.1	11	17.7	2.6	5	1.7	3.1	0	0	0	0
	14.2	11	17.7	2.6	5	1.7	3.1	0	0	0	0
	14.3	11	17.7	2.6	5	1.7	3.1	0	0	0	0
	15	12	18.6	2.6	5	1.8	3.2	0	0	0	0
	15.1	12	18.6	2.6	5	1.8	3.2	0	0	0	0
	15.2	12	18.6	2.6	5	1.8	3.2	0	0	0	0
	15.3	12	18.6	2.6	5	1.8	3.2	0	0	0	0
	16	12.9	19.5	2.6	5	1.9	3.4	0	0	0	0
	16.1	12.9	19.5	2.6	5	1.9	3.4	0	0	0	0
	16.2	12.9	19.5	2.6	5	1.9	3.4	0	0	0	0
	16.3	12.9	19.5	2.6	5	1.9	3.4	0	0	0	0
	17	13.8	20.5	2.6	5	2	3.5	0	0	0	0
	17.1	13.8	20.5	2.6	5	2	3.5	0	0	0	0
	17.2	13.8	20.5	2.6	5	2	3.5	0	0	0	0
	17.3	13.8	20.5	2.6	5	2	3.5	0	0	0	0
	18	14.7	21.4	2.6	5	2.2	3.6	0	0	0	0
	18.1	14.7	21.4	2.6	5	2.2	3.6	0	0	0	0
	18.2	14.7	21.4	2.6	5	2.2	3.6	0	0	0	0
	18.3	14.7	21.4	2.6	5	2.2	3.6	0	0	0	0
	19	15.7	22.3	2.6	5	2.3	3.7	0	0	0	0
	19.1	15.7	22.3	2.6	5	2.3	3.7	0	0	0	0
	19.2	15.7	22.3	2.6	5	2.3	3.7	0	0	0	0
	19.3	15.7	22.3	2.6	5	2.3	3.7	0	0	0	0
	20	16.6	23.2	2.6	5	2.4	3.8	0	0	0	0
	20.1	16.6	23.2	2.6	5	2.4	3.8	0	0	0	0
	20.2	16.6	23.2	2.6	5	2.4	3.8	0	0	0	0
	20.3	16.6	23.2	2.6	5	2.4	3.8	0	0	0	0
	21	17.5	24.2	2.6	5	2.5	4	0	0	0	0
	21.1	17.5	24.2	2.6	5	2.5	4	0	0	0	0
	21.2	17.5	24.2	2.6	5	2.5	4	0	0	0	0
	21.3	17.5	24.2	2.6	5	2.5	4	0	0	0	0
	22	18.5	25.1	2.6	5	2.6	4.1	0	0	0	0
	22.1	18.5	25.1	2.6	5	2.6	4.1	0	0	0	0
	22.2	18.5	25.1	2.6	5	2.6	4.1	0	0	0	0
	22.3	18.5	25.1	2.6	5	2.6	4.1	0	0	0	0
	23	19.4	26	2.6	5	2.8	4.2	0	0	0	0
	23.1	19.4	26	2.6	5	2.8	4.2	0	0	0	0
	23.2	19.4	26	2.6	5	2.8	4.2	0	0	0	0
	23.3	19.4	26	2.6	5	2.8	4.2	0	0	0	0
	24	20.3	27	2.6	5	2.9	4.3	0	0	0	0
	24.1	20.3	27	2.6	5	2.9	4.3	0	0	0	0
	24.2	20.3	27	2.6	5	2.9	4.3	0	0	0	0
	24.3	20.3	27	2.6	5	2.9	4.3	0	0	0	0
DYS458	25	21.3	27.9	2.6	5	3	4.4	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
DYS19	8	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	8.1	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	8.2	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	8.3	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	9	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	9.1	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	9.2	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	9.3	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	10	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	10.1	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
DYS19	10.2	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	10.3	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	11	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	11.1	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	11.2	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	11.3	6.2	10.2	1.5	3.7	1.1	3	9.8	17.5	3.6	10.1
	12	7.3	11.4	1.7	4	1.1	3	9.8	17.5	3.6	10.1
	12.1	7.3	11.4	1.7	4	1.1	3	9.8	17.5	3.6	10.1
	12.2	7.3	11.4	1.7	4	1.1	3	9.8	17.5	3.6	10.1
	12.3	7.3	11.4	1.7	4	1.1	3	9.8	17.5	3.6	10.1
	13	8.4	12.5	2	4.2	1.1	3	9.8	17.5	3.6	10.1
	13.1	8.4	12.5	2	4.2	1.1	3	9.8	17.5	3.6	10.1
	13.2	8.4	12.5	2	4.2	1.1	3	9.8	17.5	3.6	10.1
	13.3	8.4	12.5	2	4.2	1.1	3	9.8	17.5	3.6	10.1
	14	9.6	13.6	2.2	4.4	1.2	3.1	9.8	17.5	3.6	10.1
	14.1	9.6	13.6	2.2	4.4	1.2	3.1	9.8	17.5	3.6	10.1
	14.2	9.6	13.6	2.2	4.4	1.2	3.1	9.8	17.5	3.6	10.1
	14.3	9.6	13.6	2.2	4.4	1.2	3.1	9.8	17.5	3.6	10.1
	15	10.7	14.7	2.4	4.6	1.3	3.3	9.8	17.5	3.6	10.1
	15.1	10.7	14.7	2.4	4.6	1.3	3.3	9.8	17.5	3.6	10.1
	15.2	10.7	14.7	2.4	4.6	1.3	3.3	9.8	17.5	3.6	10.1
	15.3	10.7	14.7	2.4	4.6	1.3	3.3	9.8	17.5	3.6	10.1
	16	11.8	15.8	2.6	4.8	1.5	3.4	9.8	17.5	3.6	10.1
	16.1	11.8	15.8	2.6	4.8	1.5	3.4	9.8	17.5	3.6	10.1
	16.2	11.8	15.8	2.6	4.8	1.5	3.4	9.8	17.5	3.6	10.1
	16.3	11.8	15.8	2.6	4.8	1.5	3.4	9.8	17.5	3.6	10.1
	17	12.9	17	2.8	5.1	1.6	3.5	9.8	17.5	3.6	10.1
	17.1	12.9	17	2.8	5.1	1.6	3.5	9.8	17.5	3.6	10.1
	17.2	12.9	17	2.8	5.1	1.6	3.5	9.8	17.5	3.6	10.1
	17.3	12.9	17	2.8	5.1	1.6	3.5	9.8	17.5	3.6	10.1
	18	14	18.1	3.1	5.3	1.8	3.7	9.8	17.5	3.6	10.1
	18.1	14	18.1	3.1	5.3	1.8	3.7	9.8	17.5	3.6	10.1
	18.2	14	18.1	3.1	5.3	1.8	3.7	9.8	17.5	3.6	10.1
	18.3	14	18.1	3.1	5.3	1.8	3.7	9.8	17.5	3.6	10.1
	19	15.2	19.2	3.3	5.5	1.9	3.8	9.8	17.5	3.6	10.1
	19.1	15.2	19.2	3.3	5.5	1.9	3.8	9.8	17.5	3.6	10.1
	19.2	15.2	19.2	3.3	5.5	1.9	3.8	9.8	17.5	3.6	10.1
	19.3	15.2	19.2	3.3	5.5	1.9	3.8	9.8	17.5	3.6	10.1
DYS19	20	16.3	20.3	3.5	5.7	2	4	9.8	17.5	3.6	10.1

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
YGATAH4	7	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	7.1	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	7.2	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	7.3	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	8	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	8.1	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	8.2	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	8.3	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	9	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	9.1	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	9.2	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	9.3	7.3	14.3	2.5	5.1	1.7	1.7	0	0	0	0
	10	8.4	15.4	2.5	5.1	1.7	1.7	0	0	0	0
	10.1	8.4	15.4	2.5	5.1	1.7	1.7	0	0	0	0
	10.2	8.4	15.4	2.5	5.1	1.7	1.7	0	0	0	0
YGATAH4	10.3	8.4	15.4	2.5	5.1	1.7	1.7	0	0	0	0
	11	9.5	16.5	2.5	5.1	1.7	1.7	0	0	0	0
	11.1	9.5	16.5	2.5	5.1	1.7	1.7	0	0	0	0
	11.2	9.5	16.5	2.5	5.1	1.7	1.7	0	0	0	0
	11.3	9.5	16.5	2.5	5.1	1.7	1.7	0	0	0	0
	12	10.6	17.6	2.5	5.1	1.7	1.7	0	0	0	0
	12.1	10.6	17.6	2.5	5.1	1.7	1.7	0	0	0	0
	12.2	10.6	17.6	2.5	5.1	1.7	1.7	0	0	0	0
	12.3	10.6	17.6	2.5	5.1	1.7	1.7	0	0	0	0
	13	11.7	18.7	2.5	5.1	1.7	1.7	0	0	0	0
	13.1	11.7	18.7	2.5	5.1	1.7	1.7	0	0	0	0
	13.2	11.7	18.7	2.5	5.1	1.7	1.7	0	0	0	0
	13.3	11.7	18.7	2.5	5.1	1.7	1.7	0	0	0	0
	14	12.8	19.8	2.5	5.1	1.7	1.7	0	0	0	0
	14.1	12.8	19.8	2.5	5.1	1.7	1.7	0	0	0	0
	14.2	12.8	19.8	2.5	5.1	1.7	1.7	0	0	0	0
	14.3	12.8	19.8	2.5	5.1	1.7	1.7	0	0	0	0
	15	13.9	20.9	2.5	5.1	1.7	1.7	0	0	0	0
	15.1	13.9	20.9	2.5	5.1	1.7	1.7	0	0	0	0
	15.2	13.9	20.9	2.5	5.1	1.7	1.7	0	0	0	0
	15.3	13.9	20.9	2.5	5.1	1.7	1.7	0	0	0	0
YGATAH4	16	15	22	2.5	5.1	1.7	1.7	0	0	0	0
DYS448	13	4.3	9.6	0	0	0	0	0	0	0	0
	13.1	4.3	9.6	0	0	0	0	0	0	0	0
	13.2	4.3	9.6	0	0	0	0	0	0	0	0
	13.3	4.3	9.6	0	0	0	0	0	0	0	0
	13.4	4.3	9.6	0	0	0	0	0	0	0	0
	13.5	4.3	9.6	0	0	0	0	0	0	0	0
	14	4.3	9.6	0	0	0	0	0	0	0	0
	14.1	4.3	9.6	0	0	0	0	0	0	0	0
	14.2	4.3	9.6	0	0	0	0	0	0	0	0
	14.3	4.3	9.6	0	0	0	0	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	14.4	4.3	9.6	0	0	0	0	0	0	0	0
	14.5	4.3	9.6	0	0	0	0	0	0	0	0
	15	4.3	9.6	0	0	0	0	0	0	0	0
	15.1	4.3	9.6	0	0	0	0	0	0	0	0
	15.2	4.3	9.6	0	0	0	0	0	0	0	0
	15.3	4.3	9.6	0	0	0	0	0	0	0	0
	15.4	4.3	9.6	0	0	0	0	0	0	0	0
	15.5	4.3	9.6	0	0	0	0	0	0	0	0
	16	4.3	9.6	0	0	0	0	0	0	0	0
	16.1	4.3	9.6	0	0	0	0	0	0	0	0
	16.2	4.3	9.6	0	0	0	0	0	0	0	0
	16.3	4.3	9.6	0	0	0	0	0	0	0	0
	16.4	4.3	9.6	0	0	0	0	0	0	0	0
	16.5	4.3	9.6	0	0	0	0	0	0	0	0
	17	4.3	9.6	0	0	0	0	0	0	0	0
	17.1	4.3	9.6	0	0	0	0	0	0	0	0
	17.2	4.3	9.6	0	0	0	0	0	0	0	0
	17.3	4.3	9.6	0	0	0	0	0	0	0	0
	17.4	4.3	9.6	0	0	0	0	0	0	0	0
	17.5	4.3	9.6	0	0	0	0	0	0	0	0
	18	4.3	9.6	0	0	0	0	0	0	0	0
	18.1	4.3	9.6	0	0	0	0	0	0	0	0
DYS448	18.2	4.3	9.6	0	0	0	0	0	0	0	0
	18.3	4.3	9.6	0	0	0	0	0	0	0	0
	18.4	4.3	9.6	0	0	0	0	0	0	0	0
	18.5	4.3	9.6	0	0	0	0	0	0	0	0
	19	4.6	9.9	0	0	0	0	0	0	0	0
	19.1	4.6	9.9	0	0	0	0	0	0	0	0
	19.2	4.6	9.9	0	0	0	0	0	0	0	0
	19.3	4.6	9.9	0	0	0	0	0	0	0	0
	19.4	4.6	9.9	0	0	0	0	0	0	0	0
	19.5	4.6	9.9	0	0	0	0	0	0	0	0
	20	4.8	10.1	0	0	0	0	0	0	0	0
	20.1	4.8	10.1	0	0	0	0	0	0	0	0
	20.2	4.8	10.1	0	0	0	0	0	0	0	0
	20.3	4.8	10.1	0	0	0	0	0	0	0	0
	20.4	4.8	10.1	0	0	0	0	0	0	0	0
	20.5	4.8	10.1	0	0	0	0	0	0	0	0
	21	5.1	10.4	0	0	0	0	0	0	0	0
	21.1	5.1	10.4	0	0	0	0	0	0	0	0
	21.2	5.1	10.4	0	0	0	0	0	0	0	0
	21.3	5.1	10.4	0	0	0	0	0	0	0	0
	21.4	5.1	10.4	0	0	0	0	0	0	0	0
	21.5	5.1	10.4	0	0	0	0	0	0	0	0
	22	5.3	10.7	0	0	0	0	0	0	0	0
	22.1	5.3	10.7	0	0	0	0	0	0	0	0
	22.2	5.3	10.7	0	0	0	0	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	23.3	5.6	10.9	0	0	0	0	0	0	0	0
	23.4	5.6	10.9	0	0	0	0	0	0	0	0
	23.5	5.6	10.9	0	0	0	0	0	0	0	0
	24	5.9	11.2	0	0	0	0	0	0	0	0
	24.1	5.9	11.2	0	0	0	0	0	0	0	0
	24.2	5.9	11.2	0	0	0	0	0	0	0	0
	24.3	5.9	11.2	0	0	0	0	0	0	0	0
	24.4	5.9	11.2	0	0	0	0	0	0	0	0
	24.5	5.9	11.2	0	0	0	0	0	0	0	0
DYS448	25	6.1	11.4	0	0	0	0	0	0	0	0
DYS391	4	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	4.1	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	4.2	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	4.3	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	5	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	5.1	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	5.2	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	5.3	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	6	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	6.1	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	6.2	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	6.3	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	7	7.2	11.7	3.7	3.7	0	0	0	0	0	0
DYS391	7.1	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	7.2	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	7.3	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	8	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	8.1	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	8.2	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	8.3	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	9	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	9.1	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	9.2	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	9.3	7.2	11.7	3.7	3.7	0	0	0	0	0	0
	10	8.4	12.9	3.7	3.7	0	0	0	0	0	0
	10.1	8.4	12.9	3.7	3.7	0	0	0	0	0	0
	10.2	8.4	12.9	3.7	3.7	0	0	0	0	0	0
	10.3	8.4	12.9	3.7	3.7	0	0	0	0	0	0
	11	9.6	14.1	3.7	3.7	0	0	0	0	0	0
	11.1	9.6	14.1	3.7	3.7	0	0	0	0	0	0
	11.2	9.6	14.1	3.7	3.7	0	0	0	0	0	0
	11.3	9.6	14.1	3.7	3.7	0	0	0	0	0	0
	12	10.8	15.3	3.7	3.7	0	0	0	0	0	0
	12.1	10.8	15.3	3.7	3.7	0	0	0	0	0	0
	12.2	10.8	15.3	3.7	3.7	0	0	0	0	0	0
	12.3	10.8	15.3	3.7	3.7	0	0	0	0	0	0
	13	12	16.5	3.7	3.7	0	0	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	13.1	12	16.5	3.7	3.7	0	0	0	0	0	0
	13.2	12	16.5	3.7	3.7	0	0	0	0	0	0
	13.3	12	16.5	3.7	3.7	0	0	0	0	0	0
	14	13.2	17.7	3.7	3.7	0	0	0	0	0	0
	14.1	13.2	17.7	3.7	3.7	0	0	0	0	0	0
	14.2	13.2	17.7	3.7	3.7	0	0	0	0	0	0
	14.3	13.2	17.7	3.7	3.7	0	0	0	0	0	0
	15	14.4	18.9	3.7	3.7	0	0	0	0	0	0
	15.1	14.4	18.9	3.7	3.7	0	0	0	0	0	0
	15.2	14.4	18.9	3.7	3.7	0	0	0	0	0	0
	15.3	14.4	18.9	3.7	3.7	0	0	0	0	0	0
	16	15.6	20.1	3.7	3.7	0	0	0	0	0	0
	16.1	15.6	20.1	3.7	3.7	0	0	0	0	0	0
	16.2	15.6	20.1	3.7	3.7	0	0	0	0	0	0
	16.3	15.6	20.1	3.7	3.7	0	0	0	0	0	0
DYS391	17	16.8	21.3	3.7	3.7	0	0	0	0	0	0
DYS456	9	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	9.1	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	9.2	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	9.3	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	10	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	10.1	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	10.2	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	10.3	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	11	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	11.1	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	11.2	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	11.3	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	12	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	12.1	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	12.2	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	12.3	10.8	17.5	3.7	8.4	1	3	0	0	0	0
	13	11.9	18.6	3.7	8.4	1	3	0	0	0	0
	13.1	11.9	18.6	3.7	8.4	1	3	0	0	0	0
	13.2	11.9	18.6	3.7	8.4	1	3	0	0	0	0
	13.3	11.9	18.6	3.7	8.4	1	3	0	0	0	0
	14	13	19.7	3.7	8.4	1.2	3.2	0	0	0	0
	14.1	13	19.7	3.7	8.4	1.2	3.2	0	0	0	0
	14.2	13	19.7	3.7	8.4	1.2	3.2	0	0	0	0
	14.3	13	19.7	3.7	8.4	1.2	3.2	0	0	0	0
	15	14	20.8	3.7	8.4	1.4	3.3	0	0	0	0
	15.1	14	20.8	3.7	8.4	1.4	3.3	0	0	0	0
	15.2	14	20.8	3.7	8.4	1.4	3.3	0	0	0	0
	15.3	14	20.8	3.7	8.4	1.4	3.3	0	0	0	0
DYS456	16	15.1	21.9	3.7	8.4	1.6	3.5	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	16.1	15.1	21.9	3.7	8.4	1.6	3.5	0	0	0	0
	16.2	15.1	21.9	3.7	8.4	1.6	3.5	0	0	0	0
	16.3	15.1	21.9	3.7	8.4	1.6	3.5	0	0	0	0
	17	16.2	23	3.7	8.4	1.7	3.7	0	0	0	0
	17.1	16.2	23	3.7	8.4	1.7	3.7	0	0	0	0
	17.2	16.2	23	3.7	8.4	1.7	3.7	0	0	0	0
	17.3	16.2	23	3.7	8.4	1.7	3.7	0	0	0	0
	18	17.3	24.1	3.7	8.4	1.9	3.9	0	0	0	0
	18.1	17.3	24.1	3.7	8.4	1.9	3.9	0	0	0	0
	18.2	17.3	24.1	3.7	8.4	1.9	3.9	0	0	0	0
	18.3	17.3	24.1	3.7	8.4	1.9	3.9	0	0	0	0
	19	18.4	25.1	3.7	8.4	2.1	4.1	0	0	0	0
	19.1	18.4	25.1	3.7	8.4	2.1	4.1	0	0	0	0
	19.2	18.4	25.1	3.7	8.4	2.1	4.1	0	0	0	0
	19.3	18.4	25.1	3.7	8.4	2.1	4.1	0	0	0	0
	20	19.5	26.2	3.7	8.4	2.3	4.2	0	0	0	0
	20.1	19.5	26.2	3.7	8.4	2.3	4.2	0	0	0	0
	20.2	19.5	26.2	3.7	8.4	2.3	4.2	0	0	0	0
	20.3	19.5	26.2	3.7	8.4	2.3	4.2	0	0	0	0
	21	20.6	27.3	3.7	8.4	2.5	4.4	0	0	0	0
	21.1	20.6	27.3	3.7	8.4	2.5	4.4	0	0	0	0
	21.2	20.6	27.3	3.7	8.4	2.5	4.4	0	0	0	0
	21.3	20.6	27.3	3.7	8.4	2.5	4.4	0	0	0	0
	22	21.7	28.4	3.7	8.4	2.6	4.6	0	0	0	0
	22.1	21.7	28.4	3.7	8.4	2.6	4.6	0	0	0	0
	22.2	21.7	28.4	3.7	8.4	2.6	4.6	0	0	0	0
	22.3	21.7	28.4	3.7	8.4	2.6	4.6	0	0	0	0
	23	22.8	29.5	3.7	8.4	2.8	4.8	0	0	0	0
DYS456	23.1	22.8	29.5	3.7	8.4	2.8	4.8	0	0	0	0
	23.2	22.8	29.5	3.7	8.4	2.8	4.8	0	0	0	0
	23.3	22.8	29.5	3.7	8.4	2.8	4.8	0	0	0	0
	24	23.9	30.6	3.7	8.4	3	5	0	0	0	0
DYS390	16	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	16.1	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	16.2	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	16.3	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	17	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	17.1	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	17.2	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	17.3	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	18	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	18.1	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	18.2	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	18.3	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	19	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	19.1	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	19.2	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	19.3	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	20	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	20.1	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	20.2	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	20.3	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	21	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	21.1	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	21.2	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	21.3	8.8	11.3	2.2	2.2	1.2	1.2	0	0	0	0
	22	9.9	12.3	2.2	2.2	1.3	1.3	0	0	0	0
	22.1	9.9	12.3	2.2	2.2	1.3	1.3	0	0	0	0
	22.2	9.9	12.3	2.2	2.2	1.3	1.3	0	0	0	0
	22.3	9.9	12.3	2.2	2.2	1.3	1.3	0	0	0	0
DYS390	23	10.9	13.4	2.2	2.2	1.4	1.4	0	0	0	0
	23.1	10.9	13.4	2.2	2.2	1.4	1.4	0	0	0	0
	23.2	10.9	13.4	2.2	2.2	1.4	1.4	0	0	0	0
	23.3	10.9	13.4	2.2	2.2	1.4	1.4	0	0	0	0
	24	12	14.4	2.2	2.2	1.5	1.5	0	0	0	0
	24.1	12	14.4	2.2	2.2	1.5	1.5	0	0	0	0
	24.2	12	14.4	2.2	2.2	1.5	1.5	0	0	0	0
	24.3	12	14.4	2.2	2.2	1.5	1.5	0	0	0	0
	25	13	15.4	2.2	2.2	1.6	1.6	0	0	0	0
	25.1	13	15.4	2.2	2.2	1.6	1.6	0	0	0	0
	25.2	13	15.4	2.2	2.2	1.6	1.6	0	0	0	0
	25.3	13	15.4	2.2	2.2	1.6	1.6	0	0	0	0
	26	14	16.5	2.2	2.2	1.7	1.7	0	0	0	0
	26.1	14	16.5	2.2	2.2	1.7	1.7	0	0	0	0
	26.2	14	16.5	2.2	2.2	1.7	1.7	0	0	0	0
	26.3	14	16.5	2.2	2.2	1.7	1.7	0	0	0	0
	27	15.1	17.5	2.2	2.2	1.8	1.8	0	0	0	0
	27.1	15.1	17.5	2.2	2.2	1.8	1.8	0	0	0	0
	27.2	15.1	17.5	2.2	2.2	1.8	1.8	0	0	0	0
	27.3	15.1	17.5	2.2	2.2	1.8	1.8	0	0	0	0
	28	16.1	18.6	2.2	2.2	1.9	1.9	0	0	0	0
	28.1	16.1	18.6	2.2	2.2	1.9	1.9	0	0	0	0
DYS390	28.2	16.1	18.6	2.2	2.2	1.9	1.9	0	0	0	0
	28.3	16.1	18.6	2.2	2.2	1.9	1.9	0	0	0	0
	29	17.2	19.6	2.2	2.2	2	2	0	0	0	0
	29.1	17.2	19.6	2.2	2.2	2	2	0	0	0	0
	29.2	17.2	19.6	2.2	2.2	2	2	0	0	0	0
	29.3	17.2	19.6	2.2	2.2	2	2	0	0	0	0
	30	18.2	20.6	2.2	2.2	2.1	2.1	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
DYS438	5	3.4	5.3	2	2	1	1	0	0	0	0
	5.1	3.4	5.3	2	2	1	1	0	0	0	0
	5.2	3.4	5.3	2	2	1	1	0	0	0	0
	5.3	3.4	5.3	2	2	1	1	0	0	0	0
	5.4	3.4	5.3	2	2	1	1	0	0	0	0
	6	3.4	5.3	2	2	1	1	0	0	0	0
	6.1	3.4	5.3	2	2	1	1	0	0	0	0
	6.2	3.4	5.3	2	2	1	1	0	0	0	0
	6.3	3.4	5.3	2	2	1	1	0	0	0	0
	6.4	3.4	5.3	2	2	1	1	0	0	0	0
	7	3.4	5.3	2	2	1	1	0	0	0	0
	7.1	3.4	5.3	2	2	1	1	0	0	0	0
	7.2	3.4	5.3	2	2	1	1	0	0	0	0
	7.3	3.4	5.3	2	2	1	1	0	0	0	0
	7.4	3.4	5.3	2	2	1	1	0	0	0	0
	8	3.4	5.3	2	2	1	1	0	0	0	0
	8.1	3.4	5.3	2	2	1	1	0	0	0	0
	8.2	3.4	5.3	2	2	1	1	0	0	0	0
	8.3	3.4	5.3	2	2	1	1	0	0	0	0
	8.4	3.4	5.3	2	2	1	1	0	0	0	0
	9	3.9	5.8	2	2	1	1	0	0	0	0
	9.1	3.9	5.8	2	2	1	1	0	0	0	0
	9.2	3.9	5.8	2	2	1	1	0	0	0	0
	9.3	3.9	5.8	2	2	1	1	0	0	0	0
	9.4	3.9	5.8	2	2	1	1	0	0	0	0
	10	4.4	6.3	2	2	1	1	0	0	0	0
	10.1	4.4	6.3	2	2	1	1	0	0	0	0
	10.2	4.4	6.3	2	2	1	1	0	0	0	0
	10.3	4.4	6.3	2	2	1	1	0	0	0	0
	10.4	4.4	6.3	2	2	1	1	0	0	0	0
DYS438	11	4.9	6.8	2	2	1	1	0	0	0	0
	11.1	4.9	6.8	2	2	1	1	0	0	0	0
	11.2	4.9	6.8	2	2	1	1	0	0	0	0
	11.3	4.9	6.8	2	2	1	1	0	0	0	0
	11.4	4.9	6.8	2	2	1	1	0	0	0	0
	12	5.4	7.3	2	2	1	1	0	0	0	0
	12.1	5.4	7.3	2	2	1	1	0	0	0	0
	12.2	5.4	7.3	2	2	1	1	0	0	0	0
	12.3	5.4	7.3	2	2	1	1	0	0	0	0
	12.4	5.4	7.3	2	2	1	1	0	0	0	0
	13	5.9	7.8	2	2	1	1	0	0	0	0
	13.1	5.9	7.8	2	2	1	1	0	0	0	0
	13.2	5.9	7.8	2	2	1	1	0	0	0	0
	13.3	5.9	7.8	2	2	1	1	0	0	0	0
	13.4	5.9	7.8	2	2	1	1	0	0	0	0
	14	6.4	8.3	2	2	1	1	0	0	0	0
	14.1	6.4	8.3	2	2	1	1	0	0	0	0
DYS438	14.2	6.4	8.3	2	2	1	1	0	0	0	0
	14.3	6.4	8.3	2	2	1	1	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	14.4	6.4	8.3	2	2	1	1	0	0	0	0
	15	6.9	8.8	2	2	1	1	0	0	0	0
	15.1	6.9	8.8	2	2	1	1	0	0	0	0
	15.2	6.9	8.8	2	2	1	1	0	0	0	0
	15.3	6.9	8.8	2	2	1	1	0	0	0	0
	15.4	6.9	8.8	2	2	1	1	0	0	0	0
	16	7.4	9.3	2	2	1	1	0	0	0	0
	16.1	7.4	9.3	2	2	1	1	0	0	0	0
	16.2	7.4	9.3	2	2	1	1	0	0	0	0
	16.3	7.4	9.3	2	2	1	1	0	0	0	0
	16.4	7.4	9.3	2	2	1	1	0	0	0	0
DYS438	17	7.9	9.8	2	2	1	1	0	0	0	0
DYS392	3	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	3.1	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	3.2	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	4	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	4.1	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	4.2	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	5	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	5.1	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	5.2	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	6	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	6.1	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	6.2	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	7	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	7.1	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	7.2	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	8	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	8.1	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	8.2	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	9	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	9.1	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	9.2	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	10	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	10.1	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	10.2	9.4	12.3	5.7	10	1.6	2.4	0	0	0	0
	11	11	14	6.9	11.2	1.6	2.4	0	0	0	0
	11.1	11	14	6.9	11.2	1.6	2.4	0	0	0	0
	11.2	11	14	6.9	11.2	1.6	2.4	0	0	0	0
DYS392	12	12.7	15.6	8.1	12.4	1.9	2.6	0	0	0	0
	12.1	12.7	15.6	8.1	12.4	1.9	2.6	0	0	0	0
	12.2	12.7	15.6	8.1	12.4	1.9	2.6	0	0	0	0
	13	14.3	17.3	9.3	13.6	2.1	2.9	0	0	0	0
	13.1	14.3	17.3	9.3	13.6	2.1	2.9	0	0	0	0
	13.2	14.3	17.3	9.3	13.6	2.1	2.9	0	0	0	0
	14	16	18.9	10.5	14.8	2.4	3.2	0	0	0	0
	14.1	16	18.9	10.5	14.8	2.4	3.2	0	0	0	0
	14.2	16	18.9	10.5	14.8	2.4	3.2	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	15	17.6	20.6	11.7	15.9	2.6	3.4	0	0	0	0
	15.1	17.6	20.6	11.7	15.9	2.6	3.4	0	0	0	0
	15.2	17.6	20.6	11.7	15.9	2.6	3.4	0	0	0	0
	16	19.3	22.2	12.9	17.1	2.9	3.7	0	0	0	0
DYS392	16.1	19.3	22.2	12.9	17.1	2.9	3.7	0	0	0	0
	16.2	19.3	22.2	12.9	17.1	2.9	3.7	0	0	0	0
	17	20.9	23.9	14	18.3	3.2	3.9	0	0	0	0
	17.1	20.9	23.9	14	18.3	3.2	3.9	0	0	0	0
	17.2	20.9	23.9	14	18.3	3.2	3.9	0	0	0	0
	18	22.6	25.5	15.2	19.5	3.4	4.2	0	0	0	0
	18.1	22.6	25.5	15.2	19.5	3.4	4.2	0	0	0	0
	18.2	22.6	25.5	15.2	19.5	3.4	4.2	0	0	0	0
	19	24.2	27.2	16.4	20.7	3.7	4.5	0	0	0	0
	19.1	24.2	27.2	16.4	20.7	3.7	4.5	0	0	0	0
	19.2	24.2	27.2	16.4	20.7	3.7	4.5	0	0	0	0
	20	25.9	28.8	17.6	21.9	3.9	4.7	0	0	0	0
	20.1	25.9	28.8	17.6	21.9	3.9	4.7	0	0	0	0
	20.2	25.9	28.8	17.6	21.9	3.9	4.7	0	0	0	0
DYS392	21	27.5	30.5	18.8	23.1	4.2	5	0	0	0	0

DYS518	31	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	31.1	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	31.2	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	31.3	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	32	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	32.1	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	32.2	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	32.3	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	33	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	33.1	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	33.2	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	33.3	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	34	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	34.1	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	34.2	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	34.3	19.1	24.2	5.7	10.6	2.4	3.3	0	0	0	0
	35	20.1	25.3	5.7	10.6	2.6	3.5	0	0	0	0
	35.1	20.1	25.3	5.7	10.6	2.6	3.5	0	0	0	0
	35.2	20.1	25.3	5.7	10.6	2.6	3.5	0	0	0	0
	35.3	20.1	25.3	5.7	10.6	2.6	3.5	0	0	0	0
	36	21.2	26.3	5.7	10.6	2.8	3.7	0	0	0	0
	36.1	21.2	26.3	5.7	10.6	2.8	3.7	0	0	0	0
	36.2	21.2	26.3	5.7	10.6	2.8	3.7	0	0	0	0
	36.3	21.2	26.3	5.7	10.6	2.8	3.7	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
DYS518	37	22.2	27.4	5.7	10.6	3	3.9	0	0	0	0
	37.1	22.2	27.4	5.7	10.6	3	3.9	0	0	0	0
	37.2	22.2	27.4	5.7	10.6	3	3.9	0	0	0	0
	37.3	22.2	27.4	5.7	10.6	3	3.9	0	0	0	0
	38	23.2	28.4	5.7	10.6	3.2	4.1	0	0	0	0
	38.1	23.2	28.4	5.7	10.6	3.2	4.1	0	0	0	0
	38.2	23.2	28.4	5.7	10.6	3.2	4.1	0	0	0	0
	38.3	23.2	28.4	5.7	10.6	3.2	4.1	0	0	0	0
	39	24.3	29.4	5.7	10.6	3.4	4.2	0	0	0	0
	39.1	24.3	29.4	5.7	10.6	3.4	4.2	0	0	0	0
	39.2	24.3	29.4	5.7	10.6	3.4	4.2	0	0	0	0
	39.3	24.3	29.4	5.7	10.6	3.4	4.2	0	0	0	0
	40	25.3	30.5	5.7	10.6	3.5	4.4	0	0	0	0
	40.1	25.3	30.5	5.7	10.6	3.5	4.4	0	0	0	0
	40.2	25.3	30.5	5.7	10.6	3.5	4.4	0	0	0	0
DYS518	40.3	25.3	30.5	5.7	10.6	3.5	4.4	0	0	0	0
	41	26.4	31.5	5.7	10.6	3.7	4.6	0	0	0	0
	41.1	26.4	31.5	5.7	10.6	3.7	4.6	0	0	0	0
	41.2	26.4	31.5	5.7	10.6	3.7	4.6	0	0	0	0
	41.3	26.4	31.5	5.7	10.6	3.7	4.6	0	0	0	0
	42	27.4	32.6	5.7	10.6	3.9	4.8	0	0	0	0
	42.1	27.4	32.6	5.7	10.6	3.9	4.8	0	0	0	0
	42.2	27.4	32.6	5.7	10.6	3.9	4.8	0	0	0	0
	42.3	27.4	32.6	5.7	10.6	3.9	4.8	0	0	0	0
	43	28.4	33.6	5.7	10.6	4.1	5	0	0	0	0
	43.1	28.4	33.6	5.7	10.6	4.1	5	0	0	0	0
	43.2	28.4	33.6	5.7	10.6	4.1	5	0	0	0	0
	43.3	28.4	33.6	5.7	10.6	4.1	5	0	0	0	0
	44	29.5	34.6	5.7	10.6	4.3	5.2	0	0	0	0
	44.1	29.5	34.6	5.7	10.6	4.3	5.2	0	0	0	0
	44.2	29.5	34.6	5.7	10.6	4.3	5.2	0	0	0	0
	44.3	29.5	34.6	5.7	10.6	4.3	5.2	0	0	0	0
	45	30.5	35.7	5.7	10.6	4.5	5.4	0	0	0	0
	45.1	30.5	35.7	5.7	10.6	4.5	5.4	0	0	0	0
	45.2	30.5	35.7	5.7	10.6	4.5	5.4	0	0	0	0
	45.3	30.5	35.7	5.7	10.6	4.5	5.4	0	0	0	0
	46	31.6	36.7	5.7	10.6	4.7	5.6	0	0	0	0
	46.1	31.6	36.7	5.7	10.6	4.7	5.6	0	0	0	0
	46.2	31.6	36.7	5.7	10.6	4.7	5.6	0	0	0	0
	46.3	31.6	36.7	5.7	10.6	4.7	5.6	0	0	0	0
	47	32.6	37.8	5.7	10.6	4.9	5.8	0	0	0	0
	47.1	32.6	37.8	5.7	10.6	4.9	5.8	0	0	0	0
	47.2	32.6	37.8	5.7	10.6	4.9	5.8	0	0	0	0
	47.3	32.6	37.8	5.7	10.6	4.9	5.8	0	0	0	0
	48	33.6	38.8	5.7	10.6	5.1	6	0	0	0	0
	48.1	33.6	38.8	5.7	10.6	5.1	6	0	0	0	0
	48.2	33.6	38.8	5.7	10.6	5.1	6	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	48.3	33.6	38.8	5.7	10.6	5.1	6	0	0	0	0
	49	34.7	39.8	5.7	10.6	5.3	6.1	0	0	0	0
	49.1	34.7	39.8	5.7	10.6	5.3	6.1	0	0	0	0
	49.2	34.7	39.8	5.7	10.6	5.3	6.1	0	0	0	0
	49.3	34.7	39.8	5.7	10.6	5.3	6.1	0	0	0	0
DYS518	50	35.7	40.9	5.7	10.6	5.4	6.3	0	0	0	0
DYS570	9	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	9.1	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	9.2	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	9.3	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	10	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	10.1	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	10.2	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	10.3	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	11	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	11.1	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	11.2	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	11.3	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	12	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	12.1	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	12.2	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	12.3	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	13	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	13.1	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	13.2	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	13.3	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	14	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	14.1	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	14.2	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
	14.3	10.3	13.7	2.9	5.7	2	4.3	0	0	0	0
DYS570	15	11.1	14.5	2.9	5.7	2	4.3	0	0	0	0
	15.1	11.1	14.5	2.9	5.7	2	4.3	0	0	0	0
	15.2	11.1	14.5	2.9	5.7	2	4.3	0	0	0	0
	15.3	11.1	14.5	2.9	5.7	2	4.3	0	0	0	0
	16	11.9	15.3	2.9	5.7	2	4.3	0	0	0	0
	16.1	11.9	15.3	2.9	5.7	2	4.3	0	0	0	0
	16.2	11.9	15.3	2.9	5.7	2	4.3	0	0	0	0
	16.3	11.9	15.3	2.9	5.7	2	4.3	0	0	0	0
	17	12.7	16.1	2.9	5.7	2	4.3	0	0	0	0
	17.1	12.7	16.1	2.9	5.7	2	4.3	0	0	0	0
	17.2	12.7	16.1	2.9	5.7	2	4.3	0	0	0	0
	17.3	12.7	16.1	2.9	5.7	2	4.3	0	0	0	0
	18	13.5	16.9	2.9	5.7	2	4.3	0	0	0	0
	18.1	13.5	16.9	2.9	5.7	2	4.3	0	0	0	0
	18.2	13.5	16.9	2.9	5.7	2	4.3	0	0	0	0
	18.3	13.5	16.9	2.9	5.7	2	4.3	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	19	14.3	17.7	2.9	5.7	2	4.3	0	0	0	0
	19.1	14.3	17.7	2.9	5.7	2	4.3	0	0	0	0
	19.2	14.3	17.7	2.9	5.7	2	4.3	0	0	0	0
	19.3	14.3	17.7	2.9	5.7	2	4.3	0	0	0	0
	20	15.1	18.5	2.9	5.7	2	4.3	0	0	0	0
	20.1	15.1	18.5	2.9	5.7	2	4.3	0	0	0	0
	20.2	15.1	18.5	2.9	5.7	2	4.3	0	0	0	0
	20.3	15.1	18.5	2.9	5.7	2	4.3	0	0	0	0
	21	15.9	19.3	2.9	5.7	2	4.3	0	0	0	0
	21.1	15.9	19.3	2.9	5.7	2	4.3	0	0	0	0
	21.2	15.9	19.3	2.9	5.7	2	4.3	0	0	0	0
	21.3	15.9	19.3	2.9	5.7	2	4.3	0	0	0	0
	22	16.7	20.1	2.9	5.7	2	4.3	0	0	0	0
	22.1	16.7	20.1	2.9	5.7	2	4.3	0	0	0	0
	22.2	16.7	20.1	2.9	5.7	2	4.3	0	0	0	0
	22.3	16.7	20.1	2.9	5.7	2	4.3	0	0	0	0
	23	17.5	21	2.9	5.7	2	4.3	0	0	0	0
DYS570	23.1	17.5	21	2.9	5.7	2	4.3	0	0	0	0
	23.2	17.5	21	2.9	5.7	2	4.3	0	0	0	0
	23.3	17.5	21	2.9	5.7	2	4.3	0	0	0	0
	24	18.4	21.8	2.9	5.7	2	4.3	0	0	0	0
	24.1	18.4	21.8	2.9	5.7	2	4.3	0	0	0	0
	24.2	18.4	21.8	2.9	5.7	2	4.3	0	0	0	0
	24.3	18.4	21.8	2.9	5.7	2	4.3	0	0	0	0
	25	19.2	22.6	2.9	5.7	2	4.3	0	0	0	0
	25.1	19.2	22.6	2.9	5.7	2	4.3	0	0	0	0
	25.2	19.2	22.6	2.9	5.7	2	4.3	0	0	0	0
	25.3	19.2	22.6	2.9	5.7	2	4.3	0	0	0	0
	26	20	23.4	2.9	5.7	2	4.3	0	0	0	0
	26.1	20	23.4	2.9	5.7	2	4.3	0	0	0	0
	26.2	20	23.4	2.9	5.7	2	4.3	0	0	0	0
	26.3	20	23.4	2.9	5.7	2	4.3	0	0	0	0
DYS570	27	20.8	24.2	2.9	5.7	2	4.3	0	0	0	0
DYS437	9	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	9.1	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	9.2	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	9.3	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	10	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	10.1	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	10.2	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	10.3	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	11	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	11.1	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	11.2	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	11.3	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	12	4.3	7.6	2.2	2.2	0	0	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	12.1	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	12.2	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	12.3	4.3	7.6	2.2	2.2	0	0	0	0	0	0
	13	5.3	8.6	2.2	2.2	0	0	0	0	0	0
	13.1	5.3	8.6	2.2	2.2	0	0	0	0	0	0
	13.2	5.3	8.6	2.2	2.2	0	0	0	0	0	0
	13.3	5.3	8.6	2.2	2.2	0	0	0	0	0	0
	14	6.3	9.6	2.2	2.2	0	0	0	0	0	0
	14.1	6.3	9.6	2.2	2.2	0	0	0	0	0	0
	14.2	6.3	9.6	2.2	2.2	0	0	0	0	0	0
	14.3	6.3	9.6	2.2	2.2	0	0	0	0	0	0
	15	7.3	10.7	2.2	2.2	0	0	0	0	0	0
	15.1	7.3	10.7	2.2	2.2	0	0	0	0	0	0
	15.2	7.3	10.7	2.2	2.2	0	0	0	0	0	0
	15.3	7.3	10.7	2.2	2.2	0	0	0	0	0	0
DYS437	16	8.4	11.7	2.2	2.2	0	0	0	0	0	0
	16.1	8.4	11.7	2.2	2.2	0	0	0	0	0	0
	16.2	8.4	11.7	2.2	2.2	0	0	0	0	0	0
	16.3	8.4	11.7	2.2	2.2	0	0	0	0	0	0
	17	9.4	12.7	2.2	2.2	0	0	0	0	0	0
	17.1	9.4	12.7	2.2	2.2	0	0	0	0	0	0
	17.2	9.4	12.7	2.2	2.2	0	0	0	0	0	0
	17.3	9.4	12.7	2.2	2.2	0	0	0	0	0	0
	18	10.4	13.7	2.2	2.2	0	0	0	0	0	0
	18.1	10.4	13.7	2.2	2.2	0	0	0	0	0	0
DYS437	18.2	10.4	13.7	2.2	2.2	0	0	0	0	0	0
	18.3	10.4	13.7	2.2	2.2	0	0	0	0	0	0
	19	11.4	14.7	2.2	2.2	0	0	0	0	0	0
DYS385	5	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	5.1	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	5.2	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	5.3	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	6	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	6.1	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	6.2	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	6.3	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	7	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	7.1	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	7.2	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	7.3	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	8	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	8.1	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	8.2	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	8.3	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	9	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	9.1	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	9.2	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	9.3	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	10	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	10.1	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	10.2	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	10.3	8.9	12.5	3	9.9	1.2	1.2	0	0	0	0
	11	9.8	13.4	3	9.9	1.3	1.3	0	0	0	0
	11.1	9.8	13.4	3	9.9	1.3	1.3	0	0	0	0
	11.2	9.8	13.4	3	9.9	1.3	1.3	0	0	0	0
	11.3	9.8	13.4	3	9.9	1.3	1.3	0	0	0	0
	12	10.7	14.3	3	9.9	1.5	1.5	0	0	0	0
	12.1	10.7	14.3	3	9.9	1.5	1.5	0	0	0	0
	12.2	10.7	14.3	3	9.9	1.5	1.5	0	0	0	0
	12.3	10.7	14.3	3	9.9	1.5	1.5	0	0	0	0
DYS385	13	11.6	15.2	3	9.9	1.6	1.6	0	0	0	0
	13.1	11.6	15.2	3	9.9	1.6	1.6	0	0	0	0
	13.2	11.6	15.2	3	9.9	1.6	1.6	0	0	0	0
	13.3	11.6	15.2	3	9.9	1.6	1.6	0	0	0	0
	14	12.5	16.1	3	9.9	1.7	1.7	0	0	0	0
	14.1	12.5	16.1	3	9.9	1.7	1.7	0	0	0	0
	14.2	12.5	16.1	3	9.9	1.7	1.7	0	0	0	0
	14.3	12.5	16.1	3	9.9	1.7	1.7	0	0	0	0
	15	13.4	17	3	9.9	1.9	1.9	0	0	0	0
	15.1	13.4	17	3	9.9	1.9	1.9	0	0	0	0
	15.2	13.4	17	3	9.9	1.9	1.9	0	0	0	0
	15.3	13.4	17	3	9.9	1.9	1.9	0	0	0	0
	16	14.3	17.9	3	9.9	2	2	0	0	0	0
	16.1	14.3	17.9	3	9.9	2	2	0	0	0	0
	16.2	14.3	17.9	3	9.9	2	2	0	0	0	0
	16.3	14.3	17.9	3	9.9	2	2	0	0	0	0
	17	15.2	18.8	3	9.9	2.2	2.2	0	0	0	0
	17.1	15.2	18.8	3	9.9	2.2	2.2	0	0	0	0
	17.2	15.2	18.8	3	9.9	2.2	2.2	0	0	0	0
DYS385	17.3	15.2	18.8	3	9.9	2.2	2.2	0	0	0	0
	18	16.1	19.7	3	9.9	2.3	2.3	0	0	0	0
	18.1	16.1	19.7	3	9.9	2.3	2.3	0	0	0	0
	18.2	16.1	19.7	3	9.9	2.3	2.3	0	0	0	0
	18.3	16.1	19.7	3	9.9	2.3	2.3	0	0	0	0
	19	17	20.6	3	9.9	2.4	2.4	0	0	0	0
	19.1	17	20.6	3	9.9	2.4	2.4	0	0	0	0
	19.2	17	20.6	3	9.9	2.4	2.4	0	0	0	0
	19.3	17	20.6	3	9.9	2.4	2.4	0	0	0	0
	20	17.9	21.5	3	9.9	2.6	2.6	0	0	0	0
	20.1	17.9	21.5	3	9.9	2.6	2.6	0	0	0	0
	20.2	17.9	21.5	3	9.9	2.6	2.6	0	0	0	0
	20.3	17.9	21.5	3	9.9	2.6	2.6	0	0	0	0
	21	18.8	22.4	3	9.9	2.7	2.7	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	21.1	18.8	22.4	3	9.9	2.7	2.7	0	0	0	0
	21.2	18.8	22.4	3	9.9	2.7	2.7	0	0	0	0
	21.3	18.8	22.4	3	9.9	2.7	2.7	0	0	0	0
	22	19.7	23.3	3	9.9	2.9	2.9	0	0	0	0
	22.1	19.7	23.3	3	9.9	2.9	2.9	0	0	0	0
	22.2	19.7	23.3	3	9.9	2.9	2.9	0	0	0	0
	22.3	19.7	23.3	3	9.9	2.9	2.9	0	0	0	0
	23	20.6	24.2	3	9.9	3	3	0	0	0	0
	23.1	20.6	24.2	3	9.9	3	3	0	0	0	0
	23.2	20.6	24.2	3	9.9	3	3	0	0	0	0
	23.3	20.6	24.2	3	9.9	3	3	0	0	0	0
	24	21.5	25.1	3	9.9	3.1	3.1	0	0	0	0
	24.1	21.5	25.1	3	9.9	3.1	3.1	0	0	0	0
	24.2	21.5	25.1	3	9.9	3.1	3.1	0	0	0	0
	24.3	21.5	25.1	3	9.9	3.1	3.1	0	0	0	0
	25	22.4	26	3	9.9	3.3	3.3	0	0	0	0
	25.1	22.4	26	3	9.9	3.3	3.3	0	0	0	0
	25.2	22.4	26	3	9.9	3.3	3.3	0	0	0	0
	25.3	22.4	26	3	9.9	3.3	3.3	0	0	0	0
	26	23.3	26.9	3	9.9	3.4	3.4	0	0	0	0
	26.1	23.3	26.9	3	9.9	3.4	3.4	0	0	0	0
	26.2	23.3	26.9	3	9.9	3.4	3.4	0	0	0	0
	26.3	23.3	26.9	3	9.9	3.4	3.4	0	0	0	0
	27	24.2	27.8	3	9.9	3.6	3.6	0	0	0	0
	27.1	24.2	27.8	3	9.9	3.6	3.6	0	0	0	0
	27.2	24.2	27.8	3	9.9	3.6	3.6	0	0	0	0
	27.3	24.2	27.8	3	9.9	3.6	3.6	0	0	0	0
	28	25.1	28.7	3	9.9	3.7	3.7	0	0	0	0
	28.1	25.1	28.7	3	9.9	3.7	3.7	0	0	0	0
	28.2	25.1	28.7	3	9.9	3.7	3.7	0	0	0	0
	28.3	25.1	28.7	3	9.9	3.7	3.7	0	0	0	0
DYS385	29	26	29.6	3	9.9	3.8	3.8	0	0	0	0
DYS449	21	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	21.1	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	21.2	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	21.3	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	22	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	22.1	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	22.2	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	22.3	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
DYS449	23	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	23.1	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	23.2	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	23.3	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	24	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	24.1	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	24.2	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	24.3	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	25	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	25.1	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	25.2	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	25.3	16.3	23.6	3.9	9.3	1.9	3.9	0	0	0	0
	26	17.1	24.5	3.9	9.3	2.1	4.1	0	0	0	0
	26.1	17.1	24.5	3.9	9.3	2.1	4.1	0	0	0	0
	26.2	17.1	24.5	3.9	9.3	2.1	4.1	0	0	0	0
	26.3	17.1	24.5	3.9	9.3	2.1	4.1	0	0	0	0
	27	18	25.3	3.9	9.3	2.3	4.3	0	0	0	0
	27.1	18	25.3	3.9	9.3	2.3	4.3	0	0	0	0
	27.2	18	25.3	3.9	9.3	2.3	4.3	0	0	0	0
	27.3	18	25.3	3.9	9.3	2.3	4.3	0	0	0	0
	28	18.8	26.2	3.9	9.3	2.4	4.5	0	0	0	0
	28.1	18.8	26.2	3.9	9.3	2.4	4.5	0	0	0	0
	28.2	18.8	26.2	3.9	9.3	2.4	4.5	0	0	0	0
	28.3	18.8	26.2	3.9	9.3	2.4	4.5	0	0	0	0
	29	19.7	27	3.9	9.3	2.6	4.6	0	0	0	0
	29.1	19.7	27	3.9	9.3	2.6	4.6	0	0	0	0
	29.2	19.7	27	3.9	9.3	2.6	4.6	0	0	0	0
	29.3	19.7	27	3.9	9.3	2.6	4.6	0	0	0	0
	30	20.5	27.9	3.9	9.3	2.8	4.8	0	0	0	0
	30.1	20.5	27.9	3.9	9.3	2.8	4.8	0	0	0	0
	30.2	20.5	27.9	3.9	9.3	2.8	4.8	0	0	0	0
	30.3	20.5	27.9	3.9	9.3	2.8	4.8	0	0	0	0
	31	21.4	28.7	3.9	9.3	3	5	0	0	0	0
	31.1	21.4	28.7	3.9	9.3	3	5	0	0	0	0
	31.2	21.4	28.7	3.9	9.3	3	5	0	0	0	0
	31.3	21.4	28.7	3.9	9.3	3	5	0	0	0	0
	32	22.2	29.6	3.9	9.3	3.2	5.2	0	0	0	0
	32.1	22.2	29.6	3.9	9.3	3.2	5.2	0	0	0	0
	32.2	22.2	29.6	3.9	9.3	3.2	5.2	0	0	0	0
	32.3	22.2	29.6	3.9	9.3	3.2	5.2	0	0	0	0
	33	23.1	30.4	3.9	9.3	3.3	5.4	0	0	0	0
	33.1	23.1	30.4	3.9	9.3	3.3	5.4	0	0	0	0
	33.2	23.1	30.4	3.9	9.3	3.3	5.4	0	0	0	0
	33.3	23.1	30.4	3.9	9.3	3.3	5.4	0	0	0	0
DYS449	34	23.9	31.3	3.9	9.3	3.5	5.5	0	0	0	0
	34.1	23.9	31.3	3.9	9.3	3.5	5.5	0	0	0	0
	34.2	23.9	31.3	3.9	9.3	3.5	5.5	0	0	0	0
	34.3	23.9	31.3	3.9	9.3	3.5	5.5	0	0	0	0
	35	24.8	32.1	3.9	9.3	3.7	5.7	0	0	0	0
	35.1	24.8	32.1	3.9	9.3	3.7	5.7	0	0	0	0
	35.2	24.8	32.1	3.9	9.3	3.7	5.7	0	0	0	0
	35.3	24.8	32.1	3.9	9.3	3.7	5.7	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	36	25.6	33	3.9	9.3	3.9	5.9	0	0	0	0
	36.1	25.6	33	3.9	9.3	3.9	5.9	0	0	0	0
	36.2	25.6	33	3.9	9.3	3.9	5.9	0	0	0	0
	36.3	25.6	33	3.9	9.3	3.9	5.9	0	0	0	0
	37	26.5	33.8	3.9	9.3	4.1	6.1	0	0	0	0
DYS449	37.1	26.5	33.8	3.9	9.3	4.1	6.1	0	0	0	0
	37.2	26.5	33.8	3.9	9.3	4.1	6.1	0	0	0	0
	37.3	26.5	33.8	3.9	9.3	4.1	6.1	0	0	0	0
	38	27.3	34.7	3.9	9.3	4.2	6.3	0	0	0	0
	38.1	27.3	34.7	3.9	9.3	4.2	6.3	0	0	0	0
	38.2	27.3	34.7	3.9	9.3	4.2	6.3	0	0	0	0
	38.3	27.3	34.7	3.9	9.3	4.2	6.3	0	0	0	0
	39	28.2	35.5	3.9	9.3	4.4	6.4	0	0	0	0
	39.1	28.2	35.5	3.9	9.3	4.4	6.4	0	0	0	0
	39.2	28.2	35.5	3.9	9.3	4.4	6.4	0	0	0	0
	39.3	28.2	35.5	3.9	9.3	4.4	6.4	0	0	0	0
	40	29	36.4	3.9	9.3	4.6	6.6	0	0	0	0
	40.1	29	36.4	3.9	9.3	4.6	6.6	0	0	0	0
	40.2	29	36.4	3.9	9.3	4.6	6.6	0	0	0	0
	40.3	29	36.4	3.9	9.3	4.6	6.6	0	0	0	0
DYS449	41	29.9	37.2	3.9	9.3	4.8	6.8	0	0	0	0
DYS393	6	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	6.1	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	6.2	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	6.3	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	7	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	7.1	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	7.2	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	7.3	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	8	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	8.1	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	8.2	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	8.3	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	9	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	9.1	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	9.2	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	9.3	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	10	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	10.1	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	10.2	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	10.3	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	11	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	11.1	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	11.2	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	11.3	11.3	20.1	4	10	1.7	3.8	0	0	0	0
	12	11.3	20.1	4	10	1.7	3.8	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat			N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW		STD	LOW
	12.1	11.3	20.1	4	10	1.7	3.8	0	0	0	0	
	12.2	11.3	20.1	4	10	1.7	3.8	0	0	0	0	
	12.3	11.3	20.1	4	10	1.7	3.8	0	0	0	0	
DYS393	13	12.5	21.3	4	10	1.9	4	0	0	0	0	
	13.1	12.5	21.3	4	10	1.9	4	0	0	0	0	
	13.2	12.5	21.3	4	10	1.9	4	0	0	0	0	
	13.3	12.5	21.3	4	10	1.9	4	0	0	0	0	
	14	13.8	22.6	4	10	2.1	4.3	0	0	0	0	
	14.1	13.8	22.6	4	10	2.1	4.3	0	0	0	0	
	14.2	13.8	22.6	4	10	2.1	4.3	0	0	0	0	
	14.3	13.8	22.6	4	10	2.1	4.3	0	0	0	0	
	15	15	23.8	4	10	2.4	4.5	0	0	0	0	
	15.1	15	23.8	4	10	2.4	4.5	0	0	0	0	
	15.2	15	23.8	4	10	2.4	4.5	0	0	0	0	
	15.3	15	23.8	4	10	2.4	4.5	0	0	0	0	
	16	16.3	25.1	4	10	2.6	4.7	0	0	0	0	
	16.1	16.3	25.1	4	10	2.6	4.7	0	0	0	0	
	16.2	16.3	25.1	4	10	2.6	4.7	0	0	0	0	
	16.3	16.3	25.1	4	10	2.6	4.7	0	0	0	0	
	17	17.6	26.4	4	10	2.8	5	0	0	0	0	
	17.1	17.6	26.4	4	10	2.8	5	0	0	0	0	
	17.2	17.6	26.4	4	10	2.8	5	0	0	0	0	
	17.3	17.6	26.4	4	10	2.8	5	0	0	0	0	
	18	18.8	27.6	4	10	3.1	5.2	0	0	0	0	
	18.1	18.8	27.6	4	10	3.1	5.2	0	0	0	0	
	18.2	18.8	27.6	4	10	3.1	5.2	0	0	0	0	
	18.3	18.8	27.6	4	10	3.1	5.2	0	0	0	0	
DYS393	19	20.1	28.9	4	10	3.3	5.4	0	0	0	0	

DYS439	5	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	5.1	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	5.2	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	5.3	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	6	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	6.1	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	6.2	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	6.3	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	7	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	7.1	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	7.2	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	7.3	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	8	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	8.1	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	8.2	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	8.3	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	9	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	9.1	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	9.2	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	9.3	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	10	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	10.1	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	10.2	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	10.3	7.6	11.6	3.4	4.9	1.5	1.5	0	0	0	0
	11	8.6	12.6	3.4	4.9	1.5	1.5	0	0	0	0
	11.1	8.6	12.6	3.4	4.9	1.5	1.5	0	0	0	0
	11.2	8.6	12.6	3.4	4.9	1.5	1.5	0	0	0	0
	11.3	8.6	12.6	3.4	4.9	1.5	1.5	0	0	0	0
DYS439	12	9.6	13.6	3.4	4.9	1.5	1.5	0	0	0	0
	12.1	9.6	13.6	3.4	4.9	1.5	1.5	0	0	0	0
	12.2	9.6	13.6	3.4	4.9	1.5	1.5	0	0	0	0
	12.3	9.6	13.6	3.4	4.9	1.5	1.5	0	0	0	0
	13	10.6	14.6	3.4	4.9	1.5	1.5	0	0	0	0
	13.1	10.6	14.6	3.4	4.9	1.5	1.5	0	0	0	0
	13.2	10.6	14.6	3.4	4.9	1.5	1.5	0	0	0	0
	13.3	10.6	14.6	3.4	4.9	1.5	1.5	0	0	0	0
	14	11.6	15.6	3.4	4.9	1.5	1.5	0	0	0	0
	14.1	11.6	15.6	3.4	4.9	1.5	1.5	0	0	0	0
	14.2	11.6	15.6	3.4	4.9	1.5	1.5	0	0	0	0
	14.3	11.6	15.6	3.4	4.9	1.5	1.5	0	0	0	0
	15	12.6	16.6	3.4	4.9	1.5	1.5	0	0	0	0
	15.1	12.6	16.6	3.4	4.9	1.5	1.5	0	0	0	0
	15.2	12.6	16.6	3.4	4.9	1.5	1.5	0	0	0	0
	15.3	12.6	16.6	3.4	4.9	1.5	1.5	0	0	0	0
	16	13.6	17.6	3.4	4.9	1.5	1.5	0	0	0	0
	16.1	13.6	17.6	3.4	4.9	1.5	1.5	0	0	0	0
	16.2	13.6	17.6	3.4	4.9	1.5	1.5	0	0	0	0
	16.3	13.6	17.6	3.4	4.9	1.5	1.5	0	0	0	0
	17	14.6	18.6	3.4	4.9	1.5	1.5	0	0	0	0
	17.1	14.6	18.6	3.4	4.9	1.5	1.5	0	0	0	0
	17.2	14.6	18.6	3.4	4.9	1.5	1.5	0	0	0	0
	17.3	14.6	18.6	3.4	4.9	1.5	1.5	0	0	0	0
DYS439	18	15.6	19.6	3.4	4.9	1.5	1.5	0	0	0	0
DYS481	16	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	16.1	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	16.2	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	17	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	17.1	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	17.2	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	18	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	18.1	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	18.2	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	19	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	19.1	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	19.2	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		**N-2 base pair**		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	20	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	20.1	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	20.2	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	21	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	21.1	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	21.2	20.3	26.3	5.5	9.1	3.6	7	9.5	17.8	0	0
	22	21.6	27.6	5.5	9.1	4	7.5	9.5	17.8	0	0
	22.1	21.6	27.6	5.5	9.1	4	7.5	9.5	17.8	0	0
	22.2	21.6	27.6	5.5	9.1	4	7.5	9.5	17.8	0	0
	23	22.9	28.9	5.5	9.1	4.5	7.9	9.5	17.8	0	0
	23.1	22.9	28.9	5.5	9.1	4.5	7.9	9.5	17.8	0	0
	23.2	22.9	28.9	5.5	9.1	4.5	7.9	9.5	17.8	0	0
	24	24.2	30.2	5.5	9.1	4.9	8.4	9.5	17.8	0	0
	24.1	24.2	30.2	5.5	9.1	4.9	8.4	9.5	17.8	0	0
	24.2	24.2	30.2	5.5	9.1	4.9	8.4	9.5	17.8	0	0
	25	25.5	31.5	5.5	9.1	5.3	8.8	9.5	17.8	0	0
	25.1	25.5	31.5	5.5	9.1	5.3	8.8	9.5	17.8	0	0
	25.2	25.5	31.5	5.5	9.1	5.3	8.8	9.5	17.8	0	0
DYS481	26	26.8	32.8	5.5	9.1	5.8	9.2	9.5	17.8	0	0
	26.1	26.8	32.8	5.5	9.1	5.8	9.2	9.5	17.8	0	0
	26.2	26.8	32.8	5.5	9.1	5.8	9.2	9.5	17.8	0	0
	27	28.1	34.2	5.5	9.1	6.2	9.7	9.5	17.8	0	0
	27.1	28.1	34.2	5.5	9.1	6.2	9.7	9.5	17.8	0	0
	27.2	28.1	34.2	5.5	9.1	6.2	9.7	9.5	17.8	0	0
	28	29.4	35.5	5.5	9.1	6.7	10.1	9.5	17.8	0	0
	28.1	29.4	35.5	5.5	9.1	6.7	10.1	9.5	17.8	0	0
	28.2	29.4	35.5	5.5	9.1	6.7	10.1	9.5	17.8	0	0
	29	30.7	36.8	5.5	9.1	7.1	10.6	9.5	17.8	0	0
	29.1	30.7	36.8	5.5	9.1	7.1	10.6	9.5	17.8	0	0
	29.2	30.7	36.8	5.5	9.1	7.1	10.6	9.5	17.8	0	0
	30	32	38.1	5.5	9.1	7.5	11	9.5	17.8	0	0
	30.1	32	38.1	5.5	9.1	7.5	11	9.5	17.8	0	0
	30.2	32	38.1	5.5	9.1	7.5	11	9.5	17.8	0	0
	31	33.4	39.4	5.5	9.1	8	11.4	9.5	17.8	0	0
	31.1	33.4	39.4	5.5	9.1	8	11.4	9.5	17.8	0	0
	31.2	33.4	39.4	5.5	9.1	8	11.4	9.5	17.8	0	0
	32	34.7	40.7	5.5	9.1	8.4	11.9	9.5	17.8	0	0
	32.1	34.7	40.7	5.5	9.1	8.4	11.9	9.5	17.8	0	0
	32.2	34.7	40.7	5.5	9.1	8.4	11.9	9.5	17.8	0	0
DYS481	33	36	42	5.5	9.1	8.9	12.3	9.5	17.8	0	0
DYF387S1	29	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	29.1	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	29.2	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	29.3	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	30	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	30.1	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	30.2	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	30.3	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	31	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	31.1	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	31.2	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	31.3	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	32	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	32.1	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	32.2	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	32.3	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	33	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	33.1	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	33.2	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	33.3	9.2	17.4	3.2	8.3	2.3	6.1	0	0	0	0
	34	10	18.3	3.2	8.3	2.3	6.1	0	0	0	0
	34.1	10	18.3	3.2	8.3	2.3	6.1	0	0	0	0
	34.2	10	18.3	3.2	8.3	2.3	6.1	0	0	0	0
	34.3	10	18.3	3.2	8.3	2.3	6.1	0	0	0	0
	35	10.9	19.2	3.2	8.3	2.3	6.1	0	0	0	0
	35.1	10.9	19.2	3.2	8.3	2.3	6.1	0	0	0	0
	35.2	10.9	19.2	3.2	8.3	2.3	6.1	0	0	0	0
	35.3	10.9	19.2	3.2	8.3	2.3	6.1	0	0	0	0
	36	11.8	20.1	3.2	8.3	2.3	6.1	0	0	0	0
	36.1	11.8	20.1	3.2	8.3	2.3	6.1	0	0	0	0
	36.2	11.8	20.1	3.2	8.3	2.3	6.1	0	0	0	0
	36.3	11.8	20.1	3.2	8.3	2.3	6.1	0	0	0	0
DYF387S1	37	12.7	20.9	3.2	8.3	2.3	6.1	0	0	0	0
	37.1	12.7	20.9	3.2	8.3	2.3	6.1	0	0	0	0
	37.2	12.7	20.9	3.2	8.3	2.3	6.1	0	0	0	0
	37.3	12.7	20.9	3.2	8.3	2.3	6.1	0	0	0	0
	38	13.6	21.8	3.2	8.3	2.3	6.1	0	0	0	0
	38.1	13.6	21.8	3.2	8.3	2.3	6.1	0	0	0	0
	38.2	13.6	21.8	3.2	8.3	2.3	6.1	0	0	0	0
	38.3	13.6	21.8	3.2	8.3	2.3	6.1	0	0	0	0
	39	14.4	22.7	3.2	8.3	2.3	6.1	0	0	0	0
	39.1	14.4	22.7	3.2	8.3	2.3	6.1	0	0	0	0
	39.2	14.4	22.7	3.2	8.3	2.3	6.1	0	0	0	0
	39.3	14.4	22.7	3.2	8.3	2.3	6.1	0	0	0	0
	40	15.3	23.6	3.2	8.3	2.3	6.1	0	0	0	0
	40.1	15.3	23.6	3.2	8.3	2.3	6.1	0	0	0	0
	40.2	15.3	23.6	3.2	8.3	2.3	6.1	0	0	0	0
	40.3	15.3	23.6	3.2	8.3	2.3	6.1	0	0	0	0
	41	16.2	24.5	3.2	8.3	2.3	6.1	0	0	0	0
	41.1	16.2	24.5	3.2	8.3	2.3	6.1	0	0	0	0
	41.2	16.2	24.5	3.2	8.3	2.3	6.1	0	0	0	0
	41.3	16.2	24.5	3.2	8.3	2.3	6.1	0	0	0	0
	42	17.1	25.3	3.2	8.3	2.3	6.1	0	0	0	0
	42.1	17.1	25.3	3.2	8.3	2.3	6.1	0	0	0	0
	42.2	17.1	25.3	3.2	8.3	2.3	6.1	0	0	0	0
	42.3	17.1	25.3	3.2	8.3	2.3	6.1	0	0	0	0
	43	18	26.2	3.2	8.3	2.3	6.1	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
DYF387S1	43.1	18	26.2	3.2	8.3	2.3	6.1	0	0	0	0
	43.2	18	26.2	3.2	8.3	2.3	6.1	0	0	0	0
	43.3	18	26.2	3.2	8.3	2.3	6.1	0	0	0	0
	44	18.8	27.1	3.2	8.3	2.3	6.1	0	0	0	0
	44.1	18.8	27.1	3.2	8.3	2.3	6.1	0	0	0	0
	44.2	18.8	27.1	3.2	8.3	2.3	6.1	0	0	0	0
	44.3	18.8	27.1	3.2	8.3	2.3	6.1	0	0	0	0
	45	19.7	28	3.2	8.3	2.3	6.1	0	0	0	0
DYS533	6	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	6.1	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	6.2	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	6.3	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	7	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	7.1	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	7.2	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	7.3	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	8	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	8.1	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	8.2	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	8.3	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	9	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	9.1	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	9.2	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	9.3	8.5	14.9	5.8	13.6	1.6	1.6	0	0	0	0
	10	9.7	16.1	5.8	13.6	1.8	1.8	0	0	0	0
	10.1	9.7	16.1	5.8	13.6	1.8	1.8	0	0	0	0
	10.2	9.7	16.1	5.8	13.6	1.8	1.8	0	0	0	0
	10.3	9.7	16.1	5.8	13.6	1.8	1.8	0	0	0	0
	11	10.9	17.3	5.8	13.6	2	2	0	0	0	0
	11.1	10.9	17.3	5.8	13.6	2	2	0	0	0	0
	11.2	10.9	17.3	5.8	13.6	2	2	0	0	0	0
	11.3	10.9	17.3	5.8	13.6	2	2	0	0	0	0
	12	12.1	18.5	5.8	13.6	2.2	2.2	0	0	0	0
	12.1	12.1	18.5	5.8	13.6	2.2	2.2	0	0	0	0
	12.2	12.1	18.5	5.8	13.6	2.2	2.2	0	0	0	0
	12.3	12.1	18.5	5.8	13.6	2.2	2.2	0	0	0	0
DYS533	13	13.3	19.6	5.8	13.6	2.4	2.4	0	0	0	0
	13.1	13.3	19.6	5.8	13.6	2.4	2.4	0	0	0	0
	13.2	13.3	19.6	5.8	13.6	2.4	2.4	0	0	0	0
	13.3	13.3	19.6	5.8	13.6	2.4	2.4	0	0	0	0
	14	14.5	20.8	5.8	13.6	2.7	2.7	0	0	0	0
	14.1	14.5	20.8	5.8	13.6	2.7	2.7	0	0	0	0
	14.2	14.5	20.8	5.8	13.6	2.7	2.7	0	0	0	0
	14.3	14.5	20.8	5.8	13.6	2.7	2.7	0	0	0	0
	15	15.7	22	5.8	13.6	2.9	2.9	0	0	0	0
	15.1	15.7	22	5.8	13.6	2.9	2.9	0	0	0	0
	15.2	15.7	22	5.8	13.6	2.9	2.9	0	0	0	0
	15.3	15.7	22	5.8	13.6	2.9	2.9	0	0	0	0

RETIRED

		N-1 repeat		N+1 repeat		N-2 repeat		N-0.5 repeat		N+0.5 repeat	
Locus	Allele	STD	LOW	STD	LOW	STD	LOW	STD	LOW	STD	LOW
	16	16.9	23.2	5.8	13.6	3.1	3.1	0	0	0	0
	16.1	16.9	23.2	5.8	13.6	3.1	3.1	0	0	0	0
	16.2	16.9	23.2	5.8	13.6	3.1	3.1	0	0	0	0
	16.3	16.9	23.2	5.8	13.6	3.1	3.1	0	0	0	0
	17	18	24.4	5.8	13.6	3.3	3.3	0	0	0	0
	17.1	18	24.4	5.8	13.6	3.3	3.3	0	0	0	0
DYS533	17.2	18	24.4	5.8	13.6	3.3	3.3	0	0	0	0
	17.3	18	24.4	5.8	13.6	3.3	3.3	0	0	0	0
	18	19.2	25.6	5.8	13.6	3.5	3.5	0	0	0	0

RETIRED

41.2 Common Dye Artifacts

41.2.1 GlobalFiler: Located on the U: Drive, in the DNA Training Folder, in the GlobalFiler folder is a PDF document titled “GlobalFiler Technical Note”. This document contains artifacts identified post-developmental validation for the GlobalFiler PCR Amplification Kit.

41.2.2 Yfiler Plus: Located on the U: Drive, in the DNA Training Folder, in the Yfiler Plus folder is a PDF document titled “Yfiler Plus User Guide”. Pages 79-83 discuss artifact observation in this kit.

RETIRED

41.3 Control Profiles

41.3.1 GlobalFiler

41.3.1.1 EP1

Dye	Loci					
6-FAM	D3S1358	vWA	D16S539	CSF1PO	TPOX	
	14, 18	16, 18	12, 13	10, 11	8, 10	
VIC	Yindel	Amel	D8S1179	D21S11	D18S51	DYS391
	2	X, Y	10, 14	30	14, 16	11
NED	D2S441	D19S433	TH01		FGA	
	14	14, 15	7, 9.3		20, 22	
TAZ	D22S1045	D5S818	D13S317	D7S820	SE33	
	16	9, 11	10, 11	8, 9	17, 28.2	
SID	D10S1248	D1S1656	D12S391		D2S1338	
	14, 16	15, 15.3	17, 22		17, 23	

41.3.1.2 POS (Control 007)

Dye	Loci					
6-FAM	D3S1358	vWA	D16S539	CSF1PO	TPOX	
	15, 16	14, 16	9, 10	11, 12	8	
VIC	Yindel	Amel	D8S1179	D21S11	D18S51	DYS391
	2	X, Y	12, 13	28, 31	12, 15	11
NED	D2S441	D19433	TH01		FGA	
	14, 15	14, 15	7, 9.3		24, 26	
TAZ	D22S1045	D5S818	D13S317	D7S820	SE33	
	11, 16	11	11	7, 12	17, 25.2	
SID	D10S1248	D1S1656	D12391		D2S1338	
	12, 15	13, 16	18, 19		20, 23	

RETIRED

41.3.2 Yfiler Plus

41.3.2.1 EP1

Dye	Loci					
6-FAM	DYS576	DYS389I	DYS635	DYS389II	DYS627	
	18	13	23	29	25	
VIC	DYS460	DYS458	DYS19	YGATAH4	DYS448	DYS391
	11	16	15	12	18	11
NED	DYS456	DYS390	DYS438		DYS392	DYS518
	18	26	12		14	37
TAZ	DYS570	DYS437	DYS385 a/b		DYS449	
	18	15	12, 13		31	
SID	DYS393	DYS439	DYS481	DYF387S1		DYS533
	13	12	25	36, 37		12

41.3.2.2 Pos (Control 007)

Dye	Loci					
6-FAM	DYS576	DYS389I	DYS635	DYS389II	DYS627	
	19	13	24	29	21	
VIC	DYS460	DYS458	DYS19	YGATAH4	DYS448	DYS391
	11	17	15	13	19	11
NED	DYS456	DYS390	DYS438		DYS392	DYS518
	15	24	12		13	37
TAZ	DYS570	DYS437	DYS385 a/b		DYS449	
	17	15	11, 14		30	
SID	DYS393	DYS439	DYS481	DYF387S1		DYS533
	13	12	22	35, 37		13

RETIRED

43.1 PURPOSE:

Occasionally, a Mitochondrial DNA (mtDNA) result that was developed using a protocol that is no longer in use may need to be further evaluated for comparison purposes or to assess a match generated in CODIS.

43.2 RESPONSIBILITY:

DNA Unit Personnel authorized for Mitochondrial DNA Legacy Data Interpretation.

43.3 Comparing Sequences

43.3.1 When comparing sequences obtained from samples, only the regions with a common range will be evaluated. For example, if a partial sequence (16024-16365 and 73-284) is obtained for the evidentiary sample and a full sequence is obtained for the known sample, the comparison will be conducted on positions 16024-16365 and 73-284. In addition, sequence before and after the defined HV1 and HV2 regions will also be used for comparison purposes, provided this range is common to both samples.

43.4 Comparing Sequences with Length Heteroplasmy

43.4.1 Length heteroplasmy in HV1 most commonly arises when there is a substitution of a C for a T at position 161843. The reference type in HV1 is C_5TC_4. HV1 length heteroplasmy is not recorded in casework samples. Rather, sequences are truncated to fit the C_5TC_4 format.

43.4.2 The number of C nucleotides exhibited in samples with HV2 length heteroplasmy is highly variable and care must be exercised when making comparisons. In order for sequence concordance to be declared, a common length variant must be observed in the samples being compared. For instance, if a questioned sample has predominately 7 C residues preceding the T at position 310, but shows the presence of 8 Cs as well, it is concordant with samples containing any mixture of length variants of 7 or 8 Cs.

43.4.3 Where a sequence does not have a T at 310 and length heteroplasmy is present, it is notated 310 C and no insertions or deletions relative to rCRS at 303-315 are noted. If a sequence does not have a T at 310 and length heteroplasmy is not present, then the below guidance can be used:

Length variants in HV2 are commonly observed in the number of C residues preceding a T residue at position 310. The reference type in HV2 is C7TC5. A 309.1 C insertion in HV2 will be annotated C8TC5, and the insertions 309.1 C, 315.1 C will be annotated as C8TC6. It is possible to determine the dominant length variant in this region, as well as some or all of the minor length variants. The sequence can be notated to reflect these variants.

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43.5 Interpretation of Sequence Comparisons

43.5.1 The following interpretations are available for sequence comparisons:

43.5.1.1 Exclusion:
If the samples differ at two or more positions (excluding length heteroplasmy), they can be excluded as coming from the same source or maternal lineage.

43.5.1.2 Inconclusive:

The comparison should be reported as inconclusive if the samples differ:

a. At a single position only (whether or not they share a common length variant between positions 302-310).

b. Only by not sharing a common length variant between positions 302-310 (all other positions are concordant).

Because of the possibility of undetected heteroplasmy, additional samples may be analyzed when two sequences differ by a single base. These samples may include blood, buccal swabs, and hair. Hair fragments from a known hair standard may be combined and processed as a single known sample.

43.5.1.3 Cannot Exclude:
If samples have the same sequence or are concordant, they cannot be excluded as coming from the same source or maternal lineage.

Sequence concordance is defined as having a common DNA base at every nucleotide position, including common length variants. For example, if one sample has evidence of a C and a T at a given position, and the other has a C, they share the C in common at that position and are concordant. However, if one sample has evidence of a C and a T at a given position, and the other has a G, these sequences are not concordant.

43.6 Sequence Confirmation

43.6.1 A second qualified analyst must have previously confirmed all non-mixture sequences regardless of interpretation or destination. Confirmation involves independently assembling sequences for the NC, RB, positive control, and evidentiary samples from the analysis data. Confirmed sequence range is defined as the shortest length of sequence obtained by two analysts.

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Note: Mixture samples will not be interpreted for comparison purposes. A mixture is defined as 3 or more sites of point heteroplasmy.

43.7 Databases

43.7.1 The CODIS 9.0 database, containing 10,629 individuals, is used to determined match probability estimates. The database represents a broad geographic sampling from across the United States and covers the entire control region (16024-576). All profiles reflect the Scientific Working Group on DNA Analysis Methods (SWGDAM) mtDNA Interpretation Guidelines document dated 04/23/2019, which includes standards for sequence nomenclature. The database is maintained by the FBI Laboratory in collaboration with SWGDAM and is updated periodically.

43.8 Searching Profiles

43.8.1 The CODIS 9.0 database, containing 10,629 individuals, is used to search casework profiles in the forensic database. The range of the profile to be searched consists of the shortest range of sequence agreed upon by the analysts reviewing the data. Ambiguous base positions in both database samples and case work profiles are searched as an N (meaning the search includes all four bases at that position) and do not have any exclusionary value when compared with other sequences. Deletions are searched as a (-). Those database samples whose range(s) are fully included within the casework profile range are used in the database search result. Presently, this includes the regions 16024-16365 (HV1) and 73-340 (HV2). The database search result provides the number of database profiles that match the casework profile, as well as the number of profiles in the database that differ by up to five positions.

43.8.2 The number of C residues in samples with HV1 length heteroplasmy around positions 16183-16194 (see section 43.8) are not considered for comparison purposes.

43.8.3 All sequence polymorphisms in the sample searched are entered; however, all length variants at nucleotide positions 16193 and 309 are ignored in database searches of relevant concordant sequences. Hence, length variability in this region will not add any additional rarity to a database profile search.

43.9 Upper Bound Frequency Estimate

43.9.1 An upper bound 95% confidence interval can be calculated from the results of a database search in order to estimate the population frequency of a profile. The upper bound estimate is dependent on the size of the database, and these estimates may change as the database size changes.

43.9.2 The following formulae may be used to calculate the upper bound estimate:

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43.9.2.1 In cases where the profile has been observed in a database:

$$p + 1.96 [(p) (1-p)/N]^{1/2}$$

p = x/N, where x is the number of times a profile has been observed in a population and N is the number of profiles in that population.

43.9.2.2 In cases where the profile has <u>not</u> been observed in a database:

$$1-\alpha^{1/N}$$

α is the confidence coefficient (0.05 for a 95% confidence interval), and N is the number of individuals in the population.

43.9.3 The results of these upper bound frequency estimate calculations for the three major population groups in the United States (African-American, Caucasian, and Hispanic) are included in the report. The calculations for other groups, whose total number in the database exceeds 100 people, may be included in the report where appropriate.

43.9.4 mtDNA haplotypes are inherited independently from autosomal loci. Therefore, mtDNA haplotype frequencies may be combined with autosomal STR and Y-STR frequencies to generate an overall profile frequency estimate.

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43.10 References

Scientific Working Group on DNA Analysis Methods (SWGDAM). Interpretation Guidelines for Mitochondrial DNA Analysis by Forensic Testing Laboratories (2019) [Online]. Available: https://docs.wixstatic.com/ugd/4344b0_f61de6abf3b94c52b28139bff600ae98.pdf

43.11 Writing Reports:

Note: In the Testing Summary Section, a table will summarize the items and profile/regions which were amplified and sequenced and if a sample was entered into CODIS.

An example of the table:

Item #	Profile Obtained[1]					CODIS Entry
	GF	1A	1B	2A	2B	
1	Yes	Yes[2]	Yes[2]	Yes[2]	Yes[2]	Yes - CT and National

[1]GF = GlobalFiler STR DNA amplification kit

1A through 2B = mitochondrial DNA (mtDNA) hypervariable regions sequenced

[2]Using hypervariable regions 1 & 2 (HV1 & HV2) primers only

In the Conclusions Summary Section, a table will summarize the items, type of profile obtained (full or partial) and comparisons (if any).

An example of the table:

Item #	Description	Type	Victim (#2)
1	Trace material from hat	Full Profile	Excluded

Sequencing results will be found in the Appendix of the report.

An Example of the table:

Item	#1	#2	#3
Range (bp)	15998-16389	15998-16389	15998-16388
	16124 C	16124 C	16114 A
	16223 T	16223 T	16129 A
HV1	16319 A	16319 A	16213 A
			16223 T
			16278 T
			16355 T
			16362 C
Range (bp)	49-380	49-407	49-407
	73 G	73 G	73 G
	150 T	150 T	150 T
	152 C	152 C	152 C
HV2	263 G	263 G	182 T
	315.1 C	315.1 C	195 C
			198 T
			204 C
			263 G
			315.1 C

RETIRED

In the Conclusions Section, a table will summarize the CODIS 9.0 database search (if conducted).

An example of the table:

Group	Number of Observations	Individuals in Group	Upper Bound Frequency Estimate
African-American	0	2449	0.12% or 1 in 833
Caucasian	0	2609	0.11% or 1 in 909
Hispanic	0	2576	0.12% or 1 in 833

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43.11.1 When a CODIS 9.0 database search (containing 10,629 individuals) is conducted, the report will include a database table containing the search results of the three major population groups of the United States: African-American, Caucasian, and Hispanic. The table may also contain other applicable populations where appropriate. In addition, the report will contain the search results of any other population where a matching type is found.

Note: When making a statement about the CODIS 9.0 database search, the following will be listed: the number of individuals searched, and the sequence ranges searched. See examples below.

Note: Below are examples of case scenarios. However, it is not an exhaustive list of all possible casework scenarios. Other reporting strategies (i.e. wording and or table changes) may be used with approval of the TL.

43.11.2 Cases in which the DNA sequences are the same:

43.11.2.1 Example:

The mtDNA sequences obtained from items #1 and #2 are the same. Therefore, J. DOE (or another member of the same maternal lineage) cannot be excluded as the source of item #1. Searching the mtDNA population database (CODIS 9.0, containing 10,629 individuals, at positions 16024 – 16365 and 73 – 340) the mtDNA sequence obtained from items #1 and #2 has been observed in African American, Caucasian, and Hispanic populations as follows:

[See 43.11 for an example of the CODIS 9.0 database search table]

43.11.3 Cases in which the DNA sequences are the same, but a complete sequence was not obtained from all regions:

43.11.3.1 Example:

The mtDNA sequences obtained from items #1 (at positions 16024 – 16236 and 73 – 340 only) and #2 are the same. Therefore, J. DOE (or another member of the same maternal lineage) cannot be excluded as the source of item #1.

Searching the mtDNA population database (CODIS 9.0, containing 10,629 individuals, at positions 16024 – 16236 and 73 – 340), the mtDNA sequence obtained from items #1 and #2 has been observed in African American, Caucasian, and Hispanic populations as follows:

[See 43.11 for an example of the CODIS 9.0 database search table]

Note: Above statements identifies partial range that was obtained.

43.11.4 Cases in which the DNA sequences differ at two or more nucleotide positions (excluding length heteroplasmy):

43.11.4.1 Example:

The mtDNA sequences obtained from items #1 and #2 are different. Therefore, J. DOE is excluded as the source of item #1.

43.11.5 Cases in which the DNA sequences of the evidentiary sample and one known sample are the same and the DNA sequences of the evidentiary sample and a different known sample differ at two or more nucleotide positions (excluding length heteroplasmy):

43.11.5.1 Example:

The mtDNA sequences obtained from items #1 and #2 are the same. Therefore, J. DOE (or another member of the same maternal lineage) cannot be excluded as the source of item #1.

Searching the mtDNA population database (CODIS 9.0, containing 10,629 individuals, at positions 16024 – 16365 and 73 – 340), the mtDNA sequence obtained from items #1 and #2 has been observed in African American, Caucasian, and Hispanic populations as follows:

[See 43.11 for an example of the CODIS 9.0 database search table]

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The mtDNA sequence obtained from item #3 is different from the sequence obtained from item #1. Therefore, J. SMITH is excluded as the source of item #1.

43.11.6 Cases in which the DNA sequences in the evidentiary and known samples are concordant with sequence heteroplasmy present:

43.11.6.1 Example:

The mtDNA sequence obtained from item #1 is concordant with the mtDNA sequence obtained from item #2. Therefore, J. DOE (or another member of the same maternal lineage) cannot be excluded as the source of item #1.

Searching the mtDNA population database (CODIS 9.0, containing 10,629 individuals, at positions 16024 – 16365 and 73 – 340), the mtDNA sequence obtained from items #1 and #2, including all four nucleotides, A, C, G, and T, at position 16069, has been observed in African American, Caucasian, and Hispanic populations as follows:

[See 43.11 for an example of the CODIS 9.0 database search table]

43.11.7 Cases in which the DNA sequences from the evidentiary and known samples differ by a single nucleotide, and no evidence of a common nucleotide is present in the questioned and/or known sample at the position of difference:

43.11.7.1 Example:

The mtDNA sequence obtained from item #1 is the same as those obtained from items #2, #3, and #4, with the exception of position 160643. At this position, the presence of a thymine (T) was observed in item #1. In items #2, #3, and #4, a cytosine (C) was observed at position 160643. Due to the one base pair difference observed between item #1 and items #2, #3, and #4, the results are inconclusive as to whether J. DOE is excluded as the source of items #2, #3, and #4.

43.11.8 Cases in which there is a sample with an apparent mixture of more than one DNA sequence:

43.11.8.1 Example:

The mitochondrial DNA (mtDNA) sequence obtained from item #1 indicates the presence of a mixture of mtDNA from more than one individual. Therefore, no conclusions can be made for this item. No other mtDNA examinations were conducted for this item.

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43.11.9 Cases in which there is a sample with an apparent mixture, but a complete sequence was not obtained from all regions:

43.11.9.1 Example:

The mtDNA sequence obtained from item #1 (at positions 16024 – 16236 and 73 – 340 only) indicates the presence of a mixture of mtDNA from more than one individual. Therefore, no conclusions can be made for this item. No other mtDNA examinations were conducted for this item.

Note: Above statement identifies partial range obtained.

43.11.10 Cases in which two evidentiary samples are the same, but (1) no known samples are available for comparison or (2) they do not match the known samples tested:

43.11.10.1 Example:

The mtDNA sequences obtained from items #1 and #2 are the same. Therefore, items #1 and #2 cannot be excluded as coming from a common source. Since no known samples were submitted, no further comparisons can be made at this time.

The mtDNA sequences obtained from items #1 and #2 are the same. Therefore, items #1 and #2 cannot be excluded as coming from a common source. The mtDNA sequences obtained from items #1 and #2 are different from item #3. Therefore, J. DOE is excluded as the source of items #1 and #2.

Optional: At the analyst's discretion, mtDNA sequencing results table and database search results table can be inserted into the report for those samples that cannot be excluded as coming from a common source.

Searching the mtDNA population database (CODIS 9.0, containing 10,629 individuals, at positions 16024 – 16365 and 73 – 340), the mtDNA sequence obtained from items #1 and #2 has been observed in African American, Caucasian, and Hispanic populations as follows:

[See 43.11 for an example of the CODIS 9.0 database search table]

43.11.11 Cases in which no sequence was obtained for a sample:

43.11.11.1 Information will be captured in the Testing Summary Table.

43.11.12 Cases in which no mtDNA examination was conducted because the remains were determined to be of non-human origin:

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43.11.12.1 Example:

Dr. J. Smith examined item #1. Item #1 is not of human origin; therefore, no mitochondrial DNA examinations were conducted.

43.11.13 Cases in which the mtDNA sequence is not of the requisite quality:

43.11.13.1 Example:

The mitochondrial DNA (mtDNA) sequence obtained from item #1 was not of the requisite quality, therefore no comparisons could be made for this item.

43.11.14 Missing Persons cases:

43.11.14.1 CODIS entry will be captured in the Testing Summary Table or the Conclusions Summary Table.

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40.1 Purpose:

The primary objective reflected in this section is for the analyst to render the best interpretation of the data.

40.2 Responsibility:

DNA Section personnel.

40.3 Y-STR Analysis:

40.3.1 This procedure is a general guideline for the analysis and interpretation of Y-STR profiles when using the Yfiler Plus (YFP) PCR Amplification Kit. However, it is not an exhaustive list of all possible casework scenarios. Analyst training, experience, and judgment must be considered when reporting Y-STR profiles. Y-STR data are interpreted by evaluating the results at all loci. These procedures generally follow those outlined in the Applied Biosystems/Life Technologies GeneMapper ID-X Software version 1.5 Reference Guide.

40.3.2 When the YFP amplification kit is employed, 27 Y-STR loci; DYS576, DYS389I, DYS635, DYS389II, DYS627, DYS460, DYS458, DYS19, Y-GATA H4, DYS448 (6 base repeat), DYS391, DYS456, DYS390, DYS438 (5 base repeat), DYS392 (3 base repeat), DYS518, DYS570, DYS437, DYS385 a/b (2 loci amped with one set of primers), DYS449, DYS393, DYS439, DYS481, DYF387S1 (2 loci amped with one set of primers), and DYS533 are typed in a single PCR amplification reaction according to manufacturer's and laboratory protocols.

40.3.3 YFP amplification products are separated and detected using an ABI 3500xl Genetic Analyzer and the 3500 Series Data Collection Software 4 and analyzed with GeneMapper ID-X version 1.6 (ID-X) software.

40.3.4 For the ID-X software for YFP, the Analysis Method (YFP_CTDSS) is as outlined on pages 53-56 of Appendix A, Experimental Procedures for the CT-DSS YFP Internal Validation performed by Thermo Fisher except for the following: Under the Peak Detector Tab, the Analysis Range is Partial Range and the Start and Stop Pts will be set by the analyst for each set of data but shall include the full range of required size standard peaks (60-460), the Sizing Range is Partial Sizes with the Start Size set to 60 and the Stop Size set to 460. The Peak Detection Amplitude Thresholds will be set to B: 35, G: 55, Y: 35, R: 55, P: 55, and O: 150. For the Panel, use Yfiler_Plus_Panel_v4.1X. For the Size Standard, use GS600_LIZ_(60-460).

RETIRED

40.1 Purpose:

The primary objective reflected in this section is for the analyst to render the best interpretation of the data.

40.2 Responsibility:

DNA Section personnel.

40.3 Y-STR Analysis:

40.3.1 This procedure is a general guideline for the analysis and interpretation of Y-STR profiles when using the Yfiler Plus (YFP) PCR Amplification Kit. However, it is not an exhaustive list of all possible casework scenarios. Analyst training, experience, and judgment must be considered when reporting Y-STR profiles. Y-STR data are interpreted by evaluating the results at all loci. These procedures generally follow those outlined in the Applied Biosystems/Life Technologies GeneMapper ID-X Software version 1.5 Reference Guide.

40.3.2 When the YFP amplification kit is employed, 27 Y-STR loci; DYS576, DYS389I, DYS635, DYS389II, DYS627, DYS460, DYS458, DYS19, Y-GATA H4, DYS448 (6 base repeat), DYS391, DYS456, DYS390, DYS438 (5 base repeat), DYS392 (3 base repeat), DYS518, DYS570, DYS437, DYS385 a/b (2 loci amped with one set of primers), DYS449, DYS393, DYS439, DYS481, DYF387S1 (2 loci amped with one set of primers), and DYS533 are typed in a single PCR amplification reaction according to manufacturer's and laboratory protocols.

40.3.3 YFP amplification products are separated and detected using an ABI 3500xl Genetic Analyzer and the 3500 Series Data Collection Software 4 and analyzed with GeneMapper ID-X version 1.6 (ID-X) software.

40.3.4 For the ID-X software for YFP, the Analysis Method (YFP_CTDSS) is as outlined on pages 53-56 of Appendix A, Experimental Procedures for the CT-DSS YFP Internal Validation performed by Thermo Fisher except for the following: Under the Peak Detector Tab, the Analysis Range is Partial Range and the Start and Stop Pts will be set by the analyst for each set of data but shall include the full range of required size standard peaks (60-460), the Sizing Range is Partial Sizes with the Start Size set to 60 and the Stop Size set to 460. The Peak Detection Amplitude Thresholds will be set to B: 35, G: 55, Y: 35, R: 55, P: 55, and O: 150. For the Panel, use Yfiler_Plus_Panel_v4.1X. For the Size Standard, use GS600_LIZ_(60-460).

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40.4 Analysis of Y-STR DNA Profiles using GMID:

40.4.1 The results are evaluated for each locus. However, considering the data from all loci tested will assist in the overall interpretation of the results for each sample. See DNA SOP-6 for standard report conclusions.

40.4.2 Off-Scale Data: If too much DNA is added to the amplification reaction, the fluorescent intensity for the PCR products may exceed the linear dynamic range for detection by the instrument. Samples with off-scale peaks may exhibit raised baselines, split peaks, increased minus A peaks, excessive pull-up and/or higher than expected stutter levels, and/or amplification artifacts in one or more of the colors. Examine the peak heights in ID-X software. Any peaks greater than 30,000 RFU may be off-scale. These samples may need to be reinjected for a shorter time or diluted and re-amplified.

40.4.3 Peak identification: The laboratory's analytical threshold (AT) is dye channel specific, B: 35 RFU, G: 55 RFU, Y: 35 RFU, R: 55 RFU, P: 55 RFU, and O: 150 RFU. The identity of peaks called by ID-X is generally assigned to one of the following categories:

40.4.4 Allele Peak: A called allele has a peak height ≥ AT RFU, a fragment size that falls within the base pair range, and has the appropriate dye color for the locus. However, not all peaks ≥ AT RFU are typed as alleles. Stutter peaks, pull-up/pull-down peaks, background noise, and amplification or injection artifacts could fall above the RFU threshold, especially if the allele peaks are high. Peaks ≥ AT RFU that are not called by ID-X may be called by the analysts on a case-by-case basis. Such peaks are interpreted with caution with TL approval.

40.4.5 Detection of variant alleles: Variant alleles have been identified from many STR loci. These alleles may contain full or partial repeats. Variant alleles are labeled as Off Ladder (OL) alleles in ID-X. If a particular variant has been observed before by the DESPP CT Division of Scientific Services (CT-DSS), Thermo Fisher (Life Technologies), NIST, or in published literature, the sample does not need to be repeated. In ID-X, change the OL to the proper allele call. However, if the repeat has not been observed, the sample may be re-amplified and/or reinjected to confirm the OL allele as warranted.

40.4.6 Stutter peaks (N-2, N-3, N-4, N-5, N-6, N-8, N-9, N-10, N-12, N+2, N+3, N+4, N+5, N+6—depending on the locus): A stutter peak has a fragment length typically four base pairs smaller than the true allele (see exceptions above). If the peak height in the stutter position is less than the user-defined stutter threshold,

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the peak is considered a stutter peak and is filtered out by ID-X or manually edited by the analyst (following use of DNA QR-345). Allele-specific stutter thresholds were determined by CT-DSS during the YFP Internal Validation. Peaks in stutter positions that are below the Laboratory's stutter thresholds are not listed on the report and are not used for statistical purposes. If the height of the peak in the stutter position is greater than the CT-DSS stutter thresholds, this indicates a possible mixture, an overloaded sample, or a higher than normal stutter ratio. Additional information provided by other loci may be of assistance in these cases.

40.4.7 Minus A Peaks: The YFP amplification kit is optimized to add an extra nucleotide to the 3' end of the PCR product. The nucleotide is predominantly adenosine (A). Lack of a full A addition may be observed especially when the amount of input DNA is greater than approximately 1 ng. However, sample degradation can increase the effective concentration of a small amplicon relative to the larger amplicons, leading to minus A product at the smaller loci. Data from other loci may be of assistance in these cases. When appropriate, a smaller quantity of sample may be re-amplified.

40.4.8 Pull-up Peaks: Pull-up peaks are generally seen when excess amplification product is injected on the CE or when the spectral calibration is sub-optimal. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-up peaks are often displayed in several colors. Pull-up peaks should have approximately the same fragment size in each of the colors as the true allele peak. Additional information provided by other loci may be of assistance in these cases. When evaluating allelic peaks that may contain a pull-up (PU) contribution, an estimate of the PU contribution is subtracted from the height of the potential allelic peak to determine if the remaining peak height is sufficient for allele or asterisk designation. The estimated pull-up contribution is determined by evaluating the height of unambiguous pull-up peaks (pull-up with e.g., no stutter or allelic contribution) at each locus where pull-up is detected for a particular color.

Note: the 3500 Series Data Collection Software 4 has a pull-up correction function applied to the raw data. This function is not necessarily consistent across the entire base-pair range for the detection of peaks from amplified DNA, therefore care must be taken when identifying the percentage of signal for unambiguous pull-up peaks. Using unambiguous pull-up peak(s) in the same locus will most often be optimal. In the case where there are no unambiguous pull-up peaks for the dye channel, consider that the median percentage of pull-up

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peaks observed during the validation was 0.7% with 95.1% of the pull-up peaks being < 3% and 99.4% being < 5%.

Example: The percentage of pull-up peaks as compared to the height of the peaks causing the pull-up is calculated. (PU percentage = PU peak height/height of the peak causing the PU). This percentage is converted to an RFU value (multiply percent by height of peak causing the pull-up) which is then subtracted from the potential allelic peak in question. The resulting "subtracted" peak is called where DNA Section allele calling criteria are met. The same methodology would be applied to potential allelic peaks with a pull-down component (see below).

40.4.9 Pull-down Peaks: Pull-down peaks are generally seen when excess amplification product is run on the CE or when the spectral calibration is sub-optimal for the run. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-down peaks can be displayed in several colors. Pull-down peaks can have approximately the same fragment size, but often the pull-down peak will be slightly a larger or smaller size as compared to the true allele. Additional information provided by other loci may be of assistance in these cases.

40.4.10 Spikes: fluorescent spikes can be seen within GMID data. Spikes are typically sharp peaks caused by air bubbles or polymer crystals in a capillary, which can be displayed in different colors, and have approximately the same "fragment size". Additional inspection of the raw data or re-injection may assist in cases where spikes are believed to be present. Upon subsequent injection, spikes should not reoccur at the same position.

40.4.11 Dye Artifacts/Dye Blobs: Broad peaks may be the result of dissociated dyes or fluorescent particles that are trapped within the polymer matrix and then fluorescently excited by the laser. Additional inspection of other samples, especially negative controls, within the module may assist in interpretation in cases where dye blobs are believed to be present.

40.4.12 Amplification Artifact Peaks: Nonspecific amplification product peaks, generally with good peak morphology, may result when excessive template DNA is amplified. When appropriate, a smaller quantity of sample may be re-amplified.

40.4.13 High Background: Background fluorescence may reach an RFU level above the calling threshold. Generally such high background peaks exhibit atypical peak morphology. When appropriate, re-injection may assist with interpretation.

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40.4.14 Raised Baseline: Elevated baseline of one color over a broader (multiple bin) scan range may occur. Note: pull-down from two adjacent peaks may cause raised baseline between the pull-down peaks; the "pull-down" designation should be used for the editing notation when raised baseline is caused by pull-down.

40.5 Highly Degraded/Low Template Samples:

Highly degraded/low template samples are interpreted with caution. Degraded samples may "appear" to exhibit high stutter and greater –A at the smaller amplicons with off-scale data. In addition, degraded/low template samples may display unbalanced heterozygous alleles and locus/allele dropout. Partial profiles may be interpreted as determined by the data present for each locus. Given the possibility of stochastic allele sampling, especially with low quantity and/or degraded samples, single peaks (at loci DYS385 and DYF387S1) less than the stochastic threshold are interpreted with caution (pertains to putative haplotype assignment at that locus). To maximize the number of loci with peaks above the threshold, a sample may be run at increased injection times on the 3500. However, any off-scale data at the smaller amplicons are interpreted with caution.

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40.6 Inhibited Samples:

40.6.1 Inhibited samples may exhibit allele, locus, or complete profile dropout, unbalanced alleles at loci DYS385 and DYF387S1, and interlocus peak imbalance. To possibly overcome an inhibitor, smaller quantities of sample may be re-amplified. Quantitation data may aid in determining whether re-amplification is warranted as well as the amount of sample to be re-amplified. Before the genotyping amplification is performed, an evaluation of the Quantifiler Trio IPC for each sample is useful. The Quantifiler Trio IPC result for a sample being undetected or having an elevated C_T may indicate that a sample has an inhibitor present. If this is the case, multiple quantities or dilutions of a sample may be amplified for the original amplification.

40.6.2 Input of excessive female DNA may result in inhibition of Y-STR amplification. When appropriate, smaller quantities of sample may be re-amplified. Quantitation data may aid in determining whether re-amplification is warranted as well as the amount of sample to be re-amplified.

40.7 X-homologous peaks:

With large quantities of female DNA present in the amplified DNA extract, the Y primers may generate X-homologous or female cross-reactivity peaks. If the known female sample is amplified, any X-homologous peaks may be removed from the evidentiary DNA profile. Note: may require amplifying the same quantity of female DNA as with the evidentiary sample. The female cross-reactivity peaks are most common in the blue and red dye channels (see the YFP Developmental and CT-DSS Internal Validation results and the YFP User Guide for examples).

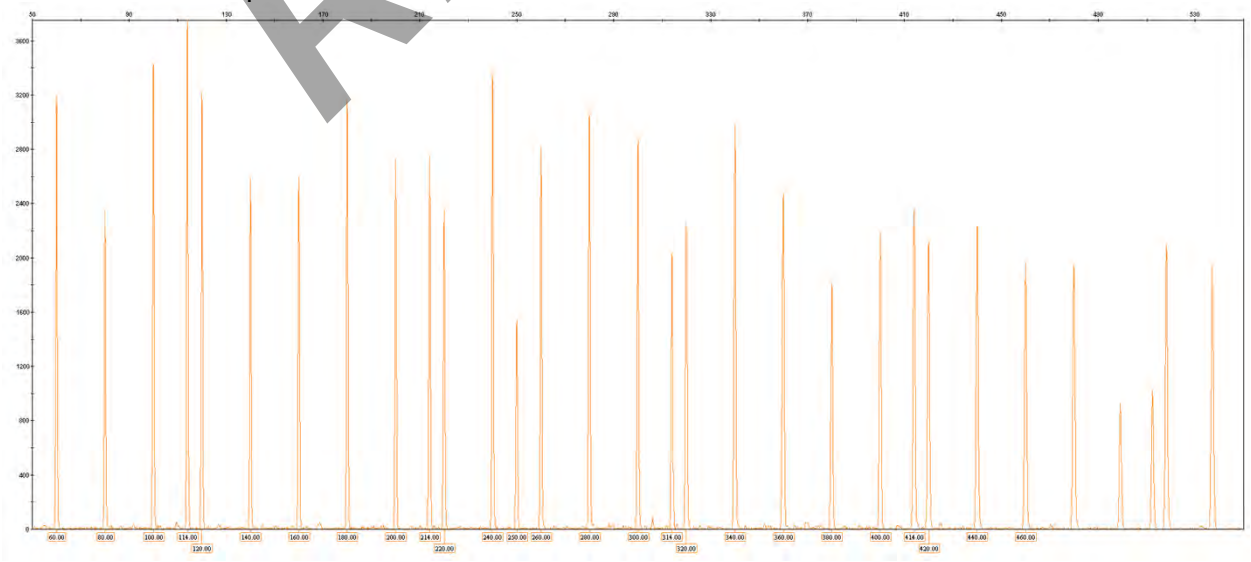
40.8 Technical Review:

The technical reviewer of a Y-STR project shall import the primary analyst's project utilizing the GeneMapper Manager. Any discrepancies in analysis should be noted and retained, but the project shall not be altered by the technical reviewer. If applicable, the analyst will make any appropriate changes to their project, to be checked by the technical reviewer.

40.9 General Analysis of Yfiler Plus Results:

40.9.1 The standard analysis parameters for samples and controls are B: 35 RFU, G: 55 RFU, Y: 35 RFU, R: 55 RFU, P: 55 RFU, and O: 150 RFU . Profiles (forensic unknowns) with peaks >10,000 RFU are interpreted with caution with approval of the TL.

40.9.2 Ensure that all required internal lane size standard (60-460) peaks are called in all samples.



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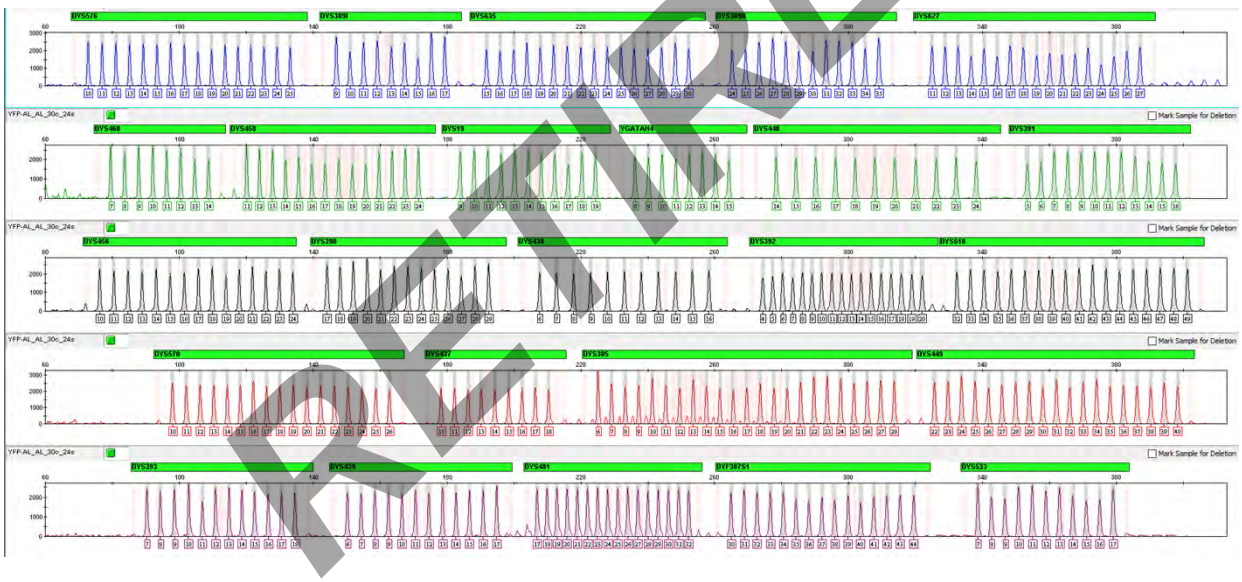
40.9.2.1 If a sample's internal size standard fails due to a low sizing quality (e.g. pull-up peaks into the size standard), the option to override the sample's size quality is available and shall be noted on the analysis worksheet. Both the primary and the second analyst must confirm that the size quality failure was not due to the loss of internal lane size standard peaks. The sample may then be analyzed as normal.

40.9.2.2 Ensure that each ladder in each injection has all allele peaks detected above the minimum threshold. If a ladder does not meet these requirements, change the sample type from allelic ladder to sample. One ladder in each injection must meet the requirements.

40.9.2.2.1 The Yfiler Plus Allelic Ladder should appear as follows:



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40.9.3 Y-STR Control Requirements:

40.9.3.1 Import the appropriate sample files including controls (RB, NEG, EP1*, POS control DNA 007) and at least one ladder. At least one set of controls (for each amplification set) must give the expected results**. If not, the amplification set must be re-injected, re-amplified, or re-extracted as warranted. When necessary, if the amplification positive control does not give the expected result, the TL may sign off on an extraction positive control that did give expected results as the amplification positive.

*With extraction sets where a male laboratory control is not available, using the YFP kit positive control is sufficient with TL approval.

40.9.3.2 Potential contamination is assessed by evaluating the evidentiary samples, RB, NEG, and positive controls. All samples within a batch will be cross-checked against each other to detect potential contamination. Analysts should use their experience and judgment during this process. An excel macro is available to assist with batch cross-comparisons.

**RB and NEG samples should not have any callable peaks. If callable peaks are detected, the set of samples may be re-run, re-amplified, or re-extracted as appropriate. If callable peaks are detected and the evidentiary samples are not affected, the set of extracted samples may be interpreted with caution with TL approval. Note that the RB may be omitted for re-amplifications using the same volume (or less) of template. The RB and NEG may be omitted for re-injections of equal or lesser time/sensitivity.

If contamination is detected and depending on factors such as the type and extent of contamination, and whether the source of the contamination can be identified, the contaminated sample(s) and/or amplification sets may be interpreted with caution in the report or deemed unsuitable for comparison following TL review and approval. Contaminated profiles that are deemed unsuitable for comparisons will be reported out as such. Contamination is tracked in a Qualtrax workflow.

40.9.3.3 **Positive Control Profiles: Ensure that all alleles were included and that the positive controls give the expected profile. The EP1 may be omitted for re-amplifications if expected results were previously generated for the extraction set. The positive controls may be omitted for re-injections.

40.9.4 Evidentiary profiles that contain elevated stutter peaks at a majority of the loci, have off-scale peaks and other artifacts visible through the electropherogram due to injecting too much and/or amplifying too much sample may be reinjected using a reduced injection time and/or using less amplified DNA. Samples may also be re-amplified using a reduced amount of template and then re-run on the CE instrument. Please refer to DNA SOP-37 YFP Amplification.

40.9.5 If a peak is labelled as off ladder (OL) or is outside the ladder region and therefore not labelled by the ID-X software, review the data to determine that it is a true microvariant or off-ladder allele. Peaks of this nature may be confirmed through re-injection or re-amplification, if necessary.

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40.9.6 The stochastic threshold (ST) is 500-700 RFU for loci DYS385a/b and DYF387S1 (see table below; ST derived from internal validation).

40.9.6.1 Per the "SWGDAM Guidelines on STR Interpretation" A stochastic threshold (ST) is defined as the "value above which it is reasonable to assume that allelic dropout has not occurred within a single-source sample". For YFP, the stochastic threshold is the peak height above which one can expect that both peaks at loci DYS385a/b and DYF387S1 would be observed. This threshold is applied to reduce the incidences of calling a false "homozygote".

Injections Time Ranges

Stochastic Threshold	3500-1, 2, 3, 5, & 6		3500-4
500 RFU	1.2 kV, 16 sec	LOW	1.2 kV, 12 sec
500 RFU	1.2 kV, 24 sec	STANDARD	1.2 kV, 18 sec
700 RFU	1.6 kV, 32 sec	HIGH	1.6 kV, 24 sec

40.9.6.2 Specific worksheets to be used for YFP amplification and injection on CE instrument are as follows:

DNA QR-324 YFP Amplification Worksheet

DNA QR-328 YFP Injection/Analysis Worksheet

40.10 Determination of Single Source or Mixture Profile & Number of Contributors

Single Source Profile Attributes: A single source profile will generally have no more than 1 allele at any locus except DYS385a/b and DYF387S1 (typically 2 alleles). Expected peak balance at DYS385a/b and DYF387S1 decreases as a function of peak height similar to that seen with autosomal loci. In rare instances, an individual may exhibit a bi/tri-allelic pattern at a locus.

When an extra-allelic pattern is identified, the results must be documented in the case file. The locus is able to be searched in the YHRD database.

40.10.1 Determination of the number of contributors (different Y lineages) to the DNA profile:

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Evidence profiles should be assessed for the number of possible contributors, data above/below the stochastic threshold at the DYS385a/b and DYF387S1 loci, peak heights of called alleles, etc., when making this determination as discussed below:

The following steps are followed to assign the likely number of lineages in the profile:

1. Review the profile as a whole, assessing the level of degradation, presence of low level peaks, noisy or clean baseline and general quality (template) of the profile.
2. Identify likely stutter peaks by reference to the YFP allele-specific stutter ratio (SR) expectations for each allele at each locus using DNA QR-345 (see also plots of SR as needed).
3. Find the locus with the highest number of unambiguous allelic peaks, A, keeping in mind that DYS385 and DYF387S1 routinely have two results. A gives the initial assessment of the likely number of contributors to the profile.
4. Review peak height balance at loci DYS385 and DYF387S1.

The detection of more than one allele per locus (other than the DYS385 and DYF387S1 loci) is generally indicative of a mixed sample. Variation in peak heights between alleles between loci and at DYS385 and DYF387S1 may assist in the interpretation of such results.

Typically, if a maximum of 2 alleles are observed at a locus (except for DYS385 and DYF387S1), the DNA results are consistent with a mixture of two male lineages. Two peak patterns (at a single locus other than DYS385 and DYF387S1) have been observed from single source samples, but they are rare. The analyst should consider the possibility of high stutter peaks, which can complicate interpretation. While counting allele peaks is critical in determining the number of contributors, the analyst must also consider that allele sharing between different haplotype lineages may result in an underestimation of the actual number of contributors. The number of lineages identified reflects the analyst's assessment of the most likely number of different haplotypes required to reasonably explain the observed profile.

A Y-STR mixture simulation tool has been validated by the Division of Scientific Services to assist in the determination of the number of haplotypes that are present in Yfiler Plus profiles. This tool may be used by analysts to demonstrate the expected amount of allele sharing at each locus per contributor number in the absence of dropout.

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Note 1: Amplification results from low-template quantities (particularly less than 100 pg) may exhibit increased stutter and peak variance. Based on the YFP/3500xl validation data, it was noted that profiles from low-template contributors may exhibit multiple high stutter peaks that can be considerably above the standard stutter thresholds (see low-template stutter thresholds). A similar impact may occur with respect to peak imbalance at DYS385 and DYF387S1. Therefore, when these low-template peaks pertaining to stutter or peak imbalance are used to infer an extra lineage, there should be data elsewhere in the profile to support this.

Note 2: The low-template stutter thresholds are not included in DNA QR-345 and the YFP panel. For each stutter type being evaluated by CT DSS, the "low" data thresholds are included in DNA SOP-41 to aid in determining the number of contributors and to highlight the increased expected stutter variance associated with low-template single lineage profiles and/or profiles with low-template contributors.

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40.11 **Qualitative Assessment of YFP Profiles—Comparisons to Knowns:**

40.11.1 Single source haplotypes: If 1 peak was called ≥ AT (except at DYS385 and DYF387S1) with nothing else detected ≥ AT at any other locus, and "Known A" has that allele, the results would be consistent with "Known A" being the source. One would consider the issue of "heterozygous" allelic peak imbalance at DYS385 and DYF387S1. Therefore, with good peak balance: the results would be consistent with "Known A" being the source of the DNA profile for item #xx. Significant peak imbalance (> 50%) at DYS385 and/or DYF387S1, may be indicative of a mixture. Note: all YFP results are utilized for comparison purposes. However, for most circumstances, only the Yfiler loci are used for statistics if the comparison is "inclusion/CBE".

40.11.1.1 For single source partial profiles with peaks ≥ AT, report a CBE interpretation when partial results are detected at DYS385 and/or DYF387S1, as long as the observed alleles are consistent with the known source.

Note: in situations where no loci have more than 1 peak ≥ AT (except 2 peaks at DYS385) [e.g. such as if there is significant peak imbalance (> 50%)], it is important to consider if the profile could be a mixture (partial composite of 2 or more individuals).

40.11.2 **Interpretation of DNA mixtures**

40.11.2.1 The results at all YFP loci are utilized for comparison purposes. However, for most circumstances, only the Yfiler loci are used for statistics if the comparison is "inclusion/CBE". These general approaches do not account for all possible mixture scenarios and no two mixtures are exactly the same.

The analyst considers the quantity of DNA amplified, the peak heights of the alleles, the extent of degradation/inhibition, the impact of stutter, potential carryover between fractions in a differential extraction, and if a known profile compared to the mixture can be objectively determined to be a contributor (e.g. intimate samples).

40.11.2.2 Factors taken into consideration when interpreting mixtures:

40.11.2.2.1 If one person can be objectively determined to be included in the mixture (e.g. consensual partner), the mixture is evaluated by a consideration of what loci have DNA profile results (≥ AT) that could not be from the known source.

40.11.2.2.2 The total number of dropouts (overall and at each locus) that would be required to still be consistent with a CBE conclusion. While allele dropout (including peaks below the stochastic threshold at DYS385 and DYF387S1), can occur at any locus, extensive validation and case experience has shown that smaller amplicons amplify more efficiently and are less affected by degradation than are larger amplicons. Therefore, the results at the smaller amplicons can be particularly diagnostic.

40.11.2.2.3 To what degree would a putative low-level contributor coincidently be included by a high-level contributor due to overlap.

40.11.2.2.4 If the analyst and reviewers feel that the above rules do not adequately apply to a specific mixture comparison, consult the TL.

40.11.3 YFP profiles will be searched against the Staff Index and documented on DNA QR-20 Staff Search Worksheet.

40.12 General Report Conclusions for Y-STR Results:

40.12.1 YFP report templates are described in DNA SOP-6. Additionally, see below examples of general statements for reports.

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40.12.2 Elimination: used when the known sample is not the source of, or a contributor to, the DNA profile detected from item xx. The known sample has alleles that are not detected in the evidentiary sample and the lack of detection is not reasonably explainable by degradation/inhibition/allele dropout (stochastic effects) within the evidentiary sample.

40.12.3 Inconclusive: No conclusion can be drawn from the comparison between the known sample and the evidentiary sample due to uncertainty. There are two reasons for inconclusive results; limited data detected, and the mixture being too complex. See 40.12.3.1 and 40.12.3.2 below:

40.12.3.1 Based on limited data: This statement is made when the data does not provide sufficient support for any conclusion regarding a comparison of a mixture to a particular known.

40.12.3.2 Mixture too complex: This statement is made for mixtures of 4 or more male lineages where the known is not eliminated.

40.12.4 Cannot be eliminated (CBE) as the source of, or a contributor to a DNA profile: This conclusion is used when there is a strong correlation between the known sample and the evidentiary sample, but some of the alleles present in the known sample are not detected in the evidentiary sample above the analytical threshold. In this instance, dropout is a reasonable explanation taking into consideration peak height data, overall quantity of template DNA, information regarding the extent of degradation, inhibition, stochastic effects, and masking by stutter or contributor ratio.

40.12.5 Inclusion: All alleles consistent with the known sample are detected (≥ AT) in the evidentiary profile at all loci where results are generated.

40.13 YFP Statistics: (see 40.14 YFP Workflow and Qualification of Loci for Statistics)

40.13.1 For single source YFP profiles, statistics are calculated, for most circumstances, at all Yfiler loci where DNA results are obtained ≥ the analytical threshold. For DYS385, if both alleles are detected, the locus is also used for statistical purposes. If only one allele is detected at DYS385, that locus can be used for statistical purposes, given that the allele is above our stochastic threshold and that the known in question is a homozygote. The YFP random match probability is calculated using the counting method. A haplotype frequency is generated by counting the number of times a haplotype has been observed in the population database divided by the total number of haplotypes in the Database. Haplotype frequencies are given an upper bound 95% confidence interval (See DNA SOP-6).

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The Y-Chromosome STR Haplotype Reference Database (YHRD) Y-STR population database (available at https://yhrd.org) is used.

40.13.1.1 On the YHRD website homepage, click "Search the Database" on the upper left of the site. From there, click on "Manually enter the haplotype/haplotypes to search for". "Yfiler" is the default for the order the loci are listed. Change the order to "Yfiler Plus". Enter the YFP profile at all loci, with a comma separating the two alleles (even if a homozygote) at DYS385 and DYF387S1. (Note only enter DYS385 if you will be using it for statistics.) After entering the YFP profile, click on the Yfiler button to search just the Yfiler loci portion of the profile. Next, click "Search". When search is complete, click "Add feature to this report" and select "National Database (with Subpopulations, current SWGDAM-compliant version). Click the "X" to close the "worldwide" results before printing page for the case jacket.

Note: for low-level profiles, especially those that have results at YFP loci that do not overlap with Yfiler, the YFP loci may be searched. Report the more discriminating statistic.

40.13.1.2 An exact upper bound 95% confidence interval (Clopper-Pearson method) is calculated from the results of a database search in order to estimate the population frequency of a haplotype using the population (United States data set: African American, Caucasian, or Hispanic) with the most common haplotype frequency. The upper bound estimate is dependent on the size of the database, and these estimates may change as the database size changes.

40.13.1.3 The formula used to calculate the 95% upper bound frequency estimate is:

$$\sum_{k=0}^{x}\binom{n}{k}p_0^k(1-p_0)^{n-k}=0.05$$

https://strbase.nist.gov/pub_pres/SWGDAM-Jan2012-YSTRs.pdf

Clopper, C. and Pearson, E. *The use of confidence or fiducial limits illustrated in the case of binomial,* Biometrika (1934) 26:404-413.

40.13.2 Y-STR haplotypes are inherited independently from autosomal loci. Therefore, Y haplotype (and cumulative haplotype) frequencies may be combined with autosomal STR RMPs or CPIs using the product rule to generate an overall profile frequency estimate. (applies to outsourced cases only with no STRmix analysis)

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40.13.3 Y-STR Mixtures: (see 40.14 YFP Workflow and Qualification of Loci for Statistics)

40.13.3.1 The expected frequency of haplotypes that could contribute to a YFP mixture is calculated using the Combined Counting Method (CCM). A cumulative haplotype frequency is generated by counting how many times each of the possible haplotypes are observed in the population Database, summing them and dividing by the total number of haplotypes in the database (i.e. cumulative haplotype frequency = x/N, where x is the number of potentially contributing haplotypes observed in a population and N is the total number of haplotypes searched in that population). An exact upper bound 95% confidence interval is then applied using the population (African American, Caucasian, or Hispanic) with the most common CCM frequency. Note: The frequency and confidence interval for a mixture profile are calculated using the software tool Y-Mix, which contains the U.S. portion of the YHRD database.

The cumulative haplotype frequency (with an upper bound 95% confidence interval) is calculated for samples with an additional allele at any locus, i.e., 2 alleles at DYS393, DYS19, DYS389II, DYS390, DYS391, or 3 alleles at DYS385.

40.13.3.2 The file for the Y-Mix software tool is located on the S: Drive. The loci in the Excel folder are in alphanumeric order. Enter the mixture profile under the appropriate YF (YFP when necessary) loci, with one row per allele. (Note: for each locus with data entered, add a "0" along with the alleles detected if a null has been seen in the database at that locus). Defaults (Limit database to samples with all the loci entered above?: Yes, Treat this profile as a single source sample?: No, Desired UCI: 95%, Use (x+1)/(N+1)?: No) are to be unchanged. Click "compare the profile to the database" when profile entry is complete. Print page when results are obtained.

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40.14 **YFP Workflow and Qualification of Loci for Statistics:**

Note: If it so happens that YFP loci will be used for statistics, the same guidelines for YF loci will follow (i.e. DYF387S1 will follow the guidelines set forth for DYS385).

Note: Loci used for statistical purposes will be noted in the allele table of the DNA report via footnote designation.

40.14.1 Evaluate data at all YFP loci (≥ AT) to determine if profile is a single haplotype or a mixture (see 40.10).

40.14.2 Qualify results for statistics at DYS385 using a ST of ≥ 500 or 700 RFU (based on injection time) and at other loci as discussed below. Statistics are calculated as described in 40.13 for 1-3 lineage profiles.

40.14.3 Compare known(s) to evidentiary profiles as in 40.11 and 40.12.

40.14.4 Single Haplotype Profile:

40.14.4.1 2 alleles detected at DYS385: use data (above & below ST) at DYS385, and other Yfiler loci where included.

40.14.4.2 1 allele detected at DYS385: (A) Qualify locus if allele is above ST and use data for statistics at other loci where included. (B) Disqualify locus for statistics if allele is below ST and use data at remaining YF loci where included. Note: the known would be eliminated as the source if the known does not have the allele found in the evidence profile.

40.14.5 Mixture of 2 Haplotypes (CCM): Evaluate results at all loci as described in 40.14.1-3.

40.14.5.1 Mixture evident at DYS385 with < 4 alleles and all alleles > ST at DYS385: qualify locus for stats and use data for statistics at other YF loci where the mixture is detected.

40.14.5.2 Mixture evident at DYS385 with < 4 alleles and some/all allele(s) below ST at DYS385: Disqualify locus for stats and use data at other loci where the mixture is detected. Note: the known (2 alleles at DYS385) would be eliminated as a contributor if the known does not have at least 1 allele found in the evidence profile. If the known has only l allele at DYS385, the known may not be eliminated depending on the results at other loci. Note that DYF387S1 is utilized in the same manner as DYS385 for qualitative comparison purposes, but only DYS385 is considered for statistics.

40.14.5.3 Mixture with 4 alleles at DYS385 and allele(s) above or below ST at DYS385: qualify locus for statistics and use data for statistics at other YF loci where the mixture is detected.

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40.14.5.4 Mixture not evident at DYS385, e.g., 2 alleles detected at DYS385 and no significant peak imbalance at DYS385 (where the minor allele is > 20% of the major allele): disqualify locus for statistics. Use data for statistics at other YF loci where the mixture is detected.

40.14.6 Mixture of 3 Haplotypes (CCM): Evaluate results at all YFP loci as described in 40.14.1-3.

40.14.6.1 Mixture with 2-4 alleles at DYS385 and all alleles > ST at DYS385: disqualify locus for stats and use data for statistics at other YFP loci where all 3 contributors are detected.

40.14.6.2 Mixture with 2-4 alleles at DYS385 and some/all allele(s) below ST at DYS385: disqualify locus for stats and use data at other YF loci where all 3 contributors are detected.

40.14.6.3 Mixture with 5 alleles at DYS385 and all alleles > ST at DYS385: qualify locus for stats and use data for statistics at other YF loci where all 3 contributors are detected.

40.14.6.4 Mixture with 5 alleles at DYS385 and some/all alleles below ST at DYS385: disqualify locus for stats and use data for statistics at other YFP loci where all 3 contributors are detected.

40.14.6.5 Mixture with 6 alleles at DYS385 and allele(s) above or below ST at DYS385: qualify locus for statistics and use data for statistics at other YF loci where all 3 contributors are detected.

40.14.6.6 Mixture not evident at DYS385: disqualify locus for statistics. Use data for statistics at other YF loci where all 3 contributors are detected.

40.15 Deconvolution of Major in 2 Male Lineage Mixtures

Note: All loci able to be deconvoluted, regardless of being a YF or YFP locus, will be deconvoluted as the major profile. The deconvolution of a major is done independently to determining which loci will be used for statistical purposes.

40.15.1 A major contributor to mixtures of 2 male lineages may be deconvoluted in lieu of the Combined Counting method (CCM) discussed in 40.14.5 with TL/DD approval as follows:

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40.15.2 Deconvolution of mixtures at all YFP loci except DYS385 and DYF387S1: A major contributor may be deconvoluted where the height of the major allele is at least 5X greater than the minor allele (minor is ≤ 20% of major). Example: if the minor allele is 100 RFU, the major allele must be ≥ 500 RFU.

40.15.3 Where only 1 allele is detected at any locus except DYS385 and DYF387S1, the "major contributor allele" must be ≥ 500 or 700 RFU, depending on the injection time (see 40.9.6.1). If not, the locus is not eligible for deconvolution.

40.15.4 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 1 allele is present, the major contributor may be deduced if the peak height is ≥ the stochastic threshold.

40.15.5 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 2 alleles are present, the "heterozygous" major contributor may be deduced if the peaks are ≥ the stochastic threshold and "heterozygous" peak balance is at least 50%.

40.15.6 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 2 alleles are present, ("homozygous" major with significant peak imbalance), the major contributor may be deduced if the major peak is ≥ the stochastic threshold and the mixture ratio at DYS385 or DYF387S1 is at least 5:1.

40.15.7 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 3 alleles are present, ("heterozygous" major): the major contributor may be deduced if both peaks are ≥ the stochastic threshold, the mixture ratio of the lowest major allele to the minor allele is at least 5:1, and "heterozygous" peak balance is at least 50%.

40.15.8 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 3 alleles are present, ("homozygous" major): the major contributor may be deduced if the major allele is ≥ the stochastic threshold and the mixture ratio of the major allele to the highest minor allele is at least 5:1.

40.15.9 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 4 alleles are present,: the major contributor may be deduced if the two highest peaks are ≥ the stochastic threshold, the mixture ratio is at least 5:1 (lowest major allele must be at least 5X greater than the highest minor allele), and "heterozygous" peak balance is at least 50%.

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40.15.10 Once deconvolution is completed, the known(s) are compared to the profile of the deconvoluted major contributor (see 40.11 & 40.12).

40.15.11 If an individual is eliminated as a potential source of the major contributor but cannot be eliminated from the overall mixture (i.e. a potential minor contributor), the CCM statistic is calculated as discussed in 40.14.5.

40.16 Deconvolution of Major in 3 Male Lineage Mixtures

Note: All loci able to be deconvoluted, regardless of being a YF or YFP locus, will be deconvoluted as the major profile. The deconvolution of a major is done independently to determining which loci will be used for statistical purposes.

40.16.1 A major contributor to mixtures of 3 male lineages may be deconvoluted in lieu of the combined counting method (CCM) discussed in 40.14.5 with TL/DD approval as follows:

40.16.2 Deconvolution of mixtures at all loci except DYS385 and DYF387S1: A major contributor may be deconvoluted where the height of the major allele is at least 5X greater than the highest minor allele (minor is ≤ 20% of major).

40.16.3 Where only 1 allele is detected at any locus except DYS385 or DYF387S1, the "major contributor allele" must be ≥ 500 or 700 RFU depending on the injection time (see 40.9.6.1). If not, the locus is not eligible for deconvolution.

40.16.4 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 1 allele is present, the major contributor may be deduced if the peak height is ≥ the stochastic threshold and the mixture ratio at all loci where the mixture is detected is at least 5:1.

40.16.5 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 2 alleles are present, the "heterozygous" major contributor may be deduced if the peaks are ≥ the stochastic threshold, the mixture ratio at all loci where the mixture is detected is at least 5:1, and "heterozygous" peak balance is at least 50%.

40.16.6 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 2 alleles are present ("homozygous" major with significant peak imbalance), the major contributor may be deduced if the major peak is ≥ the stochastic threshold and the mixture ratio at DYS385 and DYF387S1 is at least 5:1.

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40.16.7 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 3-6 alleles are present ("heterozygous" major): the major contributor may be deduced if both peaks are ≥ the stochastic threshold, the mixture ratio of the lowest major allele to the highest minor allele is at least 5:1, and "heterozygous" peak balance is at least 50%.

40.16.8 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 3-5 alleles are present ("homozygous" major): the major contributor may be deduced if the major allele is ≥ the stochastic threshold and the mixture ratio of the major allele to the highest minor allele is at least 5:1.

40.16.9 Once deconvolution is completed, the known(s) are compared to the profile of the deconvoluted major contributor (see 40.11 & 40.12).

40.16.10 If an individual is eliminated as a potential source of the major contributor but cannot be eliminated from the overall mixture (i.e. a potential minor contributor), the CCM statistic is calculated as discussed in 40.14.5.

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40.4 Analysis of Y-STR DNA Profiles using GMID:

40.4.1 The results are evaluated for each locus. However, considering the data from all loci tested will assist in the overall interpretation of the results for each sample. See DNA SOP-6 for standard report conclusions.

40.4.2 Off-Scale Data: If too much DNA is added to the amplification reaction, the fluorescent intensity for the PCR products may exceed the linear dynamic range for detection by the instrument. Samples with off-scale peaks may exhibit raised baselines, split peaks, increased minus A peaks, excessive pull-up and/or higher than expected stutter levels, and/or amplification artifacts in one or more of the colors. Examine the peak heights in ID-X software. Any peaks greater than 30,000 RFU may be off-scale. These samples may need to be reinjected for a shorter time or diluted and re-amplified.

40.4.3 Peak identification: The laboratory's analytical threshold (AT) is dye channel specific, B: 35 RFU, G: 55 RFU, Y: 35 RFU, R: 55 RFU, P: 55 RFU, and O: 150 RFU. The identity of peaks called by ID-X is generally assigned to one of the following categories:

40.4.4 Allele Peak: A called allele has a peak height ≥ AT RFU, a fragment size that falls within the base pair range, and has the appropriate dye color for the locus. However, not all peaks ≥ AT RFU are typed as alleles. Stutter peaks, pull-up/pull-down peaks, background noise, and amplification or injection artifacts could fall above the RFU threshold, especially if the allele peaks are high. Peaks ≥ AT RFU that are not called by ID-X may be called by the analysts on a case-by-case basis. Such peaks are interpreted with caution with TL approval.

40.4.5 Detection of variant alleles: Variant alleles have been identified from many STR loci. These alleles may contain full or partial repeats. Variant alleles are labeled as Off Ladder (OL) alleles in ID-X. If a particular variant has been observed before by the DESPP CT Division of Scientific Services (CT-DSS), Thermo Fisher (Life Technologies), NIST, or in published literature, the sample does not need to be repeated. In ID-X, change the OL to the proper allele call. However, if the repeat has not been observed, the sample may be re-amplified and/or reinjected to confirm the OL allele as warranted.

40.4.6 Stutter peaks (N-2, N-3, N-4, N-5, N-6, N-8, N-9, N-10, N-12, N+2, N+3, N+4, N+5, N+6—depending on the locus): A stutter peak has a fragment length typically four base pairs smaller than the true allele (see exceptions above). If the peak height in the stutter position is less than the user-defined stutter threshold,

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the peak is considered a stutter peak and is filtered out by ID-X or manually edited by the analyst (following use of DNA QR-345). Allele-specific stutter thresholds were determined by CT-DSS during the YFP Internal Validation. Peaks in stutter positions that are below the Laboratory's stutter thresholds are not listed on the report and are not used for statistical purposes. If the height of the peak in the stutter position is greater than the CT-DSS stutter thresholds, this indicates a possible mixture, an overloaded sample, or a higher than normal stutter ratio. Additional information provided by other loci may be of assistance in these cases.

40.4.7 Minus A Peaks: The YFP amplification kit is optimized to add an extra nucleotide to the 3' end of the PCR product. The nucleotide is predominantly adenosine (A). Lack of a full A addition may be observed especially when the amount of input DNA is greater than approximately 1 ng. However, sample degradation can increase the effective concentration of a small amplicon relative to the larger amplicons, leading to minus A product at the smaller loci. Data from other loci may be of assistance in these cases. When appropriate, a smaller quantity of sample may be re-amplified.

40.4.8 Pull-up Peaks: Pull-up peaks are generally seen when excess amplification product is injected on the CE or when the spectral calibration is sub-optimal. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-up peaks are often displayed in several colors. Pull-up peaks should have approximately the same fragment size in each of the colors as the true allele peak. Additional information provided by other loci may be of assistance in these cases. When evaluating allelic peaks that may contain a pull-up (PU) contribution, an estimate of the PU contribution is subtracted from the height of the potential allelic peak to determine if the remaining peak height is sufficient for allele or asterisk designation. The estimated pull-up contribution is determined by evaluating the height of unambiguous pull-up peaks (pull-up with e.g., no stutter or allelic contribution) at each locus where pull-up is detected for a particular color.

Note: the 3500 Series Data Collection Software 4 has a pull-up correction function applied to the raw data. This function is not necessarily consistent across the entire base-pair range for the detection of peaks from amplified DNA, therefore care must be taken when identifying the percentage of signal for unambiguous pull-up peaks. Using unambiguous pull-up peak(s) in the same locus will most often be optimal. In the case where there are no unambiguous pull-up peaks for the dye channel, consider that the median percentage of pull-up

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peaks observed during the validation was 0.7% with 95.1% of the pull-up peaks being < 3% and 99.4% being < 5%.

Example: The percentage of pull-up peaks as compared to the height of the peaks causing the pull-up is calculated. (PU percentage = PU peak height/height of the peak causing the PU). This percentage is converted to an RFU value (multiply percent by height of peak causing the pull-up) which is then subtracted from the potential allelic peak in question. The resulting "subtracted" peak is called where DNA Section allele calling criteria are met. The same methodology would be applied to potential allelic peaks with a pull-down component (see below).

40.4.9 Pull-down Peaks: Pull-down peaks are generally seen when excess amplification product is run on the CE or when the spectral calibration is sub-optimal for the run. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-down peaks can be displayed in several colors. Pull-down peaks can have approximately the same fragment size, but often the pull-down peak will be slightly a larger or smaller size as compared to the true allele. Additional information provided by other loci may be of assistance in these cases.

40.4.10 Spikes: fluorescent spikes can be seen within GMID data. Spikes are typically sharp peaks caused by air bubbles or polymer crystals in a capillary, which can be displayed in different colors, and have approximately the same "fragment size". Additional inspection of the raw data or re-injection may assist in cases where spikes are believed to be present. Upon subsequent injection, spikes should not reoccur at the same position.

40.4.11 Dye Artifacts/Dye Blobs: Broad peaks may be the result of dissociated dyes or fluorescent particles that are trapped within the polymer matrix and then fluorescently excited by the laser. Additional inspection of other samples, especially negative controls, within the module may assist in interpretation in cases where dye blobs are believed to be present.

40.4.12 Amplification Artifact Peaks: Nonspecific amplification product peaks, generally with good peak morphology, may result when excessive template DNA is amplified. When appropriate, a smaller quantity of sample may be re-amplified.

40.4.13 High Background: Background fluorescence may reach an RFU level above the calling threshold. Generally such high background peaks exhibit atypical peak morphology. When appropriate, re-injection may assist with interpretation.

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40.4.14 Raised Baseline: Elevated baseline of one color over a broader (multiple bin) scan range may occur. Note: pull-down from two adjacent peaks may cause raised baseline between the pull-down peaks; the "pull-down" designation should be used for the editing notation when raised baseline is caused by pull-down.

40.5 Highly Degraded/Low Template Samples:

Highly degraded/low template samples are interpreted with caution. Degraded samples may "appear" to exhibit high stutter and greater –A at the smaller amplicons with off-scale data. In addition, degraded/low template samples may display unbalanced heterozygous alleles and locus/allele dropout. Partial profiles may be interpreted as determined by the data present for each locus. Given the possibility of stochastic allele sampling, especially with low quantity and/or degraded samples, single peaks (at loci DYS385 and DYF387S1) less than the stochastic threshold are interpreted with caution (pertains to putative haplotype assignment at that locus). To maximize the number of loci with peaks above the threshold, a sample may be run at increased injection times on the 3500. However, any off-scale data at the smaller amplicons are interpreted with caution.

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40.6 Inhibited Samples:

40.6.1 Inhibited samples may exhibit allele, locus, or complete profile dropout, unbalanced alleles at loci DYS385 and DYF387S1, and interlocus peak imbalance. To possibly overcome an inhibitor, smaller quantities of sample may be re-amplified. Quantitation data may aid in determining whether re-amplification is warranted as well as the amount of sample to be re-amplified. Before the genotyping amplification is performed, an evaluation of the Quantifiler Trio IPC for each sample is useful. The Quantifiler Trio IPC result for a sample being undetected or having an elevated C_T may indicate that a sample has an inhibitor present. If this is the case, multiple quantities or dilutions of a sample may be amplified for the original amplification.

40.6.2 Input of excessive female DNA may result in inhibition of Y-STR amplification. When appropriate, smaller quantities of sample may be re-amplified. Quantitation data may aid in determining whether re-amplification is warranted as well as the amount of sample to be re-amplified.

40.7 X-homologous peaks:

With large quantities of female DNA present in the amplified DNA extract, the Y primers may generate X-homologous or female cross-reactivity peaks. If the known female sample is amplified, any X-homologous peaks may be removed from the evidentiary DNA profile. Note: may require amplifying the same quantity of female DNA as with the evidentiary sample. The female cross-reactivity peaks are most common in the blue and red dye channels (see the YFP Developmental and CT-DSS Internal Validation results and the YFP User Guide for examples).

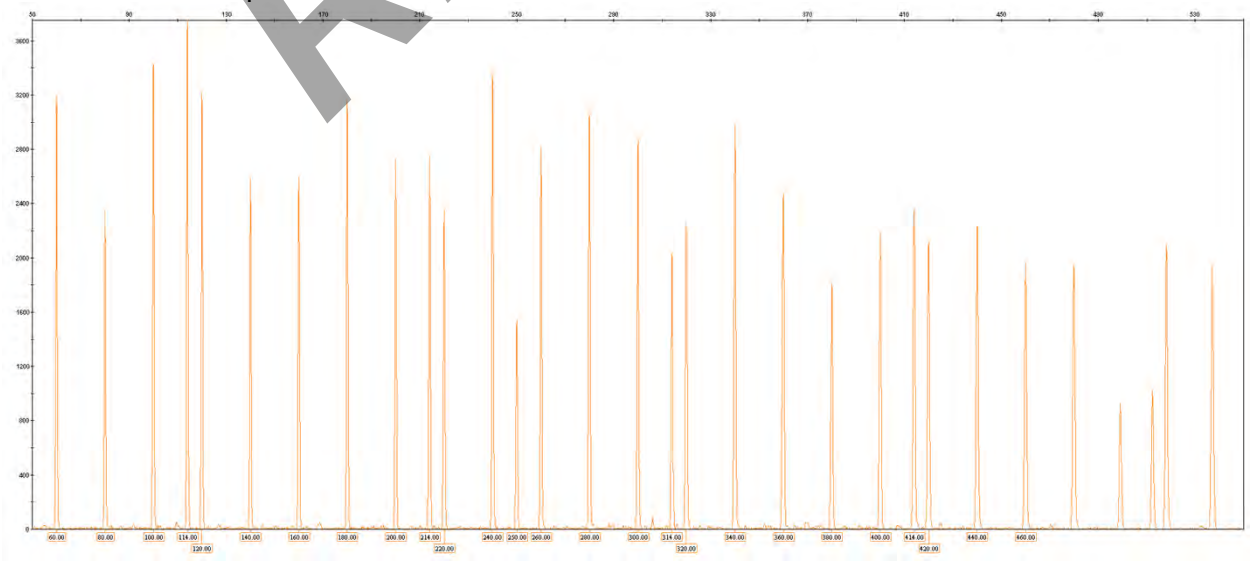
40.8 Technical Review:

The technical reviewer of a Y-STR project shall import the primary analyst's project utilizing the GeneMapper Manager. Any discrepancies in analysis should be noted and retained, but the project shall not be altered by the technical reviewer. If applicable, the analyst will make any appropriate changes to their project, to be checked by the technical reviewer.

40.9 General Analysis of Yfiler Plus Results:

40.9.1 The standard analysis parameters for samples and controls are B: 35 RFU, G: 55 RFU, Y: 35 RFU, R: 55 RFU, P: 55 RFU, and O: 150 RFU . Profiles (forensic unknowns) with peaks >10,000 RFU are interpreted with caution with approval of the TL.

40.9.2 Ensure that all required internal lane size standard (60-460) peaks are called in all samples.



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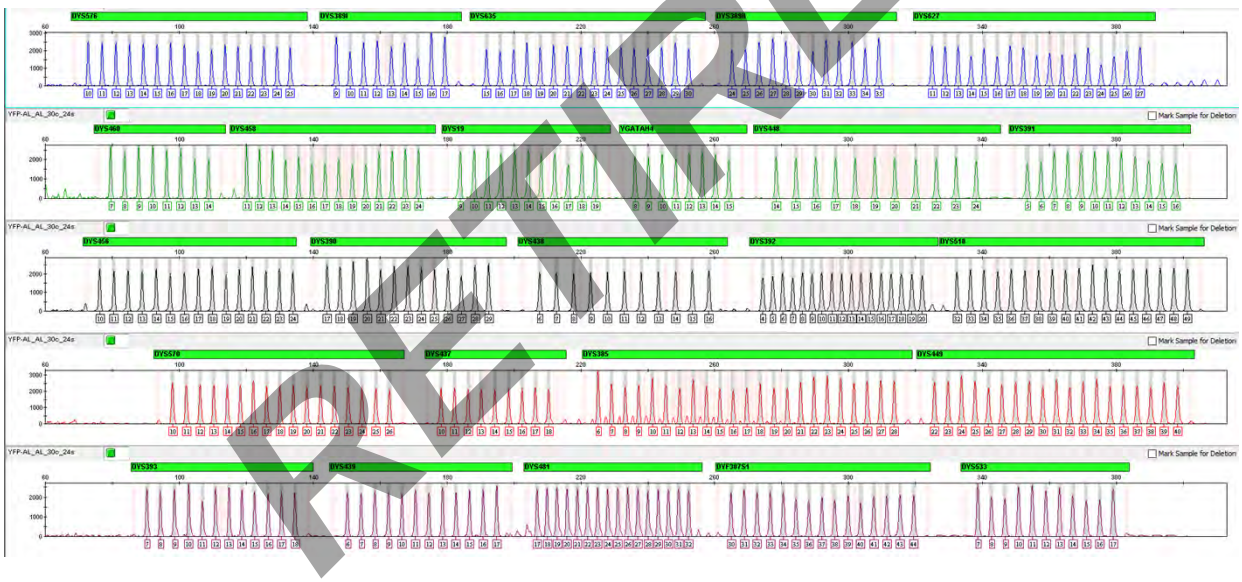
40.9.2.1 If a sample's internal size standard fails due to a low sizing quality (e.g. pull-up peaks into the size standard), the option to override the sample's size quality is available and shall be noted on the analysis worksheet. Both the primary and the second analyst must confirm that the size quality failure was not due to the loss of internal lane size standard peaks. The sample may then be analyzed as normal.

40.9.2.2 Ensure that each ladder in each injection has all allele peaks detected above the minimum threshold. If a ladder does not meet these requirements, change the sample type from allelic ladder to sample. One ladder in each injection must meet the requirements.

40.9.2.2.1 The Yfiler Plus Allelic Ladder should appear as follows:



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40.9.3 Y-STR Control Requirements:

40.9.3.1 Import the appropriate sample files including controls (RB, NEG, EP1*, POS control DNA 007) and at least one ladder. At least one set of controls (for each amplification set) must give the expected results**. If not, the amplification set must be re-injected, re-amplified, or re-extracted as warranted. When necessary, if the amplification positive control does not give the expected result, the TL may sign off on an extraction positive control that did give expected results as the amplification positive.

*With extraction sets where a male laboratory control is not available, using the YFP kit positive control is sufficient with TL approval.

40.9.3.2 Potential contamination is assessed by evaluating the evidentiary samples, RB, NEG, and positive controls. All samples within a batch will be cross-checked against each other to detect potential contamination. Analysts should use their experience and judgment during this process. An excel macro is available to assist with batch cross-comparisons.

**RB and NEG samples should not have any callable peaks. If callable peaks are detected, the set of samples may be re-run, re-amplified, or re-extracted as appropriate. If callable peaks are detected and the evidentiary samples are not affected, the set of extracted samples may be interpreted with caution with TL approval. Note that the RB may be omitted for re-amplifications using the same volume (or less) of template. The RB and NEG may be omitted for re-injections of equal or lesser time/sensitivity.

If contamination is detected and depending on factors such as the type and extent of contamination, and whether the source of the contamination can be identified, the contaminated sample(s) and/or amplification sets may be interpreted with caution in the report or deemed unsuitable for comparison following TL review and approval. Contaminated profiles that are deemed unsuitable for comparisons will be reported out as such. Contamination is tracked in a Qualtrax workflow.

40.9.3.3 **Positive Control Profiles: Ensure that all alleles were included and that the positive controls give the expected profile. The EP1 may be omitted for re-amplifications if expected results were previously generated for the extraction set. The positive controls may be omitted for re-injections.

40.9.4 Evidentiary profiles that contain elevated stutter peaks at a majority of the loci, have off-scale peaks and other artifacts visible through the electropherogram due to injecting too much and/or amplifying too much sample may be reinjected using a reduced injection time and/or using less amplified DNA. Samples may also be re-amplified using a reduced amount of template and then re-run on the CE instrument. Please refer to DNA SOP-37 YFP Amplification.

40.9.5 If a peak is labelled as off ladder (OL) or is outside the ladder region and therefore not labelled by the ID-X software, review the data to determine that it is a true microvariant or off-ladder allele. Peaks of this nature may be confirmed through re-injection or re-amplification, if necessary.

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40.9.6 The stochastic threshold (ST) is 500-700 RFU for loci DYS385a/b and DYF387S1 (see table below; ST derived from internal validation).

40.9.6.1 Per the "SWGDAM Guidelines on STR Interpretation" A stochastic threshold (ST) is defined as the "value above which it is reasonable to assume that allelic dropout has not occurred within a single-source sample". For YFP, the stochastic threshold is the peak height above which one can expect that both peaks at loci DYS385a/b and DYF387S1 would be observed. This threshold is applied to reduce the incidences of calling a false "homozygote".

Injections Time Ranges

Stochastic Threshold	3500-1, 2, 3, 5, & 6		3500-4
500 RFU	1.2 kV, 16 sec	LOW	1.2 kV, 12 sec
500 RFU	1.2 kV, 24 sec	STANDARD	1.2 kV, 18 sec
700 RFU	1.6 kV, 32 sec	HIGH	1.6 kV, 24 sec

40.9.6.2 Specific worksheets to be used for YFP amplification and injection on CE instrument are as follows:

DNA QR-324 YFP Amplification Worksheet

DNA QR-328 YFP Injection/Analysis Worksheet

40.10 Determination of Single Source or Mixture Profile & Number of Contributors

Single Source Profile Attributes: A single source profile will generally have no more than 1 allele at any locus except DYS385a/b and DYF387S1 (typically 2 alleles). Expected peak balance at DYS385a/b and DYF387S1 decreases as a function of peak height similar to that seen with autosomal loci. In rare instances, an individual may exhibit a bi/tri-allelic pattern at a locus.

When an extra-allelic pattern is identified, the results must be documented in the case file. The locus is able to be searched in the YHRD database.

40.10.1 Determination of the number of contributors (different Y lineages) to the DNA profile:

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Evidence profiles should be assessed for the number of possible contributors, data above/below the stochastic threshold at the DYS385a/b and DYF387S1 loci, peak heights of called alleles, etc., when making this determination as discussed below:

The following steps are followed to assign the likely number of lineages in the profile:

1. Review the profile as a whole, assessing the level of degradation, presence of low level peaks, noisy or clean baseline and general quality (template) of the profile.
2. Identify likely stutter peaks by reference to the YFP allele-specific stutter ratio (SR) expectations for each allele at each locus using DNA QR-345 (see also plots of SR as needed).
3. Find the locus with the highest number of unambiguous allelic peaks, A, keeping in mind that DYS385 and DYF387S1 routinely have two results. A gives the initial assessment of the likely number of contributors to the profile.
4. Review peak height balance at loci DYS385 and DYF387S1.

The detection of more than one allele per locus (other than the DYS385 and DYF387S1 loci) is generally indicative of a mixed sample. Variation in peak heights between alleles between loci and at DYS385 and DYF387S1 may assist in the interpretation of such results.

Typically, if a maximum of 2 alleles are observed at a locus (except for DYS385 and DYF387S1), the DNA results are consistent with a mixture of two male lineages. Two peak patterns (at a single locus other than DYS385 and DYF387S1) have been observed from single source samples, but they are rare. The analyst should consider the possibility of high stutter peaks, which can complicate interpretation. While counting allele peaks is critical in determining the number of contributors, the analyst must also consider that allele sharing between different haplotype lineages may result in an underestimation of the actual number of contributors. The number of lineages identified reflects the analyst's assessment of the most likely number of different haplotypes required to reasonably explain the observed profile.

A Y-STR mixture simulation tool has been validated by the Division of Scientific Services to assist in the determination of the number of haplotypes that are present in Yfiler Plus profiles. This tool may be used by analysts to demonstrate the expected amount of allele sharing at each locus per contributor number in the absence of dropout.

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Note 1: Amplification results from low-template quantities (particularly less than 100 pg) may exhibit increased stutter and peak variance. Based on the YFP/3500xl validation data, it was noted that profiles from low-template contributors may exhibit multiple high stutter peaks that can be considerably above the standard stutter thresholds (see low-template stutter thresholds). A similar impact may occur with respect to peak imbalance at DYS385 and DYF387S1. Therefore, when these low-template peaks pertaining to stutter or peak imbalance are used to infer an extra lineage, there should be data elsewhere in the profile to support this.

Note 2: The low-template stutter thresholds are not included in DNA QR-345 and the YFP panel. For each stutter type being evaluated by CT DSS, the "low" data thresholds are included in DNA SOP-41 to aid in determining the number of contributors and to highlight the increased expected stutter variance associated with low-template single lineage profiles and/or profiles with low-template contributors.

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40.11 **Qualitative Assessment of YFP Profiles—Comparisons to Knowns:**

40.11.1 Single source haplotypes: If 1 peak was called ≥ AT (except at DYS385 and DYF387S1) with nothing else detected ≥ AT at any other locus, and "Known A" has that allele, the results would be consistent with "Known A" being the source. One would consider the issue of "heterozygous" allelic peak imbalance at DYS385 and DYF387S1. Therefore, with good peak balance: the results would be consistent with "Known A" being the source of the DNA profile for item #xx. Significant peak imbalance (> 50%) at DYS385 and/or DYF387S1, may be indicative of a mixture. Note: all YFP results are utilized for comparison purposes. However, for most circumstances, only the Yfiler loci are used for statistics if the comparison is "inclusion/CBE".

40.11.1.1 For single source partial profiles with peaks ≥ AT, report a CBE interpretation when partial results are detected at DYS385 and/or DYF387S1, as long as the observed alleles are consistent with the known source.

Note: in situations where no loci have more than 1 peak ≥ AT (except 2 peaks at DYS385) [e.g. such as if there is significant peak imbalance (> 50%)], it is important to consider if the profile could be a mixture (partial composite of 2 or more individuals).

40.11.2 **Interpretation of DNA mixtures**

40.11.2.1 The results at all YFP loci are utilized for comparison purposes. However, for most circumstances, only the Yfiler loci are used for statistics if the comparison is "inclusion/CBE". These general approaches do not account for all possible mixture scenarios and no two mixtures are exactly the same.

The analyst considers the quantity of DNA amplified, the peak heights of the alleles, the extent of degradation/inhibition, the impact of stutter, potential carryover between fractions in a differential extraction, and if a known profile compared to the mixture can be objectively determined to be a contributor (e.g. intimate samples).

40.11.2.2 Factors taken into consideration when interpreting mixtures:

40.11.2.2.1 If one person can be objectively determined to be included in the mixture (e.g. consensual partner), the mixture is evaluated by a consideration of what loci have DNA profile results (≥ AT) that could not be from the known source.

40.11.2.2.2 The total number of dropouts (overall and at each locus) that would be required to still be consistent with a CBE conclusion. While allele dropout (including peaks below the stochastic threshold at DYS385 and DYF387S1), can occur at any locus, extensive validation and case experience has shown that smaller amplicons amplify more efficiently and are less affected by degradation than are larger amplicons. Therefore, the results at the smaller amplicons can be particularly diagnostic.

40.11.2.2.3 To what degree would a putative low-level contributor coincidently be included by a high-level contributor due to overlap.

40.11.2.2.4 If the analyst and reviewers feel that the above rules do not adequately apply to a specific mixture comparison, consult the TL.

40.11.3 YFP profiles will be searched against the Staff Index and documented on DNA QR-20 Staff Search Worksheet.

40.12 General Report Conclusions for Y-STR Results:

40.12.1 YFP report templates are described in DNA SOP-6. Additionally, see below examples of general statements for reports.

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40.12.2 Elimination: used when the known sample is not the source of, or a contributor to, the DNA profile detected from item xx. The known sample has alleles that are not detected in the evidentiary sample and the lack of detection is not reasonably explainable by degradation/inhibition/allele dropout (stochastic effects) within the evidentiary sample.

40.12.3 Inconclusive: No conclusion can be drawn from the comparison between the known sample and the evidentiary sample due to uncertainty. There are two reasons for inconclusive results; limited data detected, and the mixture being too complex. See 40.12.3.1 and 40.12.3.2 below:

40.12.3.1 Based on limited data: This statement is made when the data does not provide sufficient support for any conclusion regarding a comparison of a mixture to a particular known.

40.12.3.2 Mixture too complex: This statement is made for mixtures of 4 or more male lineages where the known is not eliminated.

40.12.4 Cannot be eliminated (CBE) as the source of, or a contributor to a DNA profile: This conclusion is used when there is a strong correlation between the known sample and the evidentiary sample, but some of the alleles present in the known sample are not detected in the evidentiary sample above the analytical threshold. In this instance, dropout is a reasonable explanation taking into consideration peak height data, overall quantity of template DNA, information regarding the extent of degradation, inhibition, stochastic effects, and masking by stutter or contributor ratio.

40.12.5 Inclusion: All alleles consistent with the known sample are detected (≥ AT) in the evidentiary profile at all loci where results are generated.

40.13 YFP Statistics: (see 40.14 YFP Workflow and Qualification of Loci for Statistics)

40.13.1 For single source YFP profiles, statistics are calculated, for most circumstances, at all Yfiler loci where DNA results are obtained ≥ the analytical threshold. For DYS385, if both alleles are detected, the locus is also used for statistical purposes. If only one allele is detected at DYS385, that locus can be used for statistical purposes, given that the allele is above our stochastic threshold and that the known in question is a homozygote. The YFP random match probability is calculated using the counting method. A haplotype frequency is generated by counting the number of times a haplotype has been observed in the population database divided by the total number of haplotypes in the Database. Haplotype frequencies are given an upper bound 95% confidence interval (See DNA SOP-6).

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The Y-Chromosome STR Haplotype Reference Database (YHRD) Y-STR population database (available at https://yhrd.org) is used.

40.13.1.1 On the YHRD website homepage, click "Search the Database" on the upper left of the site. From there, click on "Manually enter the haplotype/haplotypes to search for". "Yfiler" is the default for the order the loci are listed. Change the order to "Yfiler Plus". Enter the YFP profile at all loci, with a comma separating the two alleles (even if a homozygote) at DYS385 and DYF387S1. (Note only enter DYS385 if you will be using it for statistics.) After entering the YFP profile, click on the Yfiler button to search just the Yfiler loci portion of the profile. Next, click "Search". When search is complete, click "Add feature to this report" and select "National Database (with Subpopulations, current SWGDAM-compliant version). Click the "X" to close the "worldwide" results before printing page for the case jacket.

Note: for low-level profiles, especially those that have results at YFP loci that do not overlap with Yfiler, the YFP loci may be searched. Report the more discriminating statistic.

40.13.1.2 An exact upper bound 95% confidence interval (Clopper-Pearson method) is calculated from the results of a database search in order to estimate the population frequency of a haplotype using the population (United States data set: African American, Caucasian, or Hispanic) with the most common haplotype frequency. The upper bound estimate is dependent on the size of the database, and these estimates may change as the database size changes.

40.13.1.3 The formula used to calculate the 95% upper bound frequency estimate is:

$$\sum_{k=0}^{x}\binom{n}{k}p_0^k(1-p_0)^{n-k}=0.05$$

https://strbase.nist.gov/pub_pres/SWGDAM-Jan2012-YSTRs.pdf

Clopper, C. and Pearson, E. *The use of confidence or fiducial limits illustrated in the case of binomial,* Biometrika (1934) 26:404-413.

40.13.2 Y-STR haplotypes are inherited independently from autosomal loci. Therefore, Y haplotype (and cumulative haplotype) frequencies may be combined with autosomal STR RMPs or CPIs using the product rule to generate an overall profile frequency estimate. (applies to outsourced cases only with no STRmix analysis)

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40.13.3 Y-STR Mixtures: (see 40.14 YFP Workflow and Qualification of Loci for Statistics)

40.13.3.1 The expected frequency of haplotypes that could contribute to a YFP mixture is calculated using the Combined Counting Method (CCM). A cumulative haplotype frequency is generated by counting how many times each of the possible haplotypes are observed in the population Database, summing them and dividing by the total number of haplotypes in the database (i.e. cumulative haplotype frequency = x/N, where x is the number of potentially contributing haplotypes observed in a population and N is the total number of haplotypes searched in that population). An exact upper bound 95% confidence interval is then applied using the population (African American, Caucasian, or Hispanic) with the most common CCM frequency. Note: The frequency and confidence interval for a mixture profile are calculated using the software tool Y-Mix, which contains the U.S. portion of the YHRD database.

The cumulative haplotype frequency (with an upper bound 95% confidence interval) is calculated for samples with an additional allele at any locus, i.e., 2 alleles at DYS393, DYS19, DYS389II, DYS390, DYS391, or 3 alleles at DYS385.

40.13.3.2 The file for the Y-Mix software tool is located on the S: Drive. The loci in the Excel folder are in alphanumeric order. Enter the mixture profile under the appropriate YF (YFP when necessary) loci, with one row per allele. (Note: for each locus with data entered, add a "0" along with the alleles detected if a null has been seen in the database at that locus). Defaults (Limit database to samples with all the loci entered above?: Yes, Treat this profile as a single source sample?: No, Desired UCI: 95%, Use (x+1)/(N+1)?: No) are to be unchanged. Click "compare the profile to the database" when profile entry is complete. Print page when results are obtained.

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40.14 **YFP Workflow and Qualification of Loci for Statistics:**

Note: If it so happens that YFP loci will be used for statistics, the same guidelines for YF loci will follow (i.e. DYF387S1 will follow the guidelines set forth for DYS385).

Note: Loci used for statistical purposes will be noted in the allele table of the DNA report via footnote designation.

40.14.1 Evaluate data at all YFP loci (≥ AT) to determine if profile is a single haplotype or a mixture (see 40.10).

40.14.2 Qualify results for statistics at DYS385 using a ST of ≥ 500 or 700 RFU (based on injection time) and at other loci as discussed below. Statistics are calculated as described in 40.13 for 1-3 lineage profiles.

40.14.3 Compare known(s) to evidentiary profiles as in 40.11 and 40.12.

40.14.4 Single Haplotype Profile:

40.14.4.1 2 alleles detected at DYS385: use data (above & below ST) at DYS385, and other Yfiler loci where included.

40.14.4.2 1 allele detected at DYS385: (A) Qualify locus if allele is above ST and use data for statistics at other loci where included. (B) Disqualify locus for statistics if allele is below ST and use data at remaining YF loci where included. Note: the known would be eliminated as the source if the known does not have the allele found in the evidence profile.

40.14.5 Mixture of 2 Haplotypes (CCM): Evaluate results at all loci as described in 40.14.1-3.

40.14.5.1 Mixture evident at DYS385 with < 4 alleles and all alleles > ST at DYS385: qualify locus for stats and use data for statistics at other YF loci where the mixture is detected.

40.14.5.2 Mixture evident at DYS385 with < 4 alleles and some/all allele(s) below ST at DYS385: Disqualify locus for stats and use data at other loci where the mixture is detected. Note: the known (2 alleles at DYS385) would be eliminated as a contributor if the known does not have at least 1 allele found in the evidence profile. If the known has only l allele at DYS385, the known may not be eliminated depending on the results at other loci. Note that DYF387S1 is utilized in the same manner as DYS385 for qualitative comparison purposes, but only DYS385 is considered for statistics.

40.14.5.3 Mixture with 4 alleles at DYS385 and allele(s) above or below ST at DYS385: qualify locus for statistics and use data for statistics at other YF loci where the mixture is detected.

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40.14.5.4 Mixture not evident at DYS385, e.g., 2 alleles detected at DYS385 and no significant peak imbalance at DYS385 (where the minor allele is > 20% of the major allele): disqualify locus for statistics. Use data for statistics at other YF loci where the mixture is detected.

40.14.6 Mixture of 3 Haplotypes (CCM): Evaluate results at all YFP loci as described in 40.14.1-3.

40.14.6.1 Mixture with 2-4 alleles at DYS385 and all alleles > ST at DYS385: disqualify locus for stats and use data for statistics at other YFP loci where all 3 contributors are detected.

40.14.6.2 Mixture with 2-4 alleles at DYS385 and some/all allele(s) below ST at DYS385: disqualify locus for stats and use data at other YF loci where all 3 contributors are detected.

40.14.6.3 Mixture with 5 alleles at DYS385 and all alleles > ST at DYS385: qualify locus for stats and use data for statistics at other YF loci where all 3 contributors are detected.

40.14.6.4 Mixture with 5 alleles at DYS385 and some/all alleles below ST at DYS385: disqualify locus for stats and use data for statistics at other YFP loci where all 3 contributors are detected.

40.14.6.5 Mixture with 6 alleles at DYS385 and allele(s) above or below ST at DYS385: qualify locus for statistics and use data for statistics at other YF loci where all 3 contributors are detected.

40.14.6.6 Mixture not evident at DYS385: disqualify locus for statistics. Use data for statistics at other YF loci where all 3 contributors are detected.

40.15 Deconvolution of Major in 2 Male Lineage Mixtures

Note: All loci able to be deconvoluted, regardless of being a YF or YFP locus, will be deconvoluted as the major profile. The deconvolution of a major is done independently to determining which loci will be used for statistical purposes.

40.15.1 A major contributor to mixtures of 2 male lineages may be deconvoluted in lieu of the Combined Counting method (CCM) discussed in 40.14.5 with TL/DD approval as follows:

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40.15.2 Deconvolution of mixtures at all YFP loci except DYS385 and DYF387S1: A major contributor may be deconvoluted where the height of the major allele is at least 5X greater than the minor allele (minor is ≤ 20% of major). Example: if the minor allele is 100 RFU, the major allele must be ≥ 500 RFU.

40.15.3 Where only 1 allele is detected at any locus except DYS385 and DYF387S1, the "major contributor allele" must be ≥ 500 or 700 RFU, depending on the injection time (see 40.9.6.1). If not, the locus is not eligible for deconvolution.

40.15.4 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 1 allele is present, the major contributor may be deduced if the peak height is ≥ the stochastic threshold.

40.15.5 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 2 alleles are present, the "heterozygous" major contributor may be deduced if the peaks are ≥ the stochastic threshold and "heterozygous" peak balance is at least 50%.

40.15.6 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 2 alleles are present, ("homozygous" major with significant peak imbalance), the major contributor may be deduced if the major peak is ≥ the stochastic threshold and the mixture ratio at DYS385 or DYF387S1 is at least 5:1.

40.15.7 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 3 alleles are present, ("heterozygous" major): the major contributor may be deduced if both peaks are ≥ the stochastic threshold, the mixture ratio of the lowest major allele to the minor allele is at least 5:1, and "heterozygous" peak balance is at least 50%.

40.15.8 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 3 alleles are present, ("homozygous" major): the major contributor may be deduced if the major allele is ≥ the stochastic threshold and the mixture ratio of the major allele to the highest minor allele is at least 5:1.

40.15.9 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 4 alleles are present,: the major contributor may be deduced if the two highest peaks are ≥ the stochastic threshold, the mixture ratio is at least 5:1 (lowest major allele must be at least 5X greater than the highest minor allele), and "heterozygous" peak balance is at least 50%.

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40.15.10 Once deconvolution is completed, the known(s) are compared to the profile of the deconvoluted major contributor (see 40.11 & 40.12).

40.15.11 If an individual is eliminated as a potential source of the major contributor but cannot be eliminated from the overall mixture (i.e. a potential minor contributor), the CCM statistic is calculated as discussed in 40.14.5.

40.16 Deconvolution of Major in 3 Male Lineage Mixtures

Note: All loci able to be deconvoluted, regardless of being a YF or YFP locus, will be deconvoluted as the major profile. The deconvolution of a major is done independently to determining which loci will be used for statistical purposes.

40.16.1 A major contributor to mixtures of 3 male lineages may be deconvoluted in lieu of the combined counting method (CCM) discussed in 40.14.5 with TL/DD approval as follows:

40.16.2 Deconvolution of mixtures at all loci except DYS385 and DYF387S1: A major contributor may be deconvoluted where the height of the major allele is at least 5X greater than the highest minor allele (minor is ≤ 20% of major).

40.16.3 Where only 1 allele is detected at any locus except DYS385 or DYF387S1, the "major contributor allele" must be ≥ 500 or 700 RFU depending on the injection time (see 40.9.6.1). If not, the locus is not eligible for deconvolution.

40.16.4 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 1 allele is present, the major contributor may be deduced if the peak height is ≥ the stochastic threshold and the mixture ratio at all loci where the mixture is detected is at least 5:1.

40.16.5 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 2 alleles are present, the "heterozygous" major contributor may be deduced if the peaks are ≥ the stochastic threshold, the mixture ratio at all loci where the mixture is detected is at least 5:1, and "heterozygous" peak balance is at least 50%.

40.16.6 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 2 alleles are present ("homozygous" major with significant peak imbalance), the major contributor may be deduced if the major peak is ≥ the stochastic threshold and the mixture ratio at DYS385 and DYF387S1 is at least 5:1.

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40.16.7 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 3-6 alleles are present ("heterozygous" major): the major contributor may be deduced if both peaks are ≥ the stochastic threshold, the mixture ratio of the lowest major allele to the highest minor allele is at least 5:1, and "heterozygous" peak balance is at least 50%.

40.16.8 To qualify the results at DYS385 and DYF387S1 for deconvolution: where 3-5 alleles are present ("homozygous" major): the major contributor may be deduced if the major allele is ≥ the stochastic threshold and the mixture ratio of the major allele to the highest minor allele is at least 5:1.

40.16.9 Once deconvolution is completed, the known(s) are compared to the profile of the deconvoluted major contributor (see 40.11 & 40.12).

40.16.10 If an individual is eliminated as a potential source of the major contributor but cannot be eliminated from the overall mixture (i.e. a potential minor contributor), the CCM statistic is calculated as discussed in 40.14.5.

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Making the dilution series of the Human genomic DNA standard

A dilution series is made (as needed) using the Quantifiler THP DNA Standard (100 ng/µl) and the Quantifiler THP DNA Dilution Buffer supplied with the kit. The standard dilutions used for amplification/quantitation are 50 ng/µl, 5 ng/µl, 0.5 ng/µl, 0.05 ng/µl, and 0.005 ng/µl.

1. Remove the 100 ng/µl Quantifiler THP DNA Standard from the +4°C refrigerator, vortex for 20-30 seconds, and then **very** briefly pulse-spin down the contents of the tube.

2. The 50 ng/µl standard is made by diluting the 100 ng/µl stock with Quantifiler THP DNA Dilution Buffer. Mix 100 µl of the stock with 100 µl of DNA Dilution Buffer. Vortex the mixture for 10-15 seconds, then **very** briefly spin down.

3. Then mix 20 µl of the 50 ng/µl standard with 180 µl of DNA Dilution Buffer to make the 5 ng/µl standard. Vortex 10-15 seconds, then spin down.

4. Repeat step 3 for each subsequent dilution of the series substituting for the 50 ng/µl standard with the current dilution standard to make the subsequent dilution standard (i.e. use the 5 ng/µl to make the 0.5 ng/µl, the 0.5 ng/µl to make the 0.05 ng/µl, etc.).

5. The standard dilution series is stored at 2-8°C. The 100 ng/µl stock is stored with the kit at 2-8°C. The standard dilutions were stable for at least 6 weeks during the in-house validation. The expiration date on the standard dilutions will be 1 month from the preparation date. The slope, Y-intercept, and R^2 values of the standard curve of each run can be monitored for signs of degradation. A decrease in slope and/or increase in Y-intercept CT outside of acceptable ranges (see page 5 of this work instruction) could indicate degradation of the standard. Remake standard dilution, if appropriate. Document all QC testing using DNA QR-33.

Setting up the amplification reactions and running them on the 7500 instrument

1. Use DNA QR-24 Quantifiler Trio DNA Worksheet to locate the placement of samples in the 96-well block of the 7500 instrument and calculate the master mix volumes that will be needed based on the number of reactions. Fill in the sheet as necessary and print.

2. Any sample less than 250 ng/5 µl (50 ng/µl) does not need to be diluted before amplification with the Quantification kits. If a sample is suspected of being greater than 50 ng/µl, a dilution of the sample can be used for quantitation (include dilution factor in sample name or note on worksheet). Samples greater than approximately 5 ng/µl can slightly inhibit the Internal Positive Control (IPC) amplification.

3. In the PCR machine room, turn on the computer for the 7500 real-time instrument. Enter the appropriate user name and password at the logon prompt and wait till the computer has completely booted up before pressing the switch to turn on the 7500 instrument.

4. After the 7500 instrument finishes its start-up sequence, double click on the '**HID Real-Time PCR Analysis Software**' icon on the desktop to open the software. Username is GUEST. Click ok.

5. On the initial software window, click the square gray button labeled 'Quantifiler Trio' (upper left button of the 7 square buttons). If you did not use DNA-QR-24 for plate/sample setup and you are creating your plate/sample setup manually skip step 6 (immediately below) and go to the next section of this work instruction titled 'Manually creating a plate setup...', otherwise continue onto step 6.

6. Select the 'File' drop down menu and select 'Import', then browse for the plate setup file you created by using the Quantifiler Trio worksheet macro. After importing your plate setup file, type in your run name in the 'Experiment Name:' field in the Setup\Experiment Properties window. Select 'Save' from the menu bar, check that the filename is as you intended, and then click 'Save' button. The file will be saved to D:\experiments or D:\Applied Biosystems\7500\experiments depending on which 7500 instrument you are using. Alternatively, the plate setup can be done manually using the 'HID Real-Time PCR Analysis Software' (see the next section of these work instructions).

7. Return to the amp set up room and remove the Quantifiler THP PCR Reaction Mix and Quantifiler Trio Primer Mix from the Trio kit box stored in the 4°C refrigerator (amp room). Note: Minimize exposure of the Primer and Reaction Mixes to light. Mix the Reaction and Primer Mixes by vortexing the tube briefly, then briefly spin down. Make a master mix of Reaction Mix (10 ul/reaction) and Primer Mix (8 ul/reaction). It may be necessary to make additional reactions. In addition to your samples, you will need 5 reactions for the standard dilution series and 1 reaction for a No Template/Negative Control (NTC/NEG). Vortex the master mix briefly and then briefly spin down.

8. Place the 96-well Support Base (SUP-Base, for 8-tube strips tubes) or Splash Free 96-well Base (SF-Base, for optical plates) in the hood for use as a tube/plate rack. Remove the appropriate number of optical 8-tube strips OR 96-well optical plate from their sealed plastic bag packaging and place them in the appropriate SUP- or SF-Base. If using 8-tube strips, remove a packet of optical 8-cap strips from their sealed plastic bag packaging and place them in the hood, **careful not to place them on a surface that has been exposed to bleach or other contaminants that could be transferred to the 7500 instrument block during amplification**.

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Suggestion: Using the 1.5 ml tube rack or pipette tip box in the amp hood, prop up the SF-Base at an angle towards you so you can see better into the tubes or wells of the plate for pipetting. Make sure the SF-Base is supported on both sides by the tube rack so there is no contact between the bottoms of the strip tubes or plate with the tube rack.

9. Pipet 18 µl of the master mix into each tube or well being used.

10. Pipet 2 µl of each standard or sample into the appropriate tube or well. For the NTC/NEG, use 2 ul of Quantifiler DNA THP Dilution Buffer or Milli-Q purified water. Casework reagent blanks shall not be used as the Quant NTC/NEG control since they could be contaminated during extraction, but they may be quantitated as a sample.

11. If using strip tubes, label each cap strip on the **underside** of the tab at one end of the strip (**doing it this way prevents transfer of marker ink to the heated lid or block of the 7500 instrument**). Then, place a cap strip on each tube strip by gently pressing on the cap at each end of the strip. Use the ABI tool designed for pressing caps onto strip tubes to get a good seal. Avoid using gloved fingers to press the caps onto the tubes as transfer of any chemical or liquid could obscure the optically clear caps.

If you are using an optical plate, remove a piece of optically clear tape from the box, peel off the backing from the sticky side, and place the tape (sticky side down) onto the top of the plate covering all of the wells. Use the ABI tool designed to seal the tape to the plate by pressing down and dragging it across the top of the tape from side to side and top to bottom, etc. Remove the excess tape from both sides.

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12. Using two SF-Bases, balance your strip tubes or plate (there is a balance plate next to the spinner) in the 96-well plate spinner in the amp setup room and spin down your samples for 1 minute at 3700 rpm to remove bubbles produced during pipetting and to bring each sample to the bottom of the tube or well. Transfer the plate or strips, **leaving behind the base in the amp hood**, to the 7500 instrument room. **IMPORTANT! The bases in the thermal cycler room should not enter the amp setup room to prevent contamination of the amp setup room with amp product**.

13. Open the 7500 instrument tray by pressing on the right side of the tray. Make sure the appropriate tray holder is in the instrument tray, there is one for optical strip tubes and one for 96-well plates. Each tray holder is labeled as to which it is designed for. 96-well plates fit into the tray with well A1 in the upper left. Place tubes in the tray holder in the positions defined by your plate set up file. It is best if the tubes are positioned in a symmetrical pattern to prevent the heated lid (which can tilt on a cam system) from tilting to one side or the other across the tube strips.

14. In the software, under the 'Experiment Menu' in the upper left, click on the 'Run' tab. The 'Instrument Status' should read 'Connected' in the upper right. Click 'START RUN' in the upper left. The run will take approximately 1 hour to complete.

15. When the run is done, save the file and close the software. Transfer the run file from the D: Drive to U:\7500 Quant Data\your folder. Turn off the 7500 instrument and laptop computer.

Manually creating a plate setup document for your samples using the 7500 instrument laptop computer

1. Under 'Setup' → 'Experiment Properties', type in a run name in the 'Experiment Name' field. Select 'Save' from the menu bar, check that the filename is as you intended, and then click the 'Save' button. The file will be saved to D:\experiments or D:\Applied Biosystems\7500\experiments depending on which 7500 instrument you are using.

2. Under 'Setup' select 'Plate Setup'. In the 'Define Targets and Samples' window click 'Add New Sample'. Modify the name of the newly made sample appropriately. Repeat this step until all samples are added to the experiment.

3. Select the 'Assign Targets and Samples' tab or click on the 'Assign Targets and Samples' button under the 'Define Samples' window.

4. In 'View Plate Layout', select a well or wells to add a Trio Standard to. After selecting the appropriate well(s), scroll in the 'Assign Sample(s) to the Selected Wells' window. Find the standard in the list that you want to assign to the selected well(s) and left click the mouse on the box to the left of the sample name. Only one set of standards is needed for Quantifiler Trio run. Repeat the above for the NEG/NTC and casework samples. Select more than one well for sample assignment if running a sample in replicate. Once all the samples' well positions are defined, click the 'Save' button. Return to step 7 of the previous section of this work instruction.

Analyzing the run data

1. Log on to the stand-alone desktop computer using the 'dnauser' user logon with the appropriate password. Double click on the 'HID Real-Time PCR Analysis Software' icon on the desktop to open the software. Username is GUEST. Click ok. Click 'continue without connection to instrument'.

2. Click 'File' → 'Open' or select 'Open' from the menu bar, then navigate to your run file located on the U:\ drive and double-click on the file or click 'Open'. Verify 'Experiment Name:' 'your file name' and 'Kit Name:' 'Quantifiler Trio'.

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3. Click 'Analysis Settings' in the upper right corner of the software window pane and select the 'CT Settings' tab. Verify that the default settings for 'Baseline Start' and 'Baseline End' are '3' and '15' for all four targets, respectively. Verify that the 'Threshold' is '0.1' for 'T.IPC' and '0.2' for the other three targets. If no changes were needed, close the 'Analysis Settings' by clicking cancel.

4. Click the green 'Analyze' button in the upper right. Under the 'Analysis' pane on the far left, click on 'Standard Curve'. In the plate map pane on the right, select just the 5 wells containing the standards. In the 'Standard Curve' pane on the left, verify that the slopes and Y-intercepts for the three targets are in the acceptable range (see below). Verify that the R^2 values are greater than '0.98'. Based on internal validation run data, the standard curve slopes for the targets Trio Y, Large Autosomal, and Small Autosomal should be in the range -3.0 to -3.6, -3.1 to -3.6, and -3.0 to -3.5, respectively. Based on internal validation run data, the Y-intercept C_T values for the targets Trio are as follows:

7500 Instrument Number	Large Autosomal Y-Intercept	Small Autosomal Y-Intercept	Y Chromosome Y-Intercept
7500-1	24.1-25.6	25.9-27.3	25.1 – 26.7
7500-2	24.4-26.0	26.8-28.2	26.0-27.6
7500-3	24.2-25.8	26.2-27.8	25.4-27.0

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If the Y-intercept or slope values fall outside these ranges, consult with the Technical Leader for approval. Individual standards that appear to deviate from the curve significantly may be removed from the standard curve to attain improved values for the slope, Y-intercept, and R^2. To remove a standard from the curve, select the appropriate well on the plate map, right click, and select 'Omit' → 'Well'. You can remove up to 2 standards. Note what was removed on the Quantifiler Trio DNA Quantitation Worksheet (DNA-QR-24). Click the 'Analyze' button.

5. Print the three standard curves with their slopes, Y-intercepts, and R^2 values by doing the following. Select 'Print Report' from the menu bar. Uncheck 'Experiment Summary', 'Plate Layout', 'Results Table (By Well)' and 'QC Summary'. Click 'Print Report'. Change 'Print Range' to 'Pages from 1 to 3' and click 'ok'.

6. Export the results for printing with the macro by doing the following. Select all the wells of the plate map by clicking the upper left hand corner of the plate map. In the menu bar near the top, click on the 'Export' button. Make sure only 'Results' is checked in (1) and 'One File' is selected for (2). In (3), verify 'File Type' is '*.xls'. Browse to U:\7500 Quant Data\Your Folder. Click 'Open'. Click 'Start Export'. The default setting is to export all columns, do not alter this setting. Click 'Close Export Tool'. Your file will be saved to your folder as run

name_data. Click 'Close' in the menu bar to close your file. Choose not to save when asked as it will take several minutes when saving over the network. Close the software.

7. Write down any comments you feel necessary regarding your quantitation analysis on the DNA QR-24 Quantifiler Trio DNA Worksheet (e.g., the removal of a sample from the standard curve).

8. On your office computer open 'DNA-QR-22c. Click on the green 'Import Quant Trio Data' button. Browse to U:\7500 Quant Data\your folder\ and select the excel file your-run-name_data. Select 'Open' or double click on the name to import. Click '**Yes**' or 'No' to sort the samples by DNA concentration. Click 'Yes' to print a copy of the Trio results report or 'No' if you printed it previously. For each sample, the Trio results report will contain the well, sample type, small autosomal target quantity (Sm Auto), large autosomal target quantity (Lg Auto), the average autosomal quantity (Avg Auto), Y target quantity (Y), internal positive control crossing threshold (IPC Ct), degradation index if applicable (DI=Sm Auto/Lg Auto), and the male to female ratio (M:F) if applicable (M:F=1:[Sm Auto – Y]/Y). The report will display flagged values for the IPC Ct and degradation index by shading them in yellow for analyst review. The IPC Ct will be flagged for the standards if they are outside of the range 25.5 to 30.5 and for samples if their IPC Ct is >2 Ct above the average IPC Ct of the standards. The DI will be flagged if its value is ≥3. See sections 9 and 10 of this work instruction for evaluation of these values. The report will also display if the slopes, Y-intercepts, and R2 values are within the optimal ranges. Consult your lead, the Technical Leader, and/or the 'Quantifiler HP and Trio DNA Quantification Kits User Guide' if necessary.

RETIRED

9. IPC Cts that are flagged may indicate an inhibitor present in the sample. However, for our internal validation inhibitor study, there was only an effect on the genotyping amplification when the IPC Ct was undetermined. A large quantity of DNA (>5 ng/ul) can also cause the IPC Ct to increase. In addition, an inhibitor may cause the Degradation Index to be elevated as the inhibitor will typically have a greater effect on the Large Autosomal Target quantity (LA) relative to the Small Autosomal (SA) or Y Target quantities. Multiple quantities of DNA from one sample may be amplified when Taq inhibitors are present in the sample. Degradation Indexes ≥3 with a normal IPC Ct may indicate the sample is degraded.

10. For casework genomic DNA samples that exhibit degradation, different approaches should be used to determine the appropriate volume of that sample to amplify based on the Quantifiler Trio quantitation results. To reiterate, be cognizant that inhibitors will also cause a reduction in the LA creating a DI > 1, so make sure the IPC Ct does not indicate possible amplification inhibition.

A) When the SA result is such that the maximum volume of the degraded sample genomic DNA would not reach the 1 ng target for amplification, the maximum volume should be amplified.

B) If the DI is <3, the SA should be used to calculate the sample amplification volume.

Otherwise, calculate the volume needed to amplify the appropriate amount of DNA to amplify (depending on STR kit being used) for the SA, the average of the small and large autosomal target quantities (AA), and for the LA. The amount based on the SA will be the smallest volume of genomic DNA to amplify, the amount based on the LA will be the largest volume of genomic DNA to amplify, and the amount based on the AA will be in between. For Y-STR amplifications, the Y target should be used to calculate the volume needed to amplify.

C) For samples with a DI of ~3 to 7.5, using the LA to determine the volume needed for the appropriate amount of DNA to amplify (depending on STR kit being used) should give optimal amplification results.

D) For samples with DIs >~7.5 to <30, a volume in between that calculated using the LA and the AA could be used (e.g. calculate the volume needed based on the LA and the volume needed based on the AA, then add those two volumes and divide by 2).

E) When the DI is >30, use the AA to target the appropriate amount of DNA to amplify (depending on STR kit being used).

F) When the LA, and thus the DI, is undetermined, use the SA to target the appropriate amount of DNA to amplify (depending on STR kit being used).

If necessary, multiple quantities of DNA from one sample may be amplified when the sample shows evidence of degradation (see DNA SOPs 4.3.3 and 30.4.4).

11. If DNA (≥ 1pg/µL) is detected in the Trio negative control or reagent blanks, bring it to the attention of the TL. Depending on the amount detected in the negative control, the Trio results may be interpreted with caution with TL approval. Detection of less than 1pg/µL does not need TL notification.

12. Refer to the 'Quantifiler HP and Trio DNA Quantification Kits User Guide' and 'HID Real-Time PCR Analysis SoftwareUser Guide' if necessary.

13. Based on validation, for property crime cases, sample processing will be stopped when no DNA is detected. Samples stopped at the quantitation stage will be retained and reported appropriately.

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Based on validation, refer to DNA SOP-34 regarding sexual assault cases and the stop at quantitation thresholds established. Samples stopped at the quantitation stage will be retained and reported appropriately.

A notation will be made in the case record that will identify which samples were stopped at quant (DNA QR-48).

In order to monitor analytical quality, if it is determined that all evidentiary sample processing for a given extraction set is terminated the reagent blank control must be either quantified or typed in order for the evidentiary sample processing to be terminated.

RETIRED

BATCH PAPERWORK & ANALYSIS

Analyst

1) Bring all data files into GeneMarker and analyze using GF_CTDSS.
2) Save GeneMarker project to file folder that HID files are in (U:\3130 NUCLEAR DNA CASEWORK & QC\To Be Analyzed).
3) Analysts may utilize QR-342 as follows: export text file to your "Analysis Export File" in your "To Be Analyzed folder". Use exported text file to create "Supplemental GlobalFiler Stutter Filter" worksheet, DNA QR-342, to use as a guide for stutter peaks with n-2 stutter contributing. If the supplemental stutter filter is not utilized (e.g., if the profile is single source or if you feel that the filter is not needed), still print out the QR-342 worksheet and note that the batch had no N-2 stutter concerns.
4) Analyze samples, adding comments for all click-offs. Click-offs should mostly be dye-artifacts, raised baseline, pull-up peaks, and stutter peaks that have n-2 stutter contributing.
5) If a sample needs to be re-injected, or if for another reason you are not reporting out a sample, disable the sample in GeneMarker, noting the reason on QR-325 and in GeneMarker.
6) Re-save GeneMarker file when all click-offs have been made and all appropriate samples have been disabled.
7) Re-export report as a text file to your "Analysis Export File" in your "To Be Analyzed" folder, you can save over the file created in step #3. This text file will be used by the Concordance Checker, Cross-Comparison/Staff Search, Contributor Estimator, Deconvolution workbook, and Allele Table macros.
8) Run concordance checker (DNA QR-37) if positive controls are present, using text file created.
9) Print out e-grams from samples and controls.
10) Repeat steps 1 thru 9 if there are multiple injection sheets.
11) Complete batch cross-comparison.
12) For each sample project, start a new project by bringing in the same data files into GeneMarker, and analyze them using the GF_CTDSS_STRmix template. Save GeneMarker project with identical name as before, but with "STRmix" added to the end.
    - **OR** you can re-analyze your data from the current project by pressing the green play button again. A message will come up asking if you want to re-call the size standard. You want to check the box for "Call Size Again" and click on "Apply to all". Don't forget when you save this file to save it with STRmix added to the end.
13) Disable all samples that you disabled in the previous project.
14) Using your analysis on the e-gram peak tables as a guide, click off all the same peaks you just clicked off with the following exceptions (these exceptions will come up as non-concordances when you run your project comparison, to be noted in the comments section, with the exception of stutter peaks with n-2 stutter contributing, that are below the laboratory stutter threshold):

RETIRED

- If you clicked off a stutter peak because pull-up contributed to it, leave this peak in your STRmix project, provided that the peak is still greater than AT, with pull-up subtracted, and the pull-up is not responsible for the majority of the peak height.
- If you clicked off a peak as high stutter, leave this peak in your STRmix project.
- Same goes if any of these peaks are a combo of different stutters
  - There might be exceptions to this, for example, if the subtraction of pull-up brings the stutter peak below AT, STRmix should not see this peak, and if the pull-up contribution adds an unusually large amount of height to the stutter peak, it might be better for STRmix not to see such a high peak. Ask for assistance if you are unsure.
  - Peaks that you clicked off with n-2 stutter contributing, that are at or below the laboratory's stutter threshold, will remain in the STRmix project. These will not be listed as non-concordances when running the project comparison.

15) Re-save GeneMarker file when all edits have been made and all samples have been disabled.
16) Export report as a text file, same location as file created in step 3 on page 1.
17) Run DNA-QR-301 Project Comparison Tool using the exported text files for your fully analyzed project and your STRmix project.
    - If there are any discrepancies not accounted for in your analysis, you must correct them in the appropriate GeneMarker project. Then re-save the GeneMarker projects AND re-export the text files AND re-run project comparison macro.
    - Keep repeating this until the number of discrepancies can be explained by your analysis.
    - Print out this sheet for the batch paperwork; you only need the final copy in your batch.
18) Create DNA-QR-302 Contributor Estimation Worksheet for each sample with results. If it's a single source sample, or you feel you don't need the macro to assist you in determining number of contributors, you still need this sheet, but add notes to the comments area as to your determination of number of contributors.
    - Minimum number of male contributors will auto-populate on designated line.
19) Initial and date QR-4A (Batch Paperwork Review Worksheet).
20) Give batch to Technical Reviewer.

Technical Reviewer

1) Review all batch paperwork.
2) Open the analyst's fully analyzed allele table project.
3) Review samples, note any differences to the 1st Analyst. Check that:
   a. Correct analysis template is used.
   b. Analyst's assessment of size standards, ladders, controls, and potential contamination are appropriate.
   c. All called peaks appear to be true alleles.
   d. All deletions/edits made are appropriate.
   e. The project comparison QR has appropriate number of non-concordances.

RETIRED

f. You are in agreement with the number of contributor estimation.

4) Initial and date QR-4A.
5) Give batch to Administrative Reviewer.

Administrative Reviewer

1) Review all batch paperwork.
2) Initial and date QR-4A.
3) Give batch back to Analyst.

Analyst

1) Make changes to project(s), if necessary, according to the technical review. Analytical changes will require updates to allele table GeneMarker project & report text file, STRmix GeneMarker project & report text file, number of contributors worksheet, and a re-run of the project comparisons worksheet.
2) Review & make any corrections to batch paperwork and number of contributors worksheets
3) Initial and date QR-4a. Return batch to Technical Reviewer.

Technical Reviewer

1) Review any changes made by 1st analyst.
2) Add batch name to spreadsheet on U: Drive.
3) Initial and date QR-4A.
4) Return batch to Analyst.

Analyst

1) Make photocopies of injection sheets, Quant Trio Report, and halt at quant sheets for appropriate case jackets in batch. Original sample e-grams & original contributor estimator worksheets are just for case jackets, not to be in batch paperwork.
2) Scan batch paperwork (not containing sample e-grams & contributor estimator sheets) & save PDF file to U: Drive.
3) Move HID and project files from “To Be Analyzed” to “Completed” folder on U: Drive.
4) Give batch paperwork to Technical Reviewer to review.

Technical Reviewer

1) Ensure all pages scanned and PDF file on U: Drive is complete and accurate.
2) Ensure HID folders have been moved to U:\3130 NUCLEAR CASEWORK & QC\Completed.
3) Add initials to batch paperwork spreadsheet.

RETIRED

CASEWORK

Analyst

1) Enter all possible profiles into CODIS.
2) Make comparisons of all knowns to all Q's in case.
    a. Does a sample have 5+ contributors?
        i. If so, do any of the knowns CBE to the sample warrant being conditioned? If yes, does that bring it down to 4 unconditioned contributors?
            1. Yes – can interpret.
            2. No – Clear major present – can make comparison to deduced major profile. No comparisons will be made to minor components of profile.
            3. No – No clear major present – No comparisons will be made to profile.
    b. Samples that are clear eliminations are not run through STRmix, "Eliminate by Analyst" in report macro.
    c. Comparisons that are not clear eliminations, are run through STRmix.
        i. Reported out comparison based on LR calculated.
        ii. The "Sample ID" field shall contain both item # and known # (if applicable), so that there is a unique identifier in the footer of all pages when printed.
    d. If reporting out a statistic for a comparison, deconvolute in STRmix with appropriate knowns conditioned to profile.
        i. Report out comparison based on LR calculated.
        ii. If not reporting out a statistic (i.e. intimate sample with no suspect known submitted, single source sample when known expected on sample), no need to run STRmix, enter "assumed" in report workbook. STRmix may be run if necessary to determine if assuming is appropriate.
    e. Does a sample only have one allele at one locus? → inconclusive or elimination by analyst
    f. Are you unable to determine the number of contributors with reasonable scientific certainty?
        i. Clear major present – Can make comparison to deduced major profile. No comparisons will be made to minor components of profile.
        ii. No clear major present – No comparisons will be made to profile.
    g. Is there a problem with the deconvolution discovered Re: 2nd diagnostics that can't be resolved? → inconclusive or elimination by analyst
3) If a sample is deconvoluted through STRmix:
    a. The file will automatically save onto the server, under F:\results.
    b. Review the summary report. Ensure that the input file is correct. The letter "S" has previously been added to the beginning of the sample names of the STRmix input file when the project comparison macro was run.

RETIRED

c. Print out pages 1 thru 3 of the STRmix report, or more if "Per Locus Likelihood Ratios" for the three reported populations groups is on subsequent pages.
d. Scrutinize summary reports for any problems with the deconvolution.
    i. Fill out DNA-QR-303 for the 2nd diagnostics for each deconvolution. Any assumed contributors will be recorded on DNA-QR-303.
e. Move the file in the F:\results folder to your folder in F:\ results.
f. Create a folder in your folder for this case, named with the case number in format DSS-XX-XXXXXX, and move that folder into this newly created case specific folder.

4) Review case jacket, write report.
5) Give case jacket to TR.

Technical Reviewer

1) Review case jacket, comparisons and report as you normally would.
    a. If you disagree with an eliminated comparison, the 1st Analyst will have to run this sample through STRmix.
    b. Review the summary report. Ensure that the input file is correct. The letter "S" has previously been added to the beginning of the sample names of the STRmix input file when the project comparison macro was run.
2) If you need to review more of the summary report than is in the case jacket, you can find it on the STRmix server, F:\Results in the Analyst's folder, in the folder specific to this case.
3) Give case jacket to AR.

Administrative Reviewer

1) Review case jacket and report as you normally would.
2) Give case jacket back to Analyst.

Analyst

1) Make all appropriate corrections to report and case jacket.
2) If the TR disagreed with one of your elimination calls, you must run that sample through STRmix, print out appropriate pages and fill out DNA-QR-303.
    a. The report will have to be re-worked:
        i. If it is still an elimination, it is now "elimination by STRmix", and not "elimination by analyst", report wording will be different.
            1. If it is no longer an elimination, report out result based on LR.
3) Print out final copy of report.
4) If there are STRmix results, move the folder for this case from your folder on the server's F: Drive to F:\results\Completed.
5) Give case jacket to TR for correction check.

RETIRED

Technical Reviewer

1) Ensure all corrections are accurate.
2) If additional deconvolution work needed to be completed, make sure new wording in report is accurate and review all additional paperwork.
3) If there's STRmix data, check to make sure the folder has been moved to F:\results\Completed.
4) Give case jacket to AR for correction check.

Administrative Reviewer

1) Ensure all corrections are accurate.
2) Give case jacket back to analyst.

CODIS

Analyst

1) If there is a CODIS hit for a sample of yours, you will be notified by the CODIS administrator/alternate or their designee.
2) The CODIS hit might warrant the sample to be deconvoluted in STRmix. If a deconvolution of this sample has already been completed, the case jacket can be brought to the CODIS administrator/alternate or their designee.
3) If a deconvolution of this sample has not yet been completed, deconvolute the sample, making sure to condition any knowns that you assumed when writing the report.
    a. Print out page 1 of the STRmix report, fill out DNA-QR-303, and give the case jacket to your TR to review the additional paperwork. You can check, initial, and date next to 3.D. on DNA-QR-4.

Technical Reviewer

1) Review additional paperwork in case jacket.
2) Check, initial and date next to 3.D. on DNA-QR-4.
3) Give the case jacket to the AR

Administrative Reviewer

1) Review additional paperwork in case jacket.
2) Document review on DNA-QR-4.
3) Give case jacket to the CODIS administrator/alternate or their designee.

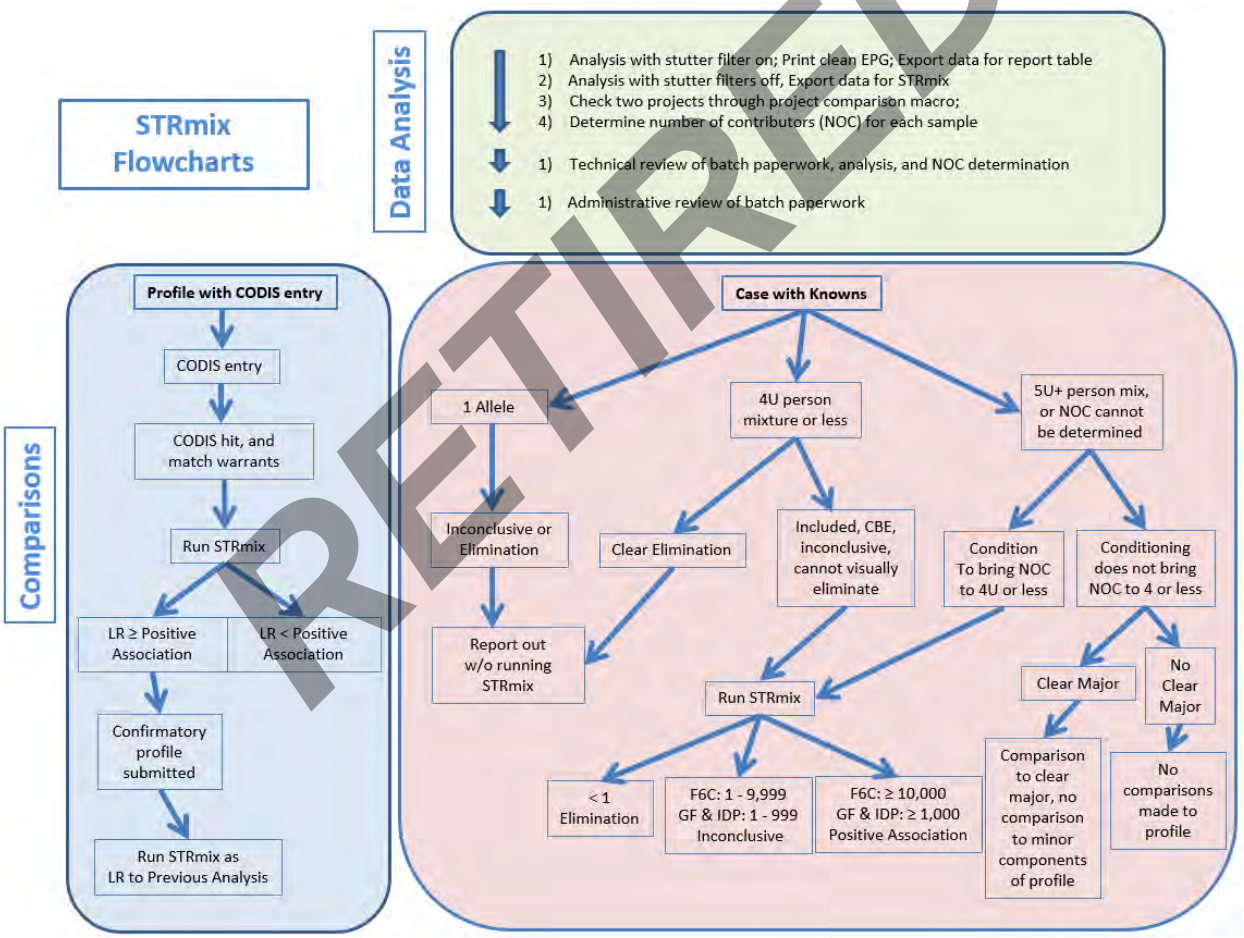
Analyst

1) When you receive your case jacket back for initials, move your STRmix folder to F:\results\completed. Check, initial, and date that you did so on DNA-QR-4.

RETIRED

OTHER

1) If a known comes in later on that warrants being conditioned, all deconvolutions need to be repeated with a new deconvolution, and a new statistic is calculated.
2) If a known comes in later that's not clearly eliminated regarding a sample that has already been deconvoluted, use the "LR to previously analysis" function of STRmix, and do not repeat the deconvolution.
3) Similarly, if you have a Q in the case, and two knowns are CBE to that sample, only deconvolute the sample once. For the 2nd known, use the "LR to previous analysis" function of STRmix.



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One-Time GeneMarker-HID (GM-HID) Setup

User Management

1. Within the GM-HID software on each analyst's computer, at least two Administrator accounts and one Analyst account are created by going to Help→ User Management. Additional accounts may be created if appropriate.
   a. An Administrator account has full privileges. Only designated staff will have Administrator access. Administrator accounts require a password.
   b. An Analyst account has the default set of privileges for this account type, with the addition of access to "Edit – View Preferences".
   c. Users designated as Administrators still need an Analyst account as well.
2. Log in as an Administrator.
3. Within the User Manager, on the "User Manager" tab, enter an Organization (ex. "CT Forensic Lab"). Check the box for "Run User Protection".
4. On the "Settings" tab, check the box for "Record Data Edit History". Click Ok.

Analysis Templates

1. Open the software and click on the folder icon in the upper left of the menu to open data. When analyzing GlobalFiler (GF) data, click on the 'Dyes' button in the lower left and make sure 6 dyes is selected. Click on 'Change Chemistry' and select 'AB'. Channel 1 should read 'Blue, 6-FAM', channel 2 should read 'Green, VIC', channel 3 should read 'Yellow, NED', channel 4 should read 'Red, TAZ', channel 5 should read 'Purple, SID', and channel 6 should read 'Orange, LIZ'. Click 'Ok'. Click on the 'Add' button in the upper right and navigate to the location of the data folder you want to import. Double click on the folder to access the .hid files. Select all or some of the files to import and click 'Open'. The files will be added to the 'Data File List'. The 'Add' button can be clicked again to add more data from other folders. When done adding data, click 'Ok' and the raw data will be added to the project.
2. Log in as an Administrator (if not already).
3. Click on the green arrow button in the toolbar menu to open the 'Run Wizard' window. Click on 'Select an existing template or create one'. Name the template 'GF_CTDSS' or 'GF_CTDSS_STRmix' depending on whether the stutter filters are applied. For known samples amplified by the GlobalFiler Express (GFE) Kit, name the template 'GFE_CTDSS_20%'. Select the panel with the same name. Set the size standard to GS600 and the standard color to 'LIZ'. The GS600 size standard should be edited using the 'Size Template Editor' to disable the following sized peaks of the standard: 20, 40, 514, 520, 540, 560, 580, and 600. After opening the editor and selecting the size standard, change the value in the 'Enabled" column from '1' to '0' for the sizes listed above. Save the changes. The analysis type should be 'HID'. Click on the 'Next' button to display the 'Data Process Options' window.
   a. As a note, the three panels contain the same settings except that the GF_CTDSS_STRmix panel has no thresholds for stutter, and the GFE_CTDSS_20% panel is programmed for diploid results.
4. For 'Raw Data Analysis' select 'Auto Range', 'Smooth', 'Superior', 'Spike Removal', 'Saturation Detection', and 'Saturation Repair'. For 'Size Call' select 'Local Southern'. For 'Allele Call' deselect 'Auto Range' and set the 'Start' to 60 and the 'End' to 502. For 'Peak Detection Threshold', leave the default

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parameters of '50', '32500', and '20' for 'Min Intensity', 'Max Intensity', and 'Percentage >, Global Max', respectively. Click on the 'Next' button to display the 'Additional Settings...' window.

5. Select 'Auto Select Best Ladder' and set 'Allow Match # Variance' to '0', 'Max Average Size Diff' to '0.40' and deselect 'Use Ladder Library'. Select 'Auto Panel Adjustment'. For the 'Positive Control Template' select 'GF'. If it is not part of the positive control list, it can be added using the 'Positive Control Template Editor' under the 'Tools' menu. For 'Allele Evaluation' set both 'Peak Scores' to zero. Leave 'Mixture Evaluation' deselected. If this is the first time you are running this 'Run Wizard' template click on the 'Save' button. Click on the 'Ok' button. The raw data will be analyzed and allele calls will be generated. The 'Data processing...' window remains open until you click 'Ok'.
6. To confirm the analysis thresholds for allele peak detection, go to the 'tools' menu and select 'Panel Editor'. In the upper left window of the editor select each of the three panel templates used for analysis in turn. The '[Ploidy]' number should be set to '0' for the two templates used for evidence and '2' for the template used only for knowns. If not, right click on the panel name and select 'edit' and change the ploidy to the appropriate value and click 'Ok'. To check peak detection thresholds and type of stutter filters used, right click on any marker name of the panel at the top center of the window and select 'Edit Marker'. The minimum 'Homozygote', 'Heterozygote', and both 'Inconclusive' intensities are dye specific and should be set to '40' RFU for Blue, '55' RFU for Green, '35' RFU for Yellow, '50' RFU for Red, and '55' RFU for Purple. The 'Max Heterozygote Imbalance (%)' should be 1% for forensic unknown profiles and 21% for knowns. The 'Min Heterozygote Imbalance (%)' should be 0% for forensic unknown profiles and 20% for knowns. The imbalance settings for knowns effectively creates a 20% marker filter in lieu of stutter filters. If changes needed to be made, select the 'Apply Homo/Hetero Settings to All Markers in this dye' option. For the stutter filter, 'Use Allele-Specific Values (From Panel)' should be selected for the GF_CTDSS and GF_CTDSS_STRmix panels and 'Use Marker-Specific Values' for the GFE_CTDSS_20% panel. If changes needed to be made, select the 'Apply Stutter Settings to All Markers' option. Click 'Ok'. If changes were applied, click the 'Save Changes' icon in the toolbar menu (**not** the 'Save Changes with Signal Info' icon).

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Viewing Preferences

1. Select 'View' from the toolbar menu then select 'Preference...'.
2. Under the 'Start up Settings' tab, select 'Wizard' and 'Show Navigator'. The default network path (C:\) for importing/opening raw data and projects and exporting reports can be modified if desired by checking the appropriate box and selecting the browse button on the right, e.g., U:\3130 NUCLEAR DNA CASEWORK & QC\To Be Analyzed\Analyst Initials.
3. Under the 'Display Settings' tab 'Allele Label', the 'Decimal Precision' should be '1'. Select 'Mark Off-Marker as 'OL'', 'Mark Off-Bin as 'OB'', and 'Set Missed Allele as a Single Line in Ladder'. Under 'Chart settings', select 'Max # of Open Charts and set to 96, Max Chart # in Page' (set to '4'), and 'Max Allele Label Layers' (set to '8'). Under 'Peak Label', select 'Height' and 'Vertical' and set the 'Position' to 'Allele Label'. For known samples, the peak height label may be deselected.
4. Under the 'Forensic' tab, set the Ladder, Positive Control, and Negative Control Identifiers to 'LADDER', 'POS', and 'NEG', respectively. Select 'Show Ladder Samples in Report', Show Control Samples in Report', and 'Mark Deleted/Edited Peaks with Symbols'. When done, click 'Ok'.

Edit Record

1. View any sample in the All Color Browser. Right click on any peak. Select "Edit Comments."
2. Add template comments for common edits. See SOP 1 Appendix 2 for designated abbreviations. Click Ok.
    a. Tip: Make the top comment template one that is not used that frequently. Entering a comment requires the analyst to select it by clicking, and as this comment is selected by default an extra click is needed to select it.
3. Right click on any peak. Select "Show Columns". Edit this list so that the following columns (and no others) are checked: Size, Height, Marker, Allele, and Allele Comments.

Print Templates

1. Select the 'Print Report' icon in the toolbar menu. Click on 'Select an Existing Template or Create One' and name the template.
2. Under the 'Standard' tab, select 'Normal', 'Selected Samples', 'Electropherogram', '6-FAM', 'VIC', 'NED', 'TAZ', 'SID', 'Hide Bins', 'Peak Table', and 'Start on Separate Page'. Under the 'Advanced' tab, select 'Print Report Header', 'Each Page', 'File Name', 'Print Markers', 'Print Alleles', 'Print Edited Peak Only', 'New Page for Each Sample' and 'Implement Y Axis Settings'. Set the 'Chart Height' to 64. Under the 'Page' tab, select 'Portrait' and set all four Margins to '0.0'. Click 'Save'. Click 'Preview'.
3. If the analyst routinely processes known samples, create a separate print template for known egrams. (Other analysts may add this template as well). Use the same settings as above with these exceptions: de-select 'Peak Table' on the 'Standard' tab, de-select 'File Name' on the 'Advanced' tab, and set the Chart Height to 37-40 depending on whether peak heights will be displayed. Ideally known egrams should be a single page; the Chart Height may be adjusted further.
4. Optionally, analysts may create additional print templates. For example, a template for controls could use the same settings as the known template but have 'Peak Table' selected with the option 'Start on Separate Page'.

Report Settings

1. Click on the "Report Settings" icon.
2. Select the following options: 'Forensics', 'Show File Name', 'Vertical', 'Exclude Sample Index', 'Exclude Report Header', 'Split Items', 'Show Allele Name', 'Show Size', 'Show Height', and 'Show Rejected Low Score Alleles'. De-select all other options. Click Ok.

Analysis in GM-HID

Note that all settings selected remain in effect until changed. If any of the above default settings are changed during analysis of a project, be sure to change them back again.

Creating a Project

1. Open GM-HID and log in using the appropriate Analyst (not Administrator) account.
2. Import all of the pertinent data files as in Step 1 under "Analysis Templates".

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3. Select 'File' and then 'Save Project' from the toolbar menu. Save the project to a network folder containing the .hid files analyzed. Type in a name for the project in the format: "Kit-3500#-BatchID-Modifier#-Any Other Details" (ex. "GF-2-MGR050317-2-JES") and navigate to the appropriate location, and click the 'Save' button. Modifier number and other details are optional. For descriptions of all options and tools for GeneMarker, please see the GeneMarker HID User Manual.
4. Click on the green arrow button in the toolbar menu to open the 'Run Wizard' window. Select the template GF_CTDSS, GF_CTDSS_STRmix, or GFE_CTDSS_20% as appropriate to your samples. Click 'Next' on the 'Template Selection' and 'Data Process' screen and 'Ok' on the 'Additional Settings' screen. If this is a re-Analysis of a previous analyzed project, select 'Call Size Again' and click 'Apply to All' when prompted
5. Note that even though allele calls have been generated, the changes to the project have not been saved. Re-save at any time during analysis.
6. Ensure that all required internal lane size standard peaks are called in all samples. Select the 'Size Calibration' icon in the toolbar menu. Select each sample in the list on the left to view the ILS trace for that sample. Verify that these GS600 standard peaks are present (60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, 460, 480, and 500) and the sizing quality score is ≥ 88 (the software flags the sizing quality of the ILS when it is <88). If a sample's internal size standard fails due to a low sizing quality (e.g. pull-up peaks into the size standard), the size standard may be successfully edited to produce a sufficient sizing quality score (see GM-HID User Manual). Alternatively, the sample may be re-injected, re-prepped and re-injected, or re-amplified.
7. Ensure that passing allelic ladders have all allele peaks detected. Ladders are listed in the sample file tree in blue type. Passing ladders will appear in bold blue type. Double-click on the first ladder and select the 'Browse by All Color' icon in the toolbar menu. Verify that the passing ladders have all allele peaks detected. With the 'Set Missed Allele as a Single Line in Ladder' flag enabled (see Step 3 under "Viewing Preferences"), allele labels for missed ladder peaks will be placed one row above allele labels of peaks that were called and there will be a red vertical line for that allele bin. Failed ladders will not be used to assign alleles to profiles.
8. To view and edit electropherograms for the samples, double click the first sample in the sample tree list. Then select the 'Browse by All Color' icon in the toolbar menu. Maximize the egram window. To view electropherograms of other samples, click in the vertical view slider on the far right to toggle up or down one sample at a time.

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<u>Editing a Project</u>

1. View each sample in the 'All Color Browser'. (See SOP 39 for details on data analysis.)
2. Select a peak by clicking on it. De-select by clicking a selected peak while holding "Ctrl", or de-select all peaks in a color at once by double-clicking elsewhere in the color.
    a. Note that once a peak has been selected, it remains selected until performing one of the actions to de-select it or closing the All Color Browser.
3. To delete a peak, select it, right-click and select 'Delete'. Multiple peaks can be deleted at once.

4. For each deleted peak in evidentiary (forensic unknown) profiles, add a comment as to the reason for deletion (for example, the designated abbreviation for the artifact causing it). Right-click the peak and select 'Edit Comments'. A list of Comment Templates appears, in which commonly used comments can be stored and selected. (See SOP 1 Appendix 2 for designated abbreviations.)
5. To confirm a red/yellow flagged peak, right click the peak and select 'Confirm'.
6. To change an allele call, right click the peak and select 'Edit Allele", and adjust the label as appropriate.
7. If it's appropriate to insert an allele, right click the peak and select 'Insert Allele'. Adjust the label if needed.
    a. Zoom in on the peak in order to insert at the precise size where the peak is highest.
8. For casework known and database samples, reasons for routine edits do not need to be recorded. Issues such as change in allele call or insertion of an allele are noted on the 3500xL analysis worksheet.
9. Disable any samples not being reported and note the reason in the Comments section on the 3500xL analysis worksheet. Use sufficient detail as to be clear to a reviewer; for example: "re-inject at max due to low peaks" or "re-prep sample- ILS failed."
10. Re-save the project when edits are complete.

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Printing Electropherograms

1. After editing is complete, close the 'All Color Browser'. Select the 'Set Axis' icon in the toolbar menu and select 'Fixed X'. Set the range from 70 to 450. Click 'Ok'.
2. Select the samples to print (all samples in the project that will use the same print template) by double-clicking to check-mark their sample name(s) in the sample tree. Select the 'Print Report' icon in the toolbar menu. Print each set of samples using the appropriate template. See SOP 23 for sample types required to print.
    a. If appropriate for a sample, chart height may be lowered to better fit data on fewer pages or raised for better view of profile details (see Step 2 under "Print Templates").
3. Should an electropherogram need to be re-printed, be sure to manually note edit changes on the peak table, or cross out the edit information for that sample from the original table if printing a new table.

Exporting a Report

1. Click on the "Save" icon next to the "Report Settings" icon.
2. For "Save as Type:", choose "text file". Save in the designated network folder for Analysis Export Files.

Reviewing a Project

1. Open GM-HID and log in using the appropriate Analyst (not Administrator) account.
2. See electropherogram headers to check that the correct Analysis Template was used.
3. When checking edits, match edited peaks onscreen (marked with an "X" or "E" symbol) to edits listed in the printed peak tables using size by holding crosshairs above edited peak.
4. To confirm that reasons for not reporting samples, as listed in Comments on the 3500xL analysis worksheet, are appropriate, re-enable disabled samples. (Do not save project.)

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BATCH PAPERWORK & ANALYSIS

Analyst

1) When a new known is submitted to the laboratory for comparison to a case previously amplified with ID or IDP, the DNA profile is generated for the known.
2) The analyst will make comparisons of that known to all questioned samples in the case. Analyst will determine if the known is eliminated as the source of/contributor to the DNA profile, included as/CBE to the source of/contributor to the DNA profile, or if the comparison is inconclusive.
3) All questioned samples to which the known has not been eliminated as the source of/contributor to must be re-analyzed, using GeneMapper ID-X, using an analytical threshold of BGYR = 50rfu. Obtain the batch paperwork or case jacket in which these samples were previously analyzed.
    a. Add all appropriate questioned samples, all associated negative controls, and all associated ladders into GeneMapper ID-X project. (These files will be in the archived data folder on the U-drive. Archived data may also be found on archived DVDs). The data can be copied to the analyst's specific "to be analyzed" folder prior to importing into GeneMapper ID-X. A new folder will need to be made for reanalysis and placed in the completed folder.
        i. The positive controls do not need to be brought in or reanalyzed, but do look at the already created profiles to verify that they had previously worked as expected.
    b. Analyze data with appropriate analysis method containing the proper range, encompassing all size standards for all samples, and BGYR amplitude thresholds set to 50. (For IDP samples, this will be the same analysis method used in the past to create the input file for the ID+ Stutter Filter Macro.) Analyze data with either "Identifiler_Plus_Panels_v1" or "Identifiler_CODIS_v1.1", depending on if the data was amplified with Identifiler Plus or Identifiler, respectively. All other settings will remain the same as the previous analysis.
    c. Save with the following file name format: Kit-AT-initialsdate-Allele. (AT is analytical threshold; date will be the current date of doing analysis.) For example, IDP-50-JBE060617-Allele. (Allele is to distinguish that this file is for the allele table.) If analyzing more than one project in a day, add -1, -2, etc. to the end of this name.
    d. Use the previous analysis as a guide for click-offs. Click-off all peaks/artifacts previously clicked off, remembering to note reason for click-off when prompted. Analyze data for possible artifacts between 50 & 74 RFU, which would not have previously been clicked off. Analyze data for stutter peaks between 50 and 75 rfu that

were not previously called, but are at or below the stutter threshold for that allele. Make and record additional click-offs appropriately on original Injection Analysis Worksheet.

e. Analyze negative controls for peaks between 25 and 49 RFUs. If any peaks are noted, record on QR sheet. Follow current SOPs when dealing with contaminated controls.
f. Print out electropherograms for negative controls and questioned samples. Compare questioned samples to previously reported out electropherogram. The only differences should be that asterisk peaks are now called peaks. All alleles should be identical. If applicable, add original batch name to all electropherograms.
g. Note that all controls/standards performed as expected on original injection sheet, by adding additional column of checks, with initials and date. "N/A" the "Cross-Comparison to Batch" line, if applicable.
h. In the "Genotypes" tab, export table (see below to create table) to the analyst specific "Analysis Export File" folder on U-drive in the "To Be Analyzed" folder. File name will have same name as project name. This text file will be used for the Contributor Estimator (QR-302), the project comparison macro (QR-301), and the Allele Table Macro.
    i. Check the following columns for the "GeneMapper ID-X Analysis Table": Sample File, Marker, Allele, Size, & Height. Set the number of alleles to 39. Uncheck the "Keep Allele, Size, Height, Area, Data point, Mutation and Comment together" box. This table will be used for the "Allele Table" on the report.
i. Using the GeneMapper ID-X Manager, export the saved project, being sure to note where exported projects are located on paperwork. Close this project.
j. Data will now need to be analyzed with stutter filters off. To do this, new panels and bins are necessary, and the creation of a new analysis method (only needs to be done once, then it will be in the GeneMapper ID-X settings, if done, proceed to k):
    i. In Panel Manager, import panels, then bins for "Identifiler_Plus_Panels_No-ST-Filter" and "AmpFLSTR_Panels_No-ST-Filter".
    ii. In "GeneMapper ID-X Manager, under "Analysis Methods" tab, click on the method previously used to analyze IDP data and click "Save As", and re-name IDP-50-noST.
    iii. Double-click the new method to open Analysis Method Editor. In "peak detector" tab, make sure all peak detection amplitude thresholds for BGYR are set to 50. In "Allele" tab, make sure bin set is the No-ST-Filter set just imported.
        1. This will need to be done for IDP and ID if analyzing data obtained from both kits.

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k. Bring in all files that were previously analyzed in step a. Re-analyze data, now with either IDP-50-noST analysis method / AmpFLSTR_Panels_No-ST-Filter panels for IDP, or ID-50-noST analysis method / AmpFLSTR_Panels_No-ST-Filter panels for ID. Using the analysis worksheet as a guide, click-off the artifact peaks (not stutter peaks) clicked off in the previous project. Remember to keep high stutter when applicable. Save file with the same name as before, but with –STRmix at end instead of -Allele, for example, IDP-50-JBE060617-STRmix. Although controls need to be brought into this project, click-offs for the controls do not need to be repeated.

l. Export the table, as done with "Allele" project. This will be the text file used for STRmix analysis and the project comparison macro.

m. Using the GeneMapper ID-X Manager, export the saved project to the same folder selected for the "Allele" project.

4) Use the "Project Comparison" tool to make sure click-offs in both projects are identical (with allowable exceptions noted in "comments" section).
5) Create DNA-QR-330 for allele table project. This will remain part of the batch paperwork.
6) Create a contributor # estimation (QR-302) sheet for each sample. Note: Generally the use of any non-artifact peaks below AT (25-49rfu) is added to this worksheet. With a single source sample, this worksheet is still used; comments are added as to the determination of number of contributors.
    a. Check the box in the "Notes" section if peaks below AT were used.
    b. The "minimum # of male contributors line" will be greyed out for IDP data.
7) Fill out new DNA QR-4A sheet(s), attach to batch (es) (if applicable) and new paperwork, and give these to Technical Reviewer for review.

Technical Reviewer

1) Import analyst's "Allele Table" project into GeneMapper ID-X then open that project. Guidance to where this project resides may be on the analysis worksheet.
2) Review questioned samples and negative controls. Note any differences. Check that:
    a. Correct analysis template is used.
    b. Analyst's assessment of size standards, ladders, controls, and potential contamination are appropriate.
    c. All called peaks appear to be true alleles.
    d. All deletions/edits made are appropriate and documented properly.
3) Review all new paperwork (sample electropherograms, control electropherograms, number of contributors sheets, project concordance sheet) and all additions to old paperwork (new edits, new controls/standard performance). Add a new set of checks in a column on the injection sheet for controls and standards, to the right the previous 2nd analysts checks, and initial and date.

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4) Fill out, and initial and date DNA QR-4A.
5) Give batch to AR.

Administrative Reviewer

1) Review all batch paperwork.
2) Fill out, initial, and date DNA QR-4A.
3) Give batch back to analyst.

Analyst

1) Review and make any corrections.
    a. If additional click-offs need to be made, or click-offs need to be undeleted, do so in both STRmix and Allele Report project. Re-save and Re-Export both projects. Re-export both text files. Rerun project comparison macro. If appropriate, re-run affected contributor number estimates.
2) Initial and date DNA QR-4A. Return batch to Technical Reviewer.

Technical Reviewer

1) Review any changes made by analyst.
2) Add batch name to spreadsheet on U-drive.
3) Initial and date DNA QR-4A.
4) Return batch to Analyst.

Analyst

1) Make photocopy of injection sheets for case jacket in batch, if applicable. This, along with the original questioned sample electropherograms, and contributor number estimator, are for the case jacket.
2) Scan batch paperwork (not containing sample electropherograms & contributor number estimator sheets) and save PDF file to appropriate folder on u-drive, if applicable.
3) Give batch paperwork to TR to review scan, if applicable.

Technical Reviewer

1) Ensure all pages scanned and PDF file on u-drive is complete and accurate, if applicable.
2) Add initials to batch paperwork spreadsheet, if applicable.

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CASEWORK

Analyst

1) Deconvolute (see DNA SOP-22) all questioned samples just reanalyzed in STRmix with appropriate knowns conditioned and appropriate knowns being compared to deconvoluted profiles. Known input files can either be exported from GeneMapper ID-X or manually created using DNA QR-304. Input files for conditioned knowns must include sizes.
    a. The file will automatically save onto the server, under F:\results.
    b. Print out pages 1 and 3 of the advanced report.
    c. Scrutinize advanced report for any problems with the deconvolution.
    d. Fill out DNA QR-303 for the 2nd diagnostics for each deconvolution.
    e. Move the file in the F:\ results folder to the analyst specific folder in F:\results.
    f. Create a folder in analyst specific folder for this case, named with the case number in format DSS-XX-XXXXXX, or ID-XX-XXXXXX, and move that folder into this newly created case specific folder.
2) Review case jacket, write report.
3) Give case jacket to TR.

Technical Reviewer

1) Review case jacket, report, and make all appropriate corrections.
    a. If there is a disagreement with an elimination comparison, the analyst re-analyzes that sample and runs the profile through STRmix. Repeat “Analysis” portion of work instructions.
2) If there is a need to review more of the Advanced Report than is in the case jacket, it can be found on the STRmix server, F:\Results in the analyst’s folder, in the folder specific to this case.
3) Give case jacket to AR.

Administrative Reviewer

1) Review case jacket, report, and make all appropriate corrections.
    a. If there is a disagreement with an elimination comparison, the analyst will re-analyze that sample and run the profile through STRmix. Repeat “Analysis” portion of work instructions.
2) If there is a need to review more of the Advanced Report than is in the case jacket, it can be found on the STRmix server, F:\Results in the analyst’s folder, in the folder specific to this case.

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3) Give case jacket back to analyst.

Analyst

1) Make all appropriate corrections to report and case jacket.
2) If either the TR or AR disagrees with an elimination call, that sample must be re-analyzed with the new AT, and run through STRmix. Repeat "Analysis" portion of Work Instruction.
    a. If the conclusion is still an elimination, the "elimination by STRmix" report wording is used, and not "elimination by analyst"; wording will be slightly different. See report template.
    b. If the conclusion is not an elimination, report out results based on LR.
3) Print out copy of final report.
4) Move the STRmix results from analyst folder to the completed folder on the F:\ drive.
5) Give the case jacket to TR for correction check.

Technical Reviewer

1) Ensure all corrections are accurate, sign report.
2) Check to make sure STRmix data has been moved to F:\Results\Completed.
3) Give case jacket to AR for correction check.

Administrative Reviewer

1) Ensure all corrections are accurate.
2) Give case jacket back to analyst.

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1. See SOP 21.7 and 21.8 for deconvolution and statistical analysis of vendor profiles. See SOP 33 for general protocols regarding the qualitative interpretation of vendor generated F6C profiles.

2. If necessary, the assigned DSS analyst may import the relevant sample files into GeneMarker HID. The analyst may review the analyzed data from the vendor but will not make any changes to the data.

3. The vendor lab analytical thresholds are described below for reference:

A) **Bode Cellmark**

Kit Color	F6C RFU	GF RFU
B	100	125
G	100	125
Y	100	125
R	100	125
P	100	125

B) **DNA Labs International**

Color	F6C RFU	GF RFU
B	84	145
G	93	197
Y	39	183
R	42	109
P	64	135

4. Determine the number of contributors as described in SOP 33. Non-artifact, sub-analytical peaks are used to assess the number of contributors ≥ the following RFU levels for each vendor lab where the level of machine noise and the quality of the profile allows:

a) **Bode Cellmark**

Kit Color	F6C RFU	GF RFU
B	50	50
G	65	60
Y	50	50
R	50	60
P	50	60

B) **DNA Labs International**

Color	F6C RFU	GF RFU
B	25	40
G	25	55
Y	25	35
R	25	50
P	25	55

5. Document Batch Paperwork review on QR-4a and Case Review on QR-4.

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Analysis/Review of Male Screen Batch Paperwork Workflow

- DNA QR-22b,32 Dilution Factor and DNA QR-313 Male Screen Concentration Worksheets will flag samples needing microcon concentration. Individuals performing the male screen will not need DNA Analyst's sign-offs during the process. (see DNA SOP 34).

-Once the male screen batch is completed, it along with the associated case jackets will be handed off to a qualified DNA Analyst as per the rotation.

-It will be the responsibility of the DNA Analyst to review the male screen batch and case jacket information and create DNA requests in JusticeTrax for all cases in the batch.

1. Review male screen batch paperwork to determine which screened samples are positive, inconclusive, or negative (see SOP 34).

    a) For screened samples that are positive or inconclusive (low quantity of human DNA) in the 1st quant run, make notes in the "Comments" section of the 1st DNA QR-24. Initial and date your comments.

    b) For screened samples that are positive, inconclusive (SA value failed to concentrate), or negative after microcon concentration, make notes on the DNA QR-313. Initial and date your notes.

    c) Make sure the Lot #s are correct and the Pos control has passed (value in the Reagent Lot Log). Initial all sheets in the male screen batch.

2. Review the case jacket information to determine what type of testing, i.e. Differential vs. Non-Differential (with or without DTT) or NFT (no further testing) for male screen negative cases, is needed for each sample. In general, most samples will be a Differential, unless it is clear that the contact is touch or saliva only. If the victim was drugged/intoxicated during the assault or a young minor, a differential should be done. There may be situations where maximizing the amount of DNA extracted is desirable, but there is a remote possibility of the presence of semen. For these situations, a Non-Differential with the addition of DTT could be performed. Make case management aware of these situations.

3. In JusticeTrax, for each male screened sample, type in the results in the Notes section of the Evidence tab: Male Screen Result, DNA Test Type, male screen batch, DNA Analyst Initials. The (#) already present in the Notes is the number of swabs for that item. These notes will aid Case Management in fielding

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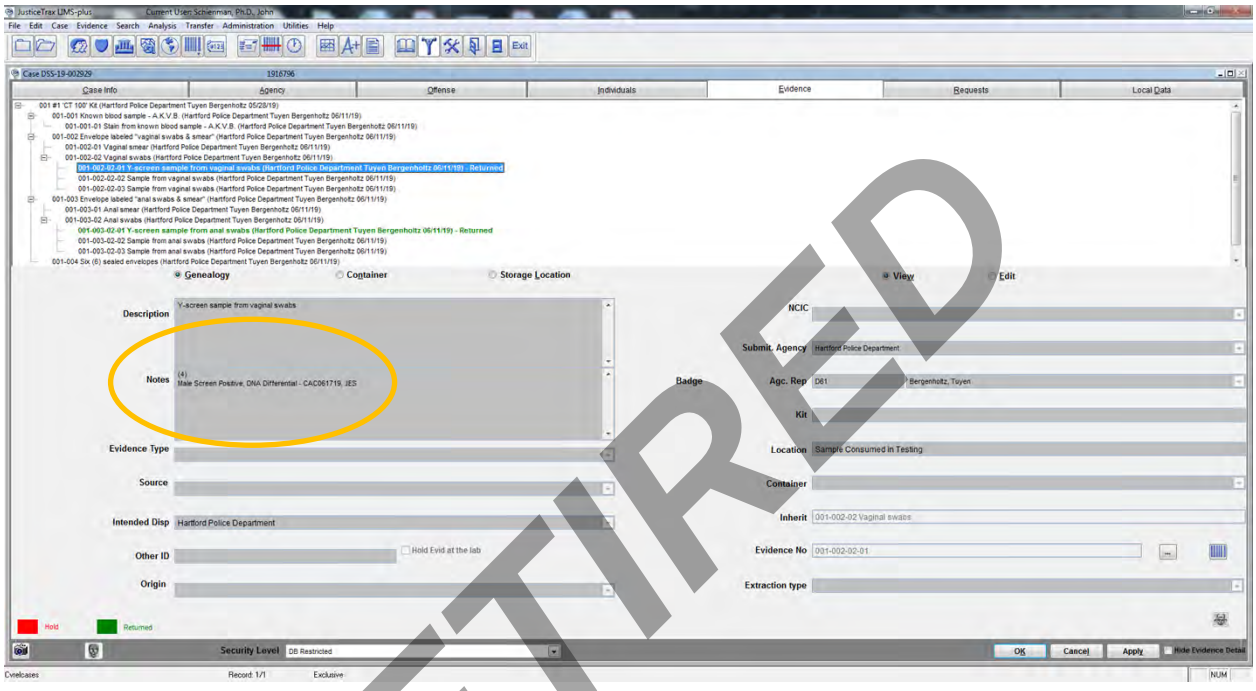
phone calls regarding DNA testing and modifying a "DNA – Consumption" request when permission for testing is received.



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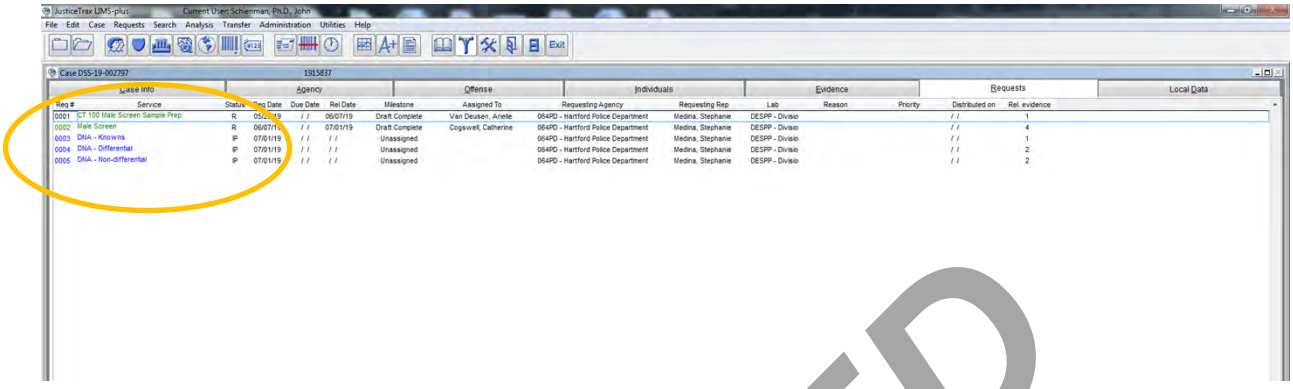
4. Under the Requests tab, add the appropriate DNA requests and related evidence, including DNA Knowns, but do not add these to the casework knowns spreadsheet at this time. Don't print the barcode for the request. There will be 3 sub-items for every sample; the already consumed Y-screen sample, one in "Freezer Storage – DNA Sample", and one in "Freezer Storage". Make sure to choose the one in "Freezer Storage – DNA Sample" as related evidence for the DNA request. The one in "Freezer Storage" is for serology testing upon request. In some cases, the victim's known sample will be the oral swab from the kit. **Check to see if a suspect has been arrested in the case, if so use the "DNA – Consumption" request in JusticeTrax.** When the permission to test is received, case management will modify the request(s) to match the evidence notes and Evidence Transfer Sheet.



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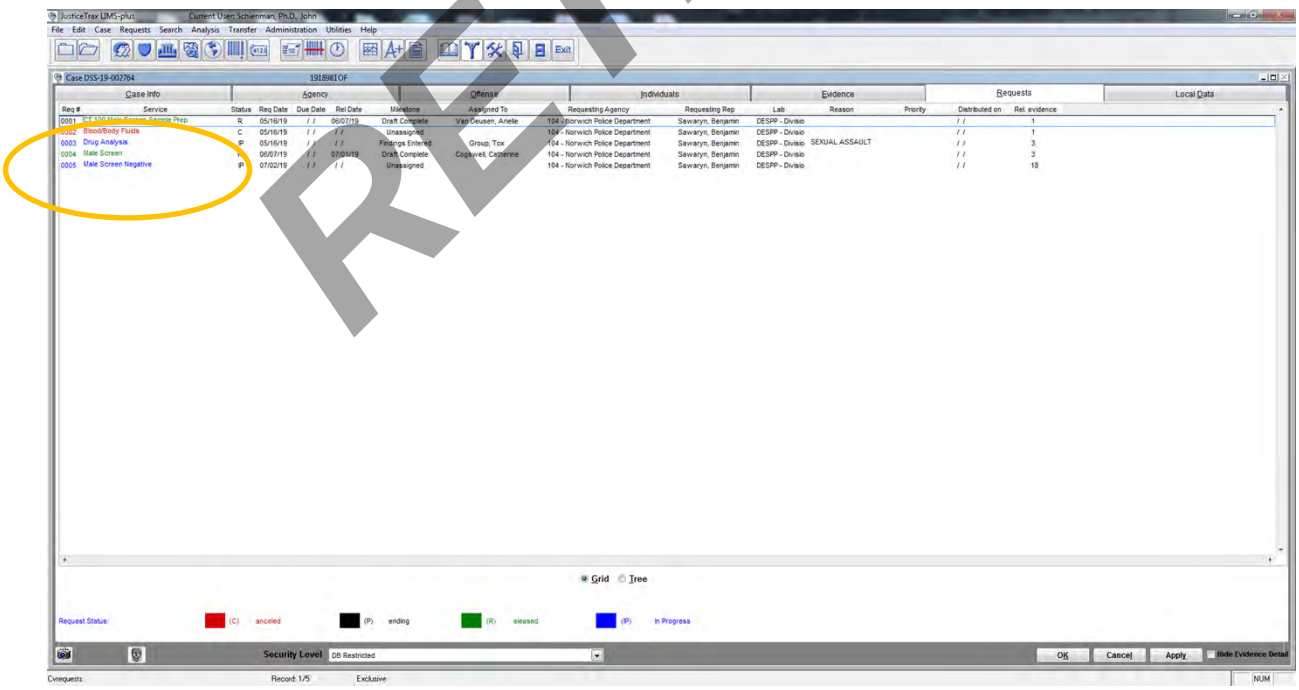
For cases with only negative male screen samples, we will be reporting out the negative results. A request still needs to be added, "Male Screen Negative", so the request can track the draft complete, technical review, and administrative review process. The samples that are in "Freezer Storage – DNA Sample" will need to be transferred to "Freezer Storage" both physically and in JusticeTrax. These will be added into batches with cases that are positive and will be moved to freezer storage by the processing analyst. CM will add a note to the batch sheet to alert the analyst.



5. On the evidence transfer sheet in the case jacket, hand-write the DNA request type in the "Evidence Type" column for each sample, i.e. DNA Differential. Also, under the "Notes" column hand-write the male screen results, e.g. Male Screen Positive, Inconclusive, or Negative. Initial and date these hand-written additions.

6. Fill out DNA QR-4E and hand off to another analyst on the rotation for TR/AR.

7. When TR is done, make copies of the Male Screen batch paperwork (DNA QR-4E, the first DNA QR-24, and DNA QR 313, if applicable) for each case jacket in the set and add the case number to each worksheet.

8. Give the set of cases to Case Management. Scan the male screen batch paperwork and save to U-Drive. File the original Male-Screen batch paperwork in the casework batch paperwork filing cabinets.

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Process Post Male-Screen

1. Once the samples have been extracted, the quant results need to be evaluated to determine which samples will go on for amplification or be halted. For any non-interpreting analyst that is processing the batch, give paperwork and case jackets to an interpreting analyst as per the rotation list to make this evaluation. The interpreting analyst will analyze the quant data, fill in the halt at quant sheet, create the amp sheet(s), print out the staff search sheet(s), add the appropriate known(s) to the casework knowns spreadsheet, and eventually own the batch.

2. Samples will follow the process outlined on the flow chart in DNA SOP-34. Things to consider:
   a. How many suspects are alleged in the narrative? Was there a consensual partner that could be a contributor? Typically 1 sample will be sent on but if there are more potential males contributing, you will want to send on more samples.
   b. Is the most probative sample not the most positive? If you have a probative sample (ex: vaginal swab) that has a male value and the ratio is sufficient but the M:F ratio is close to 1:50, you may develop a minor male but it may not be sufficient for CODIS. If this is the case, you may also want to send a sample that although not as probative, would give a potential CODIS eligible profile (ex: Dried secretion swab of neck).
   c. Are multiple orifice swabs positive for male DNA? Vaginal penetration and Anal penetration are legally different charges. If both are positive for male DNA and the ratios are sufficient,

then both should be sent. Keep in mind item 2b above and send a third sample if necessary to develop a CODIS eligible profile.

d. If the B fraction of a sample is being amplified, the A fraction will also need to be amplified for QA purposes. This is regardless of the ratio of M:F DNA in the A fraction. If the A fraction of a sample is being amplified and the B fraction does not meet the criteria, then it does NOT need to be amplified.

e. Y-STR testing is to be done on samples where a known for the suspect is already at the laboratory and the male quant value is positive. You may not need Y-STR testing if you already have samples that may generate Autosomal profiles. If you have a vaginal sample going for autosomal testing and an anal sample that is eligible for Y-STR testing then it's a good idea to amplify it. However, if you have a vaginal sample that is going for autosomal testing and a touched sample eligible for Y-STR testing, you may choose not to amplify the touched sample for Y-STR testing. If doing Y-STR testing, the chosen sample(s) may need to be concentrated first (target of 0.5ng of male DNA), but keep in mind that if it is an A fraction then it needs to be sent to Autosomal **AND** Y-STR testing for QA purposes. Amplify for autosomal DNA first, and then perform the concentration for Y-STR testing. If the sample being amplified for Y-STR is a B fraction, then the A fraction will need to be amplified for Autosomal loci.

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3. QR-48 includes 3 columns. This QR will assist the analyst in writing reports. The 3 columns are:
    a. Highest quant target >0ng/ul?—answer Y or N, as some samples are undet. for all targets
    b. Male DNA present?—if answer to (a.) is no, mark as N/A or leave blank
    c. M:F ratio >1:50?—if answer to (a. or b.) is no, mark as N/A or leave blank

4. Once you have determined which cases will have samples moving on to amplification (either autosomal and/or Y-STR), add any knowns to the knowns spreadsheet for processing. If a case has no samples moving on for amplification and it contains a non-kit submitted known, add that to the spreadsheet for itemization and retaining only. Remember to indicate if a known also needs Y-STR testing. Do not cancel any known requests. If a case is halted at quant, the known assignment will still be closed out as usual.

5. Report wording is contained within the flow chart in DNA SOP-34 and will indicate specific reasons why a sample was halted. Also refer to DNA SOP-6.

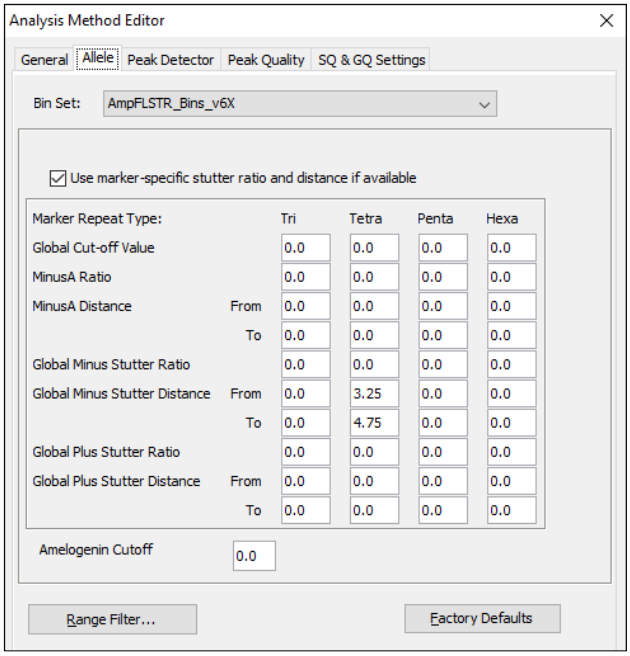
1. Open the GeneMapper ID-X version 1.6 software and login with the appropriate user name and password.

NOTE: The instructions below describe specific ways to accomplish tasks or select options in the software, but recognize these descriptions are not exhaustive. Often, there are multiple pathways of carrying out the same function, e.g. using the icon menu, text menu, or shortcut keys. Please see the GeneMapper® ID-X Software Version 1.5 Reference Guide, GeneMapper™ *ID-X* Software v1.6 User Bulletin, and the software menus for alternative options.

To verify setup:

1. Select the 'Tools' menu, then select 'Panel Manager' to verify that the panels folder 'AmpFLSTR_Panels_v6X' is present in the window in the upper-left under the 'Panel Manager' icon. Then select the 'AmpFLSTR_Panels_v6X' folder to verify that the 'AmpFLSTR_Bins_v6X' bin set is loaded in the panel. If the panel and bin set are not present, select the 'Panel Manager' icon in the upper-left window, then from the menu select 'File' and 'Import Panels'. Select 'AmpFLSTR_Panels_v6X' from the Panels folder list and select the 'Import' button. Then select the newly imported 'AmpFLSTR_Panels_v6X' folder in the upper-left window. From the menu, select 'File' and 'Import Bins'. Select 'AmpFLSTR_Bins_V6X' from the Panels folder list and select the 'Import' button. Then select 'Apply' and then 'OK'.

2. Select the 'Tools' menu, then select GeneMapper ID-X Manager. Select the 'Analysis Methods' tab. Verify the method 'YFP_CTDSS' is listed. If not, import the method from the 'Panels_Bins' folder on the U: Drive. To verify the settings of the method, select it from the list of 'Analysis Methods' and select 'Open'. Successively, select the 'Allele', 'Peak Detector', 'Peak Quality', and 'SQ & GQ Settings' tabs and verify the settings match the screen shots to the right and on the next page. If any of the settings do not match, correct them and select 'Save'. Note: the start point for the analysis partial range is listed as 2500 scans, but should be adjusted for each set of data to avoid including the amplification primer front in the analysis range so the software's auto-scaling of the Y-axis feature will adjust the sample's allele peaks to the appropriate scale.



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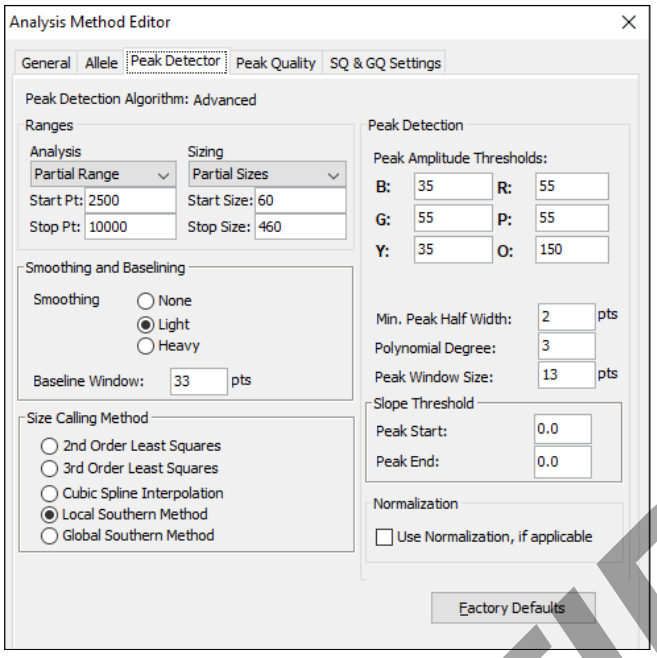

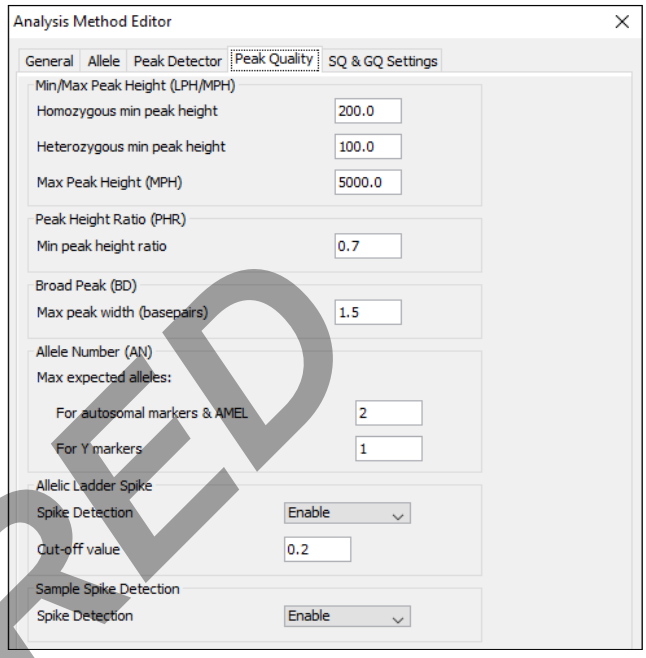

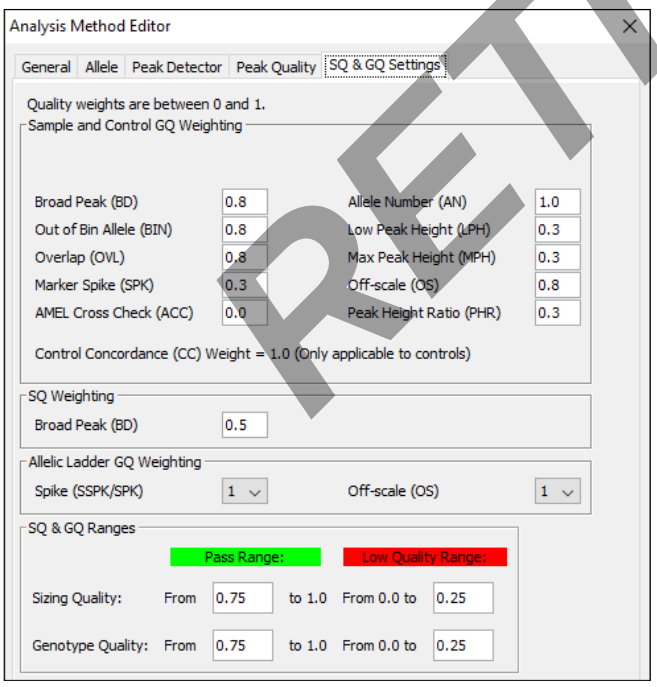

3. Select the 'Table Settings' tab and verify that 'CT-DSS Table' is listed. If not, import the method from the 'Panels_Bins' folder on the U: Drive. To verify the settings of the table, select it from the list of 'Table Settings' and select 'Open'. Select the 'Samples' tab and verify that the only 'Column Settings' selected to show are 'Sample File', 'Sample Name', 'Sample Type', 'Analysis Method', 'Panel', 'Size Standard', 'Sizing Quality Overridden (SQO)', and 'Sizing Quality (SQ)'. Sort by 'Sample File', 'Ascending', then by 'None', then by 'None'. Select the 'Genotypes' tab and verify that the only 'Column Settings' selected to show are 'Sample File', 'Marker', 'Allele', 'Size', and 'Height'. Sort by 'Sample File', 'Ascending', then by 'Marker',

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'Ascending', then by 'None'. If any of the settings do not match those below, correct them and select 'OK'.

To Analyze Data:

1. If another project is already open, clear it by selecting 'New Project' icon from the menu header. To import data files, select the 'Add Samples to Project' icon in the menu header and navigate to the data folder. Select the data folder and then select 'Add To List >>' on the lower-left and the folder will appear in the 'Samples To Add:' list on the right. Select more than one data folder if needed. Select the 'Add' button on the lower-right to add the data to your project. The samples will appear in the 'Samples' tab list.
2. Verify that the 'Table Setting:' in the menu header is set to 'CT-DSS Table'. If not, use the drop down menu to select it.
3. In the 'Sample Type' column, verify that your ladders have the 'Allelic Ladder' type and all other samples have the 'Sample' type selected. Adjust if needed.
4. For samples amplified by the Yfiler™ Plus Kit, select 'YFP_CTDSS', 'Yfiler_Plus_Panel_v4.1X, and 'GS600_LIZ_(60-460)' for the Analysis Method, Panel, and Size Standard, respectively. Tip: Select the method, panel, and size standard for the first sample in the list. Then select the 'Analysis Method' cell in the header and while holding the left mouse button drag the selection across to the 'Panel' and 'Size Standard' cells to select all three columns. Then select 'Ctrl+D' on the computer keyboard to fill down these choices to all the sample and ladders.
5. Select the green play button icon in the menu header to analyze the data with these settings. The 'Save Project' window will pop up asking for a project name. Name the project 'YFP-3500#-Batch ID-Modifier#-Any other details', e.g. YFP-2-MAP102219-2-JES. The modifier number and other details are optional. Select 'OK'. The project will be analyzed and saved to the oracle database of the software.
6. When analysis of the samples is complete, select the 'Samples' tab. The 'SQ' column will indicate the sizing quality of the ILS for each sample. A green square indicates passing, a yellow triangle indicates caution and that there may be a problem with the ILS, and a red stop sign indicates ILS failure. To view the ILS for the samples, use the left mouse button to select the cell containing '1' to the left of the first sample in the list and while holding down the left mouse button drag down the entire list of samples. Select the 'Size Match Editor' icon in the menu header and the editor will open. Selecting a sample from the list on the left will display the ILS peaks detected for that sample. A passing ILS will typically have a score of 1.0, but any value ≥ 0.75 will pass as determined by the Analysis Method's 'SQ & GQ Settings' (see screen shot above). Occasionally, spikes, spectral pullup, or other artifacts in the orange dye channel may be identified by the software as an ILS peak altering the detection of one or more of the true ILS peaks. To modify the detection of a peak, first select

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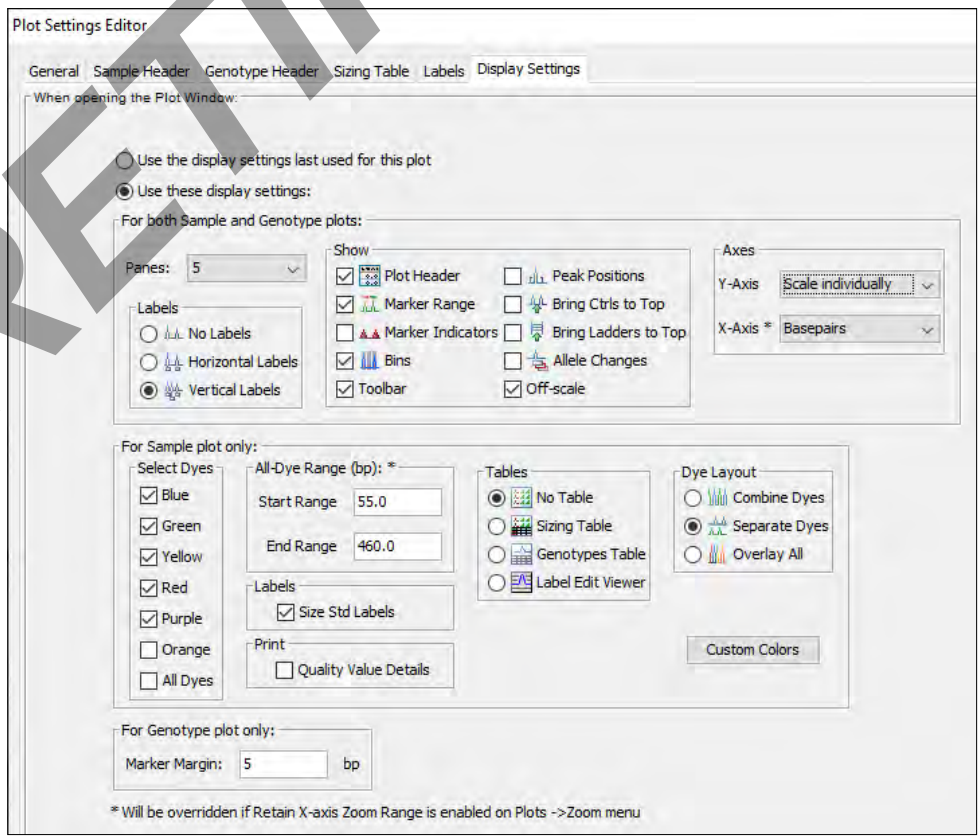
the peak with the left mouse button such that it fills with a dark blue color. Then select this blue highlighted peak with the right mouse button and select 'Add…', 'Delete', or 'Change…' as appropriate. Select the 'Apply' and then 'OK' buttons and reanalyze the sample by selecting the green play button icon in the menu header. If the 'Size Match Editor' has been used to modify the ILS for a sample, a green check will appear in the 'SQO' column for that sample. Alternatively, reinject, or replate and reinject the sample(s).

7. To view electropherograms (egram) for the allelic ladders and samples, select the sample(s) with the left mouse button and select the 'Display Plots' icon in the menu header. To alter display plot settings, select 'Tools' from the menu, then 'Plot Settings'. Select the 'Sample Header' tab and verify that 'Sample File' is the only attribute to show on the egram. Select the 'Labels' tab and verify that 'Label 1:' is 'Allele Call' for 'Assigned Allele', 'Custom Allele', and 'Allelic Ladder' and is 'Artifact label' for 'Artifact'. Verify that 'Label 2:' is 'Height' for 'Assigned Allele' and 'Custom Allele' and all other labels are 'NONE'. Verify that in the 'When opening the Plot Window' box all items are unchecked and 'Label Color:' is 'Dye Color-Border'. Select the 'Display Settings' tab and verify that it matches the screen shot below. These display settings may be modified to aid in sample analysis and editing, but the below settings should be restored prior to egram printing with the exception of the left and right margins. The margins can be narrowed to print as little of the spaces to the left and right of the marker ranges as possible. To do this, place the mouse cursor on the left side of the egram below the loci headers of any dye, but above the profile window such that the mouse cursor changes to a magnifying glass. Locate the magnifying glass to the desired left-hand margin and then press and hold the left mouse button and drag across to the



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desired right-hand margin and then release the mouse button. To undo these narrowed margins, position the mouse cursor to change it to a magnifying glass, then double click the left mouse button. For editing, it is advantageous to keep the margins wider in case there are variant alleles significantly outside the marker ranges for the smallest and largest loci of each dye color.

8. Allelic ladders that injected poorly or are missing allele calls for other reasons can be disabled by changing the 'Sample Type' for that ladder to 'Sample'.

9. Interpret and edit the egrams of the samples and controls as warranted (see DNA SOP-40). To delete or rename an allele/peak call, place the mouse cursor in the allele/peak label and then click the right mouse button. Choose 'Delete label(s)' or 'Rename Allele Label' as appropriate. If deleting, the software will ask you to confirm. After selecting 'Yes', the software window will prompt you to input a 'Reason(s). Type in the appropriate abbreviation, e.g. 'pu' for pull-up. Click 'OK'. If renaming, select 'Custom Allele Label' or 'Custom Artifact label', enter the new name and then click 'OK'. When project editing is complete, select 'File' from the menu and then select 'Save Project'.

10. To print the egrams for the controls and samples, first select the samples from the list, as previously described, that you want to print and select the 'Display Plots' icon from the menu header. Samples can be printed one at a time or as a batch. Set the desired left and right-hand margins as described above. Select 'File' from the menu and then 'Page Setup'. Select the 'Plot' tab and then select 'Small', 'Medium', or 'Large' as desired. Click 'OK'. Select 'File' from the menu and then 'Print Preview'. Based on the 'Print Preview', make adjustments to 'Page Setup' as desired and then select 'File' from the menu and then select 'Print'. Select the appropriate printer and properties and then select 'Print'. Close the egram by selecting the 'X' in the upper right-hand corner. Peak heights in the printed egram label are not required for positive controls or knowns. Access 'Plot Settings' in the 'Tools' menu as previously described and change 'Label 2:' for 'Assigned Allele' to 'NONE' to print without peak height in the label.

11. To print the sample edits, first select all the samples in the sample list for which you want to print edits. Then select 'View' from the menu, then select 'Label Edit Viewer'. A window opens with a list of the edits for each sample. Click the 'Export' button and navigate to the folder location for the raw data. Export as a 'Tab-delimited text (.txt)' file. Click 'Export'. By default, the exported file name will (and should) contain the project name. Open the exported file with DNA QR-330 and click 'Print'.

12. To export the completed project to the U: Drive, select 'Tools' from the menu header, then select 'GeneMapper™ ID-X Manager'. Select the 'Projects' tab and then select the project from the list. Click on the 'Export' button. Navigate to the folder location for the raw data and click 'Save'.

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13. To export a file for import into the Concordance checker (DNA QR-37), select the 'Genotypes' tab. Select 'File' from the menu header, then select 'Export Table'. Export the file as 'Tab-delimited text (.txt)'. Navigate to the appropriate folder on the U: Drive, then click 'Export'.

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Chemistry Unit – Case Review Checklist

Technical Reviewer Name (print): _____ **Date:** _____

Lab/Case Number: _____ **Request #:** _____	**Yes**	**No**	**N/A**
Evidence description clear and consistent with Request for Analysis (RFA) form			
Case notes are clear and reflect the item(s) tested			
Photographs have been correctly annotated (e.g., lab number, date, initials)			
Case notes, worksheets, and all user-generated documentation support the conclusion/results within the report			
Analytical data is present and support the conclusion/results within the report			
All calculations have been verified and documented as such within the casefile			
Controls (positive/negative/reference standards) and blanks were used appropriately			
All applicable procedures were followed according to documentation			
The report reflects the general testing method(s) performed on the evidence			
Chain of custody is free of discrepancies/breaks			
Corrections that result from the review process have been recorded within the LIMS (i.e., reject findings and record reason)			
Technical review milestone in the LIMS marked as 'Completed'			
Technical Reviewer's Notes (mandatory if TR milestone was rejected ; also record in the LIMS):			

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Administrative Reviewer Name (print): _____ **Date:** _____

	Yes	**No**	**N/A**
Administrative information within report corresponds to casefile documentation (e.g., Agency name, Agency representative, Agency case number)			
No spelling/grammatical errors within casefile or report			
Corrections have been appropriately documented			
Analyst's initials, date, and case number are on each page			
Evidentiary items received are described within report			
Appropriate initials and dates are on all casefile paperwork			
Technical review has been successfully completed			
Administrative review milestone in the LIMS marked as 'Completed'			
Administrative Reviewer's Notes (mandatory if AR milestone was rejected ; also record in the LIMS):			

Title: Chemical Analysis Section – Data Archiving

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1 Scope

The purpose of this document is to provide a uniform policy for the storage of raw electronic data generated by instrumentation within the Chemical Analysis Section (CAS). Data files take up a great deal of the hard disk space and cause a variety of system problems when allowed to remain on system hard drives for an extended periods of time. Therefore, a policy has been established to handle old data and to provide optional methods for permanent data archiving.

2 Principle

As stated in individual policies and protocols, hard copies of data are often printed and kept within various places (e.g., case files, instrument logbooks). Once data has been printed and filed appropriately, the printed materials are considered the 'official' copies and are deemed technical records. However, where possible, data files are kept and archived. The majority of the instrument computers within the Chemical Analysis Section (CAS) are connected to a closed network. This policy outlines the amount of time that data may be stored on an instrument computer and optional methods of data archiving. The term 'data' used in this procedure includes, but is not limited to: raw data, data file, file, directories, methods, sequences, layouts, reports, or to a location on an instrument/computer hard drive. This policy does not apply to those instruments that do not collect data to a computer or hard drive (e.g., an instrument connected directly to a plotter).

3 Equipment/Materials/Reagents

- TCP/IP Network (Transmission Control Protocol/Internet Protocol)
- CD-R or DVD-R disks (Compact Disc – Recordable ; Digital Video/Versatile Disc – Recordable)
- Thumb/Flash drives

4 Standards / Controls

Not applicable.

5 Calibration

Not applicable.

6 Sampling

Not applicable.

7 Procedure

A. Hard Drive Storage

i. Data should be kept on instrument computer hard drives for at least a period of approximately six (6) months unless it is archived sooner in order to free-up file space on hard drives. Where applicable, the performance monitoring protocols can direct instrument operators to view and record the remaining available disk space on a daily basis. Instrument operators should contact a Lead Examiner or an appropriate Deputy Director if less than 100 MB are available on instrument computers.

ii. Periodic checks should be made by Lead Examiners to ensure that data files dated older than six months on instrumental computers have been backed up, and possible removed, in order to maintain sufficient hard disk file space.

iii. It is the responsibility of the individual instrument operators to either notify someone or to know when data needs to be backed up and/or transferred.

B. Data Archiving

i. When the data stored on an instrument hard drive becomes excessive (typically indicated by the availability of less than 100 MB of free disk space), data files should be transferred (e.g., to network servers, CD-R/DVD-R, thumb drives) by either manual or automated methods.

ii. When data are older than one (1) year from creation, they should be appropriately backed up. Data can remain on hard drives if there is adequate storage space.

iii. Data located on the network servers will be backed up according to DESPP-IT (Department of Emergency Services and Public Protection – Information Technology) policy.

iv. Any data transferred to removable media (e.g., CD-R, DVD-R, thumb drives) should be appropriately labeled (e.g, dates of archiving) and stored in a central location within limited access rooms (e.g., laboratory space). Media which is not conducive to proper labeling should be stored in a manner wherein proper labeling can be applied (e.g., a thumb drive teathered to a larger label).

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v. If an individual operator, for any reason, wishes to archive data for their personal work-related use, the data can be copied (not deleted) from the hard drive, stored on a recordable medium, and they will be responsible for it's security. This type of archiving is only optional, however, and not a requirement.

vi. While Lead Examiners will do periodic checks of instrument file space, it is the responsibility of instrument operators to know when it is time to back up data and to perform the actual backing-up and/or transfer.

vii. It is the responsibility of the instrument operators to ensure all media are adequately and securely stored and labeled.

8 Calculations

Not applicable.

9 Safety

Not applicable.

10 References

DSS Quality Manual.

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Rev. #	History
1	New procedure/original issuance.

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Title: Examination and Extraction of Fire Debris Evidence

1 Introduction

The analysis of fire debris evidence for the presence of ignitable liquids (whether residual or bulk) can play a large role in law enforcement's (or other similar agency's) investigations. The detection of such compounds at a fire scene does not necessarily lead to a conclusion that a fire was incendiary in nature. Certain chemicals and types of mixtures such as gasoline, fuel oils, charcoal lighter fluids, and paint thinners can be used to accelerate or help ignite fires.

The term ignitable liquid is a set of substances which fall within certain commonly accepted classification groups of chemicals and/or mixtures. While certain liquids may, in fact, be ignited under certain parameters or within particular environments (e.g., candle wax, motor/lamp oils), they are outside the classification scheme used within this discipline and will not be included within the ignitable liquid definition. Ignitable liquids are often mixtures that contain large numbers of hydrocarbon constituents. Gasoline, for example, has over 400 chemical constituents and can be considered one of the more complex ILRs. The collection and concentration of ignitable liquids takes advantage of the volatility of their constituents. A charcoal adsorbent material is used in conjunction with a heated container to capture such chemical constituents. Volatile chemicals are adsorbed onto the charcoal and then eluted with a solvent for further analysis.

Detection limits, volatility of liquid residues, and variations of sampling techniques/methods can influence detectability. Difficulties in identifying ignitable liquids arise from their complex composition, from matrix interferences caused by materials which contain ignitable liquids (e.g., pyrolysis products of styrene from carpet backing), and from the loss of volatile constituents due to weathering or other factors. Because most evidence is post-burn, very small amounts of ignitable liquids are trying to be detected – hence the term ignitable liquid residues (ILRs). Analysis of materials for ILRs is generally performed using gas chromatography-mass spectrometry (GC/MS). A technique within mass spectrometry called ion profile analysis (or target compound analysis) can be used to compare chemical data to reference materials and standards for identification purposes. Such an identification can be considered a characterization of a material based upon physical or chemical properties that sufficiently differentiates that material from similar substances.

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2 Scope

This procedure serves as a general guideline for determining whether ignitable liquid(s) are within evidence by using certain sample preparations (e.g., extraction/elution) and tests (e.g., GC/MS). Authorized analysts who conduct such examinations within the fire debris category of testing are responsible for ensuring this procedure gets applied to applicable evidence and that results are placed within final reports. This procedure allows for the examination, extraction, and detection of ignitable liquids (ILs) within evidentiary materials. There are seven major classes of ignitable liquids (and one called miscellaneous/other) that chemicals may be identified as being part of by

using gas chromatography, mass spectrometry, extracted ion profiling (or extracted ion profile analysis), or a combination thereof within recovered fire debris evidence (or related materials). The scope of this procedure will focus on the following classifications: gasoline, distillates (aka. petroleum distillates), isoparaffinic products, aromatic products, naphthenic-paraffinic products, normal-alkane products, oxygenated solvents, and miscellaneous (aka. others-miscellaneous)).

Certain low molecular weight chemicals (e.g., alcohols) may only be detected when in sufficient quantity and not at the residue level using this procedure.

3 Principle

The techniques employed within this procedure utilize physical examination, extraction, and instrument analysis to determine whether ILRs are present within evidentiary material. In general analytes are often captured and isolated by heating evidence within a container's headspace, trapped onto highly adsorbent material, extracted using an elution solvent, and then analyzed using instrumentation (e.g., GC/MS). Evidence may be examined without the need of an adsorbent material (e.g., when samples are in sufficient quantity/concentration) and may warrant dilution prior to analysis. When necessary, and if appropriate, alternate techniques can be used for analysis (e.g., passive headspace extraction, infrared spectrophotometry, headspace-GC/MS) even though they may not be specifically described in this procedure. Use of other unit/section procedures is allowed with reasonable modifications and if approved by the unit's FSE2 (or higher).

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4 Specimens

Any type of material related to fire debris-type evidence should be able to be analyzed using this procedure. In order for heated headspace-type analyses to be performed, materials need to fit into a closed container (e.g., airtight-like metal can). Specimens needing to be diluted (e.g., suspected liquid gasoline) do not necessarily need to be in an airtight container for analyses to be performed. Evidence submitted which haven't been stored within airtight containers may need to be returned to submitting agencies due to quality control issues (e.g., contamination or loss of sample).

5 Equipment/Materials/Reagents

a. General laboratory glassware

b. Gas Chromatograph/Mass Spectrometer (GC/MS) (Agilent or equivalent)

c. Autosampler – Agilent (or equivalent)

d. GC Column - DB-5(MS), 30 m, 0.25 mm i.d., 0.25 μm film (or equivalent)

e. Heating mantles: capable of heating pint, quart, and gallon cans

f. Thermometer (reference only ; need not be certified/calibrated)

g. Vacuum source (mechanical pump or equivalent)

h. Cans: unused paint cans (e.g., pint, quart, one gallon, five gallon)

i. Pentane (reagent grade or equivalent)

j. Acetone (reagent grade or equivalent)

k. Appropriate solvent (reagent grade or equivalent)

l. Appropriate standards and controls (e.g., ASTM mix, Gasoline, Diesel, Lighter Fluid)

m. Activated charcoal tubes (SKC or equivalent)

n. Appropriate Gases (e.g., Nitrogen, Helium, Air)

o. Syringe (e.g., 0.5 mL – 2 mL, or equivalent)

p. Adhesive tape

q. Reference Standards (e.g., certified reference or purchased at store)

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6 Standards and Controls

Positive and Negative Controls:

Standards/Controls can be purchased or generated in-house (e.g., evaporated gasoline) and stored appropriately. If known materials are purchased then their stabilities should be determined by the manufacturer (e.g., expirations dates). If stability information is not available, then known materials, when stored appropriately, will be valid for one (1) year since their last verification. All standards/controls/reagents will be verified prior to be used in casework analysis and documentation will be retained. Appropriate positive and negative controls will be used with each sample or batch of samples.

a. Positive Control

If necessary, one or more of the following can be used as a positive control.

i. Hydrocarbon Mixture (e.g., GC/MS Performance Check Solution)

ii. Gasoline Reference Standard (Prepared in-house at an appropriate concentration)

iii. Other ignitable liquid standards/materials (e.g., NCFS, ASTM, NIST, purchased)

b. Negative Control

One or more of the following will be analyzed with every case and prior to each batch of extract solutions that are analyzed to ensure that extractions and instrumentation are free from contamination. If samples are concentrated under $N_{2(g)}$ then a negative control (same solvent used for the evidence sample) will be concentrated and analyzed in the same manner at least once during the case in order to demonstrate that extractions are contamination-free.

i. Solvent(s) that may have been used with sample analysis (e.g., pentane)

ii. Empty Can

iii. Activated Charcoal Tube Extract

Note-01: The above can be combined to form a negative control (e.g., empty can's heated headspace purged through a charcoal tube and extracted with pentane)

c. Blanks

An appropriate blank solvent (e.g., extraction solvent) should be analyzed in-between each sample to ensure the instrument is free from carryover and is ready for the next analysis.

7 Sampling

All submitted specimens will be analyzed and sampling is not applicable. However, if multiple specimens are submitted and they are determined that they were sampled from the same/similar area and enough positive results have been found with respect to ignitable liquid/residues, it is up to the analyst's discretion to contact the FSE2 (or higher) and/or the submitting agency representative to possibly change the number of specimens to be analyzed in the submission.

8 Procedure

a. Initial Examination

i. Observe the evidence (i.e., contents of the can/packaging)

ii. Presence of odors within samples:

- Always work in well ventilated area.
- Wafting the contents in order to evaluate the odor of ignitable liquids is not recommended due to the nature of potential hazardous chemicals within fire debris samples. However, strong ignitable liquid odors may be noted when the can has been opened without purposely smelling the sample.
- Odor of strong ignitable liquids may influence recovery method choice.

iii. Note on the worksheet a description of the material and any other appropriate observations or characteristics.

iv. The lab case number, the item number, and the analyst's initials will be on the top or side of the can/packaging.

v. If materials are present which may require additional forensic analyses (e.g., latent print or DNA), the nature of the materials and the type of testing should be considered before heating and confirmed with appropriate section analyst(s).

b. Recovery and analysis of organic compounds (use one or more of the following techniques)

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- Analyze a negative control (e.g., blank air, extraction solvent, or a can treated in the same manner as evidence) to ensure that the procedure and/or instrument is contamination-free.
- Analyze blanks (e.g., blank air, appropriate solvent) in-between all samples to ensure that there is no significant carryover.
- All instrumentation will have been successfully evaluated for quality prior to being used for casework.

i. Heated Headspace Technique

A. Punch a hole in the lid of the submitted can.

B. Cover the hole with a piece of plastic adhesive tape.

C. Heat the can until a bulge appears under the tape (or reasonable time period)

D. Remove the can from the heat source.

E. Using an appropriate syringe (e.g., 3 mL), inject 0.5 mL (0.5 cc) of heated headspace into the GC/MS.

F. Record appropriate information (e.g., approximate temperature, time period) within case notes.

ii. Adsorption/Elution (Dynamic) Technique

A. Check the outside of the can for dirt or debris. If present, clean the can to ensure that it is contamination-free.

B. Punch 1-4 evenly spaced holes around the circumference of the can near the bottom. If there is water in the can then place the holes approximately 1 inch above the water level.

C. Replace the original lid with a lid adapted to hold a charcoal tube. Snip the ends of the charcoal tube and place it into the Swagelok fitting. Insert the tube with the 'open' end in the holder.

D. Insert the can into the heating mantle (the temperature is approximate 80 °C. In some cases it may be necessary to insert a dial thermometer through the lid until it touches the contents of the can (determined by analyst).

E. Connect the end of the charcoal tube to the vacuum line, and turn on the vacuum pump.

F. After an appropriate period of time (i.e., when condensation appears in the open area of the tube or when the temperature of the contents reaches 40-50 °C) remove the charcoal tube. CAUTION: Avoid sample temperatures above 80 °C to minimize the stripping of 'lighter' components of flammable liquids.

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G. Place the charcoal tube into an appropriate glass vial for collection of the eluate.

H. Using an appropriate pipette add solvent (e.g., 1.5 mL of pentane) through the top of the charcoal tube.

I. Elute the solvent through the charcoal tube. Repeat the procedure with a second wash, if necessary.

J. If the sample extract cannot be analyzed the same day then store the extract in the refrigerator or freezer under proper seal and record necessary information within LIMS.

K. If a petroleum distillate or gasoline odor was not discernible during initial observations (or if residual amounts are suspected) then concentrate the sample by using a stream of nitrogen gas ($N_{2(g)}$) to approximately 100 µL.

L. Inject extract solution into the GC/MS and analyze. Clean instrumentation appropriately in-between samples.

M. Thoroughly clean the bottom of the modified can lid by rinsing it and the interior of the Swagelok fitting with acetone. This removes condensed water and any organics from the lid.

N. Clean and/or ventilate sampling area to ensure it is free of any possible contamination of ignitable liquid residues (liquid or gaseous) prior to analyzing additional evidence.

O. Record appropriate notes in case file (e.g., differing solvents or extraction volumes, if used).

iii. Solvent Extraction (Debris, Liquids, Residues)

A. Debris:

1. While in a hood (or appropriate clean sampling area) rinse a suitable container with pentane (or other appropriate solvent), isolate the solvent, and treat as you would when analyzing an evidentiary sample. Label the extract as a negative control.

Note-02: At times a negative control may be labeled as a solvent blank.

2. Fill the same container (as above) half way with all or a portion of the contents to be analyzed. While in a hood (or appropriate clean sampling area) add an appropriate amount of solvent, such as pentane (the amount needed will vary with the amount of debris that was submitted). Add the solvent slowly, rinsing

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the debris in the process of adding. Ensure the solvent comes into contact with all of the debris for a complete extraction to take place.

3. Filter the pentane extract solution and collect into a second clean container.
4. Concentrate (either by gentle heating or evaporation) until a small amount of solvent remains.
5. Transfer the extract solution into an appropriate container (e.g., glass vial or test tube) and concentrate as necessary using $N_{2(g)}$.
6. Analyze the extract solutions using relevant instrumentation (e.g., GC/MS). Clean instrumentation appropriately in-between samples.

B. Liquids:

1. Alternate instrumental techniques can be employed, if necessary and approved (e.g., infrared, headspace-GC/MS).
2. Dilute liquid samples by adding an appropriate amount of pentane (or other solvent). The amount is dependent on the concentration of the items to be analyzed. Each item will be evaluated by the analyst before an appropriate amount is determined.
3. Filter and concentrate, if necessary. Analyze extracts using relevant instrumentation (e.g., GC/MS). Clean instrumentation appropriately in-between samples.

C. Residues:

1. Consider using adsorption/elution
2. Rinse submitted evidence using appropriate amount of solvent (e.g., pentane).
3. Filter and concentrate, if necessary.
4. Analyze extracts using relevant instrumentation (e.g., GC/MS). Clean instrumentation appropriately in-between samples.

c. Record appropriate information within either case notes or worksheets if not captured within procedure (e.g., techniques used, solvents, volumes, concentrating/dilution volumes).

i. Reagents and steps not explicitly stated in this procedure will be recorded within examination documents (e.g., dilutions, alternate extraction solvents, appropriate standards)

ii. Lot numbers of solvents and other appropriate materials will be recorded within examination documents.

d. Sample Preservation

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After each sample is analyzed solvent is added to the sample vial and it is placed into the submitted can. The sample vial should be placed under proper seal prior to being returned with the can. Evidence may be sub-itemized, if necessary. All evidence will be properly secured and accounted for during transfers and return.

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9 Instrumental Parameters

The following are the typical operating parameters for the instrument used in this procedure. With approval from the Lead Examiner (or higher) these instrument conditions may be modified to adjust or improve the procedure. Documentation of these parameters should be included with sequence data so that any instrumental parameter changes may be recorded in case files.

GC Oven:

Initial Temp	40 °C
Initial Time	2.0 min.
Ramp	10 °C/min.
Final Temp	300°C
Hold Time	12.5 min.
Equil. Time	0.1 min.

GC Inlet/Injector:

Inj. Vol.	1 µL
Mode	Splitless
Inlet Temp.	250 °C
Total Flow	55 mL/min
Septum Purge	4 mL/min

GC Column:

Type	HP-5MS (or equivalent	Mode	Constant Flow
Length	30 m	Init. Flow	1 mL/min
Diameter	0.25 mm	Ave. Linear Velocity	36 cm/sec
Film Thickness	0.25 µm	Carrier Gas	Helium (He)

Mass Spectrometer:

Transfer Line	300 °C
Source	230 °C
Quadrupole	150 °C
Low Mass	40
High Mass	400

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10 Decision Criteria

a. Evaluation of Data

i. The total ion chromatogram (TIC) and associated spectra for each sample are reviewed using appropriate GC/MS software. Each significant chromatographic peak should be reviewed to determine if it is an analyte of interest.

ii. Preliminary identification of an analyte or mixture can be achieved by operator recognition of particular chromatographic retention times/profiles, by instrumental library providing

matching mass spectral information, use of reference standard controls for comparison, using other accepted methods of evaluation (e.g., ASTM guidelines), or a combination.

iii. A full identification occurs when the sample analyte (or mixture of analytes) is compared to a positive control (e.g., an in-house validated standard) and the following criteria are met:

A. Chromatography

Retention Times: Analyte peak retention times (or relative retention times (RRT)) are within +/- 5% between sample and positive control.

Profile: The overall chromatographic pattern profile of a mixture should compare favorably to the chromatographic profile pattern of the known. While pattern matching of extracted ion profiles rarely gives perfect correlation with reference ignitable liquids, some sort of correlation should be present.

Ratios: Based on accepted/published criteria (e.g., ASTM), certain analyte ratios within mixtures should be present.

Note-03: Exceptions may be made based on the analyte/mixture in question. For example, some weathered gasoline samples may not exactly compare in all aspects to pure gasoline and analyst experience/knowledge may factor in to decision making.

B. Mass Spectrometry

1. Mass spectra will compare favorably to mass spectra from positive controls (e.g., reference standards). Comparison of mass spectral fragment pattern ion ratios should also compare favorably but may differ based on certain factors (e.g., large concentration differences between controls and samples). Spectra may also compare favorably to known library spectra, although library spectra may have been produced under different mass spectral parameters.

2. Mass spectral reference libraries (e.g., Wiley, NCFS, in-house) will be used during the evaluation process. An internal library may be made of substances analyzed in-house and may not be in published libraries. Entries within an internal in-house library should be compared to published spectra from reliable sources prior to use in casework.

iv. To accept GC/MS data the following should be followed:

A. Blanks

1. The blank prior to the injection of the sample must not contain significant unexplainable peaks. However, due the nature of samples analyzed within the

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Unit (i.e., burnt debris) certain substances may remain on a GC column even after the method has completed and thereby elute after multiple injections have been performed. If retention times significantly differ (i.e., minutes) then column carryover is likely the issue (as opposed to injection carryover) and data may be acceptable. Consultation of an FSE2 or higher should occur in these situations if considering acceptance of data.

Note-04: Significant Peaks: To justify the existence of a significant chromatographic peak the peak's baseline signal-to-noise ratio (SNR), based on height, should equal or exceed three (3).

2. If the analyte(s) being identified in a sample is present in the blank just prior to its injection then that sample peak(s) should be ten-times (10x) greater in height than that of the analyte peak(s) from the previous blank.
3. When it is known that there is a carry-over issue with a sample then multiple blanks can be injected to minimize the carryover and only the blanks after a sample and just prior to the next sample need to be evaluated.
4. Blanks injected after a sample injection should be free of any analytes of interest. Any analytes found in the blanks may indicate that the method's temperature program was not long enough to elute all the peaks from the GC column.

B. Controls

1. Negative controls will be free of analytes of interest and no significant unexpected peaks should be present. Explainable peaks will be acceptable.
2. Positive control analytes must have acceptable chromatographic and mass spectral data.
3. Unexpected results within positive or negative control data should be reported to the Lead Examiner or higher for evaluation.

v. In cases where identifications cannot be made due to the lack of obtaining a positive control then indications can be made, if appropriate (e.g., based on mass spectral library data). Consultation with other analysts/technical reviewers or the Lead Examiner (or higher) may be appropriate in such situations.

vi. Positive controls should be analyzed contemporaneously when identifying analytes in evidentiary samples or when specifically looking for analytes in samples which result in negative results.

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Note-05: If reference standards/positive controls have been analyzed previously (i.e., not contemporaneously) then such data may be used for comparison purposes if no significant instrument/method changes have occurred (e.g., column clipping, column change, temperature program change).

vii. Documentation: Appropriate instrumental printouts, including reagent blanks and controls, will be included in the case file such that an independent reviewer would be able to readily interpret analyst conclusions.

A. All appropriate chromatograms will be retained in case files. Label the chromatographic peaks for all analytes being identified.

B. All identified analytes will have documented spectra and appropriate comparative reference spectra (e.g., from positive controls and/or spectral libraries) within the case files.

C. In samples where no analytes are identified the analyst should include the TIC along with any appropriate extracted ion chromatograms (EICs). If there are significant peaks that cannot be identified, or attributed to common artifacts, then such spectra should be included in the case file.

Note-05: Mass spectral data is considered significant if, by not including such data in a case file, it would diminish be detrimental to the quality of the examination/results.

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11 Calibration

Not applicable.

12 Uncertainty

Not applicable – this is a qualitative procedure only.

13 Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment should be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Biological specimens will be handled using universal precautions and will be treated as biohazardous. Potentially contaminated items and surfaces will be cleaned prior to use, especially. When casework samples are being processed/analyzed brown paper (or other similar barrier) should be placed in between the surface and the specimens.

14 References

a. General Laboratory (GL) Manual and Procedures

b. ASTM Standard Methods

c. GC-MS Guide to Ignitable Liquids

d. Bertsch, W., Holzer, G., and Sellers, C. S., *Chemical Analysis for the Arson Investigator and Attorney***,** Huthig Buch Verlag: Heidelberg, 1993

e. Hendrikse, J., Grutters, M., Schäfer, F. *Identifying Ignitable Liquids in Fire Debris. A Guideline for Forensic Experts*. Academic Press, 2016

f. Kelly, R. L., and Martz, R. M., *Accelerant Identification in Fire Debris by Gas Chromatography/Mass Spectrometry Techniques,* Journal of Forensic Sciences, Vol. 29, No. 3, July 1984, pp. 714-722

g. Stauffer, E., Dolan, J.A, Newman, R., *Fire Debris Analysis*, Academic Press, Burlington, MA, 2008.

h. Kelly, R. L., and Martz, R. M., *Accelerant Identification in Fire Debris by Gas Chromatography/Mass Spectrometry Techniques,* Journal of Forensic Sciences, Vol. 29, No. 3, July 1984, pp. 714-722

i. Stauffer, E., Dolan, J.A, Newman, R., *Fire Debris Analysis*, Academic Press, Burlington, MA, 2008.

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Rev. #	History
2	Slight format change. Replaced section with unit. Modified the 'Materials' section. Added a 'Standards and Controls' section specifically requiring controls to be analyzed. Re-worded the 'smell' portion of the procedure. Decreased injection volume in 'Heated Headspace' section from 1 mL to 0.5 mL. Fixed type from 180 °C to 80 °C within 'Absorption/Elution' section. Decreased amount of pentane used during extraction within 'Absorption/Elution' section from 0.5 mL to approximately 0.3 mL. Added requirement to record lot numbers and solvents within examination documents. Added a 'Revised History' section to the document.
3	Revised sections 4c and 5b to reflect that if samples are accelerant-free, the analysis of a blank after that particular sample is not required.
4	General format change throughout document ; Added sections: 'Introduction,' 'Scope,' 'Principle,' 'Specimens' ; Updated the 'Materials' section ; Updated 'Samples and Controls' section ; added 'Sampling' section ; updated 'Procedure' section to be in-line with current practices ; specified documentation needs within casefiles ; added steps to address potential cross-contamination ; Added 'Instrumental Parameters' section ; Added 'Decision Criteria' section ; updated references. Updated how to detect odors within Section 8.

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A. Purpose

The analysis of samples by scanning electron microscopy (SEM) using energy dispersive X-ray spectroscopy (EDS) is a method employed by the Chemistry Unit of the laboratory. The following procedure shall serve as a guideline for analyzing and interpreting results from sample analyses.

B. Scope

This procedure serves as a general guideline for all procedures that involve the use of SEM analyses. Application of this procedure to evidence requires knowledge of general concepts, methods of a scanning electron microscope (SEM), and understanding of an energy dispersive X-ray spectrometer (EDS). Methods requiring use of SEM/EDS require operational proficiency of each instrument. This knowledge can be obtained from the references provided, training from manufacturers or principal operators within the Unit, and schools in SEM and EDS.

C. Responsibility

Analysts authorized to use the SEM/EDS instrument.

D. Equipment/Materials/Reagents

Listed below are general supplies required for electron microscopy (EM). Additional supplies unique to a particular method are indicated within that topic.

1. Scanning Electron Microscope with Backscattered Electron (BE) detector (Hitachi S-3700N or equivalent)
2. Energy Dispersive X-ray Analyzer (EDAX Genesis or equivalent)
3. Aluminum Stubs (Electron Microscopy Services)
4. Adhesive-backed tabs or double-sided adhesive tape (Electron Microscopy Services)
5. Copper pure element reference material (Sigma-Aldrich or equivalent)

E. Standards/Controls/Reagents

A copper (Cu) pure element reference material is analyzed each day on which casework is performed in order to check the SEM and to measure the performance of the EDS system. The quality control (QC) data from the Cu reference material will be placed into the QC logbook for the instrument. This QC check should be performed prior to evidentiary analyses.

F. Calibration

Not applicable.

G. Sampling

Not applicable.

H. Procedure

1. Ensure instrument is ready for analyses. Never turn on the electronics to the EDS detector without an adequate level of $N_{2(l)}$.
2. Vent the system and open chamber.
3. Insert the sample into the specimen holder. Never touch the surfaces of the discs with bare hands - use gloves appropriately. Use tweezers around step of stubs to manipulate discs.
4. Note position of samples.
5. Close chamber and evacuate.
6. After the chamber is properly evacuated (i.e., green light) turn on the power at the main SEM console. Ensure that the filament, operating voltages, working distance, magnification, and spot size are all at their correct settings and are operating properly.
7. Ensure the correct detector is selected (backscatter electron (BSE) versus secondary electron imaging (SEI)).
8. Adjust brightness and contrast as needed in order to obtain an optimal SEM image.
9. Obtain a good overall view of the sample. The raster area of the SEM is selected in order to yield the best possible sample area. If it is not possible to select one large area, several smaller areas can be analyzed and the data can then be compared and/or summed.
10. If needed, specific particles can be magnified and analyzed.
11. Capture appropriate images, as needed.
12. Collection parameters should be captured, recorded, or noted.
13. Ensure proper collection times are selected (e.g., 2 minute live time) and collect spectral data, as needed, using the EDS detector.
14. Save spectra using appropriate filenames. Using generic filenames will overwrite spectra and data may be lost.
15. Evaluate spectra for elemental information. Final elemental peak determination must be based on user interpretation of valid energy values appropriate to each element's theoretical values.
16. Document appropriate images and spectral data (i.e., hardcopies).
17. Analyze appropriate controls (positive, negative), as necessary.
18. Turn off electronics, properly vent system, and remove sample(s).
19. System should remain under vacuum with electronics off when not in use.

I. Evaluation of Data

1. Elemental Determination:

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a. While preliminary elemental peak information can be initially obtained using automated features, final elemental peak determination must be based on user interpretation of valid energy values appropriate to each element's theoretical values.
b. Use a systematic approach to elemental identification so that misidentifications or omissions of minor peaks does not occur. This approach includes consideration of X-ray line families, spectral artifacts, escape peaks, sum peaks, and overlaps.
c. Reference lines, or energies, may be obtained from several sources including energy slide rules, computer generated 'KLM markers' that may be superimposed on the spectrum, and published tables. Additionally, manufacturers often provide an automatic element identification application. Often these aids are used to complement one another.
d. Identification can begin with high-energy peaks and major peaks. High-energy peaks will typically have less overlap than lower energy peaks within a spectrum. If a major peak is present, then a complete family of peaks should be able to be observed. Each line within the family should be labeled with elemental symbols. Spectral artifacts, including sum peaks and escape peaks associated with major peaks, should be identified and labeled.
e. As spectral interpretation alternates between the identification of major and minor peaks, the vertical (counts) scale should be adjusted to reveal the required detail. In addition to the higher energy peaks, note the presence of any lower energy families and their expected relative intensities. Individual asymmetric peaks and inconsistent peak ratios within a family may indicate a peak overlap. Elemental identification is aided by superimposing and scaling KLM reference lines on the spectrum or referencing the actual spectrum of an elemental standard. The analyst should become familiar with the characteristic pattern and relative intensities of peaks of various atomic numbers.
f. Following the identification of major components, lower intensity peaks and overlapped peaks are identified. Identification of minor elemental components is often limited by the number of characteristic peaks present in a spectrum.
g. The presence of an element can be considered unequivocal only when a distinctive unique set of lines is produced, or when a single peak occurs at an energy where it cannot be mistaken for another element or spectral artifact. Unequivocal identification may not be possible if an element is present in low concentration or if lines required for confirmation are overlapped with the lines of other elements.
h. If an automatic identification application is used, peak identification must be confirmed by the analyst.
i. If identification is unequivocal, then each individual peak should be labeled with the corresponding elemental symbol. If the identification is probable but not unequivocal, then the peak label should indicate so (e.g., by parenthesizing the elemental symbols or by putting a 'poss' for possible).
j. For documentation, spectra should be displayed on a scale that clearly demonstrates the peaks identified. Multiple scales may be necessary.

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2. Spectral Comparison:
   a. Comparisons of elemental composition can be made by the direct spectral comparison of materials. These spectra can be collected at the same time as that of the questioned material.
   b. Spectra are acquired from materials to be compared, using similar analytical conditions.
   c. Overlay spectra of materials to be compared. Vertical offset and horizontal and vertical scales should be adjusted to permit thorough spectral comparison.
   d. Depending upon their correspondence, the spectra can be characterized as similar, generally similar, and different.
      i. Spectra should be considered "similar" if they only exhibit channel-to-channel intensity variations.
      ii. Spectra should be considered 'generally similar' if they exhibit only a few slight differences. These differences may be qualitative or quantitative (intensity). Permissible qualitative differences in materials considered to be generally similar may consist of the absence of trace amounts of common elements. Permissible quantitative differences may consist of slight ratio differences in the peaks heights of major or minor elements.
   e. Spectra should be considered 'different' when they exhibit distinct and significant differences. These differences may be qualitative or quantitative. Significant qualitative differences may consist of the absence of trace amounts of rarely occurring elements or the absence of a significant peak from a more commonly occurring element. Quantitative differences may consist of significant ratio differences in the peak heights of major and minor elements.
   f. There is not an absolute distinction between the definitions, 'generally similar' and 'different.'

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J. Instrumental Conditions

1. General operating conditions can be:

Beam Voltage	Variable depending on sample (e.g., 25 kEV)
Beam Current	Adjusted to yield 30% deadtime
Live counting time	~200 seconds (bulk analysis) ~100 seconds (specific particle)
Pulse Processor time constant	Mid-range value
Spot-size	Variable
Working Distance	Variable (e.g., 10mm, 11.2mm)

2. Changes to General Operating Conditions:

   Changes to suggested instrumental conditions may be needed under the following circumstances:

   a. Beam voltage is increased when higher energy line excitation is required
   b. Beam voltage is decreased when greater spatial resolution is required
   c. The pulse processor time constant is lengthened when greater spectral resolution is required
   d. The pulse processor time constant is shortened when a greater count rate is required (e.g., for trace element analysis or construction of elemental distribution maps)
   e. Detector-to-sample distance can be reduced to increase X-ray collection efficiency
   f. Beam current is increased when the X-ray count is too low. Beam current may be increased by decreasing the condenser lens current and/or increasing the final aperture size.
   g. Beam current is decreased when the X-ray count is too low. Beam current may be decreased by increasing the condenser lens current and/or decreasing the final aperture size.

K. Decision Criteria

If spectral differences are detected, it is likely that the materials that produced them are not similar in composition; however, several alternative explanations are possible:

1. Dissimilar sample geometry.
2. Spectral collection from non-representative areas of an inhomogeneous sample.
3. X-ray fluorescence contributions from elements in adjacent structures.
4. X-ray contribution from extraneous material.
5. Contribution of carbon from a carbon SEM mount.

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L. Uncertainty of Measurement

Not applicable.

M. Limitations

1. Adhesive may transfer to the sample, adversely affecting subsequent organic examinations.
2. While considered a non-destructive technique, samples usually are not removed and reanalyzed using other methods after adhered to an SEM stub.
3. If the dimensions are limited, a sample may be difficult to orient precisely.
4. This technique is limited to solid samples.
5. X-ray fluorescence may excite characteristic X-rays from structures remote from the area visibly selected for analysis.
6. Elements present in trace concentrations, under average conditions, are generally not detected below 0.5%.

7. Although the natural X-ray line width is approximately 2eV, EDS resolution is generally no better than approximately 140eV. As a result, overlap of peaks in the EDS spectrum of materials containing several elements may occur. Some commonly occurring overlaps encountered in EDS are TiK_{α}/VK_{β}, VK_{β}/CrK_{α}, CrK_{β}/MnK_{α}, MnK_{β}/FeK_{α}, FeK_{β}/CoK_{α}, $PbM_{\alpha}/SK_{\alpha}/MoL_{\alpha}$, $BaL_{\alpha}/TiK_{\alpha}$, KK_{β}/CaK_{α}, $ZnL_{\alpha}/NaK_{\alpha}$, PK_{α}/ZrL_{α}, and $AlK_{\alpha}/BrL_{\alpha}$.
8. Similar spectra do not necessarily indicate identical composition. They are similar only down to the detection limits of this analytical method.
9. The particular methods employed for each sample examined will depend upon sample size, sample suitability, analytical request, and available accessories. Variations to sample preparation may occur.

N. Safety

1. General precautions common to EM laboratories include minimization of contact with cryogens, venting or filtering of rough pump discharge, and avoidance of direct exposure to beryllium metal.
2. Use universal precautions when handling potentially biohazardous materials. Refer to the GL *Safety Procedure* and appropriate MSDS/SDS documents for additional information. Take standard precautions for the handling of chemicals and sharp cutting instruments.

O. References

1. Hitachi S-3700N operator's manual.
2. EDAX Genesis Manual(s)
3. Bearden, J.A. "X-ray Wavelengths," *Reviews of Modern Physics*, 39 (1), No. 1, 1967.
4. Goldstein, et al. *Scanning Electron Microscopy and X-ray Microanalysis*, second edition, Plenum Press, New York, 1992.
5. Ward, D.C. "A Small Sample Mounting Technique for Scanning Electron Microscopy and X-ray Analysis," *Forensic Science Communications*, 1(2), 1999
6. Ward, D.C. and Sibert, R.W., "The Use of Vacuum Evaporation of Metals for Surface Feature Enhancement," *AFTE Journal*, 18(4), 1986.

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Rev. #	History
1	Old procedure (CH-SOP-25) was accidentally retired and this will serve as a new document with new title. Added 'Scope' section. Significant format and verbiage changes throughout document. Added more specification to the 'Procedure' section. Added the following sections to the document: Scope, Standards/Controls/Reagents, Evaluation of Data, Instrumental Conditions, Decision Criteria, Limitations, and Safety. Added more references. Added Revision History section.

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Chemistry Unit – GSR Case Review Checklist

Technical Reviewer Name (print): _____ Date: _____

Lab/Case Number: _____	**Yes**	**No**	**N/A**
Evidence description clear			
Case notes clear			
Photographs correctly annotated			
Technical data included and appropriate			
Technical data filenames appropriate – Sample			
Technical data filenames appropriate – PLANO			
Technical data filenames appropriate – Copper			
Technical data filenames appropriate – Blank			
Copy of the appropriate PLANO(s) present (annotated and printed per SOP)			
Copy of the Copper standard(s) present (annotated and printed per SOP)			
Appropriate blank(s) included			
Findings on the report backed by the technical data			
Chain of custody reviewed and appropriate			
Technical Review Milestone in JusticeTrax Acceptable		*See TR Notes*	
Technical Reviewer's Notes (if applicable ; mandatory if TR milestone was unacceptable):			

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Administrative Reviewer Name (print): _____ Date: _____

	Yes	**No**	**N/A**
Case demographics correct: Name			
Agency information			
Item numbers correct			
Description reviewed for typos			
Cross outs initialed			
Analyst's initials and case number on each page			
Administrative Review Milestone in JusticeTrax Acceptable		*See AR Notes*	
Administrative Reviewer's Notes (if applicable ; mandatory if AR milestone was unacceptable):			

Title: Analysis of primer-Gunshot Residue (pGSR) evidence

1. Introduction:

This procedure is used to analyze samples for the possible presence of primer-gunshot residue (GSR or pGSR). Propellant within ammunition is ignited by chemicals after the primer is activated from a physical strike within a firearm. Propellant within a firearm cartridge burns and generates gases that force projectiles (i.e., bullets) out of a firearm. Expended residues from the chemicals within the primer are potentially deposited on the hands of shooters and anything else in close proximity of the firearm's discharge. The detection of residues from primer discharge are based on the "Sinoxid" primer formulation which contains lead styphnate (possibly with other lead compounds), antimony sulfide, and barium nitrate. The discharge of firearms produce a range of chemicals, including residues from explosive material, oxidizers, reducing agents, sensitizers, fuels, binders, and primer. (Romolo & Margot, 2001). Collectively, the chemicals being produced can be referred to as gunshot residue (GSR). In current forensic practices, however, the term GSR refers specifically to inorganic particulate residue that is formed after firearm discharge related to primer chemicals, including metal oxides. The term "primer discharge residue" (or pGSR) is sometimes used to make this distinction more clear. The chemicals from gunshot residue can also be broken down into inorganic and organic GSR. The particles of interest within this procedure will only be inorganic, particularly those containing Ba, Sb, and/or Pb.

Gunshot primer particles can be transferred to clothing or other nearby individuals. Residues can also be transferred indirectly based on touch-to-touch interactions, a process commonly referred to as secondary transfer. Because of the possibility of secondary transfer and environmental interferences, it can be challenging to interpret analytical results, be they positive or negative. The detection of pGSR particles occurs in two stages within this procedure. The first stage is a screening portion wherein analytical software, based on certain parameters, determine possible pGSR particle(s) on a GSR stub's surface, and summarize particle information with location. The second stage involves examiner confirmation. The number of pGSR particles reported by the software during the preliminary screening portion of the exam will be limited and may be greater than the number of particles actually confirmed to have characteristics indicative of pGSR.

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2. Scope

This procedure is limited to the qualitative screening of specimens. Only inorganic particles will be analyzed. Comparison of inorganic particles to specific types of primer chemicals is possible, but prior approval is required. The number of characteristics (i.e., specific elements [barium (Ba), antimony (Sb), and lead(Pb)], shape, size) present within a sample will determine how strongly associated detected particles will be when compared to primer-gunshot residue particles. Decision criteria will be in the form recommended by the relevant forensic scientific community.

3. Principle

Inorganic residues captured on adhesive carbon-coated stubs are analyzed for the presence of particles that contain general morphology and size, along with select elemental compositions, which can be found within discharged primer ammunition particles. Using a scanning electron microscope with associated detectors, minute particles associated with gunshot residue primers can be detected. A combination of morphology, size, and elemental information is used.

4. Specimens

The analysis conducted within this procedure involves the examination of GSR stub collection devices which have been used on surfaces of evidence. Collection devices typically are stubs with cylindrical surfaces that contain carbon with adhesive. These stubs should be stored within plastic containers and are usually submitted within cardboard boxes (GSR kits). While 4-stub GSR kits from hand samplings have historically been accepted for analysis, 2-GSR stub kits are preferred. Additionally, cotton-tipped applicator swabs have been used for atomic absorption (AA)-related GSR analyses in the past, but these are not typically accepted. Transfer of AA swabs to GSR stubs is possible – however it must be understood that not all particles may transfer from the swabs to the stub surfaces. Instrumentation is used to determine if particles on GSR stubs contain certain characteristics related to primer-gunshot reside.

While the GSR kit may have a submission number, each GSR stub will have its own item number and description.

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5. Responsibility:

Analysts authorized to conduct examinations within the GSR category of testing.

6. Equipment/Materials/Reagents

a. Stubs with carbon adhesive (GSR stubs)

b. Tweezers

c. Scanning electron microscope (SEM) with secondary electron imaging (SEI), backscatter electron (BSE) and energy-dispersive X-ray Spectroscopic (EDS) detectors

d. Copper reference standard, Sigma or equivalent

e. PLANO reference standard (or equivalent)

f. Carbon coater

g. Methanol (Reagent grade)

h. Water (Deionized (DIW or equivalent)

7. Standards and Controls

Positive and Negative Controls: Can be purchased (e.g., PLANO) or generated in-house (e.g., GSR stub from known firearm discharge). Store either in SEM chamber or within plastic container – can be carbon coated. If generated in-house the controls must be verified appropriately and the documentation will be retained. Controls may be sent to an outside laboratory for further confirmation . Any documentation must be retained for QA/QC purposes. All controls will have unique identifiers (i.e., lot numbers) which can be incorporated into case notes/files.

8. Calibration

This procedure is qualitative only.

9. Sampling

Not applicable.

10. Procedure:

a. Preparation

i. Upon starting the analysis document information from the sample labels on appropriate worksheet(s).

ii. Examiners will wear gloves during sample handling and will change gloves with new samples. Tools (e.g., tweezers) will be pre-cleaned with water and methanol (or equivalent solvent) when used to handle evidence. The stems of GSR stubs will be used to avoid contamination. The touching of GSR disc adhesive surfaces with other objects will be avoided.

iii. Ensure item number information is recorded on each GSR stub base, as well as their respective containers. Label control stubs accordingly. Add lab number information to stubs, if possible.

iv. Appropriate negative and positive controls will be analyzed. A negative control GSR stub is used to monitor ambient conditions. Both a positive and negative control will be analyzed with each batch of samples.

v. Individually place stubs in proper locations using the appropriate SEM sample/stage holder and record the positions within case notes or worksheets.

vi. It is recommended to mark the side of the GSR stubs and align with the markings of the sample holder. This assists with reproducibility if GSR stubs have to be re-mounted and analyzed in the future.

vii. All stubs will be itemized (or sub-itemized) within the Laboratory Information Management System's (LIMS) JusticeTrax LIMS-Plus software.

viii. If AA swabs or other items of evidence (e.g., clothes) have been submitted, each item (or group of items if packaged together) will be sampled using GSR stubs as best as possible. The goal is to transfer any potential pGSR particles from the items to the GSR collection stubs.

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1. Since this involves residue material, examination areas will be cleaned with MeOH and lined with brown examination paper in-between each item of evidence and proper negative controls will be collected.

Note: If swabs never touch the brown paper/examination area surface, then negative controls for AA swabs will be handled differently (i.e., not be required) than negative controls for items actually resting on examination surfaces (e.g., clothing).

2. Transfer of actual fibers from swab tips or cloth-like items should be avoided.
3. GSR stubs from such samples may be carbon coated in order to reduce 'charging effects,' but this is not required.
4. If carbon coating is performed, such information and description of the carbon coating process (including instrument model information) will be recorded within the Examiner's case notes.
5. Sub-items will be created within LIMS-Plus when GSR stubs are used to sample items of evidence. Appropriate sub-item numbers and lab numbers will be recorded on (or attached to) the newly created GSR stubs, if possible. This creation will occur on the day the [GSR stub] sub-items are generated.

ix. Other samples (e.g., dirty stubs) may be carbon coated in order to reduce the amount of 'charging' interference when the GSR software evaluates samples. Carbon coater information and the process for coating will be recorded within Examiner case notes.

b. Scanning Electron Microscopy [with multiple detectors] Analysis

A scanning electron microscope (SEM) with a backscatter electron (BSE) detector, secondary electron imaging (SEI) detector, and an energy dispersive X-ray spectroscopic (EDS) detector will be used for this analysis. A combination of software and manual examination is used to render conclusions.

i. Quality Assurance/Quality Control (QA/QC):

1. Appropriate quality control (QC) reference standards will be analyzed according to the QA/QC procedure for that instrument.
2. When the instrument is ready set operating voltage, working distance, magnification, and spot size on the SEM control screen to appropriate levels.
3. Focus the microscope and adjust Brightness and Contrast to obtain good image(s).

ii. Automated preliminary analysis using BSE and EDS detectors, along with GSR software:

1. Select BSE (backscatter electron) mode on the SEM control screen and adjust appropriate brightness/contrast level and other settings.
2. Set up the parameters within the Genesis GSR analysis program.
3. Move and locate the sample stubs.
4. Enter the sample labeling information (e.g., case #, item #).

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5. All appropriate collection information (e.g., software printouts, screen capture images, case notes) will be printed and stored in the casefile.
   Any information not readily printed will be accurately described in Examiner case notes (e.g., how brightness and contrast were adjusted and specifically set for a given analysis).
   The information within the case file must be enough so that another authorized Examiner will be able to reproduce the findings within the report.
6. All stubs will be set to be scanned and analyzed automatically by the software based on morphology, intensity measurements, and preliminary elemental determination.
   Initial data from particles will be classified by the software.
   The maximum number of automatically classified 3-component particles (i.e., 3-COMP) within a GSR kit for a single person (or a set item) can be set at a user-defined number (e.g., 10) within the collection parameters for each stub.
7. The entire surface of stubs do not have to be completely analyzed. However, at least 70% of stub areas should be mapped with fields in the software. The process of how the software is set up to search each stub (i.e., the start/end path) can be user-defined. For example, if ten (10) 3-COMP particles are detected by the time only 10% of the stub has been analyzed and the analysis automatically stops, then that is acceptable if at least three (3) of the particles can be verified as containing GSR-related characteristics.

Note: If at least three (3) 3-component particles cannot be confirmed when using a limited-number of automatically classified 3-COMP particles, then more of the stub's surface will be analyzed.

8. Start the GSR analysis.
9. Select particles will be re-examined manually after preliminary analyses of all of the stubs by the software have been completed.

iii. Manual confirmation using EDS and SEI (GSR kits of the same individual, item, or area of interest)

1. When the analyses from the software are completed a result summary is created.
2. Click on a particle number to recall the stored spectrum and designated particle image.
3. If a preliminary EDS spectrum reveals the possible presence of lead, antimony, and/or barium, move the stage to the designated particle field position.
4. Collect the secondary electron image (SEI) of any particle used to render a conclusion within the report. The SEI images will contain adequate magnification, resolution, and clarity to document pGSR particles. Such images will be saved electronically with filenames unique to the case, printed, and printouts kept within the case file.
5. Re-collect a new EDS spectrum using a 100 second live time acquisition (thus revealing enough data to adequately determine if lead, antimony, and/or barium are present). Spectra used in rendering conclusions will be saved electronically with a filename unique to the case, printed, and the printouts kept within the case file.
6. Print out a result page with particle images and spectra.

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7. During the analysis of a stub within a GSR kit (or within a group of stubs for an item of evidence), if three (3) pGSR-related particles are found and confirmed (i.e., contain Ba, Sb, and Pb, and have shapes and sizes corresponding to pGSR particles), then no further examination (screening or confirmation) of other particles within that same stub will be performed.
8. During the analysis of a stub within a GSR kit (2-stub or 4-stub kit, or within a group of stubs for an item of evidence), if one stub has at least three (3) pGSR-related particles (i.e., all contain Ba, Sb, and Pb, and all have shapes and sizes of pGSR particles) then confirmation of particles from other stubs within the same kit (or within a group of stubs for an item of evidence) is not required.
9. The maximum number of particles [of the same component number] to be confirmed within a GSR kit (or within stubs of the same person/object) will be six (6).
   For example: If only one or two 3-COMP particles are identified, then no more than six (6) 2-COMP or 1-COMP particles are to be confirmed.

Note: There must be a minimum of three (3) such particles per stub in order to stop confirming additional particles. For example, if only two (2) 3-element pGSR particles were found on one stub, then the Examiner would only stop when three (3) such particles were identified on multiple stubs. No more than six (6) 3-element particles will be confirmed within a GSR kit (or similar items of evidence). If no '3-COMP' particles exist then the above steps will be followed for 2-COMP particles. Correspondingly, if no '2-COMP' particles exist then the above steps will be followed for '1-COMP' particles.

10. If submitting agencies specifically request that all stubs within a GSR kit (or within all submitted clothing) be fully analyzed, then all will be analyzed. This specific request must be documented in writing by the submitting agency and will be kept within LIMS-Plus.
11. Document results (e.g., using a worksheet) and record all appropriate case and sample information within case notes. All data printouts will contain the instrument name (i.e., SEM-01, SEM-02), date(s) of acquisition, and the Analyst(s) identifiers (i.e., initials or name) who acquired and is responsible for the data.
12. Instrumental parameters must be recorded within the examination documents (e.g., beam voltage, collection time, magnification, working distance). This should preferably be done electronically, but handwritten notes are acceptable.
13. All data (e.g., images and spectra) from particles used to render conclusions within final laboratory reports must be maintained within case files.

c. Remove GSR stubs from the SEM chamber and put each back into their original containers.

d. Properly seal and store all stubs in an approved and secure location.

11. Decision Criteria:

The following criteria are used as guidelines in determining the acceptability of the data produced in this procedure. Results from the automated GSR software analysis of stubs provides a list of possibilities which

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have select characteristics of pGSR particles. Manual acquisition of EDS spectra, along with particle imaging allow Examiners to confirm pGSR particle qualities.

a. Software results

i. The Genesis GSR software will produce a list of particles which are possibilities for GSR particles based on size, image moiety, and elemental composition (i.e., 1-component, 2-components, or 3-components).

ii. The software results will be used to select particles for confirmation purposes. Data from the software will not solely be used to render a conclusion within laboratory reports.

iii. If an evidentiary stub is negative for '3COMP' particles (i.e., presumptive positive '*characteristic* pGSR particles'), then the number of '3COMP' particles found from the software's analysis of the positive control stub (used for the batch analysis) must be at least the user-defined maximum number of automatically classified 3-component particles (e.g., ten (10) in step 10. B. ii. 6 of this procedure). If not, then the instrument will be re-optimized and the negative, postitive, and non-3-component particle-evidentiary stubs will be re-analyzed.

b. Morphology and Size

Particles identified as characteristic of or consistent with gunshot residue primer using this method are often spheroid particles, typically between 0.5 μm and 5.0 μm in diameter. The remainder are irregular in shape or vary from 0.5 μm to 100 μm + in size, or both. In general, it is not consistent with the mechanisms of pGSR formation to find particles by SEM displaying crystalline morphology. However, such particles have occasionally been observed in known primer-GSR residues. Since morphology can vary greatly, it will never be considered as the only criterion for identification of pGSR.

c. Elemental Characteristics

The elemental composition is the most diagnostic property to determine if a particle could be a pGSR particle. Within this procedure the three elements: barium (Ba), antimony (Sb), and lead (Pb) will be used to determine the possibility of a pGSR particle. However, it is understood that some primer components may not have compounds that contain such elements.

When appropriate, the elemental composition of the recovered residues can be compared with case-specific known source primer formulations. Occasionally, pGSR particles with apparent unusual elemental compositions can be encountered and elemental compositions of evidence can be compared to case-specific sources, such as cartridges or ammunition/weapon test fire deposits, if specifically requested and approved by the Deputy Director.

i. Particles classified as being <u>characteristic of</u> pGSR must contain the following elemental composition within the particle's EDS spectra: Barium, Antimony, and Lead.
These particles have compositions which are infrequently found in particles from any other source other than pGSR-related particles.

ii. Particles will be classified as being <u>consistent with</u> pGSR when they contain one (1) of the following elemental compositions:

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1. Lead and Antimony, or
2. Lead and Barium, or
3. Antimony and Barium

These particles have compositions which are also found in particles from non-firearm sources.

iii. Particles classified as commonly associated with pGSR must contain only one (1) of the following elements: Barium, Antimony, or Lead
These particles have compositions which are also found in particles from environmental sources. When such particles are found on stubs from a GSR kit or from clothing that contain 'characteristic' and/or 'consistent with' particles, then they have more significance towards pGSR-related particles. However, when no 'characteristic' and/or 'consistent with' particles are found with 'commonly associated' particles, then such particles have little significance to pGSR presence.

iv. The following classifications will be used within laboratory reports to indicate whether a residue particle possibly resulted from gunshot primer (from highest to lowest): 'characteristic of' > 'consistent with' > 'commonly associated with'. Individual elements will not be listed within reports.

d. Positive/Negative Control

Results from the data analysis of controls must correspond to the appropriate control.

i. Positive controls will have at least three (3) pGSR particles confirmed (along with supporting data and SEI images).

ii. Negative controls will have no particles that are related to pGSR which need to be confirmed.

1. If the negative control stub has one or more 3-component particles that are confirmed, then the Lead Examiner (or higher) will be notified. All stubs analyzed in the same batch will be reported as inconclusive with an explanation in the report.
2. If the negative control stub has one or more 1-component or 2-component particles that are confirmed, then the Lead Examiner (or higher) will be notified and evidentiary stubs in the same batch that have 1-COMP or 2-COMP data will be rejected and, unless at least three (3) 3-COMP particles have been confirmed, the analyses will be reported as inconclusive with an explanation in the report.

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12. Limitations

The analysis of evidence for the presence of GSR (pGSR) can provide information to an investigation. However, the presence or absence of GSR (pGSR) cannot absolutely determine whether a person discharged a firearm or was in the presence of a firearm being discharged.

While the classifications used to indicate whether a residue particle possibly resulted from gunshot primer are ranked (from highest to lowest): 'characteristic of' > 'consistent with' > 'commonly associated with,' the determination that particles found on a GSR stub are, in fact, definitively from a discharged firearm

cannot be made using this procedure.

The absence of particles either 'characteristic of,' 'consistent with,' or 'commonly associated with' pGSR does not mean that the sampled evidence was not in the close proximity of a discharged firearm. Studies have shown that evidence sampled in close proximity to a discharged firearm may not contain pGSR particles.

Some ammunition contain lead-free primers – thus causing evidentiary stubs from such firearm discharge areas to only have the possible result, 'consistent with pGSR' or 'commonly associated with pGSR' to be in final laboratory reports. Other ammunition may have other limited elements within their primers, causing similar limitations to occur within final laboratory reports.

13. References

Hitachi S-3700N Scanning Electron Microscope Operators Manual on the help menu of the program.

EDAX Genesis GSR Analysis software user's manual and movie (Appendix B).

Law Enforcement Development Group of the Aerospace Corporation. "Final Report on Particle Analysis for Gunshot Residue Detection". Prepared for the National Institute of Law Enforcement and Criminal Justice Law Enforcement Assistance Administration, U.S. Department.

Meng, H.H., Caddy, B., "Gunshot Residue Analysis-A Review", Journal of Forensic Sciences, 1977; Vol.42, No.4, pp.553-570

Wolten, et.al, "Particle Analysis for the Detection of Gunshot Residue. I: Scanning Electron Microscopy/Energy Dispersive X-ray Characterization of Hand Deposits from Firing", JFS, Vol. 24, No. 2, April 1979, pp 409-422.

Wolten, et.al, "Particle Analysis for the Detection of Gunshot Residue. II: Occupational and Environmental Particles", JFS, August 1978.

GSR Summary, Dennis Ward, FBI Academy.

ASTM Designation E 1588-17, "Standard Practice for Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy.

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Revision #	Revision History
2	Revised title. Updated Responsibility section. Updated procedure. Removed references to manuals, other SOPs, and to appendices. Added minimum number of confirmatory spectra to be analyzed and documented per sample. Added step to allow that not all stubs in a GSR kit needed to be fully analyzed if certain parameters were met. Added requirement to include instrumental parameters within case file. Added positive and negative controls. Added a 'Revision History' section to the document.
3	Removed the requirement of company-specific instruments (e.g., Hitachi, EDAX) within section C. Changed and updated section C.2.c.6. so that the number of particles to be confirmed, printed, and kept within case files could be cumulative (i.e., from an entire GSR kit) and not necessarily restricted to three (3) particles per disc/stub.
4	Edited title, removed "Purpose" section. Added "Introduction", "Scope", "Principle", and "Specimens" sections. Edited "Responsibility" section. Added "Equipment/Materials/Reagents", "Standards and Controls", "Calibration", and "Sampling" sections. Edited "Procedure" section to include detailed guidelines for handling of various types of GSR casework, utilization of the SEM and associated detectors, analyzing and confirming particles, the minimum and maximum number of particle types to be confirmed, and the necessary documentation for GSR casework. Added "Decision Criteria" and "Limitations" sections.
5	Changed the Decision Criteria section (11. A. iii) to reflect when presumptive data need to be re-collected within evidence and control stubs. Since the software stops collection after a user-defined number of 3-COMP particles are detected, a percentage of particles can't be used within the criteria. Therefore, a set number (i.e., user-defined number) of 3-COMP particles needs to be used within the decision criteria section. Updated sections 10.a.ii, 10.a.viii, 10.a.viii.5, 10.b.iii.8, . Removed section 10.b.iii.9. Added methanol and water to section 6.

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Title: Performance Monitoring Protocol (QA/QC) for the Scanning Electron Microscope (SEM) Instrument

A. Purpose

Conducting elemental analysis on samples, whether for the presence of primer-gunshot residue (GSR or pGSR) particles, for paint composition, or general information using scanning electron microscopy (SEM) instrumentation is a type of testing employed within the Chemistry Unit. The following procedure shall serve as a quality assurance/quality control guideline when analysts use the SEM instrument and associated detectors (i.e., secondary electron imaging (SEI), backscatter electron (BSE), and energy dispersive X-ray spectroscopy (EDS)) for casework. These guidelines are used to ensure that the generated data are reproducible and accurate. A copper standard is used to demonstrate that the SEM instrument can produce amble electrons, so the SEI detector can produce a clear image, and so the EDS detector can appropriately detect correct X-ray spectra and peak energy intensities. The PLANO standard is used to demonstrate that the GSR software, in conjunction with the BSE detector, can reproducibly and consistently produce quality results, so the SEI detector can produce a clear image, and so the EDS detector can adequately produce X-ray spectra of GSR-simulated particles.

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B. Scope

This procedure serves as a general guideline for evaluating SEM instruments for paint, GSR-related, and other elemental-type analyses. Once particular milestones are achieved, instruments are considered appropriate and ready to use each day. The term ‘daily’ (or day) refers to a day wherein the instruments are used for casework. A copper reference standard is analyzed daily to demonstrate that the SEM, as well as the SEI and EDS detectors, are operating as expected. A simulated GSR particle-containing reference standard (i.e., PLANO disk) is analyzed [at least] monthly to demonstrate the instrument’s ability to detect elements related to primer-gunshot residue (i.e., Ba, Sb, and Pb). The term ‘monthly’ refers to a month wherein the instruments are used for casework.

C. Responsibility

Scientists who use the SEM/EDS instrument(s)

D. Equipment/Materials/Reagents

Listed below are general supplies required for electron microscopy (EM). Additional supplies unique to a particular method are indicated within that topic.

1. Scanning Electron Microscope with Backscattered Electron (BSE) and secondary electron imaging (SEI) detectors (Hitachi, or equivalent)
2. Energy Dispersive X-ray Analyzer/Detector (EDAX or equivalent)
3. Aluminum Stubs (Electron Microscopy Services or equivalent)

4. Adhesive-backed tabs or double-sided adhesive tape (Electron Microscopy Services or equivalent)
5. Copper reference standard, 99.9% or better (Sigma-Aldrich or equivalent)
6. Gunshot Residue Control Standard (PLANO ; Ted Pella, or equivalent)

E. Standards/Controls/Reagents

1. SEM/EDS Performance Standard (i.e., copper reference standard)
   A copper (Cu) pure element reference standard has already been mounted on an aluminum SEM stub using an carbon adhesive tab. This stub reference standard needs no further preparation and is used repeatedly due to the non-destructive nature of the technique.
2. GSR Performance Standard (i.e., PLANO disk)
   A GSR reference (aka. PLANO) standard is received from the manufacturer in an already-prepared and mounted state on an SEM stub. This stub reference standard needs no further preparation and is used repeatedly due to the non-destructive nature of the instrumental technique.
3. Negative Control/Blank
   An aluminum SEM stub containing a carbon adhesive tab. This stub control does not contain any GSR-related particles and needs no further preparation. It can be used repeatedly due to the non-destructive nature of the instrumental technique. It is manipulated contemporaneously and in a manner similar to casework samples.

F. Calibration

Not applicable.

G. Sampling

Not applicable.

H. Procedure

1. Ensure instrument is ready for analysis. Check for error readings and/or error indicator lights.
2. Vent and open the sample chamber.
3. Insert sample(s) into the specimen holder. Never touch the surfaces of the discs with bare hands - use gloves appropriately. Use tweezers around step of stubs to manipulate discs.
4. Close chamber and evacuate.
5. After the chamber is properly evacuated (i.e., green light), turn on the filament (i.e., power at the main SEM console). Ensure that the filament, operating voltages, magnification, and other parameters are all at their correct settings and are operating properly.
6. Daily QA/QC:
   Examine the SEM/EDS Performance Standard (i.e., copper reference standard) each day that the instrument is used for casework.

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Note: If samples are analyzed overnight and the SEM sample chamber has not been opened, then the QA/QC procedure need not be performed prior to completing analyses.
However, once the SEM sample chamber has been opened, the Daily QA/QC must be performed and documented for the day prior to additional casework being analyzed.

a. Adjust brightness and contrast, as needed, in order to obtain a good overall view of copper.

b. Focus the SEM on a flat portion of the copper standard, preferable the same place each time (e.g., upper right hand corner). The stub should be oriented on the SEM platform in the same (or as close as possible) direction each time it is placed into the SEM chamber.

c. Set up the parameters according to the Instrumental Conditions section (e.g., collection time, magnification). Other parameters will be set as close to possible as previous QA/QC analyses (e.g., spot intensity, working distance).

d. The parameters will be electronically recorded within each EDS spectrum (e.g., sample info including lot #, working distance, acquisition magnification, live time (100 seconds), beam voltage (25 keV), instrument label (SEM-01, SEM-02), and initials of analyst).

e. Each filename will be of the format: Cu Std_SEM-##_XXX_YYYY-MM-DD (where ## is the instrument number, XXX are the analyst's initials, and YYYY-MM-DD are the Year-Month-Day, respectively).

f. Record/print the copper spectrum from 0 keV to 25 keV.
The energy values from each of copper's four (4) peaks will be labeled both by energy level (i.e., keV) and intensity (unit-less), at the apices of the peaks (i.e., Cu L_l, Cu L_α, Cu K_α, and Cu K_β).

g. Any significant peaks (other than C, O) will be labeled (if element is known) according to the energy value at peak apices. Peak intensities are not required to be documented, however.

7. Monthly QA/QC:
Examine the GSR Performance Standard (i.e., PLANO disk) and the Negative Control/Blank stubs on the first day of each month that the instrument is used for casework.

a. The same method and parameters will be used as would be done when analyzing GSR particles within casework.

b. The parameters and other important information (e.g., sample info including lot #, magnification, beam voltage (25 keV), instrument label (SEM-01, SEM-02), initials of analyst) will be electronically recorded within each data file, if possible. Any parameters not able to be electronically stored will be written on paperwork.

c. An image of at least one (1) GSR-related particle within the PLANO disk (using the SEI detector) will be included in the GSR Performance Standard paperwork.

d. Confirmation elemental data for the image in the form of an EDS spectrum (showing the presence of barium, antimony and lead) will be acquired with at least 100 live time seconds, be printed, and be included in the QA/QC binder with the rest of the data for that day.

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e. Images and data will be saved and printed. Filenames will be in the format: PLANO_SEM-##_XXX_YYYY_MM_DD (where ## is the instrument number, XXX are the analyst's initials, and YYYY-MM-DD are the Year-Month-Day, respectively).

8. Ensure appropriate images have been printed.
9. Ensure appropriate collection parameters have been captured, recorded, and/or noted.
10. Ensure spectra have been saved using appropriate filenames. Generic filenames will not be used – this is done to prevent spectra from being overwritten and data being lost.
11. The system should remain under vacuum with electronics off when not in use for an extended period of time.
12. Ensure the logbook contains all QA/QC activity and information, along with date and operator's initials, and a final determination has been made regarding operability of the instrument (i.e., pass/fail).
13. The analyst performing this procedure each day (or month) will ensure all QA/QC printouts are properly secured in the appropriate QA/QC binder prior to leaving for the day, and prior to allowing another analyst to perform casework.
14. Any significant environmental impacts on the instrument (e.g., power interruption, service to instrument) will require that both the Daily and Monthly QA/QC be performed prior to casework.

I. Decision Criteria (Evaluation of Data)

1. SEM/EDS Performance Standard – Daily
   Evaluate the Cu standard's image and X-ray spectra for elemental information using the parameters listed within this document. Final elemental peak determination will be based on EDS energy values appropriate to copper peak's theoretical value(s).

   a. Image quality will be evaluated but image printouts are optional.

   b. If an automatic identification application is used, peak identification must be confirmed by the analyst.

   c. Compare the SEM/EDS Performance Standard's X-ray spectrum with the spectrum from the previous day (or other spectra). Determine if a significant energy value or signal intensity values have changed. Overlay and print the two spectra using the same scale, if possible. The daily SEM/EDS Performance Standard's X-ray spectrum must appear generally similar to the previous day's spectrum, including high-to-low energy peak ratios, Gaussian peak shapes, peak-width-at-1/2-maximum (PWHM) distance, and absence of any spectral artifacts. Changes in the low-to-high peak intensity ratio may indicate problems in the detector. A peak intensity change > 20% of the previous day's value(s) will be considered significant. A peak energy change > 30eV (+/- 0.03 keV) of the previous day's value(s) will be considered significant.

   d. If significant energy value changes are found, or if additional unwanted peaks are discovered, corrective measures must be taken. If issues can't be resolved it will be documented in the appropriate QA/QC logbook, the Lead Examiner (or higher) will be notified, and a

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determination on instrument operability and acceptance will be made. Changes in any of the observed performance criteria may indicate that the instrument be evaluated by the manufacturer's service personnel (either in person or via phone support).

2. Gunshot Residue Control Standard (PLANO) – Monthly
   Evaluate for GSR-related particles using the same methods for casework. Both backscatter electron and secondary electron (particle imaging) data will be used in the evaluation.
   a. The image printout of at least one (1) GSR-simulated particle that contains all three elements – Ba, Sb, and Pb will show expected morphology of a GSR particle.
   b. If an automatic identification application is used (i.e., GSR software), print out all necessary data as would be done for casework.
   c. Compare resulting data with the previous month's (or other) data to determine if significant changes have occurred. A significant drop is considered (>20% loss in the number of GSR particles detected). In cases of significant particle detection drop, the Lead Examiner (or higher) will be notified. A drop of >20% may indicate the need for vendor service.
   d. If significant changes are noted, corrections will be instituted to resolve the problem(s). If problems can't be resolved, the unit's Lead Examiner (or above) will be notified and a determination on instrument operability acceptance will be made collectively.

3. Negative Control/Blank – Monthly
   Evaluate for GSR-related particles using the same methods for casework. Appropriate detection (particle imaging) will be used in the evaluation.
   a. Evaluate for the presence of GSR-related particles using the same methods for casework.
   b. If particles contain any combination of the three elements – Ba, Sb, and Pb then the SEM instrument, as well as the surrounding sample preparation area(s), will be thoroughly cleaned. A new Negative Control/Blank sample will be prepared, re-analyzed, and evaluated. Continued detection of GSR-related particles will result in the Lead Examiner (or above) being notified. Further actions will be decided and documented in the appropriate instrument logbook.
   c. All appropriate documentation will be kept in the corresponding instrument logbook.

4. Other Evaluation(s) – Annually (as needed)
   Preventive Maintenance: If a vendor annual system preventive maintenance agreement exists for either the SEM or any of its detectors, the vendors will perform as per the agreement. This will usually include cleaning and calibration of the instrument and/or its components – both mechanically and electronically. All maintenance (as well as problems) will be recorded within the appropriate instrument log book. Documentation from the service company will also be kept in the appropriate instrument book. Instrument software upgrades and/or changes will be included in the appropriate logbook.

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J. Instrumental Conditions

General operating conditions can be:

Beam Voltage	25 kV
Spot Intensity	Adjusted to yield ~10,000 cps
Live counting time	100 seconds (Cu analysis ; Confirmatory Plano elements)
Working Distance	~10mm (optimal EDS X-ray collection)
Magnification	200x
Area of Cu Analyzed	Upper right quadrant
Analysis Mode Setting	Area Full Window

L. Uncertainty of Measurement

Not applicable.

M. Limitations

1. This technique is limited to solid samples.
2. X-ray fluorescence may excite characteristic X-rays from structures remote from the area visibly selected for analysis.
3. Elements present in trace concentrations, under average conditions, are generally not detected below 0.5%.

N. Safety

Use universal precautions when handling potentially biohazardous materials. Refer to the GL *Safety Procedure* and appropriate MSDS/SDS documents for additional information. Take standard precautions for the handling of chemicals and sharp cutting instruments.

O. References

1. Hitachi S-3700N operator's manual.
2. EDAX Genesis Manual(s)
3. Bearden, J.A. "X-ray Wavelengths," *Reviews of Modern Physics*, 39 (1), No. 1, 1967.
4. Goldstein, et al. *Scanning Electron Microscopy and X-ray Microanalysis*, second edition, Plenum Press, New York, 1992.
5. Ward, D.C. "A Small Sample Mounting Technique for Scanning Electron Microscopy and X-ray Analysis," *Forensic Science Communications*, 1(2), 1999

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6. Ward, D.C. and Sibert, R.W., "The Use of Vacuum Evaporation of Metals for Surface Feature Enhancement," *AFTE Journal*, 18(4), 1986.

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Revision #	History
3	Significant format and verbiage changes throughout document. Added more specification to the 'Procedure.' Added other sections (e.g., Decision Criteria, Instrumental Conditions, and Limitations. Added a Revision/History section.

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SEM/EDS RESULTS

Lab Case #: _____ Date:_____

Item #: _____

SAMPLE(S)	ALTERNATE LABEL	ELEMENT(S)
LEFT HAND		
RIGHT HAND		

LEFT PALM		
LEFT BACK		
RIGHT PALM		
RIGHT BACK		

BLANK SEM STUB		Elements associated with GSR ☐ No ☐ Yes: _____

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Technical and Administrative Review Checklist – OCME Specific

Case Number:_____

Technical Review	
Draft report reviewed: Evidence Description Appropriate Appropriate Report Format Findings supported by documentation Uncertainty Statement included if appropriate All items reported or listed as not analyzed Names of appropriate analysts on report	
Results reviewed Appropriate identifications made Chromatography acceptable if applicable Batch review appropriately documented	
Appropriate Calibrators/Controls and blanks used	
Calculations checked if applicable	
Review of Case information to determine if further work is required	
Technical Reviewer/Date	
Administrative Review	
Case Demographics acceptable Name of Submitting Agency Name of ME (report to) Lab and agency case numbers correct	
Per batch analyst's initials or signature on each page	
Technical reviewer initialed above	
Milestone sticker completed	
Cross outs or changes properly annotated, no obliterations	
Administrative Reviewer	
Final Review	
Electronic signature of technical reviewer and analysts on report	
Report Emailed to OCME	
Reviewer/Date	

Notes:

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Laboratory Number: _____

Technical Review	Yes	No	N/A
Evidence description clear / consistent with request for analysis form			
Case notes clear			
Photographs included /correctly annotated (case, item number, date)			
Weight calculation(s) checked			
Weight of group reported			
Weight of items analyzed reported			
Weight with/without packaging correctly reported			
Uncertainty calculation correct & reported as needed			
Substances reported are supported by technical data			
Appropriate blanks used (blanks bracket samples)			
Appropriate (+ and -) controls run/included			
Appropriate standards run/included and lot #			
Appropriate methods listed on report			
Chain of custody from JusticeTrax reviewed and logical			
Drug form (rock/powder/pm) appropriate			

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Technical Reviewer: _____

Date: _____

Administrative Review

Demographic information correct: Name			
Agency			
Agency case number			
Submission number(s)			
Cross outs initialed/dated (no obliterations)			
Description reviewed for typos			
Item numbers consistent			
Analysts initials and case number on all pages			

Administrative Reviewer: _____

Date: _____

Notes:

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A. **Purpose**:

Within this document are general guidelines that are important for maintaining the integrity and forensic defensibility of the work performed within the Controlled Substances (CS) Unit. A controlled substance is generally a drug or chemical whose manufacture, possession, or use is regulated by a government entity (e.g., illicitly used drugs or prescription medications). Evidence is usually submitted for two (2) purposes – weight and qualitative examination. Currently quantitative analyses (other than weight measurements) are not conducted within the CS Unit. Comparisons of analyte quantities within evidence, as long as numerical values are not reported, can be considered qualitative and may be performed. Such requests usually come from evidence that has been suspected of being tampered or adulterated and accompanies known exemplars for comparison purposes.

B. **Scope:**

This document includes general information and describes the workflow related to the overall process of evidentiary examinations conducted within the CS Unit. Only qualitative analyses are conducted and quantitation (i.e., purity determination) is not currently perform. Comparisons of relative purities (without quantitation), however, are sometimes performed in order to determine of submitted evidence is consistent with being tampered or adulterated. Definitions related both to the CS Unit and other Units can be found within the appendix of this document

C. **Responsibility**:

Analysts assigned to the Controlled Substance Unit.

D. **Safety**:

Proper PPE must be worn whenever analyzing drug evidence. At a minimum proper ventilation (e.g., use of a powder-safe hood) will be used when potential hazards for drug exposure exist. Eye protection (e.g., safety glasses) must be worn when there is a potential for eye injury. It will be the responsibility of the employee to determine when proper eye protection will be used.

E. **Procedure**:

1. All analysts must work in a manner that provides safe working conditions for themselves and their co-workers.
2. Analysts should wear lab coats and disposable gloves will be worn while analyzing evidence. Gloves will be changed in-between different items of evidence, as well as between the use of

reference materials, so as to avoid contamination. Lab coats will be changed when there is an indication of contamination or when such activities warrant changing (e.g., residue analyses).

3. All evidence transfers are documented in LIMS-Plus when they occur. Personal identification numbers (PINs) are used in conjunction with staff [person-to-person] evidentiary transfers.
    a. In the event that an aliquot of evidence is split and transferred to a second analyst, a sub-item will be created in LIMS-Plus. The chain of custody will be maintained for all portions of evidence if said evidence is to be analyzed or returned.
    b. If an error is found within the chain of custody for a case then that information will be brought to the attention of the appropriate Lead Examiner (or higher). Certain QA actions will be taken to correct and document the issue.
4. All cases are opened, inventoried, and closed by analysts with the assistance of a witness, when needed.
5. A witness will verify the contents of the submission both during initial inventory and during the closing of a case.
6. Only one case should be opened and/or closed by an analyst at a time. This practice is in place to avoid mixing-up case materials.
7. All evidence sampling and transfers will include appropriate labeling (e.g., laboratory number and item identifiers).
8. Photographs may be taken of evidence, as appropriate. Photographs can be used to supplement case notes and allow analysts to minimize written notes. All photographs, whether used by an analyst during their examination(s) or not, will be retained within appropriate storage locations according to their case file(s). While hardcopies only need to be made which are necessary for a case, all digital photos must be retained.
9. Ink will be used for all casework documentation (Blue pens are preferred and red ink is discouraged).
10. Examination areas, surfaces, utensils, and any other such equipment will be pre-cleaned (e.g., methanol, ethanol, acetone) prior to an underlayment barrier (e.g., brown paper or equivalent) being placed down when analyzing all evidence in order to prevent contamination. Such cleaning is especially important when examining/analyzing drug residue evidence. For drug residue evidence disposable equipment (e.g., spatulas, pipettes) should be used and surfaces should be pre-tested for possible contamination prior to evidence being examined/placed down, whenever possible.
11. Evidence lockers are usually assigned to individual analysts and can contain evidence, aliquots of evidence, extracts of evidence, and other similar case materials. All such evidentiary materials will be stored under proper seal and kept locked when not being actively analyzed. All evidence within these lockers will be placed under proper evidence seal, when possible. Tamper-evident tote tags

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should be used on the outside of evidence lockers (along with handwritten inked initials on the tags) at the end of a workday to ensure integrity of stored evidence inside a locker. Any incident where an analyst feels evidence or integrity of evidence may be of concern is required to contact the appropriate Unit Lead (and higher) for necessary QA action.

12. All non-common abbreviations used within worksheets and case notes should be defined within appropriate case documentation.
13. Analysts will follow laboratory procedures for case analyses. Significant deviations are changes in procedures which could adversely affect the results of experiments. If significant deviations from procedures are necessary then analysts must discuss the issue(s) with the appropriate Unit Lead (and higher) and obtain pre-approval using the QA process (such approval can additionally be documented in case notes). Minor deviations are changes in a procedure which do not adversely affect the results of an experiment. The analyst must document such deviations in the case file notes.
14. Laboratory areas (both examination and instrumental) will be kept clean. This is not only good laboratory practice, but it is also done to avoid case contamination and any exposure to chemicals, drugs, and/or biological materials.
15. All supplies and reagents will be stored in a manner so as to ensure that they are suitable for use and to preserve their integrity.
16. Consumable materials will be ordered to meet the needs of the laboratory testing that is performed. When orders are placed for items that can affect the quality of the testing, the ordered items must meet the needs of the test. When receiving items from a vendor analysts must verify the contents of what was received prior to using such materials in casework.
17. All reagents must be properly labeled and secured in a manner so as to ensure that they are suitable for use in case analyses. Analysts will store and maintain reagents with special handling requirements in a manner that meets said requirement. Manufacturer's information (e.g., MSDS, SDS) should be consulted when analysts are unsure of storage (e.g., refrigerator, solvent cabinet), use, or potential hazards.
18. All reagents and reference materials (with the exception of certified reference materials) that are used in case analyses must be validated before use with casework. In general, a reagent is deemed acceptable for casework use if the reagent gives results that are as expected whether it be for positive and negative use.
19. All standards must be stored to maintain their integrity. Manufacturer's guidelines should be followed.
20. Standards used for comparison purposes can be evaluated/validated at the same time that evidence is analyzed.

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21. All instruments will be verified as operable and pass operating criteria prior to being used for casework.
22. Instruments should be maintained appropriately to maintain quality.
23. Instruments that do not meet daily QA requirements and which can't be corrected to meet said requirements will be marked as out of service and won't be used for casework until appropriate QA requirements are met.

General Case Flow in the CS Unit:

24. Cases are initially received from submitting agencies in the Evidence Receiving Unit (ERU) and then transferred the CS Unit. Evidence must be under proper seal during all transfers between sections.
25. A case file and paperwork is prepared for each case and barcode and milestone labels are usually included.
26. Case(s) are assigned to an analyst through LIMS-Plus and analysts transfer evidence from a storage location to themselves using LIMS-Plus.
27. Analysts will document and verify that evidence is under proper seal and has not been tampered since being transferred from ERU through the use of a witness.
28. The analyst will open a case with a witness so verification of evidence can be performed. Documentation of this action is recorded appropriately.
29. The inventorying of a case is documented using the appropriate Unit form(s).
30. If a discrepancy is found the analyst will note it and, depending on its nature, may halt the analysis until the issue can be resolved. If the issue jeopardizes the quality of the evidence, work will be halted on the case, the situation will be recorded within case documentation, and the Unit Lead (or higher) will be informed. Evidence Receiving Unit or Case management Unit personnel should be notified if administrative discrepancies exist. When an analyst contacts a submitting agency concerning a discrepancy they will record this communication and its resolution within appropriate documentation (e.g., case notes, entry in LIMS-Plus).
31. Once the inventory is acceptable and without issue an analyst can work independently.
32. Labels are made for the case and appropriate analyses will be conducted according to established procedures.
33. Weights are taken based on the case and following the guidance in procedures. Aliquots are taken based on case needs and following the guidance in procedures. Aliquots and evidence not actively being worked will be stored under proper seal within an evidence locker. Appropriate chain of custody will be maintained at all times.

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34. Case paperwork is compiled (e.g., notes, instrument data sheets, worksheets) during analyses. Case notes describe the evidence and details of the analyst's observations and work. Pictures can be used to supplement case notes and it is acceptable to annotate case pictures with necessary information – just as long as critical information is not blocked. Case notes must be legible and can be recorded using the appropriate forms.
35. Two (2) samples are usually obtained from each piece of evidence and identifications are generally made when results from both samples are in agreement. This usually occurs when either two portions or two samples both result in the same conclusion. Findings should be recorded on appropriate forms.
36. Upon completing the analyses for a case conclusions are made based on the compiled data. Appropriate controls will have been used to ensure quality work (reference standards will have been used for positive controls and analyte-free materials will have been used for negative controls). Findings are entered into LIMS-Plus and the case is marked, 'Draft Complete.' The draft report should be placed in the case jacket and will be reviewed after a technical reviewer is assigned in LIMS-Plus.
37. Any corrections resulting from the technical review (TR) will be noted (e.g., within the appropriate area in LIMS-Plus). If corrections warrant a continuation of the TR then the reviewer will select 'Reject Findings' within LIMS-Plus and return the case to the analyst with enough explanation as to why the case was sent back for corrections. A discussion is always recommended so adequate communication between analyst and reviewer is ensured.
38. Prior to return of evidence to the Evidence Receiving Unit the case is re-inventoried and closed (sealed) by the analyst in the presence of a witness. Documentation of this action is recorded appropriately.
39. The evidence can be transferred into storage in the "Cases in Review" location until all the reviews have been completed.
40. After TR is successful the same process occurs with administrative review (AR) – along with the same method of communication and documentation within LIMS-Plus.
41. Anytime disagreements cannot be resolved during TR and/or AR, then an appropriate Unit Lead (or higher) will be consulted. Resolution to disagreements are usually accomplished either at the Deputy Director level or prior.
42. After all reviews are successful a final report is generated. The original report is maintained electronically within LIMS-Plus.
43. The analyst can make copies (e.g., 3) of the report and these copies along with the evidence can get transferred back to the Evidence Receiving Unit. Once of the report copies should get a sub-item within LIMS-Plus. This is for tracking purposes when the report copy is transferred to the submitting agency from ERU.

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44. A supplemental report may need to be issued when additional evidence or additional work on evidence is performed (often at the request of the submitting agency). This supplemental report will be completed and issued having the same level of technical and administrative reviews as was done with the original report. It will be clearly marked as being a supplemental report and reference will be made to the original report (e.g., date of original report). Only supplemental results should be included in the supplemental report. It is not necessary to include results from the original report.
45. A revised (aka. amended) report is used when corrections are needed within the original report. This report will be completed and issued having the same level of technical and administrative reviews as was done with the original report. It will be clearly marked as being a revised (or amended) report and reference will be made to the original report (e.g., date of the original report). The before and after changes will be included within the revised report so that the reader will adequately know what was changed from the original report.
46. Distribution of supplemental and/or amended reports should be similar to original reports (i.e., trackable through sub-itemization in LIMS-Plus).
47. The report case file may be filed appropriately.

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APPENDIX – Abbreviations and Definitions:

ACET: Acetone
Amph: Amphetamine
APAP: Acetaminophen ; Paracetamol ; N-acetyl-para-aminophenol
BB: Borate Buffer
BBX: Borate Buffer Extraction
Benzo: Benzodiazepine
bl: Blood
BM: Botanical Material
bndl: Bundle
cap: Capsule
CFB: Cocaine Free Base
CH: Cystolithic-like Hairs
CH P/A: Cystolithic-like Hairs present or absent
CHEP: Cyproheptadine
cmpd: Compound
coc: Cocaine
cont: Containing/Container
CPB: Clear Plastic Bag
CRM: Certified Reference Material
CS: Controlled Substance(s)
CSF: Cocaine Salt Form
CZLB: Clear Zip Lockable Bag
DS: Daily Standard
DSS: Division of Scientific Services
ea: Each
ECO: Evidence Control Officer
Encl: Enclosed
env: Envelope
EPB: Evidence Plastic Bag
ETOAc ; EACT: Ethyl Acetate
EtOH: Ethanol
evid: Evidence
FA: Fatty Acid
FTIR: Fourier Transform Infrared Spectrophotometry
g: Grams
GC: Gas Chromatography
GCMS: Gas Chromatography/Mass Spectrometry

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GHB: Gamma Hydroxybutyrate ; Gamma-Hydroxybutyric Acid
HPLC/MS ; LC/MS: High Performance (Pressure) Liquid Chromatography/Mass Spectrometry
HS: Heat Sealed
Insol: Insoluble
IPA ; iPrOH: Isopropanol
IR: Infrared
IS: Internal Standard
K: Kilogram
KPBC: knotted plastic bag corners
KT: knot tied
liq: liquid
LIMS-Plus ; JT ; JTRAX: JusticeTrax® laboratory information management system (LIMS) software
LSD: Lysergic Acid Diethylamide
6-MAM ; 6-AM: 6-Monoacetylmorphine
Mar: Marihuana ; Marijuana
ME: Manilla-like Envelope
MeOH: Methanol
meth: Methamphetamine
mg: Milligram
mL: Milliliter
MS: Mass Spectrometry
NCS: No Controlled Substances
NDD: No Drugs Detected
Neg: Negative
NS: Not Scheduled
Num: Numerous
OCP: One Clear Plastic
op: Opioids
P1: Portion One
P2: Portion Two
p: Powder
PCP: Phencyclidine ; Phenylcyclohexylpiperidine
pdr: Powder
PE: Petroleum Ether
PKG: Package/Packaging
plb: Plastic Bag
PM: Plant Material
Pos: Positive
PPE: Personal Protective Equipment
Proc: Procedure

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pwdr: Powder
PZB: Plastic Ziplockable Bag
QAQC ; QA/QC: Quality Assurance/Quality Control
QA: Quality Assurance
QC: Quality Control
rel: Related
res: Residue
RS: Rock Substance
Sol ; Sol'n: Solution
STD: Standard
RT: Retention Time
tbt: Tablet
tab: Tablet
THC: Tetrahydrocannabinol
Total U ; TU: Total Uncertainty
TX ; tox: Toxicology
TX Bag: Toxicology Convenience Bag
Uncer ; U: Uncertainty
ur: Urine
UV: Ultra Visible
UV/VIS: Ultraviolet/Visible Spectrophotometry
vol: Volume
WE: Weighing Events
wt: Weight
ZLB ; zip: Ziplockable Bag

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Proper Seal: Packaging of evidence such that the material contained within the packaging is free from sample loss, contamination, and/or un-noticed deleterious change.

Visible Residue: A term used to describe an amount of a solid material that is visible within evidence and is in a quantity too small to be adequately isolated and weighed (e.g., less than a milligram of solid, a few particles of solid powder).

Non-Visible Residue: A term that can be used to describe evidence where no solid drug-like material is visible but which non-visible particles of potential analyte may be present.

Reference Standards: materials (usually pure) that are commonly purchased and which meet certain traceability requirements. Product information (e.g., purity, lot number, expiration date) are usually found on reference standard containers. These are sometimes referred to as pure standards and are commonly used to verify the identity of a detected analyte.

DEA Exempt Standard: Chemicals purchased or made in a concentration of 1mg/mL or less.

"Like" items: evidence within a case that have similar physical appearance, including packaging and contents.

Sample Selection: A process wherein evidentiary "Like" items, or portions of said items, are selected to be analyzed. There is no assumption of homogeneity – only that the "Like" items may contain the same material(s). Any reported analytical results will only be from items that were actually selected and tested. Results are only considered related to the whole population of the submitted evidence when all of the items have been analyzed.

Sampling Plan: A process wherein evidentiary items are analyzed and their results represent the entire contents based on a statistical plan. This plan can be used with multiple item populations and can be used to determine the number of samples an analyst needs to analyze in order for an inference about the whole submission (population) to be made. The CS Unit does not currently use a sampling plan. However, in the event that a customer requires a result which represents the entire submission's population, a Bayesian statistical approach will be used (e.g., SWGDRUG Sampling Plan) and use of the plan will be documented in the case.

Analyst: a scientist who has certain authorizations, such as to handle and analyze evidence in a particular discipline or Unit. They are typically responsible for the security of the evidence they handle, analytical work they perform, and reporting of results.

Toxicology Convenience Bag: a bag or container used by the laboratory for convenience. These are labeled as "TX" to demonstrate that they were added to the case by the laboratory.

Witness: An employee or authorized analyst within the DSS laboratory that can act as a verifier of an action. Although the witness may not be responsible for analytical work, they are responsible for verifying that an activity took place (e.g., evidence was sealed, number of items of evidence were received/returned, weight value was transcribed and/or recorded correctly).

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Rev. #	History
6	Added guidance under section E.3, added a and b concerning itemization of aliquots and addressing chain of custody issues.
7	Updated Section A with more description of purpose. Moved Section C (Definitions) to the Appendix section. Added a section titled Scope. General reformatting of document. Updated Section C with more definitions (DSS, LIMS-Plus, Proper Seal, Residues, Reference Standards, Sample Selection, Sampling Plan, Analyst, Witness). Added powder-safe hood to Section D. Updated Section E regarding changing of PPE, usage of LIMS-Plus, information within LIMS-Plus, gave more clarification throughout section, updated retaining all photographs, emphasized cleaning of equipment/area, gave more instruction on use of evidence lockers and tamper evident tote tags for outside of lockers, described more QA aspects during case examinations, added description of positive/negative control usage, added description of administrative functions of case file documentation, emphasized chain-of-custody and proper seal of evidence/aliquots, described what needs to be done in LIMS-Plus during TR/AR rejections, general updates throughout section. Combined sections E and F (removed 'General Information') ; Created an appendix section and added definitions and more acronyms/abbreviations.

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Title: Gas Chromatography/Mass Spectrometry (GC/MS)

A. **Introduction**:

Gas Chromatography/Mass Spectrometry (GC/MS) can be used to provide qualitative identification of analytes and, when applicable, quantitative information. The GC/MS instrumentation provides both retention time and fragmentation pattern information for analytes. Comparison of data (samples versus positive controls) provides the basis for qualitative identification. Using GC/MS data analytes can be tentative identified using library spectra for comparison. Confirmatory identification can be achieved by comparing both chromatographic and mass spectral data with known reference materials. Although the GC/MS is a single instrument it is considered two (2) separate orthogonal analytical techniques.

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B. **Responsibility**:

All analysts who are authorized to perform casework ; preferably within the Drug Chemistry discipline.

C. **Definitions/Acronyms**:

QA/QC: Quality Assurance/Quality Control
m/z: mass-to-charge ratio
GC/MS: Gas Chromatography/Mass Spectrometry
CHEP: Cyproheptadine
EtOAc: Ethyl Acetate
MeOH: Methanol
$CHCl_3$: Chloroform
Instrument Check Solution: a substance put into a GC/MS sample that demonstrates the instrument had an acceptable injection and is working as expected. This is generally CHEP and the solvent is usually methanol, but other compounds or solvents can be used, if needed.

D. **Equipment/Materials/Reagents**:

1. General laboratory glassware
2. Gas chromatograph with mass spectral detector (GC/MS)), Agilent (or equivalent)
3. Perfluorotributylamine (PFTBA, FC-43) (Agilent or equivalent)
4. Capillary Column: (Restek™ Rxi™-5ms, 30m, 0.25mm i.d., 0.25µm, or equivalent)
5. Chloroform (reagent grade or equivalent)
6. Cyproheptadine (CHEP) (reagent grade or equivalent)

7. CHEP/MeOH (0.001% ; 10ppm): ~10mg of CHEP in ~1000mL of methanol (Instrument check solution ; need not be quantitatively accurate)
8. CHEP/EtOAc, (0.001%, 10ppm): ~10mg of CHEP in ~1000mL of ethyl acetate (Instrument check solution ; need not be quantitatively accurate)
9. Ethyl Acetate (reagent grade or equivalent)
10. THC: Tetrahydrocannabinol
11. Methanol (reagent grade or equivalent)
12. Various glassware and materials (variable suppliers)
13. Daily Standard: Fentanyl, Oxycodone, Phencyclidine (PCP), Cocaine, delta-9-THC, Heroin – in a CHEP solution. This is a mixture of drugs made from reference standards which can represent samples found within casework. The composition may change based on the needs of the unit but, if changed, should be documented within the instrument QA/QC log books.
14. Combo Control: amphetamine and trazadone reference standards in a CHEP solution.

E. **Preparation of Performance Test Solution, Controls (Standards):**

When preparing the stock Daily Standard Mix record the volumes used and lot numbers on the Daily Standard Stock Solution Reagent Log sheet (See CS-7.3). The log sheet is maintained in a log book within the Unit.

1. Daily Standard Mix (aka: Performance Test Mix)

The concentrations of analytes within the Daily Standard Mix should be:

Analyte	Stock Solution Concentration	Volume of Stock Solution	Total (or Final) Volume of CHEP solvent	Final Concentration of Analyte in Mix
Cocaine	1 mg/mL	100 µL (0.10 mL)	10 mL	10 µg/mL (10 ppm)
Fentanyl	1 mg/mL	100 µL (0.10 mL)		10 µg/mL (10 ppm)
Heroin	1 mg/mL	300 µL (0.30 mL)		30 µg/mL (30 ppm)
Oxycodone	1 mg/mL	100 µL (0.10 mL)		10 µg/mL (10 ppm)
PCP	1 mg/mL	100 µL (0.10 mL)		10 µg/mL (10 ppm)
THC	1 mg/mL	75 µL (0.75 mL)		7.5 µg/mL (7.5 ppm)

a. The date the solution is made will include the lot number assigned to the appropriate Daily Standard Mix solution. For traceability purposes, this date/lot number should be on any aliquots of the stock solution that will be retained for future use. The date the Daily Standard Mix solution was placed in service/use will be documented on form CS-7.3.

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b. Since this solution will be assessed each day of use no expiration date needs to be listed on the Daily Standard Mix's container. If it is determined that the stock solution is no longer acceptable (either due to contamination or compound breakdown) then the stock solution, and any aliquots thereof, will be properly disposed.

Note-01: The Daily Standard Mix is re-verified each time GC/MS results are considered acceptable. The solution's expiration date is one (1) year from the last date of verification.

2. Positive and Negative Controls

a. Reference Standards (aka. Positive Control Solutions)

Solutions made from either solids or pre-made solutions (usually purchased as reference standard materials) which contain specific analytes (or multiple analytes) and are prepared in an appropriate solvent(s). Concentrations of these analyte solutions should be less than 100 µg/mL (100 ppm) for each analyte and be appropriate for comparing to analytes within unknown samples. Documentation of how such control solutions were prepared will be recorded and filed, as needed.

b. Residue Positive Control Solutions

Solutions of specific analytes (or multiple analytes) can be prepared in an appropriate solvent. Concentrations of these analyte solutions should be less than 50 µg/mL (50ppm) for each analyte and be appropriate for comparing unknown sample analytes of residual concentrations. Documentation of how such controls were prepared will be recorded and filed, as needed.

c. Control Materials (Positive or Negative)

Known substances (preferably a commercial product from a known vendor or from a known source) that can be used for comparison purposes. Historically these materials have referred to plant materials, rock materials, powders, residues, and foods from known sources – all with appropriately-known sourcing information (i.e., where obtained).

Positive Control materials are usually those which either contain (or which have been added/spiked with) known analytes. Examples of such positive control materials include foods spiked with drugs. These materials (usually extracts) should be analyzed in a manner similar to, and contemporaneously with, unknown samples for comparison purposes.

Negative control materials can be matrix-type materials that are similar to unknown samples and which do not contain analytes of interest (i.e., drugs) or which have not been spiked. Such controls are usually extracted/analyzed in a manner similar to, and contemporaneously with, unknown samples.

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F. **Procedure**:

1. Set Up of GC/MS and Evaluation:

   a. GC/MS Daily check:

   Prior to an instrument being used for casework on any given day an air/water check, tune, and daily standard solution evaluation are performed. A check list can be utilized for documentation and to demonstrate that an instrument is acceptable for use. The checklist should be filed in the QA/QC maintenance log book for each instrument. (See CS-7.2)

Note-02: If QA/QC instrument log books become digital then an equivalent method of storage can be on electronic devices.

Note-03: If the instrument is still running a sequence from the night [previous day], instrument evaluation can be done at the conclusion of the sequence and no interruption is necessary simply because the days have changed).

Note-04: The term 'daily' only refers to the day an instrument is used for casework or similar type of work (e.g., procedure validation).

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i. Tuning of Mass Spectrometer:

(a) Generally autotunes are performed the day instruments are used for casework, but tuning more frequently is acceptable.

Note-05: Quick tunes have in the past been acceptable but are no longer preferred.

(b) Tune Parameter Review:

(i) Correct mass assignments (m/z 69, 219, and 502) +/- 0.2

(ii) Peak widths (acceptable range 0.45-0.65)

(iii) Relative ion abundances (69 at 100%, 219 > 20%, and 502 > 1%)

(iv) EM voltages are recorded to help track the condition of the source.

Note-06: Relative Ion Abundances within autotunes are instrument dependent. The above criteria was set to allow acceptability among all GC/MS instruments within the Unit. The data within each autotune printout should be compared to previous autotune data *for the same instrument* to help determine if significant changes have occurred and instrument attention is necessary.

ii. Air and Water Checks:

These are performed each day of use to verify that there are no leaks in the GC system. The ions corresponding to water (m/z 18), nitrogen (m/z 28), and carbon dioxide (m/z 44) are checked to verify that values are less than 10% of m/z 69 and to track any trend that may suggest there is a leak.

iii. Daily Standard Mix (Qualitative)

At a minimum this usually consists of Cocaine, fentanyl. heroin, oxycodone, PCP, and THC in a CHEP solution (other appropriate drugs may accompany, if necessary). This mix is analyzed each day to verify that there are no unexplainable shifts in expected analyte retention times and that mass spectra are consistent to what is expected. The printout of the daily standard is maintained in a file in the instrumentation room (or equivalent).

Note-07: The Daily Standard Mix analytes may be altered based on the requirements of the Unit.

Note-08: The Daily Standard Mix is re-verified each time GC/MS results are considered acceptable. The solution's expiration date is one (1) year from its last day of verification.

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iv. Instrumentation Evaluation

In the event of a problem within one or more of the evaluation parameters (air/water check, tune, or daily standard), analyst(s) will work to determine the cause of the failure prior to analyzing casework. This may include the need for basic maintenance. If the problem is corrected the check sheet is marked as acceptable for use and any troubleshooting or solutions should be documented. If the problem still occurs and the instrument should not be used, it will be marked appropriately as not acceptable for use. Analysts will work with the Lead Examiner to determine the issue with the out-of-service instrument and try to correct the issue. An appropriate 'Out-of-Service' sign will be placed on the instrument to indicate that it will not be used for casework.

All appropriate printouts (e.g., air/water checks, tunes, and the documentation of any maintenance performed) are located in maintenance (QA/QC) log books (or equivalent) for each instrument. Printouts of daily standards are kept in a file within the instrumentation room.

The following parameters are reviewed and documented on the GC/MS Daily QA/QC Maintenance Log (CS7.2)

(a) Evaluation of GC/MS: Expected analytes within the Daily Standard Mix (i.e., Target Peaks) will be present. Other detected analytes (i.e., Non-Targeted peaks) should not be present. If non-targeted analytes are present then they must be explainable (e.g., 6-MAM peak due to the breakdown of heroin)

(b) Evaluation of GC/MS: The ratio of abundance of heroin to CHEP should not decrease less than 20% from the previous day's acceptable data.

(c) Evaluation of GC: The retention times of each of the Daily Standard Mix analytes will not deviate by +/- 5% when compared to respective previously acceptable performance standard solution chromatographic data.

(d) Evaluation of MS: The mass assignments from the mass spectra of the components within the Daily Standard Mix must compare favorably (i.e., similar in peak profile) to known spectra and to previously analyzed spectra.

b. Instrument Maintenance:

Analysts may perform routine maintenance if trained. No non-routine maintenance (e.g., change or clip column, venting instrument) should be performed on an instrument without notification to the Lead Examiner. All maintenance (routine or otherwise) will be documented within the instrument QA/QC maintenance logbook.

i. Septa: Analysts can determine if septa need to be changed based on the results of instrument evaluation (i.e., air/water, Daily Standard Mix (or other sample)).

ii. Liners: Analysts can determine if liners need to be changed based on the results of instrument Daily Standard Mix (or other sample) evaluation. Decreased sensitivity for heroin (or other analytes) and/or the presence of unexpected peaks may indicate the need to change a liner.

iii. Liner seals: Analysts can determine if liner seals need to be changed based on the results of instrument evaluation (i.e., Daily Standard Mix (or other sample)).

iv. Clipping column/Changing columns: Analysts can determine if column maintenance is necessary based on the results of instrument evaluation (i.e., Daily Standard Mix (or other sample)). Loss of sensitivity or the presence of unexpected peaks may indicate the need for column clipping or changing.

v. Switching/changing filaments: Loss of current or complete/partial signal loss indicates a possible need for a filament change.

vi. Cleaning the source: An increase in electron-multiplier (EM) voltage values may indicate that the source needs to be cleaned.

vii. Other Maintenance: as required due to instrument function and based on the evaluation of instrument performance results.

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c. Instrument Checks: Most samples prepared for GC/MS injection contain CHEP (cyproheptadine) in methanol or in an appropriate solvent. Samples (blank, sample, or control/standard) are placed in CHEP and the CHEP acts as an internal performance check during each sample injection.

(i) Blanks:

Solutions with no unexpected analytes. These are mainly used to verify that there is no carryover in-between injections (e.g., case samples).

1. A blank should be one of the first samples analyzed in a set of samples. This ensures the system is free of possible contaminates.
2. Blanks will be analyzed between different sample types in the same case.
3. Blanks should be analyzed at a minimum of every five (5) injections if multiple like-items from a single case are analyzed.
4. Blanks will be analyzed in-between different cases.

(ii) Controls:

1. Negative Control:
   Prepared in the same manner and at the same time as samples and with no analytes other than CHEP.
2. Positive Control:
   Usually prepared in a similar solvent matrix as samples and contain known analytes (along with CHEP) which can be used to compare to samples.

(iii) Additional Instrument Checks:

The CHEP and positive controls are used to show that the instrument is working properly as determined by the expected retention time and mass spectral fragmentation patterns. The addition of CHEP to samples allows analysts to determine if valid injections were made successfully.

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Note-09: The internal CHEP can be made in solvents other than methanol if analytes of interest are not soluble in methanol or if methanol causes artifacts to be formed (e.g., designer amphetamines or derivatizations).

2. Sample Preparation:

Samples for analysis can range from a variety of matrices (e.g., powder, rock, residue, plant material, pill, tablet). Samples limited in size or amount/type of material can be rinsed (or extracted) with solvent (or otherwise prepared). Some of these samples may be considered

residues based on the limited amount of material that is present. Such materials include, but are not limited to, empty syringes, other types of drug paraphernalia, plant materials, tablets, liquids, and food stuffs. (See SOP CS-4).

a. Sample log: samples which are to be run by GC/MS are listed on a GC/MS Sample Position Worksheet (see CS-7.1). This is a map of sample position on the auto sampler. It is acceptable for GC/MS vials to only be labeled with the vial position (this is considered properly labeled), since this map will allow the identity of the sample to be tracked. The sequence printout can also be used to track the sample vials as long as the printouts are checked and documented.

b. Solid Samples (e.g., Powder or Rock):

   i. Add approximately 1 mL of solvent (e.g., CHEP/MeOH or CHEP/EtOAc, as appropriate) to properly labeled containers (e.g., GC/MS vials or test tubes).

   ii. Add ~1 mg sample (e.g., using a new applicator stick or clean spatula) to the vial (or test tube), mix, and ensure sample adequately dissolves into solution.

   iii. Cap the vials (or test tubes) in preparation for analysis.

   iv. Prepare appropriate blanks and controls (containing CHEP) for the analysis and in a similar manner as sample preparation. The negative control solution containing CHEP can also be considered a reagent blank.

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Note-10: Because of the potential for variable sample concentrations, dilutions may be necessary to adjust concentrations of sample solutions so adequate data can be produced. Controls will be analyzed in the same manner as samples whenever possible. Dilutions of controls may be necessary. Documentation of any changes (e.g., dilutions) should occur.

c. Residue samples (including samples extracted or otherwise prepared so that they are now in a residual form)

   i. To the dried residue (often previously prepared in a GC/MS vial) add 1 mL of appropriate solvent (e.g., CHEP in MeOH or CHEP in EtOAc, as appropriate) and cap.

   ii. Prepare appropriate blanks and controls for the analysis.

Note-11: Because of the variable nature of residue samples, analyst discretion will be employed in this process regarding dilutions or concentrating samples. Unusual or exceptional cases should be discussed with the Lead Examiner or Deputy Director to ensure method applicability.

d. Tablets: tablet samples can be analyzed direct or as a residue if an extraction method was used.

   i. If directly analyzing then prepare samples as a Powder/Rock sample listed above. If analyzing samples after an extraction was performed then analyze samples as residue samples listed above.

   ii. Prepare appropriate blanks and controls for the analysis.

   iii. Manufactured tablets with an identifiable imprint will be analyzed by searching for drug information using the logo and reputable literature, whether hardcopy or online. Information obtained will be documented in the case file along with references. Such evidence may additionally be analyzed by other techniques (e.g., FTIR, GC/MS) for confirmation purposes, if necessary. Evidentiary tablet analyses can be stopped after information is obtained through a logo search only if tablets are not suspected to be illicitly produced and upon approval from either the Lead Examiner or higher. Subsequent reports shall convey the appropriate limitations to a logo-only search.

3. GC/MS Analysis:

   a. Each GC/MS is checked and evaluated on each day of use and prior to the analysis of any samples. If an instrument is still analyzing a sequence from the night before then QA/QC evaluation can be done at the conclusion of the sequence. Once an instrument is marked as acceptable for use it can then be considered acceptable for the day's casework unless proven otherwise. (see CS-7.2)

   b. It is acceptable to have changes to the temperature programs, as needed, for specific cases as long as the appropriateness of the change is demonstrated by properly analyzing blanks and controls. Any variations beyond a simple temperature hold in the temperature method will be documented in the case file, either by a notation or a printout of the temperature method. It is a good practice to print hardcopies of methods along with each sequence and place into case files. Vial placements can be verified by using the sequence printouts prior to analyses.

   c. Samples prepared as above should be injected using the appropriate GC/MS method (e.g., 'Drugs.m' or 'COMBO.m').

      i. The 'Drugs.m' method is appropriate for most routine samples. This includes plant extractions, most tablets, rocks, and powder samples. This method is not appropriate for most steroids since those usually contain late-eluting substances. It is also not recommended for some designer methamphetamine and amphetamine-type analytes since those are low molecular weight substances and require a slower rate in GC-oven temperature ramping.

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ii. The 'Combo.m' method is a combined method that encompasses both early and late eluting analytes. This method is generally used for powder, tablet, and rock samples that are negative by the 'Drugs.m' method. This method is a longer method that will often detect early eluting substances such as amphetamines and late eluting compounds such as trazadone. When using this method an appropriate 'Combo-type' positive control should be analyzed.

iii. Copies/printouts of methods are maintained with each instrument so that instrument parameters can be recalled for specific sample sequences at any time. If a new method is developed a copy of that method will be maintained similarly.

iv. When there is an indication of the type of substance present (such as through a tablet imprint) the standards books can be consulted to see which temperature program is appropriate for the compound of interest.

d. Autosampler Sequence: After entering the sequence of samples, blanks, and controls into the instrument software, the information can either be written on the "GC/MS Sample Position Worksheet" (CS-7.1) or printed out. These sheets act as maps of the auto sampler so sample can be cross-referenced with data printouts. They can be kept on a clip board next to each specific instrument. Periodically these are removed from the clipboards and filed appropriately.

i. Vial position, method program, sample identification and instrument operator are entered into the sequence. The exact order of controls, reagent blanks, and samples may vary with each sequence, but generally the order of samples to be analyzed is:

(a) Negative control

(b) Positive control

(c) Blank

(d) Samples (usually no more than five (5) like samples are to be analyzed without a blank being injected)

Blanks or negative controls must be analyzed (unless approved by Lead or higher):
1) In-between different cases, or
2) In-between differing sample matrixes within a single case, or
3) In-between like sample matrixes if packaged in separate evidence bags.

(e) Blank

(f) Additional positive control reference standards, as needed.

Note-12: The Daily Standard Mix is acceptable for use as a positive control.

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(i) Positive control reference standards are analyzed for the comparison of retention times (RT) and mass spectra (known to unknown). Even though a known positive control may have been analyzed, published spectra should be used and accompany analyte printouts from samples (i.e., library searching spectra) when possible.

(ii) Positive control reference standard solutions should be analyzed within 24 hours of the original injection and should be appropriate to the method that was used. However, if nothing has changed with the instrument or method that would affect the results (e.g., clipping column or temperature changes) then data from controls greater than a 24-hour period are acceptable, within reason.

4. Evaluation/Interpretation of Data:

   a. The total ion chromatogram (TIC) and associated spectra for each sample are reviewed using appropriate GC/MS software. Each significant chromatographic peak should be reviewed to determine if it is an analyte of interest (i.e., a controlled substance).

Note-13: Significant Peaks: To justify the existence of a significant chromatographic peak the peak's baseline signal-to-noise ratio (SNR), based on height, should equal or exceed three (3).

   b. Preliminary identification of an analyte is achieved by either operator recognition of a particular spectral profile or by the instrumental library providing matching spectral information.

   c. An identification is considered "confirmed" when the sample analyte is compared to a positive control (e.g., an in-house validated standard) and the following three (3) criteria are met:

      i. Chromatography

         Retention Times: Analyte peak retention times (e.g., relative retention times (RRT)) are within +/- 5% between sample and positive control.

Note-14: Exceptions may be made based on the analyte in question. For example, a wider window of acceptance (e.g., +/- 10%) may be accepted for steroids or non-controlled substances that do not chromatograph well (e.g., stanozol and acetaminophen).

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ii. Mass Spectrometry

(a) Mass spectra will compare favorably to mass spectra from positive controls. Comparison of mass spectral fragment pattern ion ratios should also compare favorably but may differ based on certain factors (e.g., large concentration differences between controls and samples). Spectra may also compare favorably to known library spectra, although library spectra may have been produced under different mass spectral parameters.

(b) Mass spectral reference libraries (e.g., Wiley, AAFS, SWGDRUG, internal) will be used during the evaluation process. The internal library is made of substances analyzed in-house and may not be in the published libraries. Entries within the internal in-house library should be compared to a published spectra from a reliable source prior to use in casework.

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Note-15: In some situations an analyte may be identified in a report by 'mass spectral match only' when an in-house standard is not available but a published reference spectra is available and is used for confirmation. It will be made clear in the report that the identification was made by using only mass spectral data and not chromatographic data (i.e., only one instrumental technique). This will generally only be used for non-controlled substances where GC/MS spectra are consistent with published reference data.

Note-16: Non-controlled substances, whether mixed with controlled substances or not, do not have to be identified. However, if analytes are found within a sample which are indicative of a 'cutting agent' and their chromatographic peaks are greater than peaks from controlled substances (or other analytes of interest), then the 'cutting agent' (or similar) analyte may be identified if deemed important by the analyst and proper controls are used. Contact an FSE2 or higher if uncertain whether to identify an analyte or not.

5. Acceptance of Data:

a. To accept sample GC/MS data the following must be true:

i. The blank prior to the injection of the sample must not contain significant unexplainable peaks. However, due the nature of samples analyzed within the Unit (e.g., plant materials or oil based samples), certain non-controlled substances may remain on a GC column even after the method has completed and thereby elute after multiple injections have been performed. If retention times significantly differ (i.e., minutes) then column carryover is likely the issue (as opposed to injection carryover)

and data may be acceptable. Consultation of an FSE2 or higher should occur in these situations if considering acceptance of data.

(a) If the analyte being identified in the sample is present in the blank prior to its injection then the abundance of the peak in the blank (height or area) must be less than 5% of the abundance of the CHEP peak, or the sample's analyte peak should be greater than ten-times (10x) that of the analyte peak from the previous blank.

(b) When it is known that there is a carry-over issue with a sample then multiple blanks can be injected to minimize the carryover and only the blanks after a sample and just prior to the next sample need to be evaluated.

(c) Blanks injected after a sample injection should be free of any analytes of interest. Any analytes found in the blanks may indicate that the method's temperature program was not long enough to elute all the peaks from the GC column.

ii. Controls must be acceptable.

(a) Negative controls will be free of analytes of interest and no significant unexpected peaks should be present. Explainable peaks will be acceptable.

(b) Positive control analytes must have acceptable chromatographic and mass spectral data with the unknown sample analytes for an identification to occur.

(c) Unexpected results within positive or negative control data should be reported to the Lead Examiner or higher for evaluation.

b. Documentation: Appropriate instrumental printouts, including reagent blanks and controls, will be included in the case file such that an independent reviewer would be able to readily interpret analyst conclusions.

i. The TIC (Total Ion Chromatogram) will be retained in case files. Label the chromatographic peaks for all analytes being identified and for the CHEP peak. Indication whether the retention times are acceptable should occur (e.g., *RT OK or RT* √).

(a) Every significant peak will be evaluated. Indications that peaks have been searched will be done either with a check mark or other method.

(b) Analysts may choose to label non-reported/non-confirmed analytes. This can be done by labeling the peak and putting the label in parenthesis or by making some other notation indicating it the analyte peaks have not been confirmed.

ii. All identified analytes will have documented spectra and appropriate comparative reference spectra (e.g., from positive controls and/or spectral libraries) within the case files.

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iii. In samples where no analytes are identified the analyst should include the TIC along with any appropriate extracted ion chromatograms (EICs). If there are significant peaks that cannot be identified, or attributed to common artifacts, then such spectra should be included in the case file.

iv. In cases where identifications cannot be made due to the lack of obtaining a positive control, indications can be made , if appropriate (e.g., based on mass spectral library data).

v. Positive controls should be analyzed contemporaneously when identifying analytes in evidentiary samples or when specifically looking for analytes in samples which result in negative results.

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GC/MS Common Temperature Programs (may be changed, if needed):

Temperature program	Drugs			Combo		
Initial temp	90° C			60° C		
Initial Time	0.00 min			0.00 min		
Ramps	rate	temp	Final time	rate	temp	time
Rate/final	30.0	300	7.50	20	200	0.0
				30	300	20.0
Post temp	0°c			280°c		
Post time	0.00			0		
Run Time	~14.5 min			~32.3 min		
Front inlet						
Mode	Pulsed splitless			Pulsed splitless		
Initial temp	250°c			250°c		
Pressure	9.32psi			8.19 psi		
Pulse pressure	40.0psi			40.0psi		
Pulse time	0.50 min			0.50 min		
Split Ratio	n/a			n/a		
Split Flow	n/a			n/a		
Total flow	48.1mL/min			53.4 ml/min		
Gas type	Helium			Helium		
Injection volume	1 microliter			1 microliter		
Post injection washes	Solvent A -3 Solvent B - 3			Solvent A -3 Solvent B - 3		
Tune file	ATUNE			ATUNE		
Acquisition mode	Scan			SCAN		

Solvent delay	4.95 minutes			3.73 min		
	Low mass	High Mass	Threshold	Low mass	High Mass	Threshold
	44	550	500	44	550	500
	MS Quad 150°c max 200°c			MS Quad 150°c max 200°c		

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G. **References**:

1. Clark's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids, and Post-Mortem Materials, The Pharmaceutical Society of Great Britain.
2. The Drug Identification Bible
3. Baselt, R.C., *Disposition of Toxic Drugs and Chemicals in Man*, 7th ed., Biomedical Publications: Foster City, California, 2004.
4. Moffat, A.C., *Isolation and Identification of Drugs*, 2nd ed., Pharmaceutical Press: London, 1986.

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Rev. #	History
3	Made slight grammatical and format modifications. Added a 'Revised History' section to the document. Updated logo searching part of the procedure. Strike-outs removed and color uniformly set as black. Replaced 'section' with 'unit.' Replaced section supervisor with Lead Examiner. Summarized steps so that they were not wordy. Replaced Laboratory with Unit. Corrected CHEP error from 1% to 0.001% concentration. Removed the option that the Daily Standard Mix solution's concentrations can be altered based on analyst's decision. Added that no maintenance should be done on instruments without Lead Examiner notification.
4	Added title to document. Changed Purpose to Introduction. General formatting changes throughout document. Removed 'Unit' within responsibility section. Updated definitions section. Updated 'Reagents' section to include other materials and instruments. Added 'Preparation of Performance Test Solution, Controls, and Standards' section and described how Performance Mix is prepared. Updated various parts of the 'Procedure' section to align with current practice and to improve clarity. Defined what a significant peak was and updated evaluation/decision criteria to reflect current practices. Updated tune parameter and methods. Updated 'References' section.

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A. Purpose:

The primary use of the Fourier-Transform Infrared spectrophotometer (FTIR) in this procedure is for the identification of drugs and controlled substances. It is particularly useful in differentiating the different forms of cocaine (i.e., cocaine base and cocaine hydrochloride). The FTIR instrument can be used in the identification of a variety of solid, liquid, and gas samples (e.g., nitrous oxide). Analysis of drugs and other compounds by FTIR is considered a structurally elucidating confirmatory technique.

The FTIR is used in conjunction with the gas chromatograph/mass spectrometer (GC/MS) in order to identify cocaine in samples. The use of water solubility testing and FTIR analysis can be used to differentiate the different forms of cocaine.

Historically the distinction of cocaine free base (CFB) from cocaine salt form (CSF), or a mixture of the two forms, was important prior to 2005. This was because the criteria weights and associated penalties associated with the possession of these drugs were set at 0.5grams for CFB and 28.35 grams (1 ounce) for CSF. Currently the criteria weights and associated penalty changes are set at 0.5 ounces for both forms of the drug, so differentiation of the two forms is not always necessary.

B. Safety:

The FTIR utilizes a laser which should not be directly viewed. As with any electrical device there is a chance of electrical shock if not handled properly. Do not perform maintenance on this instrument unless trained to do so. A laboratory coat will be worn while working with the FTIR instrument while analyzing casework samples.

C. Responsibility:

All analysts examining samples for the presence of drugs, controlled substances, or other chemicals.

D. Materials and Equipment:

Fourier-transform infrared (FTIR) spectrophotometer (Perkin-Elmer or equivalent)

Horizontal Attenuated Total Reflectance (ATR) Accessory (Pike, KRS-5, ZnSe, or equivalent)

Benzocaine (Reagent grade)

Polystyrene (ThermoNicolet or equivalent)

E. Procedure:

1. Analysis: Using ATR:

   The FTIR used in conjunction with a HATR (aka. ATR) accessory. The ATR accessory allows for samples to be analyzed directly without sample preparation. Other FTIR accessories (e.g., microscope, gas cell) may be used for casework if they have been validated.

   a. Perform the QA/QC check on the FTIR daily – that is, on the day it is to be used for sample analysis. A polystyrene (PS) reference and benzocaine standard are analyzed and all printouts should be placed into the instrument logbook.

   b. The energy of the light source should be monitored to verify that there has not been a significant energy decrease. Any energy decrease may indicate the need to replace the source. If the source is left unchanged it could result in loss of sample sensitivity.

   c. If the QA/QC check has problems or the instrument operability fails, document the failure in the instrument log book and notify the section supervisor, or designee.

   d. Clean the ATR platform with methanol (or other appropriate solvent) to remove any materials.

   e. Collect a background spectrum before acquiring a sample spectrum. A background spectrum can be saved and used if multiple samples will be analyzed during a relatively short time period. If desired, a background spectrum can also be collected directly after sample collection instead of before sample acquisition.

   f. Thorough cleaning of the ATR accessory usually results in no carryover. Collection a blank spectra in-between casework samples is necessary when residual analytes are analyzed. The blank ensures that the sampling platform is clean and void of carryover.

   g. Where applicable, collect positive control samples after unknown samples have been analyzed.

   h. Save all data (saving interferogram data is recommended, but optional)

   i. Perform data analysis (e.g., library searching, spectral comparisons, spectral subtractions)

   j. Interpretation of Data:

      i. Cocaine form determination:

      Cocaine freebase (CFB): ~2945cm^{-1}, ~1734cm^{-1} (doublet peak), ~1706cm^{-1} ,~712cm^{-1}.

      Cocaine salt form (CSF): similar to CFB, however there will be an additional peak at ~730cm^{-1}. Additionally for CSF, slight shifts can occur at the 2945cm^{-1}, 1734cm^{-1}, and 1706cm^{-1} ranges.

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ii. Spectra should compare favorably (whether determining the form of cocaine or whether helping determine the identity of a drug).

iii. While library searches are helpful, it is the responsibility of the analyst to make the final determination as to what drug or chemical was detected.

iv. When comparing spectra it is best to overlay the spectra in absorbance mode as opposed to transmittance mode.

2. Analysis: Using Gas Cell

The gas cell is a chamber which allows a sample that is in a gaseous state to be sampled and analyzed on the FTIR (e.g., nitrous oxide).

a. Remove the HATR and attach the gas cell accessory to the FTIR. The gas cell is a closed glass cylinder with 2 stopcocks (these are utilized to fill and purge the cell).

b. Collect a background spectra using the glass cell without sample.

c. Purge the cell with air (or $N_{2(g)}$).

d. Analyze a blank using the gas cell and ensure the cell is contamination-free.

e. Purge the gas cell with unknown gas by opening both stop cocks (so that it is an open system) for an adequate amount of time. Stop the purging process and close the stopcocks.

f. Perform an analysis.

g. Purge the cell with air (or $N_{2(g)}$) and ensure that it is contamination-free.

h. Use a sampling plan similar to other types of drug analyses (i.e., if only one sample then analyze 2x ; if all evidence is indistinguishable, sample only two (2) pieces of evidence and stop if results are same).

i. Analyze positive control(s) in a similar manner.

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F. Evaluation:

The FTIR is a very stable instrument and requires very little maintenance. The maintenance performed includes daily (as used), and as needed items.

1. Day of use: Analyze a PS standard and a benzocaine standard
2. Compare the spectra to previously acquired spectra to confirm that there were no shifts or changes. Major differences indicate problems with the instrument or the accessory.
3. If needed, compare spectra to library spectra. Consult supervisor, or designee, if shifts in spectra, changes in spectra, or problems are observed.

4. Print necessary spectra and place in the instrument's maintenance logbook.
5. Change desiccant as needed. This is performed by opening the cover of the instrument removing the cage which contains the desiccant and placing fresh desiccant bags in the cage. The cage is then returned to the instrument and the software can be updated to show the change. If applicable, this can be done as part of the service contract by an Perkin-Elmer Engineer.
6. Adjust HATR as needed. This should only be performed by appropriately trained personnel and as needed when there's a drop in the energy level or after replacing the HATR. The HATR alignment can be adjusted to maximize the energy levels of the light source.

G. References:

Operating the Perkin-Elmer Spectrum 100 FT-IR Spectrophotometer, operator's manual.

Spectrum 100 FT-IR User's Guide; Perkin-Elmer, 2008

Spectrum 100 FT-IR Help Topics; Instrument Validation.

Spectrum 100 FT-IR Help Topics; Performing an instrument validation

Clark's Isolation and Identification of Drugs in pharmaceuticals, body fluids, and post-mortem materials, The Pharmaceutical Society of Great Britain.

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Rev. #	History
4	General formatting changes throughout document. Changed the primary use of the instrument from cocaine form differentiation to chemical, drug, and controlled substance identification. Sampling plan for gases similar to other drug analysis sampling plan. Made service contract maintenance optional. Updated history section of document.

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Toxicology Technical and Administrative Review Checklist: Case number: _____

	Alcohol	Drug	Supplemental
	Check if acceptable if not acceptable add notation		
Technical Review			
Draft report reviewed: · Evidence Description appropriate · Appropriate Report format · Reports supported by Documentation · Uncertainty statement included when appropriate			
Screening Results Reviewed			
HS/GC, GC/MS or LC/MS results reviewed · appropriate identifications made · chromatography acceptable · batch review appropriately documented			
Appropriate Calibrator/Controls and Blanks used			
Calculations checked if applicable			
Review of evidence submission form/EMIT/reported findings to determine if further work is required			
Technical Reviewer/Date			
Administrative Review			
Case demographics acceptable: · Name of Submitting Agency · Subjects name/address · Lab case number/Agency case number			
An analyst's initials or signature on all pages in case file			
Technical Reviewer Initialed above			
Milestone sticker completed			
Cross outs or changes are properly annotated / no obliterations			
Administrative Reviewer/Date			
Final Review			
Final Report Signed, dated and analyst title present			
Report Mailed/Faxed (for DFSA prepared to forward to Evidence Receiving)			
Reviewer/Date			

Notes:

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Blood:	Serum:
Urine:	Vitreous:
Other:	

Test	Batch# / Reviewed & Approved by:	Date	Analyst	Item	Results		
EMIT					Neg	Amp Barb Bz Coc THC Op PCP Md Oxy Bup	EtOH - / +
					Neg		EtOH - / +
					Neg	THC Barb	
Volatiles							
WAN/BDS							
LC/MS							
Opioids							
Benzos							
THC							
Coc/BE							

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Case Notes:

Title: TX 5: Case Documentation and Review

1.0 PURPOSE:

The purpose of this procedure is to establish the processes for preparation, review, storage, and retention of case documents within the Toxicology Unit. This procedure applies to all personnel involved in the daily practices of the Toxicology Unit.

2.0 DEFINITION:

2.1 Analyst (FSE or Chemist): one who is assigned to the unit and usually has the designation of Forensic Science Examiner (FSE). Other designations of analysts may include Chemist or Principal Chemist in their titles. Analysts are authorized to perform tests within the unit and analyze evidence. Various analysts have degrees of responsibility in the preparation of case documentation and participation in review processes.

2.2 Batch Reviewer (aka: Batch Technical Reviewer): one who has the knowledge, skills, and authorization to adequately review sequences and resulting data from batches of samples that were collected during an analytical experiment. While complete knowledge of the area being reviewed is required, it is recommended that batch reviewers be competent in the operation of the technique that is being reviewed. Batch reviewers are authorized based on thorough evaluation of their knowledge, skills, and abilities within each area that they are allowed to review. Authorized analysts who produce data and who have been deemed competent in the practical operation of instrumentation through casework are deemed authorized to perform batch/technical reviews. The batch reviewer cannot be the analyst/author of the batch.

2.3 Technical Reviewer: one who has the technical knowledge, skills, and authorization to adequately review the science within the data of case files.

2.3.1 Final Technical Reviewer:
Ensures the technical accuracy for the totality of the case that is under review. The technical reviewer can, but is not required to, review data that has been previously batch reviewed (e.g., blanks, controls, calibrators, and related samples). Aside from previously-reviewed batch data, the remaining analytical data must be technically reviewed to ensure that the reported findings are supported and valid. Technical reviewers cannot review their own data. It is permissible to have multiple technical reviewers on a case (e.g., when the main technical reviewer isn't authorized to review all of the analytical procedures within a case and another reviewer is needed to perform a technical review on specific aspects of the case file). Persons performing technical reviews will determine whether the appropriate examinations have been performed, that the

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conclusions were consistent with the documented data and that they were within the limitations of the discipline, and that there was sufficient supporting documentation for the conclusion(s) of the report. As verifier, the technical reviewer will independently confirm reported conclusions by reviewing or examining relevant information including, but not limited to, data, notes, and charts.

2.3.2 Batch Technical Reviewer
Batch reviewers are analysts who have the knowledge and authorization to evaluate the technical accuracy of batches/sequences as a whole for specific types of analyses.

2.4 Administrative Reviewer: one who has the knowledge, skills and authorization to adequately review case files for conformance to administrative quality. In general administrative reviews can be performed by laboratory support personnel or by anyone assigned by the Unit Lead to complete the task. Administrative reviewers need not be analysts or a qualified examiners/scientists. An administrative reviewer will ensure that reports are clear, concise, accurate, complete (with respect to the requests that were submitted), in compliance with DSS general laboratory (GL) procedures, and verify that a technical review has been performed. Additional duties of an administrative reviewer may include, but not be limited to, obtaining handwritten signatures from analysts (when needed), physical sending of copies of report, and filing.

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3.0 PROCEDURE

3.1 Preparation of case jacket:
When evidence is transferred to the Toxicology Unit it should be transferred with the related case paperwork. Case jackets (various colored file folders) are used to hold paperwork for each case submission. Multiple case folders should be used for different submissions of the same case (same laboratory numbers can have multiple case file folders).

3.1.1 A self-adhesive milestone label on the front of the file folder and a case summary sheet TX-5.3 allows for information to be readily seen for each case file. Use of the milestone labels are for reference only and are not considered administrative documents. The TX-5.3 case summary sheet, when used, will be considered an examination document.

3.1.2 Barcode labels for each item submitted within a case should be placed on file folders. These labels list the number of submissions (items) that are related to that case jacket.

3.1.3 Colors of the case jackets:
Red file folders are used for fatality-type [antemortem] cases
Yellow folders are used for drug-facilitated sexual assault-type cases (DFSA)
Green folders are used for proficiency test cases
Blue folders are used for postmortem-type cases
Manila-colored folder are used for driving under the influence-type (DUI) [antemortem] and other cases

3.1.4 Administrative paperwork can either be placed within the case file folder or can be found within the JusticeTrax laboratory information management system (LIMS).

3.2 Case jackets containing paperwork for all active cases (i.e., those where reports have yet to be released) should be stored within the laboratory space. When cases have been completed (i.e., all reports have been released), case file folders and the folders are placed within locked filing cabinets. As space availability dictates, the completed case file folders may be archived and moved to another secure storage location. Electronic reports need not be printed and kept within case file folders as they are stored on the LIMS.

3.3 Batches: Analyses in the Toxicology Unit are often performed as part of a batch (or sequence). Batches usually include multiple case samples, calibrators, and control solutions. Batches are used for efficiency purposes wherein multiple items of evidence are analyzed within one sequence. Either copies of relevant batch data sheets and batch summary information are to be filed within each applicable case file folder or within LIMS. The completed batch/sequence paperwork will include at minimum (if applicable): calibrators, controls, blanks, instrumental parameter/sequence printouts, and proof of successful batch technical review. Batch paperwork will be filed in a secure location (e.g. Toxicology Unit laboratory space) and will be trackable (i.e., a list, file, or other type of system exists wherein cases/lab numbers are associated/linked to batches).

3.4 Reviews

3.4.1 Technical Reviews

3.4.1.1 Batch Technical Reviews: Instead of conducting separate technical reviews on each case, each batch of data can be 'Batch Reviewed' by a Batch [Technical] Reviewer. Batch reviews are performed after the analyst who set up the batch/sequence has evaluated their data. All batch reviews of data need to be clearly identified on the batch/sequence paperwork. After batch technical reviews are complete, each case must contain a cross-reference to each batch/sequence.

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If there are problems with batches/sequences and a batch review fails (i.e., the batch doesn't meet acceptance criteria), the failure must be easily identifiable within the batch paperwork and reasons for the failure (e.g., annotated on the batch coversheet) must be present. As with all batch paperwork, cases must be cross-referenced to all batches, either within case file folders or within LIMS.

3.4.1.2 Technical Review (Final): Final reviews of cases are performed by authorized analysts, Unit Leads, or other designees (e.g., Director, Deputy Director) after all analytical work has been completed and "Draft Reports" have been done. A technical review checklist (form TX 5.1, 'Technical and Administrative Review Checklist' or TX 5.2 'Technical and Administrative Review OCME Case Specific') is used as a guide. The information on the milestone stickers on the case file folder jackets are used for convenience but are not considered administrative notes. Technical reviews are done to ensure the technical accuracy of the data within each case file folder. Documentation that batch reviews have been done must be found within case file folders or within LIMS. The data/information within batches are not required to be technically reviewed during the final technical review because such reviews have already occurred. Final technical reviews must ensure that any batches within case file folders have been reviewed and were acceptable relevant to the cases in question. Any data/information not already technically reviewed will be assessed during a final technical review.

Final Technical Reviews will ensure:

- Evaluation of case documentation for consistency and accuracy among analytical batch data, other analytical data, and all reported findings.
- Manual calculations will be reviewed and documentation of such reviews (e.g., initials of reviewer) should be evident.
- All uncertainty statements are correct.
- Appropriate testing has been performed and relevant questions have been answered.
- Problems or limitations in casework are clearly explained in case files (e.g., case notes) and, when applicable, within reports.
- Appropriate checklists are used during technical reviews (e.g., TX-5.1, TX-5.2)
- Ensure that reviewers of batches have documented their reviews (e.g., initialing the coverpage of the batch).

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- All notes/comments from technical reviewers will occur on the appropriate checklists. Technical reviewers should not add any information to casefile paperwork (administrative or examination documents). Corrective (or other) comments should be limited to only being placed directly on Draft Report pages and/or on review checklists.
- Submit case back to appropriate analyst(s) for any corrections/additions that are needed.
- Submit case files for Administrative Reviews upon acceptance of technical reviews
- Appropriate milestones within LIMS will be updated.

3.4.2 Administrative Review: Administrative reviews are performed after completion of final technical reviews and before reports are released. Administrative reviewers are individuals who will ensure that administrative information is accurate, free from clerical errors, and abide by certain quality assurance/quality control guidelines. Administrative reviewers should use the appropriate checklist portion of form TX 5.1 (Toxicology Technical and Administrative Review Checklist) or TX 5.2 (Technical and Administrative Review Checklist- OCME Specific) when conducting reviews.

Administrative Reviews will ensure accuracy regarding:

- Information within reports corresponds to what was requested within Request For Analysis (RFA) forms.
- Name of Submitting Agency (Name of medical examiner for OCME cases)
- Subject's Name (if applicable)
- Subject's initials for sexual assault cases
- No subject name on OCME cases
- Subject's address (DUI cases only)
- Case number correct
- Agency case number correct
- Other demographics (as appropriate to the case)
- Grammatical and/or clerical aspects of the case (any minor errors may be changed by Unit Lead or equivalent)
- DSS case number (Lab #) is correct on all Administrative and Examination Documents (this can be added by the Administrative Reviewer, if needed)
- Appropriate analyst initials are on all Examination Documents
- Final technical reviewers must have initialed, at a minimum, appropriate technical and administrative review worksheets (TX-5.1 or TX-5.2)

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- Any administrative errors or changes shall be documented in either case file folders or within LIMS. If handwritten, all additions or changes will be initialed by the person making said additions/changes.
- All notes/comments from administrative reviewers will occur on the appropriate checklists. Reviewers should not add any information to case file paperwork (administrative or examination documents). Corrective (or other) comments should be limited to only being placed directly on Draft Report pages and/or on review checklists.
- Appropriate milestones should be completed on case file folders, but these are for convenience and are not considered administrative documents.
- Appropriate milestones within LIMS will be updated.
- If required for distribution, the Administrative Reviewer will print the final report
- Distribution of reports may be performed by the Administrative Reviewers or their designee.

3.5 Ethanol Conversion Reports.
Ethanol conversion cases are maintained electronically in JusticeTrax (JT). Technical and Administrative reviews will be documented in JT under milestones. No casefiles or case file folders will be maintained within the Toxicology Unit for these types of requests. These reports do not fall under guidelines of 3.1 (above). Conversion reports will contain technical and administrative reviews just like other casework. When finalized, conversion reports will be faxed, mailed, or sent out electronically.

3.6 Final Reports

3.6.1 If reports contain handwritten signatures of analysts, the original report will be kept with case file folders and will be securely stored (e.g., in Toxicology Unit cabinets).

3.6.2 If reports are generated with electronic signatures, original reports are stored in JusticeTrax and copies may be included within case file folders.

3.7 Issuance of Reports

3.7.1 DUI Cases

3.7.1.1 A copy of the report is sent (e.g., U.S. Postal Service) to the subject/source.

3.7.1.2 Additional copies of reports are sent (e.g., fax, electronic, mail) to the submitting agency and others, as appropriate (e.g., State's Attorney, DMV).

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3.7.1.3 If reports need to be corrected, any new report will be clearly marked as being revised along with an explanation as to why revisions were necessary. Both the incorrect and the corrected information will be listed so customers are fully aware of changes within reports.

3.7.1.4 If additional information needs to be added to reports, any new reports will be clearly marked as being supplemental and description(s) as to what the addition(s) were will be included in all supplemental reports.

3.7.1.5 Documentation that reports have been sent will be either included in case file folders or within LIMS.

3.7.2 Postmortem Cases for the Office of the Chief Medical Examiner (OCME)

3.7.2.1 Reports will be sent electronically, but other means may be necessary.

3.7.2.2 Documentation that reports have been sent will be either included in case file folders or within LIMS.

3.7.2.3 Filenames for electronic copies of reports should contain the initials of the medical examiner associated with the case (e.g., DSS-16-00123 JG for Dr. James Gill)

3.7.3 Sexual Assault Cases (or other cases)

3.7.3.1 Two copies of the report are made.

3.7.3.2 The report is itemized in LIMS following the guidance of GL-11 'Records Control' and is transferred to the Evidence Receiving Unit for distribution to the customer.

3.7.3.3 A copy of the report can be either stored in the case file folder or in LIMS.

3.8 Documents generally found in the case jacket (or may be found electronically in LIMS)

- Copy of reports (Draft Report, Released Report)
- Administrative Documents
  - Review sheets (Technical and Administrative)
  - Request for Analysis (RFA) form
  - Medical reports
- Examination Documents
  - Case Summary Sheet
  - Analytical Data – including Batch information (e.g., Batch Summary Sheets)

3.9 Record Retention – refer to GL-11 (Records Control)

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Rev. #	History
6	Added verbiage and updated formatting within the entire document. Added to definitions section and clarified roles of reviewers. Simplified color definitions and added OCME type of case. Made milestone labels not required, but useful. Storage of case documents can either occur in file folders and/or in JusticeTrax (JT). Clarified information regarding batches (e.g., batch review process, retrieval of batch information, linking batch information to case information). Clarified definitions of 'reviews' within document. Simplified Ethanol Conversion Report section. Added ability to document sending out of reports to be electronic within JT. Removed unnecessary verbiage throughout document. Added OCME reporting information. Added History section.
7	3.1.2 changed items to submissions 3.2 removed the need to print the final report and place in case file 3.4.2 updated to allow for reports not to be printed, maintained electronically and allowing for person other than the Administrative Reviewer to distribute case reports.

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Ethanol Conversions

1 Introduction

The measurement of ethanol (EtOH) in a person's body for instances involving legal matters (e.g., driving under the influence (DUI)) can be performed within breath or within body fluids (e.g., whole blood, urine, blood-plasma/serum). When samples are sent to the Division of Scientific Services (DSS) laboratory for toxicological testing, the specimens are usually in the form of whole blood or urine. There are times, however, when blood samples are drawn within medical facilities and the blood is analyzed for medical purposes. Subsequent medical reports on the blood samples often have ethanol concentration values listed as plasma-ethanol concentration instead of whole-blood ethanol concentration. Since legal statutes are worded to reflect whole-blood ethanol concentrations, mathematical conversions are often needed on the values within the medical reports in order for the ethanol quantitation values to be useful within the judicial system.

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2 Scope

Quantitative values indicating serum ethanol concentrations found within medical reports will be mathematically converted to equivalent whole-blood ethanol concentration values using a currently accepted method within the forensic toxicology discipline. No chemical analyses of specimens will be conducted within this procedure. Numerical values used for conversions will be solely based on blood-serum values and are typically received from medical (or similar) documentation. Since it is highly unlikely that an original medical report will be sent in for a blood-ethanol conversion request, the term 'medical report' in this procedure is synonymous with medical report copy.

Serum alcohol to blood alcohol conversion can be performed either using a range on conversion factors or using a single conversion factor. The conversion factor used within this procedure (1.16) is an average of the low (1.12) and high (1.18) conversion values found reported within reference articles with an administrative addition of 0.01 (e.g., $(1.12 + 1.18)/2 = 1.15 + 0.01 = 1.16$).

3 Principle

A blood-serum ethanol concentration value to be converted to a whole-blood ethanol concentration can be submitted either electronically or by paper copy. The value to be converted must be in the form of a valid medical (or similar) report. Oral requests of conversions will not be performed unless properly approved by the Deputy Director, or higher.

4 Equipment/Materials/Reagents

a. JusticeTrax program

b. Calculator (or equivalent)

5 Preparation of Reagents, Standards, and/or Controls

Not applicable

6 Calibration

Not applicable

7 Sampling

Not applicable

8 Procedure

8.1 Valid documentation

Only one (1) blood-ethanol conversion will be done per laboratory report. Multiple medical reports of individuals will be handled as separate requests and each will have their own DSS laboratory report. The page of the medical report which contains the value to be converted should be from a medical facility. The applicable documentation must contain the [medical] facility's name, address, and the patient's name. The complete medical report need not be submitted for a blood-ethanol conversion to be performed.

8.2 Correct name(s)

Ensure that the patient's name (i.e., suspect) has been entered correctly into the Laboratory Information Management System (LIMS) and that the name within the medical documentation concurs with JusticeTrax (JT) data. If multiple medical report pages are received, the serum value to be converted must be on a page which contains the [correct] patient(i.e., suspect) name. Initials, patient identification numbers, or any identifier other than the patient's name will not be accepted as valid identification.

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8.3 Ethanol value to be converted

Find the correct value to be converted within the medical report (i.e., serum). Ensure that the value is for ethyl alcohol and that the units are milligrams (mg) per deciliter (dL) which is sometimes listed as: mg/dL or MG/DL. If the ethanol concentration value is not in a mg/dL unit format, mathematically convert the value to a value equivalent to a 'mg/dL' unit format. Show calculations so that they may be reviewed for accuracy. This can be done directly on the medical report and will be considered case notes.

8.4 Conversion (ensure value is truncated and only 2 decimal places)

Convert the serum ethanol concentration value to a whole blood ethanol concentration value in gram percent (g%) units. This will be done automatically using the JT system. If the incoming value for serum ethanol is in a concentration format other than what is typically submitted, the Examiner may manually convert the calculation prior to entering the value into the JT system.

For verification purposes or when necessary (e.g., when JT is unavailable), the calculation can be done by multiplying the serum ethanol (mg/dL) value by 0.000862 (or by dividing the serum (mg/dL) value twice: once by 1.16 (to convert serum EtOH to whole blood EtOH), and then again by 1000 (to convert from mg to g)). This will give a resulting whole blood ethanol value in units of gram per 100 milliliters (g/100mL), which is equivalent to the unit of gram percent (g%, or gm%).

$$\frac{28\text{mg}}{\text{dL}} \times 0.000862 = \frac{0.02\text{g}}{100\text{mL}} = 0.02\text{g\%} = 0.02\text{gm\%}$$

If the serum ethanol value is reported in units of gram per deciliter (g/dL), the conversion can be done by dividing that number by 1.16. This will give a resulting whole blood ethanol value in units of gram per 100 milliliters (g/100mL), which is equivalent to the unit of gram percent (g%, or gm%).

$$\frac{0.333\text{g}}{\text{dL}} \div 1.16 = \frac{0.28\text{g}}{100\text{mL}} = 0.28\text{g\%} = 0.28\text{gm\%}$$

All final ethanol concentration values will be truncated to the second decimal place (i.e., hundredths place). Conventional rounding rules will not be applied to these mathematical

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conversions. The number of significant digits is not the same as the number of decimal places. If a plasma ethanol value consists of a number with more than three decimal places, then the converted value will still only be to two decimal places.

For any value within the submitted medical documentation which has a range associated with it (e.g., <), the converted value will carry the same range (e.g., <28mg/dL converts to <0.02gm%).

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9 Uncertainty of Measurement

The uncertainty of measurement should be found within the serum measurement values from the medical documentation. No uncertainty will be reported resulting from the single point conversion within this procedure.

The report will have the following statement, "The conversion from serum ethanol to blood ethanol concentration found within this report is based on a single point conversion factor, as opposed to a conversion factor range."

10 Limitations

Not applicable

11 Safety

Not applicable

12 References

M.T. Barnhill, Jr., D. Herbert, D.J. Wells, Journal of Analytical Toxicology, Vol. 31, January/February, 2007

P.M. Rainey, Relation Between Serum and Whole-Blood Ethanol Concentrations, Clin. Chem., 39/11, 2288-2292, 1993

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Wiley and Sons: New York, 1991.

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Drummer, O.H., The Forensic Pharmacology of Drugs of Abuse, page 302.

RETIRED

Revision #	Revision History
1	New Document
2	Removed the 'Issue Date' from the 'Revision History' section of the document.

RETIRED

Title: General Approach to Toxicology Cases

1. Scope

This procedure provides general guidelines for the receipt, analysis, and disposition of toxicological evidence for cases assigned to the Toxicology Unit (TU) within the Chemical Analysis Section (CAS) of the Division of Scientific Services' (DSS) forensic laboratory. These guidelines are based on recommendations set forth within a number of reference documents such as, but not limited to:

- DSS laboratory Quality Manual procedures
- ANSI National Accreditation Board (ANAB) AR3125 – Forensic Science Testing and Calibration Laboratories Accreditation Requirements
- Society of Forensic Toxicologists (SOFT)/American Academy of Forensic Sciences (AAFS) Forensic Toxicology Laboratory Guidelines
- American Board of Forensic Toxicology (ABFT) Laboratory Accreditation Manual
- Drug Abuse Handbook
- Poison Detection in Human Organs
- Introduction to Forensic Toxicology
- Principles of Forensic Toxicology, Handbook of Analytical Toxicology

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2. Equipment/Materials/Reagents

Not applicable. Guidance for preparing reagents and the utilization of instruments/equipment may be found in specific toxicological procedures.

3. Standards and Controls

Not applicable. Guidance for preparing standards and controls (e.g., positive and negative) may be found within specific toxicological procedures.

4. Calibration

Not applicable. Guidance for calibration may be found within specific instrumental procedures.

5. Sampling

The most common types of toxicological specimens for antemortem analyses are urine and blood (sometimes plasma or serum will be submitted). For postmortem analyses blood, urine, and/or vitreous humor are often analyzed (if plasma/serum are submitted they may be

treated like blood specimens and will be analyzed accordingly). Prior to sampling containers should be inverted or swirled to ensure homogeneity. If blood samples are found to be clotted, such clots may need to be removed or homogenized/broken-up (as best as possible) before sampling occurs. The determination of whether blood clots will be removed or analyzed and the process for ensuring homogeneity of clotted samples will be done on an as-needed basis and will involve a Lead Examiner or higher. Appropriate case notes will be taken for documentation of events.

Blood samples may be received in multiple tubes. When blood has been reportedly collected at the same time interval and from the same location/person, and has been in the same type of test tube (i.e., grey top), it may be considered the same item and be given the same item number during accessioning (e.g., Item #: 001-001 – blood within two (2) red top tubes). In these circumstances the tubes can be further labeled to distinguish the tubes from one another. Any sampling from the separate tubes will be described within applicable case note documents by the analyst(s) who handles the evidence. Situations where it is not clear whether multiple tubes of blood should be considered one item or multiple items will involve the Lead Examiner or higher for a decision to be made. Any clarification or justification for such actions and/or decisions will be appropriately recorded.

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6. Procedure

6.1. Sample Collection, Receipt, and Transfer

The proper selection, collection, and submission of biological specimens for toxicological analyses is important for the scientifically sound interpretation of analytical data. There are recommended minimal volume amounts for specific types of specimens in order to adequately perform routine toxicological examinations (e.g., human performance cases: blood = 15 mL ; urine = 100 mL). However, specimens may be limited and submitting agencies may not be able to obtain recommended minimal amounts. In cases where limited sample amounts are received by the DSS laboratory, the type and amount of specimen may influence which analyses will be performed. In such situations analysts will work with Lead Examiners, and possibly the submitting agencies, to decide which analytical path will be pursued.

When blood is analyzed for containing certain drug classes (e.g., cocaine, ethanol) it is advantageous if the specimen is mixed with chemicals so as to enhance the stability of analytes. This can be accomplished by using specimen tubes that contain preservatives and/or anticoagulants. For most toxicology cases, the preferred collection tube is a grey-top Vacutainer® which contains a mixture of sodium fluoride and potassium oxalate. Expiration dates on such tubes are only for vacuum integrity and do not reflect on the quality of the tube or its components after samples have been captured within the container.

Containers with specimens collected from living persons should be found labeled with appropriate information (e.g., source name, time/date of collection). Biological specimens should be submitted to the DSS laboratory in separate individual packages, under proper [evidentiary] seal, with appropriate warning labels, under a single communication (i.e., Request for Analysis (RFA) form), and should be submitted in a refrigerated or frozen condition. Typically such evidence is found submitted within some sort of kit and/or paperwork (e.g., DUI kit, CT400 kit, DPS-0009-C, copy of medical records).

Such evidence will typically be received through the Evidence Receiving Unit of the DSS laboratory. Proper seal of evidence refers to a condition of packaging which ensures evidence is prevented from cross contamination, there is no sample loss, and any attempt at deleterious change of the evidence would be noticeable.

After initially receiving a toxicology case the evidence will be inventoried and information will be entered into LIMS-Plus (JusticeTrax (JT)). The information can include, but not be limited to: type of specimen(s) received, any labeling present on the containers, the approximate specimen amounts, and any damage to or leaks from the containers. The specimen containers will be labeled with appropriate information from LIMS-Plus (e.g., item number(s) and the Laboratory/Case Number). Any problems or issues with evidence will be addressed at the time of inventory, will be documented appropriately, and can involve a Lead Examiner, Assistant Director, Deputy Director, or designee. Pictures may be taken to document the evidence and any picture should contain the lab number, the date, and the analyst's initials. Pictures will be included within appropriate case files (LIMS-Plus or hardcopy). Contributors may be contacted, if appropriate, for minor issues or discrepancies within cases, and will be contacted for major issues within cases (e.g., sample loss or sample integrity issues).

Evidence transfers will involve adequate chain-of-custody (CoC) documentation and will be documented within the laboratory information management system (LIMS) software (e.g., LIMS-Plus). If evidence is transferred to multiple analysts for the purpose of aliquoting samples then evidence does not necessarily need to be under proper seal between such transfers (evidence must be free of sample loss and free of possible contamination). Any transfer of evidence possession needs to be tracked with respect to CoC documentation to ensure that proper accountability is recorded among analysts. If aliquoted portions of a sample are to be consumed during analysis then each portion can either be considered the same item from which it originally came (and the same item number can be used) or it can be sub-itemized (usually describing why it was used). If a different analyst will be taking control of an aliquoted portion then sub-itemization will be performed.

For example: suppose Item #001-001 blood is to be tested for volatiles by Analyst A and will then be tested for drugs by Analyst B. In this scenario Analyst A takes possession of Item #001-001 and aliquots 0.5 mL of blood into a headspace vial for their own work. To assist their co-worker Analyst A then aliquots 0.5 mL of Item

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#001-001 blood into a test tube for future drug screening by Analyst B. The aliquoted [0.5 mL] drug screen sample will be sub-itemized by Analyst A as Item #001-001-01 (description may be: 0.5 ml BZ/OP Quant aliquot). Analyst B comes at a later time and physically takes Item #001-001-01, appropriately updating the information within LIMS-Plus as a transfer Item #001-001-01 from the refrigerator to themselves. If there was no more of Item#001-001-01 and it was to be consumed during the drug analysis portion, then the aliquoted Item #001-001-01 would be updated within LIMS-Plus as being consumed in analysis.
In the unusual circumstance that an extract from a specimen will be retained (e.g., all the blood has been consumed and the extract needs to be saved), then the extract will be sub-itemized (e.g., Item #: 001-001-01-01) and recorded within the CoC of LIMS-Plus. Extracts from situations wherein biological evidence has been exhausted should be retained until directed otherwise.

6.2. Sample Storage

Due to the nature of biological material, specimens will be kept refrigerated or frozen and under proper seal when not under active examination. Refrigerators and freezers are located in the Toxicology Unit for evidence storage and shall be appropriately secured within a lockable system. Other areas, if acceptable for evidence storage, may be used to store evidence (e.g., refrigerators and freezers in other areas of the laboratory) and will have similar lockable systems. Use of tamper-evident devices will be employed (e.g., evidence tape, security tags).

6.3. Analytical Schemes for Toxicology Testing

Forensic toxicological examinations are conducted on specimens in order to detect a wide range of drugs and/or other substances. The Lead Examiner, Assistant Director, Deputy Director, or designee will be responsible for evaluating cases prior to reports being issued in order to ensure that all requests have been completed/addressed, good case management has occurred, and quality has been maintained. Refer to specific case types for more guidance. If evidence with limited volume or multiple types of evidence are received (e.g., multiple blood tube types, multiple serum tubes), a lead Examiner (or higher), possibly involving the submitting agency's representative, should be consulted so as to create an analytical scheme directing what evidence will be analyzed so as to maximize efficiency and completeness.

Pertinent case histories should be reviewed during both the accessioning process and when cases are being completed (i.e., technical reviews). For most routine DUI-related cases analysts should only need a brief understanding of the history within requests. Certain cases (e.g., sexual assaults) may require a more thorough review of case history so that adequate analytical plans are developed and followed. Professional judgment will be used to determine the sequence of tests which will be performed. The general analytical schemes within this document can be used for

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guidance and, in general, should be followed. Lead Examiners, the Assistant Director, and/or the Deputy Director will be consulted for guidance when necessary.

Suspected DUI Cases: Many examinations within the Unit involve antemortem specimens from driving under the influence (DUI) investigations wherein volatiles and/or impaired-driving drugs are suspected to be present. Submitting agency representatives often request that ethanol/drugs be detected, identified, and possibly quantitated. Such analytes within blood samples may be quantitated, but quantitation within urine specimens will be limited to volatile compounds (e.g., ethanol, methanol, acetone, isopropanol). Biological evidence submitted from motor vehicle accident investigations will be treated the same, whether they involve injuries, are from fatalities, or where DUI is simply either suspected or needs to be ruled-out.

Blood-ethanol determinations and/or recreational/prescription drug analyses are usually requested within DUI-related submissions. Drug analyses are typically sought when ethanol is found to be low (or has not been detected at all) from breathalyzer examinations and/or drug usage has been suspected (e.g., DRE evaluations, drug paraphernalia within vehicle found, suspect admissions). Depending on sample matrices, relevant drug-positive findings can be presumptively reported or can be confirmed (and/or quantitated). The search for and/or quantitation of drugs within blood samples will often depend on ethanol results, agency representative requests, and other factors. When breathalyzer refusals have occurred then ethanol and/or drug analyses are sought. In submissions where ethanol findings from samples are high (i.e., equal to or over 0.10 g% blood alcohol content (BAC)), analyses for drugs may be omitted from analytical schemes unless specifically requested. In such cases samples will be saved and preserved accordingly in case future drug analysis requests are received.

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Unless sample volumes are limited, samples received from hospitals should be analyzed as if they were never previously analyzed. Data from previous examinations of submitted samples can be evaluated and can influence analytical schemes. Lead examiners (or higher) should be involved in unusual cases or when uncertainty exists in how to proceed during the scheduling of tests.

Because customers often request that ethanol results be reported as soon as possible (e.g., for Per Se hearings at the Department of Motor Vehicles (DMV)), reports containing volatile-only results (or volatile and presumptive drug results) within DUI-related cases can be issued separate from reports that contain drug confirmations and/or quantitations. While drug reports that are issued after ethanol reports are supplemental reports, it is not a requirement that they be titled as such. Tables can be found which help summarize the general analytical schemes for DUI case specimens (see figures). If non-biological evidence is submitted within a DUI investigation (e.g., liquid from a bottle seized within a vehicle), the Deputy Director, along with Lead Examiners, will determine which Unit and/or personnel will perform the examinations.

Suspected DFSA cases: Other types of cases within the Toxicology Unit may involve biological evidence from suspected drug-facilitated sexual assault (DFSA)-type investigations. Evidence from these cases will often involve the submission of both urine (recommended) and blood samples. These cases involve analyzing samples for recreational drugs, ethanol, central nervous system depressants (e.g., benzodiazepines, barbiturates), and hallucinogenic compounds. Additional information (e.g., from CT-100 sexual assault kits) may be needed. All DFSA cases will involve confirmatory analytical techniques in addition to any presumptive techniques which may be used. Reports involving presumptive testing-only should not be issued for DFSA-type cases. See below for a generalized analytical scheme. Toxicological analyses will begin within sixty (60) days of the request date for all DSFA-type cases in order to conform to statutes related to sexual assault evidence.

Postmortem cases: Specimens from deceased individuals may be received (e.g., from the Office of the Chief Medical Examiner (OCME) or another entity) and will usually be limited to homicides, motor vehicle accidents, and traumatic suicides (when deemed appropriate by the OCME). Specimens from these cases usually include blood, urine, and/or vitreous humor. If applicable, a memorandum of understanding (MOU) should be followed with regards to case management and regarding which analytes are to be screened, confirmed, and/or quantitated. All confirmations and quantitations not involving drugs/metabolites listed within the MOU should be vetted through a Lead Examiner or higher before allowing additional work to be scheduled or performed.

Other Cases: While the majority of submissions within the DSS laboratory's Toxicology Unit will be from suspected DUI, suspected DFSA, or postmortem type cases, this procedure doesn't preclude other types of cases involving human biological materials to be examined within the Unit (e.g., serum-conversions, proficiency tests).

As new technology is acquired general analytical schemes may change for the different types of cases that are submitted. Some techniques may be substituted, as necessary, and presumptive and confirmatory analyses may be combined into single steps during examinations. While repetition of sample analyses should be done to ensure accuracy, such practices may not always be feasible (e.g., limited sample, time-sensitive cases). Situations where ethanol tests were not conducted or drug quantitations were not performed may be warranted, but must be documented within case files as to why such decisions were made.

6.3.1. Urine

Suspected DUI Cases: Urine samples from suspected driving under the influence (DUI) cases are usually submitted when either breathalyzer results are unavailable or when it is suspected that ethanol may not be the sole contributing factor within the investigation. The analytical scheme for urine

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samples from suspected DUI cases can involve qualitative/quantitative volatile analyses and qualitative-only analyses for all other drugs. However, if breathalyzer results indicate that little-to-no ethanol played a factor in certain DUI investigations, then the analysis for drugs in those cases may be the only requests that are necessary.

When a submitting agency is unclear as to what may be causing impairment of an individual, the following analytical scheme is often performed: a volatile analysis, a chromatographic/mass spectrometric (GC/MS or LC/MS) analysis, and/or a presumptive screening (e.g., immunoassay). Given certain situations (e.g., when ethanol is identified at blood alcohol-equivalent concentrations equal to or greater than 0.100 g%), drug analyses may not be required and ethanol-only reports may be issued. In such situations documentation within case files will explain why drug requests were not performed. Submitting agencies will have an opportunity to request further [drug] analyses by contacting the DSS laboratory. Information within incoming documents may guide analytical plans. If breathalyzer results are known and indicate that requesting agency representatives only want drug analyses to be performed, then analytical plans can be developed to exclude volatile analyses and only focus on drug analyses. In such situations adequate documentation must exist which explains why certain analyses were (or were not) performed.

If ethanol was determined to be less than 0.1 g% (or not reported at all) within a specimen and no drugs were detected during a screening examination, then further [confirmatory] testing may need to commence before a final report is released. However, if confirmatory-type analyses were performed then no further testing may be necessary. Because of the way Connecticut statutes have been written and because other entities need ethanol results in a timely fashion (e.g., DMV Per Se hearings), reports only indicating quantitative volatile results may be issued separately from drug reports. Quantitation of drugs (other than ethanol) within urine samples will not be done unless prior approval is obtained from a Lead Examiner or higher.

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Suspected Drug-Facilitated Sexual Assault (DFSA) Cases: Urine is the recommended type of sample for these cases and should be obtained from the victim as soon as possible. Urine specimens in these cases will be qualitatively tested in order to detect possible past-ingestion of drugs and/or alcohol. For gamma-hydroxybutyric acid (GHB), urine collection times must be less than twelve (12) hours from the suspected drugging/incident. Ethanol should not be analyzed within urine specimens if urine specimen collection times are greater than forty-eight (48) hours after the incident. Samples that have been collected five (5) days after the alleged incident will not be analyzed without prior approval from either the Lead Examiner, Assistant Director, or the Deputy

Director. Quantitation of drugs within urine samples will not be done unless prior approval is obtained from the Lead Examiner or higher.

Postmortem Cases: Urine samples may be submitted and analyzed to supplement findings from other sample matrices (e.g., 6-monoacetylmorphine (6-MAM) for determination of heroin usage). Such samples may also be analyzed for other classes and types of drugs. Additionally in these situations, such urine specimens may be qualitatively analyzed for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine/metabolites, fentanyl/metabolites, methadone/metabolites, opiates, oxycodone/oxymorphone, and phencyclidine (e.g., immunoassay or LC/MS).

6.3.2. Blood

Suspected DUI Cases: While not as frequent as urine, blood samples from DUI-related cases are preferred since such samples tend to give information relating to influence of chemicals at a time close to an occurrence of an incident. Based on the request and based on case history, analyses of these samples can begin with quantitative detection for volatiles. Presumptive screening for drugs-of-abuse [qualitative] can occur. Given certain situations (e.g., when ethanol is identified at concentrations equal to or greater than 0.100 g%), drug analyses may not be required and ethanol-only reports may be issued. In such situations documentation within case files will explain why drug requests were not performed. Submitting agencies will have an opportunity to request further [drug] analyses by contacting the DSS laboratory. If confirmatory-type analyses were performed then no further testing may be necessary. Because of the way Connecticut statutes have been written and because other entities need ethanol results in a timely fashion (e.g., DMV Per Se hearings), reports only indicating quantitative volatile results may be issued separately from drug reports. Typically only toxicologically significant/relevant compounds should be quantitated within blood specimens. Determination of whether compounds should be quantitated can be based on the original request and, if necessary, based on communication with the submitting agency's representative. It may be appropriate to report analytes as qualitative-only or to report them as 'greater than' a certain value (semi-quantitative). Consultation with a Lead Examiner (or higher) will be done when analysts are uncertain whether quantitations should be performed or how reports should list results.

Suspected Drug-Facilitated Sexual Assault (DFSA) Cases: These cases may call for screenings of blood for recreational drugs, ethanol, and other central nervous system depressants (e.g., benzodiazepines, barbiturates). Targeted screens for certain DFSA drugs (e.g., GHB, flunitrazepam) may also be necessary. Relevant drugs within blood specimens are quantitated based on the

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original request and, if need be, based on further communication with the submitting agency's representative. Specimens should not be analyzed for GHB if their collection times are greater than twelve (12) hours after the time of drugging/incident and ethanol should not be analyzed within specimens if their collection times are greater than twenty-four (24) hours after the incident unless approval from a Lead Examiner or higher has been obtained. Urine samples that have been collected five (5) days after the alleged incident and blood samples that have been collected after two (2) days will not usually be analyzed. Blood may be tested if more than twenty-four (24) hours lapse between the time of incident and the collection time in order to be able to better interpret the significance of positive urine findings (or if target drugs are known to have longer half-lives). Because DFSA-type cases can be unusual and non-routine, consultation with a Lead Examiner or higher should be considered when determining which tests be included during the development of an analytical plan or scheme.

Postmortem Cases: When death is delayed after an incident, antemortem blood evidence is often preferred because results from these specimens can give information prior to post-hospital intervention. Typically only limited drugs/metabolites will be sought within evidence from these cases (i.e., OCME according to the MOU). Determination of whether compounds should be quantitated can be based on the MOU or communication with the submitting agency's representative. It may be appropriate to report analytes as qualitative-only or to report them as 'greater than' a certain value (semi-quantitative). Consultation with a Lead Examiner (or higher) will be done when analysts are uncertain whether quantitations should be performed or how reports should list results.

6.3.3. Vitreous Humor/Fluid:

Postmortem Cases: Vitreous fluid samples may be submitted and analyzed to supplement findings from other sample matrices. When positive ethanol findings within blood specimens occur, confirmation of ethanol will be achieved through the quantitative analyses of associated vitreous fluid specimens. When morphine is identified in a blood sample and there is no associated urine sample submitted with the case, the vitreous sample will be qualitatively analyzed for 6-acetylmorphine (aka: 6-monoacetylmorphine (6-MAM)), to indicate possible heroin use.

6.4. Analytical Schemes for Screening Tests

Analytical schemes can begin with the use of screening protocols for drug class determination. Toxicological screening techniques can include, but are not limited to, immunoassays, extractions, and/or headspace analyses using selective detectors on gas chromatographs. A list of analytes that can be screened within the Toxicology Unit

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using immunoassay and/or headspace gas chromatography can be seen in Table 1. Additional analytes may be added, as necessary.

Table 1: Analytes which can be Routinely Screened for within DUI Cases

Ethanol	Benzodiazepines	Cocaine	Oxycodone
Amphetamines	Buprenorphine	Methadone	Phencyclidine (PCP)
Barbiturates	Cannabinoids (Δ^9-THC)	Opioids	

Alternatively, confirmatory techniques (e.g., gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS)) or similar instrumentation can be used to screen samples for drugs/metabolites. When agreed upon by a submitting agency, analyses may be limited to only select analytes (e.g., OCME's MOU). Table 2 lists drugs/metabolites which have been agreed, based on an MOU, to be commonly screened during the analysis of OCME samples. Even though LC/MS is a confirmatory technique certain experiments may be used within the instrument which allow it to rapidly examine specimens and thus be considered a screening tool. When a confirmatory technique is used in lieu of a screening technique, and the experiment contains sufficient information to qualitatively identify compound(s), analysis of a second aliquot for confirmation purposes is not required. If necessary, the Lead Examiner or higher should be consulted to determine if further confirmatory testing is needed.

The purpose of screening techniques, especially in DUI cases, is to help rule-out the possible presence of analytes at certain detectable limits. When screening results are negative it indicates that certain analytes/classes of analytes are not detected. When screening results are positive it indicates that certain analytes/classes of analytes are present. Depending on the screening technique further testing may be warranted. Screening, sometimes referred to as presumptive testing, should have minimum detection limits for analytes of interest that include therapeutic concentration ranges for drugs. The selection of the screening technique(s) usually depends upon the case type, the amount of available specimen, and what questions need to be answered for the submitting agency.

There are some situations wherein urine samples may only be analyzed by a screening technique and a report will be issued listing presumptive results. In those situations, when presumptively positive, a second aliquot of urine can be analyzed to ensure accuracy in the findings. Presumptive-positive drug results (e.g., from immunoassay data) within urine samples may be reported without confirmatory analyses being performed. In those situations a qualifying statement will accompany the report advising the reader that such results were only presumptive.

6.5. Analytical Schemes for Confirmatory Tests (see figures)

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In the case of LC/MS and GC/MS, chromatography and mass spectrometry are considered two orthogonal techniques. Additionally, a different MS experiment may also be considered an orthogonal technique (e.g., full scan versus MS/MS versus selected ion monitoring (SIM)). Often, confirmatory tests for targeted analytes are more specific than screening assays which are used for initial detection. All confirmatory tests will include the use of positive and negative controls for the analyte(s) of interest. Positive control(s), however, need not be analyzed within the same batch/sequence as unknown samples as long as the positive control(s) are analyzed under the same analytical conditions and conform to quality control criteria found within specific experimental methods. When a screening technique indicates the possible presence of an analyte in a specimen (e.g., urine), confirmation of the identity of the analyte in a second aliquot from the same specimen is acceptable. Whenever possible and practical, the use of mass spectrometry is recommended as a confirmatory technique.

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Table 2: Analytes Routinely Screened and/or Quantitated within Postmortem Cases

11-Hydroxy Delta-9 THC	Desalkylflurazepam	Methylephedrine
6-Monoacetylmorphine$_{(free)}$	Diazepam	Midazolam
Acetone	Dihydrocodeine / Hydrocodol$_{(free)}$	Morphine$_{(free)}$
Alpha-Hydroxyalprazolam	Diphenhydramine*	Norbuprenorphine$_{(free)}$
Alprazolam	EDDP	Nordiazepam
7-Aminoclonazepam	Ephedrine/Pseudoephedrine	Norfentanyl
Amobarbital	Estazolam	Norpropoxyphene
Amphetamine	Etizolam	Oxazepam
Barbiturates	Fentanyl	Oxycodone$_{(free)}$
Benzoylecgonine	Flurazepam	Oxymorphone$_{(free)}$
Blood Alcohol Concentration$_{(BAC)}$	Hydrocodone$_{(free)}$	Pentobarbital
Buprenorphine$_{(free)}$	Hydromorphone$_{(free)}$	Phencyclidine
Butabarbital	Hydroxyethylflurazepam	Phendimetrazine
Butalbital	Hydroxytriazolam	Phenmetrazine
Cannabinoids	Isopropanol	Phenobarbital
Chlordiazepoxide	Lorazepam	Phentermine
Clobazam	MDA	Phenylpropanolamine
Clonazepam	MDMA	Propoxyphene
Cocaethylene	MDEA	Secobarbital
Cocaine	Methadone	Selegiline
Codeine$_{(free)}$	Methamphetamine	Temazepam
Delta-9 Carboxy THC	Methanol	Triazolam
Delta-9 THC	Methorphan (Dextro/Levo)*	Zolpidem

*May only be detected when using LC/MS (detection by immunoassay dependent on concentration)

When urine samples are positive for volatiles after presumptive testing is done (i.e., EMIT), then confirmatory testing for volatiles is usually performed. Depending on the situation and the amount of volatiles (e.g., ethanol) found within the specimen, further analyses for volatiles/drugs may be performed. Documentation must be present which explains decisions as to why the analysis of certain analytes was excluded.

While evidence from sexual assault cases may initially be analyzed by presumptive techniques, confirmatory techniques are usually used for these cases, when applicable. In cases where the presumptive technique also provides confirmatory results (e.g., accurate mass-LC/MS) additional [confirmatory] analyses may not be required. Limited sample or other situations may influence the number of tests that will be applied to certain evidence. Good laboratory practice and sound judgement will be used by analysts when evaluating and deciding on analytical plans. The Lead Examiner (or higher) will be notified of any unusual situations or when potential problems exist with analytical schemes.

6.6. Analytical Schemes for Quantitations (see figures)

Quantitation is performed on analytes based on importance to a case (as determined by case history, specimen volume, specimen type, and toxicological significance) and based on agreements with submitting agencies (e.g., MOU with OCME). Appropriate internal standards (e.g., deuterated) and controls (positive/negative) are used within each batch/sequence. Calibrator solutions (i.e., calibrators) need not be analyzed within the same batch/sequence as unknown samples as long as the control(s) are analyzed under the same analytical conditions and conform to quality control criteria found within specific experimental methods. A lead Examiner (or higher) should be consulted if utilizing calibration data for quantitations wherein such data is greater than thirty (30) days.

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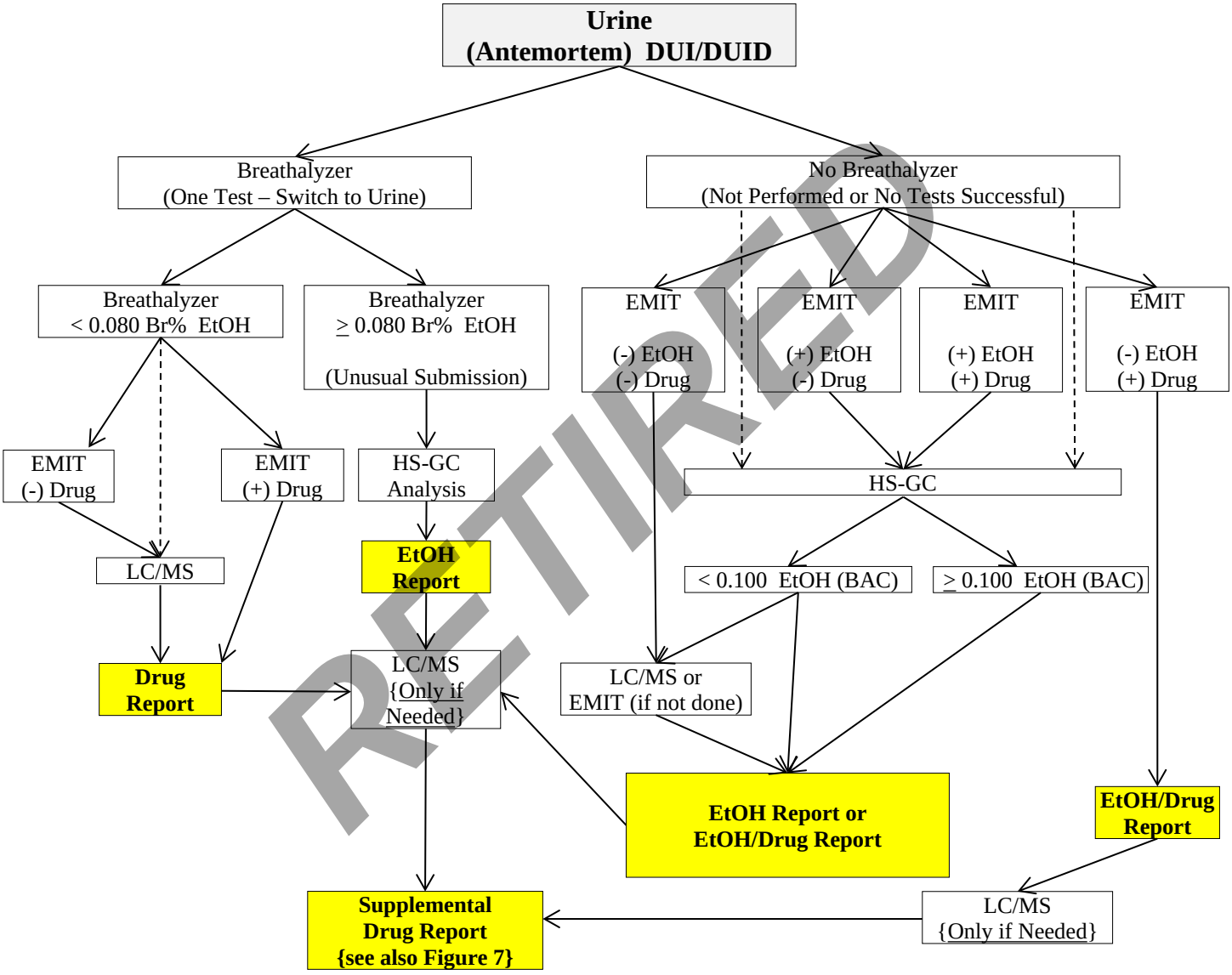
Figure 1: Analytical Scheme for DUI Antemortem Cases (Urine)



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Note-01: The term HS-GC throughout this document can mean either HS-GC(FID) or HS-GC/MS(FID)

Figure 2: Analytical Scheme for DUI Antemortem Cases (Blood)

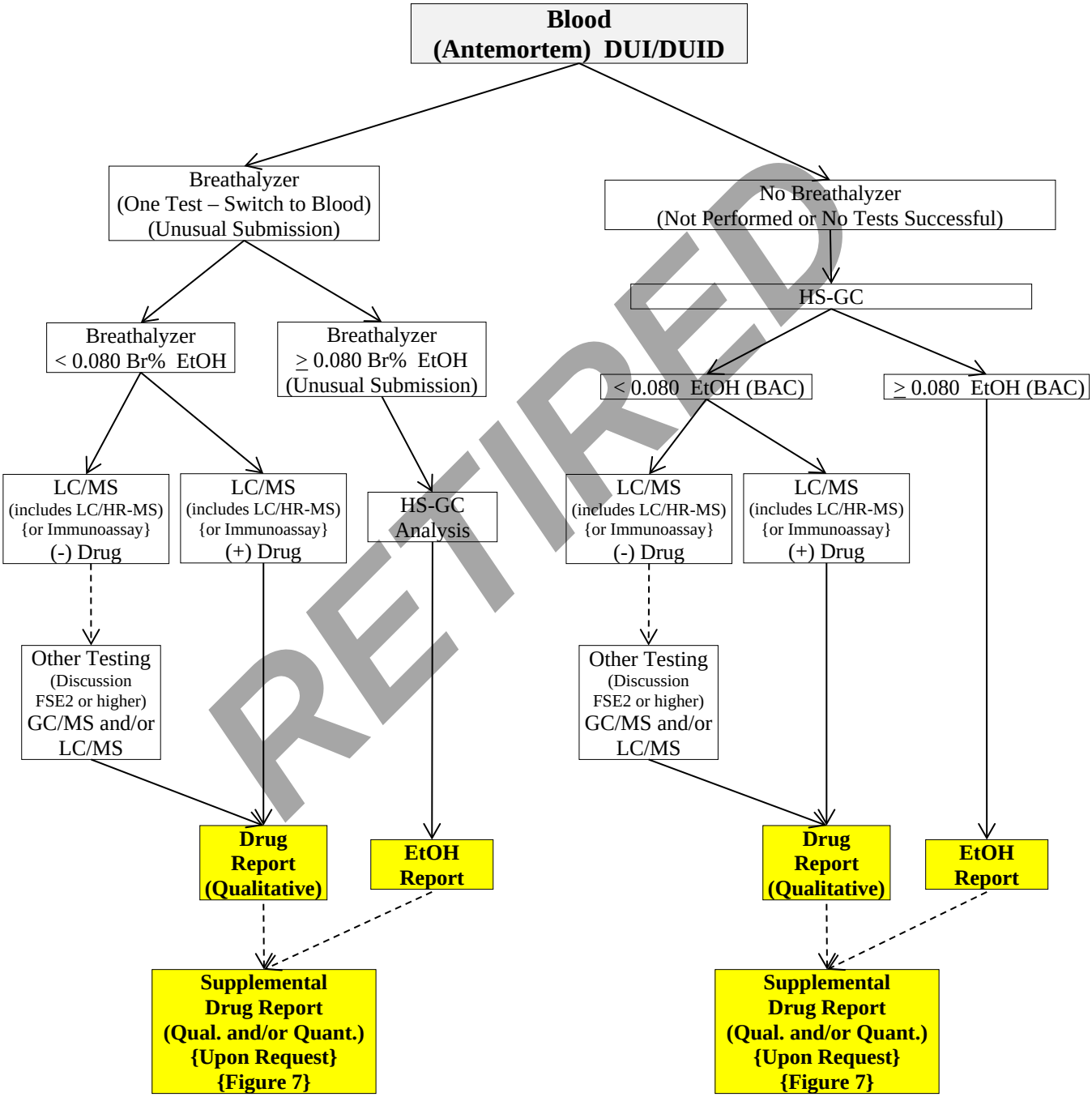

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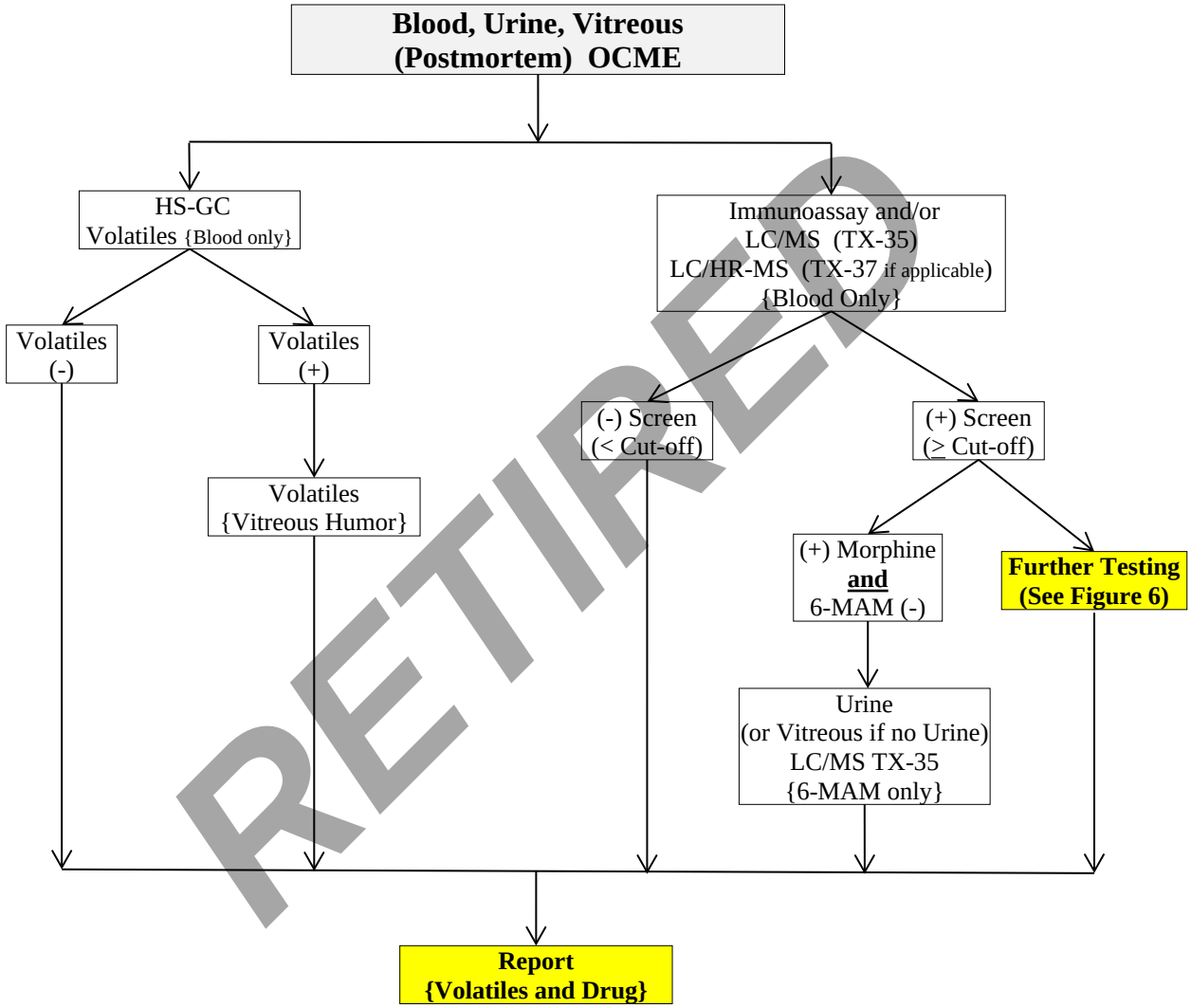
Figure 4: Analytical Scheme for Postmortem Cases (Blood, Urine, Vitreous Fluid)



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Note-02: Variations in this (and other) schemes are allowed depending on sample volume, customer requests, and other reasons deemed toxicologically applicable. Such variations will be recorded in appropriate case file and/or batch paperwork.

Figure 5: Analytical Scheme for Antemortem Cases (Supplemental/Confirmation – Urine)

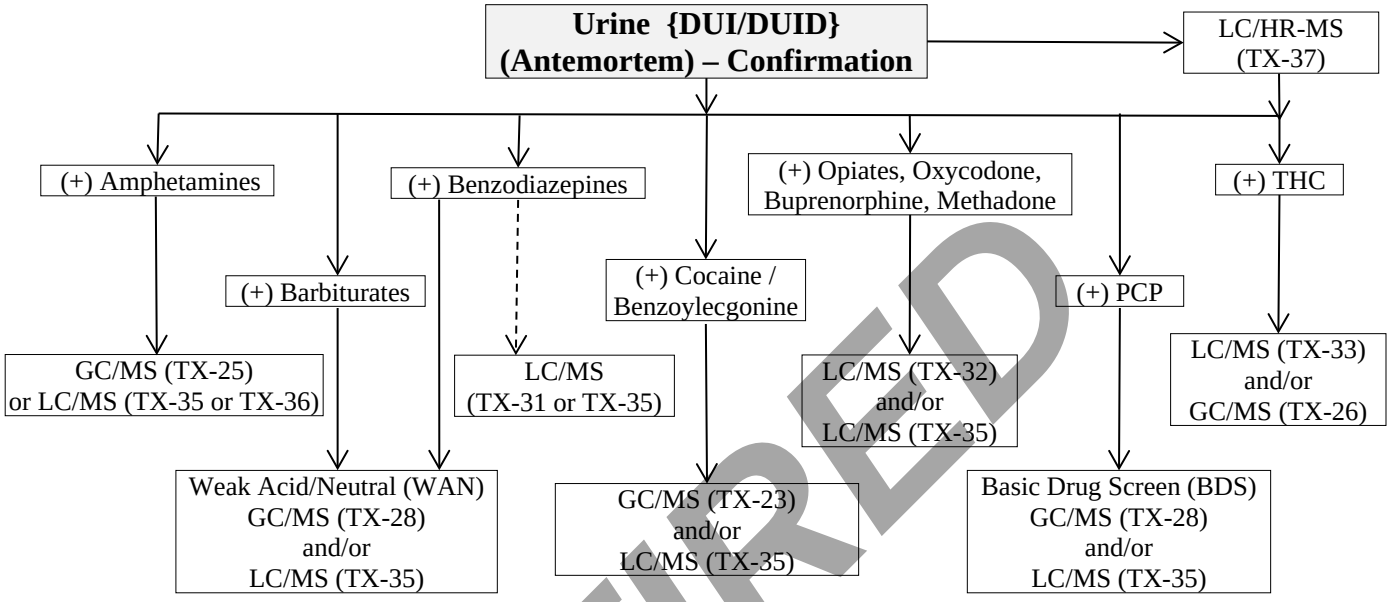

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Figure 6: Analytical Scheme for Postmortem Cases (Further Testing Confirmation – Blood)

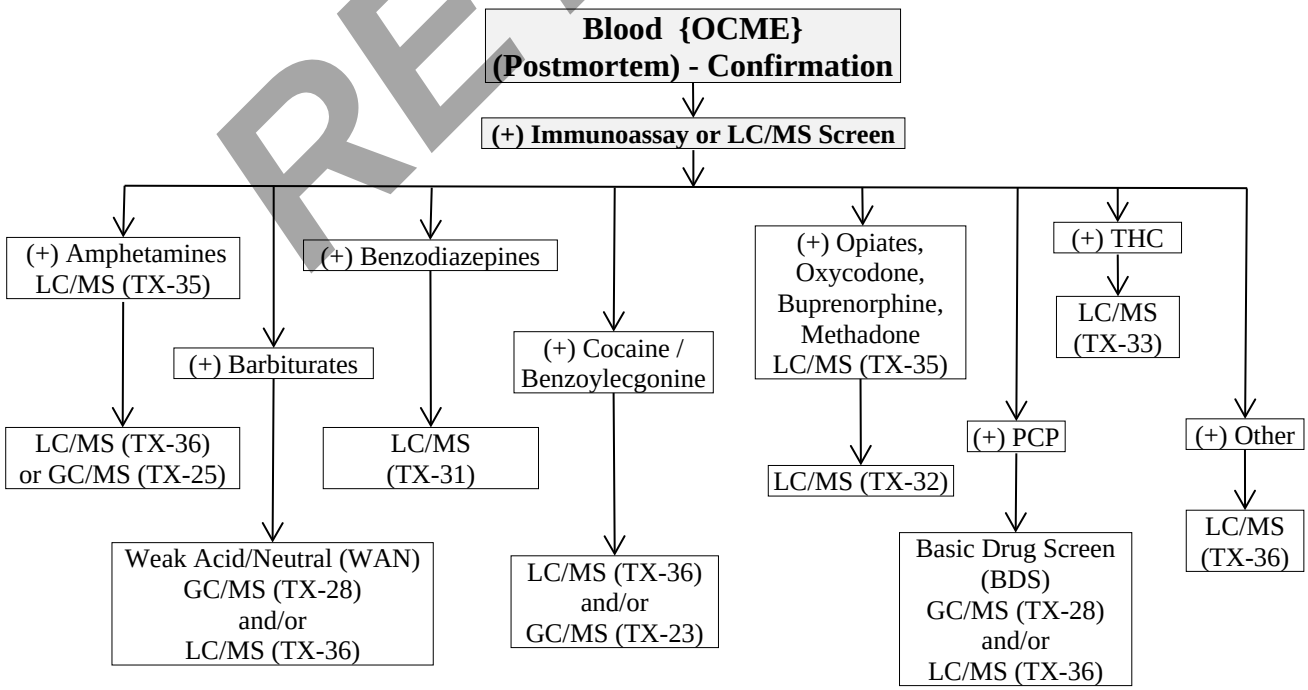
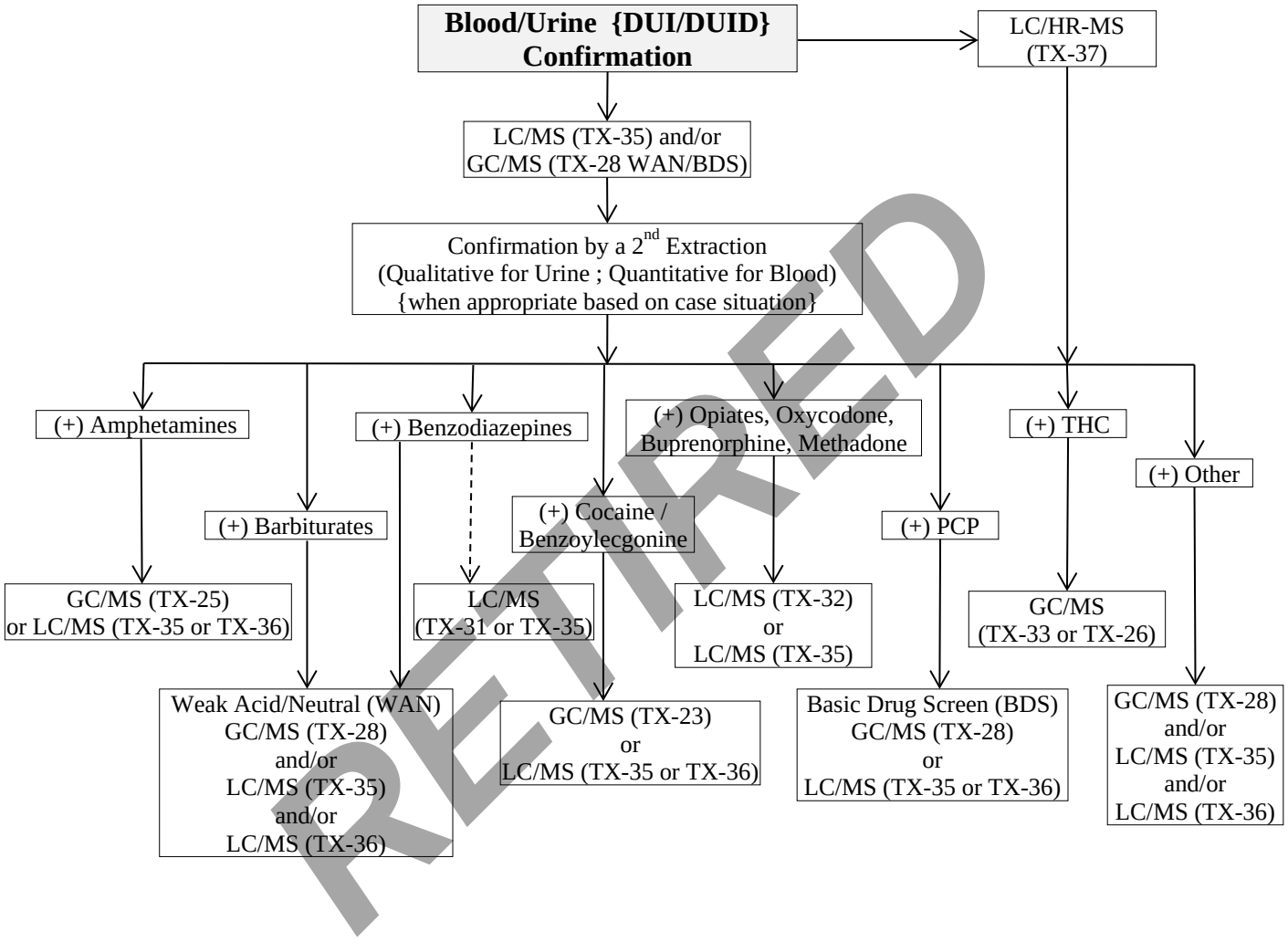


Figure 7: Analytical Scheme for DUI Antemortem Cases (Confirmation – Blood/Urine)



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7. Determining if the Calibration is Acceptable (Batch Acceptance)

Acceptable results of a calibration may vary. However, the following general guidelines should be considered when determining its acceptability:

Note-03: Guidance for batch acceptance within this procedure will be used, however decision criteria within separate procedures may override this guidance.

7.1. Chromatographic peak integration is reviewed to verify that peaks in calibrators, controls and unknowns are being similarly integrated. Automated integration processes available in instrumental software packages are used whenever possible. However, occasionally it is necessary to manually integrate a peak. When manual

integration is performed this will be made apparent to the reviewer of the data either by automated markings applied by the software package or by a note on the data printout.

7.2. A multi-point calibration curve should have a correlation coefficient of at least 0.98 and the calibrators should bracket the response of the case specimen(s).

7.3. When using a multi-point calibration curve, the line generated from the linear (or non-linear) regression can be used to determine what the 'measured amount' of analyte is within the calibrator solutions. This measured amount should be within ±20 % of the actual amount. If any calibrator solution falls outside of this amount then they may be dropped as long as at least four (4) non-zero calibrators remain and the case sample(s) and positive control sample(s) are still bracketed by the remaining calibration curve points.

7.4. All quantitative positive control solutions within batches should have values derived from the calibrations that are within ±20 % of their target values. For a quantitative result to be reportable it must be bracketed within the batch sequence by at least two (2) positive controls that meet the acceptance criteria (i.e., +/-20%). Because batches often contain multiple analytes associated with multiple case samples, if a specific positive control solution within a batch is not within the acceptable expected value then the Lead/Supervisor (or higher) will be consulted to determine what specific sample quantitations may be reported and/or when a batch, as a whole, can be deemed acceptable. All such consultations and their reasons/outcomes will be clearly documented (e.g., within the batch summary sheet).

7.5. If all positive control solutions in a batch are not within the +/-20 % expected range then the batch will be rejected for quantitation purposes but may still be used for qualitative evaluation. In general, any consistently failed positive control solutions will be brought to the attention of the Lead/Supervisor for determination of quality control activities and batch/case impact.

7.6. Calibration and control results may be reviewed as a batch. A batch summary should be provided to a technical reviewer along with all calibration and control data from that set. The technical reviewer will be an analyst who did not participate in the analysis of the batch (non-analysis activities such as starting an instrument sequence or correcting simple instrumental operations is not considered participation in a batch). The technical reviewer will ensure that the batch meets all applicable quality control guidelines. The batch summary sheet, the instrument method printout, the sequence printout, all corresponding calibrator and control data printouts, and any other pertinent records will be stored with batches. A copy of the signed batch summary sheet will be included in applicable case records so cross reference to batch documents is possible.

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7.7. Internal standard recovery of batches will be monitored by taking the average of the internal standard areas for the calibrators (or the calibrators and controls). Internal standard recoveries for individual unknown samples should fall within 50% and 200% of the calculated average of the calibrators (or calibrators and controls). If the internal standard recovery is outside of this range then the reason for this will be investigated and quantitative results will not be reported until a valid reason is identified. All reasons should be documented in appropriate case files or batch documents.

8. Uncertainty

Procedural uncertainty is reported with all quantitative results and should be evaluated annually. The overall uncertainties can be calculated based on an evaluation of control performance (variance). Documentation of the calculation of uncertainty can be maintained in a notebook in the laboratory. Included in this notebook should be documentation (or appropriate references) of reference materials and equipment utilized in the procedure that could affect the quantitative accuracy of the method. For analytes where there is no historical data to determine uncertainty, and the analyte does not fall into a class of drugs where uncertainty has been established, uncertainty can be reported based on the response of batch controls.

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9. Reagent Validation

When a new lot of a solvent or reagent is needed they will be appropriately validated prior to use. Analysts will verify that reagents/solutions worked as expected and that there were no interfering or unexpected results observed. Documentation for the acceptability will be appropriately retained (e.g., within batch documentation, logbook). A solution is considered validated and acceptable for use when the solution is shown to have worked in the procedure, and blanks/negative controls are shown not to contain contaminants that will interfere with the overall findings. Documentation should include recording the validation of the reagent on the batch runsheet and having the batch reviewed. Completing the Reagent Logbook, includes stating the name of the reagent/solution, lot number, the date made by who, and the date it was validated. If created in-house, the lot number should include information related to date made and preparer's initials (e.g., MPR07212016). If the same solution is made using different reagents or at different times during the same day, lot numbers can be differentiated using hyphenated letters (e.g., JJ07042016-a, JJ07042016-b). Solutions may be marked with a colored sticker (e.g., green) according to which method the solution is validated for, the date validated (the batch date), and the analyst's initials.
For materials that are purchased in multi-bottle lots, once one bottle from the lot is found to be acceptable for a particular use, all the bottles in the lot can also be marked as validated for that use (or related use, as appropriate). Bottles with working solutions will be labeled with appropriate information (e.g., solution name, lot number (if generated in-house will include data and initials of preparer), expiration date (if applicable and if different than the

standard one year from date of validation), initials (if different from in-house lot number), colored label (e.g., green) with validation information, applicable safety information. When applicable, quality control solutions (e.g., controls. calibrators) should be purchased. If solutions need to be made in-house, good quality assurance practices will be employed (e.g., varying reagent lot numbers, varying calibrator solutions, recording trend lines, verifying newly prepared solutions against older solutions)

10. Abbreviations

The following abbreviations may be found in the procedures and case notes within the Toxicology Unit:

DUI	Driving Under the Influence
DOC	Department of Corrections
DFSA	Drug Facilitated Sexual Assault
EtOH	Ethanol
MeOH	Methanol
IPA	Isopropanol
RT	Retention time
EMIT	Enzyme Multiplied Immunoassay Technique
GC MS	Gas Chromatography Mass Spectroscopy
IR	Infrared
UV	Ultraviolet
LC MS	Liquid Chromatography Mass Spectroscopy
TX	Toxicology
CS	Controlled Substances
WAN	Weak Acid Neutral
BDS	Basic Drug Screen
BL	Blood
UR	Urine
CHEP	Cyproheptadine
DI H2O	De ionized Water
Neg	Negative
NDD	No Drugs Detected
SA	Sexual Assault
Coc	Cocaine
BE	Benzoylecgonine
Op	Opioids
THC	Delta-9-Tetrahydrocannabinol
COOH-THC	Carboxy-delta-9-tetrahydrocannabinol
THC-OH	11-Hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC, OH-THC)
Benzo	Benzodiazepine

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SMA	Sympathomimetic Amines
GHB	Gamma-hydroxybutyrate or gamma-hydroxybutyric acid
OCME	Office of the Chief Medical Examiner
MOU	Memorandum of Understanding
Volatiles	Methanol, Ethanol, Acetone, Isopropanol

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11. References

DSS Quality Manual

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Handbook of Analytical Toxicology, Cravey, R. and Baselt, R. Eds., Biochemical Publications: Davis, CA, 1981

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Rev. #	History
3	Updated verbiage and formatting within the entire document. Inserted figures and tables to summarize schemes. Inserted information about OCME samples. Removed unnecessary verbiage throughout document. Added schematic figures to summarize methodology. Added History section.
4	Updated verbiage, grammar, and formatting within the entire document. Added 'Assistant Director' throughout document. Updated to allow for either LC/MS Screening or Immunoassay techniques as an initial screening tool. Clarified that the use of LC/MS as a screening tool may not require further analysis. Updated flow charts to include LC/MS Screen and other LC/MS Quantitative methods. Removed bias from consideration of Uncertainty. Added limitation statement within Table 2. Updated section 6.5 and the figures.
5	General unknown drug screen of comprehensive drug screen added. Discussion about postmortem cases added. Wording added to allow the extraction process and instrumentation analysis to be performed by separate analysts. Added abbreviations PM and OCME. Definition for work product expanded. Definitions for basic drugs of abuse screen and comprehensive drug screen added. Updated verbiage and formatting within the entire document. Inserted figures and tables to summarize schemes. Inserted information about OCME samples. Removed unnecessary verbiage throughout document. Added schematic figures to summarize methodology.
6	Fixed grammar and spelling errors throughout document. Assistant Director position added throughout document. Added statement about LC/MS analyses in paragraph 1 of section 6.3.1. Changed wording in paragraph 1 of section 6.3.1. Added "other than ethanol" to statement in last sentence of paragraph 1 of section 6.3.1. Changed "customer" to "submitting agency's representative" in paragraph 2 of section 6.3.2. Updated postmortem cases section under suction 6.3.2. Updated Section 6.4 and added paragraph about situations in which urine samples may only be analyzed by a screening technique. Added a brief statement under Table 2 in Section 6.5. Added LC/MS to Figures 1, 2, and 3. Updated Figure 4 to combine immunoassay and LC/MS screen. Added LC/MS to figures 5 and 6. Changed THC confirmations within figures 5 and 6. Updated Figure 7 to include LC/MS. Updated format and added MOU abbreviation in section 9.

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7	Updated itemization numbers to reflect new format change. Updated analytical plans for DUI cases. Updated wording and other material within sections 5 and 6.1. Information regarding expiration dates added in section 6.1. Changed JT to LIMS-Plus throughout document. Updated wording in Section 6.1 for clarity. Added use of tamper-evident devices within section 6.2. Moved paragraph about case history from section about postmortem cases in section 6.3 and moved to section 6.3. Added information under suspected DUI cases in sections 6.3, 6.3.1, and 6.3.2. Updated sections 6.3 and 6.4 regarding minimum detection limits for analytes of interest, including therapeutic ranges. Added statement about documentation to section 6.5 and added the ability to use older calibration data for quantitations. Updated figures throughout document.
8	Updated Figure 2 ('+ Drug' corrections in flow chart). Updated title. Made changes to Figures 5, 6, & 7.
9	Added section describing batch acceptance criteria (Section 7).

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1.0 INTRODUCTION

The Enzyme-Multiplied Immunoassay Technique (EMIT) assay is an immunological screening method. An enzyme that is bound to a drug/analyte is used in the procedure. The enzyme is capable of catalyzing a substrate and indirectly causing a reaction wherein a chemical absorbance change occurs. When an enzyme-labeled drug/analyte is mixed with a sample that already contains the target drug (along with the appropriate substrate and oxidizing agent), the amount of absorbance within the resulting mixture will be proportional to the amount of drug/analyte within the sample. In the detector-part of the instrument, radiation of a fixed intensity is passed through a solution and the transmitted light's intensity is measured and compared. The absorbance of the radiation will be [roughly] proportional to the amount of drug/analyte within the sample. When no drug/analyte is present in a sample, most of the enzyme-labeled drug/analyte is bound to antibodies and the enzyme is rendered inactive. Enzyme-substrate reactions within EMIT cause an indirect absorbance-change event by causing NAD^+ (nicotinamide adenine dinucleotide) to convert to NADH (hydrogenated form). Instrumentation uses monochromatic light to measure the amount of NAD^+ that is converted to its reduced form, NADH. The NADH absorbs ultraviolet light more than NAD^+. The more drug/analyte in the sample, the greater the absorbance of radiation when light is passed through the sample. The technique is considered homogenous since all reagents are mixed together and no rinsing of samples occurs.

Ethyl alcohol can also be analyzed using this instrument. However, the ethanol assay is based on an enzymatic reaction only and not due to an immunological interaction. Alcohol dehydrogenase (ADH) catalyzes the oxidation of ethyl alcohol to acetaldehyde, while NAD^+ is subsequently converted to its NADH reduced form. A change in absorbance (i.e., production of NADH) results when ethanol and ADH react. The increase in absorbance of light is directly proportional to the concentration of ethyl alcohol.

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2.0 SCOPE

Enzyme multiplied immunoassay techniques (EMITs) have the advantage of rapidly screening small volumes of biological samples for the presence of drugs. The use of an automated system allows fast and reproducible screening tests. All immunoassay tests are only presumptive and may be subject to false positive or false negative results. For confirmatory purposes, any results obtained through the use of this procedure must be accomplished through the use of a structurally elucidating analytical technique (e.g., gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS)). Other analytes/classes of drugs may be added to this procedure provided that their reagents/kits have been validated prior to use. This technique is generally geared towards the analysis of urine for the possible presence of drugs and/or ethanol. Blood, serum, or plasma can also be used within this procedure for drug analysis, but additional sample preparation will be needed. The EMIT procedure will not be used for ethanol analysis within blood, serum, or plasma samples.

3.0 PRINCIPLE

In each assay, samples (e.g., urine or blood extract) are mixed with reagents (e.g., Reagent 1 and Reagent 2) in a certain sequence. No washing of solutions is performed (as in ELISA – enzyme-linked immunosorbent analysis) and all the reactions occur within one homogeneous mixture. After a certain amount of time, monochromatic ultraviolet radiation is passed through each solution and intensities are compared in order to determine if any absorbance changes have occurred. Reagent 1 usually contains a substrate, a UV-detection compound, and a drug/antigen-specific antibody. Reagent 2 usually contains an enzyme-labeled drug/antigen.

If a sample is positive (i.e., the solution contains the drug/analyte being tested), antibody/antigen reactions will occur that are specific for the drug/analyte of interest. The majority, if not all, of the antibody sites will be taken up by the sample's drugs/antigens. When Reagent 2 is added to the solution, an enzyme-labeled drug/antigen is introduced. Because all of the antibody sites have been taken, the enzyme-labeled drug/antigen will not bind and will be free-floating in solution. The enzyme-part of the enzyme-labeled drug/antigen will react with free-floating substrate molecules and acts as a catalyst. When an enzyme catalyzes a substrate, the reaction causes NAD^+ to be reduced to NADH. Unlike substrates, enzymes are not consumed so they continue to multiply the reaction by constantly causing NADH to be produced until no more substrate remains. Since NADH absorbs ultraviolet radiation, the instrument will report a relatively high absorption value. The amount of drug in a sample will be proportional to the amount of ultraviolet light that is absorbed by the homogeneous solution.

If a sample is negative (i.e., the solution contains no drug/analytes specific to that particular test), antibody/antigen reactions will not occur when Solution 1 is added to the sample. All of the antibody sites will be free and open. When Reagent 2 is added to the solution, an enzyme-labeled drug/antigen is introduced and will be able to react with free-floating antibodies. As a result the enzyme will not be able to react with the substrate and the light-absorbing agent (NAD^+) will not reduce to NADH. Since NADH absorbs ultraviolet radiation, the amount of ultraviolet light that is absorbed by the homogeneous solution will be minimal and the instrument will report a relatively low absorption value.

Quantitation can occur using EMIT technology when solutions that contain varying known amounts of specific analytes are analyzed contemporaneously with unknown samples. A resulting calibration graph comparing analyte concentration with instrumental absorbance values can used to calculate unknown sample concentrations. Control samples can ensure that quality results are produced from calculations. Within this procedure quantitative data can be obtained from ethanol analyses within urine samples. However, these values are not officially reported and are typically used only for informational purposes when comparing EMIT data to future analytical analyses (i.e., headspace-gas chromatography), if needed.

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4.0 SPECIMENS

4.1 Urine: Approximately 650 µL.

4.2 Blood: 1 mL
Within this procedure, serum and plasma will be treated the same as blood with respect to sample preparation/extraction. If limited volumes are encountered, seek guidance from the Lead Examiner and/or the Deputy Director.

4.3 Other matrices: Depends on controls and validation. Examiners should consult the Lead Examiner and/or the Deputy Director prior to analyzing other matrices.

While this procedure has been validated for blood and urine specimens, other matrices may be substituted and may be analyzed. This can be done provided that appropriate controls have been analyzed and, if necessary, appropriate validation has occurred for the particular matrix.

Evidence should be stored refrigerated when not under examination. It is acceptable for an analyst to individually transfer aliquots of samples into temporary containers (e.g., test tubes) even if the EMIT testing will not be performed on the same date. This occurrence should be documented within the case notes and/or on the batch paperwork. Aliquots will be treated in the same manner as the original evidence samples (i.e., prevented from cross-contamination, degradation, and/or sample loss), can remain in the control of the analyst if placed under proper seal, and do not need be sub-itemized. It is acceptable for the analyst, in the process of aliquoting multiple case samples (i.e., creating a batch), to put the boxes of evidence in one convenience container and seal the container for overnight storage in an evidence refrigerator. In such cases the evidence can remain in the analyst's possession according to the chain of custody. In these circumstances all evidence will be considered in the control of the analyst listed within the chain of custody.

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Work lists for EMIT can be generated through JusticeTrax (JT). Specimens comprising an analytical batch can be obtained from the JT work list.

Some evidence (e.g., suspected Driving Under the Influence (DUI) cases) is maintained in the Toxicology Unit for a limited time period (e.g., approximately 8 weeks after the last toxicology report for the submission has been released). In the absence of notification from the contributor, or of any legal actions or reason to maintain the samples, such evidence can be discarded in an appropriate medical waste disposal container. Samples from fatality cases (i.e., those cases wherein someone has died as a result of the accident), samples from cases that have pending legal actions, and samples from cases where specific requests have been made for retention of evidence can be held and stored indefinitely, or until directed by appropriate authorities.

5.0 EQUIPMENT / MATERIAL / REAGENTS

5.1 Viva-E EMIT analyzer workstation and related equipment and supplies (e.g., sample cups) (Siemens or equivalent)

5.2 Sorvall ST16 (Thermo Scientific)

5.3 Standard laboratory glassware, plastic ware, and equipment (e.g., test tubes)

5.4 EMIT II Plus Assay Kits: (Siemens or equivalent)

Multidrug Kits	Urine Cut-Off (ng/mL) ; Calibrator/Ctrl Kits
Amphetamine (9C309UL ; 10445420)	1000 (Level L3)
Barbiturates (9D029UL ; 10445422)	200 (Level L3)
Benzodiazepine (9F029UL ; 10445429)	200 (Level L3)
Cocaine (9H029UL ; 10445437)	300 (Level L3)
Methadone (9E029UL ; 10445426)	300 (Level L3)
Opiate (9B309UL ; 10445416)	300 (Level L1)
PCP (9J029UL ; 10445441)	25 (Level L3)
Cannabinoid (9N029UL ; 10445469)	100 (Level L4)
Ethanol (9K309UL ; 10445452)	0.010 – 0.600 g/dL (see 5.7)

5.5 Immunalysis Assay Kits: (Siemens or equivalent)

Kit	Urine Cut-Off (ng/mL) ; Calibrator/Ctrl Kits
Oxycodone (302UR-0100 ; 10991156)	300 (see 5.8)
Buprenorphine (336 UR-0100 ; 10991178)	5 (see 5.8)

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5.6 EMIT II Calibrator/Control Kits (Siemens or equivalent)

Level 0: 9A509UL (10445406) Cal 0 Control Kit – Negative Control

Level 1: 9A529UL (10445407) Multidrug Cal 1 Calibrator Kit (used for opiates only)

Level 3: 9A569UL (10445409) Multidrug Cal 3 Calibrator Kit

Level 4: 9A589UL (10445410) Multidrug Cal 4 Calibrator Kit (used for THC only)

Level 5: 9A609UL (10445411) Multidrug Cal 5 Calibrator Kit – Positive Control

5.7 Ethyl Alcohol (Siemens or equivalent)

Negative Calibrator: 9K029UL (10445445)

100mg/dL Calibrator: 9K059UL (10445448)

Low Control (0.036 - 0.044): 9K049UL (10445447)

High Control (0.270 - 0.330): 9K079UL (10445449)

5.8 Immunalysis (Siemens or equivalent)

Calibrator (Oxycodone ; 300): C302UR-10-1-300 (10991160)

Low (Neg.) & High (Pos.) Control (Oxycodone ; 225 & 375): C302UR-10-2-300 (10991161)

Calibrator (Buprenorphine ; 5ng/mL): C336 UR-10-1 (10991180)

Low (Neg.) & High (Pos.) Controls (Buprenorphine ; 3.75 & 6.25): C336 UR-10-2 (10991181)

5.9 Liquichek Urine Toxicology Controls (Bio-Rad or equivalent)

Level S1E: contains target analytes

Level S2E: contains target analytes

Level S3: used for Cannabinoid Only (125ng/mL)

5.10 Reference standard solutions (Cerilliant or equivalent)

5.11 Needle Rinse Solution (Sodium Hypochlorite solution) (ACS Grade or equivalent)

5.12 System Solution (3203-063 ; 10445247) (Siemens or equivalent)
Used to flush EMIT system and avoid bacterial growth
Fill water container with ~10 L deionized water and 25 mL of system solution

5.12 Hydrochloric Acid (0.1N HCl) (ACS Grade or equivalent)

5.13 System Solution (Siemens or equivalent)

5.13 Methanol (MeOH) (ACS Grade or equivalent)

5.14 Acetonitrile (CH_3CN or ACN) (ACS Grade or equivalent)

5.15 Water (Deionized (DIW), Millipore, or equivalent in-house supply)

5.16 Whole blood (Blood bank or equivalent)

5.17 Methanolic hydrochloride (1% MeOH-HCl) – can be prepared by diluting 100 µL of concentrated HCl into 10 mL of methanol. Stable for at least one (1) month at refrigerator temperature

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6.0 STANDARDS / CONTROLS

6.1 Working Standard Solution (for blood procedure only)

6.1.1 Mixture of ten (10) analytes in methanol used to make the positive control and calibrator/cut-off blood solutions.

6.1.2 Two (2) sets of the Working Standard Solution should be made:
- One Working Standard Solution should be made and used for preparation of the blood positive control solution.
- The second Working Standard Solution should be made by another Examiner, if available, and should be used for the preparation and the blood calibrator/cut-off solution.

6.1.3 Each Working Standard Solution can be prepared by adding 5mL of MeOH to a volumetric flask (or equivalent), adding the appropriate amounts of drug analytes (see table below), and diluting to volume (10mL) with MeOH.

Table: Summary for preparation of the working standard solutions*

Drug	Reference Standard	Volume of Reference Standard (µL)	Concentration of Working Standard Solution (in 10mL of MeOH) (µg/mL)
Methamphetamine	1.0 mg/mL	50	5
Secobarbital	1.0 mg/mL	5	0.5
Diazepam	1.0 mg/mL	5	0.5
Benzoylecgonine	1.0 mg/mL	10	1
THC	1.0 mg/mL	10	1
Morphine	1.0 mg/mL	20	2
Methadone	1.0 mg/mL	20	1
Phencyclidine (PCP)	1.0 mg/mL	1	0.1
Buprenorphine	100 µg/mL	5	0.05
Oxycodone	1.0 mg/mL	5	0.5

*(Note: Two (2) separate lot preparations of this solution should be made – One solution used for blood Positive Control and the other used for blood Calibrator/Cut-off)
(Stable when stored in freezer for 1 year (or 1 year from last verification))

RETIRED

6.2 Control Solutions

6.2.1 Negative Control Urine
Level 0: 9A509UL (10445406) Cal 0 Control Kit

6.2.2 Positive Control Urine
Level 5: 9A609UL (10445411) Multidrug Cal 5 Calibrator Kit

6.2.3 System Check Positive Control Urine
These solutions only need to be used when the system is calibrated
Liquichek Urine Toxicology Controls (Bio-Rad):
Level S1E: contains target analytes

Level S2E: contains target analytes
Level S3: used for Cannabinoid Only (125ng/mL)

6.2.4 Negative Control Blood:
Drug-free blood.

6.2.5 Positive Control Blood
Composed of a mixture of ten (10) analytes in blood - prepared from the methanolic Working Standard Solution.
Can be prepared by adding 1mL of negative control blood to a test tube, adding 100µL of the MeOH Working Standard Solution, capping, and mixing well.

Table: Summary for preparation of the blood positive control solution

Drug	**Volume of Working Std. (µL)**	**Concentration of Blood Positive Control (in 1mL of blood) (ng/mL)**
Methamphetamine	100	500
Secobarbital		50
Diazepam		50
Benzoylecgonine		100
THC		100
Morphine		200
Methadone		200
Phencyclidine (PCP)		10
Buprenorphine		5
Oxycodone		50

(Stable when stored in freezer for 1 year (or 1 year from last verification))

RETIRED

6.3 Calibrator/Cut-Off Solutions

6.3.1 Urine Calibrator Solutions
Use EMIT II Calibrator/Control Kits
Level 1: Multidrug Cal 1 Calibrator Kit (used for opiates only)
Level 3: Multidrug Cal 3 Calibrator Kit (everything but opioid and THC)
Level 4: Multidrug Cal 4 Calibrator Kit (used for THC only)

6.3.2 Blood Calibrator Solutions
Composed of a mixture of ten (10) analytes in blood - prepared from the methanolic Working Standard Solution.

Can be prepared by adding 1mL of negative control blood to a test tube, adding 50µL of the MeOH Working Standard solution, capping, and mixing well.

Table: Summary for preparation of the blood calibrator/cut-off Solution

Drug	Volume of Working Std. (µL)	Concentration of Blood Calibrator/Cut-Off (in 1mL of blood) (ng/mL)
Methamphetamine	50	250
Secobarbital		25
Diazepam		25
Benzoylecgonine		50
THC		50
Morphine		100
Methadone		100
Phencyclidine (PCP)		5
Buprenorphine		2.5
Oxycodone		25

(Stable when stored in freezer for 1 year (or 1 year from last verification))

RETIRED

7.0 VALIDATION OF REAGENTS

Validated reagents can be marked with a green dot/sticker which indicates that the reagents used for the specific batch of EMIT samples have been validated. Materials (e.g., controls, calibrators/cut-offs, etc.) are considered validated when all samples produce results as expected.

8.0 PROCEDURE (The following steps can be used for an analysis)

8.1 Instrument Preparation

8.1.1 Perform system refill:

8.1.1.1 Remove the rotor and cuvette covers and open instrument door.

8.1.1.2 Remove all covers from reagents in the reagent rotor.

8.1.1.3 Ensure that needle rinse and HCl bottles are full.

8.1.1.4 Ensure that tubes in positions W and B are filled with water and needle rinse solution appropriately.

8.1.1.5 Inspect wash arms, mixers, and cuvette rotors. Ensure that the system solution containers are filled adequately and that waste containers have enough available space so they don't get full during analyses.

8.1.1.6 Check and ensure that there are adequate amount of reagents present in the reagent bottles prior to initiating instrument operation.

8.1.1.7 Check syringes and tubing for bubbles.
If necessary, tap where needed to remove bubbles.

8.2 Instrument Calibration (Urine)

The EMIT instrument should be calibrated once per week and per manufacturer's instruction. Calibration should be performed after any system change, including reagent refill, solution lot change, or bottle change. Bottles should be changed for new reagent lot numbers. It is important to not overfill bottles. Reagent(s) should be filled only up to the shoulder of the bottles. For urine samples, drugs of abuse calibration utilizes levels 0, 1, 3, and 4 of the Calibrator/Control kits and ethanol calibration utilizes negative and 100mg/dL calibrators.

When calibrator or control solutions do not have enough volume remaining to adequately analyze a batch of samples, it is not recommended for calibrator/control solutions of differing lot numbers to be combined. However, if this needs to happen, the Deputy Director will be consulted and the system should be recalibrated so that the new solution(s) can be validated. Additionally, since these new solutions will no longer be comprised of just one lot number, a new analyst-generated lot number will be created (e.g., MPR03022016). Information listing the lot numbers of each of the newly created solution will be recorded and associated with the analyst-created lot number. All information should be kept either in sample batch documents and/or in the case file(s).

8.2.1 Place labeled sample cups or test tubes in appropriate positions on the sample rotor.

8.2.2 Pipet and load calibrators and controls as follows:

Table: General guide for position placement – alternate positions are permissible.

Position of Sample Cup/Test Tube	Description	Approximate Volume (µL)
C1	Level 0 Calibrator/Control	500
S2	Level 1 Calibrator	300
S4	Level 3 Calibrator	500
S5	Level 4 Calibrator	300
S7	Ethanol Level 0.0 Calibrator	200
S8	Ethanol Level 0.1 Calibrator	200

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8.2.4 If needed, clear the previous run positions on the sample rotor.

8.2.5 Load calibrators and controls on the instrument.

8.2.6 Start calibration on EMIT instrument.

8.2.7 Evaluation of Urine Calibrator/Cut-Off Solutions:
At the completion of each calibrator analysis, the instrument will pause until the calibrator solution result is accepted by the operator. One or more of the following should be valid in order for a calibrator solution to be deemed 'acceptable:'

8.2.7.1 An analyte's calibrator/cut-off solution is acceptable if replicate absorbance values are within ± 5% of each other.

8.2.7.2 An analyte's calibrator/cut-off solution is acceptable if the "Reported" absorbance value is within ± 5% of the "Reported" absorbance value for the previously analyzed batch's calibrator/cut-off solution.

8.2.7.3 An analyte's calibrator/cut-off solution can be acceptable if its "Reported" absorbance value is within ± 5% of the mean "Reported" absorbance value from the past ten (10) "Reported" values for that calibrator/cut-off solution.

Note: "Reported" values are the average of the "Replicate" values for a particular calibrator.

If a calibrator is not accepted, the operator should either re-analyze the same calibrator or place a new aliquot of the calibrator in the instrument and re-analyze.

If a calibrator solution is not acceptable based on the above criteria, even after the operator re-analyzes the calibrator solution, then a Lead Examiner (and possibly a service engineer) should be contacted. If justified, the Deputy Director may allow the Examiner to accept the calibrator result for an analyte on a provisional basis. When this occurs, the Deputy Director must document and justify their decision within the affected case file(s) and within the batch documentation. In these situations, all positive and negative controls must work as expected. If any of the subsequent controls don't perform as expected, and the calibrator solutions had problems passing quality control measures, then the entire batch will be considered a failure and remediation measures will need to be implemented (i.e., service engineer notified).

8.3 Instrument Calibration (Blood)

Blood Calibrator/Control Solutions:

Blood calibrator solutions will not be used as official calibrators within the instrument software. They will be analyzed as samples (see Sample Analysis section) and their absorbance values will be used during calibration evaluations.

Evaluation of Blood Calibrator/Cut-Off Solutions:

Evaluation is not done like it is with the evaluation of urine calibrator/cut-off solutions (i.e., prior to sample analysis). See section later in procedure regarding blood calibrator/cut-off solution evaluation.

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8.4 Sample Analysis

8.4.1 Urine Samples

8.4.1.1 For drug analyses, only one (1) urine sample per individual should be analyzed. If two (2) or more urine samples were submitted for the same individual, analyze the earliest sample. The other sample(s) should be analyzed only when necessary. If the other samples are to be analyzed, the Examiner should document the reasoning for this in the case file(s). Questions on whether to consume limited-volume samples should be directed to the Lead Examiner or the Deputy Director prior to analysis.

8.4.1.2 For ethanol analyses, both urine samples should be analyzed.

8.4.1.3 Urine samples are usually analyzed without any additional preparation (i.e., neat). Any problems with turbidity or sample degradation should be dealt with on an individual basis. Any sample clean-up or sample preparation steps will be recorded in the case notes and/or case file(s).

8.4.1.4 If needed, centrifuge the urine sample and isolate the supernatant into an EMIT test tube. Alternate clean-up procedures are acceptable, when needed. If utilized, these types of clean-up procedures need to be documented and placed within the case notes.

8.4.1.5 Analyze the urine sample(s) and the urine controls (positive and negative) using the EMIT instrument.

8.4.1.6 The urine Liquichek controls (i.e., S1E, S2E, S3) need only be analyzed during calibration. They are used only as a system check and not for a sample batch control check. Thus, if the urine calibration has been established for the week, then subsequent analyses of the S1E, S2E, and S3 controls do not have to be done.

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Table: General guide for volumes of calibrators/controls/samples

Description	Approximate Amounts (µL)
Level 0 Control/Calibrator	500
Level 1 Calibrator	300
Level 3 Calibrator	500
Level 4 Calibrator	300
Oxycodone Low Control	100
Oxycodone High Control	100
Buprenorphine Low Control	100
Buprenorphine High Control	100
Ethanol Level 0.0 (Negative) Cal	200
Ethanol Level 0.10 Calibrator	200
Ethanol Low Control	250
Ethanol High Control	250
S1E Liquichek	500
S2E Liquichek	500
S3 Liquichek	250
Level 5 Control	600
Case samples	600

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8.4.2 Blood (Serum/Plasma) Samples

8.4.2.1 Only one (1) sample per individual should be analyzed.

8.4.2.2 If two (2) or more samples were submitted for the same individual, analyze the earliest sample. The other sample(s) should be analyzed only when necessary. If the other samples are to be analyzed, the Examiner should document the reasoning for this in the case file(s). Questions on whether to consume limited-volume samples should be directed to the Lead Examiner or the Deputy Director prior to analysis.

8.4.2.3 Analyze the appropriate controls (positive and negative) and the appropriate blood calibrant/cut-off solutions using the EMIT instrument.

8.4.2.4 The blood calibrant/cut-off solutions should be analyzed twice using the EMIT instrument (similar to the urine calibrators being analyzed in replicate).

8.4.2.5 Blood samples are subjected to a protein precipitation procedure prior to analysis.

8.4.2.5.1 Individually pipette 1.0 mL of each [blood] specimen into an appropriately labeled test tube

8.4.2.5.2 Add 2 mL acetonitrile to each tube and cap

8.4.2.5.3 Vortex-mix each sample for at least 30 seconds

8.4.2.5.4 Centrifuge samples for at least 5 minutes using at least 800 rpm

8.4.2.5.5 Individually decant [top] solvent layer from each specimen and place into appropriately labeled test tubes or cups

8.4.2.5.6 Individually add 25 µL of 1% MeOH-HCl to each tube

8.4.2.5.7 Vortex-mix

8.4.2.5.8 Evaporate to dryness using $N_{2(g)}$ at $\leq$ 40° C

8.4.2.5.9 Reconstitute each sample with 500µL* water

8.4.2.5.10 Vortex-mix for at least 30 seconds

*Note: Alternatively, such as when limited volumes of samples are encountered, 250µL of water can be used for reconstitution. However, a cup needs to be used instead of a test tube during analysis. This volume must be documented within the batch documentation and the case file(s). The Lead Examiner should be consulted and calibrator/cut-off concentrations may need to be adjusted.

8.4.2.6 Blood samples are not tested for ethanol using EMIT.

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9.0 EVALUATION

9.1 Urine calibrator and control evaluation

9.1.1 Print a calibration sheet with cut-off values from the main menu.
See the previous section for evaluation of urine calibrator/cut-off solutions.

9.1.2 Check the Level 0 and the S1E (when appropriate) controls and ensure that the EMIT software reported their results as negative.
Samples should not be analyzed until control criteria are met.

9.1.3 Evaluate the sample batch at the completion of the sequence.
Each analyte should be checked to ensure that the EMIT flagged the correct result.
Any problems with certain analyte assays will be addressed individually and on a case-by-case basis.

9.1.4 Control Acceptable Results:
All positive urine control's absorbance values should read above their respective calibrator's/cut-off's absorbance values and should flag as positive, as appropriate, by

the EMIT instrument software.
When analyzed, the S2E control and the S3 [cannabinoid] control should flag positive.
Note: the word 'appropriate' was used due to different positive control solutions being analyzed as controls for various analytes.

All negative urine control's absorbance values should read below their respective calibrator's/cut-off's absorbance values and should flag as negative by the EMIT instrument software.
When analyzed, the S1E control should flag negative.

9.1.5 Samples are usually analyzed as a batch (or group) and utilize common controls and calibrators for quality control purposes. Because substantial paperwork would have to be replicated if controls and calibrator data were included within each sample's case file, the complete paperwork [batch packet] can be kept separately (e.g., within the Toxicology Unit). This batch packet should contain all evaluation (i.e., Quality Control (QC)) documentation. Specific results for each case are filed in their appropriate case file(s). The QC results can be printed from the EMIT instrument. Batch packet data is usually transferred to long-term storage after a year since passed its collection date.

Note: Due to varying analyte concentrations, the above criteria may not always be met for some analytes within batches. Because this is a presumptive screening assay the batch may, depending on the magnitude and nature of the failure, be accepted by the Deputy Director or Technical Lead(s). If the batch is accepted in these circumstances, appropriate documentation must justify the acceptance decision and such documentation will be kept in the case file(s) and/or batch data. For urine samples, if the above criteria are not met and there are positive findings by the EMIT, the replicate urine samples must fully pass QC evaluation set forth in this document in order for a valid result to be reported.

9.2 Blood calibrator and control evaluation:

Calibration/cut-off absorption values within EMIT analyses of blood samples are lower than corresponding EMIT absorption values for urine samples. Because of this, the EMIT instrument's urine calibration/cut-off data can't be used when analyzing blood samples within this procedure. Absorbance values from blood calibrator solutions will be used as cut-off values when evaluating blood samples. Assessment for blood samples will be based on the comparison of a sample's absorbance reading and each calibrator/cut-off solution's absorbance reading for a particular analyte.

9.2.1 Calibrator solutions should be analyzed in replicate – similar to urine calibrators. One or more of the following should be valid in order for a calibrator solution to be deemed 'acceptable:'

9.2.1.1 An analyte's calibrator/cut-off solution is acceptable if replicate absorbance values are within ± 5% of each other.

9.2.1.2 An analyte's calibrator/cut-off solution is acceptable if the average absorbance value is within ± 5% of the average absorbance value for the

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previously analyzed batch's calibrator/cut-off solution.

9.2.1.4 An analyte's calibrator/cut-off solution can be acceptable if its average absorbance value is within ± 5% of the mean of average absorbance values from the past ten (10) average values for that calibrator/cut-off solution.

9.2.2 Control Acceptable Results:
All positive blood control's absorbance values should read above their respective calibrator's/cut-off's absorbance values

All negative blood control's absorbance values should read below their respective calibrator's/cut-off's absorbance values and should flag as negative by the EMIT instrument software.

Note: Most blood positive control samples will [erroneously] indicate a negative 'Reportable' result from the EMIT software because the software will be using urine calibration/cut-off data during its evaluation process. These results should be disregarded and manual evaluation by the analyst must occur.

9.2.3 Blood sample batches can be utilized. However, since the calibration data stored within the EMIT instrument is for urine samples, blood calibration/cut-off solutions need to be analyzed contemporaneously with each batch of blood samples. However, when sample-batches are utilized, the complete paperwork [batch packet] can be kept separately (e.g., within the Toxicology Unit). This batch packet should contain all evaluation (i.e., Quality Control (QC)) documentation. Specific results for each case are filed in their appropriate case file(s). For blood analyses, the urine QC results should not be printed from the EMIT instrument. Batch packet data is usually transferred to long-term storage after a year past its collection date.

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10.0 INTERPRETATION AND REPORTING OF RESULTS

10.1 Urine Samples (non-Ethanol):

The EMIT instrument will indicate that an analyte is possibly present within a sample based on comparison of absorption values between the sample and the calibration/cut-off solutions.

Positive Results: Occurs when the instrument flags an analyte as being possibly present within a sample. Results from analytes whose absorbance values are greater than, or equal to, the corresponding analyte's calibration/cut-off absorbance values will trigger this response. Urine samples which are EMIT positive may need to be re-sampled and re-analyzed for specific analytes.

Negative Results: Occurs when the instrument flags an analyte as being not detected within a sample. Results from analytes whose absorbance values are lower than the corresponding analyte's calibration/cut-off absorbance values will trigger this response. Any analyte for a drug class having a negative result will not be reported as possibly being present within a

presumptive report (if that type of report is issued).

Elevated Results: Elevated results occur when samples have absorption values significantly greater than negative control absorption values, are close to the cut-off absorption values, but are not equal to (or greater than) the absorption values for the calibration/cut-off concentrations of a particular analyte. While confirmation techniques can be employed to confirm presence/absence of analyte(s) and while elevated values can be noted within case files, samples with elevated results will be treated as 'negative results' (see above). It is at the Examiner's discretion whether confirmatory procedures be used for the sample.

10.2 Blood Samples:

For this procedure, the EMIT instrument is automatically using urine calibration data when evaluating blood samples. The urine calibration/cut-off solutions have absorbance values that are significantly higher than those for corresponding blood samples. When blood samples (i.e., blood extract solutions) are analyzed within this procedure, determination of the possible presence or absence of an analyte will be performed manually by the Examiner and not by the software.

For post mortem cases (or OCME ante mortem cases) an enzyme immunological technique will be performed for Barbituates and Marihuana related compounds.

Instrument-generated Positive Results: Any positive result automatically reported from the EMIT software will indicate that the sample has an analyte concentration greater than, or equal to, that within a corresponding urine calibration/cut-off solution. In these situations, the analyte concentration in the blood sample will be greater than that within the urine calibration/cut-off solution(s). Any positive results for a drug class should be reported in a presumptive test report, if that type of report is issued.

Positive Results: Occurs when a sample's absorbance value for an analyte is greater than, or equal to, the absorbance value for the corresponding analyte's calibration/cut-off solution. Any positive results for a drug class will be used as presumptive information when deciding whether to conduct further examinations on the specimen. If there is an inadequate amount of sample and EMIT results are the only results that are available, and a report needs to be issued, any positive findings will be reported with the necessary caveat that the technique is a presumptive one and is not confirmatory.

Negative Results: Occurs when results from analytes whose absorbance values are lower than the corresponding analyte's calibration/cut-off absorbance values. Any analyte for a drug class having a negative result will not be reported as possibly being present within a presumptive report (if that type of report is issued). Any negative results for a drug class will be used as presumptive information when deciding whether to conduct further examinations on the specimen. If there is an inadequate amount of sample and EMIT results are the only results that are available, and a report needs to be issued, any negative findings will be reported with the necessary caveat that the technique is a presumptive one and is not confirmatory.

Elevated Results: Elevated results are considered Negative Results. They occur when

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samples have absorption values significantly greater than negative control absorption values, are close to the cut-off absorption values, but are not equal to (or greater than) the absorption values for the calibration/cut-off concentrations of a particular analyte. While confirmation techniques can be employed to confirm presence/absence of analyte(s) and while elevated values can be noted within case files, samples with elevated results, if reported, will be treated as 'negative results' (see above).

10.3 See case workflows in SOP TX-19 for analytical guidance, based on, sample type and offense.

11.0 LIMITATIONS

The use of EMIT for the screening of forensic samples is considered presumptive and not confirmatory. False positive results can occur due to cross-reactivity. Negative results will occur when analyte concentrations are at levels below calibration/cut-off concentrations. In cases where EMIT data indicates the presence of an analyte, confirmatory procedures will be performed when an identification is required. Quantitative values will not be reported and are typically used for comparison purposes only (e.g., HS-GC data).

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12.0 MAINTENANCE

12.1 Weekly Needle Rinse: May be performed after running samples or prior to initiating a new week's usage.

12.1.1 Remove covers from reagent and sample rotors

12.1.2 Remove covers from needle rinse solution and 0.1N HCl bottles

12.1.3 The operator must ensure that the needle rinse solution test tube (Position W on the sample ring) and the needle rinse solution bottle does not get too low. The test tube should be complete filled before starting the needle rinse.

12.2 Bi-annual Preventative Maintenance: Siemens Service personnel

12.3 Rotor Change: Cuvette Rotor can be changed every 10,000 tests. This is normally performed by Siemens Service Personnel. However, if analyst needs to change they should refer to the Operator's manual for directions.

13.0 SAFETY

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment should be utilized. This includes gloves, safety glasses, and protective clothing (e.g., lab coat), as appropriate. Biological specimens subject to the analytical procedure should be handled using universal precautions. Potentially contaminated items and surfaces should be cleaned and disinfected prior to any further use.

14.0 REFERENCES

Dade Behring; "Viva-E Operators Manual" Dade Behring, Inc. P.O. Box 6101 Glasgow Business Community, Newark Delaware 19714

Blood Immunoassay Screen for Drugs of Abuse using the Olympus AU 400. West Chester County Department of Laboratories and Research: Division of Forensic Toxicology.

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Rev. #	History
3	Changed title. Significant format and verbiage changes throughout document. Added blood matrix sample-specific analyses as well as new calibrator/cut-off information. Updated product identifier information. Changed the requirement for the Bio-Rad Liquichek Urine positive controls to be analyzed with every batch to only when the calibrant/cut-off solutions are analyzed. Removed the requirement that all urine samples from the same individual must be analyzed. Removed and updated tables throughout document. Added the use of both cups and test tubes for analyses (sample volume dependent). Removed specific work instructions from document. Updated evaluation and reporting criteria. Added History section to document.
4	Added guidance for the analysis of post mortem blood samples. Removed tables under 10.2, added statement that post mortem cases will be screened for Marihuana and Barbs by an immunological technique, and added 10.3 refering the user to SOP TX-19 for workflows. Updated sections 8.2 (combining of calibrator/control solutions of differing lot numbers), 9.2.2, and 9.2.3, 10.1, 10.2, and 11.0.

1. Introduction

This procedure is primarily used to detect, identify, and quantitate ethanol and other volatile compounds within biological matrices (e.g., blood, urine, vitreous humor). The majority of samples being analyzed by this method are related to driving under the influence (DUI) investigations. Presumptive screening for ethanol within such samples can be accomplished using an Enzyme-Multiplied Immunoassay Technique (EMIT) instrument. Even though the analysis for ethanol using the EMIT instrument is a non-immunoassay experiment, any samples that are screened positive by this instrumentation will be analyzed using this procedure for confirmation. Additionally, samples suspected of containing volatile components other than ethanol (e.g., methanol, acetone, isopropanol) can be also be analyzed using this procedure.

2. Scope

This procedure allows for the screening, quantitation, and confirmation of ethanol, methanol, acetone, and isopropanol within biological samples. Other matrices can be utilized using this procedure once the procedure has been properly validated. The procedure does not necessarily need to be updated when used with new matrices. Additional matrices can be used and chemicals other than the four previously listed can be sought, if needed, using this procedure when proper quality assurance/quality control (QA/QC) measures are utilized and decision criteria are met.

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3. Principle

This procedure involves the analysis of samples for volatile compounds, (e.g., methanol, ethanol, acetone, isopropanol, and similar compounds), utilizing a headspace-gas chromatographic (HS-GC) or headspace-gas chromatographic/mass spectrometric (HS-GC/MS) method. Samples are typically in an aqueous environment, are sealed within air-tight vials, and are examined using a headspace analysis-type sampling technique. Volatile components within aqueous solutions, when placed in closed heated environments, reach an equilibrium between their vapors and their solutions. Vapor phases are analyzed with appropriate columns and detectors (e.g., HS-GC/FID, HS-GC/MS, dual column technique). Volatile components of solutions are identified and can be subsequently quantitated using calibration graphs, reference standards, and internal standards.

The headspace technique is based on various gas laws which state that when a volatile liquid in solution (e.g., ethanol) comes into contact with a closed air space, an equilibrium forms between the liquid phase and the headspace of that solution. At a constant temperature the concentration of the volatile liquid within the headspace is directly proportional to its concentration within the liquid phase. This procedure describes a way to separate, identify, and quantitate, if necessary, analytes (i.e., volatiles) from biological (or other) matrices.

4. Specimens

Any aqueous sample may be suitable for this analysis including, but not limited to: blood, serum, urine, vitreous humor. Blood sample collection tubes containing proper anticoagulant and preservative (i.e., 'gray-tops' containing potassium oxalate and sodium fluoride) should be used. However, any airtight tubes will be accepted. Containers for urine and other sample types should be within sealed, preferably airtight, glass or plastic (e.g., polypropylene) containers. All samples should be received with proper documentation, have been properly sealed (i.e., prevent sample loss, contamination, or deleterious change), and have been properly stored. Once samples are received within the Toxicology Unit all corrections regarding proper packaging or evidence integrity will be corrected prior to being properly stored within either a refrigerator or freezer.

When available, 0.4 mL of specimen is typically used within this procedure (2 aliquots of specimen, 200 µL each). Specimens with elevated analyte concentrations (e.g., high ethanol concentrations based on EMIT data) may require dilution with water in order to ensure that results are within the linear range of the calibration graph used in the procedure (i.e., 0.01 g% to 0.4 g%).

If sample amount is limited then modifications to this procedure are allowed (e.g., analyzing only one aliquot, dilution). Additionally, specimens other than biological materials may be analyzed using this procedure. However, if either of these situations occur then either the Unit Lead or higher needs to document approval within the applicable case file(s) prior to analysis.

5. Equipment/Materials/Reagents

5.1 General laboratory glassware (e.g., Class A glassware)

5.2 Headspace autosample vials (e.g., 20 mL) with appropriate seals/caps and crimper.

5.3 Pipettes (e.g., automatic pipettor-diluter), or equivalent

5.4 Vortex mixer

5.5 Headspace-gas chromatograph with flame ionization detectors (HS-GC(FID)), Shimadzu (or equivalent) or Headspace-gas chromatograph with mass spectral detector (HS-GC/MS)), Agilent (or equivalent)

5.6 Capillary Column #1: (Rtx-BAC1 ; Restek 18003, 30m, 0.32mm i.d., 1.8 µm, or equivalent)

5.7 Capillary Column #2 (Rtx-BAC2 ; Restek 18002, 30m, 0.32mm i.d., 1.2 µm, or equivalent)

5.8 Acetone (HPLC grade, or equivalent)

5.9 Deionized water (DIW, Millipore, or equivalent)

5.10 Ethanol (EtOH ; anhydrous 200 proof USP, or equivalent)

5.11 $Ethanol_{(aq)}$ (80 $mg/dL_{(aq)}$; Certified Reference Material (CRM) Standard, Cerilliant E-030, or equivalent)

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5.12 Multicomponent Mixture (MeOH, EtOH, Acetone, IPA ; 0.1 $g\%_{(aq)}$; 100 $mg/dL_{(aq)}$; 1 $mg/mL_{(aq)}$; Certified Reference Material (CRM), Cerilliant A-056, or equivalent)

5.13 Isopropanol (IPA ; HPLC grade, or equivalent)

5.14 Methanol (MeOH ; HPLC grade, or equivalent)

5.15 n-Propanol (NPA ; HPLC grade, or equivalent)

5.16 Sodium Azide (NaN_3 ; Baker, or equivalent)

5.17 Volatile Mixture (MeOH, EtOH, Acetone, IPA ; in water
0.01 $g\%_{(aq)}$, 0.025 $g\%_{(aq)}$, 0.05 $g\%_{(aq)}$, 0.2 $g\%_{(aq)}$, 0.4 $g\%_{(aq)(aq)}$;
Certified Reference Material (CRM), Cerilliant (A-127), or equivalent))

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6. Preparation of Calibrators, Controls and Standard Stock Solutions

The externally-prepared ethanol positive control CRM at 0.080 g% $(w/v)_{(aq)}$ will be analyzed within batches – regardless if internal or external solutions are utilized. If this CRM is unavailable then the Lead Examiner (or higher) will be notified and, upon written approval, an equivalent positive control can be used. This will be documented in the notes within each batch.

Externally-prepared calibrator and control solutions will be primarily used. Comparable in-house solutions will be used when external solutions are not available.
For example, a typical batch will include the following (not in any particular order):

a) Externally-Prepared [5-Point] Volatile [CRM] Calibrator Solutions:
(0.010 g%, 0.025 g%, 0.050 g%, 0.20 g%, 0.40 g%)

b) Externally-Prepared Ethanol [CRM] Positive Control Solution (0.08 g%)

c) Externally-Prepared Volatile [CRM] Positive Control Solution (0.10 g%)

d) Negative Control Solution (Externally prepared or In-House)

e) Unknown Samples

New lots of reagents, calibrator solutions, control solutions, and other types of solutions will be analyzed and verified for quality (and documented) prior to being used on casework samples for reporting purposes. Such new lots will typically be analyzed along with older (previously verified) reagents/solutions so that they can be quality-compared and verified as being acceptable for use.

Note-01: Additional positive controls (other than what are listed) can be used, whether they be externally purchased or internally prepared. Different external calibrator solutions can be used, if necessary, but both FSE2 and DD (or higher) approval is required prior to batch analysis.

Note-02: Lot numbers on labels for solutions/reagents internally generated will be of the format: month, day, year, analyst's initials, and a sequential number: {Example: 08112018MPR}

If multiple lots are made on the same date by the same analyst then a sequential number will be added: {Example: 08112018MPR-01, 08112018MPR-02}

Note-03: Throughout the procedure different volumes/amounts can be used as long as the same final concentration is obtained. If different volumes are used, however, such differences will be noted on the batch summary sheet under the 'Notes' section. The FSE2 (or higher) should be notified just to make sure nothing in procedure will be negatively impacted.

6.1 Check balance using calibrated weights on the day of solution preparation. Record appropriate checks accordingly.

Externally-Prepared Volatile Calibrator Solutions:

6.2 Externally-Prepared [5-Point] Volatile [CRM] Calibrator Solutions
(Volatile Mixture: $MeOH_{(aq)}$, $EtOH_{(aq)}$, $Acetone_{(aq)}$, $IPA_{(aq)}$)
(0.010 g% (w/v), 0.025 g% (w/v), 0.050 g% (w/v), 0.20 g% (w/v), 0.40 g% (w/v))

6.2.1 Carefully open, individually transfer liquids into autosample vials, cap, and securely seal. The solutions should be: 100 µg/mL, 250 µg/mL, 500 µg/mL, 2000 µg/mL, and 4000 µg/mL mixtures of MeOH, EtOH, Acetone, and Isopropanol in 1.2 mL ampules. No dilutions are necessary.

6.2.2 Cap and seal after use.

6.2.3 Label appropriately and any unused solutions will be refrigerated.

Externally-Prepared Positive Control Solutions:

6.3 $Ethanol_{(aq)}$ [CRM] Positive Control Solution ($EtOH_{(aq)}$; 0.080 g% $(w/v)_{(aq)}$)

6.3.1 Carefully open and transfer liquid into an autosample vial

6.3.2 Cap and seal after use.

6.3.3 Label the vial appropriately.

6.3.4 Store refrigerated.

6.4 Externally-Prepared Volatile [CRM] Positive Control Solution
($MeOH_{(aq)}$, $EtOH_{(aq)}$, $Acetone_{(aq)}$, $IPA_{(aq)}$; 0.10 g% $(w/v)_{(aq)}$)

6.4.1 Carefully open the ampule and transfer the liquid into an autosample vial.

6.4.2 Cap and seal after use.

6.4.3 Label the vial appropriately.

6.4.4 Store refrigerated.

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Internal Standard (I.S.) Solutions:

6.5 n-Propanol [$NPA_{(aq)}$] Internal Standard (I.S.) Stock Solution (5.6 g% $(w/v)_{(aq)}$; 7.0 % $(v/v)_{(aq)}$)

6.5.1 Transfer 7.0 mL of n-propanol into a 100 mL volumetric flask, dilute to volume with DIW, and mix well. (Note: density of NPA is 0.803 g/mL)

6.5.2 Transfer to an amber bottle (or equivalent) and properly label.

6.5.3 This solution will be tightly capped and stored refrigerated. It is stable for at least one (1) year from the date of its last verification.

6.6 n-Propanol [$NPA_{(aq)}$] Internal Standard Working Solution (0.011g% $(w/v)_{(aq)}$; 1.4 % $(v/v)_{(aq)}$)

6.6.1 Transfer 4.0 mL of the $NPA_{(aq)}$ I.S. Stock Solution into a 2.0 L volumetric flask, dilute to volume with DIW, and mix well.

6.6.2 Transfer to an amber bottle (or equivalent) and properly label.

6.6.3 This solution will be tightly capped and can be stored at room temperature. It is stable for at least one (1) year from the date of its last verification.

In-House (Internal) Volatile Calibrator Solutions (used when External Solutions are not available):

6.7 In-House [3-Point] Volatile Calibrator Solutions
($MeOH_{(aq)}$, $EtOH_{(aq)}$, $Acetone_{(aq)}$, $IPA_{(aq)}$)
(0.02 g% (w/v), 0.10 g% (w/v), 0.30 g% (w/v))

6.8 Volatile Calibrator Stock Solution (VCSS ; 1.0 g% $(w/v)_{(aq)}$ each of MeOH, EtOH, Acetone, IPA)

6.8.1 Accurately weigh 5.000g (+/- 0.002g) of acetone, methanol, isopropanol, and ethanol. Quantitatively transfer into a 500 mL volumetric flask with DIW.
Add 0.1 g of sodium azide, dilute to volume with DIW, and mix well.

6.8.2 Transfer the VCSS to an amber bottle (or equivalent) and properly label with lot number and other information (a properly capped volumetric flask with parafilm is considered equivalent).

6.8.3 This solution will be tightly capped and stored refrigerated. It is stable for at least one (1) year from the date of its last verification.

6.9 Low Volatile Calibrator Solution (LVCS ; 0.02 g% $(w/v)_{(aq)}$; 0.020 g/100 mL)

6.9.1 Using a volumetric pipette add 2 mL of the 1.0 g% (w/v) Volatile Calibrator Stock Solution to a 100 mL volumetric flask, dilute to volume with DIW, and mix well.

6.9.2 Transfer to an amber bottle (or equivalent) and properly label.

6.9.3 This solution will be tightly capped and stored refrigerated. It is stable for at least one (1) year from the date of its last verification.

6.10 Medium Volatile Calibrator Solution (MVCS ; 0.1 g% $(w/v)_{(aq)}$; 0.10 g/100 mL)

6.10.1 Using a volumetric pipette add 10 mL of the 1.0 g% (w/v) Volatile Calibrator Stock Solution to a 100 mL volumetric flask, dilute to volume with DIW, and mix well.

6.10.2 Transfer to an amber bottle (or equivalent) and properly label.

6.10.3 This solution will be tightly capped and stored refrigerated. It is stable for at least one (1) year from the date of its last verification.

6.11 High Volatile Calibrator Solution (HVCS ; 0.3 g%$(w/v)_{(aq)}$; 0.30 g/100 mL)

6.11.1 Using volumetric pipettes add 30 mL of the 1.0 g% (w/v) Volatile Calibrator Stock Solution to a 100 mL volumetric flask, dilute to volume with DIW, and mix well.

6.11.2 Transfer to an amber bottle (or equivalent) and properly label.

6.11.3 This solution will be tightly capped and stored refrigerated. It is stable for at least one (1) year from the date of its last verification.

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<u>In-House Positive Control Solutions</u>:

6.12 In-House Ethanol Positive Control Solution ($EtOH_{(aq)}$; 0.5 g% $(w/v)_{(aq)}$; 0.50 g/100mL)

6.12.1 Accurately weigh 5.000g (+/- 0.002g) of ethanol and quantitatively transfer into a 1000 mL volumetric flask with DIW containing 0.2 g of sodium azide.
Dilute to volume with DIW and mix well.

6.12.2 Transfer to an amber bottle (or equivalent) and properly label.

6.12.3 This solution will be tightly capped and stored refrigerated. It is stable for at least one (1) year from the date of its last verification.

6.13 In-House Volatile Mix Positive Control Solution
($MeOH_{(aq)}$, $EtOH_{(aq)}$, $Acetone_{(aq)}$, $IPA_{(aq)}$; 0.10 g% $(w/v)_{(aq)}$; 0.10 g/100 mL)

6.13.1 Prepare in same manner as the Volatile Calibrator Stock Solution (VCSS ; 1.0 g% $(w/v)_{(aq)}$ each of MeOH, EtOH, Acetone, IPA) except use reagents from a different source (or lot #) if available. Transfer the newly prepared VCSS to an amber bottle (or equivalent) and properly label as In-House Volatile Mix Positive Control Stock Solution (VPCSS). This solution will be tightly capped and stored refrigerated (a properly capped volumetric flask with parafilm is considered equivalent). It is stable for at least one (1) year from the date of its last verification.

6.13.2 Using a volumetric pipette add 10.0 mL of the newly prepared VPCSS (1.0 g% $(w/v)_{(aq)}$) to a 100 mL volumetric flask, dilute to volume with DIW, and mix well.

6.13.3 Transfer to an amber bottle (or equivalent) and properly label (a properly capped volumetric flask with parafilm is considered equivalent).

6.13.4 This solution will be tightly capped and stored refrigerated. It is stable for at least one (1) year from the date of its last verification.

Negative Control Solution:

6.14 Negative Control Solution (0.00 g% $(w/v)_{(aq)}$ of MeOH, EtOH, Acetone, IPA)

6.14.1 Place known DIW into a 1000 mL volumetric flask containing 0.2 g of sodium azide. Dilute to volume with DIW and mix well. Alternate variations of preparing this solution are acceptable (e.g., 250 mL of water with 50 mg sodium azide).

6.14.2 Transfer into an amber bottle (or equivalent) and properly label.

6.14.3 This solution will be tightly capped and stored refrigerated. It is stable for at least one (1) year from the date of its last verification.

7. Procedure

7.1 Place all specimens and solutions at a room temperature environment for approximately thirty (30) minutes.

7.2 Prepare or acquire necessary calibrator and control solutions.

7.3 Label vials for calibrators and controls as seen below (variations are acceptable):

7.3.1 Blank DIW (no internal standard)

7.3.2 Negative Control Solution (contains I.S.)

7.3.3 Volatile Calibrator (e.g., External [CRM] 0.010 g%)

7.3.4 Volatile Calibrator (e.g., External [CRM] 0.025 g%)

7.3.5 Volatile Calibrator (e.g., External [CRM] 0.050 g%)

7.3.6 Volatile Calibrator (e.g., External [CRM] 0.20 g%)

7.3.7 Volatile Calibrator (e.g., External [CRM] 0.40 g%)

7.3.8 Blank DIW (no internal standard)

7.3.9 Positive Control EtOH Solution (e.g., External Ethanol [CRM] 0.08 g%)

7.3.10 Volatile Positive Control (e.g., External 0.10 g%)

7.3.11 Blank DIW (no internal standard)

RETIRED

7.3.12 Evidentiary Samples ($X_i \ldots X_n$) ; will be made in duplicate.
Two (2) aliquots (200 µL) of each evidentiary item (i.e., blood, urine, vitreous) will be placed into separate headspace vials.

7.3.13 Volatile Positive Control (e.g., External 0.10 g%)
Will be analyzed after each full set of twelve (12) evidentiary items or less (each replicate specimen vial is considered an evidentiary item). The frequency of the number of samples analyzed before a Volatile Positive Control is analyzed can be altered with the Unit Lead's (or higher) permission.

7.3.14 Volatile Positive Control (e.g., External [CRM] 0.10 g%) ; next-to-last sample in batch.

7.3.15 Blank DIW ; last sample in batch.

Note-04: Internally prepared calibrators are acceptable if external calibrators are unavailable.

Note-05: If multiple Examiners are adding samples to the same batch then each Examiner must prepare and submit their own negative control and positive control solutions.
Negative control solutions can either be analyzed together in the batch or be placed prior to each Examiner's set of samples. Blank DIW will be placed in-between each Examiner's set of samples when multiple Examiners are adding samples to the same batch.

Sample Preparation (Automatic Pipetting of Samples, Calibrators, and Controls):

Pipetter/Diluter Preparation/Priming:

7.4 Turn on the Pipetter/Diluter and check for bacterial contamination in solutions

7.5 Place the inlet tubing into the NPA Internal Standard working solution bottle making sure the end of the tubing is well below the level of the liquid.

7.6 Remove the dispenser probe from its holder and place the tubing from the probe in an empty waste container/flask.

7.7 Press the prime switch and liquid will be dispensed from the probe at this time. The system's lines will be primed. Continue to cycle until the bubbles have disappeared from the lines.

RETIRED

Sample preparation with Pipetter/Diluter:

7.8 Use the automated pipettor and place its probe into blank DIW, draw up 200 µL, dispense into a waste container, and perform the between-sampling rinse. Dry the probe tip with a clean wipe as needed.

7.9 Place the probe into the Internal Standard Working Solution and press the Pipette Activation Button (PAB) once to aliquot 1 mL of the $NPA_{(aq)}$.

7.10 Place the probe into the sample to be analyzed (i.e., negative control, calibrator, positive control, unknown sample) and press the PAB again to draw up 200 µL.

7.11 Place the probe inside the appropriately labeled autosample vial and press the PAB once to dispense.

7.12 Place the probe into a waste container and press the PAB once more to dispense the between-sampling rinse into the waste container. Dry the probe tip with a clean wipe as needed.

7.13 Place a headspace cap on the vial and crimp-seal tightly. Take this time to verify that the proper sample was transferred into the correspondingly labeled headspace vial.

7.14 Repeat the internal standard/sample dispensing into head-space vials for all calibrators, controls, and samples. This can be done according to the order stated earlier under this 'procedure' section.

Note-06: If automatic pipetter is not functioning then manual pipetting is permitted. Adequate adjustments will be performed by analysts so that this procedure can be followed. Lead Examiner (or higher) can be sought for assistance, if necessary.

RETIRED

Setting-up Instrument with Samples:

7.15 Ensure air and hydrogen are flowing and ensure detectors (flame ionization detector (FID) and/or mass spectrometer (MS) are operational.

7.16 Ensure the appropriate instrumental quality assurance/quality control (QA/QC) procedures were performed. The instrument must have passing QA/QC results prior to preparing and loading of samples.

7.17 Enter the sequence order for the method Batch Table.

7.18 Place the labeled headspace vials in the appropriate order within the instrument.

7.19 Enter the following dilution factors:
0.769 for urine, 0.862 for serum, and 1.000 for blood and vitreous samples.

Note-07: OCME samples do not contain dilution factors.

7.20 Save the Batch Table to the day's date.

7.21 Prior to analyzing samples:
Print the sequence list. Check that the physical placement of the headspace vials and the vial positions within the instrument's sequence list match. Once the check has been completed place an indication of the sample check (e.g., 'sequence checked' or 'sequence verified') on the sequence page along with Analysts initials and date.

7.22 Print the instrument method and include both the method and the sequence printouts with the batch documents.

7.23 Begin the sequence and analyze the samples.

7.24 After the batch has completed:
Re-check that the physical placement of the headspace vials and the vial positions within the instrument's sequence list match. Once the re-check has been completed place an indication of the sample re-check (e.g., 'sequence re-checked post analysis' or 'sequence re-verified post analysis') on the sequence page along with Analysts initials and date.

8. Instrumentation

Instrumental Parameters:

The following are the typical operating parameters for the instrument used in this procedure. With approval from the Lead Examiner and Deputy Director (or higher), the instrument conditions may be modified to adjust or improve the procedure. Documentation of such changes must be included with batch data so that any instrumental parameter change can be associated with data and casework until this procedure has been updated.
The appendix contains an abbreviated version of the procedure. The checklist can be used by analysts. Any changes within the instrumental parameters, if listed on the checklist, will be reflected on the checklist by the analyst filling it out.

RETIRED

HS-GC(FID):
The following parameters can be used. Some values may change due to slight variability (e.g., flow rates). Significant differences must be approved by Unit Lead (or above) prior to changing.

AOC-5000	Parameters		AOC-5000	Parameters
Cycle	HS-inj		**Syringe Temperature**	75 C
Syringe	2.5 mL-HS		Fill Speed	500 µL/sec
Sample Volume	850 µL		Pull Up Delay	500 msec
Incubation Temperature	50 C		Inject To	GC Injector 1
Incubation Time	4 min.		Injection Speed	1 mL/sec
Agitation Speed	300 rpm		Pre-Injection Delay	500 msec
Agitation On	3 sec.		Post-Injection Delay	0 msec
Agitation Off	1 sec.		Syringe Flushing	25 sec.
Oven Temp	55°C		**Injection Port SPL1**	
Sample Line Temp	150°C		Injection Mode	Split
Transfer Line Temp	150°C		Carrier Gas	He
Shaking Level	1		Flow Control Mode	Velocity
Multi Injection Count	1		Pressure	136.6 kPa
Multi Injection Count	1		Total Flow	35.0 mL/min.
Pressurizing Gas Pressure	50.0 kPa		Column Flow	5.00 mL/min.
Equilibrating Time	15.00 min.		Linear Velocity	65.1 cm/sec.
Pressurizing Time	0.15 min.		Purge Flow	10.0 mL/min
Pressure Equilib. Time	0.35 min.		Split Ratio	4.0
Load Time	0.50 min.		Injection Volume (mL)	850 µL
Autosampler (HS-20)	**Parameters**		**Autosampler (HS-20)**	**Parameters**
Load Equilib. Time	0.25 min.		Needle Check	Yes
Injection Time	0.50 min.		Action Leak Check Error	Continue
Needle Flush Time	0.50 min.		Action - No vial on tray	Skip
GC Cycle Time	4.51 min.			
Check GC Ready	On		**Column Oven**	
Ext. GC Ready Check Limit	10 min.		Initial Temperature	50.0°C
Check System Ready	On		Equilibration Time	0.0 min
Ext. Sys. Ready Check Limit	45 min.		GC Run Time	4 min
Analysis Mode	Constant		Ramp Rate	0°C/min
			Final Temp.	50.0
			Hold Time	4.00

* or equivalent

RETIRED

HS-GC(FID) (Cont'd):

Column Information	FID 1	FID 2
Column Names	**Rtx-BAC1**	**Rtx-BAC2**
Column Cat. #	18003	18002
Film Thickness	1.80 µm	1.20 µm
Column Length	30.0 m	30.0 m
Inner Diameter	0.32 mm	0.32 mm
Column Max Temp	260°C	260°C
Detector	**FID 1**	**FID 2**
Temperature	260°C	260°C
Sampling Rate	40 msec.	40 msec.
Stop Time	4.00 min	4.00 min
Delay Time	0.00 min	0.00 min
Subtract Detector	None	None
Makeup Gas	He	He
Makeup Flow	30.0 mL/min	30.0 mL/min
H2 Flow	40.0 mL/min	40.0 mL/min
Air Flow	400.0 mL/min	400.0 mL/min

Note: Slight variations in the instrument programs are acceptable when analyte separation is demonstrated in the controls and calibrators are acceptable. However these variations will be approved (by FSE2 or higher) prior to changing and such changes will be documented within case/batch notes.

RETIRED

HS-GC(FID/MS):
The following parameters can be used. Some values may change due to slight variability (e.g., flow rates). Significant differences must be approved by Unit Lead (or above) prior to changing.

Autosampler	Parameters		Autosampler	Parameters
GC Cycle Time	6.5 min.		Sample Vial Penetration	15 mm
Sample Volume	0.5 mL		Sample Vial Penet. Speed	50 mm/sec
Incubation Time	5 min.		Sample Aspiration Rate	12 mL/min
Incubation Time Increment	0 min.		Sample Post Asp. Delay	1 sec.
Heat Agitator	On		Inlet Penetration Depth	45 mm
Incubation Temperature	50 C		Inlet Penet. Speed	50 mm/sec
Heat Syringe	On		Pre-Inject Time Delay	0.5 sec.
Pre-Injection Flush Time	5 sec.		Inject Flow Rate	10 mL/min.
Agitator Speed	250 rpm		Post Injection Delay	0.5 sec
Agitator On Time	5 sec.		Flush Time	10 sec.
Agitator Off Time	5 sec.		Continuous Flush	Off
GC	**Parameters**		**Injector**	**Parameters**
Run Time	6.5 min.		Mode	SPLIT
Post Run Time	0 min.		Split Ratio	10:1
Oven Equilibration Time	0 min.		Split Flow	46.049 mL/min.
Max. Oven Temperature	250 C		Injector Heater	250 C
Initial Temperature	47 C		Injector Pressure	9.5 psi
Hold Time	6.5 min.		Injector Total Flow	53.654 mL/min.
Post Run Temperature	100 C		Septum Purge Flow	3 mL/min.
			Gas Saver	On
Transfer Line	**Parameters**			
Temperature	200 C			

RETIRED

HS-GC(FID/MS) (Cont'd):

Column Information	Agilent	Agilent
Column Names	DB-BAC-1 Ultra Inert (Col. #2)	DB-BAC-2 Ultra Inert (Col. #3)
Column Cat. #	123-9334UI	123-9434UI
Film Thickness	1.8 µm	1.2 µm
Column Length	30 m	30 m
Inner Diameter	320 µm	320 µm
Column Max Temp	260°C	260°C
Pressure (initial)	7.9 psi	7.9 psi
Pressure (post run)	3.8589	3.8589 psi
Flow	2.6857 mL/min.	1.5733 mL/min.
Ave. Velocity	59.637 cm/sec.	27.599 cm/sec.
Holdup Time	0.8384 min.	1.8117 min.
Detector	**MS**	**FID (Front Detector)**
Temperature (Source)	230 C	250 C
Temperature (Quad)	150 C	–
Sampling Rate	–	20 Hz (Signal #1)
Makeup Gas	–	He
Makeup Flow	–	25 mL/min.
H_2 Flow	–	30 mL/min.
Air Flow	–	400 mL/min.
Ion Source	EI	–
Fixed Electron Energy	70 eV	–
Solvent Delay	1 min.	–
Gain Factor	2	–
EM Saver	False	–
Acquisition Type	Scan	–
Start Mass	29 m/z	–
End Mass	200 m/z	–
Threshold	0	–
Scan Speed	781 (N=3) Scan Speed	–

Note: Slight variations in the instrument programs are acceptable when analyte separation is demonstrated in the controls and calibrators are acceptable.

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9. Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. Retention time (chromatographic characteristic), peak shape, and signal-to-noise (aka: integratable peaks) are used as the basis for peak detection. In most cases all of the criteria below will be met in order to detect the appropriate volatile analytes within specimens. Only analytes found within evidentiary specimens of batches will need to be evaluated. For example, if twenty-five (25) unknown samples are analyzed and ethanol is the only volatile analyte detected at reportable limits, then the other volatiles which are in the controls and calibrators need not be processed nor evaluated.

9.1 Chromatography:

Chromatographic peaks will possess good chromatographic quality (i.e., Gaussian peak shape, reasonable peak width, distinguishing signal-to-noise). In order for a chromatographic peak to be deemed acceptable it will compare favorably to corresponding chromatographic peaks within known samples (i.e., positive controls).

9.1.1 Retention Time (RT):

The relative retention time of a chromatographic peak (i.e., compared to the internal standard's retention time) will be within ±0.1 minute of the relative retention time obtained from analysis of a reference standard or positive control.

9.1.2 Signal-to-Noise Ratio (SNR):

To justify the existence of a peak, its baseline signal-to-noise ratio (SNR) will be automatically integrated (or exceed 3). Further, the baseline signal for the peak of interest will be at least 10-fold greater than that for any observed peak (corresponding to the analyte of interest) at a similar retention time in a negative control or blank injected prior to that sample. Peak area or peak height can be used to determine SNR.

9.1.3 Mass Spectrometry

In order for analytes to be reported, correct mass spectra for each analyte will be present. Ion ratios should compare favorably to ion ratios from applicable reference standards.
Ion ratios are calculated and compared to ensure that analytes have strong enough data to support an identification. Comparisons of ion ratios is performed using the following:

9.1.3.1 Any ion signal greater than 15% of the most intense ion signal in a background-subtracted mass spectrum will normally be considered a *significant ion*.

9.1.3.2 *Diagnostic ions* are significant ions in an analyte's mass spectrum that are characteristic of that particular analyte. Determination of diagnostic ions depends upon knowledge of the chemical structure of a component under investigation and may therefore only be determined from mass spectra of known standards. There is not a universally accepted standard for determining diagnostic ions.

9.1.3.3 Using an analyte's [background-subtracted] m/z base peak (e.g., 31 for EtOH) and comparable start and end times, get the areas of the extracted ion chromatographic

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(EIC) peaks (this includes the base peak along with about three (3) other diagnostic ions (e.g., 45, 29, 27 for EtOH) within the positive control.

9.1.3.4 Take the ratios of the areas from each diagnostic ion's EIC peak and ratio to the area of the analyte's EIC base peak within the positive control. These [positive control] analyte ion ratios will be used to compare with ion ratios from other samples.

9.1.3.5 Repeat the above two steps for other samples in the batch.

9.1.3.6 Use the criteria below to determine if an analyte should be reported.

Generally speaking and using the following criteria, analytes within unknown samples should be reported if their ion ratios correspond to known reference analytes having similar ion ratios:

Expected [Set] Ion Ratio	Allowance
>50%	10%
20% – 50%	20%
<20%	5%

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Example:

Ethanol Mass Spectral Comparison

		Base Peak	Diagnostic Ions (m/z)		
Specimen		**31**	**45**	**29**	**27**
Pos. Ctrl. Ethanol	response	4948620	3604926	816328	1195924
Filename-10.D	ion ratio	100.0	72.8	16.5	24.2
Item 001-01	response	151084	106130	24627	39021
Filename-18.D	ion ratio	100.0	70.2	16.3	25.8
	pass / fail	**PASS**	**PASS**	**PASS**	**PASS**
Item 001-02	response	9879947	7196655	1637126	2430409
Filename-20.D	ion ratio	100.0	72.8	16.6	24.6
	pass / fail	**PASS**	**PASS**	**PASS**	**PASS**

Note-08: This comparison can be performed automatically within data processing software.

9.2 Batch Acceptance:

9.2.1 The batch must have completed with no unexplained interruptions and no unexplained errors. Consult the Lead Examiner (or higher) if unsure whether to continue processing the batch.

9.2.2 All calibrator solutions that have been analyzed during a batch (and their resulting data) will be included in data processing and sample evaluation. No calibrators within a batch can be selectively eliminated from the resulting calibration graph within out DD approval and documentation explaining why and how such elimination is statistically allowable.

9.2.3 No analytes of interest will have been detected in the Negative Control.

9.2.4 All applicable analytes of interest within positive controls, as well as internal standards, will be identified during data processing.

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Note-09: Analytes of interest are considered those compounds that are being reported.

9.2.5 Calibration Check (EtOH-only): Quantitative results of the External Ethanol [CRM] ; 0.080 g%) Positive Control Solution must be within +/- 5% of its target value (range: 0.076 g% – 0.084 g%). Results from the CRM data are recorded in the appropriate Quality Control (QC) chart spreadsheet.

9.2.6 Calibration Checks (MeOH, EtOH, Acetone, IPA): Quantitative EtOH results within the Volatile Positive Control Solution must be within +/- 5% of its target value (i.e., 0.10 g% ; range: 0.095 g% – 0.105 g%) and quantitative MeOH, acetone, and IPA results within the Volatile Positive Control Solution must be within +/- 10% of their target values (i.e., 0.10 g% ; range: 0.090 g% – 0.110 g%). Results from these data are recorded in the appropriate Quality Control (QC) chart spreadsheet.

9.2.7 Calibration Checks (other volatiles): Quantitative results from other positive controls (other than from ethanol and the volatile controls) which may have been analyzed along with associated calibration solutions shall be within +/- 10% of their targeted values. Results from these data are recorded in the appropriate Quality Control (QC) chart spreadsheet.

9.2.8 Other than the n-propyl alcohol (NPA internal standard), no significant peaks will be present in the negative control sample. A significant peak is an analyte of interest with an extrapolated concentration $\geq$ 0.005 g% .
{Blank and Carryover Check}

Note-10: Significant peaks are considered peaks which are automatically integrated and which impact the quality of the results.

9.2.9 Analytes below the lowest calibrator will not be quantitatively reported. However if such analytes are chromatographically acceptable (and toxicologically significant) then they may be reported qualitatively.

9.2.10 Column (BAC1) Sample Replicate Evaluation (for EtOH only):

9.2.10.1 Analyte's empirical concentration is ≤ 0.03 g%:
The difference between the BAC1 column results for each aliquot replicate should be ≤ 20 %. Seek FSE2 (or higher) guidance to determine if samples need to be re-analyzed or if acceptance of current value is allowed – document in batch case notes.

9.2.10.2 Analyte's empirical concentration is between 0.03 g% and 0.08 g%:
The difference between the BAC1 column results for each aliquot replicate should be ≤ 10 %. Seek FSE2 (or higher) guidance to determine if samples need to be re-analyzed or if acceptance of current value is allowed – document in batch case notes.

9.2.10.3 Analyte's empirical concentration is ≥ 0.08 g%:
The difference between the BAC1 column results for each aliquot replicate should be ≤ 5 %. Seek FSE2 (or higher) guidance to determine if samples need to be re-analyzed or if acceptance of current value is allowed – document in batch case notes.

9.2.10.4 If blood specimens are clotted and the above criteria are not met then the samples should be centrifuged and be analyzed as serum samples. Seek FSE2 (or higher) guidance to determine if samples need alternative processing.

9.2.10.5 If the above criteria are not met and it is not due to clotting, then a Unit Lead or higher will be consulted and the samples may need to be repeated. Record within the case notes of the batch and, if necessary, within affected casefile notes.

9.2.11 Dual Column Evaluation:

9.2.11.1 Any reportable analyte must be able to be identified by relative retention times (i.e., peak times compared to the internal standard) on both GC columns.

9.2.11.2 All reportable analyte GC peaks should be totally resolved. Extracted ion chromatography (mass spectral data) will be used if co-elution is an issue.

9.2.11.3 In order to be reportable, analytes must have quantitative values from both GC columns (BAC1 and BAC2) that agree within +/- 10 %.

9.2.12 Unknown Sample Compound Detection:

This procedure is intended to be used for both qualitative and quantitative purposes of select analytes (e.g., ethanol). The majority of other unknown compounds will only be qualitatively reported. If necessary, other analytes may be quantitated (e.g., acetone).

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9.2.13 Reporting Limit (RL):

9.2.13.1 Only analytes at or above the lowest calibrator solution will be reported quantitatively. Analytes that have been automatically integrated from the processing software but whose concentrations are below the lowest calibrator solution may be reported qualitatively. For quantitative purposes the reporting limit for volatiles will be 0.02 g%.

9.2.13.2 If analytes have quantitation values greater than the highest calibrator solution then dilution and re-analysis should occur. Variations in reporting terminology can also be used if appropriate and approved by the Unit Lead or higher (e.g., Item #01: Ethanol ≥ 0.40 g%).

9.2.13.3 Blood samples with ethanol concentrations greater than the highest calibrator solution (e.g., > 0.40 g%) will be reanalyzed using appropriate dilutions if accurate quantitations above that highest calibrator's concentration are needed. Information regarding how the dilutions were prepared will be noted within the batch paperwork. If unsure of the proper dilution consult the Unit Lead (or higher).

9.2.13.4 For urine samples with converted (i.e., blood-equivalent) ethanol concentrations ≥ 0.30 g%, appropriate dilutions and re-analyses are necessary if the highest calibrator solution is 0.40 g% (> 0.23 g% in In-House calibrants are used). Notes describing how the dilutions were prepared will be in the batch paperwork. If unsure of the proper dilution then consult the Unit Lead (or higher).

9.2.13.5 For serum samples with converted (i.e., blood-equivalent) ethanol concentrations ≥ 0.34 g%, appropriate dilutions and re-analyses are necessary if the highest calibrator solution is 0.40 g% (> 0.25 g% in In-House calibrants are used). Notes describing how the dilutions were prepared will be in the batch paperwork. If unsure of the proper dilution then consult the Unit Lead (or higher).

9.2.13.6 Based on toxicological significance, samples with volatile analytes that are found to be outside the calibration ranges will be assessed to determine if they need to be diluted and repeated or if they can simply be reported either qualitatively or greater than the highest calibrator. The Unit Lead (or higher) will be consulted for such instances and notes will be kept within batch documentation.

9.2.13.7 Other causes where samples are not able to be repeated (e.g., limited sample) will be noted within batch and casefile paperwork. The FSE2 and higher will be consulted on how to phrase report so that such instances are captured within the report as to why certain QA/QC actions were not performed.

9.2.13.8 Samples which have analytes that elute after a GC run has completed (e.g., past 4 minutes) may need to be reanalyzed using longer GC run times.

9.2.13.9 Samples which contain significant carry-over peaks (i.e., contamination into the next sample) may need their samples re-analyzed. Consult Unit Lead (or higher)

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when these situations occur. Documentation in case files will occur to explain rationale of decision of whether to re-analyze or not.

9.2.14 Data Processing:

9.2.14.1 Quantitation of the headspace-GC data may be performed automatically by the software. This is often done using macros. While quantitation is normally performed using BAC1 column data, if applicable and if necessary, GC data processing using BAC2 data can be performed. Acceptability of data using BAC2 data will be at the discretion of the FSE2 (or higher) and will be documented in case notes.

9.2.14.2 Quantitative calculations are based on comparisons of chromatographic peak areas between analytes and the internal standard (i.e., n-propanol).

9.2.14.3 Peak area response ratios from the calibrant solutions are calculated and compiled to create a linear calibration graph (y-axis is Area Ratio and x-axis is ethanol concentration). A best-fit line ($y = mx+b$) is created from the calibration graph which does not include the origin as a point.

9.2.14.4 Calibrator solutions only need to be analyzed once per instrumental sequence/batch.

9.2.14.5 Calibrator and control solution data should be evaluated prior to analyzing evidentiary samples to ensure that quality control measures have been satisfied and the calibration graph is acceptable.

9.2.15 Other Criteria:

Blank and Carryover Check: Other than the n-propyl alcohol (NPA internal standard), no significant peaks will be present in the negative control sample. If any reportable target analytes are present in the blanks or negative control samples then they should be at concentrations below 0.005g%.

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Note-11: Toxicologically significant refers to whether the reporting of an analyte would reasonably have an impact to the customer.

9.2.16 Calibration Linearity: All calibration lines are considered acceptable if their associated correlation coefficients (r^2) are ≥ 0.99. The calibration lines for each analyte are independently evaluated as follows:

9.2.17 If a calibration line is not acceptable then the batch is rejected for that particular analyte and case samples will not be quantitatively reported until a new calibration line is acquired and deemed acceptable.

9.2.18 Troubleshooting, instrument evaluation, and/or preparation of new calibration solutions, if necessary, will be done prior to analysis of samples.

9.2.19 If some of the repeated ethanol positive control values are found to be outside their designated acceptable limits then casework samples that were analyzed prior-to and directly-after (i.e., those analyzed in-between bracketed controls) will be re-analyzed, if possible. Consult Unit Lead (or higher) for guidance, if necessary.

9.2.20 Other than ethanol, if volatile positive control analyte's results are found to be outside designated acceptable limits then consult Unit Lead (or higher) for guidance on how to proceed.

9.2.21 Appropriate corrective action(s) may need to be done before casework samples are reported if multiple control values are found to be unacceptable (e.g., repeat entire batch after performing instrument QA/QC). Consult Unit Lead (or higher) for guidance, if necessary.

10. Reporting of Results

The following criteria are used as guidelines in determining how to report results using this procedure.

10.1 Analyte levels less than the reportable limit will be reported as '[Analyte(s)] not reportable' (or similar language). Further description of when such phrases are used will be included in reports so as to convey to readers that analytes may be present at concentrations lower than their reportable limits.

10.2 For evidentiary biological samples where volatile analytes were quantitated and were within the reportable limits (RL):

10.2.1 The reported value will be an average of the two (2) BAC1 column replicate concentrations.

10.2.2 Whole blood concentration numbers will be truncated to three (3) decimal digits (i.e., 3 decimal places).

10.2.3 Urine sample ethanol concentration values will be converted to whole blood equivalent ethanol values (i.e., BAC equivalent) using a conversion ratio of 1.3–to–1 (i.e., 0.769).

10.2.4 Serum sample ethanol concentration values will be converted to whole blood equivalent ethanol values (i.e., BAC equivalent) using a conversion ratio of 1.16–to–1 (i.e., 0.862).

RETIRED

Note-12: For OCME cases, ethanol concentrations within samples will not be converted.

10.3 Ethanol concentration values within non-biological samples will not have any conversions performed and will be reported by weight (i.e., g%). Reporting an ethanol concentration within non-biological samples will involve converting the weight concentration value to a volume concentration value using an ethanol density of 0.789 g/mL, thus getting a volume-volume percent concentration. (Example: (0.080 g% / 0.789 g/mL) = 0.10 % (v/v))

10.4 Uncertainty of measurement will be reported based on the most current calculated uncertainty value for this procedure.

10.5 The format for reporting volatile analyte concentrations will be: y +/- U (where y is the measured quantity value and U is the expanded uncertainty).

10.6 A confidence interval of at least 95% will be used.

10.7 Measurement uncertainty will be reported to three (3) decimal digits (i.e., 3 decimal places) because listing this number with only two (2) decimal digits would cause uncertainty values of zero (i.e., 0.00) to be reported. Such values would cause confusion for customers. Because the uncertainty value must have the same number of decimal digits as the measured quantity, three (3) decimal digits will be used for both the concentration value and the associated measurement uncertainty within this procedure.

RETIRED

11. Quality Assurance/Quality Control (QA/QC)

11.1 Each sample is evaluated for acceptability according to the sample and run acceptance criteria specified above.

11.2 Sensitivity: The limit of quantitation (LOQ) for this procedure has been administratively set to be 0.010 g% (w/v) for ethanol, methanol, isopropanol, and acetone. The reporting limit has been administratively set to be at 0.020 g% (w/v) for the same analytes for this procedure. Other volatile compounds will have LOD and LOQ values listed within their validation documents. The limit of detection (LOD) for ethanol, methanol, isopropanol, and acetone for this procedure has been administratively set to at least 0.010 g% (w/v).

11.3 Specificity: Because of the dual column concept, no potentially interfering substances have been identified using this procedure. All samples are analyzed using two (2) separate columns (using differing polarity chemistries) as well as two (2) different detectors (flame ionization and mass spectrometry).

11.4 Accuracy: The use and practice of analyzing positive controls contemporaneously with evidentiary samples ensures that correct volatile compounds are detected and reported.

11.5 Precision: Positive control concentrations are empirically calculated, stored within a spreadsheet (i.e., QC charts) and tracked for uniformity and repeatability/reproducibility.

11.6 Linearity: Best-fit calibration lines between the ranges of 0.010g% to 0.40g% for ethanol, methanol, isopropanol, and acetone are recorded and evaluated within each batch.

11.7 Sample Verification: All vial sequences and vial positions are checked both before and after samples are analyzed by the instrument. Evidence, when enough is available, is aliquoted and sampled at least two-times within each batch.

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12. Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment will be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Biological specimens will be handled using universal precautions and will be treated as biohazardous. Potentially contaminated items and surfaces will be cleaned prior to use.

13. References

Reed, D. and Cravey, R.H. (1971) A Quantitative Gas Chromatographic Method for Alcohol Determination. J. Forensic Sci. Soc. 11:263

Karnitis, L. and Porter L.J. (1971) A Gas Chromatographic Method for Ethanol in Vapors of Biological Fluids. J. Forensic Sci. 16:318-322

Jones, A.W. and Schubereth, J. (1989) Computer-aided Headspace Gas Chromatography Applied to Blood Alcohol Analysis: Importance of Online Process Control. J. Forensic Sci. 34:1116-1127

Baselt, R.C., *Disposition of Toxic Drugs and Chemicals in Man*, 7th ed., Biomedical Publications: Foster City, California, 2004.

Moffat, A.C., *Isolation and Identification of Drugs*, 2nd ed., Pharmaceutical Press: London, 1986.

Dubowski, K.M., *Manual for Analysis of Ethanol in Biological Liquids*, 1977.

Garriott, James, *Medicolegal Aspects of Alcohol*, 3rd ed., Lawyers and Judges Publishing: Tucson, AZ, 1996.

Levine, B.; Smith, M.L.; Smialek, J.E.; Caplan, Y.H. *J For Sci*. 1993, *38*, 663-667.

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Appendix:
Bench Method:
Volatile Analysis by HS-GC(FID/MS)

The following is an abbreviated version of this procedure and a pdf-version can be used.
This form may be used at the bench level by the analyst performing the procedure.

Negative Control [volatile-free]$_{(aq)}$	**Lot:**	
Positive Controls$_{(aq)}$ **Externally Prepared / In-House**	**Volatile Mix:** **Ethanol:**	**Lot:** **Lot:**
n-Propanol Internal Standard	**Lot:**	
Calibrator Solutions$_{(aq)}$ **Externally Prepared / In-House** **(circle one)**	**Calib. #1:** **Calib. #2:** **Calib. #3:** **Calib. #4:** **Calib. #5:**	**Lot:** **Lot:** **Lot:** **Lot:** **Lot:**
Other:	**Type:** **Type:** **Type:** **Type:** **Type:**	**Lot:** **Lot:** **Lot:** **Lot:** **Lot:**

Procedure:

	For Blood or Urine (or Other Matrix) Specimens
Ensure gas flows are adequate and instrument is warmed-up and ready for analyses (QA/QC is complete)	
Place samples, controls, and calibrators at room temperature for ~ 30 minutes	
Prepare or acquire calibrator/control solutions	
Label vials appropriately (calibrators, controls, evidence samples, blanks)	
Prepare automatic Pipetter/Dilutor and prime (if using)	
Prepare samples (i.e., 1 mL of I.S.(n-propanol$_{(aq)}$) + 200 µL sample)	
Between-sample rinse (if automatic Pipetter/Dilutor is used)	
Tightly cap headspace vials	
Repeat dispensing as necessary	
Create sequence within instrument software	
Ensure methods and parameters are correct	
Ensure appropriate dilution factors are set (OCME do not get dilution factors)	
Save the batch table to today's date	
Verify accuracy of sequence/vial placement (prior to analysis):	
Verify accuracy of sequence/vial placement (post analysis):	

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Rev. #	History
3	Updated verbiage and formatting within the entire document. Appendices were updated with correct information and formatting was changed. Added 'Blank DIW' (that doesn't contain an internal standard) within the Automatic Pipetting of Samples, Calibrators, and Controls step. Updated instrumental parameters including adding separate HS-GC-2 parameters. Combined the volatile stock solution to one mixture (Volatile Working Solution). Renamed old 'Blank' to be 'Negative Control' (contains internal standard). Replaced 'Conditioner' to '0.02 g% Volatile Working Solution' within the instrumental sequence. Changed the calibration requirements to not include the origin (0-point). Added a GC ramp pre-conditioning step, if necessary, within the Instrumental Analysis section. Added HS-GC-2 information within the Instrumental Analysis section. Changed 'Run Evaluation' to Decision Criteria' and updated that section. Requirements within 'Sample Acceptance Criteria' section was updated. Throughout document: Eliminated much redundancy, Director changed to Deputy Director, Supervisor changed to Lead Examiner. Previous cross-outs were removed and font color within entire document was changed to black.
4	6.1.3 Results for the EtOH CRM standard must be within 5% of its target 0.08% value, range 0.076 – 0.084%. 6.1.4.4 Accuracy and Precision: All positive control solution quantitation results must agree within 10% of the statistical mean of their respective concentration. Added 0.1g% Ethanol Control - directions to prepare. Added statement that controls and calibrators will be made from different stock solutions. Clarified statement on when dilutions are needed. Added statement that the analysts will use the EMIT results for urine samples as a guide to determine if a sample must be diluted.
5	Updated the SOP to allow for a combined volatile mixture for the calibration of Ethanol, Methanol, Acetone and Isopropanol. Removed the use of ethanol only controls. Changed the suggested sequence. Updated the acceptability guidance to be for all the volatile compounds not just ethanol. Added guidance on the reporting of results. (Sections 3.3, 4.0, 5.3, 6.1.3, 6.2, and 6.3)
6	Section 3.3.5 Positive Control changed to Positive Ethanol Control. Section 6.3 Updated reporting to include reporting volatiles and

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	specialized needs of OCME cases.
7	Updated verbiage and formatting within the entire document. Added new set of externally prepared and In-House calibrators and positive control solutions, updated number of positive controls. Updated measurement uncertainty format and explained need for three (3) significant digits within reportable concentrations. Included GC/MS instrumentation and updated GC-FID parameters. Updated the Decision Criteria section to include MS. Updated appendix. Added description of mass spectral comparison. General format rearrangement throughout document.

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1.0 PRINCIPLE:

This procedure describes the analysis of aqueous samples for volatile compounds, (methanol, ethanol, acetone, isopropanol, and analogous compounds), utilizing a headspace gas-chromatographic method. Samples are diluted first with deionized water at a 1 to 100 ratio using class A volumetric pipettes and flasks and further diluted with an aqueous solution using N-propanol as an internal standard... Next, they are sealed in vials for headspace analysis. Volatile components in the heated aqueous phase diffuse into, and reach equilibrium with the vapor phase. An aliquot of the vapor phase is injected into the gas chromatograph (simultaneously onto two separate columns), which separates the analytes as a function of their chemical characteristics. Separated components from each column are identified by retention time, and quantitated by response on Flame-Ionization Detectors. Quantitation is based on a one-point calibration using the peak area ratio between the analyte and the internal standard.

2.0 SPECIMEN:

Samples requiring analysis for ethanol and other volatile compounds have their associated case jacket kept in the office in Toxicology sextion. Any aqueous sample may be suitable for this analysis, including (but not limited to) Beer, Wine, Spirits, and other containers with suspected ethanol. All other samples should be sealed and stored in appropriate airtight glass or polypropylene containers under refrigeration if possible. If not analyzed immediately, preserved liquid samples should be refrigerated and may be stored (sealed) for up to 12 months.

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2.1 All evidence transfers, either between individuals or between an individual and a storage location must be documented on the Chain of Custody for the case, either in the LIMS, or on hard-copy COC document maintained in the Case Jacket.

2.2 When not in the sampling or aliquot process, samples in the toxicology section must be stored in a secure and locked area.

2.3 Samples must be maintained in such a manner so that they are protected from contamination or deleterious change. Depending on the nature of the sample, this may mean refrigeration or freezing when not in the analytical process.

2.4 When samples are finished being analyzed, samples in the toxicology section must be maintained “Under Proper Seal.” This is interpreted to mean that the sample, or a container in which the sample is kept is sealed with tamper-evident tape with the initials and date of the person placing the seal on the seal as well.

3.0 MATERIALS AND EQUIPMENT:

3.1 Equipment:

3.1.1 Suitable Gas Chromatograph with autosampler for headspace; equipped with dual FID detectors, Rtx-BAC1 and Rtx-BAC2 30m capillary columns. (0.53 x 3 um; Restek 18000 & 18001) or equivalent.

3.1.2 Automatic Pipetter-Diluter (200 microliter & 2 ml syringes).

3.1.3 20 ml headspace autosampler vials with appropriate seals and aluminum caps, and crimper.

3.1.4 General Laboratory Glassware and Equipment.

3.2 Reagents:

3.2.1 Ethanol (EtOH; Baker; anhydrous 200 proof USP or equivalent)

3.2.2 Deionized water (DIW; Millipore or equivalent In-House supply)

3.2.3 Acetone (Baker HPLC grade or equivalent)

3.2.4 n-Propanol (NPA; Baker HPLC grade or equivalent)

3.2.5 Methanol (MeOH; Baker HPLC grade or equivalent)

3.2.6 Isopropanol (IPA; Baker HPLC grade or equivalent)

3.2.7 Aqueous EtOH Certified Reference Standard. (Cerilliant Corp. or better)

3.3 Preparation of Calibrators, Controls and Standard Stock Solutions:

(Balance used for the preparation of solutions must be checked by calibrated weights on the day of solution preparation)

3.3.1 1% EtOH Standard Stock Solution

3.3.1.1 Accurately weigh 5.000 g of anhydrous EtOH, and quantitatively transfer to a 500 ml Class A volumetric flask with DIW, Add 0.1 g of Sodium Azide. Q.S. with DIW and mix well.

3.3.1.2. Label the standard with the appropriate identification and safety labels (Analyte, concentration, preparer, date prepared validation date and analyst's initials).

3.3.1.3 Document preparation on a "Calibration/Control Standard Preparation Form," and file in the Standards Preparation Logbook.

3.3.1.4 This solution should be stored refrigerated, and is stable for at least 1 year.

3.3.2 1% Acetone Standard Stock Solution

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3.3.2.1 Accurately weigh 5.000 g of Acetone, and quantitatively transfer to a 500 ml Class A volumetric flask with DIW. Add 0.1 g of Sodium Azide. Q.S. with DIW and mix well.

3.3.2.2 Label the standard and document preparation as above. This solution should be stored refrigerated, and is stable for at least 1 year.

3.3.3 1% MeOH Standard Stock Solution

3.3.3.1 Accurately weigh 5.000 g of Methanol, and quantitatively transfer to a 500 ml Class A volumetric flask with DIW. Add 0.1 g of Sodium Azide. Q.S. with DIW and mix well.

3.3.3.2 Label the standard and document preparation as above. This solution should be stored refrigerated, and is stable for at least 1 year.

3.3.4 Isopropanol Standard Stock Solution

3.3.4.1 Accurately weigh 5.00 g of IPA, and quantitatively transfer to a 500 ml Class A volumetric flask with DIW. Add 0.1g of Sodium Azide. Q.S. with DIW and mix well.

3.3.4.2 Label the standard and document preparation above. This solution should be stored refrigerated, and is stable for at least 1 year.

3.3.5 Cal. (0.10 g/100ml EtOH.)

3.3.5.1 Using a class A volumetric pipette, add 10 ml of the 1% EtOH standard stock solution to a 100 ml class A volumetric flask. Q.S. with DIW and mix well by inversion.

3.3.5.2 Label the standard and document preparation as above. This solution should be stored refrigerated, and is stable for at least 1 year.

3.3.6 0.10 g/100 ml EtOH In-House Control

3.3.6.1 Weigh 2.00 g of ethanol, quantitatively transfer to a two liter class A volumetric flask with DIW. Q.S. with DIW and mix well.

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3.3.6.2 Decant the contents of the flask into a large amber glass bottle, label appropriately. This solution should be stored refrigerated, and is stable for at least 1 year.

3.3.7 Internal Standard Stock (nPA) Solution

3.3.7.1 Transfer ~ 7 ml of NPA to a 100 ml volumetric flask. Q.S. with DIW and mix well.

3.3.7.2 Label the standard and document preparation as above. This solution may be stored at room temperature, and is stable for at least one year.

3.3.8 Internal Standard Working Diluent Solution

3.3.8.1 Dilute 4.0 ml of NPA Stock Solution to a final volume of 2000 ml with DIW, mix thoroughly. Decant the contents of the 2000 ml flask into a large, amber bottle.

3.3.8.2 Label the standard and document preparation as above. This solution may be stored at room temperature, and is stable for at least one year.

3.3.9 Certified Reference Standard Solution

3.3.9.1 Store in air tight container after opening.

Note: (Cerilliant or equivalent) Certified Reference Material solutions expire 1 year from date of manufacture. These solutions may only be used for 6 months after date of first use. Expiration date of the Calibrator check solution in current use, along with the lot number, target value, and acceptable ranges are detailedon page 2 of the Volatile Batch Summary Review Form.

3.3.10 Volatile Calibrator Solution.

3.3.11 Using a class A volumetric pipette, add 10 ml each of the 1% MeOH, IPA and Acetone standard stock solutions to a 100 ml class A volumetric flask. Q.S. with DIW and mix well by inversion.

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3.3.12 Label the standard and document preparation as above. This solution may be stored at room temperature, and is stable for at least one year.

3.3.13 Volatile Control Solution.

3.3.13.1.1.1 Using a class A volumetric pipette, add 10 ml each of the 1% MeOH, IPA and Acetone standard stock solutions to a 100 ml class A volumetric flask. Q.S. with DIW and mix well by inversion.

3.3.13.1.2 Label the standard and document preparation as above. This solution may be stored at room temperature, and is stable for at least one year.

3.4 Validation of Reagents:

Reagents and controls are validated by use in analytical batches. Acceptable performance is documented by CRM and In House Control performance Validated reagents are Marked with a green dot, detailing the specific procedure for which the reagent was validated, and the batch on which that process was documented. Newly prepared reagents may be evaluated for validity on an analytical batch, prior to any consideration of sample results. Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as validation of reagents. Reagents so validated are marked with a green sticker as noted above. Preparation of reagents, and their validation is documented in the Toxicology Section Reagent Preparation Validation Logbook, Maintained in the Toxicology laboratory.

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4.0 PROCEDURE; Sample Analysis

4.1 Each sample is diluted 1 ml of sample to 100 mls with DI H2O using a class A volumetric flask and pipette.

Note 1: All biological specimens must be handled with care, and considered as Bio-Hazardous; "Universal Precautions" for handling biological specimens must be observed at all times, as outlined in the Laboratory Safety Manual.

Note 2: Prior to the withdrawal of aliquots, Guth Calibration Check Solution, Calibrator, Control and Internal Standard solutions should be removed from the refrigerator, and allowed to stand at room temperature for at least 30 min.

Note 3: Departure from procedures as specified in this SOP is not anticipated. Should an issue arise that may require such a departure, the issue must be raised with Quality Manager

and/or the Director. If the proposed change will not present a change of a magnitude that would require validation, the change may be approved, and the Director will modify and re-issue the SOP accordingly.

Any such procedural changes would be subject to the review process afforded by the quality control measures of the analytical scheme described herein. Hence, any modification or change that produces an unexpected deleterious effect on the analytical procedure would be expected to trigger analysis or batch failure in the QC review stages.

4.1 Pipetter/Diluter - Preparation/Priming:

4.1.1 Turn on the Pipetter/Diluter.

4.1.2 Place the inlet tubing in the NPA diluent solution bottle, making sure the end of the tubing is well below the level of the liquid.

4.1.2.1 Remove the dispenser probe from its holder and place the tubing from the probe in an empty waste container/flask.

4.1.3 Press the prime switch; Liquid will be dispensed from the probe at this time, as the system primes the lines. Cycle until bubbles have disappeared from lines.

4.2 Set-Up for Sample Preparation

4.2.1 Scroll to [Run an Existing Method], then press [Select].
4.2.2 Highlight [ETHANOL], and then press [Select].
4.2.3 The instrument will ask for confirmation of syringe sizes; Press [Confirm].
4.2.4 The instrument will ask for initialization; Press [Confirm].
4.2.5 Headspace Vial Labeling;

Note: All controls and samples are run in duplicate.

4.2.5.1 Label vials for calibrators and controls as follows:
System conditioner (any level calibrator or control),
Calibrator (0.10%) 12.7%: 1
Negative control: 1
CRM (certified reference material): 2
In House Control 0.1% Ethanol : 2
0.3% Calibrator/Control Ethanol: 2
0.02% Calibrator/Control Ethanol: 2
Volatile Control: 2
Samples: 2 (up to 15 cases)

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In House Control 0.1% Ethanol: 2

Note: Each analyst contributing samples to a batch must prepare an additional set of controls

4.2.5.2 For each case, label 2 vials for each Submission.

Note: For every 15 cases, another set of ethanol controls (2) must be prepared.

4.3 Preparation of Calibrators, Guth and Controls

4.3.1 Automatic Pipetting of Samples, Calibrators and Controls

4.3.1.1 Place the probe in the solution to be sampled.
4.3.1.2 Press the Pipette Activation Button (PAB) once to aliquot 1 ml of diluent/internal standard solution.
4.3.1.3 Press the PAB to draw up 200 µl of the sample, calibrator or control.
4.3.1.4 Place the probe inside the appropriately labeled autosampler vial.
4.3.1.5 Press the PAB once to dispense the aliquots into the autosampler vial.
4.3.1.6 Press the PAB once more to dispense the rinse between sampling into a waste container.
4.3.1.7 Place a headspace cap on the vial and crimp - seal.
4.3.1.8 Proceed with the next sample, calibrator or control, starting with step number 1.
4.3.1.9 Repeat for all calibrators, controls and samples.
4.3.1.10 Place the vials sequentially into the sampling carriage Headspace Autosampler in the sequence detailed below:

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Sample #	Contents
1	System Conditioner (any calibrator or control)
2	Calibrator 1 (0.10 g/%)
3	Blank DI Water 1 carry over check
4	CRM Rep 1
5	CRM Rep 2
6	0.1 g% EtOH In-house Control Set 1 Rep 1
7	0.1 g% EtOH In-house Control Set 1 Rep 2
8	0.3 g% EtOH In-house Control Set 1 Rep 1
9	0.3 g% EtOH In-house Control Set 1 Rep 2
10 - X	Samples; (each in duplicate)
X+1	0.02 g% EtOH In-house Control Set 1 Rep 1 as needed
X+2	0.02 g% EtOH In-house Control Set 1 Rep 2 as needed

4.4 Clean-Up of Automatic Pipetter/Diluter

4.4.1 Press [Escape] (ESC) from the current method.

4.4.2 Press [ESC] again to get to the main menu.

4.4.3 Press the prime switch; Liquid will be dispensed from the probe at this time.

4.4.4 Place the probe tubing into a waste reservoir and run thru 3 cycles.

4.4.5 Return prime switch to original postion.

4.4.6 Turn the instrument off and place the probe in the probe holder on the side of the pipetter/diluter.

5.0 INSTRUMENTAL ANALYSIS

5.1 Gas Chromatograph Setup:

5.1.1 Turn the air and hydrogen valves counterclockwise for the FID's

5.1.2 In the software program which controls the gas chromatograph, enter the sequence order for the method.

5.1.3 Save the sequence using the date in the file name,

5.2 Autosampler Configuration:

5.2.1 Place the vials in the proper order on the carousel for the autosampler and from the touch pad enter the proper to and from vial numbers and also the proper method . Select run.

5.3 Calibration/Quantitation

5.3.1 Data reduction of the headspace - GC run is performed by the chemstation

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.Quantitative calculations are based on a comparison of the analyte to I.S. peak area response ratio for the controls and samples, to a linear calibration curve (y = mx+b) established from similar ratios from the known calibrator solutions. The software method is programmed to produce a linear one-point calibration curve, including the origin as a point. The value of the 0.1% calibrator is 12.7%. This value is based on the specific gravity of Ethanol which is 0.79. Using the equation:

$$0.1\% \div 0.79 \times 100 = 12.658\%$$

Each headspace run is independently calibrated. Corrective action, including instrument troubleshooting and/or preparation of new calibration solutions should be undertaken prior to repeat analysis of samples.

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6.0 RUN EVALUATION

6.1 Run Acceptance Criteria

6.1.1. Run Completion: The batch must have been injected without unexplained interruption, and no significant instrumental errors can have occurred.

6.1.2 Blank and Carryover Check: No significant integrated peaks (other than the internal standard) should be noted in the blank sample. Target analytes present in the Carryover check must be at concentrations below the maximum allowable (0.005 g%). Both checks are documented on p. 2 of the Batch Summary Form.

6.1.3 Calibration Check (Accuracy & Precision): EtOH results for both replicates of the Certified calibration Check solution must be within 5% of the target value, as detailed on the Volatile Batch QC Review Form (page 2). Replicates must agree within 5%, and are similarly documented.

6.1.4 Calibration Linearity: The correlation coefficient of the calibration curve must be greater than or equal to 0.99, and is documented on page 2 of the Batch Summary Form.

6.1.5 0.1 g% EtOH Control (Accuracy & Precision): EtOH results for both replicates (per set, if applicable) of the In-House 0.1 g% control solution must be within 10% of the validation value, as detailed on the "Volatile Batch QC Review Form (page 2).

6.1.6 Precision: All Calibrator and Control duplicate analysis results must agree within 5%.

6.1.7 If the run is rejected. Corrective action, including instrument troubleshooting, proper documentation and/or preparation of new calibration solutions should be undertaken prior to repeat analysis of samples.

6.2 Sample Acceptance Criteria

6.2.1 Chromatography must be acceptable for all reported analytes in sample chromatograms, on the quantitation column (Baseline resolution on 1 side, at least 90% resolved from any adjacent peaks.)

6.2.2 Relative retention times for any identified analyte in control or samples must be within 0.1 min of the corresponding retention time for the analyte in the calibration solution. (This is automatic, as a function of the instrument qualitative ID window.)

6.2.3 Any reportable analyte must have been identified by the software by retention time on both columns, and the quantitative values must agree within 20%, as detailed on page 1 of the Batch Summary Form.

6.2.4 Duplicate quantitative results for any reportable analyte must be within 5% of each other, as detailed on page 1 of the Batch Summary Form (with the exception of low level autopsy cases, per analyst discretion).

6.2.5 DUI Samples with Ethanol concentrations > 0.4 g% must be re-analyzed with dilution. Post-mortem samples with such concentrations may be accepted at the discretion of the Director.

6.3 Analytical Review; A review of the batch is performed by an analyst other than the batch operator, checking the run and sample acceptance criteria as noted above. Reviews are documented on the batch summary forms.

6.4 Reporting of Results; Quantitative Results from analyses passing the sample acceptance criteria described above may be reported as follows: Only values > 0.01 g/% are reported, as the three-place average for the two cases replicate analyses, truncated to two decimal digits.

Procedural Uncertainty is reported with all quantitative results, and is calculated and tabulated annually for each analytical method, (See SOP TX-19 section 6.3).

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7.0 Quality Assurance/Quality Control

7.1 Run and Sample Evaluation, Operator and Analytical Review; Each run is evaluated according to both the Sample and Run Acceptance Criteria specified above. Run acceptance is documented by both the operator, and a second, independent reviewer. Similarly, each sample is evaluated for acceptability according to the Sample acceptance Criteria specified above.

7.2 Report Administration and Final Review:

7.2.1 Prior to any result being issued, each case file is subjected to an technical and administrative review (in accordance with ASCLD guidelines) to ensure that appropriate documentation is present in the file, that results were generated from

appropriately reviewed and accepted analytical batches, and that results have been correctly transcribed from batch summary sheets and final reports

7.2.2 Prior to the final sign-off by the Director, each case is subjected to a final review. This review is designed to ensure that appropriate testing has been done, and that the results in the case file have been generated in a forensically defensible manner.

7.3 Sensitivity: Sensitivity of the method has been documented by performance on the external PT program (CAP, NHTSA, ODOH) between the ranges of 0.02 g/% - 0.30 g/%.

7.4 Specificity: Specificity of the method has been documented by performance on the external PT program and the volatile controls, containing potentially interfering substances. All samples are analyzed on two separate columns, of differing polarity. No substances interfering with any target analyte at the appropriate retention time on both columns have been observed to date.

7.5 Accuracy: Accuracy of the method is checked on each batch by the analysis of Certified Reference material, in addition to the control materials.

7.6 Precision: Precision of the method is evaluated for each result and documented on the Batch summary spreadsheet. Precision of reported values in ensured by the requirement that any reportable result agree with its replicate analysis to within 5%.

7.7 Linearity: Linearity of the Calibration Curve for the range of 0.0 - 0.3 g/100 ml is evaluated on each instrument run, and is required to be > 0.99 (r**2).

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7.8 Performance Testing Samples: This laboratory participates in three regular, outside Proficiency Testing Programs;

College of American Pathologists- Alcohol Proficiency

7.9 Verification of Vial Sequence: The vial sequence is checked both prior to and after the injection of samples when the auto injector is used. The check after the injection of samples is documented on the run summary sheet.

Results from these proficiency-testing programs are reviewed upon receipt. Any significant problem with a PT result is addressed by the Chief Toxicologist/Laboratory Director. Any such problems and corrective/remedial action are documented in the PT notebook for the department.

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7.10 A Batch summary sheet will be produced with each batch. Data on each batch should include fields such as: Sample name, Batch ID (Date of Batch), analysts who generated data, matrix, analyte found (and concentration if applicable), controls run with the batch and results obtained. If controls do not meet the criteria, the batch can be rejected as a whole or by a case by case basis. The supervisor is notified and proper action is taken to correct any problem. Batches and/or cases shall be repeated as needed.

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8.0 References:

Reed, D. and Cravey, R.H. (1971) A Quantitative Gas Chromatographic Method for Alcohol Determination. J.Forensic Sci. Soc. 11:263

Karnitis, L. and Porter L.J. (1971) A Gas Chromatographic Method for Ethanol in Vapors of Biological Fluids. J. Forensic Sci. 16:318-322

Jones, A.W. and Schubereth, J. (1989) Computer-aided Headspace Gas Chromatography Applied to Blood Alcohol Analysis: Importance of Online Process Control. J. Forensic Sci. 34:1116-1127

A. Sources of error:

Uncertainty in alcohol determination is defined as 2X the standard deviation of the CRM, + 2x the standard deviation of the average of 20 laboratory determinations of that CRM reference value, + the procedural bias (defined as the difference of means between the CRM reference value, and the Laboratory determined value), expressed as a percentage.

Appendix I:

GC/Headspace temperature program specifications

Parameter			
Initial temp	50° C		
Hold Time	3.00min		
Ramps Rate/final temp/final time	rate	temp	time
	00.0	50	5.0
Post temp	50°c		
Post time	0.00		
Run Time	5.0 min		
Front inlet			
Mode	split		
Initial temp	180°c		
Pressure	10.0 psig		
Total flow			
Gas type	Helium		
Injection time	0.03 min		
Detector temp	300°c		
Plenum temp	100°c		

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Example of controlled Liquid Volatile batch document TX Liquid-1. Batch documents can vary based on nature of batch.

Forensic Toxicology Laboratory

Liquid Ethanol Batch Summary

Batch ID:3-18-11

Analyst(s): mwa

Vial	Matrix	Ethanol Conc. % Col. A Rep 1	Col. A Rep 2	Col. B Rep 1	Col. A Avg.	Col. A % Diff.	B:A Delta %	Other Volatiles: (Note units)
0.10/12.7% Conditioner	Water	11.755		11.760				
0.10/12.7% Cal	Water	12.700		12.700				
Blank	Water	0.000		0.000				
0.30/38.1% Control	Water	40.108	39.964	40.204	40.0360	0.36	-0.42	
TX-11-368 diet coke	liquid	0.000	0.000	0.000	0.0000	#DIV/0!	#DIV/0!	
TX-11-315-1 unopened coke	liquid	0.000	0.000	0.000	0.0000	#DIV/0!	#DIV/0!	
TX-11-315-1 1/4 full coke	liquid	0.000	0.000	0.000	0.0000	#DIV/0!	#DIV/0!	
TX-11-315-1 diet Dr. Pepper	liquid	0.000	0.000	0.000	0.0000	#DIV/0!	#DIV/0!	
TX-11-315-2 yellow	liquid	9.767	9.801	9.707	9.7840	-0.35	0.79	
CRM 0.08/10.16 %	Water	10.020	10.072	10.057	10.0460	-0.52	-0.11	
0.10/12.7% CTL	Water	12.617	12.631	12.527	12.6240	-0.11	0.77	
0.02/2.54% CTL	Water	2.303	2.300	2.298	2.3015	0.13	0.15	
			Acceptable Limits:			+/- 5%	+/- 20%	

Vial position verified prior to sample removal: _____

Analyst Run Review: _____ Date: _____

Comments: _____

Analytical Review: Run Acceptable?: _____ Date: _____

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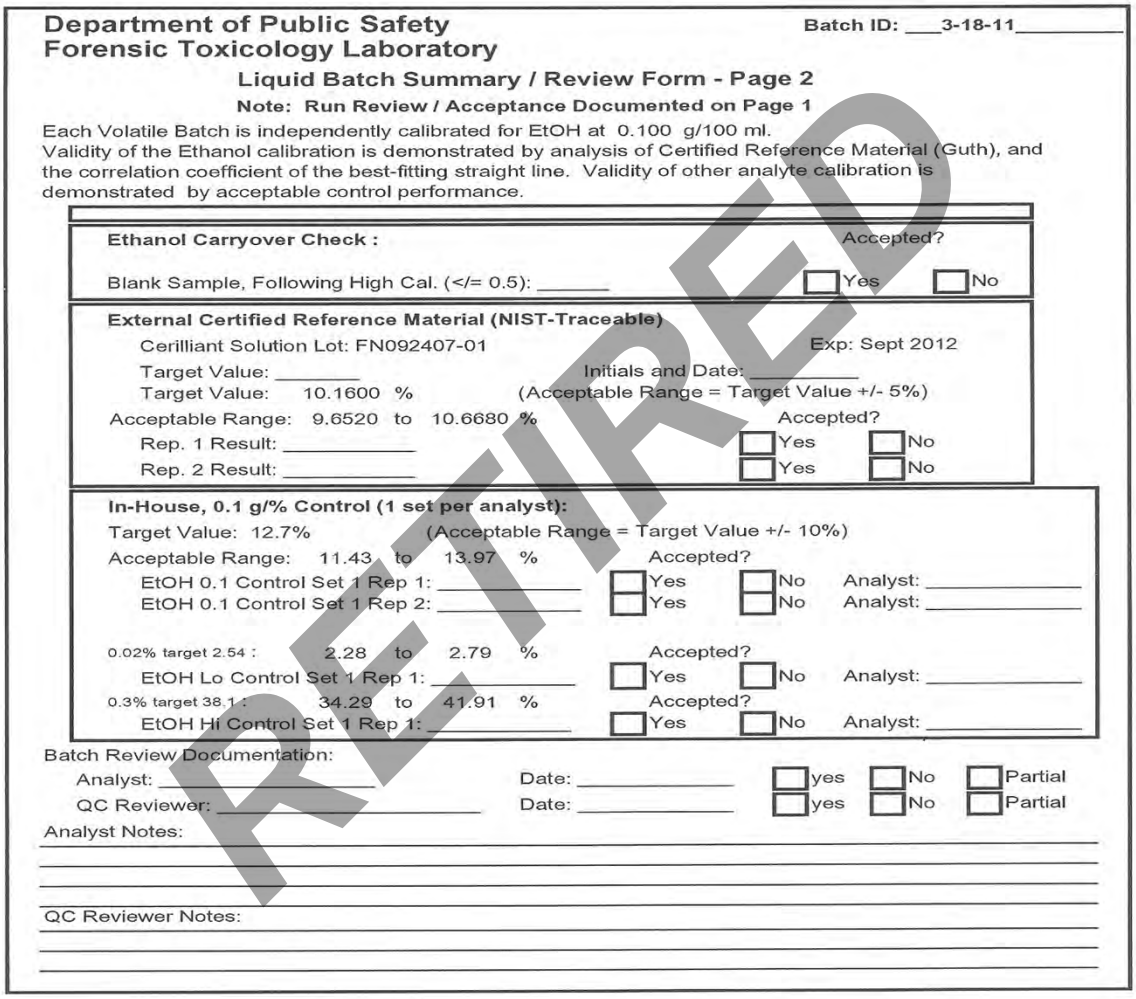
Example of controlled Liquid Volatile batch document TX Liquid-2. Batch documents can vary based on nature of batch.



Department of Public Safety
Forensic Toxicology Laboratory

Batch ID: ___3-18-11_____

Liquid Batch Summary / Review Form - Page 2

Note: Run Review / Acceptance Documented on Page 1

Each Volatile Batch is independently calibrated for EtOH at 0.100 g/100 ml.
Validity of the Ethanol calibration is demonstrated by analysis of Certified Reference Material (Guth), and the correlation coefficient of the best-fitting straight line. Validity of other analyte calibration is demonstrated by acceptable control performance.

Ethanol Carryover Check : Accepted?

Blank Sample, Following High Cal. (</= 0.5): _____ ☐ Yes ☐ No

External Certified Reference Material (NIST-Traceable)

Cerilliant Solution Lot: FN092407-01 Exp: Sept 2012

Target Value: _____ Initials and Date: _____

Target Value: 10.1600 % (Acceptable Range = Target Value +/- 5%)

Acceptable Range: 9.6520 to 10.6680 % Accepted?

Rep. 1 Result: _____ ☐ Yes ☐ No

Rep. 2 Result: _____ ☐ Yes ☐ No

In-House, 0.1 g/% Control (1 set per analyst):

Target Value: 12.7% (Acceptable Range = Target Value +/- 10%)

Acceptable Range: 11.43 to 13.97 % Accepted?

EtOH 0.1 Control Set 1 Rep 1: _____ ☐ Yes ☐ No Analyst: _____

EtOH 0.1 Control Set 1 Rep 2: _____ ☐ Yes ☐ No Analyst: _____

0.02% target 2.54 : 2.28 to 2.79 % Accepted?

EtOH Lo Control Set 1 Rep 1: _____ ☐ Yes ☐ No Analyst: _____

0.3% target 38.1 : 34.29 to 41.91 % Accepted?

EtOH Hi Control Set 1 Rep 1: _____ ☐ Yes ☐ No Analyst: _____

Batch Review Documentation:

Analyst: _____ Date: _____ ☐ yes ☐ No ☐ Partial

QC Reviewer: _____ Date: _____ ☐ yes ☐ No ☐ Partial

Analyst Notes:

QC Reviewer Notes:

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1. Introduction

Gamma-hydroxybutyrate, or gamma-hydroxybutyric acid, (GHB) is a central nervous system depressant agent acting as a GABA-receptor agonist. It is endogenous to humans, beef, and some vegetation. In humans it can be produced from succinic semialdehyde by the action of an NADPH-dependent aldo-keto reductase. The compound can be used as a psychotropic agent and has been referred to as a drug-facilitated sexual assault (DFSA) drug. It is categorized as an illegal drug, but can be used to treat conditions such as insomnia, depression, and alcoholism. Endogenous levels of GHB are generally below 1 mg/L, but higher levels may result as a consequence of oxidation of endogenous GABA (gamma-aminobutyric acid). When exogenous ingestion is suspected, forensic toxicological samples (e.g., blood, urine) may contain GHB levels well above 10 mg/L.

2. Scope

This method is primarily intended to be used for the identification and quantitation of GHB within appropriate specimens (e.g., blood, urine). It involves using solid phase extraction (SPE) columns. Derivatization is applied using a siloxyl-containing agent, N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) to allow GHB to be separated and detected by gas chromatography/mass spectrometry (GC/MS).

3. Principle

Blood and urine samples are the two most common type of toxicological specimens for requests involving the detection and quantitation of GHB. Other liquids (e.g., adulterated beverages) may be appropriate samples for GHB testing by this method, but dilution should be considered. Following the addition of a deuterated analog as an internal standard, samples are extracted with acetone coupled with a SPE column. If present within a sample, GHB is collected and taken to dryness, followed by derivatization with BSTFA. The sample is analyzed by GC/MS in the electron-impact mode (EI).

4. Specimens

This procedure typically uses 200 µL of blood, urine. Other liquids may be analyzed, but dilution and variation of the sample volume may be necessary.

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5. Equipment/Materials/Reagents

Note: Reagents are ACS grade or better unless otherwise specified

A. GC/MS and associated data station/computer (HP6890/5973 or equivalent)
B. Glass test tubes 16 x100mm (or equivalent)
C. 1.5 mL polypropylene flat top microcentrifuge tubes (or equivalent)
D. Pipette (5-20 µL) with disposable tips (or equivalent)
E. Pipette (20-200 µL) with disposable tips (or equivalent)
F. Pipette (0.1-1.0 mL) with disposable tips (or equivalent)
G. Vortex mixer
H. Micro-vial centrifuge
I. Evaporator with nitrogen (or equivalent)
J. SPE vacuum manifold or positive pressure manifold (or equivalent)
K. Clean Screen® GHB extraction column: (UCT, Inc., Part#: CSGHB203 or equivalent)
L. Heating block
M. Acetone
N. Methanol
O. Ammonium hydroxide, concentrated
P. Ethyl acetate
Q. N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% TMCS (trimethylchlorosilane)
R. DI Water (Distilled or Deionized)
S. Disodium phosphate (Na_2HPO_4)
T. Monosodium phosphate (NaH_2PO_4)
U. pH paper
V. GHB-D_6 sodium salt solution (Cerilliant G-006, 1.0 mg/mL as salt): (or equivalent)
W. GHB sodium salt solution (Cerilliant G-001, 1.0 mg/mL as salt): (or equivalent)
X. Utak GHB dried whole blood control (or equivalent)
Y. Utak GHB dried urine control (or equivalent)
Z. Control blood (Blood Bank or equivalent)
AA. Control Urine (purchased or obtained in-house)

6. Reagent Preparation

Reagent preparation and validation is documented in the Toxicology "Reagent Preparation/Validation Logbook" maintained in the Toxicology Unit.

<u>Phosphate Buffer (~0.1M, pH ~ 6.0)</u>:

1. Dissolve 1.70g Na_2HPO_4 and 12.14g NaH_2PO_4 in 800 mL D.I. Water;
2. Dilute to 1000mL using water and mix
3. Using pH paper ensure that the pH is ~6.0

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GHB Elution Solvent - (1%(v/v) NH_4OH in MeOH):

1. Combine 1 mL of concentrated ammonium hydroxide with 99 mL of methanol using a volumetric glassware (e.g., graduated cylinder)
2. Mix well

7. Standards & Controls

Preparation of all control solutions is documented in the "Calibrator and Control Preparation Log" (can be maintained in the Toxicology Unit's - wet laboratory area).

Reference Standards:

1. Na-GHB-D_6 Stock Solution (Cerilliant G-006, 1.0 mg/mL as salt) :
   MW (Na-GHB-D_6) = 132.12 MW (GHB-D_6) = 110.13
2. Na-GHB Stock Solution (Cerilliant G-001, 1.0 mg/mL as salt):
   MW (Na-GHB) = 126.09 MW (GHB) = 104.10

Internal Control Solutions:

1. GHB-D_6 Working Internal Solution (100 µg/mL)
   a. Dilute 240µL of Na-GHB-D_6 Stock Solution (1.0 mg/mL) to 2 mL with water*
   b. If stored refrigerated it is stable for at least 1 year
2. GHB Working Internal Solution (100 µg/mL):
   a. Dilute 242µL of Na-GHB Stock Solution (1.0 mg/mL) to 2 mL with water*
   b. If stored refrigerated it is stable for at least 1 year

*Note: MeOH can be substituted for H_2O and Internal Control Solutions can then be stored in a freezer.

External Control Solutions:

1. Blood Control (10 µg/mL GHB)
   a. Reconstitute Utak dried whole blood control with 5 mL water using a volumetric pipette
   b. Cap and let sit 10-15 minutes
   c. Gently swirl 3-4 minutes or mix on rotator until all particles are dissolved into a homogeneous mixture. Swirl gently each time before an aliquot is removed to ensure the solution is homogeneous.

   Note: An alternate to using the Utak dried whole blood product is to use GHB-free whole blood and add an appropriate amount of known GHB to make a 10 µg/mL GHB control blood solution.
2. Urine Control (20 µg/mL GHB and 100 µg/mL GHB)
   a. Reconstitute Utak dried urine controls (20 µg/mL and 100 µg/mL) with 5 mL water using a volumetric pipette
   b. Cap and let sit 10-15 minutes

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c. Gently swirl 3-4 minutes or mix on rotator until all particles are dissolved into a homogeneous mixture. Swirl gently each time before an aliquot is removed to ensure the solution is homogeneous.

Note: An alternate to using the Utak dried urine product is to use GHB-free urine and add an appropriate amount of known GHB to make 20 µg/mL and 100 µg/mL GHB urine control solutions.

3. Negative Control Blood: GHB-free human blood, (whole blood or reconstituted from dried blood)
4. Negative Control Urine: GHB-free human urine, (intact urine or reconstituted from dried urine)

Note: Controls should be prepared from reference materials that have a different source (e.g., lot number or supplier) than those used to prepare calibrator solutions.

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8. Calibration

Preparation of all control solutions is documented in the "Calibrator and Control Preparation Log" (can be maintained in the Toxicology Unit's - wet laboratory area).

Table 1 shows the concentrations and volumes used for the preparation of calibration solutions in water or other matrix for GHB analysis.

Table 1: Sample calibrator preparation

Calibrator level (µg/mL GHB)	Total Volume	Sample Added	Volume of GHB Working Control Solution (100µg/mL)
0	200 µL	200 µL Urine	0 µL
0	200 µL	200 µL Blood	0 µL
2	200 µL	196 µL Water	4 µL
5	200 µL	190 µL Water	10 µL
10	200 µL	180 µL Water	20 µL
25	200 µL	150 µL Water	50 µL
50	200 µL	100 µL Water	100 µL
100	200 µL	0 µL Water	200 µL

Table 2 shows the concentrations and volumes for controls

Table 2: Sample calibrator preparation

Control level (µg/mL)	Volume	QC used
10	200 µL	Utak GHB 10 whole blood
20	200 µL	Utak GHB 20 urine
100	200 µL	Utak GHB 100 urine

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9. Procedure

A. Pipet 200 µL of sample into properly labeled 1.5 mL plastic centrifuge tubes

B. Add 25 µL of GHB-D_6 Working Internal Solution (100 µg/mL) to all tubes

C. Add 1.0 mL of acetone to each tube; cap, and vortex-mix each tube for ~15 seconds

D. Centrifuge all tubes for ~5 minutes

E. Transfer using a pipette (with disposable tip) 800 µL of the top acetone layer into a properly labeled test tube

F. Evaporate the organic phase to dryness using a stream of nitrogen gas at ~50 °C

G. Reconstitute the dried extract with 200 µL of 0.1M phosphate buffer (pH ~ 6.0)

H. Vortex-mix for ~15 seconds

I. Condition the Clean Screen® GHB extraction column**: (CSGHB203 or equivalent)
   1. 1 x 3 mL Methanol
   2. 1 x 3 mL D.I. water
   3. 1 x 1 mL 0.1 M Phosphate buffer (pH ~ 6.0)
   4. Aspirate at 3 inches of Hg or less to prevent sorbent drying

   **Note: If smaller columns are used, adjust volumes accordingly

J. Apply the sample by pipetting (Eppendorf or Rainin) all reconstituted samples to properly labeled SPE columns

K. Elute GHB:
   1. Place clean test tubes into the collecting manifold
   2. Add 1 mL of CH_3OH/NH_4OH (100:1) to each SPE column
   3. Collect the extract

L. Evaporate the extract to dryness at ~60 °C using a stream of dry nitrogen

M. Reconstitution and Derivatization:
   1. Add 100 µL of ethyl acetate

2. Vortex-mix and transfer into GC vials that contain inserts
3. Add 25 µL of BSTFA with 1% TMCS to GC vials, cap, and mix
4. Heat all samples, calibrators, and controls for 10 minutes at ~70 °C in a dry-block incubator
5. Allow solutions within GC vials to cool
6. Analyze 1 µL of solutions using GC/MS

N. Quantitation can be done by using SIM mode mass spectral (MS) data:

Compound	Primary Ion	Secondary	Tertiary
GHB-D_6-di-TMS	239	240	241
GHB-di-TMS	233	234	235

Note: Batches for GHB should follow the format indicated below: Bracket casework with controls at the beginning and end of sequence. For long runs control(s) should be added in the middle of the batch. The following 'Batch Format' should be used:

Calibrators
Blank negative urine control
Blank negative blood control
Positive Control(s) beginning
Solvent blank
Samples
Positive Control(s)

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10. Instrument Conditions

The following parameters can be used for this analysis:

A. Gas Chromatograph Parameters

Oven parameters		Inlet and Carrier parameters		Column Parameters	
Initial Temperature	80 °C	Injection Mode	Pulsed splitless	Type	RTX-1
Hold time	1.00 min	Inlet temperature	250 °C	Length	30 m
Ramp rate 1	15 °C /min	Pressure	9.29 psi	Internal dia.	0.25 mm
Temperature 2	100 °C	Pulse pressure	35 psi	Film thickness	0.25 µm
Ramp rate 2	25 °C/min	Pulse time	0.50 min	Flow mode	Constant
Temperature 3	175°C	Purge flow	40.0 mL/min	Flow rate	1.0 mL/min
Ramp rate 3	40 °C/min	Purge time	0.50 min		
Final temperature	325 °C	Total flow	44.0 mL/min		
Post temperature	80 °C	Gas saver	On (helium)		
Total run time	9.08 min	Saver flow	20.0 mL/min		
		Saver time	0.80 min		
		Injection volume	1 µL		

B. Mass Spectrometer Parameter

Ionization mode	Electron Impact		Source temperature	230 °C
Acquisition mode	SIM		Transfer line temperature	280 °C
m/z group	233, 234, 235, 239, 240, 241		Solvent delay	3 min

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11. Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. In general, compound identification should be based on a comparison of the chromatography and mass spectrometry for the analyte peak of interest with data from a contemporaneously analyzed reference standard or extracted positive control. In most cases all of the following should be met in order to identify GHB within a specimen:

A. Chromatography:
The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution.

B. Retention time:
The retention time of the peak should be within ±5 % of the retention time (relative or absolute)

obtained from injection of a reference standard or extracted positive control of GHB.

C. Signal-to-Noise:
To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio should exceed 3. Further, the baseline signal for the peak from the sample of interest should be at least 10 fold greater than that for any observed peak at a similar retention time in a negative control that's being analyzed within the same batch.

D. Mass Spectrometry:
The mass spectrum of the analyte of interest should match that of the appropriate reference standard or an extracted positive control. The ion ratios for both qualitative ions must be within ±20 % of the corresponding ratio of calibrator sample.

E. Quantitations:
Calibration for each batch is done independently. No sample analysis should use a historical calibration curve. Linearity of the calibration curve is demonstrated within each batch, with R^2>0.95 as an acceptable criteria. Quantitation acceptability of the batch is also assessed by matrix specific controls which can be house made or/and commercially obtained. In house GHB controls (10 µg/mL, 25 µg/mL, 100 µg/mL) or commercially available external controls (e.g., UTAK blood 10 µg/mL GHB, urine 20µg/mL GHB to 100 µg/mL GHB) are typically used. Quantitation is calculated based on the primary ion's abundance.

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12. Limitations

A. Limit of Detection: 2 µg/mL for GHB
B. Limit of Quantitation: 5 µg/mL for GHB
C. Accuracy and precision: Accuracy is expressed as a mean (absolute value) percentage difference between mean quantitative value of specific controls and the target value. Precision is expressed as the coefficient of variation (CV) of that value.

13. Reporting of results

Urine samples with GHB present at a concentration above 10 µg/mL should be reported as positive for GHB. Blood samples with GHB present at a concentration above 5 µg/mL should be considered as positive for GHB and a quantitative value should be reported. The numbers are due to the fact that GHB is an endogenous compound.

14. References

"A solid phase method for gamma-hydroxybutyrate (GHB) in blood, urine, vitreous or tissue without conversion to gamma-butrylactone (GBL)", Developed by Joseph A. Crifasi, Saint Louis University health science center; Published on UCT website. https://www.unitedchem.com/sites/default/files/GHB-

GBL_in_Blood_2.pdf

"A solid phase extraction method for the determination of gamma-hydroxybutyrate (GHB) in urine without conversion to gamma-butrylactone (GBL)", Developed by Chester J. Kitchen, Thomas F. August and Michael Telepchak. US patent number 6,156,431.

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Rev. #	History
2	Formatting and verbiage changes throughout. Section 7 – replaced methanol with water. Changed ethylene glycol internal standard to GHB-D6. Added multipoint calibration and external controls (Utak or in-house controls). SPE columns were added to procedure. Changed title of SOP to 'Gamma-Hydroxybutyric Acid (GHB) Analysis.'

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1.0 PRINCIPLE

Cocaine is rapidly metabolized in the body by hydrolysis of the methyl ester group, to form benzoylecgonine (BE) with a cocaine half-life of ~90 min. Because of the rapid breakdown, BE is usually the major detectable product of cocaine ingestion in biological fluids. In many cases, the parent compound, cocaine, will not be detected at all. This procedure details the process by which the presence of cocaine, cocaethylene and/or BE may be confirmed in urine, blood or other aqueous fluids. Cocaine, cocaethylene or BE present in the sample is extracted onto a solid phase extraction column. Co-extracting materials are washed from the column, and extracted cocaine and/or BE is subsequently eluted. The eluent is evaporated, and any BE present is derivatized with BSTFA (N,O-bis trimethylsilyl trifluoroacetamide)/1% TMCS (Trimethylchlorosilane). The presence of any cocaine, cocaethylene and/or derivatized BE in the sample is qualitatively evaluated by 3-ion SIM, using retention time and 2 qualitative ions ratios as the basis for identification, in comparison to co-extracted calibrator and control samples.

Based on the DSS's MOU with the state OCME, Cocaethylene will be evaluated for post mortem samples and related ante mortem samples were the screening method indicates presence of the compound.

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2.0 SPECIMEN

A. Urine and Blood can be analyzed by this method. Other sample matrices may be also be appropriate to run, consult with Unit Lead for guidance.
B. JusticeTrax can be used to generate a worklist for specimens requiring confirmation for BE/Cocaine.
C. All evidence transfers, either between individuals or between an individual and storage location must be documented on the Chain of Custody for the case, either in the LIMS, or on hard-copy COC document maintained in the Case Jacket.
D. When not in the sampling or aliquot process samples will be maintained in locked storage within the Toxicology unit.
E. Samples must be maintained in such a manner so that they are protected from contamination or deleterious change. Depending on the nature of the sample, this may mean refrigeration or freezing when not in the analytical process.
F. When analysis of samples in the Toxicology unit is complete, they must be maintained "Under Proper Seal." Refer to SOP TX-19 for further guidance.
G. Samples are maintained in the Toxicology Section for a minimum of 8 weeks after case is completed, in the absence of notification of any legal action, or other reason to maintain the samples. Samples from fatalities and sexual assaults are

maintained indefinitely by the DSS. Cases with requests for retention are maintained by the laboratory based on the request. Upon request from the courts or submitting agency the evidence will be returned to the submitting agency.

3.0 EQUIPMENT:

A. GC/MS and associated data station/computer (HP6890/5973, Agilent Technologies 7890A, AT-7890B, AT-5975 or 9577 or equivalent)
B. General laboratory glassware and equipment
C. Solid phase extraction manifold and associated vacuum equipment
D. Analytical evaporator
E. UCT; ZSDAU020 “Clean Screen” extraction columns (or equivalent)

4.0 **REAGENTS**:

A. **Reagents available as stock items:**
   1. Methanol (MeOH; Baker HPLC grade or better)
   2. Deionized water (DIW; 2-megohm In-House supply)
   3. Hydrochloric Acid (HCl; Baker Reagent Grade, or equivalent)
   4. Isopropanol (IPA) (Baker HPLC grade or better)
   5. Methylene Chloride (Baker HPLC grade or better)
   6. Ammonium Hydroxide(NH_4OH; Baker Reagent Grade, or equivalent)
   7. Ethyl acetate (ETOAc; Baker HPLC grade or better)
   8. BSTFA with 1% TMCS
   9. Blank Blood (may be acquired from a hospital blood bank, or the American Red Cross or similar source)
   10. Sodium Phosphate Dibasic (Na2HPO4).
   11. Sodium Phosphate Monobasic (NaH2PO4).Drug free urine

B. **Reagents prepared in the Toxicology Laboratory:**
   1. Methylene chloride/isopropanol/ammonium hydroxide (39/10/1mL = 50mL total; or equivalent ratio; Prepared fresh each day of use)
   2. 0.1 M Phosphate Buffer pH 6
      a. Combine 48.6 g Na_2HPO_4 and 6.8 g NaH_2PO_4
      b. QS to 4000 ml using DWI0.1 M HCl (8.4 ml conc. HCl/1000 mL H2O)
   3. 0.1 M HCl
      a. To 400 mL DIW in a volumetric flask
      b. Add 8.4 mL concentratred HCl
      c. Mix and QS to 1000 ml

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d. Storage: room temperature in glass or plastic. Stability: 12 months
    a. Note: Reagent Preparation and Validation is documented in the Toxicology "Reagent Preparation/Validation Logbook" maintained in the Toxicology unit.

C. **Stock Calibrator and Control Solutions:** Comprised of benzoylecgonine, cocaethylene and cocaine (1 mg/mL; Cerilliant, Lipomed, Grace, or equivalent manufacturer)
    1. Working Standard Solution 10ug/mL:
        a. Into a 10 mL volumetric flask partially filled with methanol
        b. Pipette 100 ul of each reference standard (1mg/mL)
        c. QS with methanol and mix
        d. Store in freezer or refrigerator
        e. Stability for ~1 year, it must be revalidated after that year
    2. Diluted Working standard solution 1.0 ug/mL:
        a. Into a 10 mL volumetric flask partially filled with methanol
        b. Pipette 1 mL of working stock solution 10 ug/mL
        c. QS with methanol and mix
        d. Store in freezer or refrigerator
        e. Stability for ~1 year, it must be revalidated after that year

D. **Internal Stock Standards:** Comprised of benzoylecgonine-D3, and cocaine- D3 (1 mg/mL; Cerilliant, Lipomed, Grace, or equivalent manufacturer)
    1. Working Internal Standard: (10 ug/mL Cocaine D3, 25 ug/mL Benzoylecgonine D3)
        a. Into a 10 mL volumetric flask partially filled with methanol
        b. Pipette 100 ul of each Cocaine D3 (1mg/mL)
        c. Pipette 250 ul of Benzoylecgonine D3 (1 mg/mL)
        d. QS with methanol and mix
        e. Store in freezer or refrigerator
        f. Stability for ~1 year, it must be revalidated after that year

E. **Controls:**
    1. Urine Qualitative: the target concentration for the positive urine control is 250 ug/ml.
        a. Pipette 25 uL of the working standard solution (10 ug/mL) into 1.0 mL blank matrix
        b. Continue to follow sample preparation procedure
    2. Blood Quantitative:
        a. Refer to table in procedure section, continue to follow sample preparation procedure

F. **Quality Controls:**

Calibrators and controls must be independently prepared from a separate initial

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dilution or obtained from other sources. When available commercial reference controls will be purchased from an outside vendor. If commercial controls are not available, In-house controls should be prepared from a different provider. When only one supplier is available, a lot different from the calibrator should be used. At the least, when there is only one source, a separate preparation, different from the calibration standard (prepared by a 2nd analyst) should be used.

1. Urine Control DAU LC 2, Product # 50703, Utak Laboratories Valencia, CA 91355
    a. Remove cap from vial
    b. Reconstitute control material by adding exactly 10.0 mL of DIW, using a 10 mL volumetric pipette
    c. Replace cap and let sit 10 – 15 minutes
    d. Swirl gently 3 – 4 minutes to ensure homogeneous mixture
    e. Swirl gently each time an aliquot is removed to ensure a homogeneous mixture
    f. Handle and test the control material in the same manner as case specimens
    g. For quantitative assays, record the results of the controls obtained on the Excel Quality Control Chart on the DSS S drive, that describes statistical limits for the test method and the particular lot of the control material
    h. Store reconstituted control material refrigerated at 2-8°C, stable for 25 days after reconstitution

G. **Validation of Reagents**:

1. Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as validation of reagents. Validated reagents are marked with a green dot/sticker, detailing the specific procedure for which the reagent was validated, and the batch on which that process was documented. Newly prepared reagents may be evaluated for validity on an analytical batch, prior to any consideration of sample results Reagents so validated are marked with a green sticker as noted above. Preparation of reagents, and their validation is documented in the Toxicology unit Reagent Preparation Validation Logbook, maintained in the Toxicology laboratory.

5.0 PROCEDURE

A. **Sample Preparation:**

1. Blood:

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a. Pipette 1 mL of sample into an appropriately labeled 16 x 100 borosilicate culture tube.
    a. Analysts may dilute samples based on screening results. When less than 1 mL of sample is used the sample will be broughtup to 1 mL with phosphate buffer.
b. Add 20 uL of the Working Internal Standard
c. Add 3 mL phosphate buffer (pH 6.0)
d. Sonicate for 15 minutes
e. Centrifuge 10 minutes at ~ 5000 rmp

2. Urine (1mL)
    a. Pipette 1 ml of sample into an appropriately labeled 16 x 100 borosilicate culture tube
    b. Add 20 uL of the Working Internal Standard
    c. Add 3 mL phosphate buffer (pH 6.0)
    d. Mix
3. Urine (100 uL)
    a. Pipette 100 ul of sample into a 2nd appropriately labeled 16 x 100 borosilicate culture tube
    b. Add 20 uL of the Working Internal Standard
    c. Add 3 mL phosphate buffer (pH 6.0)
    d. Mix

4. Blood Quantitation: The chart below may be used as a guide, pipette 1 mL of sample, blank, calibrator and control to each appropriately labeled 16 x 100 screw top culture tube.

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Calibration Concentration ng/ mL	uL Working Standard (10 ug/mL)	uL diluted Working Standard (1.0 ug/mL)	Blank blood pipette uL
0 blank	0	0	1000
20	--	20	1000
50	--	50	1000
100	--	100	1000
200	20		1000
500	50		1000
1000	100		1000
QC Concentration	uL Stock Control (10 ug/mL)		

250	25		1000
	Utak Control		
Utak low	500		500

5. Label SPE tubes to correspond with each culture tube. Load into the manifold.

6. Condition the columns:
   a. 1 x 3 ml methanol; aspirate
   b. 1 x 3 ml DIW; aspirate
   c. 1 x 1 ml 0.1 M phosphate buffer pH 6; aspirate
   d. DO NOT LET COLUMN GO DRY!

7. Transfer contents of each tube to the appropriately labeled SPE tube, and allow gentle drop wise flow until the level reaches the top of the column bed.

8. Wash column:
   a. 1 x 2 ml DIW; drain (vacuum assist)
   b. 1 x 2 ml 0.1 M HCL; drain (vacuum assist)
   c. 1 x 3 ml methanol; drain (vacuum assist)
   d. Dry column (10 minutes at > 10 inches Hg)

9. Position appropriately labeled 13x100 test tubes under each SPE column, with clean thru tip inside collection test tube.

10. Elute analytes from SPE columns by adding 3 ml methylene chloride/ Isopropanol/ammonium hydroxide (39/10/1) to each SPE tube. Collect at 1-2 ml/minute.

    a. Note: Elution solvent must be prepared fresh the day of use.

11. Remove receiver tubes and evaporate to dryness at < 40° C using a gentle flow of Nitrogen.

12. Reconstitute residue with 150 ul of ethyl acetate and transfer to GC/MS vial with limited volume insert. Add 25 micro liters BSTFA (with 1% TMCS), cap and reserve for GC/MS analysis (no evaporation).

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13. Note: Cases requiring reanalysis due to high concentrations (i.e. initial analysis is greater than the high control), should be diluted with phosphate buffer as appropriate. The initial quantitative values may be used as a guide for the dilution process the dilution process shall be documented in the case jacket.

14. Note: Departure from procedures as specified in this SOP is not anticipated. Should an issue arise that may require such a departure, the issue must be reviewed and approved by with the Unit Lead and Deputy Director. Depending on the nature of the deviation DSS customers may need to be consulted, see GL-20 for guidance.

6.0 CHROMATOGRAPHY AND MASS SPECTROSCOPY

A. Instrument and Setup: See Appendix 1.
   1. GC/MS/Auto sampler: (Hewlett-Packard 6890/5973, or equivalent)
   2. Column: 30M RTX-5 (MS) or 30M RTX-1 (MS) (0.25 mm ID; 0.25 micron film) (or equivalent)
   3. Injector Temp. 250 °
   4. Detector Temp. 300 °
   5. Oven (initial): 150°, 20 ° /min to 280 °, (4.50 min hold).
   6. 10.0 minutes total run time
   7. 1 ul injection
   8. Method: cocbe.m

B. Injection sequence: Samples are injected on the GC/MS in the following general sequence:
   1. Urine Qualitative:
      a. Solvent blank
      b. Positive urine calibrator/standard 250 ng/mL (i.e. In-house control)
      c. Urine blank
      d. Utak control
      e. Case samples
      f. End batch with calibrator or control

   2. Blood Quantitation Multipoint Calibrators:
      a. Solvent blank
      b. Priming calibrator
      c. Blood blank
      d. Calibrator 1 (20 ng/mL)
      e. Calibrator 2 (50 ng/mL)

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f. Calibrator 3 (100 ng/mL)
g. Calibrator 4 (200 ng/mL)
h. Calibrator 5 (500 ng/mL)
i. Calibrator 6 (1000 ng/mL)
j. Blood blank
k. In-House and/or UTAK controls
l. Case samples
m. Run set of low and high controls midway through the batch when appropriate for longer batches.
n. End batch with a set of controls

3. File name – Include case number (including year) in each sample data file.

4. Verify the sequence, verify the vial positions in the auto sampler tray match the sequence.

C. Detection and Identification:

1. Determination of the presence of Cocaine, cocaethylene and/or BE in the sample extract are identified by appearance and ratio of the 3 ions characteristic of each species at the appropriate retention time, hence both retention time (a GC characteristic) and fragmentation pattern and ratio (MS characteristics) are used as the basis of qualitative identification.
2. For a positive identification of either cocaine, cocaethylene or BE, the retention time must be within 5% of the corresponding analyte in the calibrator injection, and the ion ratios for both qualitative ions must be within 20% of the corresponding ratio calibrator sample.
3. Qualitative identification of each analyte is independent.

D. Calibration:

1. Calibration is not performed on urine samples
2. Calibration for each batch is done independently through the use of method specific controls.

E. Quantitation:

1. Quantitation is accomplished by the comparison of the response ratio of the analyte in a specific sample, to the response ratios of the calibrators as expressed as a “standard curve”. The concentration of the analyte in the sample is then extrapolated, from the standard curve, and corrected for any dilution that may have been performed to facilitate the analysis of

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relatively concentrated blood samples.

2. For multi-point calibrations, the criteria for acceptability of the calibration and for individual calibrators is that when the values are read back against the final calibration curve they should generally be within +/- 20% of their value. A slightly wider acceptance value (e.g. +/-25% or 30%) is acceptable for calibrators that approach the LOQ of the assay.

3. Note: The available quantitative range of this procedure is (in terms of amount of drug injected on the instrument (thereby allowing for appropriate calculation of diluted samples) is defined for , and validated on each batch by the high and low control, and the acceptable performance of each.

4. The criteria for a valid calibration GC/MS linear regression "r_2" value for a 3 point curve is ≥ 0.98 using non-deuterated internal standards. A significant change in the slope of the calibration line, monitored between runs, may indicate that corrective action needs to be taken.

5. When more than 3 calibration points are used, one point may be removed if it failed to fall into the acceptable quantitative range. If two or more points need to be removed consult with the Unit Lead if any results can be accepted. On a case-by-case basis, results may be reported qualitatively or semi-quantitative.

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7.0 QUALITY CONTROL AND RUN EVALUATION

A. Verification of Vial Sequence; Vial sequence is checked both before and after the injection of samples. The check after the samples are injected is documented on the run summary sheet.

B. Chromatography - Evaluation and Acceptance Criteria: Chromatographic quality is evaluated for each peak. While general guidelines are that a peak should be symmetrical and be resolved to baseline on at least one side with 90% resolution on the other side, significant departures from those guidelines may be experienced with forensic samples. In many cases, chromatographic quality will warrant rejection of the chromatographic run, or specific samples, by the operator. Any such action should be clearly documented on the batch summary

sheet. Questionable chromatographic peak shape, resolution, or other problems with chromatography can be discussed with the Unit Lead or Deputy Director.

C. Evaluation of Potential Carryover: Carryover in the chromatographic quality is evaluated by injection of a blank sample immediately following the calibrator. Carryover of greater than 2% of cocaine, cocaethylene or BE requires batch rejection, and remedial action for the instrument (e.g. replacement of injector insert, new septum and perhaps column trim or even replacement. Demonstrated carryover, if less than 2%, will require operator consideration with regard to the potential for effects on specific samples, and may require re-extraction of specific samples. Carryover is further evaluated on a per sample basis by the requirement that quantitative results between replicates agree within 20%. Any significant carryover effect should cause the first of the two replicate samples to exceed the second by an amount in excess of the 20% differential. If not, any carryover may be considered inconsequential. In practice, when a question of potential carryover exists, the potentially affected sample replicates may be repeated at the end of the batch.

D. Evaluation of Controls: Positive and negative controls are evaluated to allow for procedural batch acceptability.

E. Qualitative Results: Controls must demonstrate the target analyte with acceptable chromatography and spectral characteristics.

F. Quantitative: Each batch must have at least 10% controls including a positive and negative. The controls can be re-injected in the middle and end of the batch to demonstrate the stability of the calibration. Acceptable results are the mean or target +/- 20% or +/- 2 standard deviations.

1. Levy-Jennings charts will be used monitor assay performance precision over time.

G. Internal standard abundance should be similar throughout the batch.

H. Linearity: Linearity of the calibration curve is demonstrable in each batch, for each analyte as a function of linear regression and quantitative results of control materials.

I. Sensitivity – Limits of Detection (LOD) and Quantitation (LOQ): For the purposes of this procedure, the LOD and LOQ are defined as equal to the lowest concentration of the lowest control. Qualitative Identification and/or Quantitative

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analysis below the concentration of the low control may be accepted on a case-by-case basis with the concurrence of the analyst, technical reviewer, Unit Lead and Deputy Director. LOD should be at least 3 times the signal to noise ratio, LOQ should be at least 10 times the signal to noise ratio.

J. Accuracy and Precision: Precision of the procedure is evaluated on a yearly basis, by repeat analyses of control or PT materials. Accuracy is expressed as a mean (absolute value) percentage difference between mean quantitative value of 10 reps of the specific control, and the target value. Precision is expressed as the CV of that value.

K. Specificity: Specificity is a function of both the resolution of target analyte during the analytical process, and the mass spectral fragmentation that analyte molecules undergo during the instrumental analysis. There has been no report of any material other than WAN and Basic drugs that elutes within 5% if the retention time of known standard materials, and produce the same fragmentation ions and ratios.

RETIRED

8.0 REPORTING OF RESULTS:

A. Once the batch is completed and the data is complied, the batch undergoes a batch review (refer to SOP TX-5 for guidance). Once the batch is accepted the results are entered into JusticeTrax.
B. Wherever possible, analytical results must be reviewed with reference to whatever case history or other information is available.
C. Procedural Uncertainty is reported with all quantitative results, and is calculated and tabulated annually for each analytical method, (See SOP TX-19 section 6.3).

9.0 SOURCES OF ERROR: The utilization of 3-ion SIM methodology, with reference to procedural, controls and calibrators yields qualitative drug identification with essentially no uncertainty. Urine drug analyses are reported only as qualitative results.

10.0 REFERENCES

A. Clarke's Isolation and Identification of Drugs. 2nd Edition
B. UCT United Chemical Technologies: Solid phase extraction methods

Appendix I:

GC/MS temperature program specifications. These are general parameters that may be adjusted based on instrument used or maintenance performed on any given instrument. Proper response of calibrators and controls work to demonstrate the appropriateness of the method.

Temperature program	COCBE.M		
Parameter			
Initial temp	150° C		
Initial Time	0.00min		
Ramps Rate/final temp/final time	rate	temp	time
	25.0	280	3.5
Post temp	280°c		
Post time	0.50		
Run Time	10.0 min		
Front inlet			
Mode	Pulsed split less		
Initial temp	250°c		
Pressure	13.7 psi		
Pulse pressure	30psi		
Pulse time	1.0 min		
Total flow	54.1mL/min		
Gas type	Helium		
Injection volume	1 microliter		
Post injection washes Solvent A / Solvent B	Solvent A -2 Solvent B - 2		
Tune file	ATTUNE		
Acquisition mode	SIM		
Solvent delay	3.8 minutes		
M/Zs	182, 185, 198, 201, 303, 306		
M/Zs	240, 243, 256, 259, 361, 364		
M/Zs	196, 317, 82		
Dwell	20		
MS Quad	150°c max 200		
MS Source	230°c max 250		

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Rev. #	History
3	Corrected typographical errors Loosened Intrument acquisition parameters and column choice restictions. Updated blood calibration levels and choice of In-house or commercial controls.

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1.0 PURPOSE:

Sympathomimetic Amines (Amphetamines, and its structural analogues; “SMA’s”) can cause profound behavioral changes and have high potential for abuse due to both chemical and psychological dependence. MDMA and MDA are metabolized to Methamphetamine and Amphetamine respectively. SMAs are water soluble and excreted in the urine. Blood and urine samples, screening positive for SMAs, are extracted using a solid phase extraction column, co-extracting materials are washed from the column and any SMAs that may be present are eluted using a methylene chloride/isopropanol/NH4OH mixture. After evaporation of the solvent, the extracted drug is derivatized with TFAA. Derivatized samples are analyzed by GC/MS.

The presence of SMAs is qualitatively evaluated by 3-ion SIM using retention time, and 2 qualitative ion ratios as the basis for identification, in comparison to co-extracted calibrator and control samples.

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2.0 SPECIMEN

A. JusticeTrax can be used to generate a worklist for specimens requiring confirmation for BE/Cocaine.

B. All evidence transfers, either between individuals or between an individual and a storage location must be documented on the Chain of Custody for the case, either in the LIMS, or on hard-copy COC document maintained in the Case Jacket.

C. When not in the sampling or aliquot process samples will be maintained in locked storage within the Toxicology unit.

D. Samples must be maintained in such a manner so that they are protected from contamination or deleterious change. Depending on the nature of the sample, this may mean refrigeration or freezing when not in the analytical process.

E. When analysis of samples in the Toxicology unit is complete, they must be maintained “Under Proper Seal.” Refer to SOP TX-19 for further guidance.

F. Samples are maintained in the Toxicology Section for a minimum of 8 weeks after case is completed, in the absence of notification of any legal action, or

other reason to maintain the samples. Samples from fatalities and sexual assaults are maintained indefinitely by the DSS. Cases with requests for retention are maintained by the laboratory based on the request. Upon request from the courts or submitting agency the evidence will be returned to the submitting agency.

3.0 EQUIPMENT:

A. GC/MS and associated data station/computer (HP6890/5973, Agilent Technologies 7890A, AT-7890B, AT-5975, 5977 or equivalent)
B. General laboratory glassware and equipment
C. Solid phase extraction manifold and associated vacuum equipment.
D. Analytical evaporator (Zymark Turbovap or equivalent)
E. UCT; ZSDAU020 “Clean Screen” extraction columns (or equivalent)

4.0 REAGENTS

A. **Reagents available as stock items** (Sigma or J.T. Baker Reagent Grade or equivalent unless otherwise specified):
    1. Methanol (Baker HPLC grade or equivalent)
    2. Ammonium Hydroxide (Baker reagent grade or equivalent)
    3. Ethyl acetate (Baker HPLC grade or equivalent)
    4. Methylene Chloride (Baker reagent grade or equivalent)
    5. Isopropanol (Baker reagent grade or equivalent)
    6. Triflouroacetic anhydride (TFAA) (Pierce or equivalent)
    7. Blank Blood (may be acquired from a hospital blood bank, or American Red Cross or other similar source)
    8. Drug free urine
    9. Deionized water (DIW; Millipore or equivalent In-House supply)
    10. Glacial Acetic acid (Baker reagent grade or equivalent)
    11. Sodium Phosphate Dibasic (Na2HPO4).
    12. Sodium Phosphate Monobasic (NaH2PO4).
    13. Hydrochloric acid (Baker reagent grade or equivalent)

B. **Reagents prepared in the Toxicology Laboratory**:
    1. <u>1 M Acetic acid:</u>
        a. Add 50 ml of DIW to a volumetric flask
        b. Add 28.6 ml of glacial acetic acid
        c. Mix, QS to 500 ml
    2. <u>0.1 M Acetic Acid</u>
        a. Add 50 ml 1 M acetic acid to a volumetric flask

RETIRED

b. Mix QS to 500 mL DIW (or equivalent ratio)

3. 0.1 M Phosphate Buffer pH 6
   a. Combine 48.6 g Na_2HPO_4 and 6.8 g NaH_2PO_4
   b. QS to 4000 ml using DWI

4. 1% Methanolic acid
   a. To 50 ml of DWI in a volumetric flask
   b. Add 1 ml HCl
   c. Mix, QS to 100 ml Methanol

5. Methylene chloride/Isopropanol/ammonium hydroxide (39/10/1)
   a. Prepare volume as appropriate
   b. Note: must be prepared fresh each day of use

C. Calibration Standards / In-house Control Standards/Internal Standard

Preparation of all calibrator and control solutions is documented in the 'Calibrator and Control Preparation' book, maintained in the Toxicology unit.

1. Stock Calibrator and Control Solutions:
   a. Comprised of Amphetamine, Methamphetamine, MDA, and MDMA. Reference material standards target analyte drug stocks are obtained from Sigma/Aldrich, Cerrilient, Lipomed, Grace or other equivalent manufacturers. In the form of 1 mL ampules of 1.0 mg/ml or 100 ug/mL.
   b. Composition of the calibration/control (analyte/concentration) will be dependent on the need of the casework.
2. Working Standard Solution 10 ug/mL
   a. Into a 10 mL volumetric flask partially filled with methanol
   b. Pipette 100 ul of each reference standard (1 mg/ml)
   c. QS with methanol, mix
   d. Store in freezer/refrigerator
   e. Stable for approximately 1 year, then it must be revalidated after that year

3. Diluted Working Standard Solution 1.0 ug / mL
   a. Into a 10 ml volumetric flask partially filled with methanol
   b. Pipette 1 mL of working stock solution 10 ug/mL
   c. QS with methanol, mix

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d. Store in freezer/refrigerator
e. Stable for approximately 1 year, then it must be revalidated after that year

4. Internal Stock Standards:
Comprised of Amphetamine D6 or related deuterated SMA; (Cerrilient, Lipomed, Grace or other equivalent)
a. Working Internal Standard SMA Deuterated SMA: 100 ng/mL
b. Into a 10 mL volumetric flask partially filled with methanol
c. Pipette 1 ml of each reference standard (1 mg/mL)
d. QS with methanol, mix
e. Store in freezer/refrigerator
f. Stable for approximately 1 year, then must be revalidated after that year

D. Controls

1. Urine Qualitative
a. The target concentration for the positive urine control is 500 ng/mL
b. Pipette 50 ul of the working standard solution (10 ug/mL) into 1.0 mL blank matrix
c. Continue to follow sample preparation procedure.

2. Blood Quantitative
a. Refer to table in procedure section
b. Continue to follow sample preparation procedure

3. Quality Controls
Calibrators and controls must be independently prepared from a separate initial dilution or obtained from other sources. When available commercial references, controls will be purchased from an outside vendor. If commercial controls are not available, in-house controls should be prepared from a different provider. When only one supplier is available, a lot different from the calibrator should be used. At the least, when there is only one source, a separate preparation, different from the calibration standard may be used.

4. Urine Control DAU LC2, product #50703, UTAK Laboratories or equivalent
a. Remove cap from vial

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b. Reconstitute control material by adding 10 mL of DWI, using a 10 mL volumetric pipette
c. Replace cap and let sit 10-15 minutes
d. Swirl gently 3-4 minutes to ensure homogenous mixture
e. Swirl gently each time an aliquoted is removed to ensure a homogenous mixture
f. Assay control material in the same manner as case specimens
g. For quantitative assays, record the results obtained on the Excel quality control chart in the DSS S drive.
h. Store reconstituted control material refrigerated at 2-8°C, stable for 25 days after reconstitution.

E. Validation of Reagents: Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as validation of reagents. Validated reagents are marked with a green dot, detailing the specific procedure for which the reagent was validated, and the batch on which that process was documented. Newly prepared reagents may be evaluated for validity on an analytical batch, prior to any consideration of sample results. Reagents so validated are marked with a green sticker as noted above. Preparation of reagents, and their validation is documented in the Toxicology Section Reagent Preparation Validation Logbook, maintained in the Toxicology unit.

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5.0 Procedure

A. Sample Preparation Blood/Urine:

1. Pipette 1 mL into an appropriately labeled 16 x 100 borosilicate culture tube
2. Add 20 ul of SMA deuterated working internal standard.
3. Add 3 mL phosphate buffer (pH 6.0)
4. Mix
   a. Note: If blood sample proceed to steps 5.1.5 and 5.1.6
5. Sonicate for 15 minutes
6. Centrifuge 10 minutes at ~5000 rpm
7. The chart below may be used as a guide, pipette 1 mL of sample, blank, calibrator and control to each appropriately labeled 16 x 100 screw top culture tube.

Calibrator Concentration ng/mL	uL Working standard (10 ug/mL)	uL diluted Working Standard (1 ug/mL)	Blank Blood uL

o-blank	0	0	1000
20	-	20	980
50	-	50	950
100	-	100	900
200	20		980
500	50		950
1000	100		900
QC Concentration	uL Stock Control (10 ug/mL)		
250	25		975
	Utak Control		
UTAK Low	500		500

8. Label SPE tubes to correspond with each culture tube. Load in the manifold.
   a. Note: Cases requiring reanalysis due to high concentrations (i.e. initial analysis is greater than the high control), should be diluted with 0.1 M pH 6.0 Phosphate buffer as appropriate. The initial quantitative values may be used as a guide for the dilution process The dilution process shall be documented in the case jacket.
   b. Note: This procedure utilizes controls prepared (spiked) in blank blood and/or urine (as appropriate to batch makeup) as follows: Each quantitative assay must incorporate a high and low control for each analyte. Blood matrix controls may be used to validate urine results, but not the reverse. Controls are prepared by addition of SMA drugs from validated stock solutions to blank sample matrix aliquots, prior to extraction, (details in Procedure below). Acceptable quantitative control performance is target value +/- 20%.

B. Extraction:
1. Condition the columns:
   a. 1 x 3 mL methanol; drain (vacuum assist)
   b. 1 x 3 mL DIW; drain (vacuum assist)
   c. 1 x 1 mL 100 mM Phosphate buffer; drain (vacuum assist)
   d. DO NOT LET COLUMN GO DRY!

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2. Transfer contents of each tube to the appropriately labeled SPE tube, and allow gentle drop wise flow until the level reaches the top if the column bed.
3. Wash column
   a. 1 x 2 mL DIW; (vacuum assist)
   b. 1 x 2 mL 100mM Acetic acid; (vacuum assist)
   c. 1 x 3 mL Methanol; (vacuum assist)
   d. Dry column (15 minutes at > 10 inches Hg)
4. Position appropriately labeled 13x100 test tubes under each SPE column, with clean thru tip inside collection test tube.
5. Elute analytes:
   a. 1 x 3 mL CH2Cl2/Isopropanol/NH4OH (78:20:2).
   b. Collect Eluate at 1 to 2 mL/minute.
   c. Add 1 drop of 1% methanolic HCl to each calibrator, control, sample and blank tubes.
6. Remove receiver tubes and evaporate to dryness at < 40° C using a gentle flow of Nitrogen (Turbovap).
7. Reconstitute residue with 150 ul of ethyl acetate and transfer to GC/MS vial with limited volume insert. Add 10 micro liters TFAA (Trifluoroacetic anhydride).
   a. Note: Do not evaporate TFAA solution
   b. Note: Departure from procedures as specified in this SOP is not anticipated. Should an issue arise that may require such a departure, the issue must be reviewed and approved by with the Unit Lead and Deputy Director. Depending on the nature of the deviation DSS customers may need to be consulted, see GL-20 for guidance.

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6.0 Chromatography and Mass Spectroscopy

A. Instrument and Setup. (see appendix 1 below):
1. GC/MS/Auto sampler: (Hewlett-Packard 6890/5973, or equivalent)
2. Column: 30M RTX-5 or 30M RTX-1 (MS) (0.25 mm ID; 0.25 micron film) (or equivalent).
3. Inj. Temp. 250 °
4. Det. Temp. 300 °
5. Oven (initial): 80°, 25 ° /min to 300 °, (1.50 min hold).
6. 13.80 min total run time
7. 1 ul injection

B. Injection sequence; Samples are injected on the GC/MS generally in the following sequence:

1. Urine Qualitative
   a. Solvent blank
   b. Positive urine calibrator/standard 500 ng/mL (i.e. In-house control)
   c. Urine blank
   d. Utak control
   e. Case samples
   f. End batch with calibrator or control

C. Blood Quantitation Multipoint Calibrators: Number of calibrators and concentration can change based on analyte of interest and the expected therapeutic range.
1. Solvent blank
2. Priming calibrator
3. Blood blank
4. Calibrator 1 (20 ng/ml)
5. Calibrator 2 (50 ng/mL)
6. Calibrator 3 (100 ng/mL)
7. Calibrator 4 (200 ng/mL)
8. Calibrator 5 (500 ng/mL)
9. Calibrator 6 (1000 ng/mL)
10. Blood blank
11. In-house and or UTAK Low control (if available)
12. Case samples
13. Run set of low and high controls midway through the batch as appropriate for longer batches)
14. End batch with a set of controls.

D. File name – Include case number (including year) in each sample data file.
E. Verify the sequence, verify the vial positions in the auto sampler tray match the sequence.

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7.0 Detection and Identification:

A. Determination of the presence of SMAs in the sample extract is by appearance and ratio of the 3 ions characteristic of each species at the appropriate retention time. Hence, both retention time (a GC characteristic) and fragmentation pattern and ratio (MS characteristics) are used as the basis of qualitative identification. Retention time must be within 5% of the

corresponding analyte in the calibrator injection, and the ion ratios for both qualitative ions must be within 20% of the corresponding ratio calibrator sample. Qualitative identification of each analyte is independent.

B. Calibration:

1. Calibration is not performed on urine samples
2. Calibration for each batch is done independently through the use of method specific controls.

C. Quantitation:

1. Quantitation is accomplished by the comparison of the response ratio of the analyte in a specific sample, to the response ratios of the calibrators as expressed as a “standard curve”. The concentration of the analyte in the sample is then extrapolated, from the standard curve, and corrected for any dilution that may have been performed to facilitate the analysis of relatively concentrated blood samples.
2. For multi-point calibrations, the criteria for acceptability of the calibration and for individual calibrators is that when the values are read back against the final calibration curve they should generally be within +/- 20% of their value. A slightly wider acceptance value (e.g. +/-25% or 30%) is acceptable for calibrators that approach the LOQ of the assay.
3. Note: The available quantitative range of this procedure is (in terms of amount of drug injected on the instrument (thereby allowing for appropriate calculation of diluted samples) is defined for , and validated on each batch by the high and low control, and the acceptable performance of each.
4. The criteria for a valid calibration GC/MS linear regression “r_2” value for a 3 point curve is ≥ 0.98. A significant change in the slope of the calibration line, monitored between runs, may indicate that corrective action needs to be taken.
5. When more than 3 calibration points are used, one point may be removed if it failed to fall into the acceptable quantitative range. If two or more points need to be removed consult with the Unit Lead if any results can be accepted. On a case-by-case basis, results may be reported qualitatively or semi-quantitative.

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8.0 QUALITY CONTROL AND RUN EVALUATION:

A. Verification of Vial Sequence; Vial sequence is checked both before and after the injection of samples. The check after the samples are injected is documented on the run summary sheet.

B. Chromatography - Evaluation and Acceptance Criteria; Chromatographic quality is evaluated for each peak. General guidelines are that peaks should be symmetrical, and resolved to baseline on at least one side, with 90% resolution on the other side. Significant departures from those guidelines may be experienced with forensic samples. In many cases, chromatographic quality will warrant rejection of the chromatographic run, or specific samples, by the operator. Any such action should be clearly documented on the batch summary sheet. Questionable peak shape, resolution, or other problems with chromatography can be discussed with the Unit Lead or Deputy Director.

C. Evaluation of Potential Carryover; Carryover in the chromatogram quality is evaluated by injection of a blank sample immediately following the calibrator. Carryover of greater than 2% (10 ng/mL) requires batch rejection, and remedial action for the instrument (e.g. replacement of injector insert, new septum and perhaps column trim or even replacement). Demonstrated carryover lf less than 2% will require operator consideration with regard to the potential for effects on specific samples, and may require re-extraction of specific samples. In practice, when a question of potential carryover exists, coming from the previous case sample containing a high concentration of an analyte, the potentially affected sample replicates may be repeated at the end of the batch.

D. Evaluation of Controls: Positive and negative controls are evaluated to allow for procedural batch acceptability.

E. Qualitative: Control results are documented and evaluated on the batch summary sheet.

F. Qualitative Results: Controls must demonstrate the target analyte with acceptable chromatography and spectral characteristics.

G. Quantitative: Assays must have controls to verify the calibration and to monitor its stability. Each batch must have at least 10% controls including a positive and negative. The controls can be re-injected in the middle and end

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to demonstrate the stability of the calibration. Acceptable results are the mean target +/-20% or +/-2 standard deviations.

H. Linearity: Linearity of the calibration curve is demonstrable in each batch, for each analyte as a function of quantitative results of control materials.

I. Sensitivity- LIMITS OF DETECTION (LOD) and QUANTITATION (LOQ): For the purposes of this procedure, the LOD and LOQ are defined as equal to the lowest concentration of the lowest control. Qualitative Identification and/or Quantitative analysis below the concentration of the low control may be accepted on a case by case basis with the concurrence of the analyst, technical reviewer, and Unit Lead.

J. Accuracy and Precision: Precision of the procedure is evaluated on a batch by batch basis or by repeat analyses of control or PT materials. Accuracy is expressed as a mean (absolute value) percentage difference between mean quantitative value of 10 reps of the specific control, and the target value. Precision is expressed as the CV of that value

K. Specificity: Specificity is a function of both the resolution of target analyte during the analytical process, and the mass spectral fragmentation that analyte molecules undergo during the instrumental analysis. There has been no report of any material either than SMAs that elute within 5% if the retention time of known standard materials, and produce the same fragmentation ions and ratios.

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9.0 Reporting of Results: SMA runs are performed as part of GC/MS batches, containing controls and calibrators.

A. Once the batch is completed and the data is complied, the batch undergoes a batch review (refer to SOP TX-5 for guidance). Once the batch is accepted the results are entered into JusticeTrax. Wherever possible, analytical results must be reviewed with reference to whatever case history or other information is available.

B. Procedural Uncertainty is reported with all quantitative results, and is calculated and tabulated annually for each analytical method, (See SOP TX-19 section 6.3).

10.0 SOURCES OF ERROR

A. The utilization of 3-ion SIM methodology, with reference to procedural, controls and calibrators yields qualitative drug identification with essentially no uncertainty. Urine drug analyses are reported only as qualitative results.

11.0 REFERENCES:

A. Clarke's Isolation and Identification of Drugs. 2nd Edition
B. UCT United Chemical Technologies "Solid phase extraction methods

Appendix I:

GC/MS temperature program specifications. These are general parameters that may be adjusted based on instrument used or maintenance performed on any given instrument. Proper response of calibrators and controls work to demonstrate the appropriateness of the method.

Parameter			
Initial temp	80° C		
Initial Time	0.00min		
Ramps Rate/final temp/final time	rate	temp	time
	25.0	300	5.00
Post temp	80°c		
Post time	0.00		
Run Time	13.8 min		
Front inlet			
Equilibration time	0.5 min		
Mode	Pulsed split less		
Initial temp	250°c		
Constant Flow	1.0 mL/ min		
Pressure	9.34 psi		
Pulse pressure	35 psi		
Pulse time	0.50 min		
Purge flow	40.0 mL/ min		
Total flow	44.1 mL/min		
Gas type	Helium		
Injection volume	1microliter		
Post injection washes Solvent A / Solvent B	Solvent A -2 Solvent B - 2		
Tune file	ATUNE.U		
Acquisition mode	SIM		
Solvent delay	3.3 min		
MS Quad	150°c max 200		

RETIRED

MS Source	230°c max 250

Rev. #	History
3	Corrected typographical errors Loosened Instrument acquisition parameters and column choice restrictions. Updated choice of using In-house or commercial controls.

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1.0 PRINCIPLE

The principle psychoactive ingredient in Marihuana is Delta-9-Tetrahydrocannabinol (THC). This compound is metabolized in the body to form the metabolite, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH), which may be further metabolized by liver glucuronidation. THC-COOH is somewhat water soluble and it, along with the glucuronide conjugate are excreted in the urine. Forensic urine samples are therefore treated with aqueous sodium hydroxide and heated to hydrolyze the glucuronide conjugate. Post-hydrolysis urine samples are acidified with glacial acetic acid, and any free THC, or THC-COOH present in the sample is extracted onto a solid phase extraction column. Co-extracting materials are washed from the column, and THC/THC-COOH is subsequently eluted. Following evaporation of the elution solvent, the extracted drug is derivatized with BSTFA (N,O, -bis trimethylsilyl trifluoroacetamide) with 1% TCMS (Trimethylchlorosilane). The derivatized sample extracts are analyzed by GC/MS.

Qualitative identification of THC-COOH is based on retention time and ion ratios for 3 tons compared to the corresponding ion ratios from a calibrator extracted and run in the same batch. Concentration of each analyte is determined by a calibration using the peak area ratio between the analyte and the internal standard, using d3-analogue of the analyte as the internal standards. Each GC/MS run is assessed separately; the processed data is evaluated using control and blank samples, and a documented batch review process by both the analyst and technical reviewer. Matrix-specific (urine) positive and negative controls are extracted and analyzed in each analytical batch the concentration of any THC-COOH present in the sample is qualitatively evaluated by 3-ion SIM, using retention time and 2 qualitative ions as the basis for identification. This procedure may be used quantitatively via construction of a multi-point calibration curve for the analyte(s) of interest.

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2.0 SPECIMEN

Urine samples requiring confirmation for THC-COOH are scheduled in JusticeTrax (JT) from which a worklist can be generated.

2.1 All evidence transfers, either between individuals or between an individual and a storage location must be documented on the Chain of Custody for the case in JusticeTrax.

2.2 When not in the sampling or aliquot process samples will be maintained in locked storage within the Toxicology unit.

2.3 Samples must be maintained in such a manner so that they are protected from contamination or deleterious change. Depending on the nature of the sample, this may mean refrigeration or freezing when not in the analytical process.

2.4 When analysis of samples in the Toxicology unit is complete, they must be maintained "Under Proper Seal." Refer to SOP TX-19 for further guidance.

2.5 Samples are maintained in the Toxicology Section for a minimum of 8 weeks after case is completed, in the absence of notification of any legal action, or other reason to maintain the samples. Samples from fatalities and sexual assaults are maintained indefinitely by the DSS. Cases with requests for retention are maintained by the laboratory based on the request. Upon request from the courts or submitting agency the evidence will be returned to the submitting agency.

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3.0 EQUIPMENT:

Equivalent equipment may be used.

- GC/MS and associated data station/computer
- General laboratory glassware and equipment
- Solid phase extraction manifold and associated positive pressure or vacuum equipment
- Analytical evaporator
- UCT; ZSTHC020 "Clean Screen"
- pH paper strips, range 0-14

4.0 REAGENTS:

4.1 Reagents available as stock items:

Equivalent or better grade reagents may be used.

- Methanol (HPLC grade or equivalent)
- Glacial Acetic Acid (Baker reagent grade or equivalent)
- Hexane (HPLC grade or equivalent)
- Deionized water (DIW; Millipore 2-megohm In-House supply)
- Sodium Hydroxide (Baker reagent grade or equivalent)
- Ethyl acetate (HPLC grade or equivalent)
- Acetonitrile (Baker reagent grade or equivalent)

- BSTFA with 1% TMCS
- Drug free urine
- THC-COOH Stock: 1 mL vial 1.0 mg/mL (Cerilliant)
- THC-COOH deuterated Stock: 1 mL vial 1.0 mg/mL (Cerilliant)
- Sodium Phosphate Dibasic (Baker reagent grade or equivalent)
- Sodium Phosphate monobasic (Baker reagent grade or equivalent)

4.2 Reagents prepared in the Toxicology Laboratory:

4.2.1 10 N NaOH; dissolve 200 g NaOH/500 mL DIW

4.2.2 0.1 M HCl; dilute 8.4 mL conc. HCl to 1000 mL DIW

4.2.3. 0.1 M HCl / Acetonitrile (70:30)

4.2.4. Hexane / Ethyl Acetate (50:50)

4.2.5 0.1 M phosphate buffer pH 6.0; combine 48.6 g Na_2HPO_4 and 6.8 g NaH_2PO_4 dilute to 4000 mL using DIW.

Note**:** Reagent Preparation and Validation is documented in the toxicology "Reagent Preparation/Validation Logbook" maintained in the Toxicology section. Validation is addressed in section 4.5, below.

4.3 Calibrators and Internal Standard: (Obtained from an approved vendor)

Note: Preparation of all calibrator and control solutions is documented in the "Calibrator and Control Preparation Log" (maintained in the Toxicology Wet Laboratory).

4.3.1 d3-THC-COOH internal standard 10 ng/µL (Cerilliant or equivalent)
Dilute 250 µL of 1.0 mg/mL reference standard into 25 mL methanol

4.3.2. THC-COOH Calibrator stock solution 10 ng/µL (Cerilliant or equivalent)
Dilute 100 µL of 1.0 mg/mL reference standard into 10 mL methanol

Note: Equivalent ratios may be used; this will be documented in the 'Calibrator and Control Preparation Log'.

4.4 Controls:

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4.4.1 #1 THC-COOH Control stock solution 10 ng/ µL (Lipomed or equivalent) Dilute 100 µL of 1.0 mg/mL reference standard into 10 mL methanol

4.4.2 #2 THC-COOH Control stock solution (THC-COOH) 1 ng/ µL (Lipomed or equivalent). Dilute 25 µL of 1.0 mg/mL reference standard into 25 mL methanol

4.4.3 This procedure utilizes controls prepared in house spiked into urine

Urine: THC-COOH Low Control: 10 ng/mL
High Control: 200 ng/mL
Calibrator: 100 ng/mL

Each run will incorporate a high and low control. Controls are prepared by addition of THC-COOH from validated stock solutions to blank sample matrix aliquots, prior to extraction, (details in Procedure below). Acceptable control performance is target value +/- 20% of the expected value.

4.5 Validation of Reagents

Validated reagents are marked with a green dot (sticker), detailing the specific procedure for which the reagent was validated, and the batch on which that process was documented. Newly prepared reagents may be evaluated for validity on an analytical batch, prior to any consideration of sample results. Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as validation of reagents. Reagents so validated are marked with a green sticker as noted above. Preparation of reagents and their validation is documented in the Toxicology Section Reagent Preparation Validation Logbook, maintained in the Toxicology laboratory.

5.0 PROCEDURE

5.1 Batch Format: Analytical batches for THC-COOH confirmation should follow the format indicated below: Note that samples are analyzed undiluted and diluted by a factor of two. Other dilution factors may be used based on the needs of the case. The immunoassay findings can be used as a guide for choosing a dilution factor.

5.2 Label a 16 x100 screw cap culture tube for each, blank, calibrator, control, and

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sample. Similarly label a SPE extraction tube and load in the manifold.

Pipet blank, standards and control according to table into 5 mL of blank urine.

	Working Standard #1 10 ng/uL µL to add	Final conc.
blank	0	
Calibrator	50	100 ng/mL

	Working Control #2 1.0 ng/uL µL to add	Working Control #1 10 ng/mL µL to add	Final conc.
Low QC	50		10 ng/mL
High QC		100	200 ng/mL

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5.3 Using a validated dispensing pipette, place 5.0 mL and 2.5 mL aliquots of urine into the properly labeled test tubes.

Note: Urine with observable turbidity can be filtered with a syringeless filter or centrifuged for approximately 3 to 5 minutes @ 5200 rpm.

Note: Samples should be diluted with 0.1M pH 6.0 Phosphate buffer. Samples requiring dilution as a function of concentration greater than the upper limit of quantitation should be diluted as appropriate.

5.4 Using a validated dispensing pipette, add 25 µL of deuterated internal standard stock solution to each sample replicate, blank, calibrator and control tube.

5.5 Hydrolysis of Urine Samples

5.5.1 Add 100 µL of 10 M sodium hydroxide to each tube and then cap.

5.5.2 Heat tubes for 30 min. in 60°C water bath, allow to cool.

5.5.3 Adjust the pH to ~3.5 +/- 0.5 with ~1 mL glacial acetic acid, check with pH test strips. Proceed to step 5.6

5.6 Extraction

Condition the columns:

- 1 x 3 mL methanol; drain to hazardous waste stream (1-2 mL/min)
- 1 x 3 mL DIW; drain to non-hazardous waste stream (1-2 mL/min)
- 1 x 1 mL 0.1 M HCl; drain non-hazardous waste stream (1-2 mL/min)
- DO NOT LET COLUMN GO DRY!

5.6.1 Transfer contents of each tube to the appropriately labeled SPE tube, and allow gentle drop wise flow until the level reaches the top if the column bed.

5.6.2 Wash column:

- 1 x 2 mL DIW; drain to nonhazardous waste stream (1-2 mL/min)
- 1 x 2 mL 0.1 M HCl/acetonitrile (70:30); drain to hazardous waste stream (1-2 mL/min)
- Dry column (15 minutes at > 10 inches Hg)

5.6.3 Position appropriately labeled 13 x100 test tubes under each SPE column; check that extraction column tip is positioned inside collection test tube.

5.6.4 Elute analyte from SPE columns by adding 3 mL hexane/ethyl acetate (50:50). Collect at 1-2 mL/minute.

5.6.5 Remove receiver tubes and evaporate to dryness at < 40º C using a gentle flow of heated nitrogen.

5.7 Reconstitute residue with 150 µL of ethyl acetate and transfer to GC/MS vial with limited volume insert. Add 25 µL BSTFA (with 1% TMCS), cap and reserve for GC/MS analysis. Note**:** Do not evaporate BSTFA solution

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6.0 CHROMATOGRAPHY AND MASS SPECTROSCOPY

6.1 Instrument and Setup: See appendix 1.

GC/MS/Auto sampler: (Hewlett-Packard 6890/5973, or equivalent)
Column: 30M RTX-1 or 30M RTX-5 (MS) (0.25 mm ID; 0.25 micron film) (or equivalent)
Inj. Temp. 250 ° C
Det. Temp. 280 ° C
Oven (initial): 160° C, 30 ° /min to 300 ° C, (2.50 min hold).
7.67 min total run time
1 µL injection

6.2 Injection sequence**;** samples are injected on the GC/MS in the following sequence:

Before running casework check that the instrument is in proper working condition (acceptable for use); refer to SOP TX-29 for further guidance.

- Matrix blank
- Control Low
- Calibrator
- Control High
- Reinject matrix blank
- Samples rep diluted
- Samples rep undiluted
- Inject ethyl acetate solvent blanks between cases
- End run batch with reinjecting the high control
- For longer runs, reinject the low control in the middle of the batch

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6.3 DETECTION AND IDENTIFICATION

Determination of the presence of THC-COOH in the sample extract is identified by appearance and ratio of the 3 ions characteristic of each species at the appropriate retention time, hence both retention time (a GC characteristic) and fragmentation pattern and ratio (MS characteristics) are used as the basis of qualitative identification. For a positive Identification of THC-COOH, the retention time must be within 5% of the analyte in the calibrator injection, and the ion ratios for both qualitative ions must be within 20% of the corresponding ratio calibrator or control.

6.4 CALIBRATION AND QUANTITATION

6.4.1 Calibration

Calibration is accomplished by the incorporation into the sample procedures of a blank sample of the matrix being analyzed that has known quantities (100 ng/mL for THC-COOH in urine) in addition to the deuterated internal standard. The response of the system to this calibrator, and the assumption of a 0 response to a 0 concentration, defines a run-specific standard curve that is used as the basis for the quantitative calculation in all controls and samples. The system is therefore single-point calibration, multi-point controls.

6.4.2 Quantitation

Quantitation is accomplished by the comparison of the response ratio of the analyte in a specific sample, to the response ratios of the calibrators as expressed as a standard curve. The concentration of the analyte in the sample is then extrapolated from the standard curve, and corrected for any dilution that may have been performed to facilitate the analysis of relatively concentrated samples.

Note: Departure from procedures as specified in this SOP is not anticipated. Should an issue arise that may require such a departure, the issue must be reviewed and approved by with the Unit Lead and Deputy Director. Depending on the nature of the deviation DSS customers may need to be consulted, see GL-20 for guidance.

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7.0 QUALITY CONTROL AND RUN EVALUATION

7.1 Verification of Vial Sequence: The vial sequence is checked both prior to and after the injection of samples when the auto injector is used. The check after the injection of samples is documented on the run summary sheet.

7.2 Chromatography - Evaluation and Acceptance Criteria: Chromatographic quality is evaluated for each peak. While general guidelines are that a peak should be symmetrical, and be resolved to baseline on at least one side, with 90% resolution on the other side, significant departures from those guidelines may be experienced with forensic samples. In many cases, chromatographic quality will warrant rejection of the chromatographic run, or specific samples, by the operator. Any

such action should be clearly documented with the batch. Questionable chromatographic peak shape, resolution, or other problems with chromatography can be discussed with the Deputy Director or Unit Lead.

7.3 Evaluation of Potential Carryover

Carryover in the chromatographic quality is evaluated by injection of a blank sample immediately following the calibrator. Carryover of greater than 2% (40 ng/mL) requires batch rejection, and remedial action for the instrument (e.g. replacement of injector insert, new septum and perhaps column trim or even replacement). Demonstrated carryover of less than 2% will require operator consideration with regard to the potential for effects on specific samples, and may require re-extraction of specific samples. Carryover is further evaluated on a per sample basis by comparing quantitative results between sample replicates. When a question of potential carryover exists, the potentially affected sample replicates may be repeated (reinjected) at the end of the batch.

7.4 Control Results: For the batch to be considered acceptable the negative blank should be blank and a positive control should be positive.

7.5 Internal Standard: The sample preparation extraction efficiency and GC/MS instrument performance can be demonstrated through the internal standard abundances. They should be consistent throughout the batch. The abundance should be about the same as the 100ng/mL calibrator. The batch or samples within the batch should be repeated when the abundance of the internal standard is too low and not acceptable.

7.6 LINEARITY: Linearity of the calibration curve is demonstrable in each batch as a function of quantitative results of control materials.

7.7 SENSITIVITY- LIMITS OF DETECTION (LOD) and QUANTITATION (LOQ):
For the purposes of this procedure, the LOD and LOQ are defined as equal to the lowest concentration of the lowest control

7.8 ACCURACY AND PRECISION: Accuracy is expressed as a mean (absolute value) percentage difference between mean quantitative value of the specific control, and the target value. Precision is expressed as the CV of that value.

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7.9 SPECIFICITY: Specificity is a function of both the resolution of target analyte during the analytical process, and the mass spectral fragmentation that analyte molecules undergo during the instrumental analysis. There has been no report of any material other than THC-COOH that elutes within 5% of the retention time of known standard materials, and produce the same fragmentation ions and ratios.

7.10 REPORTING OF RESULTS: THC-COOH runs are performed as part of GC/MS batches, containing controls and calibrators. The complete batch packets are in the Toxicology Laboratory. Refer to SOP TX-5 for guidance on batch preparation, case reporting and distribution. Qualitative results are reported for urine samples.

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8.0 SOURCES OF ERROR

The utilization of 3-ion SIM methodology, with reference to procedural, controls and calibrators yields qualitative drug identification. Interfering substances, if present, can be detected when retention times shift or ion ratios are outside their limits. If interfering substances are suspected, the urine samples maybe diluted further to minimize their impact. Urine drug analyses are reported only as qualitative results.

9.0 REFERENCES

Clarke's Isolation and Identification of Drugs. 2nd Edition

UCT United Chemical Technologies
Solid phase extraction methods

Appendix I:

GC/MS temperature program specifications. These are general parameters that may be adjusted based on instrument used or maintenance performed on any given instrument. Proper response of calibrators and controls work to demonstrate the appropriateness of the method.

Temperature program			
Parameter			
Initial temp	160° C		
Initial Time	0.00 min		
Ramps Rate/final temp/final time	rate	temp	time
	30.0	300	2.5 min
Post temp	300°c		
Post time	0.50 min		
Run Time	7.67 min		
Front inlet			
Mode	Pulsed split less		
Injector temp	250°c		
Pressure	12.9 psi		
Pulse pressure	25 psi		
Pulse time	0.50 min		
Purge to split vent	40.0 mL/min		
Total flow	44 mL/min		
Gas type	Helium		
Injection volume	1 microliter		
Post injection washes Solvent A / Solvent B	Solvent A -2 Solvent B - 3		
Tune file	ATUNE		
Acquisition mode	SIM		
Solvent delay	6.25 minutes		
M/Zs group 1 Start time 0.00	374, 371, 476, 473, 491, 488		
Dwell	20		
MS Quad	150°c		
MS Source	230°c		

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Rev. #	History
3	Loosened Instrument acquisition parameters and column choice restrictions.

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1.0 PRINCIPLE

Blood and urine samples, that require examination for “Weak Acid,” Neutral and/or Basic Drugs (WAN/BDS) by GC/MS, are extracted using mixed-bed solid phase extraction columns.

Weakly Acidic or Neutral (WAN) drugs are extracted into a hexane/ethyl acetate and basic drugs that may be present are extracted into a methylene chloride/isopropanol/ ammonium hydroxide mixture After evaporation of the solvents, the extracted drug(s) are analyzed by GC/MS in Scan mode.

Qualitative identification of the WAN and/or Basic Drugs by GC/MS is based on retention time and ion ratios for 3 ions compared to the corresponding ion ratios from a calibrator run in the same batch, or “full-scan” spectra fragmentation pattern compared to a reference library based fit. Matrix-specific (blood and/or urine) positive and negative controls are extracted and analyzed in each analytical batch. Cyproheptadine and ethinamate are used as internal standards for basic and acid neutral drugs. The presence of WAN and Basic Drugs may be confirmed in urine, blood or other aqueous fluids.

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2.0 SAFETY

This procedure is carried out in a laboratory environment, and standard safety procedures appropriate for such an environment should be utilized, including (minimally) gloves, safety glasses and protective clothing (lab coat). Biological specimens subject to the analytical procedure should be handled using universal precautions. Potentially contaminated items and surfaces should be cleaned and disinfected prior to any further use

3.0 SPECIMEN

A. Urine

B. Blood

C. Serum

D. Other Matrices e.g. meat, tissue, liquids

1. Work lists are generated through JusticeTrax. Specimens comprising an analytical batch are obtained from LIMS system work list, additional samples can be added to the worklist that were not generated by LIMS

2. All evidence transfers, either between individuals or between an individual and storage location must be documented on the Chain of Custody for the case in JusticeTrax.

3. When not in the sampling or aliquot process, samples in the Toxicology unit will be maintained in locked storage within the Toxicology unit.

4. Samples must be maintained in such a manner so that they are protected from contamination or deleterious change. Depending on the nature of the sample, this may mean refrigeration or freezing when not in the analytical process.

5. When analysis of samples in the Toxicology unit is complete, they must be maintained "Under Proper Seal." Refer to SOP TX-19 for further guidance.

6. Samples are maintained in the Toxicology Section for a minimum of 8 weeks after the case is completed, in the absence of notification of any legal action, or other reason to maintain the samples. Samples from fatalities and sexual assaults are maintained indefinitely by the DSS. Cases with requests for retention are maintained by the laboratory based on the request. Upon request from the courts or the submitting agency the evidence will be returned to the submitting agency.

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4.0 EQUIPMENT:

Note: Comparable or equivalent equipment may be used.

A. GC/MS and associated data station/computer (Hewlett-Packard 6890/5973, Agilent Technologies 7890A, AT-7890B or equivalent)

B. General laboratory glassware and equipment

C. Solid phase extraction manifold

D. Analytical evaporator

E. Water Bath

F. UCT; ZSDAU020 “Clean Screen” solid-phase extraction tubes

G. Centrifuge

H. Sonicator

I. Vortex

J. pH paper

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5.0 REAGENTS:

A. **Reagents available as stock items**: (Sigma or J.T. Baker Reagent Grade or equivalent unless otherwise specified)

1. Methanol (CH_3OH)
2. Ammonium hydroxide (NH_4OH)
3. Ethyl acetate ($C_4H_8O_{2)}$
4. Hexane ($CH_3(CH_2)_4CH_3$)
5. Methylene chloride (CH_2Cl_2)
6. Isopropanol (C_3H_8O)
7. Sodium phosphate dibasic (Na_2HPO_4)
8. Sodium phosphate monobasic ($NaH_2\ PO_4$)
9. Sodium acetate trihydrate ($CH_3COONa•_3H_2O$)
10. Hydrochloric acid (HCl)

B. **OTHER REAGENTS:**

1. β-Glucuronidase (p. vulgata ; Sigma or equivalent)

2. Blank Blood (may be acquired from a blood bank)

3. Drug free urine (donated in-house)

4. Morphine glucuronide 1.0 mg/mL (Alltech, Sigma or equivalent)

5. Deionized water (DIW; Millipore or equivalent In-House supply)

C. **REAGENTS PREPARED IN THE TOXICOLOGY LABORATORY:**

Volumes may be proportionally changed.

1. 0.1 M PHOSPHATE BUFFER PH 6.0 1 LITER

   a. Combine 1.7 g sodium phosphate dibasic Na_2HPO_4 and 12.14 g sodium phosphate monobasic NaH_2PO_4

   b. QS to 1000 mL using DIW

   c. Check pH with pH paper.

   d. Storage: room temperature in glass or plastic.

2. 1.0 M ACETIC ACID 500 mL

   a. **T**o approximately 400 mL DIW in a graduated cylinder,

   b. Add 28.6 mL glacial acetic acid

   c. Mix, QS to 500 mL

   d. Storage: room temperature in glass or plastic.

3. **0.1 M ACETIC ACID 500 mL**

   a. Dilute 50 mL 1.0 M acetic acid to 500 mL with DIW

   b. Mix. Storage: room temperature in glass or plastic.

4. **1.0 M ACETATE BUFFER (PH 5.0) 500 mL**

   a. To approximately 400 mL DIW in a graduated cylinder,

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b. Dissolve 42.9 g sodium acetate trihydrate in approximately 400 mL DIW

c. Add 10.4 mL glacial acetic acid $C_2H_4O_2$

d. Dilute to 500 mL with DIW

e. Mix. Check pH with pH paper, adjust pH to approximately 5.0 with 1.0 M acetic acid if needed.

f. Storage: room temperature in glass or plastic. Inspect daily for contamination or turbidity.

5. **0.1 M ACETATE BUFFER (PH 5.0)**

a. Dilute 20 mL 1.0 M acetate buffer to 200 mL with DIW

b. Mix. Check pH with pH paper.

c. Store at: room temperature in glass or plastic.

6. **ELUTION SOLVENT: METHYLENE CHLORIDE/ISOPROPANOL/AMMONIUM HYDROXIDE (39/10/1)**

Note: Adjust volume as needed for the total number of tubes; 3 mL needed for each tube. Must be prepared fresh each day of use.

Example. To make 150 mL of solution:

a. First combine 30 mL isopropanol and 3 mL ammonium hydroxide into a 200 mL graduated cylinder with stopper cap

b. Then add 117 mL methylene chloride (prevents buildup of pressure)

c. Cap and mix.

d. Note: Dispose of unused elution solvent mixture in the halogenated/chlorinated waste stream container.

7. **B -GLUCURONIDASE, (5,000 F UNITS/ML) IN 0.1 M ACETATE BUFFER (PH 5.0)**

Example: Calculate activity for each lot of β-Glucuronidase as follows:

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(Lot specific, value from bottle label) e.g. 1,439,000 -glucuronidase units/g solid

$$\frac{5{,}000 \text{ Units/mL}}{x \text{ mg/mL}} = \frac{1{,}439{,}000 \text{ Units}}{1000 \text{ mg}} \qquad x = 3.47 \text{ mg/mL}$$

a. Prepare daily for use, make slight excess for each batch, each 1 mL sample requires 2500 F units. Add 0.5 mL of β -Glucuronidase to each tube.

b. Example: for 48 total tubes prepare 25 mL

c. To make 25 mL:

i. Weigh out 87 mg β –Glucuronidase solid. Add to 25 mL of 0.1 M acetate buffer (pH 5.0)

ii. Dissolve before use by swirling gently.

8. **1% METHANOLIC HCL 100 ML**

a. Dilute 1 mL Concentrated HCl into 100 mL methanol.

b. Store at room temperature in glass or plastic.

c. Inspect daily before use for contamination. If any visible bacterial growth is present, discard and make fresh.

D. **VALIDATION OF REAGENTS:** Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as validation of reagents. Validated reagents are documented on the method run sheet. Newly prepared reagents may be evaluated for validity on an analytical batch, prior to any consideration of sample results. . Preparation of reagents is documented in the Toxicology Section Reagent Preparation Validation Logbook, maintained in the Toxicology laboratory**.**

Generally reagents are considered acceptable for use for 1 year from the date they are validated (unless noted). Reagents may be revalidated after 1 year to

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demonstrate continued acceptability, the expiration date will be extended for 1 year, this will be documented as listed above.

6.0 CALIBRATION STANDARDS/IN-HOUSE CONTROL STANDARDS/INTERNAL STANDARDS:

A. Note: Preparation of all calibrator and control solutions is documented in the "Calibrator and Control Preparation Log" (maintained in the Toxicology Wet Laboratory).

B. Stock Calibrator and Control solutions: Comprised of various drugs as needed; (Alltech, Cerilliant or equivalent; 1 mg/mL). Composition of the Calibrator (analyte and concentration) will be dependent on the current patterns of observed drugs, and normal levels at which those drugs are observed. (E.g., Phenobarbital cal = 5.0 mg/L, Ketamine cal = 0.5 mg/L)

1. Reference material standards are obtained from Sigma/Aldrich, Cerilliant, Lipomed, Grace or other equivalent manufacturers. 1 mL ampules at concentrations of 1.0 mg/mL or 100 ug/ mL or as available by the provider.

C. Internal Standards**:**

1. Cyproheptadine
2. Ethinamate

D. Working Standard Solution 10 ug/mL

1. Into a 10 mL volumetric flask partially filled with methanol
2. Pipette 100 uL of each reference standard (1 mg/mL)
3. QS with methanol, mix
4. Store in freezer/refrigerator
5. Generally 1 vial will be made containing the WAN analytes and 1 containing the Basic analytes (see components below).

E. Diluted Working Standard Solution 1.0 ug/mL

1. Pipette 100 uL of Working Standard Solution (10 ug/mL) into a borosilicate culture tube

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2. Add 900 uL of methanol

3. Cap and Vortex

4. Store in freezer/refrigerator

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F. Internal Stock Standards

1. Cyproheptadine ~0.1 mg/mL in methanol 250 mL
   a. In a 250 mL volumetric flask, weigh out ~32. mg of cyproheptadine hydrochloride sesquihydrate
   b. Dissolve in and QS to volume with methanol
   c. Store in freezer/refrigerator

2. Ethinamate ~0.2mg /mL in methanol 100 mL
   a. In a 100 mL volumetric flask, weigh out ~20 mg of ethinamate
   b. Dissolve in and QS to volume with methanol
   c. Store in freezer/refrigerator)

3. Working Internal Standard Solution (Cyproheptadine 5ug /mL, Ethinamate 10 ug/mL)
   a. To a 100 mL volumetric flask partially filled with methanol
   b. Pipette 5mL of stock 0.1 mg/mL cyproheptadine
   c. Pipette 5mL of stock 0.2 mg/mL ethinamate
   d. QS to volume with methanol
   e. Store in freezer/refrigerator

4. Urine and Blood Qualitative
   a. The target concentration for the positive urine and blood calibrator/standard/control is 500 ng/mL
   b. Pipette 50 uL of each the WAN and Basic Working Standard Solution (10ug /mL) into 1.0 mL blank matrix
   c. Continue to follow sample preparation procedure

5. Blood Quantitative
   a. Refer to table in Procedure section
   b. Continue to follow sample preparation procedure

6. Weak Acid Neutral Drugs Reference Standards

S.No.	Standard	Concentration of standard	Manufacturer
1	butalbital	1.00 mg/ml	Cerilliant
2	carisoprodol	1.00 mg/ml	Cerilliant
3	ibuprofen	1.00 mg/ml	Cerilliant
4	lorazepam	1.00 mg/ml	Cerilliant
5	meprobamate	1.00 mg/ml	Cerilliant
6	oxazepam	1.00 mg/ml	Cerilliant
7	pentobarbital	1.00 mg/ml	Cerilliant
8	phenobarbital	1.00 mg/ml	Cerilliant
9	phenytoin	1.00 mg/ml	Cerilliant
10	secobarbital	1.00 mg/ml	Cerilliant
11	talbutal	1.00 mg/ml	Grace
12	temazepam	1.00 mg/ml	Cerilliant
13	topiramate	1.00 mg/ml	Cerilliant

7. Basic Drugs Reference Standards

	Standard	Concentration of Standard	Manufacturer
1	Amphetamine	1.00 mg/ml	Cerilliant
2	Methamphetamine	1.00 mg/ml	Cerilliant
3	3,4-MDA	1.00 mg/ml	Alltech
4	3,4-MDMA	1.00 mg/ml	Cerilliant
5	alprazolam	1.00 mg/ml	Cerilliant
6	amitriptyline	1.00 mg/ml	Cerilliant
7	bupropion	1.00 mg/ml	Cerilliant
8	chlorpheniramine	1.00 mg/ml	Cerilliant
9	citalopram	1.00 mg/ml	Alltech
10	clonazepam	1.00 mg/ml	Cerilliant
11	cocaine	1.00 mg/ml	Cerilliant
12	codeine	1.00 mg/ml	Cerilliant
13	cyclobenzaprine	1.00 mg/ml	Cerilliant
14	desipramine	1.00 mg/ml	Cerilliant
15	dextromethorphan	1.00 mg/ml	Cerilliant
16	diazepam	1.00 mg/ml	Cerilliant
17	diltiazem	1.00 mg/ml	Cerilliant
18	diphenhydramine	1.00 mg/ml	Cerilliant
19	doxepin	1.00 mg/ml	Cerilliant

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20	doxylamine	1.00 mg/ml	Cerilliant
21	fluoxetine	1.00 mg/ml	Cerilliant
22	haloperidol	1.00 mg/ml	Cerilliant
23	hydrocodone	1.00 mg/ml	Cerilliant
24	hydroxyzine	1.00 mg/ml	Alltech
25	ketamine	1.00 mg/ml	Cerilliant
26	meperidine	1.00 mg/ml	Cerilliant
27	methadone	1.00 mg/ml	Cerilliant
28	methaqualone	1.00 mg/ml	Cerilliant
29	methylphenidate	1.00 mg/ml	Alltech
30	nordiazepam	1.00 mg/ml	Cerilliant
31	nortriptyline	1.00 mg/ml	Cerilliant
32	oxycodone	1.00 mg/ml	Cerilliant
33	phencyclidine	1.00 mg/ml	Cerilliant
34	phentermine	1.00 mg/ml	Cerilliant
35	sertraline	1.00 mg/ml	Cerilliant
36	tramadol	1.00 mg/ml	Cerilliant
37	trazodone	1.00 mg/ml	Cerilliant
38	venlafaxine	1.00 mg/ml	Cerilliant
39	zolpidem	1.00 mg/ml	Cerilliant
40	propofol	1.00 mg/ml	Cerilliant
41	hydromorphone	1.00 mg/ml	Cerilliant
42	oxymorphone	1.00 mg/ml	Cerilliant
43	EDDP	1.00 mg/ml	Cerilliant
44	Ranitidine	1.00 mg/ml	Cerilliant
45	BE	1.00 mg/ml	Cerilliant

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Note: Analytes can be added (or removed) from these mixtures as needed. For specific targeted quantitative analyses, only the analytes of interest need to be added to the calibrator and control solutions.

Manufacturers listed are suggested. Equivalent manufacturers can be used as needed.

7.0 QUALITY CONTROLS

A. Calibrators and controls must be independently prepared from a separate initial dilution or obtained from other sources. When available commercial reference controls will be purchased from an outside vendor. If commercial controls are not available, In-house controls should be prepared from a different provider. When only one supplier is available, a lot different from the calibrator should be used. At the least, when there is only one source, a separate preparation, different from the

calibration standard (prepared by a 2nd analyst) should be used.

1. Urine Control DAU LC 2, Product # 50703, Utak Laboratories Valencia, CA 91355

   a. Prepare the UTAK control per the product insert.
   b. Swirl gently each time an aliquot is removed to ensure a homogeneous mixture
   c. Handle and test the control material in the same manner as case specimens
   d.
   e. Store reconstituted control material refrigerated at 2-8°C, stable for 25 days after reconstitution

2. Morphine 3-β-D-glucuronide working solution 10 ug/mL - optional
   a. Pipette 100 uL of stock 1 mg/mL reference morphine 3-β-glucuronide into a 10 mL volumetric flask
   b. QS with methanol
   c. Stable 1 year when stored tightly capped in freezer (-0°C)
3. Urine Control Morphine 3-β-D-glucuronide. (2.0 ug/mL glucuronide, 1.24ug/mL free morphine) - optional

   For hydrolysis batches

   a. Pipette 200 uL of 10 ug/mL morphine 3-β-glucuronide solution to the Hydrolysis Control tube
   b. Add 800 uL blank urine

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8.0 PROCEDURE

A. Sample Preparation
   1. Blood
      a. Pipette 1 mL blood into an appropriately labeled 16 x 100 borosilicate culture tube
      b. Add 3 mL 0.1M phosphate buffer (pH= 6.0)
      c. Add 2 mL DIW Water
      d. Add 100 uL of working internal standard solution.
      e. Mix, Sonicate for 15 minutes
      f. Centrifuge ~8 minutes at ~ 5000 rpm
      g. Go to 'Extraction' step below
         i. Note: Transfer supernatant before pellet dissipates

2. Urine Hydrolysis (optional)
   a. Pipette 1.0 mL of each urine sample into an appropriately labeled tube
   b. Add 100 uL of working internal standard solution.
   c. Add 0.5 mL of 5,000 F units/ mL β-glucuronidase to each tube
   d. Mix/vortex, Incubate for a minimum of 3 hours or overnight at 60°C in water bath
   e. Cool to room temperature
   f. Add 3 mL 0.1M phosphate buffer (pH= 6.0)
   g. Centrifuge for ~8 minutes at ~5000 rpm (if required due to the nature of the sample)
   h. Go to Extraction step below
3. Urine (Not Hydrolyzed) and UTAK Control
   a. Pipette 1 mL of each urine sample into an appropriately labeled borosilicate culture tube. Turbid samples may be filtered prior to this step.
   b. Add 3 mL 0.1 M phosphate buffer (pH= 6.0)
   c. May add 2 mL of DWI to match the blood volume (optional)
   d. Add 100 uL of working internal standard solution
   e. Mix; (if running blood and urine samples together these can be sonicated with the blood samples).
   f. Centrifuge ~8 minutes at ~5000 rpm (if required due to the nature of the sample).
   g. Go to Extraction step below.

      Note: Cases requiring reanalysis due to high concentrations (i.e. initial analysis is greater than the high control), should be diluted with 0.1 M pH 6.0 Phosphate buffer as appropriate. The initial quantitative values may be used as a guide for the dilution process The dilution process shall be documented in the case jacket (for quantitative casework only).

4. Quantitative Test: Example of preparation of calibrators and controls.

   a. According to the table below, pipette 1 mL of sample, blank, calibrator and control to each appropriately labeled screw top culture tube. (Calibrators may not be extracted if using a historical calibration curve).

Calibrator Concentration ng/mL	uL Working Standard (10 ug/mL)	uL diluted Working Standard (1.0 ug/mL)	Blank Blood Pipette uL

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0-blank	0	0	1000
50	----	50	1000
100	----	100	1000
200	20		1000
500	50		1000
1000	100		1000
QC Concentration	uL Stock Control (10 ug/mL)		
200	20		1000
1000	100		1000
	Utak Control		
Utak Low	500		500
Utak High	1000		0

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B. Extraction

1. Condition the Clean Screen® Extraction columns:
   a. 1 x 3 mL methanol; drain to hazardous solvent waste stream
   b. 1 x 3 mL DIW; drain to regulated biohazard waste
   c. 1 x 1 mL 0.1M Phosphate buffer pH 6; drain to regulated biohazard waste.
      Note: Aspirate or drain at <3 inches Hg to prevent sorbent drying
   d. DO NOT LET COLUMN GO DRY!

2. Apply sample:
   a. Transfer prepared sample (supernatants of prepared blood and /or urine samples), to the appropriately labeled, conditioned SPE tube, and allow gentle drop wise flow until the level reaches the top of the column bed. Elute at 1 mL/minute.

3. Wash column:
   a. 1 x 2 mL Phosphate buffer; drain to regulated biohazard waste
   b. 1 x 2 mL 0.1 M Acetic acid; drain to regulated biohazard waste
   c. Dry column (~5 minutes at > 10 inches Hg)

4. Position appropriately labeled clean borosilicate collection test tubes under each SPE column, with tip inside collection test tube.

5. Elute Acid analytes:
   a. 1 x 1 mL Hexane
   b. 1 x 3 mL Ethyl acetate
   c. Collect Eluent at 1 to 2 mL/minute

6. Remove receiver tubes and evaporate to dryness at < 40 C using a gentle flow of nitrogen.

7. Reconstitute residue with 150 uL of ethyl acetate and transfer to GC/MS vial with limited volume insert, cap and reserve for GC/MS analysis. Refrigerate vials if extracts won't be injected on the instrument on the same day. Stable at least one week when tightly capped.

8. Wash column:

   a. 1 x 3 mL methanol; aspirate drain to hazardous solvent waste stream.

9. Dry column (15 minutes at > 10 inches Hg)

   a. Position another set of clean appropriately labeled borosilicate test tubes under each SPE column, with tip inside collection test tube.

10. Elute Basic analytes: Elution Solvent Methylene chloride/IPA/NH_4OH (39:10:1)
    a. 1 x 3 mL Methylene chloride/IPA/NH_4OH (39:10:1)
    b. Collect Eluent at 1 to 2 mL/minute.

11. Add 1 drop of 1% methanolic HCl to each tube

12. Remove receiver tubes and evaporate to dryness at < 40 C using a gentle flow of Nitrogen

13. Reconstitute residue with 150 uL of ethyl acetate and transfer to GC/MS vial with limited volume insert, cap and reserve for GC/MS Analysis. Refrigerate vials if extracts won't be injected on the instrument on the same day. Stable at least one week when tightly capped.

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a. Note: Departure from procedures as specified in this SOP is not anticipated. Should an issue arise that may require such a departure, the issue must be reviewed and approved by with the Unit Lead and Deputy Director. Depending on the nature of the deviation DSS customers may need to be consulted, see GL-20 for guidance.

9.0 CHROMATOGRAPHY AND MASS SPECTROSCOPY

A. Instrument and Setup:

1. GC/MS/Auto sampler:(Hewlett-Packard 6890/5973, Agilent Technologies 7890A/5975C, 7890B/5977A)

2. Column: 30M RTX-5MS or 30M RTX-1MS (0.25 mm ID; 0.25 micron film) – or equivalent

3. Injection Temperature 250 º Detector Temperature 280 º

4. Oven (initial): 80º, 25 º /min to 300 º, (7.0 min hold).

5. 16.8 min total run time, 1 uL injection

6. Scan range 40 to 470 amu

7. Method: Drugsmwa.M; Method Details Appended (Appendix II)

8. Prior to running case work, the GC/MS instrument suitability must be acceptable (see SOP TX-29 for guidance).

B. Batch Format: Qualitative batches require a positive and negative control. Quantitative batches require known calibrators and controls, case samples should be bracketed by acceptable controls. Analytical batches for WAN and Basic drug confirmations may generally follow the format indicated below. Inject 1 uL into the GC/MS using the following sequence.

1. Urine Qualitative (example of sequence):

a. Solvent blank

b. Positive urine calibrator/standard 0.500 ug/mL (i.e. In-house control)

c. Urine blank

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d. Morphine Glucuronide Control (Hydrolysis Batches Only)

e. Utak control

f. Solvent blank

g. Case samples

h. End batch with calibrator or control

2. Blood Quantitation multipoint calibrators Note: Number of calibrators and concentration can change based on analytes of interest and the expected therapeutic and toxic range.

a. Solvent blank

b. Priming Calibrator

c. Blood blank

d. Calibrator 1 (50 ng/mL)

e. Calibrator 2 (100 ng/mL)

f. Calibrator 3 (200 ng/mL)

g. Calibrator 4 (500 ng/mL)

h. Calibrator 5 (1000 ng/mL)

i. Blood blank

j. Utak control(s)

k. Low control (100 ng/mL)

l. High control (1000 ng/mL)

m. Solvent blank

n. Case sample

o. Run a control midway through the batch for longer sequences

p. End batch with a control

3. File name - Include case number (including year) in each sample data file; example 16-2103-1A1

4. Verify the sequence; verify the vial positions in the tray match sequence.

10.0 DETECTION AND IDENTIFICATION

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A. MS Analysis Criteria for EI (Electron Ionization) ion source fragmentation and detection of nominal mass spectral measurements.

Determination of the presence of WAN and Basic drugs in the sample extract are identified by evaluation of full spectra, and appearance and ratio of the 3 ions characteristic of each species at the appropriate retention time, hence both retention time or relative retention time (a GC characteristic) and fragmentation pattern and ratio (MS characteristics) are used as the basis of qualitative identification.

1. For a positive identification of drugs:

   a. All of the diagnostic ions present in the reference standard must be present in the unknown.

   b. There may be additional ions in the 'unknown' spectrum due to minor interferences that cannot be removed by background subtraction, but all of the diagnostic ions present in the reference spectrum must be present in the 'unknown' unless absent due to low absolute abundance.

   c. Relative abundances of the diagnostic ions, as well as retention times or relative retention times must always be considered in designating a "positive" match. The retention time or relative retention time must be within 2% of the corresponding analyte, run on the same instrument.

2. When selected ion monitoring is used for identification and/ or quantitation, it must compare ion ratios and retention times between calibrators, controls and unknowns. Whenever possible, three ions must be monitored for the analyte and two ions for the internal standard. The qualifying ions must be +/- 20% relative to a calibrator included in the same run. It is realized that for some analytes it may be difficult to choose multiple ions. Some spectra may contain only one or two ions that are > 5% relative abundance. Therefore, additional or complimentary methods of identification may be used.

3. Qualitative identification of each analyte is independent.

   a. Note: Qualitative identification of analytes with abundance < 5% of the I.S. abundance should be considered with great care, and evaluation of significance based on the (presumably) low concentration. Detection of metabolites from parent drugs detected adds confidence in the identification of drugs.

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4. The internal standard recovery in the extracted sample is monitored. If the internal standard recovery is substantially reduced, recoveries less than 50% or greater than 200% relative to the calibrators or controls, the quantitative value must be investigated on a case-by-case basis as to whether reporting a quantitative result is appropriate.

11.0 CALIBRATION AND QUANTITATION

Note**:** Quantitation is not performed on urine analyses.

A. Calibration: Calibration for each batch and fraction (WAN/BDS) may be performed independently or historical curves may be used. When historical curves are used the analyst will assure that there have been no major changes to the instrument since the calibration curve was analyzed. Typically curves will be updated monthly (as needed). Controls extracted with the sample batch will be used to assess the appropriateness of the historical calibration curve.

The positive control is the 0.5 ug/mL calibrator with internal standard spiked into drug-free urine or blood. The negative control is the internal standard spiked into drug-free urine or blood.

B. Quantitation: Quantitation is accomplished by the comparison of the response ratio of the analyte in a sample, to the response ratios of the calibrators as expressed as a standard curve. The concentration of the analyte in the sample is then extrapolated from the standard curve, and corrected for any dilution that may have been performed to facilitate the analysis of relatively concentrated samples.

1. Note: The available quantitative range of this procedure is limited to the calibration range. The lowest standard that meets quantitative acceptability criteria and peak identification criteria is the LOQ. Analytes that only meet peak identification criteria may be reported as detected ≤ LOD. LOD is usually the lowest calibrator. Analytes detected that are above the highest calibrator may be reported as greater than the highest calibrator or control. If a quantitative level is needed, the sample will be reanalyzed at a diluted concentration so that it falls within the calibration curve limits.

2. The criteria for a valid calibration GC/MS linear regression "r^2" value for a 3 point curve is ≥ 0.98 using non-deuterated internal standards. A significant change in the slope of the calibration line, monitored between runs, may indicate that corrective action needs to be taken.

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3. When more than 3 calibration points are used, one point may be removed if it failed to fall into the acceptable quantitative range. If two or more points need to be removed consult with the Unit Lead if any results can be accepted. On a case-by-case basis, results may be reported qualitatively or semi-quantitative.

12.0 QUALITY CONTROL AND RUN EVALUATION

A. Verification of Vial Sequence: The vial sequence is checked both prior to and after the injection of samples when the auto injector is used. The check after the samples are injected is documented on the batch summary sheet. (See appendix for an example of the batch summary sheet).

B. Chromatography - Evaluation and Acceptance Criteria: Chromatographic quality is evaluated for each peak. While general guidelines are that a peak should be symmetrical and be resolved to baseline on at least one side with 90% resolution on the other side, significant departures from those guidelines may be experienced with forensic samples. In many cases, chromatographic quality will warrant rejection of the chromatographic run, or specific samples, by the operator. Any such action should be clearly documented on the batch summary sheet. Questionable chromatographic peak shape, resolution, or other problems with chromatography can be discussed with the Unit Lead or Deputy Director.

C. Evaluation of Potential Carryover: Carryover in the chromatogram is evaluated by injection of a blank sample immediately following the calibrator. Carryover of greater than 2% requires batch rejection, and remedial action for the instrument (e.g. replacement of injector insert, new septum and perhaps column trim or even replacement). Demonstrated carryover of less than 2% will require operator consideration with regard to the potential for effects on specific samples, and may require re-extraction of specific samples. In practice, when a question of potential carryover exists coming from the previous injection of case sample containing a high concentration of an analyte, the potentially affected sample may be repeated at the end of the batch.

D. Evaluation of Controls: Positive and negative controls are evaluated to allow for procedural batch acceptability.

E. Qualitative Results: Controls must demonstrate the target analyte with acceptable chromatography and spectral characteristics.

F. Quantitative Each batch must have at least 10% controls including a positive and

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negative. The controls can be re-injected in the middle and end of the batch to demonstrate the stability of the calibration. Acceptable results are the mean or target +/- 20% or +/- 2 standard deviations.

1. Levy-Jennings charts will be used monitor assay performance precision over time.

G. Internal standard abundance should be similar throughout the batch.

H. Linearity: Linearity of the calibration curve is demonstrable in each batch, for each analyte as a function of linear regression and quantitative results of control materials.

I. Sensitivity – Limits of Detection (LOD) and Quantitation (LOQ): For the purposes of this procedure, the LOD and LOQ are defined as equal to the lowest concentration of the lowest calibrator or control. LOD should be at least 3 times the signal to noise ratio, LOQ should be at least 10 times the signal to noise ratio.

J. Accuracy and Precision: Precision of the procedure is evaluated on a yearly basis, by repeat analyses of control or PT materials. Accuracy is expressed as a mean (absolute value) percentage difference between mean quantitative value of 10 reps of the specific control, and the target value. Precision is expressed as the CV of that value.

K. Specificity: Specificity is a function of both the resolution of target analyte during the analytical process, and the mass spectral fragmentation that analyte molecules undergo during the instrumental analysis. There has been no report of any material other than WAN and Basic drugs that elutes within 5% if the retention time of known standard materials, and produce the same fragmentation ions and ratios.

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13.0 REPORTING OF RESULTS:

Once the batch is completed and the data is complied, the batch undergoes a batch review (refer to SOP TX-5 for guidance).

Results of completed batches are entered into JusticeTrax.

Wherever possible, analytical results must be reviewed with reference to whatever case history or other information is available.

Procedural Uncertainty is reported with all quantitative results, (See SOP TX-19 section 6.3).

14.0 SOURCES OF ERROR

The utilization of 3-ion SIM and/or full scan methodology, with reference to procedural, controls and calibrators yields qualitative drug identification with essentially no qualitative uncertainty. Urine drug analyses are reported only as qualitative results.

15.0 REFERENCES

Clarke's Isolation and Identification of Drugs. 2nd Edition

UCT United Chemical Technologies: Solid phase extraction methods

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Appendix II:
GC/MS temperature program specifications

Parameter			
Initial temp	80° C		
Initial Time	1.00 min		
Ramps Rate/final temp/final time	rate	temp	time
	25.0	300	7.00
Post temp	80°c		
Post time	0.00		
Run Time	16.8 min		
Front inlet			
Equilibration time	0.5 min		
Mode	Pulsed split less		
Initial temp	250°c		
Constant Flow	1.0 mL/ min		
Pressure	8.56 psi varies		
Pulse pressure	35 psi		
Pulse time	0.50 min		
Purge flow	40.0 mL/ min		
Total flow	43.4 mL/min		
Gas type	Helium		
Injection volume	1 microliter		
Post injection washes Solvent A / Solvent B	Solvent A -3 Hexane Solvent B 3 Ethyl acetate		
Tune file	ATUNE.U		
Acquisition mode	Scan		
Solvent delay	2.5 min		
Low mass	40		
High mass	470		
Threshold	250		
MS Quad	150°c max 200		
MS Source	230°c max 250		

* changes to the above program may occur, this is acceptable if controls work as expected.

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Batch Name & Number:				**Batch summary**
	Analyst	Date	Test	Comments
Setup				
Samples Aliquoted				
Instrument # & Run Date				
Tune acceptable	Yes No			
Suitabilty acceptable				
Daily Standard	Yes No			
External Standard	Yes No			
New Reagents for Validation				
Vial Position Verified by				
QC- Qualitative				
QC negative acceptable	Yes No			
QC positive acceptable	Yes No			
Analyst Comments:				
Batch Reviewer Comments:				
Batch Acceptable	Yes No			
Batch Reviewer Initials/ Date				
State of Connecticut Department of Emergency Services and Public Protection				
Division of Scientific Services				
Once printed this version is no longer controlled.Use Qualtrax for the most current version.				

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Rev # History

5 Added chemical names in place of chemical formulas in multiple places. Add pH paper to Equipment list. To reagent preparation removed stability information and added to check pH with pH paper where appropriate. Added general statement that reagents are acceptable for use for 1 year, unless noted and may be revalidated after 1 year. Removed the need to mark reagents with green stickers, added that the validation information will be on the run sheet. Clarified that there are separate working standard solutions for the WAN and the BDS analytes. Corrected information for preparation of the IS, including the final concentrations. Removed Morphine Glucoronide from the BDS standard solution, made optional. Removed how to reconstitute UTAK, added to refer to manufacturer's product insert. Removed IS concentration information that was incorrect from procedure section. Corrected the Blank Blood volume

used for quantitative test set up. Added minor clarification information throughout document. Removed need to compare calibrators to themselves. Noted that the batch sheet was an example.

RETIRED

Title: Benzodiazepine Quantitative/Qualitative Analysis using Liquid Chromatography/Mass Spectrometry (LC/MS) (TX 31)

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1. Introduction

Benzodiazepines and the functionally related "Z drugs" (e.g., Zolpidem and Zopiclone) are drugs whose properties include anti-anxiety, hypnotic, and sedation. Benzodiazepines are class of anti-anxiety agents which interfere with the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Their effects include anti-anxiety, sedation, sleep, muscle relaxation, and anticonvulsant activity. Long-term benzodiazepine therapy can be associated with addiction and withdrawal symptoms. While benzodiazepines are considered safer than drugs in the barbiturate class, there is still significant potential for abuse and dependence with continued use. Samples that require confirmation by liquid chromatography/mass spectrometry (LC/MS) are extracted from buffered, diluted sample aliquots by adsorption onto solid phase extraction (SPE) columns. Benzodiazepines/Z-drugs that may be present are then eluted from the SPE columns, dried, and reconstituted before being analyzed using an LC/MS system. The detection of these types of drugs is determined using single point calibration cut off values. Quantitations are performed using a multipoint calibration graph with deuterated internal standards. Matrix-specific (blood and/or urine as needed) positive and negative controls are extracted and analyzed in each analytical batch. The presence of Benzodiazepines/Z-drugs may be confirmed in urine, blood or other aqueous fluids.

2. Scope

This procedure can be used for the qualitative and/or quantitative determination of biological specimens for the presence of select drugs and/or metabolites (e.g., alprazolam, α-hydroxyalprazolam, clonazepam, 7-aminoclonazepam, lorazepam, diazepam, nordiazepam, oxazepam, temazepam, Zolpidem, midazolam, flunitrazepam, 7-aminoflunitrazepam, flurazepam, N-desalkylflurazepam, triazolam, and zolpiclone). The addition of analytes to this group may not necessitate a complete validation to be performed of this procedure, but rather a verification that the analytes can be detected reproducibly and at a certain detection limit(s).

3. Principle

Biological specimens are analyzed for the presence of drugs of abuse (DoA) and/or their metabolites by extraction using solid phase extraction (SPE) columns. Final extracts are analyzed by LC/MS using ESI and can involve a combination of selected reaction monitoring (SRM) and full scan analysis modes.

4. Specimens

This procedure uses biological fluid(s) such as blood, urine, serum, and/or plasma. Qualitative analyses are usually limited to urine, blood, serum/plasma, and and/or vitreous samples while quantitative analyses are usually limited to blood (or serum/plasma). Blood sample collection tubes containing proper anticoagulant and preservative (i.e., 'gray-tops' containing potassium oxalate and sodium fluoride) should be used. Containers for urine and other sample types should be within sealed plastic (e.g., polypropylene) containers, however other containers may be acceptable. All samples should be received with proper documentation, have been properly sealed (i.e., prevent sample loss, contamination, or deleterious change), and have been properly stored. Once samples are received within the Toxicology Unit they will be properly stored within either a refrigerator or freezer. Typically 0.5 mL of sample is consumed during the analysis but varying volumes may be used, as necessary. Dilution of samples due to limited specimen or due to suspicion of high drug or metabolite concentration is acceptable.

5. Equipment/Materials/Reagents

5.1 General laboratory glassware

5.2 Disposable borosilicate test tubes (e.g., 16 x 100 mm, round bottom, borosilicate glass with Teflon caps)

5.3 Vortex mixer

5.4 Sonicator

5.5 Automatic pipettes (with disposable tips)

5.6 Positive pressure solid phase extraction device – SPEWare Cerex, 48 sample (or equivalent)

5.7 Sample concentrator with nitrogen – SPEWare Cerex 48 heated (or equivalent)

5.8 Centrifuge

5.9 pH paper (or equivalent)

5.10 Trace B Extraction Columns – SPEWare (or equivalent)

5.11 Liquid Chromatograph/Mass Spectrometer (Shimadzu LCMS-8030, or equivalent)

5.12 HPLC column – Kinetex, phenyl hexyl, 2.6 µm, 100Å, 50 mm x 4.6 mm (Phenomenex), or equivalent)

5.13 Pre-Column – SecurityGuard ULTRA Cartridge UHPLC Kinetex for 4.6mm ID Columns (Phenomenex or equivalent)

5.14 Autosample vials (LC/MS grade 1.8mL or equivalent)

5.15 Acetic acid, glacial ($CH_3COOH_{(l)}$, Reagent grade or equivalent)

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5.16 Ammonium formate (NH_4CHOO, Reagent grade or equivalent)

5.17 β-Glucuronidase (p. vulgata ; Sigma or equivalent)

5.18 Formic acid (HCOOH, Reagent grade or equivalent)

5.19 Methanol (MeOH, Reagent grade, LC/MS Grade, or equivalent)

5.20 Sodium acetate trihydrate ($NaCH_3COO \cdot 3H_2O$, Reagent grade or equivalent)

5.21 Sodium bicarbonate ($NaHCO_3$; Reagent grade or equivalent)

5.22 Sodium carbonate (Na_2CO_3 ; Reagent grade or equivalent)

5.23 Water (Mobile Phase) (LC/MS Grade ; Optima or equivalent)

5.24 Certified Reference Materials (alprazolam, α-hydroxyalprazolam, clonazepam, 7-aminoclonazepam, diazepam, desmethyldiazepam, lorazepam, oxazepam, temazepam, Zolpidem ; 1 mg/mL) (Cerilliant, Lipomed, or equivalent)

5.25 Benzodiazepine Control Mixtures (UTAK ; Blood (#12100) ; Urine (#12090)) or equivalent

5.26 Benzodiazepine Multi-Component CRM Mixtures (Cerilliant B-033-1ML) or equivalent

5.27 Ammonium $Formate_{(aq)}$: ($NH_4CHOO_{(aq)}$; 5M ; 31.5% (w/v)): Can be prepared by dissolving 3.15 g of ammonium formate in 10 mL of water. Stable for at least one (1) year in glass container when refrigerated.

5.28 Sodium $Acetate\ Buffer_{(aq)}$ (0.07M ; $NaCH_3COO$; pH~4.5): Can be prepared by combining 5.86 g of sodium acetate with 3.24 mL of glacial acetic acid in a 1 L volumetric cylinder and diluting to volume with water. Stable for at least one (1) year in glass container while at room temperature.

5.29 Sodium $Bicarbonate_{(aq)}$ (0.1M ; $NaHCO_3$; 0.84% (w/v) ; pH~8): Can be prepared by dissolving 4.2 g of sodium bicarbonate in water within a 500 mL volumetric flask and diluting to volume with water. Stable for at least one (1) year in glass container while at room temperature.

5.30 Sodium $Carbonate_{(aq)}$ (0.1M ; Na_2CO_3 ; 1.1% (w/v) ; pH~11): Can be prepared by dissolving 5.3 g of sodium carbonate in water within a 500 mL volumetric flask and diluting to volume with water. Stable for at least one (1) year in glass container while at room temperature.

5.31 $Bicarbonate/Carbonate\ Buffer_{(aq)}$ (pH~9): Can be prepared by transferring 0.1M sodium $bicarbonate_{(aq)}$ solution into a beaker and checking pH (should be pH~8). Adjust the pH to ~9 using the 0.1M sodium $carbonate_{(aq)}$ solution. Stable for at least one (1) year in glass container while at room temperature.

5.32 Solid Phase Extraction Elution Solution (SPEES) (e.g., 50 mL ; ~2 mL needed per SPE sample): {Dichloromethane (CH_2Cl_2) : IPA : NH_4OH (80 : 18 : 2)}.
Can be prepared by adding 9 mL of isopropanol to 1 mL of ammonium hydroxide within a 50 mL

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volumetric cylinder. To this mixture add 40 mL of methylene chloride and mix. This solution will be prepared when needed for use.

5.33 $MeOH_{(aq)}$ (20% (v/v)): Can be prepared by adding 80 mL of water and 20 mL of methanol to a 100 mL volumetric cylinder and mixing. Stable for at least one (1) month in glass container when refrigerated.

5.34 Methanol-HCl (1% (v/v)): Can be prepared by dissolving 10 µL of hydrochloric acid into 990 µL of methanol (or in different volume with an equivalent ratio). Stable for at least one (1) month in glass container when refrigerated. Verify pH is ≤ 3 prior to use.

5.35 Mobile Phase A – 0.01 %(v/v) $HCOOH_{(aq)}$ and 5mM $NH_4CHOO_{(aq)}$:
Can be prepared by mixing 50 µL of formic acid with 0.5 mL of 5M ammonium formate in a 500 mL volumetric cylinder, diluting to volume with [Mobile Phase] water, and mixing well. Store in glass at room temperature. Stable for at least one (1) week while in a closed state.
{Note: 5mM $NH_4CHOO_{(aq)}$ is equivalent to 0.0032 % (w/v) $NH_4CHOO_{(aq)}$.}

5.36 Mobile Phase B – (MeOH or CH_3OH). Store in glass at room temperature – stable indefinitely in a closed state at room temperature.

Note-01: Volumes can be adjusted using appropriate ratios to account for the number of samples that are to be extracted (~2 mL are needed for each extraction sample).

Note-02: Unless otherwise noted the above solutions are stable for at least one (1) year when stored in a refrigerator/freezer from either their creation or from their last verification. Check clarity and check pH (when indicated as having a pH value) for all solutions prior to use.

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6. Standards and Controls

Positive and Negative Controls and Calibrators:
Can be purchased (e.g., UTAK Labs) or generated in-house (e.g., spiked/un-spiked blank blood, spiked/un-spiked blank urine, spiked/un-spiked vitreous (or water)). Store frozen, refrigerated, or obtain fresh. If purchased, the stability should be determined by the manufacturer. Appropriate positive and negative controls will be extracted and analyzed with each assay or batch. Externally-prepared controls and calibrators are preferred, when feasible. Internally-prepared controls will be used only when externally-prepared controls are not available. When possible, control and calibrator solutions will be matrix-matched.

Internal Standard Analytes:
Purchased from suitable vendor(s) in solutions or neat solids (e.g., 0.1 mg/mL solution). Storage and stability is determined by manufacturer(s). Additional or alternative deuterated compounds may be used. If purchased solutions vary in concentration than what has typically been used, analysts may adjust the preparation volumes to account for differences. Any variations will be noted on solution preparation

worksheets and, if such worksheets are not linked to case files, variations will be noted in case files (e.g., notes, summary sheets).

Preparation of Calibrators, Controls, Standards:

Internal Standard(s)

6.1 Internal Standard (I.S.) Working Solution (Diazepam–D_5 ; 250 ng/mL):
Can be prepared by adding 250 µL of the 100 µg/mL Diazepam-D_5 into a 100 mL volumetric flask and diluting with MeOH. Mix and store refrigerated in glass. Stable for at least 1 year.

Note-03: Additional internal standard analytes can be used but must be documented within batch notes:
Amphetamine-D_6
Oxycodone-D_3
Benzoylecgonine-D_3

6.2 [Optional] Instrument Performance Standard Mix Solution: ((Diazepam-D_5) ; 10 ng/mL)

This is optional and can be used to evaluate the instrument in addition to the performance check solution for the LC/MS instrument.

Dilute the I.S. Working Solution 1:25 with water to make a 10 ng/mL final concentration. This can be done by mixing 20 µL of the I.S. Working Solution, combining it with 500 µL of deionized water, placing it into an autosample vial, and capping. This solution will be prepared when needed for use.

Note-04: Other analytes within this [Optional] I.S. Solution (if present) are not evaluated – just the Diazepam-D_5 analyte is used for evaluation purposes.

Controls

6.3 Negative Control Blood:

Purchased (e.g., Diagnostics Products Corporation, UTAK Labs), donated (e.g., American Red Cross, hospital blood bank), or in-house demonstrated negative control blood (e.g., from a proven blank specimen). Store frozen or refrigerated. If purchased or donated, stability should, when possible, be determined by manufacturer.

6.4 Negative Control Urine:

Purchased, acquired (e.g., Office of the Chief Medical Examiner (OCME)), or in-house demonstrated to be negative. Store refrigerated or frozen.

6.5 Negative Control [Other Matrix]

Synthetic or in-house negative controls [other matrix] which are analyte-free. Store refrigerated or obtain fresh. If purchased, stability should be determined by manufacturer. A negative control [other matrix] should be extracted and analyzed with each appropriate assay or batch.

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6.6 Positive Control Blood (Externally prepared ; 100 ng/mL)

Lyophilized whole blood containing select analytes can be used (e.g., UTAK Benzodiazepines Plus 100 ng/mL ; Product #12100 ; approximately 23 analytes). These are usually reconstituted with water to a certain volume (e.g., 5 mL). Follow manufacturer's recommendation for preparation. Prepare fresh and mix adequately. Store reconstituted control material refrigerated. Stability (e.g., 25 days) should be listed by manufacturer but can be longer if verified prior to use.

6.7 Positive Control Urine (Externally prepared ; 100 ng/mL)

Lyophilized urine containing select analytes can be used (e.g., UTAK Benzodiazepines Plus 100 ng/mL ; Product #12090 ; approximately 25 analytes). These are usually reconstituted with water to a certain volume (e.g., 5 mL). Follow manufacturer's recommendation for preparation. Prepare fresh and mix adequately. Store reconstituted control material refrigerated. Stability (e.g., 25 days) should be listed by manufacturer but can be longer if verified prior to use.

Note-05: The externally-prepared Urine positive control can be used as a positive control for other matrices if they have a similar make-up (e.g., water in both vitreous and urine).

6.8 Positive Control Blood and Urine Solutions (In-House prepared ; 100 ng/mL ; e.g., from Lipomed) (Only used when External Positive Controls are not available)
{alprazolam, α-hydroxyalprazolam, clonazepam, 7-aminoclonazepam, diazepam, nordiazepam, lorazepam, oxazepam, temazepam, Zolpidem}

6.8.1 In-House Positive Control Stock Solution (100 µg/mL ; e.g., Lipomed ; 10 analytes above)

Combine 100 µL of the following ten (10) 1 mg/mL reference standards (e.g., from Lipomed) into properly labeled screw-capped test tubes and mix (total volume = 1 mL):

alprazolam	α-hydroxyalprazolam	clonazepam	7-aminoclonazepam	diazepam
desmethyldiazepam	lorazepam	oxazepam	temazepam	Zolpidem (tartrate)

6.8.2 In-House Positive Control Working Solution (2.0 µg/mL ; 10 analytes (above))

Can be prepared by adding 100 µL of the In-House Positive Control Stock Solution (100 µg/mL) into a 5 mL volumetric flask that contains some methanol and bringing to volume with methanol. Tightly cap, mix, protect from light, properly label, and store in freezer. Stable for at least six (6) months.

6.8.3 In-House Positive Control Blood Solution (100 ng/mL ; 10 analytes (above))

Can be prepared by adding 50 µL of the In-House Positive Control Working Solution (2.0 µg/mL) to 500 µL of blank blood. Tightly cap, mix, protect from light, properly label, and store in freezer. Stable for at least six (6) months.

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6.8.4 In-House Positive Control Urine Solution (100 ng/mL ; 10 analytes (above))

Can be prepared by adding 50 µL of the In-House Positive Control Working Solution (2.0 µg/mL) to 500 µL of blank urine. Tightly cap, mix, protect from light, properly label, and store in freezer. Stable for at least six (6) months.

6.8.5 In-House Positive Control [Other] (100 ng/mL ; 10 analytes (above))

Can be prepared by adding 50 µL of the In-House Positive Control Working Solution (2.0 µg/mL) with 500 µL of appropriate matrix. Tightly cap, mix, protect from light, properly label, and store in freezer. Stable for at least six (6) months.

Calibrators

Solutions used for calibrations should be prepared using as few dilutions as possible and will be from certified reference materials (CRM). While this procedure lists specific analytes, equivalent reference standards may be substituted, if needed. Calibrators should be from different suppliers than those used for control solutions, when possible. If the same supplier is used for both, then calibrator and control solutions should be prepared from solutions containing differing lot numbers. When purchased standards are not 1 mg/mL concentrations, when different analytes are prepared as calibrators, or when the number of analytes differ, then appropriate adjustments will be made and a second Analyst should confirm calculations, dilutions, and other preparatory work so as to ensure quality. Such changes will be recorded within appropriate case notes, batch preparation documents, and/or reagent log books. Older calibration curves may be used and fresh calibrators do not need to be prepared for every new batch (see note below).

6.9 Benzodiazepine Calibrator Stock Sol'n (10 component calibrator mix ; 100 µg/mL) {e.g., alprazolam, α-hydroxyalprazolam, clonazepam, 7-aminoclonazepam, diazepam, desmethyldiazepam, lorazepam, oxazepam, temazepam, Zolpidem}

6.9.1 Combine 100 µL of the following ten (10) 1 mg/mL reference standards (e.g., from Cerilliant) into properly labeled screw-capped test tubes and mix (total volume = 1 mL):

alprazolam	α-hydroxyalprazolam	clonazepam	7-aminoclonazepam	diazepam
desmethyldiazepam	lorazepam	oxazepam	temazepam	Zolpidem (tartrate)

6.9.2 Tightly cap, protect from light, and store in freezer.

6.9.3 Stable for at least six (6) months when stored in freezer.

6.10 Benzodiazepine Calibrator Working Solution-01 (WS-01 ; 10 component mixture ; 2000 ng/mL)

6.10.1 Add 100 µL of the Benzodiazepine Calibrator Stock Solution (100 µg/mL) into a properly labeled 5 mL volumetric flask that contains some methanol.

6.10.2 Dilute to volume with methanol, tightly cap, mix, protect from light, and store in freezer.

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6.10.3 Stable for at least six (6) months when stored in freezer.

6.11 Benzodiazepine Calibrator Working Solution-02 (WS-02 ; 10 component mixture ; 200 ng/mL)

6.11.1 Add 500 µL of the WS-01 solution (2000 ng/mL) into a properly labeled 5 mL volumetric flask that contains some methanol.

6.11.2 Dilute to volume with methanol, tightly cap, mix, protect from light, and store in freezer.

6.11.3 Stable for at least six (6) months when stored in freezer.

6.12 Blood Calibrator Solutions

Combine the following Calibrator Working Solutions (WS-01 and WS-02) to 500 µL of matrix (blood and/or urine) to appropriately labeled screw-capped test tubes according to the table, tightly cap, and mix:

Calibrator Concentration	Volume of Calibrator Working Sol'n-01 (WS-01 ; 2 µg/mL)	Volume of Calibrator Working Sol'n-02 (WS-02 ; 200 ng/mL)	Volume of Matrix
5 ng/mL	–	12.5 µL	500 µL
10 ng/mL	–	25 µL	500 µL
20 ng/mL	–	50 µL	500 µL
50 ng/mL	–	125 µL	500 µL
100 ng/mL	25 µL	–	500 µL
200 ng/mL	50 µL	–	500 µL
500 ng/mL	125 µL	–	500 µL

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Note-06: Pre-made CRM mixtures containing benzodiazepines can be used and diluted appropriately to make alternative calibrator solutions (e.g., using 250 µg/mL ; Cerilliant ; B-033-1ML). Appropriate quality measures will occur (as stated above) in such situations.

Note-07: A new calibration curve is not required for each batch. Historical calibration curves can be used. The validation of the curve will be demonstrated through the application of positive controls extracted with each batch of samples. When there have been substantial instrument changes (e.g., a new column installed) then a new calibration curve should be prepared and used.

7. Procedure

7.1 Prepare all necessary solutions (e.g., calibrators, controls, reagents, mobile phases).

7.2 Individually add 0.5 mL of evidentiary samples into properly labeled screw-capped test tubes. Within each batch of samples ensure that the correct samples transfer into the correct tubes. Cap after each sample transfer or otherwise reduce the risk of cross-contamination between samples (e.g., by moving tubes after each addition).

7.3 Add 0.5 mL of control matrix solutions into properly labeled screw-capped test tubes.

7.4 Individually add 100 µL of internal standard (Diazepam–D_5, 250 ng/mL) into each sample test tube (25 ng of Diazepam–D_5), cap, and briefly vortex-mix (e.g., ~10 seconds).

7.5 Individually add 1 mL of 0.10 M sodium acetate buffer (pH 4.5) to each tube.

7.6 For blood samples only: Add 500 µL of DI water to each blood sample.

7.7 Cap, briefly vortex-mix, and then centrifuge **all** tubes for ~10 min at ~5000 rpm.

7.8 Appropriately label Trace B solid phase extraction (SPE) columns to correspond with each sample.

7.9 Place labeled Trace B extraction columns in the SPE column rack in the appropriate order and position the plastic waste tray (labeled "Methanol") underneath the SPE column rack.

7.10 Condition each SPE column sequentially with:

7.10.1 Methanol (1 mL)

7.10.2 Drain (≈ 3 psi) to solvent hazardous waste stream

7.11 Remove the plastic waste tray labeled "Methanol" and replace with the plastic tray labeled "Biohazardous/Buffers" (non-hazardous regulated waste stream) underneath the SPE column rack.

7.12 Rinse each SPE column sequentially with:

7.12.1 Water (1 mL)

7.12.2 Drain (≈ 3 psi) to non-hazardous regulated waste stream

7.13 Individually and carefully transfer samples to the centers of the appropriate SPE columns. Avoid any sediment found at the bottoms of the test tubes and avoid splashing.

7.14 Allow gentle drop-wise flow to the non-hazardous regulated waste stream via gravity. Use some pressure (e.g., ≤3 psi), if necessary, to express samples through the columns.

7.15 Sequentially perform the following wash/rinse on each SPE column:

7.15.1 Bicarbonate/Carbonate $Buffer_{(aq)}$ (1 mL ; pH~9)

7.15.2 Drain (~3 psi) to non-hazardous regulated waste stream

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7.15.3 Water (1 mL)

7.15.4 Drain (~3 psi) to non-hazardous regulated waste stream

7.16 Dry the columns for ~10 minutes using maximum pressure (e.g., between 60-80 psi).

7.17 During this 10 minute window (or earlier) actions such as the labeling the collection tubes (or autosample vials) and placing them in the appropriate order into the SPE collection rack to prepare for elution collection can be performed.

7.18 Once the SPE columns are dry replace the plastic waste tray with the SPE collection rack containing the collection tubes (or autosample vials).

7.19 Important: Ensure proper placement of collection tubes and that each corresponds to correctly labeled SPE column.

7.20 Elute the SPE columns:

7.20.1 Elution #1:

7.20.1.1 Add 1.0 mL of the Solid Phase Extraction Elution Solution (80:18:2 CH_2Cl_2:IPA:NH_4OH) to each SPE column.

7.20.1.2 Set the flow at ~2-4 mL/min for optimal recovery.

7.20.1.3 Collect eluent into appropriately labeled tubes.

7.20.2 Elution #2:

7.20.2.1 Add 1.0 mL of the Solid Phase Extraction Elution Solution (80:18:2 CH_2Cl_2:IPA:NH_4OH) to each SPE column.

7.20.2.2 Set the flow at ~2-4 mL/min for optimal recovery.

7.20.2.3 Collect eluent into appropriately labeled tubes.

7.21 Remove the top SPE column rack and transfer the collection rack from the SPE manifold to the sample concentrator. Ensure concentrator parts are clean and free of contamination.

7.22 Add one (1) drop of MeOH-$HCl_{(aq)}$ and evaporate all samples to dryness at ~<40^0 C.

7.23 If samples are not to be immediately analyzed they should be tightly capped, protected from light, and placed in a refrigerator or freezer under proper evidentiary seal.

Note-08: The word ‘immediately’ refers to instrumental sequences being started on the same day.

7.24 Reconstitute each collection tube (or autosample vial) with the same volume of $MeOH_{(aq)}$ (20% (v/v)) – no more than 150 µL.

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All calibrators, controls, and samples must be reconstituted with the same volume of MeOH. If the volume is different from 150 µL then this must be recorded within the appropriate documentation (e.g., case notes, summary sheets).

7.25 If not collecting into autosample vials, individually transfer elution solutions into properly labeled auto sampler vials containing sample inserts.

Setting-up Instrument with Samples:

7.26 Ensure the appropriate instrumental quality assurance/quality control (QA/QC) procedures were performed. The instrument must have passing QA/QC results prior to preparing and loading of samples.

7.27 Prepare the sequence and enter the samples in the appropriate order. Negative controls will be analyzed prior to evidentiary samples. Calibrators can be analyzed either before or after evidentiary samples. Blank samples (i.e., those containing just $MeOH_{(aq)}$ (20% (v/v))) should be analyzed in-between evidentiary samples to avoid carry-over and will be analyzed after the highest calibrator is injected.

7.28 Place the labeled autosample vials in the appropriate order within the instrument.

7.29 Save the sequence to the day's date. Ensure lab number, operator/analyst's name(s), and instrument name are recorded within each sample.

7.30 Sequence Verification:

7.30.1 Print the sequence list.

7.30.2 Check that the physical placement of the autosample vials and the vial positions within the instrument's sequence list match.

7.30.3 Once the check has been completed place an indication of the sample check (e.g., 'sequence checked' or 'sequence verified') on the sequence page along with Analysts initials and date.

7.31 Print the instrument method and include both the method and the sequence printouts with the batch documents.

7.32 Begin the sequence and analyze the samples.

7.33 After the batch has completed:
Re-check that the physical placement of the autosample vials and the vial positions within the instrument's sequence list match. Once the re-check has been completed place an indication of the sample re-check (e.g., 'sequence re-checked post analysis' or 'sequence re-verified post analysis') on the sequence page along with Analyst's initials and date.

7.34 Analyze extract solutions using LC/MS.
Blank solutions (i.e., solvent, mobile phase) will be analyzed in-between samples and after highest calibrator solution.

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8. Instrumental Parameters

The following are the typical operating parameters for the instrument used in this procedure. With approval from the Lead Examiner and Deputy Director (or higher), the instrument conditions may be modified to adjust or improve the procedure. Documentation of such changes must be included with batch data so that any instrumental parameter change can be associated with data and casework until this procedure has been updated.
The appendix contains an abbreviated version of the procedure. The checklist can be used by analysts. Any changes within the instrumental parameters, if listed on the checklist, will be reflected on the checklist by the analyst filling it out.

HPLC Parameters:

Mobile Phase A: 0.01 %(v/v) $HCOOH_{(aq)}$ and 5mM $NH_4CHOO_{(aq)}$
Mobile Phase B: $MeOH_{(l)}$ (neat)
HPLC Column: Kinetex, 2.6 µm Phenyl-Hexyl, 100 Å, 50 mm x 4.6 mm (Phenomenex)
Column Temperature: 40 °C
Constant Flow: 0.6 mL/min.
Autosampler Temperature: 15 °C
Injection Volume: 10 µL
Needle Wash: 500 µL before and after aspiration

Gradient Program: Initial 20% B ; Flow 0.6 mL/min

Time (min.)	Mobile Phase A [0.01 %(v/v) $HCOOH_{(aq)}$ + 5mM $NH_4CHOO_{(aq)}$] (%)	Mobile Phase B $MeOH_{(neat)}$ (%)
Initial	80	20
1.00	80	20
2.00	55	45
3.00	55	45
3.50	40	60
9.00	40	60
11.00	25	75
11.50	0	100
12.50	0	100
12.51	80	20
15.00	STOP	STOP

Mass Spectrometer Parameters:

Ionization Source	Electrospray	**Polarity**	Positive Ion
Ion Spray Potential	+4.5 kV	**Heat Block**	400 °C

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Nebulizer Gas	Nitrogen	**Nebulizer**	3 L/min
Drying Gas	Nitrogen	**Drying Gas**	15 L/min.
Scan Type	MRM (&/or Full Scan)	**Resolution**	Unit
Desolvation Line (DL)	250 °C		

Transition Ions Monitored

Using the LabSolutions® optimization program, the following transitions were identified:

Drug/Metabolite	Precursor Ion	Quantification Ion	Reference Ion(s)
7-Aminoclonazepam	285.60	121.10	222.10, 250.05
7-Aminoflunitrazepam	283.90	135.15	227.05, 121.20
Alprazolam	308.45	281.10	205.10, 165.15
α-Hydroxyalprazolam	324.85	297.10	216.05, 205.05
Clonazepam	315.60	270.0	214.00, 241.00
Desalkylflurazepam	288.60	139.90	226.15, 165.00
Diazepam	284.90	154.10	91.10
Flunitrazepam	313.60	268.05	239.10, 183.00
Flurazepam	387.50	315.00	317.00, 288.00
Lorazepam	321.80	275.95	230.00, 195.00
Midazolam	325.60	291.10	223.05, 249.05
Nordiazepam	270.70	140.00	165.00, 208.10
Oxazepam	286.80	241.05	104.05, 77.05
Oxazepam	462.90	286.85	241.10, 269.10
Temazepam	300.60	255.00	177.00, 193.10
Temazepam	476.70	301.00	254.95, 283.10
Triazolam	342.80	308.05	315.00, 239.00
Zolpidem	307.60	235.20	236.15, 263.15
Zopiclone	388.95	245.05	217.00, 112.05
Diazepam-D_5	289.85	154.00	198.10, 227.00

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9. Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. Retention time (chromatographic characteristic), fragmentation pattern and qualified ion ratios (mass spectrometric characteristics), and other characteristics are used as the basis for detection and identification. In most cases all of the criteria below should be met in order to identify the appropriate drugs within biological specimens.

9.1 Chromatography

All chromatographic peaks for the analytes of interest should show good chromatographic characteristics, with reasonable peak shape, width, and resolution. For low concentrations of an analyte (e.g., ≤5 ng/mL), there may be transitions that are not optimal. In order to be determined as acceptable, a chromatographic peak in a sample should compare favorably to the same analyte's chromatographic peak in a known sample which has been analyzed on the same system and in the same, or subsequent, analytical timeframe. Additionally, the following two criteria should be met:

9.1.1 Retention Time (RT)

The retention time of a peak of interest should be within 0.1 minute of, or +/- 3% relative (relative or absolute) to, the retention time of a reference standard (i.e., calibrator or positive control).

Note-09: Instrumental evaluation of retention time using +/-0.1 minute as a criteria is acceptable. Using ±2 minute window is also acceptable for sample/control evaluation.

If analyzed, the retention times of the analytes within the Procedural Performance Solution should be within ±2 % of their previously analyzed times and/or to a set acceptable value. Minor changes in mobile phase percentage may account for slight retention time shifts.

The following table can be used for approximate retention times:

Analyte	Retention Time (min)	Analyte	Retention Time (min)
7-Aminoclonazepam	4.29	Alprazolam	7.07
Zolpidem	5.26	Temazepam	7.38
Clonazepam	6.35	Nordiazepam	7.63
Lorazepam	6.42	Diazepam-D_5	8.75
α-Hydroxyalprazolam	6.54	Diazepam	8.83
Oxazepam	6.58		

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9.1.2 Signal-to-Noise Ratio (SNR)

To justify the existence of a peak, its baseline signal-to-noise ratio (SNR) will exceed 3. Furthermore, the baseline signal for the peak of interest will be at least 10-fold greater than that for any observed peak (corresponding to the analyte of interest) at a similar retention time in a negative control or blank sample that was injected just prior to that sample.

9.2 Mass Spectrometry

In order for the MS to be considered in good operating condition, the correct mass assignments for each of the analytes in the performance standard should be present. Independent MS/MS experiments are conducted for each analyte.

Analysts will review transition ion ratios and use them for identifications. Determination of the presence of target analytes in sample extracts are identified by appearance and ratio of product ions that are characteristic of analytes at appropriate retention times. In this manner, both retention times, mass spectral fragmentation patterns, and ion ratios are used as the basis for qualitative identification. For the identification of an analyte to be made retention times of chromatographic peaks should be within 0.1 minute of corresponding analytes from reference standards (i.e., calibrators or positive controls). However, since ion rations are not infallible, the meeting of this criteria is not mandatory.

Ion ratios should compare favorably to ion ratios from reference standards or an extracted positive control at a comparable concentration (e.g., positive control). Generally, ion ratios are within the following limits:

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Expected [Set] Ion Ratio	Allowance
>50 %	20%
20% - 50%	25%
<20%	30%

9.3 Batch Acceptance:

In order for a batch to be acceptable:

9.3.1 No analytes of interest will be detected in the Negative Control.

9.3.2 Chromatography evaluation (above) should be met.

9.3.3 Mass spectral evaluation (above) should be met. The 100 ng/mL positive control will be utilized to set expected ion ratios for applicable analytes.

9.3.4 Significant carry-over will be brought to the attention of the lead Examiner to determine if evidentiary samples have been negatively impacted. If so, re-analyses will occur and sample re-extraction may be necessary. Appropriate case documentation will accompany these instances within affected case files to record events.

Note-10: An analyte is considered applicable if it is important to the batch. Analytes of interest are considered those compounds that are being reported.

9.3.5 All applicable analytes of interest within Positive Controls, as well as internal standards, will be identified.

9.3.6 Quantitative results of positive controls must fall within ±20% of the analyte's target concentration.

9.3.7 Statistical data of positive controls should be recorded and evaluated within appropriate charts. If controls fall out of acceptable range then applicable controls should be discarded and new controls utilized.

9.4 Unknown Sample Compound Detection:

This procedure is intended to be used for quantitative purposes. However, if enough data is present, confirmations of analytes not quantitated can be reported if a different chromatographic or mass spectrometric experiment is employed (or with approval of Lead Examiner and Deputy Director (or higher)).

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10. Calibration

Calibration is accomplished by the addition of known amounts of analytes (in addition to an internal standard) into matrix-matched blank samples. A calibration graph is generated from the resulting data and used as the basis for the quantitation of unknown samples. Blood samples are typically the only matrix quantitated so only a blood calibration graph for each drug and/or metabolite is used with each batch. A correlation coefficient should be ≥ 0.990 when using deuterated internal standards. If the correlation coefficient is lower than 0.990 then approval from the appropriate lead analyst or higher must occur and, if deemed acceptable, justification must accompany the applicable data. If using non-deuterated internal standards a correlation coefficient ≥ 0.98 is acceptable. While a calibration curve for each drug and/or metabolite may be analyzed with each batch, a previously established (or "historical") calibration curve may be used. Following linear regression, reprocessed calibrators generally should be within 20% of their

target value, but this is not mandatory. Calibrators will not be removed from calibration graphs without approval from the lead Examiner or higher. Such removal of calibration points will be appropriately documented within batches and within each case file (e.g., case notes, summary sheets). Calibration data are used for the analysis of each batch and are done independently.

Samples resulting in concentrations higher than the highest calibrator should be diluted (approval not to dilute must be obtained by the Unit Lead or higher). Such instances may involve reporting results as 'greater than the highest calibrator' may be acceptable and would avoid dilution and re-analysis. The lower limit of quantitation (LLOQ) is the concentration of the lowest calibrator. The upper limit of quantitation (ULOQ) is the concentration of the highest calibrator. Extrapolation of the calibration graph beyond the LLOQ and ULOQ values can be done with permission of the lead Examiner, but must be documented within each applicable case file. The urine control is used for qualitative purposes only but, if necessary, it can be quantitated based on the blood calibration curve.

Calibration graphs and quantitative results are obtained by comparing chromatographic peak responses [or peak areas] to corresponding peak responses [areas] from internal standards. Calibration graphs are created and equations are obtained by plotting peak area ratios against corresponding calibrant concentrations for each analyte of interest. Based on resulting calibration equations concentrations of select analytes within evidentiary samples are obtained based on corresponding ratios of analyte peak areas to internal standard areas.

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11. Uncertainty

Some sources of measurement uncertainty in this procedure may include:

- Historical random uncertainty of repeated measurements
- Accuracy of the pipette(s) used to deliver sample volumes and/or calibrators
- Uncertainty in the concentration(s) of calibration standards and/or positive controls
- Instrument performance
- Human error

Uncertainty information for this procedure/instrument has been determined and can be included when quantitation values are reported.

It has been established that no known inferences are present within the calibrators or controls. Ion suppression or enhancement and potential interferences from other analytes has not been found for the common drugs and metabolites typically seen within casework.

12. Limitations

Limits of Detection (LOD), Lower Limits of Quantitation (LLOQ), Upper Limits of Quantitation (ULOQ)

Analyte	LOD – Urine (ng/mL)	LOD – Blood (ng/mL)	LLOQ – Blood (ng/mL)	ULOQ – Blood (ng/mL)
7-Aminoclonazepam	5*	5*	5*	500**
7-Aminoflunitrazepam	5*	5*	5*	500**
Alprazolam	5*	5*	5*	500**
α-Hydroxyalprazolam	5*	5*	5*	500**
Clonazepam	5*	5*	5*	500**
Desalkylflurazepam	5*	5*	5*	500**
Diazepam	5*	5*	5*	500**
Flunitrazepam	5*	5*	5*	500**
Flurazepam	5*	5*	5*	500**
Lorazepam	5*	5*	5*	500**
Midazolam	5*	5*	5*	500**
Nordiazepam	5*	5*	5*	500**
Oxazepam	5*	5*	5*	500**
Oxazepam	5*	5*	5*	500**
Temazepam	5*	5*	5*	500**
Temazepam	5*	5*	5*	500**
Triazolam	5*	5*	5*	500**
Zolpidem	5*	5*	5*	500**
Zopiclone	5*	5*	5*	500**

*Administratively set – values may actually be lower

**Administratively set – values may actually be higher

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13. Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment should be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Biological specimens will be handled using universal precautions and will be treated as biohazardous. Potentially contaminated items and surfaces will be cleaned prior to use, especially. When casework samples are being processed/analyzed brown paper (or other similar barrior) should be placed in between the surface and the specimens.

14. References

14.1 Screening and Confirmation of Benzodiazepines in Blood by Electrospray LCMSMS. West Chester County Department of Laboratories and Research: Division of Forensic Toxicology.

14.2 Solid-Phase Extraction Method for Trace B Columns. SPEWARE: Baldwin Park, CA.

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Appendix:

Bench Method: Benzodiazepine Quantitative/Qualitative Analysis using LC/MS

The following is an abbreviated version of this procedure and a pdf-copy can be used at the bench level by analysts.

Negative Control [drug-free] Blood	**Lot:**
Negative Control [drug-free] Urine (or Other)	**Lot:**
Positive Control Blood	**Lot:**
Positive Control Urine (or Other)	**Lot:**

Procedure:

	For Blood Specimens	
Prepare all necessary solutions (e.g., calibrators, controls, reagents, mobile phases).		
Add 0.5 mL sample/negative control/positive control into properly labeled screw-capped test tubes (e.g., 16 x 100 mm)		
	Add 100 µL of I.S. Working Sol'n to each sample (25 ng Diazepam–D_5):	**Lot:**
Add 1 mL of Sodium Acetate $Buffer_{(aq)}$ (0.1M ; pH 4.5) to each test tube.		**Lot:**
Add 500 µL of water to each blood sample, cap and vortex-mix samples, sonicate, Centrifuge.		
Label Trace-B extraction [SPE] columns appropriately.		**Lot:**
Place labeled Trace-B extraction columns in the SPE column rack in the appropriate order.		
Add 1 mL MeOH (to condition SPE), drain (~3 psi)		**Lot:**
Add 1 mL of water (to rinse each SPE column), then drain (~3 psi)		**Lot:**
Individually transfer samples/controls/calibrators to the center of their respective [properly labeled] SPE columns. Avoid sediment / splashing. Allow to migrate through SPE column via gravity. Apply small pressure, if needed (≤ 3psi).		
To each SPE column:		
	Wash with 1 mL of Bicarbonate/Carbonate buffer (pH ~9), then drain (~3 psi)	**Lot:**
	Rinse with 1 mL water, then drain (~3 psi)	**Lot:**
Dry the columns for 10 minutes using maximum pressure (e.g., between 60-80 psi)		
During 10 minute window (or earlier) actions such as proper labeling, placement in rack, preparation of SPEES can occur.		
The Solid Phase Extraction Elution Solution (SPEES) ratio is 80:18:2 CH_2Cl_2:IPA:NH_4OH.		
	Methylene Chloride (CH_2Cl_2)	**Lot:**
	Isopropyl Alcohol (2-Propanol ; IPA)	**Lot:**
	Ammonium Hydroxide (NH_4OH)	**Lot:**
After ~10 minutes replace plastic waste tray with SPE rack containing collection tubes. Ensure they are properly placed.		
Elute columns with 2x with 1 mL of Elution Solution. Flow using gravity (≤3 psi if needed)		**Lot:**
Transfer collection rack from SPE manifold to sample concentrator		
Add ~1 drop of $MeOH\text{-}HCl_{(aq)}$ and evaporate to dryness (~<40 ˚C) ; Store refrigerated, protected from light, and properly sealed if not immediately analyzed.		
Reconstitute each extract sample with 150 µL of 20% $MeOH_{(aq)}$; only if analyzing immediately		**Lot:**
Load samples, set up sequence, printout sequence/method, verify sequence w/ initials, and analyze using LC/MS		

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Appendix (Cont'd):

Specimen Procedure (Cont'd)

Analyze by LC/MS:

Instrument: LC/MS	Instrument Identifier:
HPLC Column: Phenomenex ; Kinetex Phenyl-Hexyl ; 100 Å ; 2.6 µm ; 50 mm x 4.6 mm):	Manufacturer: Lot:
Mobile Phase A: [0.01 %(v/v) $HCOOH_{(aq)}$ + 5mM $NH_4CHOO_{(aq)}$]	Lot:
Mobile Phase B: MeOH (CH_3OH) – 100%	Lot:

Gradient Program:
Initial 20% B ; Flow 0.6 mL/min

Time (min.)	%B
Initial	20
1.00	20
2.00	45
3.00	45
3.50	60
9.00	60
11.00	75
11.50	100
12.50	100
12.51	20
15.00	STOP

Mass Spectrometer Parameters

Ion Source	Electrospray
Spray Voltage	+4.5
Polarity	Positive
Nebulizer Gas	Nitrogen (3 L/min.)
Drying Gas	Nitrogen (15 L/min.)
Scan Type	MRM (&/or Full Scan)
Heat Block	400 °C
Resolution	Unit

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Revision #	Revision History
4	Section V.I E. Calibration removed requirement to compare the calibrators to the calibration curve generated. Section V. replaced autosampler vial with collection tube in multiple places. V.21 changed the reconstitution volume from 520 to 150 ul. Added line 22 Individually transfer solutions to properly labeled auto sampler vials containing sample inserts.
5	Use of pH meters removed. Added pH paper to equipment list and added check of pH for 0.1M sodium acetate buffer.
6	Updated information on the preparation of standards and controls allowing for them to be made up in methanol and stored frozen as opposed to making them each day of use. Added statement about use of primary purchased standards that are not 1mg/mL.
7	Title change. Reformatting throughout document. Minor wording changes throughout introduction/scope/principle/specimens-parts of procedure. Added statement about using externally-prepared controls before internally-prepared controls, when possible. Updated reagents, preparation of calibrators and controls, decreased number of controls, ensured use of external calibrator solutions and controls over internally-prepared solutions, edited procedure for clarity, added MeOH-HCl step prior to evaporation to dryness. Added verification of sequence step and printing out of documents. Added need to get approval and to document record any changes from SOP. Organized instrumental parameters, updated decision criteria, updated Calibration and Uncertainty, and Limitation sections. Updated Appendix for method summary.

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I. INTRODUCTION

Opioids include both natural opiate alkaloids (*e.g.,* morphine, codeine) as well as their synthetic derivatives (*e.g,.* hydromorphone, oxycodone, fentanyl). These drugs exert their effects through binding at the mu, kappa, and delta opioid receptors producing sedation and analgesia. Opioids are widely prescribed for pain management, but there is a high potential for abuse and dependence with continued use. In addition, their increasing presence in illicit drug markets makes these drugs of important forensic significance.

Samples that require identification/confirmation by LC-MS/MS are extracted from a buffered, diluted sample aliquot by adsorption onto a solid phase extraction column. Drugs that may be present are then eluted from the SPE column, dried, and reconstituted before injection onto the LC-MS/MS system.

The detection of each specific analyte is determined by single point calibration cut off for urines. Bloods are quantitated with a multipoint calibration using deuterated opioid internal standard(s).

Matrix-specific (blood and/or urine as needed) positive and negative controls are extracted and analyzed in each analytical batch. The presence of opioids or other pain management drugs may be confirmed in urine, blood or fluids.

A. Method Targets

Codeine, morphine, hydrocodone, hydromorphone, oxycodone, oxymorphone, 6-monoacetyl morphine (6-MAM), buprenorphine, norbuprenorphine, naloxone, fentanyl, and norfentanyl, tramadol, N-desmethyltramadol, and O-desmethyltramadol. Analytes may be added or removed, as necessary.

Compounds may be added or eliminated from this list upon toxicologist review.

B. Safety

This procedure is carried out in a laboratory environment and standard safety procedures should be utilized, including (minimally) safety glasses and lab coat when deemed necessary. Biological specimens subject to the analytical procedure should be handled using universal precautions. Potentially contaminated items and surfaces should be disinfected prior to and after use.

D. Specimen Requirements

1. 0.5 mL blood or urine

II. MATERIALS

A. Equipment

1. General laboratory glassware
2. Vortex, Thermolyne Maxi Mix 1 or equivalent
3. Sonicator, Fisher-Scientific or equivalent (for blood samples)
4. Centrifuge, Beckman TJ-6 or equivalent
5. Trace B Extraction Columns - SPEWARE (Baldwin Park, CA) ; (or equivalent)
6. SPEWARE CEREX System-48 Solid phase extraction manifold ; (or equivalent)
7. SPEWARE CEREX System-48 Sample Concentrator ; (or equivalent)
8. Shimadzu LC/MS/MS System (or equivalent):
    a. Degasser: Shimadzu DGU-20A
    b. Pumps: 2 Shimadzu LC-20AD Prominence
    c. Autosampler: Shimadzu SIL 20AC Prominence
    d. Column Oven: CTO-20A
    e. Pre-Column: SecurityGuard ULTRA Cartridge UHPLC Phenyl for 4.6mmID Columns (Phenomenex)
    f. Column: Kinetex Phenyl Hexyl (Phenomenex)
    g. Detector: Shimadzu LCMS-8030 Mass Spectrometer
    h. Controller: Shimadzu CBM-20A
    i. Data Station: Shimadzu LabSolutions software

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B. Reagents available as stock items:

Sigma or J.T. Baker reagent grade or equivalent unless specified

1. Methanol (CH_3OH): Fisher Optimum LCMS Grade, Burdick Jackson pesticide grade, or equivalent
2. Deionized water (DIW): Milli-Q, LCMS grade, or equivalent
3. Drug-free urine or blood (Drug free blood or equivalent)

4. Formic Acid (HCOOH) ; Reagent Grade or equivalent
5. Ammonium formate (NH_4HCO_2) ; Reagent Grade or equivalent
6. Glacial acetic acid (CH_3COOH) ; Reagent Grade or equivalent
7. Sodium acetate trihydrate ($NaCH_3COO{\cdot}3H_2O$) ; Reagent Grade or equivalent
8. Sodium carbonate (Na_2CO_3) ; Reagent Grade or equivalent
9. Sodium bicarbonate ($NaHCO_3$) ; Reagent Grade or equivalent
10. UTAK LC-2 Control – UTAK Laboratories (Valencia, CA), or equivalent
11. UTAK Whole Blood Pain Management 100 Control – UTAK Laboratories (Valencia, CA), or equivalent
12. β-Glucuronidase (*P. vulgata* ; Sigma or equivalent)

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C. Drug Standard Solutions – Cerilliant Corporation (Austin, TX)

Target Analytes	Concentration
Codeine	1.0 mg/mL
Morphine	1.0 mg/mL
Hydrocodone	1.0 mg/mL
Hydromorphone	1.0 mg/mL
Oxycodone	1.0 mg/mL
Oxymorphone	1.0 mg/mL
6-Monoacetylmorphine (6-MAM)	1.0 mg/mL
Buprenorphine	1.0 mg/mL
Norbuprenorphine	1.0 mg/mL
Naloxone	1.0 mg/mL
Fentanyl	1.0 mg/mL
Norfentanyl	1.0 mg/mL
Tramadol	1.0 mg/mL
N-desmethyltramadol	1.0 mg/mL
O-desmethyltramadol	1.0 mg/mL

D. Deuterated Drug Standard Solution – Cerilliant Corporation (Austin, TX)

Codeine-D3	1.0 mg/mL
Morphine-D3	1.0 mg/mL
Hydrocodone-D3	1.0 mg/mL
Hydromorphone-D3	1.0 mg/mL
Oxycodone-D3	1.0 mg/mL
Oxymorphone-D3	1.0 mg/mL
Buprenorphine-D4	0.1 mg/mL
Tramadol-C^{13}-D3	0.1 mg/mL

E. Drug Standard Solutions – Lipomed Inc. (Cambridge, MA)

Target Analytes	**Concentration**
Codeine	1.0 mg/mL
Morphine	1.0 mg/mL
Hydrocodone	1.0 mg/mL
Hydromorphone	1.0 mg/mL
Oxycodone	1.0 mg/mL
Oxymorphone	1.0 mg/mL
6-Monoacetylmorphine (6MAM)	1.0 mg/mL
Buprenorphine	1.0 mg/mL
Norbuprenorphine	1.0 mg/mL
Naloxone	1.0 mg/mL
Fentanyl	1.0 mg/mL
Norfentanyl	1.0 mg/mL
Tramadol	1.0 mg/mL
N-desmethyltramadol	1.0 mg/mL
O-desmethyltramadol	1.0 mg/mL

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F. Reagents prepared in the Toxicology Laboratory

1. <u>5M Ammonium formate</u>:

a. Dissolve 3.15 g of ammonium formate in 10 mL volumetric flask.

b. Q.S. to 10 mL with DIW. Stable for one year.

2. **Mobile Phase A (H_20 with 5mM ammonium formate and 0.01% formic acid)**:
   a. Add 0.5 mL 5M Ammonium formate and 0.05 mL formic acid to a 500 mL volumetric flask.
   b. Q.S. to 500 mL with high purity water.
   c. Transfer solution to a glass bottle reserved for LC/MS use only. Stable for one week.
   d. The above instructions make 500 mL of mobile phase; adjust volumes of reagents accordingly if requiring a different final
   e. check pH when necessary

3. **0.1M Sodium Acetate buffer (pH ~4.5):**
   a. Combine 5.86 g of sodium acetate trihydrate and 3.24 mL glacial acetic acid in a 1000 mL stoppered graduated cylinder.
   b. Q.S. to 1000 mL with deionized water.
   c. Store in glass container at room temperature (25^{o}C)
   d. check pH when necessary
   e. Stable for one year
   f. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.

4. **1.0 M Acetate buffer (pH ~5.0)**
   a. To approximately 400 mL DIW in a graduated cylinder,
   b. Dissolve 42.9 g sodium acetate trihydrate in approximately 400 mL DIW
   c. Add 10.4 mL glacial acetic acid $C_2H_4O_2$
   d. Dilute to 500 mL with DIW
   e. Mix. Check pH, adjust pH to 5.0 ± 0.1 (if using pH meter- otherwise pH~5) with 1.0 M acetic acid
   f. Storage: room temperature in glass or plastic. Stability: 6 months
   g. Inspect daily for contamination.

5. **0.1 M Acetate Buffer (pH ~5.0)**

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a. Dilute 20 mL 1.0 M acetate buffer to 200 mL with DIW

b. Mix. Store at: room temperature in glass or plastic. Stability: 6 months

6. **β -Glucuronidase, (5,000 F units/mL) in 0.1 M Acetate Buffer (pH ~5.0)**
Prepare daily for use, make slight excess for each batch, each 0.5 mL sample requires 1250 F units. Add 250 uL of β -Glucuronidase to each tube.

Example: for 40 total tubes prepare 10 mL
Calculate activity for each lot of β-Glucuronidase as follows:
(Lot specific, value from bottle label)
e.g. 1,439,000 -glucuronidase units/g solid
5,000 Units/mL = 1,439,000
x mg 1000 mg
1. x = 3.47 mg/mL

5,000 Units/mL = 1,439,000
x mg 1000 mg
x = 3.47 mg/mL

To make 10 mL
Weigh out 34.7 mg β –Glucuronidase solid. Add to 10 mL of 0.1 M acetate buffer (pH ~5.0)
Dissolve before use by swirling gently.
Make fresh daily as needed for each batch

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7. **0.1M Sodium Carbonate (pH ~8.0):**
   a. Add 5.3 g of sodium carbonate to a 500 mL volumetric flask.
   b. Q.S. to 500 mL with deionized water.
   c. Check pH; should be 8.0 ±0.2 if using pH meter- otherwise pH~8
   d. Store in glass container at room temperature (25^{o}C)
   e. Stable for one year
   f. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.
8. **0.1M Sodium Bicarbonate (pH ~11.0)**:
   a. Add 4.2 g of sodium bicarbonate to a **separate** 500 volumetric flask.

b. Q.S. to 500 mL with deionized water.

c. Check pH. Should be around 11.0 ±0.2 (if using pH meter- otherwise pH~11).

d. Store in glass container at room temperature (25^{o}C)

e. Stable for one year

f. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.

g. check pH when necessary

9. Bicarbonate Buffer pH~9.0: Mixture of 0.1 M Sodium Carbonate and 0.1 M Bicarbonate Solutions

a. Into a beaker containing the 0.1M Sodium Carbonate solution (lower pH solution)

b. Check pH. Adjust with the 0.1M Sodium Bicarbonate solution (higher pH solution) until a pH of 9.0 ± 0.2 is reached (if using pH meter- otherwise pH~9).

c. Store in glass container at room temperature (25^{o}C)

d. Stable for one year

e. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.

10. Reconstitution Mixture: 20% Methanol in Deionized Water

a. Into a 100 mL graduated cylinder with a cap, add 80 mL of deionized water.

b. Add 20 mL methanol.

c. Cap and shake. Store at room temperature; stable for 6 months.

d. Before use check for clarity; if cloudy, discard and prepare fresh.

III. PREPARATION OF STANDARDS

A. <u>Cerilliant Opiate Stock Standard Mix</u>

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Note: Equivalent reference standards may substituted if needed. Prepared calibrators and controls should be made from standards from different manufacturers. When purchased standards (including deuterated) are not 1 mg/mL appropriate adjustments will be made and these will be annotated in the preparation log book. In these cases a second person should confirm the calculations.

1. Into a borosilicate glass screw-top culture tube, **add 100 µL** of the following 1mg/mL Cerilliant reference standards:

Hydrocodone	Hydromorphone	Oxycodone	Oxymorphone	6-MAM

2. Into the same culture tube**, add 50 µL** of the following 1mg/mL Cerilliant reference standards:

Buprenorphine	Norbuprenorphine	Fentanyl	Norfentanyl

3. Add 300 µL methanol
4. Store in freezer (e.g., ≤ -10°C)
5. Stable for 6 months when tightly capped.
7. Final concentrations:

100 µg/mL- Hydrocodone, Hydromorphone Oxycodone, Oxymorphone, 6-MAM

50 µg/mL - Buprenorphine, Norbuprenorphine, Fentanyl, Norfentanyl

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B. <u>Cerilliant Opiate Working Standard</u>

1. Into a 10 mL volumetric flask, **add 20 µL** of the following 1 mg/mL Cerilliant reference standards:

Codeine	Morphine	Tramadol	N-desmethyl-tramadol	O-desmethyl-tramadol

2. Add 40 uL of mixed Cerilliant Opiate Stock Standard.
3. Q.S. to 10 mL with methanol.
4. Protect from light and tightly cap.
5. Store in freezer (≤-10°C).
6. Stable for 6 months.
7. *Final concentrations:*

2000 ng/mL – Codeine, Morphine, Tramadol, N-desmethyltramadol, O-desmethyltramadol

400 ng/mL – Oxycodone, Oxymorphone, Hydrocodone, Hydromorphone

200 ng/mL – Buprenorphine, Norbuprenorphine, Fentanyl, Norfentanyl

C. Working Internal Standard Mix

1. To a 50 mL volumetric flask, add 100 µL of each 1 mg/mL Cerilliant reference deuterated opiates.
2. Add 250 µL of each 100 µg/mL deuterated standard.
3. Q.S. to 50 mL with methanol.
4. Store in freezer (e.g., ≤ -10°C)
5. Stable for 6 months when tightly capped.
6. Final concentrations:

2.0 µg/mL- Codeine-D3, Morphine D3, Oxycodone-D3, Oxymorphone-D3, Hydrocodone-D3, Hydromorphone-D3

500 ng/mL- Buprenorphine-D4, Tramadol-C^{13}-D3

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IV. PREPARATION OF CONTROLS

Note: Alternative controls, both commercial and in-house, to those listed below may be employed at the analyst's discretion. When purchased standards are not 1 mg/mL appropriate adjustments will be

made and these will be annotated in the preparation log book. In these cases a second person should confirm the calculations.

A. **In-House Stock Control (Lipomed and/or Grace)**

1. Into a borosilicate glass screw-top culture tube, **add 100 µL** of the following 1mg/mL Lipomed/Grace reference standards:

Hydrocodone	Hydromorphone	Oxycodone	Oxymorphone	6-MAM

2. Into the same culture tube, **add 50 µL** of the following 1mg/mL Lipomed/Grace reference standards:

Buprenorphine	Norbuprenorphine	Fentanyl	Norfentanyl

3. Add 300 µL methanol
4. Store in freezer (e.g., ≤ -10°C)
5. Stable for 6 months when tightly capped.
6. Final concentrations:

100 ug/mL - Hydrocodone, Hydromorphone, Oxycodone, Oxymorphone, 6-MAM

50 µg/mL- Buprenorphine, Norbuprenorphine, Fentanyl, Norfentanyl

B. **Opiate Working Control**

1. Into a 10 mL volumetric flask, **add 20 µL** of the following 1mg/mL Lipomed/Grace reference standards:

Codeine	Morphine	Tramadol	N-desmethyl-tramadol	O-desmethyl-tramadol

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2. Add 40 uL of mixed In-House Stock Control (Lipomed/Grace).
3. Q.S. to 10 mL with methanol.
4. Store in freezer (e.g., ≤ -10°C)
5. Stable for 6 months when tightly capped.
6. Final concentrations:

2000 ng/mL- Codeine, Morphine, Tramadol, N-desmethyltramadol O-desmethyltramadol

400 ng/mL- Hydrocodone, Hydromorphone, Oxycodone, Oxymorphone, 6-MAM

200 ng/mL- Buprenorphine, Norbuprenorphine, Fentanyl, Norfentanyl

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C. **External Controls**

1. External controls will come from UTAK or equivalent commercially prepared samples. Reconstitute UTAK dried whole blood controls with 5 mL DI water using a volumetric pipette or equivalent.
    a. Cap and let sit 10-15 minutes
    b. Gently swirl 3-4 minutes or mix on rotator until all particles are dissolved into a homogeneous mixture, swirl gently each time an aliquot is removed to ensure a homogeneous mixture.

Note: Standards and controls can be assessed and re-validated after the established expiration date.

D. **Negative Controls**

1. Negative control: Drug-free human urine or drug-free human blood.

V. PROCEDURE

A. Label clean screw cap tubes appropriately with blank, calibrator, control and case number designations.

B. Prepare calibrator and control samples, according to tables below. Note a new calibration curve is not required for each batch. Historical calibration curves can be used, the appropriateness of the curve will be demonstrated through the application of controls extracted with each batch of samples. New calibration curves should be extracted when there have been substantial instrument changes (i.e. a new column installed).

a. Blood Calibrators

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Standard Concentrations (ng/mL)			Opiate Working Standard Volume to Add:	Blank Blood Volume to Add:
Buprenorphine Norbuprenorphine Fentanyl Norfentanyl	Hydrocodone Hydromorphone Oxycodone Oxymorphone 6-MAM	Codeine Morphine Tramadol N-desmethyltramadol O-desmethyltramadol		
Blank			0 µL	500 µL
1	2	10	2.5 µL	500 µL
2	4	20	5 µL	500 µL
5	10	50	12.5 µL	500 µL
10	20	100	25 µL	500 µL
20	40	200	50 µL	500 µL
50	100	500	125 µL	500 µL
100	200	1000	250 µL	500 µL
200	400	2000	500 µL	500 µL

b. In-House Blood Controls

Control Concentrations (ng/mL)			Opiate Working Control Volume to Add:	Blank Blood Volume to Add:
Buprenorphine Norbuprenorphine Fentanyl Norfentanyl	Hydrocodone Hydromorphone Oxycodone Oxymorphone 6-MAM	Codeine Morphine Tramadol N-desmethyltramadol O-desmethyltramadol		
5	10	50	12.5 µL	500 µL
20	40	200	50 µL	500 µL
100	200	1000	250 µL	500 µL

c. In-House Urine Control

Control Concentrations (ng/mL)			Opiate Working Control Volume To Add:	Blank Urine Volume to Add:
Buprenorphine Norbuprenorphine Fentanyl Norfentanyl	Hydrocodone Hydromorphone Oxycodone Oxymorphone 6-MAM	Codeine Morphine Tramadol N-desmethyltramadol O-desmethyltramadol		
5	10	50	12.5 µL	500 µL

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d. Commercial Controls (optional)

Control Concentration	Pipette Volume To Add:	Blank Matrix Volume to Add:
UTAK PMWB dilution x4	125 µL	375 µL
UTAK PMWB	500 µL	0 µL

C. **Blood and Urine sample preparations**

1. Add 0.5 mL case specimen, blood or urine, to appropriate labeled tubes.
2. Add 100 µL of deuterated IS mix to each tube.

Urine Total conjugated and unconjugated

Note: All urines do not need hydrolysis, on a case by case basis the analyst may decide which samples to test for total and free analytes.

Add 250 µL of β –Glucuronidase in 0.1M acetate buffer, pH 5.0 to hydrolyze urine

Heat for 3 hours at ~60°C in water bath. Cool tubes to room temperature.

3. Add 1 mL of 0.10 M sodium acetate buffer (pH ~4.5) to each tube.
   a. **Blood sample preparations:** Add 500 µL DI water to each blood tube.

      Note: Adding 500uL DI water to urine samples is not detrimental.
4. Cap tubes, then vortex each tube for 10 seconds.

   Blood sample preparations: Sonicate blood samples for ~15 minutes.

   Note: Sonicating urine samples is not detrimental and may or may not be done.
5. Centrifuge **all** tubes for ~8 min at about 5200 rpm.
6. Label SPE columns to correspond with each screw-top culture tube.
7. Place labeled Trace B extraction columns in the SPE column rack in the appropriate order. Position plastic waste tray labeled “Methanol” underneath SPE column rack.
8. Condition each column sequentially with 1 mL methanol ; drain (≈3 psi) to Solvent “Hazardous Waste” stream
9. Remove plastic waste tray labeled “Methanol” and replace with plastic tray labeled “Biohazardous/Buffers” ; 1 mL DI water ; drain (≈ 3 psi) to “Non-Hazardous” regulated waste stream
10. Carefully transfer the sample to the center of the SPE column. Avoid splashing and/or transferring any sediment found at the bottom of the tube.
11. Wash each SPE column sequentially with:
    a. 1 mL bicarbonate buffer (pH ~9.0); drain (≈ 3 psi) to non-hazardous regulated waste stream

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b. 1 mL DI water; drain (≈ 3 psi) to non-hazardous regulated waste stream

12. Dry the columns for 10 minutes using maximum pressure, between 60-80 psi.

13. During this 10 minute window (or earlier), label collection tubes and place in the appropriate position in the SPE collection rack underneath the corresponding SPE column; prepare the elution solvent.

14. The elution solvent ratio is 80: 18: 2 Dichloromethane: IPA: NH_4OH. (Adjust volume prepared for the number of samples, minimize excessive hazardous waste, 2 mL needed for each tube)

    Add the IPA and NH_4OH together first before adding the dichloromethane portion (following this order prevents unsafe buildup of gases).

15. After ~10 minutes, replace plastic waste tray with SPE collection rack containing labeled tube in order corresponding to SPE columns.

16. Elute column with two 1.0 mL aliquots of 80:18:2 Dichloromethane:IPA:NH_4OH into the appropriate autosampler vial. Flow at 2-4 mL/min to optimize recovery.

17. Remove top SPE column rack and transfer collection rack from SPE manifold to sample concentrator.

18. Evaporate all tubes to dryness at <40^{o} C.

19. Reconstitute each slotted screw cap vial with 150 µL or less of the starting mobile phase of 20% methanol in DI water. All calibrators, controls and samples must be reconstituted with the same volume of starting mobile phase. If the volume is different from 150 ul note the volume.

20. Individually transfer solutions to properly labeled auto sampler vials containing sample inserts.

21. Analyze by liquid chromatography/mass spectrometry (LC/MS).

VI. INSTRUMENT PARAMETERS (may need to be changed when necessary)

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A. LC Parameters

1. Shimadzu Prominence LC-20 System

a.	**Flow**	0.6 mL/min
b.	**Autosampler Temperature**	15°C
c.	**Injection Volume**	10 µL
d.	**Needle Wash**	500 µL; before and after aspiration
e.	**Column**	Kinetex 2.6um Phenyl-Hexyl 100A 50 x 4.6 cm
		Manufactured by Phenomenex (Torrance, CA)
f.	**Oven Temperature**	40°C
g.	**Gradient**	

Time (min)	Mobile Phase B (Methanol)	Mobile Phase A (0.01% Formic Acid in Water)
Initial	5%	95%
2.50	45%	55%
4.50	45%	55%
5.50	95%	5%
7.50	95%	5%
7.51	5%	95%
9.00	STOP	STOP

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B. M/S Acquisition Parameters: The following conditions can be adjusted if needed based on availability of gases.

Interface	DUIS (APCI and ESI)
DL Temperature	250°C
Nebulizing Gas	2 L/min
Drying Gas	15 L/min

C. Transition Ions Monitored and Retention Times (Times are approximate and may vary)

Analytes may be added or deleted as needed.
Using the LabSolutions optimization software**,** the following transitions were identified:

Drug/Metabolite	Precursor Ion	Quantification Ion	Reference Ion(s)	Retention Time (min)

Codeine	299.7	58.1	165.1	3.05
Codeine D3	303.0	61.0	128.0	3.22
Morphine	286.1	165.1	181.1	2.30
Morphine D3	288.7	201.1	165.0	2.40
6-MAM	328.0	164.95	58.1	3.31
Oxycodone	315.9	298.15	241.1	3.28
Oxycodone D3	319.0	301.15	129.1	3.41
Oxymorphone	301.9	284.15	227.1	2.43
Oxymorphone D3	305.0	287.1	230.1	2.53
Hydrocodone	299.8	199.1	171.0	3.40
Hydrocodone D3	302.75	199.05	170.9	3.50
Hydromorphone	285.9	185.0	157.0	2.47
Hydromorphone D3	289.0	185.05	157.0	2.68
Buprenorphine	468.2	55.2	84.5	6.55
Buprenorphine D4	472.0	59.1	88.15	6.62
Norbuprenorphine	414.1	57.1	101.1	5.30
Fentanyl	336.75	188.15	105.1	6.28
Norfentanyl	233.1	84.1	55.0	3.93
Tramadol	264.0	58.15	30.20, 246.20	4.07
N-desmethyltramadol	250.15	44.00	232.30	4.23
O-desmethyltramadol	250.15	58.10	30.15	3.29
Tramadol-C^{13}-D3	268.20	58.10		4.05

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D. **Detection and Identification**:

All chromatography, peak integrations, and transition ion ratios used for identifications will be reviewed.

Determination of the presence of target analytes in the sample extract are identified by appearance and ratio of product ions that are characteristic of each drug at the appropriate retention time. In this manner, both retention time (an LC characteristic) and fragmentation pattern and ratio (an MS characteristic) are used as the basis for qualitative identification. For the identification of an analyte to be made, the retention time of the chromatographic peak should be within 0.1 minute of the corresponding analyte in the calibrator sample and the ion ratios should be within certain limits (see the following table). Initially the 100 ng/mL Cerilliant Standard sample can be utilized to set the expected ion ratios, however it is recognized that

some ion ratios are concentration dependent. As such, ratios may be set on a case by case basis using a standard with a concentration close to the concentration of the analyte of interest in the case.

Expected (Set) Ion Ratio	Allowance
> 50%	20%
20-50%	25%
< 20%	30%

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E. **Calibration**:

Calibration is accomplished by the addition of a known amount of analytes (in addition to the internal standard) into a blank sample of the matrix that is tested. The response of the system to this calibration defines the standard curve that is used as the basis for the quantitative calculations for controls and samples. The system for blood samples is "multi-point calibration, multi-point control."

While a calibration curve for each drug and metabolite may be analyzed with each batch, a previously established (or "historical") calibration curve may be used. The calibration correlation coefficient must be ≥ 0.990 when using deuterated internal standards. When using non-deuterated internal standards a correlation coefficient ≥ 0.98 is acceptable. A calibrator may be removed to attain a correlation coefficient of ≥ 0.990. Samples resulting in concentrations higher than the highest calibrator should be diluted (approval not to dilute must be obtained by the Unit Lead or higher). The lower limit of quantitation (LOQ) is the concentration of the lowest calibrator. The urine control will be quantitated based on the blood calibration curve.

F. Quantitation:

Quantitation is accomplished by the comparison of the response ratio of the analyte and the internal standard in a specific sample relative to the response ratios of the calibration curve. The concentration of the analyte in the sample is then extrapolated from the standard curve.

VII. RESULTS INTERPRETATION

Positive results will be reported only when:

1. Analyte identification is based on at least two transitions with relative abundances within +/- 20 % of the target, relative to a calibrator.
2. Retention times are within 0.1 min, or +/- 3% relative to a calibrator analyzed in same batch.
3. Qualitative results have at least a 3x signal to noise (S/N) ratio.
4. The integration of the analyte peak has acceptable symmetrical shape and chromatography.
5. All MRMs show peaks at the appropriate retention times.
6. Quality control sample results are acceptable.

VIII. QUALITY CONTROL

A. Criteria for Quantitative Results

1. Statistics will be maintained on all controls and evaluated.
2. Results of controls must fall within ±20% of an analyte's target concentration.
3. All results are recorded.

B. Verification of Vial Sequence: The vial sequence is checked prior to and following injection of samples. These checks will be documented.

C. Evaluation of Potential Carryover: Potential carryover will be determined by a blank sample being analyzed after the highest calibrator. In addition, a solvent blank will be run after each sample to ensure there is no carryover in-between samples.

D. Linearity: Linearity of the calibration curve is demonstrated as a function of the correlation coefficient (r^2) and based on the quantitative results of controls.

E. Sensitivity (LOD, LOQ): For the purposes of this procedure the limit of quantitation (LOQ) is defined as equal to the lowest concentration of the lowest calibrator. The Limit of Detection (LOD) must have a response of at least 3 times the signal to noise ratio and have acceptable ion ratios.

F. Specificity: Specificity is a function of both the resolution of target analyte during the analytical process and the mass spectral fragmentation that analyte molecules undergo during the instrumental analysis.

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IX. QUALITY ASSURANCE

Quality Assurance is provided by the following multi-layer program:

1. The LC/MS analysis is thoroughly checked by the instrument operator, including vial position on the auto sampler, prior to and following the injection of samples.
2. The LC/MS data is reviewed and includes an evaluation of the following:
    a. Positive and Negative Controls
    b. Chromatographic Characteristics
    c. Transcription Errors
3. Reported results are checked against the supporting data.

X. SOURCES OF ERROR

It has been established that no known interferences are present in the calibrators/controls. Ion suppression or enhancement and potential interferences from other analytes have not been found for the common drugs and metabolites typically seen in casework. Both ante mortem and post mortem samples seem to provide comparable results, but matrix effects may occur within some samples and may be a source of error.

XI. References

A. A Comparison of the Validity of Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Tandem Mass Spectrometry Analysis of Urine Samples for Morphine, Codeine, 6-Acetylmorphine, and Benzoylecgonine; Peter R. Stout, Nichole D. Bynum, John M. Mitchell, Michael R. Baylor and Jeri D. Ropero-Miller; J.Anal Toxicol (2009) 33 (8): 398-408
B. Opiates. Connecticut Division of Scientific Services: Toxicology Laboratory.
C. Solid-Phase Extraction Method for Trace B Columns. SPEWARE: Baldwin Park, CA.

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Revision #	History
4	Slight grammatical changes throughout document. Added approximates throughout document where necessary Added to section 1.A. Added 'or equivalent' to 'Materials' section. Updated sections II.F.2, II.F.3, II.F.4, II.F.5, II.F.6, , II.F.7,, II.F.8, and II.F.9 regarding pH checks. Updated III.A.4, III.C.4, IV.A.4, IV.B.4, and V.C regarding temperatures. Updated table in V.B. regarding how much blank blood volume to add. Updated V.C.20 regarding injection volume. Updated VI.D regarding identification criteria. Updated VI.E, VII, VIII.C, VIII.D, IX, and X. Added History section to document. Updated section E. Calibration to remove the need to check calibrators against the generated calibration curve. Section V. changed auto sampler vial to collection tube in multiple locations in section. (V.19) Changed the volume of reconstitution solution from 520 ul to 150 ul. Added line 20 Individually transfer solutions to properly labeled auto sampler vials containing sample inserts.
5	Updated process for preparing controls and calibrators allowing them to be made in methanol and stored in the freezer. Also added a guidance about use of primary purchased standards at a concentration other than 1 mg/mL.

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Title: Analysis of Biological Specimens for Cannabinoids by Liquid Chromatography/Mass Spectrometry (LC/MS)

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1.0 Scope

Marijuana, obtained from the Cannabis sativa plant, is a commonly abused illicit drug. It is typically dried and smoked. The chemical Δ^9-tetrahydrocannabinol (THC) primary psychoactive component of marijuana. Another chemical, 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) is a major active metabolite of THC and 11-nor-9-carboxy- Δ^9-tetrahydrocannabinol (THC-COOH) is a major inactive metabolite of THC.

This procedure is used to confirm and quantitate THC and it's primary metabolites in blood specimens. When appropriately validated, it can also be used to qualitatively identify these compounds within other matrices (e.g., vitreous humor, plasma, serum).

2.0 Principle

Biological specimens are assayed for the presence of THC, THC-OH, and THC-COOH. A deuterated internal standard solution is added to specimens which contain deuterated analogs to the analytes of interest. Extraction of analytes occurs through the use of polymeric solid phase extraction (SPE) technology. Eluted solutions are analyzed using high performance liquid chromatography/mass spectrometry (HPLC/MS or LC/MS) including multiple reaction mode (MRM) detection.

3.0 Specimens

This procedure uses a biological fluid (e.g., blood, serum, plasma). Typically, 0.5mL of sample is used during the analysis, but differing volumes may be necessary. Dilution of samples due to limited specimen or due to suspicion of high drug or metabolite concentrations (as possibly indicated by immunoassay results) is acceptable.

4.0 Equipment/Materials/Reagents

a. General laboratory glassware

b. Disposable borosilicate test tubes (e.g., 16 x 100 mm, round bottom, borosilicate glass with Teflon caps)

c. Vortex mixer

d. Automatic pipettes (with disposable tips)

e. Positive pressure solid phase extraction device (e.g., Cerex, or equivalent)

f. Sample concentrator with nitrogen (e.g., Cerex 48 heated, or equivalent)

g. Centrifuge

h. Liquid Chromatograph/Mass Spectrometer (Shimadzu LCMS-8030, or equivalent)

i. HPLC column (Kinetex 2.6µ, C18, 100Å, 50 mm x 2.1 mm (Phenomenex), or equivalent)

j. Pre-Column – SecurityGuard ULTRA Cartridge UHPLC Kinetex for 4.6mm ID Columns (Phenomenex or equivalent)

k. Autosample vials with inserts (LC/MS grade 1.8mL or equivalent)

l. Acetic acid, glacial (Reagent grade or equivalent)

m. Acetonitrile (CH_3CN or ACN, Optima grade or equivalent)

n. Ammonium acetate (NH_4OAc ; Reagent Grade or equivalent)

o. Ammonium hydroxide (NH_4OH, Reagent grade or equivalent)

p. Ethyl acetate (HPLC grade or equivalent)

q. Hexane (HPLC grade or equivalent)

r. Hydrochloric acid – concentrated (HCl ; Reagent grade or equivalent)

s. Methanol (MeOH, Reagent grade or equivalent)

t. Water (H_2O, Millipore, Deionized (DIW) or equivalent)

u. $NH_4OAc_{(aq)}$ (5M or 38.5% (w/v): Can be prepared by dissolving 3.85 g of NH_4OAc in 10mL of water. Stable for at least two (2) months in refrigerator.

v. SPE Conditioning-Solution – $HCl_{(aq)}$ (0.1M or 0.83% (v/v)) – Can be prepared by slowly adding 8.3 mL of $HCl_{(conc)}$ to water within a 1 L volumetric flask. Dilute to volume with water. Stable for one (1) year at room temperature. This solution can be stored in a re-pipetter bottle.

w. SPE Sample Wash Mixture – H_2O:CH_3CN:NH_4OH (85:15:1): Can be prepared by combining 85mL of deionized H_2O, 15 mL of CH_3CN, 1 mL NH_4OH and mix well. Store in glass at room temperature. Should be prepared fresh.

x. Elution Solvent (THC, THC-OH) – Ethyl acetate – stable indefinitely.

y. Elution Mixture (THC-COOH) – Hexane:Ethyl Acetate:Acetic Acid (80:18:2): Can be prepared by combining 80 mL of hexane, 18 mL of ethyl actetate and 2 mL of acetic acid and mixing well. Store in glass at room temperature. Stable for at least one (1) month.

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z. Mobile Phase A – $NH_4OAc_{(aq)}$ (5mM or 0.04% (w/v)): Can be prepared by diluting 1mL of the 5 mM (38.5% (w/v)) $NH_4OAc_{(aq)}$ in 1 L of water (1:1000 dilution) and mixing well. Store in glass at room temperature. Stable for one (1) week.

aa. Mobile Phase B – (ACN or CH_3CN). Store in glass at room temperature – stable indefinitely.

bb. Δ^9-tetrahydrocannabinol (THC) Reference Standard (1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

cc. Δ^9-tetrahydrocannabinol-D_3 (THC-D_3) Reference Standard (0.1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

dd. 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) Reference Standard (1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

ee. 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol-D_3 (THC-COOH-D_3) Reference Standard (0.1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

ff. 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) Reference Standard (1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

gg. 11-hydroxy-Δ^9-tetrahydrocannabinol-D_3 (THC-OH-D_3) Reference Standard (0.1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

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5.0 Standards and Controls

Negative Control Solution: See below.

Positive Control Solution: Can be obtained from an outside source or prepared in-house. If prepared in-house, reference standards from different manufacturers or from different lot numbers should be used when making controls and calibrator solutions.

a. Stock Deuterated Internal Standard (IS) Mix Solution –
((THC-D_3 + THC-OH-D_3 + THC-COOH-D_3 (1 µg/mL each))):
Can be prepared by individually combining 100 µL each of 0.1 mg/mL solutions of THC-D_3, THC-OH-D_3, and THC-COOH-D_3 into a 10 mL volumetric flask and diluting to volume (10 mL) with MeOH.
Alternatively can be prepared, if using 1 mg/mL solutions of the reference standards, only add 10 µL each of the reference standards to a 10 mL volumetric flask and diluting to volume with MeOH.

b. Working Deuterated Internal Standard (IS) Mix Solution –
((THC-D_3 + THC-OH-D_3 + THC-COOH-D_3 (0.1 µg/mL each))):
Can be prepared by diluting the Stock Deuterated Internal Standard (IS) Mix Solution 1:10 with MeOH (e.g., 1 mL of Stock IS Mix Solution in a total volume of 10 mL of MeOH).

c. Working Standard Solution for Controls (High) –
((THC + THC-OH + THC-COOH (1 µg/mL each))):
Can be prepared by individually combining 25 µL each of the 1 mg/mL purchased referernce standard solutions of THC, THC-OH, and THC-COOH into a 25 mL volumetric flask and diluting to volume (25 mL) with MeOH.

d. Working Standard Solution for Controls (Low) –
((THC + THC-OH + THC-COOH (0.1 µg/mL each))):
Can be prepared by diluting the Working Standard Solution (High) 1:10 with MeOH.
This can be done by transferring 25 µL of the Working Standard Solution (High) into 25 mL volumetric flask and diluting to volume (25 mL) with MeOH.

e. Working Standard Solution for Calibrators (High) –
((THC + THC-OH + THC-COOH (1 µg/mL each))):
Can be prepared by individually combining 25 µL each of the 1 mg/mL purchased referernce standard solutions of THC, THC-OH, and THC-COOH into a 25 mL volumetric flask and diluting to volume (25 mL) with MeOH. Should be from different lot number, manufacturer, or prepared by a different analyst compared to the Working Standard Solution for Controls (High).

f. Working Standard Solution for Calibrators (Low) –
((THC + THC-OH + THC-COOH (0.1 µg/mL each))):
Can be prepared by diluting the Working Standard Solution (High) 1:10 with MeOH.
This can be done by transferring 25 µL of the Working Standard Solution (High) into 25 mL volumetric flask and diluting to volume (25 mL) with MeOH. Should be from different lot number, manufacturer, or prepared by a different analyst compared to the Working Standard Solution for Controls (Low).

g. High Positive Control (Hi-Ctrl) Blood
(THC + THC-OH + THC-COOH (50 ng/mL)):
Can be prepared by adding 25 µL of the Working Standard Solution (High ; 1 µg/mL)) to a screw-capped test tube containing 0.5 mL of blank blood. Store refrigerated in glass. Stable for at least one (1) month. [See table 1]

h. Low Positive Control (Lo-Ctrl) Blood
(THC + THC-OH + THC-COOH (5 ng/mL)):
Can be prepared by adding 25 µL of the Working Standard Solution (Low ; 0.1 µg/mL)) to a screw-capped test tube containing 0.5 mL of blank blood. Store refrigerated in glass. Stable for at least one (1) month. [See table 1]

i. Positive Control [Other Matrix]
(THC + THC-OH + THC-COOH (5 ng/mL)):
Can be prepared by adding 25 µL of the Working Standard Solution (Low ; 0.1 µg/mL)) to a

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screw-capped test tube containing 0.5 mL of blank [other matrix (e.g., serum, plasma, vitreous fluid)]. Store refrigerated in glass. Stable for at least one (1) month.

j. Calibrator Solutions (THC + THC-OH + THC-COOH (1, 5, 50, 200 ng/mL))
Can be prepared by using either the Working Standard Solution (Low) or Working Standard Solution (High) according to Table 2.

k. Negative Control [Othr Matrix]:
Synthetic or in-house negative control [other matrix]. Store refrigerated or obtain fresh. If purchased, stability should be determined by manufacturer. A negative control [other matrix] should be extracted and analyzed with each appropriate assay or batch.

l. Negative Control Blood:
Purchased (e.g., Diagnostics Products Corporation, UTAK Labs), donated (e.g., American Red Cross), or in-house negative control blood (e.g., from a proven blank specimen). Store frozen, refrigerated, or obtain fresh. If purchased or donated, stability should be determined by manufacturer. A negative control blood should be extracted and analyzed with each blood assay or batch.

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Table 1: Control (In-House) Solutions

Pipette into 0.5 mL Neg. Ctrl. Blood or [Other Matrix] [drug-free]				
Level	**THC THC-OH THC-COOH (ng/mL)**	**Volume of Working Std. Solution for Control (Low) (0.1 µg/mL) (µL)**	**Volume of Working Std. Solution for Control (High) (1 µg/mL) (µL)**	**Volume of Neg. Ctrl. Blood or [Other Matrix] [drug-free] (mL)**
Negative Ctrl.*	–	–	–	0.5 (Blood or [Other Matrix])
Positive Ctrl.*	5	25	–	0.5 [Other Matrix]
Low Blood	5	25	–	0.5 Blood
High Blood	50	–	25	0.5 Blood

*If [Other Matrix] samples are not being analyzed, controls will not be necessary.

6.0 Calibration

This procedure may be used quantitatively through the construction of a multipoint calibration graph for the analyte(s) of interest.

Table 2: Calibrator Solutions

Pipette into 0.5 mL Neg. Ctrl. Blood [drug-free]				
Level	**THC THC-OH THC-COOH (ng/mL)**	**Volume of Working Std. Solution for Calibrator (Low) (0.1 µg/mL) (µL)**	**Volume of Working Std. Solution for Calibrator (High) (1 µg/mL) (µL)**	**Volume of Neg. Ctrl. Blood [drug-free] (mL)**
1	1	5	–	0.5
2	2	10	–	0.5
3	5	25	–	0.5
4	20	100	–	0.5
5	50	–	25	0.5
6	100	–	50	0.5

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7.0 Sampling

Not applicable.

8.0 Procedure

The Appendix contains an abbreviated version of this procedure. This form may be used at the bench by analysts who perform the procedure.

8.1. Prepare calibrator and control solutions according to tables 1 and 2.

8.2. Add 0.5mL of unknown specimens to properly labeled test tubes.

8.3. Add 100 µL of the Working Deuterated Internal Standard (IS) Mix Solution [10 ng of each component] to each test tube and mix.

8.4. Slowly add cold (e.g., -20 °C) ACN to each blood sample while vortex-mixing for at least 30 seconds. This should cause a protein precipitation to occur. This step can be skipped for [Other Matrix] specimens.

8.5. Cap and centrifuge each tube (e.g., ~6 minutes at ~5200 RPM).

8.6. Add 2 mL of 0.1M $HCl_{(aq)}$ into each SPE column.

8.7. Individually add supernatant from each sample into SPE columns that already contain the 0.1M $HCl_{(aq)}$ and mix with pipette. Be careful not to transfer protein sediments.

8.8. Allow solutions to migrate through SPE columns via gravity. If needed, apply a small amount of pressure to push solutions through SPE columns.

8.9. Wash SPE columns with 1 mL of the SPE Sample Wash Mixture (H_2O:ACN:NH_4OH (85:15:1)) via gravity. If needed, apply a small amount of pressure to push solutions through SPE columns.

8.10. Dry the SPE columns for ~5-8 minutes using high pressure (60-80 psi). Do not over-dry (not longer than 8 minutes).

8.11. Elute SPE columns with 2 mL of ethyl acetate (Elution Solvent for THC and THC-OH) at the rate of ~0.5 mL/min. and into properly labeled test tubes. Increase the pressure briefly and expel remaining elution solvent from the SPE sorbent beds and from the Luer tips.

8.12. Dry the SPE columns for 2 minutes using high pressure (60-80 psi).

8.13. Elute SPE columns with 2 mL of Elution Mixture for THC-COOH (Hexane:Ethyl Acetate:Acetic Acid (80:18:2)) at the rate of ~0.5 mL/min. into the same test tubes as were used for the THC and THC-OH extracts. Increase the pressure briefly and expel remaining elution solvent from the SPE sorbent beds and from the Luer tips.

8.14. Evaporate the combined sample extracts to dryness (e.g., $N_{2(g)}$ at <40°C).

8.15. Dissolve the dried residue in 50 µL ACN and vortex-mix the solutions to solubilize the residues from the surfaces of the collection tubes.

8.16. Add 50 µL of water to each test tube and vortex-mix. Total volume of each solution should be ~100 µL.

8.17. Individually transfer solutions to properly labeled autosample vials containing volume inserts.

8.18. Analyze solutions by LC/MS instrumentation.

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9.0 Instrumental Conditions

The following are the typical operating parameters for the instrument used in this procedure. The instrument conditions may be modified to adjust or improve the procedure. The appendix contains an abbreviated version of the procedure and this checklist can be used by analysts.

HPLC Parameters:

HPLC Column: Phenomenex, Kinetex, 2.6µ, C18, 100 Å, 50 mm x 2.1 mm

Mobile Phase A: $NH_4OAc_{(aq)}$ (5mM or 0.04% (w/v))
Mobile Phase B: Acetonitrile (neat)
Column Temperature: 40 °C
Constant Flow: 0.5 mL/min.
Autosampler Temperature: 15 °C

Gradient Program: Initial 20% B Flow 0.5 mL/min

Time (min.)	**Mobile Phase A $NH_4OAc_{(aq)}$ – (5mM ; 0.04% (w/v)) (%)**	**Mobile Phase B Acetonitrile (100%) (%)**
Initial	80	20
0.25	80	20
1.75	10	90
2.40	10	90
2.41	80	20
4.00	80	20

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Mass Spectrometer Parameters:

Ionization Source	Electrospray	Polarity	Negative Ion
Ion Spray Potential	-3.5 kV	Heat Block	500 °C
Nebulizer Gas	Nitrogen	Nebulizer	2.5 L/min
Drying Gas	Nitrogen	Drying Gas	10 L/min.
Scan Type	MRM (&/or Full Scan)	Resolution	Unit
Desolvation Line	300 °C		

Multiple Reaction Montioring (MRM) Transition Ions:

Compound	Presursor (m/z)	Product (m/z)	Pause Time (msec)	Dwell Time (msec)	Q1 Pre-Bias (V)	Coll. Energy (V)	Q3 Pre-Bias (V)
THC	313.20	245.05	3	10	10	28	27
THC	313.20	191.20	3	10	10	30	21
THC	313.20	203.10	3	10	14	31	13
THC-OH	329.20	268.15	3	15	13	28	19
THC-OH	329.20	172.90	3	15	16	31	18
THC-COOH	342.20	325.25	3	15	11	20	23
THC-COOH	342.20	191.15	3	15	15	33	22
THC-COOH	342.20	299.20	3	15	19	23	20
THC-D_3	316.10	194.20	3	15	15	35	15
THC-OH-D_3	332.30	271.20	3	15	15	35	15
THC-COOH-D_3	346.20	248.30	3	15	15	35	15

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10.0 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. Retention time (chromatrographic characteristic), fragmentation pattern and qualified ion ratios (mass spectrometric characteristics), and other characteristics are used as the basis for detection, identification, and quantitiation. In most cases, all of the criteria below should be met in order to identify the appropriate cannabinoids within biological specimens.

10.1. Chromatography

Analyze solutions by LC/MS instrumentation.

All chromatographic peaks for the analytes of interest should show good chromatographic characteristics, with reasonable peak shape, width, and resolution. For low concentrations of an analyte (<5 ng/mL), there may be transitions that are not optimal. In order to be determined as acceptable, a chromatographic peak in an unknown sample should compare

favorably to the same analyte's chromatographic peak in a known sample which has been analyzed on the same system and in the same, or subsequent, analytical timeframe. Additionally, the following two criteria should be met:

10.1.1. Retention Time

The retention time of the peak should be within ±2% of the retention time (relative or absolute) obtained from injection of a reference standard or positive control.

10.1.2. Signal-to-Noise

To justify the existence of a peak, it's baseline signal to noise ratio should exceed 3. Further, the baseline signal for the peak from the sample of interest should be at least 10 fold greater than that for any observed peak at a similar retention time in a negative control or blank sample injected prior to that sample.

10.2. Mass Spectrometry

Independent MS/MS experiments are conducted for each analyte. Ion ratios should compare favorably to ion ratios from reference standards or an extracted positive control at a comparable concentration. Generally ion ratios are within the following limits:

Expected [Set] Ion Ratio	Allowance
>50 %	20%
20% - 50%	25%
<20%	30%

RETIRED

11.0 Calibration

Calibration is accomplished by the addition of known amounts of analytes (in addition to the internal standard) into matrix-matched blank samples. A run-specific calibration graph is generated from the resulting data and used as the basis for the quantitation of unknown samples. A calibration graph for each drug and metabolite is analyzed with each batch and a correlation coefficient should be ≥ 0.990 when using deuterated internal standards. If the correlation coefficient is lower than 0.990 then approval from the appropriate lead analyst or Deputy Director must occur and, if deemed acceptable, justification must accompany the applicable data. It is acceptable to use historical calibration curves when there are no significant instrument changes.

Controls compared to the generated curve should be +/-20% of the expected value. Controls outside of this range may be excepted with review by the unit Lead.

12.0 Uncertainty of Measurment

The critical sources of measurement uncertainty in this procedure include:

- Historical random uncertainty of repeated measurements
- Accuracy of the pipette used to deliver the sample
- Accuracy of the pipette used to deliver the calibrators
- Uncertainty in the concentration of the calibration standards
- Precision of the delivery of the internal standard

When quantitative results that are determined from this procedure are included in a report, applicable measurment uncertainties will be estimated. Information used to derive uncertainty measurements should be tracked electronically.

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13.0 Limitations

Limit of Detection (LOD), Limit of Quantitation (LOQ), Upper Limit of Quantitiation (ULOQ)

Cannabinoids	LOD – **Urine (ng/mL)	LOD – Blood (ng/mL)	LOQ – Blood (ng/mL)	ULOQ – Blood (ng/mL)
THC	**5***	**1**	**2**	**100**
THC-COOH	**5***	**1**	**1**	**100**
THC-OH	**5***	**1**	**2**	**100**

*Administratively set – values may actually be lower
**Unconjugated – analytes simply spiked in urine

14.0 Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment should be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Biological specimens will be handled using universal precautions and will be treated as biohazardous. Potentially contaminated items and surfaces will be cleaned prior to use, especially. When casework samples are being processed/analyzed brown paper (or other similar barrior) should be placed in between the surface and the specimens.

15.0 References

FBI Laboratory, Analysis of Cannabinoids from Biological Specimens by LC/MS/MS

D.M. Schwope, K.B. Scheidweiler, M.A. Huestis. Direct quantification of cannabinoids and cannabinoid glucuronides in whole blood by liquid chromatography-tandem mass spectrometry. Analytical Bioanalytical Chemistry. 401(4):1273-1283 (2011).

Monroe County MEO, 11-Nor-9-Carboxy-Δ^9-Tetrahydrocannabinol,11-Hydroxy Tetrahydocannabinol and Δ^9-Tetrahydrocannabinol Quantitation In Blood.

SPEware Corporation, Extraction of Δ^9-Tetrahydrocannabinol (THC) and its Metabolties from Whole Blood for LC-MS Confirmations using Cerex® THC Extraction Columns, 3mL, 35mg

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Appendix:
Bench Method: Analysis of Cannabinoids from Biological Specimens by LC/MS/MS

The following is an abbreviated version of this procedure.
This form may be used at the bench level by the analyst performing the procedure.

Pipette into 0.5 mL drug-free blood/[other matrix]	**Lot:**		
Standard Lot:	**Standards**		
Level	**THC THC-OH THC-COOH (ng/mL)**	**Volume of Working Std. Solution for Calibratos (Low) (0.1 µg/mL) (µL)**	**Volume of Working Std. Solution for Calibrators (High) (1 µg/mL) (µL)**
1	1	5	–
2	2	10	–
3	5	25	–
4	20	100	–
5	50	–	25
6	100	–	50

Control Lot:		**In House Controls**		
Level	**THC THC-OH THC-COOH (ng/mL)**	**Volume of Working Std. Solution for Controls (Low) (0.1 µg/mL) (µL)**	**Volume of Working Std. Solution for Controls (High) (1 µg/mL) (µL)**	**Volume of Neg. Ctrl. Blood or [Other Matrix] [drug-free] (mL)**
Negative Ctrl.	–	–	–	0.5 (Blood or [Other Matrix])
Positive Ctrl.	5	25	–	0.5 [Other Matrix]
Low Blood	5	25	–	0.5 Blood
High Blood	50	–	25	0.5 Blood

*If [Other Matrix] samples are not being analyzed, certain controls will not be necessary.

RETIRED

Appendix (Cont'd):

Specimen Procedure	
Add 0.5 mL standard/control/sample into properly labeled 16 x 100 mm test tubes	
Add 100 µL of working internal standard solution (1ug/mL)	**Lot:**
Slowly add 1 mL cold acetonitrile (ACN) while vortexing. Mix/vortex ~1 min	
Cap, centrifuge ~6min at ~5200 rpm	
Add 2 mL of 0.1 M HCl to the SPE column.	**Lot:**
Load sample onto SPE column, mixing into 0.1 M HCl (e.g., Polycrom THC 682-0353). Apply pressure as needed.	**Lot:**
Wash with 1.0 mL (85/15/1) DIW, ACN, NH_4OH Prepare fresh	
NH_4OH	**Lot:**
Acetonitrile	**Lot:**
Dry column~ 5 to 8 min. at full pressure. Do not over dry	
Elute with 2.0 mL Ethyl Acetate (THC, THC-OH fraction)	
Ethyl Acetate	**Lot:**
Dry column for 2 min at full pressure (Do not over dry)	
Elute with 2 mL (80:18:2) Hexane:Ethyl Acetate:Acetic Acid) (THC-COOH fraction)	
Hexane	**Lot:**
Ethyl Acetate	**Lot:**
Acetic Acid	**Lot:**
Evaporate combined fractions to dryness at 40 °C under nitrogen (Do not overdry)	
Dissolve the dried residue in 50 µL ACN. Mix/vortex tube rinsing sides of the tube to solubilize the residue from the surface of the collection tube.	
Acetonitrile	**Lot:**
Add 50 µL DIW to tube. Mix/vortex and ensure sides of tubes are rinsed.	
Transfer to appropriately labeled LC autosample vials.	

RETIRED

Appendix A (Cont'd):

Specimen Procedure (Cont'd)

Analyze by LC/MS/MS:

Instrument: Shimadzu 8030	Instrument Identifier:
HPLC Column: Phenomenex Kinetex C18 ; 100Å ; 2.6µm ; 50mm x 2.1 mm):	Lot:
Mobile Phase A: 5mM (0.04% NH_4OAc)	Lot:
Mobile Phase B: ACN (CH_3CN) – 100%	Lot:

Gradient Program: Initial 20% B Flow 0.5 mL/min

Time (min.)	%B
0.25	20
1.75	90
2.40	90
2.41	20
4.00	Stop

MRM Transitions:

Analyte	Precursor (m/z)	Product (m/z)	Pause time (msec)	Dwell time (msec)	Q1 Pre-Bias (V)	Coll. Energy	Q3 Pre Bias (V)
THC	313.20	245.05	3	10	10	28	27
THC	313.20	191.20	3	10	10	30	21
THC	313.20	203.10	3	10	14	31	13
THC-OH	329.20	268.15	3	15	13	28	19
THC-OH	329.20	172.90	3	15	16	31	18
THC-COOH	342.20	325.25	3	15	11	20	23
THC-COOH	342.20	191.15	3	15	15	33	22
THC-COOH	342.20	299.20	3	15	19	23	20
THC-D_3	316.10	194.2	3	15	15	35	15
THC—OH-D_3	332.30	271.20	3	15	15	35	15
THC-COOH-D_3	346.20	248.30	3	15	15	35	15

RETIRED

Rev. #	History
1	New procedure for blood or other appropriate matrices.

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Title: Exclusionary Drug Screening by Liquid Chromatography/Mass Spectrometry (LC/MS)

1 Introduction

A rapid high performance liquid chromatography/mass spectrometry (LC/MS) method using electrospray ionization (ESI) can be used to qualitatively screen biological specimens (e.g., blood, urine) for common drugs of abuse, as well as some other drugs. The amount of a detected analyte can be estimated by comparing the response (peak area) for an analyte to that for a corresponding internal standard. Positive findings can be confirmed by using either a second technique or a second aliquot of sample. Confirmation can also be achieved during quantitative analyses using appropriate procedures.

2 Scope

This procedure is limited to the qualitative screening of biological specimens for the presence of cocaine and metabolites, opioids, and benzodiazepines and other drugs (see list of target analytes and limits of detection).

3 Principle

Biological specimens are analyzed for the presence of drugs of abuse (DoA) and/or their metabolites by extraction using solid phase extraction (SPE) columns. Final extracts are analyzed by LC/MS using ESI and can involve a combination of selected reaction monitoring (SRM) and full scan analysis modes.

4 Specimens

This procedure uses biological fluid(s) such as blood, urine, serum, and/or plasma. Typically 0.5 mL of sample is consumed during the analysis but varying volumes may be used, as necessary. Dilution of samples due to limited specimen or due to suspicion of high drug or metabolite concentration is acceptable.

5 Equipment/Materials/Reagents

a. General laboratory glassware

b. Disposable borosilicate test tubes (e.g., 16 x 100 mm, round bottom, borosilicate glass with Teflon caps)

c. Vortex mixer

d. Sonicator

e. Automatic pipettes (with disposable tips)

f. Positive pressure solid phase extraction device – SPEWare Cerex, 48 sample (or equivalent)

g. Sample concentrator with nitrogen – SPEWare Cerex 48 heated (or equivalent)

RETIRED

h. Centrifuge

i. pH meter (or equivalent)

j. Trace B Extraction Columns – SPEWare (or equivalent)

k. Liquid Chromatograph/Mass Spectrometer (Shimadzu LCMS-8030, or equivalent)

l. HPLC column – Kinetex, phenyl hexyl, 2.6 µm, 100Å, 50 mm x 4.6 mm (Phenomenex), or equivalent)

m. Pre-Column – SecurityGuard ULTRA Cartridge UHPLC Kinetex for 4.6mm ID Columns (Phenomenex or equivalent)

n. Autosample vials (LC/MS grade 1.8mL or equivalent)

o. Acetic acid, glacial ($CH_3COOH_{(l)}$, Reagent grade or equivalent)

p. Ammonium formate (NH_4CHOO, Reagent grade or equivalent)

q. β-Glucuronidase (p. vulgata ; Sigma or equivalent)

r. Formic acid (HCOOH, Reagent grade or equivalent)

s. Methanol (MeOH, Reagent grade, LC/MS Grade, or equivalent)

t. Morphine glucuronide (Morphine-3-β-glucuronide or Morphine-6-β-glucuronide) (Alltech, Sigma, or equivalent)

u. Sodium acetate trihydrate ($NaCH_3COO{\cdot}3H_2O$, Reagent grade or equivalent)

v. Sodium bicarbonate ($NaHCO_3$; Reagent grade or equivalent)

w. Sodium carbonate (Na_2CO_3 ; Reagent grade or equivalent)

x. Water (Mobile Phase) (LC/MS Grade ; Optima or equivalent)

y. Ammonium $Formate_{(aq)}$: ($NH_4CHOO_{(aq)}$; 5M ; 31.5% (w/v)): Can be prepared by dissolving 3.15 g of ammonium formate in 10 mL of water. Stable for at least one (1) year in glass container when refrigerated.

z. Sodium Acetate $Buffer_{(aq)}$ ($NaCH_3COO$; pH~4.5): Can be prepared by combining 5.86 g of sodium acetate with 3.24 mL of glacial acetic acid in a 1 L volumetric cylinder and diluting to volume with water. Stable for at least one (1) year in glass container while at room temperature.

aa. Sodium $Bicarbonate_{(aq)}$ (0.1M ; $NaHCO_3$; 0.84% (w/v) ; pH~8): Can be prepared by dissolving 4.2 g of sodium bicarbonate in water within a 500 mL volumetric flask and diluting to volume with water. Stable for at least one (1) year in glass container while at room temperature.

bb. Sodium $Carbonate_{(aq)}$ (0.1M ; Na_2CO_3 ; 1.1% (w/v) ; pH~11): Can be prepared by dissolving 5.3 g of sodium carbonate in water within a 500 mL volumetric flask and diluting to volume with water. Stable for at least one (1) year in glass container while at room temperature.

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cc. Bicarbonate/Carbonate Buffer$_{(aq)}$ (pH~9): Can be prepared by transferring 0.1M sodium bicarbonate$_{(aq)}$ solution into a beaker and checking pH (should be pH~8). Adjust the pH to ~9 using the 0.1M sodium carbonate$_{(aq)}$ solution. Stable for at least one (1) year in glass container while at room temperature.

dd. Solid Phase Extraction Elution Solution (e.g., 50 mL):
{Dichloromethane (CH_2Cl_2) : IPA : NH_4OH (80 : 18 : 2)}.
Can be prepared by adding 9 mL of isopropanol to 1 mL of ammonium hydroxide within a 50 mL volumetric cylinder. To this mixture add 40 mL of methylene chloride and mix. This solution will be prepared when needed for use.

Note: The volume should be adjusted to account for number of samples that are to be extracted (2 mL are needed for each extraction sample).

ee. $MeOH_{(aq)}$ (20% (v/v)): Can be prepared by adding 80 mL of water and 20 mL of methanol to a 100 mL volumetric cylinder and mixing. Stable for at least one (1) month in glass container when refrigerated.

ff. Methanol-HCl (1% (v/v)): Can be prepared by dissolving 10 µL of hydrochloric acid into 990 µL of methanol (or in different volume with an equivalent ratio). Stable for at least one (1) month in glass container when refrigerated. Verify pH is ≤ 3 prior to use.

gg. Hydrolysis Sodium Acetate Buffer$_{(aq)}$ (0.1 M ; $NaCH_3COO$; pH~5): Can be prepared by combining first making a 1M Sodium Acetate Buffer Solution: 42.9 g of $NaCH_3COO{\cdot}3H_2O_{(s)}$ with 400 mL of water. Add 10.4 mL of $CH_3COOH_{(l)}$ in a 500 mL volumetric cylinder and diluting to volume with water. Store in glass container at room temperature. Stable for at least one (1) year at room temperature. Check clarity and pH can be checked prior to use. Use 1M acetic acid to adjust pH to 5.0 (+/- 0.1). The 0.1M Sodium Acetate Buffer Solution can be made by diluting 20 mL of the 1M Sodium Acetate Buffer Solution in 200 mL water.

hh. β-Glucuronidase (in 0.1M $NaCH_3COO$ buffer solution ; pH~5): Prepare daily for use. The concentration of the enzyme buffer solution should be 5000 Units/mL. The volume of enzyme solution per [0.5 mL] urine specimen should be 0.5 mL. Each 0.5 mL urine sample requires 2500 enzyme F-Units to ensure adequate enzymatic hydrolysis. A slight excess of solution should be made to ensure enough is available for all of the samples.
Note: β-Glucuronidase has different activities based on the lot that is used. See enzyme activity information on individual bottles (i.e., Units/gram of material).

Example: Ten (10) urine specimens (0.5 mL each) that need hydrolyzing means there will need to be 5 mL of enzyme solution total. If the activity of the β-Glucuronidase is 1,439,000 Units/g, then the amount of β-$G_{(s)}$ needed is:
[(5 mL) x (5000 β-G Units/mL) x (1 g β-G/1,439,000 β-G Unit) x 1000 mg/g] = 17.4 mg
Dissolve 17.4 mg β-$G_{(s)}$ in 5 mL 0.1M $NaCH_3COO$ buffer solution (pH~5).

ii. Mobile Phase A – 0.01 %(v/v) $HCOOH_{(aq)}$ and 5mM $NH_4CHOO_{(aq)}$:
Can be prepared by mixing 50 µL of formic acid with 0.5 mL of 5M ammonium formate in a 500 mL volumetric cylinder, diluting to volume with [Mobile Phase] water, and mixing well.

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Store in glass at room temperature. Stable for at least one (1) week while in a closed state. {Note: 5mM $NH_4CHOO_{(aq)}$ is equivalent to 0.0032 % (w/v) $NH_4CHOO_{(aq)}$.}

jj. Mobile Phase B – (MeOH or CH_3OH). Store in glass at room temperature – stable indefinitely in a closed state at room temperature.

Note: Unless otherwise noted, the above Reagent solutions are stable for at least one (1) year from either their creation or from their last verification. Check clarity and check pH (when indicated as having a pH value) for all solutions prior to use.

6 Standards and Controls

Positive and Negative Controls:
Can be purchased (e.g., UTAK Labs) or generated in-house (e.g., spiked blank blood, spiked blank urine, spiked vitreous). Store frozen, refrigerated, or obtain fresh. If purchased, the stability should be determined by the manufacturer. Appropriate positive and negative controls will be extracted and analyzed with each assay or batch.

Internal Standard Components:
Purchased from suitable vendor(s) in solution or neat solids (e.g., 0.1 mg/mL solution). Storage and stability is determined by manufacturer(s). Additional or alternative deuterated compounds may be used. If purchased solutions vary in concentration from above, analysts may adjust the preparation volumns to account for differences. Any variations will be noted on solution preparatation worksheets.

a. Internal Standard (I.S.) Working Solution (Diazepam–D_5 ; 250 ng/mL):
Can be prepared by adding 250 µL of the 100 µg/mL Diazepam-D_5 into a 100 mL volumetric flask and diluting with MeOH. Store refrigerated in glass. Stable for at least 1 year.

b. Morphine-β-glucuronide Stock Solution (0.1 mg/mL)
(Either morphine-3-β-glucuronide or morphine-6-β-glucuronide)
Purchased as 0.1 mg/mL solutions. Storage and stability is determined by manufacturer.

c. Morphine Glucuronide Control Working Solution (2.5 µg/mL):
Can be prepared by diluting 250 µL of the Morphine-β-glucuronide Stock Solution with $MeOH_{(aq)}$ (1:1 MeOH:H_2O) to a final volume of 10 mL. Store in glass in a freezer.
Stable for at least 6 months.

d. Hydrolysis Control Urine (~75 ng/mL morphine; ~125 ng/mL morphine glucuronide):
Can be prepared by adding 25 µL of the Morphine Glucuronide Control Working Solution (2.5 µg/mL) to 500 µL of Negative Control Urine on the day of analysis.

e. Optional Instrument Performance Standard Mix: ((Diazepam-D_5) ; 10 ng/mL)

This is optional and can be used to evaluate the instrument in addition to the performance check solution for the LC/MS instrument.

Dilute the I.S. Working Solution 1:25 with water to make a 10 ng/mL final concentration. This can be done by mixing 20 µL of the I.S. Working Solution, combining it with 500 µL of

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deionized water, placing it into an autosample vial, and capping. This solution will be prepared when needed for use.

Note: Other analytes within the solution are not evaluated – just the Diazepam-D_5 analyte.

f. Negative Control Blood:
Purchased (e.g., Diagnostics Products Corporation, UTAK Labs), donated (e.g., American Red Cross, hospital blood bank), or in-house demonstrated negative control blood (e.g., from a proven blank specimen). Store frozen or refrigerated. If purchased or donated, stability should, when possible, be determined by manufacturer.

g. Negative Control Urine (or other matrix):
Purchased, acquired (e.g., Office of the Chief Medical Examiner (OCME)), or in-house demonstrated to be negative. Store refrigerated or frozen.

h. Negative Control [Other Matrix]:
Synthetic or in-house negative control [other matrix]. Store refrigerated or obtain fresh. If purchased, stability should be determined by manufacturer. A negative control [other matrix] should be extracted and analyzed with each appropriate assay or batch.

i. Positive Control:
If prepared in-house, can be prepared by adding 250 µL of UTAK DAU Level 2 solution to 250 µL blank matrix (e.g., blood, urine) and mixing. If a non-routine matrix is analyzed, then dilute with 250 µL of appropriate material. The resulting 0.5 mL positive control sample will contain (at least) the following analytes:
Methylenedioxymethamphetamine (MDMA), Temazepam, Benzoylecgonine (BE), Methadone, Methaqualone, Codeine, Phencyclidine (PCP), Nortriptyline. Prepare fresh and mix adequately.

Note-01: If other drugs are to be used when preparing the In-House Cut-Off Positive Control Mixtures then a minor deviation (or other paperwork) will be prepared prior to analysis and will accompany all batch work and case files.

Note-02: Unless otherwise noted, the above standard and control solutions are stable for at least one (1) year from either their creation or from their last verification. If solutions are used as purchased (i.e., without modification), storage and stability will be determined by manufacturer.

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7 Calibration

This procedure is qualitative only.

8 Sampling

Not applicable.

9 Procedure

The Appendix contains an abbreviated version of this procedure. This form may be used at the bench by analysts who perform the procedure. If SPE cartridges are in a rack and are not removed during the extraction process and are always in a fixed position then labeling of SPE cartridges is optional, regardless of what is written in the following steps or in the Appendix. However, the vials which collect the final eluent must be properly labeled.

9.1. Specimen Preparation:

9.1.1. Add 0.5 mL of each sample to properly labeled test tubes.
Appropriate positive and negative controls will be analyzed with each batch of samples.

9.1.2. Add 100 µL of the I.S. Working Solution into each sample (25 ng each)

9.2. Urine Specimens Only (De-conjugate):
Note: Approval from Lead Examiner or Deputy Director needs to be obtained if urine specimens are to be hydrolyzed.

9.2.1. Add 250 µL of β–Glucuronidase in 0.1 M acetate buffer (pH ~ 5) to hydrolyze any conjugated drugs within urine samples.

9.2.2. Heat for 3 hours at 60 ˚C and then cool specimens to room temperature.

9.3. Add 1 mL of the Sodium Acetate Buffer (pH ~4.5) to each specimen.

9.4. Add 500 µL of water – cap and vortex-mix samples.
Sample test tubes may all be open during this step.

9.5. Sonicate for at least 15 minutes (not required for urine samples).

9.6. Centrifuge all samples (e.g., 8 minutes at ~5200 RPM)

9.7. Label Trace-B extraction [SPE] columns appropriately

9.8. Place labeled Trace-B extraction columns in the SPE column rack in the appropriate order. Position plastic waste tray underneath SPE column rack. {for Solvent Waste}

9.9. Condition each column with 1 mL MeOH, then drain (≈ 3 psi). {for Solvent Waste}

9.10. Remove solvent tray and replace with biohazardous tray.

9.11. Add 1 mL of water to each SPE column, then drain (≈ 3 psi) to biohazardous tray.

9.12. Individually transfer samples to the center of their respective [properly labeled] SPE column. Avoid splashing and/or transferring any sediment found at the bottom of the tube.

9.13. Allow samples to migrate through SPE column via gravity. Apply small pressure, if needed (< 3psi).

9.14. To each SPE column:

9.14.1. Wash with 1 mL of Bicarbonate/Carbonate buffer (pH~9), then drain (≈ 3 psi) to biohazardous waste.

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9.14.2. Wash with 1 mL water, then drain (≈ 3 psi) to Biohazardous waste.

9.15. Dry the columns for ~10 minutes using maximum pressure (e.g., between 60-80 psi).

9.16. During this 10 minute window (or earlier) label autosampler vials and place in the appropriate position in the SPE collection rack underneath the corresponding SPE column. Prepare the elution solvent.

9.17. The elution solvent ratio is 80:18:2 CH_2Cl_2:IPA:NH_4OH.
(Approximately 2 mL will be needed for each tube).

9.18. After ~10 minutes, replace plastic waste tray with the SPE collection rack containing appropriately labeled autosample vials. Ensure that each vial is placed under their corresponding SPE column for elution.

9.19. Elute columns with two (2) – 1 mL aliquots of the Solid Phase Extraction Elution Solution. Collect eluent into appropriate autosampler vials using gravity or using low pressure (<3 psi).

9.20. Remove top SPE column rack and transfer collection rack from SPE manifold to sample concentrator.

9.21. Add ~1 drop of 1% MeOH-HCl to each tube.

9.22. Evaporate all eluents to dryness at <40 ˚C.

9.23. If samples are not to be immediately analyzed they should be placed in a refrigerator or freezer under proper evidentiary seal.

Note: The word 'immediately' refers to instrumental sequences being started on the same day.

9.24. Reconstitute each extract sample with 500 µL of $MeOH_{(aq)}$ (20% (v/v)) when ready for instrumental analysis.

9.25. Check turbidity of solution. Remove undissolved particulates and treat as necessary (e.g., filtration or centrifugation). Controls (negative and positive) will be treated in same manner as samples.

9.26. Analyze extract solutions using the LC/MS instrument.
Blank solutions (i.e., solvent, mobile phase) will be analyzed in-between samples.

10 Instrumental Parameters

The following are the typical operating parameters for the instrument used in this procedure. With approval from the Lead Examiner and Deputy Director (or higher), the instrument conditions may be modified to adjust or improve the procedure. Documention must be included with batch data so that any instrumental parameter changes can be associated with data and casework until this procedure has been updated.
The appendix contains an abbreviated version of the procedure. The checklist can be used by analysts. Any changes within the instrumental parameters, if listed on the checklist, will be reflected on the checklist by the analyst filling it out.

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HPLC Parameters:

Mobile Phase A: 0.01 %(v/v) $HCOOH_{(aq)}$ and 5mM $NH_4CHOO_{(aq)}$
Mobile Phase B: $MeOH_{(l)}$ (neat)
HPLC Column: Kinetex, 2.6 µm Phenyl-Hexyl, 100 Å, 50 mm x 4.6 mm (Phenomenex)
Column Temperature: 40 °C
Constant Flow: 0.6 mL/min.
Autosampler Temperature: 15 °C
Injection Volume: 10 µL
Needle Wash: 500 µL before and after aspiration

Gradient Program: Initial 5% B ; Flow 0.6 mL/min

Time (min.)	**Mobile Phase A [0.01 %(v/v) $HCOOH_{(aq)}$ + 5mM $NH_4CHOO_{(aq)}$] (%)**	**Mobile Phase B $MeOH_{(neat)}$ (%)**
Initial	95	5
0.5	95	5
3.00	55	45
4.5	55	45
5.5	40	60
7.5	40	60
10.10	25	75
10.50	5	95
12.50	5	95
12.51	95	5
15.00	STOP	STOP

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Mass Spectrometer Parameters:

Ionization Source	Electrospray	**Polarity**	Positive Ion
Ion Spray Potential	+4.5 kV	**Heat Block**	400 °C
Nebulizer Gas	Nitrogen	**Nebulizer**	2 L/min
Drying Gas	Nitrogen	**Drying Gas**	15 L/min.
Scan Type	MRM (&/or Full Scan)	**Resolution**	Unit
Desolvation Line	250 °C		

11 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. Retention time (chromatographic characteristic), fragmentation pattern and qualified ion ratios (mass spectrometric characteristics), and other characteristics are used as the basis for detection and identification. In most cases all of the criteria below should be met in order to identify the appropriate drugs within biological specimens.

11.1. Chromatography

All chromatographic peaks for the analytes of interest should show good chromatographic characteristics, with reasonable peak shape, width, and resolution. For low concentrations of an analyte (<5 ng/mL), there may be transitions that are not optimal. In order to be determined as acceptable, a chromatographic peak in an unknown sample should compare favorably to the same analyte's chromatographic peak in a known sample which has been analyzed on the same system and in the same, or subsequent, analytical timeframe. Additionally, the following two criteria should be met:

11.1.1. Retention Time (RT)

The retention time of a peak of interest should be within ±2 % of the retention time (relative or absolute) of a reference standard or positive control.

Note: Instrumental evaluation of retention time using +/-0.1 minute as a criteria is acceptable. Using ±2 minute window is also acceptable for sample/control evaluation.

If analyzed, the retention times of the analytes within the Procedural Performance Solution should be within ±2 % of their previously analyzed times and a set value for acceptance. Minor changes in mobile phase percentage may account for slight retention time shifts.

11.1.2. Signal-to-Noise Ratio (SNR)

To justify the existence of a peak, its baseline signal-to-noise ratio (SNR) will exceed 3. Furthermore, the baseline signal for the peak of interest will be at least 10-fold greater than that for any observed peak (corresponding to the analyte of interest) at a similar retention time in a negative control or blank sample that was injected just prior to that sample.

11.2. Mass Spectrometry

In order for the MS to be considered in good operating condition, the correct mass assignments for each of the analytes in the performance standard should be present. Independent MS/MS experiments are conducted for each analyte. Ion ratios should compare favorably to ion ratios from reference standards or an extracted positive control at a comparable concentration. Generally, ion ratios are within the following limits:

RETIRED

Expected [Set] Ion Ratio	Allowance
>50 %	20%
20% - 50%	25%
<20%	30%

11.3. Batch Acceptance:

In order for a batch to be acceptable:

11.3.1. No analytes of interest will be detected in the Negative Control.

Note-01: An analyte is considered applicable if it is important to the batch. For example: OCME cases are not interested in methaqualone and nortriptyline, so these would not be considered as applicable analytes of interest for such samples.

Note-02: Analytes of interest are considered those compounds that are being reported.

11.3.2. All applicable analytes of interest within Positive Controls, as well as internal standards, will be identified.

11.3.3. For urine batches, morphine will be detected in the hydrolysis positive control.

11.4. Unknown Sample Compound Detection:

This procedure is intended to be used for qualitative screening purposes. However, if enough data is present, confirmations can be reported if a different chromatographic or mass spectrometric experiment is employed (or with approval of Lead Examiner and Deputy Director (or higher)).

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12. Calibration

Not applicable.

13. Uncertainty

Not applicable – this is a qualitative procedure only.

14. Limitation

14.1. Limits of Detection (LOD)

This information should be found in validation paperwork.

14.2. Specificity

No known interferences. This procedure will generally be used as a screening technique. All forensically significant positive findings should be confirmed by a second technique unless alternative situations apply (e.g., Memorandum of Understanding (MOU), directive from customer, approval of Deputy Director (or higher)).

Note: A second technique could include a different LC/MS instrument or using the same instrument with a different chromatographic or mass spectrometric experiment.

15. Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment should be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Biological specimens will be handled using universal precautions and will be treated as biohazardous. Potentially contaminated items and surfaces will be cleaned prior to use, especially. When casework samples are being processed/analyzed brown paper (or other similar barrier) should be placed in between the surface and the specimens.

16. References

Screening and Confirmation of Benzodiazepines in Blood by Electrospray LCMSMS. West Chester County Department of Laboratories and Research: Division of Forensic Toxicology.

Solid-Phase Extraction Method for Trace B Columns. SPEWARE: Baldwin Park, CA.

A Comparison of the Validity of Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Tandem Mass Spectrometry Analysis of Urine Samples for Morphine, Codeine, 6-Acetylmorphine, and Benzoylecgonine; Peter R. Stout, Nichole D. Bynum, John M. Mitchell, Michael R. Baylor and Jeri D. Ropero-Miller; J.Anal Toxicol (2009) 33 (8): 398-408

Exclusionary Drug Screen by UPLC-ESI-FTMS, FBI Laboratory.

Benzodiazepine Quant in Blood LCMSMS procedure, Toxicology Unit, Division of Scientific Services.

Processing of Opioids by LCMSMS procedure, Toxicology Unit, Division of Scientific Services.

Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities – 2017 Update, Logan, et. al, Journal of Analytical Toxicology, 42, 2, 63-68

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Appendix:
Bench Method:
Exclusionary Drug Screening by Liquid Chromatography/Mass Spectrometry (LC/MS)

The following is an abbreviated version of this procedure and a pdf-version can be used.
This form may be used at the bench level by the analyst performing the procedure.

Negative Control [drug-free] Blood/[other matrix]	**Lot:**
Positive Control Blood/[other matrix]	**Lot:**

Procedure:

	For Blood Specimens	
Add 0.5 mL sample/negative control/positive control into properly labeled test tubes (e.g., 16 x 100 mm)		
Add 100 µL of I.S. Working Sol'n to each sample (25 ng each drug):		
	Diazepam-D_5 (250 ng/mL)	**Lot:**
Deconjugate urine specimens (approval needed):		
	Enzyme	**Lot:**
	Hydrolysis Sodium Acetate Buffer solution (pH~5) – heat for 3 hours at 60 ˚C	**Lot:**
Add 1 mL of Sodium Acetate $Buffer_{(aq)}$ (pH 4.5) to each test tube.		**Lot:**
Add 500 µL of water to each test tube, cap and vortex-mix samples, sonicate, if blood. Centrifuge.		
Label Trace-B extraction [SPE] columns appropriately.		**Lot:**
Place labeled Trace-B extraction columns in the SPE column rack in the appropriate order.		
Add 1 mL MeOH (to condition SPE), drain (≈3 psi)		**Lot:**
Add 1 mL of water (to wash each SPE column), then drain (≈ 3 psi)		**Lot:**
Individually transfer samples/controls to the center of their respective [properly labeled] SPE column. Avoid sediment / splashing. Allow to migrate through SPE column via gravity. Apply small pressure, if needed (< 3psi).		
To each SPE column:		
	Wash with 1 mL of Bicarbonate buffer (pH ~9), then drain (≈ 3 psi)	**Lot:**
	Wash with 1 mL water, then drain (≈ 3 psi)	**Lot:**
Dry the columns for 10 minutes using maximum pressure (e.g., between 60-80 psi)		
During this 10 minute window (or earlier) label autosample vials, place in SPE rack. Prepare the elution solvent.		
The Solid Phase Extraction Elution Solution ratio is 80:18:2 CH_2Cl_2:IPA:NH_4OH. (Approximately 2 mL per each tube).		
	Methylene Chloride (CH_2Cl_2)	**Lot:**
	Isopropyl Alcohol (2-Propanol ; IPA)	**Lot:**
	Ammonium Hydroxide (NH_4OH)	**Lot:**
After ~10 minutes, replace plastic waste tray with SPE rack containing autosample vials. Ensure vials are properly placed.		
Elute columns with two (2), 1 mL aliquots of the Elution Solution Flow using gravity (<3 psi if needed)		**Lot:**
Transfer collection rack from SPE manifold to sample concentrator		
Add ~1 drop of MeOH-$HCl_{(aq)}$ and evaporate to dryness (<40 ˚C) ; Store refrigerated and properly sealed if not immediately analyzed		
Reconstitute each extract sample with 500 µL of 20% $MeOH_{(aq)}$; only if analyzing immediately		**Lot:**
Analyze by the LC/MS instrument		

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Appendix (Cont'd):

Specimen Procedure (Cont'd)

Analyze by LC/MS/MS:

Instrument: Shimadzu 8030	Instrument Identifier:
HPLC Column: Phenomenex ; Kinetex Phenyl-Hexyl ; 100 Å ; 2.6 µm ; 50 mm x 4.6 mm):	Lot:
Mobile Phase A: [0.01 %(v/v) $HCOOH_{(aq)}$ + 5mM $NH_4CHOO_{(aq)}$]	Lot:
Mobile Phase B: MeOH (CH_3OH) – 100%	Lot:

Gradient Program:
Initial 5% B ; Flow 0.6 mL/min

Time (min.)	%B
Initial	5
0.50	5
3.0	45
4.5	45
5.5	60
7.5	60
10.10	75
10.50	95
12.50	55
12.51	5
15.00	STOP

Mass Spectrometer Parameters

Ion Source	Electrospray
Spray Voltage	+4.5
Polarity	Positive
Nebulizer Gas	Nitrogen (2 L/min.)
Drying Gas	Nitrogen (15 L/min.)
Scan Type	MRM (&/or Full Scan)
Heat Block	400 °C
Resolution	Unit

RETIRED

Rev. #	History
1	New procedure for urine, blood or other appropriate matrices.
2	Updated the concentration of the Internal Standard to 250 ng/mL, also remove the use of Amobarbital-D_5 and Ethinimate as part of the IS solution. Uppdated the instrument parameters to match current parameters. Updated the IS information on the run sheet. Appropriate positive and negative controls will be extracted and analyzed with each assay or batch. Approval needed from Lead Examiner and Deputy Director if instrument conditions/parameters are changed – documention of charnge(s) must be included with batch data ; appendix must contain any changes, if listed. Section 11.1.1: Retention times of the analytes within the Procedural Performance Solution should be within ±2 % of their previously analyzed times and a set value for acceptance. 14.2: A confirmation could include using a different LC/MS instrument or using the same instrument with a different chromatographic or mass spectrometric experiment. Updated verbiage throughout to be similar to TX 37 SOP. Added direction describing when analytes can be confirmed using this procedure.

RETIRED

I. INTRODUCTION

The LCMSMS can be used to confirm and quantitate most analytes including illicit drugs, prescription drugs, over the counter pharmaceuticals and other drugs.

Samples that require confirmation and/or quantitation by LCMSMS are extracted from a buffered, diluted sample aliquot by adsorption onto a solid phase extraction (SPE) column. Drugs that may be present are then eluted from the SPE column, dried, and reconstituted before injection onto the LCMSMS system.

The detection of each specific analyte is determined by single point calibration cut off for urines. Bloods are quantitated with a multipoint calibration using deuterated internal standard(s) or other internal standard that is appropriate for the analyte.

Matrix-specific (blood and/or urine as needed) positive and negative controls are extracted and analyzed in each analytical batch. The presence of drugs may be confirmed in urine, blood, vitreous fluid or other fluids.

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A. Method Targets

This is a non-targeted quantitation method. The analyte to be quantitated will have been identified by the LCMS screen method, a GCMS method or an immunoassay method.

B. Safety

This procedure is carried out in a laboratory environment and standard safety procedures should be utilized, including (minimally) safety glasses and lab coat when deemed necessary. Biological specimens subject to the analytical procedure should be handled using universal precautions. Potentially contaminated items and surfaces should be disinfected prior to and after use.

D. Specimen Requirements

1. 0.5 mL blood, urine or other fluid (in general urine will be confirmation only)

II. MATERIALS

A. Equipment

1. General laboratory glassware
2. Vortex, Thermolyne Maxi Mix 1 or equivalent

3. Sonicator, Fisher-Scientific or equivalent (for blood samples)
4. Centrifuge, Beckman TJ-6 or equivalent
5. Trace B Extraction Columns - SPEWARE (Baldwin Park, CA)
6. SPEWARE CEREX System-48 Solid phase extraction manifold
7. SPEWARE CEREX System-48 Sample Concentrator
8. Shimadzu LC/MS/MS System consisting of (or the equivalent of):
    a. Degasser: Shimadzu DGU-20A
    b. Pumps: 2 Shimadzu LC-20AD Prominence
    c. Autosampler: Shimadzu SIL 20AC Prominence
    d. Column Oven: CTO-20A
    e. Pre-Column: SecurityGuard ULTRA Cartridge UHPLC Phenyl for 4.6mmID Columns (Phenomenex) or equivalent.
    f. Column: Kinetex Phenyl Hexyl (Phenomenex)
    g. Detector: Shimadzu LCMS-8030 Mass Spectrometer
    h. Controller: Shimadzu CBM-20A
    i. Data Station: Shimadzu LabSolutions software

B. Reagents available as stock items: Sigma or J.T. Baker reagent grade or equivalent unless specified

1. Methanol (CH_3OH): Fisher Optimum LCMS Grade or Burdick Jackson pesticide grade
2. Deionized water (DIW): Milli-Q or LCMS grade
3. Drug-free urine or blood (Drug free blood may be purchased or obtained through a hospital blood bank or equivalent)
4. Formic Acid (HCOOH)
5. Ammonium formate (NH_4HCO_2)
6. Glacial acetic acid (CH_3COOH)
7. Sodium acetate trihydrate ($NaCH_3COO{\cdot}3H_2O$)
8. Sodium carbonate (Na_2CO_3)

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9. Sodium bicarbonate ($NaHCO_3$)

10. External control such as UTAK LC-2 Control – UTAK Laboratories (Valencia, CA)

    It is noted that an external control may not be available for all analytes that need quantitation in these cases an in-house control made from a different lot or by a second analyst will be used.

12. β-Glucuronidase (*P. vulgata*; Sigma or equivalent)

C. Drug Standard Solutions - Standards will be used based on the analyte to be quantitated or confirmed.

a. Drug Standard Solutions -Cerilliant Corporation (Austin, TX) – or equivalent
b. Deuterated Drug Standard Solution – Cerilliant Corporation (Austin, TX) or equivalent
c. Drug Standard Solutions – Lipomed Inc. (Cambridge, MA) or equivalent

D. Reagents prepared in the Toxicology Laboratory

1. <u>5M Ammonium formate</u>:

a. Dissolve 3.15 g of ammonium formate in 10 mL volumetric flask.
b. Q.S. to 10 mL with DIW. Stable for one year, stored in the refrigerator.

2. <u>Mobile Phase A (H_20 with 5mM ammonium formate and 0.01% formic acid)</u>:

a. Add 0.5 mL <u>5M Ammonium formate</u> and 0.05 mL formic acid to a 500 mL volumetric flask.
b. Q.S. to 500 mL with high purity water.
c. Transfer solution to a glass bottle reserved for LC/MS use only. Stable for one week.
d. The above instructions make 500 mL of mobile phase; adjust volumes of reagents accordingly if requiring a different final volume.

3. <u>0.1M Sodium Acetate buffer (pH 4.5):</u>

a. Combine 5.86 g of sodium acetate trihydrate and 3.24 mL glacial acetic acid in a 1000 mL stoppered graduated cylinder.

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b. Q.S. to 1000 mL with deionized water.

c. Store in glass container at room temperature (25^{o}C)

d. Stable for one year

e. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.

4. <u>1.0 M Acetate buffer (pH 5.0)</u>

a. Dissolve 42.9 g sodium acetate trihydrate in approximately 400 mL DIW

b. Add 10.4 mL glacial acetic acid $C_2H_4O_2$

c. Dilute to 500 mL with DIW

d. Mix. Check pH, adjust pH to 5.0 ± 0.1 with 1.0 M acetic acid if needed.

e. Storage: room temperature in glass or plastic. Stability: 6 months

f. Inspect daily for contamination.

5. <u>0.1 M Acetate Buffer (pH 5.0)</u>

a. Dilute 20 mL 1.0 M acetate buffer to 200 mL with DIW

b. Mix. Store at room temperature. Stability: 6 months

6. <u>β -Glucuronidase, (5,000 F units/mL) in 0.1 M Acetate Buffer (pH 5.0)</u>

Prepare daily for use, make slight excess for each batch, each 0.5 mL sample requires 1250 F units. Add 250 µL of β -Glucuronidase to each tube.

Example: for 40 total tubes prepare 10 mL
Calculate activity for each lot of β-Glucuronidase as follows:
(Lot specific, value from bottle label)
e.g. 1,439,000 -glucuronidase units/g solid
5,000 Units/mL = 1,439,000
x mg 1000 mg
1. x = 3.47 mg/mL

5,000 Units/mL = 1,439,000
x mg 1000 mg
x = 3.47 mg/mL

To make 10 mL

Weigh out 34.7 mg β –Glucuronidase solid. Add to 10 mL of 0.1 M acetate buffer (pH 5.0)

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Dissolve before use by swirling gently.
Make fresh daily as needed for each batch

7. **<u>0.1M Sodium Carbonate (pH 8.0):</u>**
   a. Add 5.3 g of sodium carbonate to a 500 mL volumetric flask.
   b. Q.S. to 500 mL with deionized water.
   c. Using a validated pH meter, check pH; should be 8.0 ±0.2.
   d. Store in glass container at room temperature (25^{o}C)
   e. Stable for one year
   f. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.
8. **0.1M Sodium Bicarbonate (pH 11.0)**:
   a. Add 4.2 g of sodium bicarbonate to a **separate** 500 mL volumetric flask.
   b. Q.S. to 500 mL with deionized water.
   c. Using a validated pH meter, check pH; should be around 11.0 ±0.2.
   d. Store in glass container at room temperature (25^{o}C)
   e. Stable for one year
   f. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.

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9. **Bicarbonate Buffer pH 9.0: Mixture of 0.1 M Sodium Carbonate and 0.1 M Bicarbonate Solutions**

a. Into a beaker containing the 0.1M Sodium Carbonate solution (lower pH solution)
b. Using a validated pH meter, check pH, adjust with the 0.1M Sodium Bicarbonate solution (higher pH solution) until a pH of 9.0 ± 0.2 is reached.
c. Store in glass container at room temperature (25^{o}C)
d. Stable for one year
e. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.

10. Reconstitution Mixture: 20% Methanol in Deionized Water

a. Into a 100 mL graduated cylinder with a cap, add 80 mL of deionized water.

b. Add 20 mL methanol.

c. Cap and shake. Store at room temperature; stable for 6 months.

d. Before use check for clarity; if cloudy, discard and prepare fresh.

RETIRED

III. PREPARATION OF STANDARDS:

Whenever possible, calibrator and control solutions will be made from a different lot and/or by separate analysts using different pipettes.

A. Cerilliant Standard Mix or Single Component Standard:

Note: Equivalent reference standards may be substituted if needed. Whenever possible, prepared calibrators and controls should be made from standards from different manufacturers or different lots.

1. Based on the analyte to be quantitated the analyst will prepare a stock solution at the needed concentration. The Unit Lead should be consulted if the analyst is unsure of the appropriate range for the specific drug.
2. When multiple drugs are to be quantitated a mixed standard stock solution can be made.
3. Methanol or other appropriate solvent will be used as the diluent. The volume of standard to be made will be based on the need of the analysis.
4. Document the dilution preparation as part of the batch documentation and in the appropriate standard preparation notebook.
5. Store sealed in a freezer
6. Stable for 6 months when tightly capped.
7. Final concentrations will be dependent on the analyte to be quantitated.
8. A “Working Calibration Solution” can be made by making further dilutions based on the calibration curve required.

9. General Guidance: the dilutions below may be used as guidance for obtaining required concentrations.

Stock Solution			
Starting Concentration of CRM*	**Desired Concentration**	**Amount of Standard**	**Solvent**
1 mg/mL	100 µg/mL	**50 µL**	**450 µL**
	50 µg / mL	**25 µL**	**475 µL**
100 µg /mL	50 µg / mL	**250 µL**	**250 µL**

*Certified Reference Material

Working Solutions			
Starting Concentration of Stock Solution	Desired Concentration	Amount of Standard	QS to (with appropriate Solvent)
100 µg/mL	5000 ng/ mL	250 µL	5 mL
100 µg/mL	2000 ng/ mL	100 µL	5 mL
100 µg/mL	1000 ng/ mL	50 µL	5 mL
50 µg / mL	1000 ng/ mL	100 µL	5 mL
5000 ng/ mL	1000 ng / mL	1000 µL	5 mL
2000 ng/mL	400 ng /mL	1000 µL	5 mL
2000 ng/mL	200 ng/mL	500 µL	5 mL
1000 ng/mL	100 ng/mL	500 µL	5 mL
1000 ng/mL	200 ng/mL	1000 µL	5 mL

RETIRED

C. <u>Working Internal Standard Mix</u>

1. It is preferable to have the internal standard response in a range similar to the mid to lower end of the calibration curve.
2. Starting Guide for preparation, this can be adjusted as needed based on the analyte of interest.
    a. To a 50 mL volumetric flask, add 100 µL of each 1 mg/mL Cerilliant reference deuterated standard or 250 µL of each 100 µg / mL deuterated standard (other concentrations may be used as required).
        i. It is desirous to use a deuterated standard of the analyte to be quantitated; however other appropriate deuterated compounds can be used.
    b. Q.S. to 50 mL with methanol.
    c. Store in freezer (≤ -10°C)
    d. Stable for 6 months when tightly capped.
    e. Final concentrations : 2 µg /mL for standards with a starting concentration of 1 mg/mL and 500 ng/mL for standards with a starting concentration of 100 µg /mL

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IV. PREPARATION OF CONTROLS

Note: Alternative controls, both commercial and in-house, to those listed below may be employed at the analyst's discretion.

A. **<u>In-House Stock Control (Lipomed)</u>:** In-house controls will be used whenever possible.

Note: Equivalent reference standards may substituted if needed. Prepared calibrators and controls should be made from standards from different manufacturers.

1. Based on the analyte to be quantitated the analyst will prepare a stock solution at the needed concentration. The Unit Lead should be consulted if the analyst is unsure of the appropriate range for the specific drug.
2. The chart above may be used as guidance in preparing solutions in the needed concentrations.
3. When multiple drugs are to be quantitated a mixed standard stock solution can be made.
4. Methanol or other appropriate solvent will be used as the diluent. The volume of standard to be made will be based on the need of the analysis.

5. Document the dilution preparation as part of the batch documentation.
6. Store sealed in a freezer
7. Stable for 6 months when tightly capped.
8. Final concentrations will be dependent on the analyte to be quantitated.
9. A 'Working Control Solution" can be made through further dilutions made based on the calibration curve required.

B. **External Controls:** External controls may be used but are not required.

1. External controls may be used. These may come from UTAK or equivalent commercially prepared samples.

C. **Negative Controls**

1. Negative control: Drug-free human urine or drug-free human blood.

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V. PROCEDURE

A. Label clean screw cap tubes appropriately with blank, calibrator, control and case number designations.

B. Prepare calibrator and control samples according to tables below based on the appropriate calibration range for the analyte of interest:

1. Blood Calibrators: prepare using 500 µL of Blank blood spiked with the appropriate volume of calibration solution. (See the chart below).

   Note: Calibration curves should be created in a range appropriate to the analyte of interest. Consult the Unit Lead for guidance. A minimum of 4 calibrators should be extracted.

2. In-House Blood Controls: prepare using 500 µL of blank blood spiked with the appropriate volume of stock control solution. (See the chart below).

Note: A minimum of 2 controls will be used one that is below the mid-point of the curve and one that is above the mid-point of the curve. This is to demonstrate condition at the high and low range of the curve.

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Starting Concentration	Desired Concentration (ng/mL)	Volume to Add (µL)	Volume of Blank Blood µL
100 ng/ mL	5	**25**	**500**
	10	**50**	**500**
	100	**500**	**500**
200 ng / mL	10	**25**	**500**
	20	**50**	**500**
	100	**250**	**500**
	200	**500**	**500**
400 ng/mL	20	**25**	**500**
	100	**125**	**500**
	200	**250**	**500**
2000 ng / mL	200	**50**	**500**
	500	**125**	**500**
	1000	**250**	**500**

3. <u>In-House Urine Control</u> :

   The in-house stock solution will be used and the Chart above can be used as guidance in the preparation of these levels.

4. Commercial Controls (optional):

Control Concentration	**Pipette Volume To Add:**	**Blank Matrix Volume to Add:**
UTAK dilution x4	125 µL	375 µL
UTAK	500 µL	0 µL

C. **Blood and Urine sample preparations**

1. Add 0.5 mL case specimen, blood or urine, to appropriate labeled tubes.
2. Add 100 µL of deuterated IS to each tube. (A different volume may be required based on the IS used)
    i. **Urine Total conjugated and unconjugated**

        Note: Not all urines require hydrolysis. The analyst may decide which samples to test for total and free analytes based on the needs of the case.

        1. Add 250 µL of β –Glucuronidase in 0.1M acetate buffer, pH 5.0 to hydrolyze urine
        2. Heat for 3 hours at 60^{o}C in water bath. Cool tubes to room temperature.
3. Add 1 mL of 0.10 M sodium acetate buffer (pH 4.5) to each tube.
    i. **Blood sample preparations:** Add 500 µL DI water to each blood tube.

        Note: Adding 500 µL DI water to urine samples is not detrimental.
4. Cap tubes, then vortex each tube for 10 seconds.
    i. **Blood sample preparations:** Sonicate blood samples for 15 minutes.

    Note: Sonicating urine samples is not detrimental.
5. Centrifuge **all** tubes for 8 min at about 5200 rpm.
6. Place the Trace B extraction columns in the SPE column rack in the appropriate order. Position plastic waste tray labeled “Methanol” underneath SPE column rack.
    i. Condition each column sequentially with 1 mL methanol; drain (≈3 psi) to Solvent “Hazardous Waste” stream
7. Remove plastic waste tray labeled “Methanol” and replace with plastic tray labeled “Biohazardous/Buffers”
8. 1 mL DI water; drain (≈ 3 psi) to “Non-Hazardous” regulated waste stream
9. Carefully transfer the sample to the center of the SPE column. Avoid splashing and/or transferring any sediment found at the bottom of the tube.

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10. Wash each SPE column sequentially with:
    i. 1 mL bicarbonate buffer (pH 9.0); drain (≈ 3 psi) to non-hazardous regulated waste stream
    ii. 1 mL DI water; drain (≈ 3 psi) to non-hazardous regulated waste stream
11. Dry the columns for 10 minutes using maximum pressure, between 60-80 psi.
12. During this 10 minute window (or earlier), label autosampler LC vials and place in the appropriate position in the SPE collection rack underneath the corresponding SPE column; prepare the elution solvent.
13. The elution solvent ratio is 80: 18: 2 Dichloromethane: IPA: NH_4OH. (Adjust volume prepared for the number of samples, minimize excessive hazardous waste, 2mL needed for each tube)
    i. Add the IPA and NH_4OH together first before adding the dichloromethane portion (following this order prevents unsafe buildup of gases).
14. After 10 minutes, replace plastic waste tray with SPE collection rack containing labeled collection tubes in order corresponding to SPE columns.
15. Elute column with two 1.0mL aliquots of 80:18:2 Dichloromethane:IPA:NH_4OH into the appropriate collection tube. Flow at 2-4 mL/min to optimize recovery.
16. Remove top SPE column rack and transfer collection rack from SPE manifold to sample concentrator.
17. When target compounds are sympathomimetic amines add 25ul of 1% HCl to each sample.
18. Evaporate all samples to dryness at $<40^{o}$ C.
19. Reconstitute each tube with 150 µL or less of 20% methanol in DI water. All calibrators, controls and samples must be reconstituted with the same volume of solution. If the volume is different from 150 ul note the volume.
20. Individually transfer solutions to properly labeled auto sampler vials containing sample inserts.
21. Analyze by LC/MS/MS.

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VI. INSTRUMENT PARAMETERS

A. LC Parameters

1. Shimadzu Prominence LC-20 System

a. Flow	0.6 mL/min
b. Autosampler Temperature	15°C
c. Injection Volume	10 µL
d. Needle Wash	500 µL; before and after aspiration
e. Column	Kinetex 2.6um Phenyl-Hexyl 100A 50 x 4.6 cm
	Manufactured by Phenomenex (Torrance, CA)
f. Oven Temperature	40°C
g. Gradient	

Time (min)	Mobile Phase B (Methanol)	Mobile Phase A (0.01% Formic Acid in Water)
Initial	5%	95%
2.50	45%	55%
4.50	45%	55%
5.50	95%	5%
7.50	95%	5%
7.51	5%	95%
9.00	STOP	STOP

RETIRED

B. M/S Acquisition Parameters: The following conditions can be adjusted if needed based on availability of gases.

Interface	DUIS (APCI and ESI)
DL Temperature	250°C
Nebulizing Gas	2 L/min
Drying Gas	15 L/min

C. Transition Ions Monitored and Retention Times:

Transition ions to be monitored and analyte retention times will be dependent of the analyte(s) of interest. A neat standard should be run prior to performing a calibration on an analyte not previously run by the instrument to determine the retention time and transition ions to be monitored. LabSolutions optimization software will be used.

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D. Detection and Identification: The analyst will review all chromatography, peak integrations, and transition ion ratios used for identifications.

Determination of the presence of target analytes in the sample extract are identified by appearance and ratio of product ions that are characteristic of each drug at the appropriate retention time. In this manner, both retention time (an LC characteristic) and fragmentation pattern and ratio (an MS characteristic) are used as the basis for qualitative identification. For the identification of an analyte to be made, the retention time of the chromatographic peak must be within 0.1 minute of the corresponding analyte in the calibrator sample as well as having ion ratios that are within the following limits. Initially the neat Cerilliant Standard, diluted as appropriate, will be utilized to set the expected ion ratios, however it is recognized that some ion ratios are concentration dependent. As such, ratios may be set on a case by case basis using a standard with a concentration close to the concentration of the analyte of interest in the case.

Expected (Set) Ion Ratio	Allowance
> 50%	20%
20-50%	25%
< 20%	30%

E. Calibration: Calibration is accomplished by the addition of a known amount of analytes (in addition to the internal standard) into a blank sample of the matrix that is tested. The response of the system to this calibration defines the standard curve that is used as the basis for the quantitative calculations for controls and samples. The system for blood samples is “multi-point calibration, multi-point control.”

While a calibration curve for each drug and metabolite may be analyzed with each batch, a previously established (or "historical") calibration curve may be used. The calibration correlation coefficient must be ≥ 0.990 when using deuterated internal standards. When using non-deuterated internal standards a correlation coefficient ≥ 0.98 is acceptable. A calibrator may be removed to attain a correlation coefficient of ≥ 0.990. Samples resulting in concentrations higher than the highest calibrator should be diluted (approval not to dilute must be obtained by the Unit Lead or higher). The lower limit of quantitation (LOQ) is the concentration of the lowest calibrator. The urine control will be quantitated based on the blood calibration curve.

F. Quantitation: Quantitation is accomplished by the comparison of the response ratio of the analyte and the internal standard in a specific sample relative to the response ratios of the calibration curve. The concentration of the analyte in the sample is then extrapolated from the standard curve.

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VII. RESULTS INTERPRETATION

A. Positive results will be reported only when:

1. Analyte identification is based on at least two transitions with relative abundances within +/- 20 % of the target, relative to a calibrator.
2. Retention times are within 0.1 min, or +/- 3% relative to a calibrator analyzed in same batch.
3. Qualitative results have at least a 3x signal to noise (S/N) ratio.
4. The integration of the analyte peak has acceptable symmetrical shape and chromatography.
5. All MRMs show peaks at the appropriate retention times.
6. Quality control sample results are acceptable.

VIII. QUALITY CONTROL

A. Criteria for Quantitative Results

1. Statistics will be maintained on all controls.
2. Results of controls must fall within ±20% of the analytes' target concentration.

3. All results are recorded.

B. Verification of Vial Sequence: The vial sequence is checked prior to and following injection of samples. These checks will be documented.

C. Evaluation of Potential Carryover: Potential Carryover will be determined by a blank sample after the highest calibrator. In addition, a solvent blank will be run after each sample to ensure there is no carryover between samples.

D. Linearity: Linearity of the calibration curve is demonstrable for each analyte as a function of r^2 correlation coefficient and quantitative results of control materials.

E. Sensitivity (LOD, LOQ): For the purposes of this procedure the limit of quantitation (LOQ) is defined as equal to the lowest concentration of the lowest calibrator. The Limit of Detection (LOD) must have a response of at least 3 times the signal to noise ratio and have acceptable ion ratios.

F. Specificity: Specificity is a function of both the resolution of target analyte during the analytical process and the mass spectral fragmentation that analyte molecules undergo during the instrumental analysis.

IX. QUALITY ASSURANCE

A. Quality Assurance is provided by the multi-layer program described below:

1. The LC/MS analysis is thoroughly checked by the instrument operator. This includes the review of all data, including the determination of run acceptability and a check of vial position (prior to and following the injection of samples).
2. The LC/MS data (batch) is reviewed and signed off by a reviewer (batch reviewer) distinct from the operator; this review includes an evaluation of qualitative and quantitative (where applicable) results containing:
    a. Control Results
    b. Chromatographic Characteristics
    c. Transcription Errors
3. An overall case review by a Final Technical Reviewer.

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X. SOURCES OF ERROR

It has been established that no known interferences are present in the calibrators/controls. Ion suppression or enhancement and potential interferences from other analytes have not been found for the common drugs and metabolites typically seen in casework.

XI. References

A. A Comparison of the Validity of Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Tandem Mass Spectrometry Analysis of Urine Samples for Morphine, Codeine, 6-Acetylmorphine, and Benzoylecgonine; Peter R. Stout, Nichole D. Bynum, John M. Mitchell, Michael R. Baylor and Jeri D. Ropero-Miller; J.Anal Toxicol (2009) 33 (8): 398-408
B. Solid-Phase Extraction Method for Trace B Columns. SPEWARE: Baldwin Park, CA.

RETIRED

Revision #	History
2	Updated VI.E Calibration removing the need to compare calibrators to the generated calibration curve. Section V replaced autosample vial with collection tubes in multiple areas. V.19 changed the volume of reconstitution solution from 520 ul to 150 ul. Added V.20 to transfer to an autosampler with an insert.

Title: (TX-37 SOP) Drug Screening and Confirmation by Liquid Chromatography/High Resolution-Mass Spectrometry (LC/HR-MS)

RETIRED

1 Introduction

A rapid liquid chromatographic method using electrospray ionization (ESI) combined with high resolution accurate mass (HRAM) spectrometry (LC/MS) can be used to qualitatively screen biological specimens (e.g., blood, urine, vitreous fluid) for the presence of common drugs of abuse (DoA) or other compounds. The amount of a detected analyte can be estimated by comparing the responses (relative peak areas) for an analyte to that for a corresponding internal standard. Positive findings can be confirmed by using either a second technique or a second aliquot of the same sample. Confirmation can also be achieved during quantitative analyses using appropriate procedures.

2 Scope

This procedure is limited to the qualitative screening of biological specimens for the presence of cocaine, barbiturates, opioids, and benzodiazepines, their respective metabolites, and other drugs (see list of target analytes and limits of detection). Other target compounds can be added, as needed, once validated.

3 Principle

Biological specimens are analyzed for the presence of drugs and/or their metabolites using solid phase extraction (SPE) columns. Final extracts are analyzed by LC/MS using ESI and can involve a combination of selected reaction monitoring (SRM) and full scan analysis modes.

4 Specimens

This procedure uses biological fluid(s) such as blood, urine, serum/plasma, vitreous fluid. Typically 0.5 mL of sample is consumed during the analysis. Serum and plasma samples will be considered synonymous to, and treated the same as, blood samples.
Varying volumes may be used, as necessary, but such changes shall be documented within technical records (e.g., worksheets, case notes). For example, dilution of samples due to limited specimen or due to suspicion of high drug or metabolite concentration or sample volumes over 0.5 mL are acceptable, given prior approval from the Technical Lead (or higher).

5 Equipment/Materials/Reagents

a. General laboratory glassware

b. Disposable borosilicate test tubes (e.g., 16 x 100 mm, round bottom, borosilicate glass with Teflon caps)

c. Vortex mixer

d. Sonicator

e. Automatic pipettes (with disposable tips)

f. Positive pressure solid phase extraction device – Cerex, 48 sample (or equivalent)

g. Sample concentrator with nitrogen – Cerex 48 heated (or equivalent)

h. Centrifuge

i. pH paper (Micro Essentials Lab ; or equivalent)

j. Trace B Extraction Columns – Tecan (or equivalent)

k. Liquid Chromatograph/High Resolution-Mass Spectrometer (LC/HR-MS) – Q-Exactive (or equivalent)

l. HPLC column – Accucore C18, 2.6 µm, 50 mmx3 mm (Phenomenex or equivalent)

m. Pre-Column – Accucore Defender guard 2.6 µm, 10 mm x 3 mm (Phenomenex or equivalent)

n. Autosampler vials (LC/MS grade 1.8mL or equivalent)

o. Acetic acid, glacial ($CH_3COOH_{(l)}$, Reagent grade or equivalent)

p. Ammonium formate (NH_4CHOO, LC/MS grade or equivalent)

q. Ammonium Hydroxide (NH_4OH, Reagent grade or equivalent)

r. Formic acid (HCOOH, LC/MS grade or equivalent)

s. Hydrochloric Acid ($HCl_{(conc)}$; Reagent Grade or equivalent)

t. Isopropanol (i-PrOH, IPA, 2-Propanol, Reagent grade or equivalent)

u. Methanol (MeOH, Reagent grade, LC/MS Grade, or equivalent)

v. Methylene Chloride (CH_2Cl_2, Reagent grade or equivalent)

w. Sodium acetate trihydrate ($NaCH_3COO \cdot 3H_2O$, Reagent grade or equivalent)

x. Sodium bicarbonate ($NaHCO_3$; Reagent grade or equivalent)

y. Sodium carbonate (Na_2CO_3 ; Reagent grade or equivalent)

z. Water (H_2O ; Millipore, Deionized (DIW), or equivalent)

aa. Water (Mobile Phase) (LC/MS Grade ; Optima or equivalent)

bb. Ammonium $Formate_{(aq)}$: ($NH_4CHOO_{(aq)}$; 5M ; 31.5% (w/v)): Can be prepared by dissolving 3.15 g of ammonium formate in 10 mL of water. Stable for at least one (1) year in glass container when refrigerated.

cc. Sodium $Acetate\ Buffer_{(aq)}$ ($NaCH_3COO$; pH~4.5): Can be prepared by combining 5.86 g of sodium acetate with 3.24 mL of glacial acetic acid in a 1 L volumetric cylinder and diluting to

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volume with water. Stable for at least one (1) year in glass container while at room temperature.

dd. Sodium Bicarbonate$_{(aq)}$ (0.1M ; $NaHCO_3$; 0.84% (w/v) ; pH~8): Can be prepared by dissolving 4.2 g of sodium bicarbonate in water within a 500 mL volumetric flask and diluting to volume with water. Stable for at least one (1) year in glass container while at room temperature.

ee. Sodium Carbonate$_{(aq)}$ (0.1M ; Na_2CO_3 ; 1.1% (w/v) ; pH~11): Can be prepared by dissolving 5.3 g of sodium carbonate in water within a 500 mL volumetric flask and diluting to volume with water. Stable for at least one (1) year in glass container while at room temperature.

ff. Bicarbonate/Carbonate Buffer$_{(aq)}$ (pH~9): Can be prepared by transferring 0.1M sodium bicarbonate$_{(aq)}$ solution into a beaker and checking pH (should be pH~8). Adjust the pH to ~9 using the 0.1M sodium carbonate$_{(aq)}$ solution. Stable for at least one (1) year in glass container while at room temperature.

gg. Solid Phase Extraction Elution Solution (e.g., 50 mL):
{CH_2Cl_2 : IPA : NH_4OH (80 : 18 : 2)}.
Can be prepared by adding 9 mL of isopropanol to 1 mL of ammonium hydroxide within a 50 mL volumetric cylinder. To this mixture add 40 mL of methylene chloride and mix. This solution will be prepared when needed for use.

Note: The volume should be adjusted to account for number of samples that are to be extracted (2 mL are needed for each extraction sample)

hh. MeOH$_{(aq)}$ (20% (v/v)): Can be prepared by adding 80 mL of water and 20 mL of methanol to a 100 mL volumetric cylinder and mixing. Stable for at least one (1) month in glass container when refrigerated.

ii. Methanol-HCl (1% (v/v)): Can be prepared by dissolving 10 µL of hydrochloric acid into 990 µL of methanol (or in different volume with an equivalent ratio). Stable for at least one (1) month in glass container when refrigerated. Verify pH is $\leq$ 3 prior to use.

jj. Mobile Phase A – 0.01 %(v/v) HCOOH$_{(aq)}$ and 5mM $NH_4CHOO_{(aq)}$:
Can be prepared by mixing 50 µL of formic acid with 0.5 mL of 5M ammonium formate in a 500 mL volumetric cylinder, diluting to volume with [Mobile Phase] water, and mixing well. Store in glass at room temperature. Stable for at least one (1) week while in a closed state.
{Note: 5mM $NH_4CHOO_{(aq)}$ is equivalent to 0.0032 % (w/v) $NH_4CHOO_{(aq)}$.}

kk. Mobile Phase B – (MeOH or CH_3OH). Store in glass at room temperature – stable indefinitely in a closed state at room temperature.

Note: Unless otherwise noted, the above Reagent solutions are stable for at least one (1) year from either their creation or from their last verification. Check clarity and check pH (when indicated as having a pH value) for all solutions prior to use.

6 Standards and Controls

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Positive and Negative Control Solutions: Can be purchased (e.g., UTAK Labs) or generated in-house (e.g., spiked blank blood, spiked blank urine, spiked vitreous). Store frozen, refrigerated, or obtain fresh. If purchased, the stability should be determined by the manufacturer. If not enough vitreous fluid is available for control solution preparation(s), water can be used in its place (document accordingly in case notes).

Internal Standard Components: Purchased from Cerilliant or other suitable vendors (e.g., 0.1 mg/mL solutions or neat solid). Storage and stability is determined by manufacturer(s). Additional or alternative deuterated compounds may be used.

a. Internal Standard (I.S.) Working Solution:
(Diazepam-D_5 + Butalbital-D_5 + Meprobamate-D_3 + Morphine-D_3 + Oxycodone-D_3):

(Diazepam-D_5 at 250 ng/mL ; Butalbital-D_5 at 1000 ng/mL ; Meprobamate-D_3 at 1000 ng/mL ; Morphine-D_3 at 250 ng/mL ; Oxycodone-D_6 at 250 ng/mL)

Can be prepared by placing the following into a 100 mL volumetric flask containing methanol:
Diazepam-D_5 reference standard solution: 250 µL of a 100 µg/mL
Butalbital-D_5 reference standard solution: 1 mL of a 100 µg/mL
Meprobamate-D_3 reference standard solution: 1 mL of a 100 µg/mL
Morphine-D_3 reference standard solution: 250 µL of a 100 µg/mL
Oxycodone-D_6 reference standard solution: 250 µL of a 100 µg/mL

Dilute all of the above to volume [100 mL] with MeOH. Store in glass in a freezer.

Note-01: If other deuterated drugs are used as internal standards, documentation will accompany affected batches and casefiles (e.g., minor deviation form).

Note-02: As long as the final concentrations are met, variations in volumes, concentrations, and/or sets of reference standard solutions are allowed.

b. Optional Instrument Performance Standard Mix: ((Diazepam-D_5) ; 10 ng/mL)

This is optional and can be used to evaluate the instrument in addition to the performance check solution for the LC/MS instrument.

Dilute the I.S. Working Solution 1:25 with water to make a 10 ng/mL final concentration. This can be done by mixing 20 µL of the I.S. Working Solution, combining it with 500 µL of deionized water, placing it into an autosample vial, and capping. This solution will be prepared when needed for use.
Note: Other analytes within the solution are not evaluated – just the Diazepam-D_5 analyte.

c. Negative Control Blood:
Purchased (e.g., Diagnostics Products Corporation, UTAK Labs), donated (e.g., American Red Cross, hospital blood bank), or in-house demonstrated negative control blood (e.g., from a proven blank specimen). Store frozen or refrigerated. If purchased or donated, stability should, when possible, be determined by manufacturer.

RETIRED

Negative Control Urine (or other matrix):
Purchased, acquired (e.g., Office of the Chief Medical Examiner (OCME)), or in-house demonstrated to be negative. Store refrigerated or frozen.

d. In-House Cut-Off Positive Control Mixture (Blood, Urine):
Can be prepared by making analyte concentrations initially within methanol and then within the appropriate matrix according to the following table below. Concentrations are prepared based on purchased standard concentrations and diluted accordingly. The final mixture(s) of analytes will be in matrix-appropriate solutions.
Note: The urine In-House Cut-Off Positive Control Mixture will be used for vitreous humor samples.

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In-House Cut-Off Positive Control Mixture (1 mg/mL stock soln's) (except where noted)	Volume to Add	Dilution #1 ('Stock A') (ng/mL) (in 100 mL of MeOH)	Dilution #2 ('Stock B') (ng/mL) (in 10 mL of MeOH)	Amount to add into 0.5 mL of matrix	In-House Cut-Off Positive Control Mixture (ng/mL) (0.5 mL of matrix)
Buprenorphine (100 µg/mL)	20 µL	20	–		1
Fentanyl (100 µg/mL)	20 µL	20	–		1
6-MAM	10 µL	100	–		5
Citalopram	10 µL	100	–		5
Oxymorphone	10 µL	100	–		5
Pseudoephedrine	10 µL	100	–		5
Bupropion	20 µL	200	–		10
Cocaine	20 µL	200	–		10
Fluoxetine	20 µL	200	–		10
Lorazepam	20 µL	200	–		10
Methorphan	20 µL	200	–		10
Morphine	20 µL	200	–	25 µL	10
Oxycodone	20 µL	200	–		10
PCP	20 µL	200	–		10
Sertraline	20 µL	200	–		10
Amphetamine	40 µL	400	–		20
Methadone	40 µL	400	–		20
Nordiazepam	40 µL	400	–		20
Venlafaxine	40 µL	400	–		20
Benzoylecgonine	100 µL	1000	–		50
Diphenhydramine	100 µL	1000	–		50
Tramadol	100 µL	1000	–		50

RETIRED

Lidocaine	100 µL	–	10,000		500
Meprobamate	100 µL	–	10,000		500
Secobarbital	100 µL	–	10,000		500

e. In-House Cut-off Verification Mixture (Blood, Urine):
Can be prepared by making analyte concentrations within the In-House Cut-Off Positive Control Mixture (Blood, Urine) at three times (3x) their listed levels. Concentrations are prepared based on purchased standard concentrations and diluted accordingly. The final mixture of analytes will be in a matrix solution.
Note: The urine In-House Cut-off Verification Mixture will be used for vitreous humor samples.

f. Positive Control Mixture (Blood, Urine, Vitreous):
When possible external positive control(s) will be used. These can be prepared by using an appropriate vendor's product (e.g., UTAK DOA Level 2 (blood), PM 100 (blood), DAU High Cutoff 2 (urine), PM Plus Low (urine), Miscellaneous Panel Level 1 (urine)). Only analytes which have concentrations listed at or above three-times (3x) the listed levels within the In-House Cut-Off Positive Control Mixture analyte concentration will be evaluated.
In-house positive control mixtures will be used when external controls are not feasable. At least five (5) analytes found within the In-House Cut-Off Positive Control Mixture (at concentrations at least three-times (3x) their levels) will be used for quality control (QC) evaluation. When possible, control solutions will be matrix-matched.

The Positive Control Mixture will be prepared as needed and its preparation recorded within appropriate case file documents (i.e., case notes, batch paperwork).

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Notes (applicable to Section 6):

Note-01: For vitreous samples 6-monoacetylmorphine (6-MAM ; 6-AM) is the only analyte evaluated. Control solutions for vitreous samples can be a different, but similar, matrix (e.g., water).

Note-02: Control solutions containing 6-MAM levels lower than three-times (3x) the In-House Cut-Off Positive Control Mixture concentration are allowed.

Note-03: If other drugs are used when preparing the In-House Cut-Off Positive Control Mixtures then a minor deviation (or other paperwork) will accompany all batch work and case files.

Note-04: Unless otherwise noted the above standard and control solutions are stable for at least one (1) year from either their creation or from their last verification. If solutions are used as purchased (i.e., without modification), storage and stability will be determined by manufacturer.

7 Calibration

This procedure is qualitative only.

8 Sampling

Not applicable.

9 Procedure

The Appendix contains an abbreviated version of this procedure. This form may be used at the bench by analysts who perform the procedure. If SPE cartridges are in a rack and are not removed during the extraction process and are always in a fixed position, then labeling of SPE cartridges is optional, regardless of what is written in the following steps or in the Appendix. However, the vials which collect the final eluent must be properly labeled.

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a. Appropriate cleaning (e.g., methanol or bleach solution) will be done on table/bench surfaces before each batch of samples is analyzed. Prior to casework samples being opened (or analyzed while in an exposed state), brown paper (or other similar barrier) will be placed on top of the table/bench surface to minimize/prevent contamination.

b. Specimen Preparation (Blood, Urine, Vitreous):

   i. Add 0.5 mL of each unknown sample to a properly labeled test tube.
   Only one unknown sample will be open during transfer. Test tubes containing unknown samples will be capped or their tube positions changed after each individual transfer.

   ii. Prepare 0.5 mL of each control (Negative, External Positive, In-House Cut-Off Positive) to their own properly labeled test tubes.
   The appropriate matrix will be used based on the samples being analyzed (i.e., blood, urine, vitreous).

   iii. Add 100 µL of the I.S. Working Solution (Diazepam-D_5 + Butalbital-D_5 + Meprobamate-D_3 + Morphine-D_3 + Oxycodone-D_6) into each unknown and control sample.
   This is equivalent to 25 ng of Diazepam-D_5, 100 ng of Butalbital-D_5, 100 ng of Meprobamate-D_3, 25 ng of Morphine-D_3, and 25 ng of Oxycodone-D_6 all being added to each [0.5 mL] sample.

   Note: Sample test tubes may all be uncapped during this step.

c. Add 1 mL of the Sodium Acetate buffer (pH ~4.5) to each specimen.
Sample test tubes may all be open during this step.

d. Add 500 µL of water – cap and vortex-mix samples.
Sample test tubes may all be open during this step.

e. Sonicate, if needed, for at least 15 minutes (required for blood samples).

f. Centrifuge all samples (e.g., 8 minutes at ~5200 RPM)

g. Label Trace-B extraction [SPE] columns appropriately

h. Place labeled Trace-B extraction columns in the SPE column rack in the appropriate order. Position plastic waste tray underneath SPE column rack. {for Solvent Waste}

i. Condition each column with 1 mL MeOH, then drain (≈ 3 psi). {for Solvent Waste}

j. Remove solvent tray and replace with biohazardous tray

k. Add 1 mL of water to each SPE column, then drain (≈ 3 psi) to biohazardous tray.

l. Individually transfer samples to the center of their respective [properly labeled] SPE column. Avoid splashing and/or transferring any sediment found at the bottom of the tube.

m. Allow samples to migrate through SPE column via gravity. Apply small pressure, if needed (< 3psi).

n. To each SPE column:

   i. Wash with 1 mL of Bicarbonate/Carbonate buffer (pH~9), then drain (≈ 3 psi) to biohazardous waste.

   ii. Wash with 1 mL water, then drain (≈ 3 psi) to Biohazardous waste.

o. Dry the columns for ~10 minutes using maximum pressure (e.g., between 60-80 psi).

p. After ~10 minutes, replace plastic waste tray with the SPE collection rack containing appropriately labeled autosampler vials. Ensure that each vial is placed under their corresponding SPE column for elution.

q. Elute columns with two (2) – 1 mL aliquots of the Solid Phase Extraction Elution Solution. Collect eluent into appropriate autosampler vials using gravity or using low pressure (<3 psi).

   Note: The Solid Phase Extraction Elution Solution ratio is 80:18:2 CH_2Cl_2:IPA:NH_4OH. (Approximately 2 mL will be needed for each tube).

r. Remove top SPE column rack and transfer collection rack from SPE manifold to sample concentrator.

s. Add ~1 drop of 1% MeOH-HCl to each tube.

t. Evaporate all eluents to dryness at <40 ˚C.

u. If samples are not to be immediately analyzed they should be placed in a refrigerator or freezer under proper evidentiary seal.

   Note: The word ‘immediately’ refers to instrumental sequences being started on the same day.

v. Reconstitute each extract sample with 500 µL of $MeOH_{(aq)}$ (20% (v/v)) when ready for instrumental analysis.

w. Check turbidity of solution. Remove undissolved particulates and treat as necessary (e.g., filtration or centrifugation). Controls (negative and positive) will be treated in same manner as samples.

x. Analyze extract solutions using LC/HR–MS.
Blank solutions (i.e., solvent, mobile phase) will be analyzed in-between samples.

RETIRED

10 Instrumental Parameters

The following are the typical operating parameters for the instrument. The instrument conditions may be modified slightly to adjust or improve the procedure as needed. The appendix contains an abbreviated version of the procedure and this checklist can be used by analysts. Any changes in the instrumental parameters for a batch will be reflected in the instrumental parameter documents that accompany data.

HPLC Parameters:

Mobile Phase A: 0.01 %(v/v) $HCOOH_{(aq)}$ and 5mM $NH_4CHOO_{(aq)}$
Mobile Phase B: $MeOH_{(l)}$ (neat)
HPLC Column: Accucore C18, 2.6 µm, 50 mm x 3 mm (Thermo Scientific)
Column Temperature: 40 °C
Flow Rate: 0.7 mL/min
Autosampler Temperature: 15 °C
Injection Volume: 10 µL
Needle Wash: 80 µL before and after aspiration

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LC Gradient Program: Initial 5% B ; Flow 0.7 mL/min

Time (min.)	Mobile Phase A [0.01 %(v/v) $HCOOH_{(aq)}$ + 5mM $NH_4CHOO_{(aq)}$]	Mobile Phase B $MeOH_{(neat)}$
Initial	95 %	5 %
0.2	95 %	5 %
2.5	85 %	15 %
5.2	5 %	95 %
5.7	5 %	95 %
5.8	95 %	5 %
7.8	STOP	STOP

MS

Parameter	Value		Parameter	Value
Method duration	~8 min		Method duration	~8 min
Mode	ESI, Full scan MS, MS/MS		Mode	ESI, Full scan MS, MS/MS
Polarity	Positive		Polarity	Negative
Resolution	70,000		Resolution	70,000
AGC Target	1e6		AGC Target	1e6
Maximum IT	50 ms		Maximum IT	50 ms
Scan Ranges	1		Scan Ranges	1
Scan Range	80-500 m/z		Scan Range	80-500 m/z
Spectrum Data	profile		Spectrum data type	profile

Note: See instrument method for additional parameters

RETIRED

11 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. Retention time (chromatographic characteristic), fragmentation pattern, and qualified ion ratios (mass spectrometric characteristics) are used as the basis for detection. In most cases all of the criteria below should be met in order to detect the appropriate drugs within biological specimens.

a. Chromatography

The data from the performance check solutions for the LC/MS instrument, when analyzed, will contain peaks that possess good chromatographic quality (i.e., peak shape, peak width, signal-to-noise).

All chromatographic peaks for any reportable analytes of interest should show good chromatographic characteristics with reasonable peak shape, width, and resolution. In order to

be deemed acceptable as a chromatographic peak analyte peaks will compare favorably to corresponding chromatographic peaks within known samples.
Additionally, the following two criteria will be met:

RETIRED

i. Retention Time (RT)

The retention time of the chromatographic peak will be within ±0.2 minutes of the retention time obtained from injection of a reference standard or positive control.

ii. Signal-to-Noise Ratio (SNR)

To justify the existence of a peak, its baseline signal-to-noise ratio (SNR) will exceed 3. Further, the baseline signal for the peak of interest will be at least 10-fold greater than that for any observed peak (corresponding to the analyte of interest) at a similar retention time in a negative control or blank injected prior to that sample.

iii. Reporting Limit (RL)

Only analytes at or above a certain cut-off level will be reported.

A comparison of chromatographic peak area ratios (i.e., analyte-to-internal standard) between unknown samples compared to matrix-matched analytes found within control solutions will be used to determine when analytes will be reported. These peak area ratios will subsequently be termed, 'ratios.'
Note: Vitreous samples don't have to be compared to matrix-matched control solutions.

Analytes whose ratios are at or above the ratios from the same analytes within the In-House Cut-Off Positive Control Mixture will be reported.

Analytes not found within the In-House Cut-Off Positive Control Mixture whose ratios are at or above the ratios from the same drug class of analytes (e.g., amphetamines, opiates) within the In-House Cut-Off Positive Control Mixture will be reported.

Analytes not found within the In-House Cut-Off Positive Control Mixture which are outside the drugs and/or drug classes of those analytes within the In-House Cut-Off Positive Control Mixture will be reported when compared to an appropriate control. The comparison can either be from an extraction or from an unextracted positive control solution that contains an appropriate internal standard. Analytes who have ratios at or above the selected cut-off concentration ratios will be reported. Selected cut-off concentrations shall be determined from the Technical Lead (or higher) and will be documented within the batch and/or technical records (e.g., worksheet, case notes).
Note: Analyses of positive controls need not be done at the same time as unknown samples.

b. Mass Spectrometry (MS)

i. High Resolution Accurate Mass

In order for the MS instrument to be considered in good operating condition, the correct mass assignment for the internal standard will be present. The molecular ion will be present at the apex peak for each analyte with a tolerance of 5 mmu.

ii. Product Ion/Fragmentation Mass Spectrometry (MS/MS)

RETIRED

In order for the MS to be considered in good operating condition, the correct mass assignments for each reported analyte will be present. Independent MS/MS experiments are conducted for each analyte and ion ratios should compare favorably to ion ratios from reference standards (or extracted positive controls at comparable concentrations). Generally, ion ratios are within the following limits:

Expected [Set] Ion Ratio	Allowance
>50 %	20%
20% - 50%	25%
<20%	30%

RETIRED

c. Batch Acceptance:

A batch of samples will be analyzed with appropriate controls. The decision of whether to accept the data within a batch will be determined by the following:

No analytes of interest should be detected within Negative Control samples. For this procedure, detected analytes of interest are defined as any analytes being listed in a final report (i.e., ratios greater than or equal to corresponding positive control (or class) ratios).

All batches containing reportable analytes shall have positive control data corresponding to each analyte. A reportable analyte is considered one which is toxicologically significant. When in doubt of whether an analyte is reportable or not, the Technical Lead (or higher) will be consulted. If an analyte is detected but not reported, documentation of this (along with a reason) will be included within the batch paperwork and/or within technical records (e.g., worksheet, case notes).

Internal standards within samples will contain acceptable chromatographic and mass spectral qualities (as defined above). Individual samples that don't contain acceptable relevant internal standard data will not be accepted and will need to be repeated. For example, if an analyte is an alkaline drug and the alkaline internal standard data is acceptable, but the weak acid and/or neutral internal standard data are unacceptable, the analyte in question can be considered reportable – but the Technical Lead will be consulted and documentation as to why it is acceptable will be included within the batch paperwork and/or within technical records (e.g., worksheet, case notes).

d. Screening versus Confirmation:

This procedure can provide presumptive (screening) results when only a single aliquot of sample is analyzed and a single mass spectrometric experiment is utilized. Confirmatory results will include either:

i. Analyzing a second aliquot (which includes re-extraction) using the same instrument method, and/or

ii. Analyzing the same sample extract using a different instrumental method (i.e., different chromatographic method or mass spectral technique). A different chromatographic method would include changing chromatographic columns or mobile phases such that analyte retention times differ.
A different mass spectral technique would include variations in mass spectral experiments (e.g., MS/MS). Such experiments can be included within the same instrumental method, thus allowing one aliquot of specimen to be simultaneously analyzed by two (2) mass spectral experiments (e.g., LC/HRMS (MS/MS)).

Any questions or concerns that are not addressed in this procedure regarding whether data should be used as screening versus confirmation will be answered or addressed by the Unit Lead (or higher). Decisions regarding whether to report analytes as presumptive positive or as confirmatory due to unusual circumstances (e.g., analytes not producing significant product ions or isobaric compounds having the same retention time) will be based on the above criteria in conjunction with the Unit Lead (or higher). Such decisions will be documented within case files.

Negative samples can be reported based on the single [screening] analysis.

Processing of additional analytes using high-resolution mass spectral data (i.e., M+H) can be performed using extracted ion chromatography. This is useful in attempts to detect other potential analytes of interest that are not within the instrument method. Alternatively, multiple injections of sample extracts may be performed using additional instrumental methods so as to identify other analytes of interest.

When an analyte that is not found in the positive control is detected within a sample, the sample will be compared to a known control, if possible, for retention time and mass spectral verification (both HR-MS and MS/MS). A general description of how to prepare such analytes can be found within the Reporting Limit (RL) section below (with respect to concentrations). If positive controls for detected analytes are not available, the Technical Lead (or higher) will be consulted for guidance. Decisions will be documented within batch paperwork and/or within technical records (e.g., worksheet, case notes).

Note: When analytes are known to give potential false positive results using a particular experiment (e.g.., amphetamine using only HR-MS), then simply analyzing a second aliquot using the same experimental method will not be done for confirmation purposes ; and alternate experiment(s) will be employed for confirmatory results to be reported.

RETIRED

12. Uncertainty

Not applicable – this is a qualitative procedure only.

13. Limitation

a. Limits of Detection (LOD)
This information will be found in the validation paperwork for the instrument.

Extraction LOD for analytes are administratively set to be at least at cut-off concentrations within this procedure.

b. Reporting Limits (RL):
Administratively set analyte concentrations within positive control solutions (e.g., In-House Cut-Off Positive Control Mixture). The same concentrations will be used for all biological matrix types (e.g., blood, urine, vitreous) and are based on recommended blood level concentrations related to driving while impaired data and literature values (e.g., therapeutic concentrations).

c. Specificity
No known interferences have been found within biological specimens.

d. Experimental
There are a limited number of analytes (i.e., ~44) which can be detected during the MS/MS experiment within an instrumental method. Alternate instrumental experiments can be used for the detection of additional analytes. Refer to the specific instrumental methods for actual list of analytes. However, full scan data is still collected (i.e., M+H data) and extracted ion chromatography during data processing can be applied to help in the detection of analytes that are not found within instrumental experiments.

RETIRED

14. Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment will be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Biological specimens will be handled using universal precautions and will be treated as biohazardous. Potentially contaminated items and surfaces will be cleaned prior to use.

15. References

Solid-Phase Extraction Method for Trace B Columns. SPEWARE: Baldwin Park, CA.

Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities – 2017 Update, Logan, et. al, Journal of Analytical Toxicology, 42, 2, 63-68

Appendix:
Bench Method:
Drug Screening and Confirmation by LC/HR-MS

The following is an abbreviated version of this procedure and a pdf-version can be used.
This form may be used at the bench level by the analyst performing the procedure.

Negative Control [drug-free] (Blood, Urine, Vitreous)	**Lot:**
External Positive Control (Blood, Urine, Vitreous)	**Lot:**
In-House Cut-Off Positive Control (Blood, Urine, Vitreous)	**Lot:**

Procedure:

	For Blood or Urine (or Other Matrix) Specimens	
Add 0.5 mL sample/negative control/positive control into properly labeled test tubes (e.g., 16 x 100 mm)		
Add 100 µL of I.S. Working Sol'n to each sample/control :		
	Diazepam-D_5 + Butalbital-D_5 + Meprobamate-D_3 + Morphine-D_3+ Oxycodone-D_3	**Lot:**
Add 1 mL of Sodium Acetate Buffer$_{(aq)}$ (pH ~4.5) to each test tube.		**Lot:**
Add 500 µL of water to each test tube, cap and vortex-mix samples, sonicate, if blood. Centrifuge.		
Label Trace-B extraction [SPE] columns appropriately.		**Lot:**
Place labeled Trace-B extraction columns in the SPE column rack in the appropriate order.		
Add 1 mL MeOH (to condition SPE), drain (≈3 psi) to Solvent Waste tray		**Lot:**
Add 1 mL of water (to wash each SPE), drain (≈ 3 psi) to Biohazardous tray		**Lot:**
Individually transfer samples/controls to the center of their respective [properly labeled] SPE column. Avoid sediment / splashing. Allow to migrate through SPE column via gravity. Apply small pressure, if needed (< 3psi).		
To each SPE column:		
	Wash with 1 mL of Bicarbonate/Carbonate buffer (pH ~9.0), then drain (≈ 3 psi) to Biohazardous waste	**Lot:**
	Wash with 1 mL water, then drain (≈ 3 psi) to Biohazardous waste	**Lot:**
Dry the columns for ~10 minutes using maximum pressure (e.g., between 60-80 psi)		
During this 10 minute window (or earlier) label autosampler vials, place in SPE rack. Prepare the elution solvent.		
The Solid Phase Extraction Elocution Solution ratio is 80:18:2 CH_2Cl_2:IPA:NH_4OH. (Approximately 2 mL per each tube).		
	Methylene Chloride (CH_2Cl_2)	**Lot:**
	Isopropyl Alcohol (2-Propanol ; IPA)	**Lot:**
	Ammonium Hydroxide (NH_4OH)	**Lot:**
After ~10 minutes, replace plastic waste tray with SPE rack containing autosampler vials. Ensure vials are properly placed.		
Elute columns with two (2), 1 mL aliquots of the Elution Solution Flow using gravity (<3 psi if needed)		**Lot:**
Transfer collection rack from SPE manifold to sample concentrator		
Add ~1 drop of MeOH-$HCl_{(aq)}$ and evaporate to dryness (<40 ˚C) ; Store refrigerated and properly sealed if not immediately analyzed		**Lot:**
Reconstitute each extract sample with 500 µL of 20% $MeOH_{(aq)}$; only if analyzing immediately		**Lot:**
Check turbidity of solution. Remove undissolved particulates and treat as necessary.		
Analyze by HR-LC/MS		

RETIRED

Appendix (Cont'd):

Specimen Procedure (Cont'd)

Analyze by LC/HR-MS:

Instrument: Q-Exactive	Instrument Identifier:
HPLC Column: Accucore C18, 2.6 µm, 50 mm x 3 mm (Thermo Scientific)	Lot:
Mobile Phase A: [0.01 %(v/v) $HCOOH_{(aq)}$ + 5mM $NH_4CHOO_{(aq)}$]	Lot:
Mobile Phase B: MeOH (CH_3OH) – 100%	Lot:

Gradient Program:
Initial 5% B Flow 0.7 mL/min

Time (min.)	%B
Initial	5
0.2	5
2.5	15
5.2	95
5.7	95
5.8	5
7.8	STOP

Mass Spectrometer Parameters

Ion Source	Positive and Negative ion
Mode	ESI ; Full Scan ; MS/MS
Resolution	70,000
Range	100-500 m/z
Max ionization time	50 ms
AGC	1E+06

AGC = Automatic Gain Control

RETIRED

Rev. #	History
1	New procedure for urine, blood or other appropriate matrices.

RETIRED

SOP 1 General Toxicology BrAC unit

PURPOSE :
The conviction and or disposition of drunk drivers from Connecticut streets and highways provides important public safety benefits. In 2013 CT justice department reported 8200 DUI arrest, to include those residents killed or injured in alcohol-related crashes.

Chemical testing to determine the concentration of alcohol in the breath of persons involved in traffic violations is a critical component of the state's efforts to control drunk driving. To enforce the state's driving under the influence (DUI) laws Blood, breath, and urine. The Connecticut Department of Emergency Service and Public Protection Division of Scienctific Services forensic laboratory goal is to ensure the competency of the 124 polices department BrAC instrument operators and Instructors in CT and the qualifications of each officer and equipment use to accuractely evaluate breath alcohol in subjects determined to be driviing under the influence.

The BrAC instrument used in Connecticutis the Alcotest 9510 (Draeger) and the Intoxilyzer 5000. The two methods approved by State Statue Sec. 14-227a page 13 are Infrared and Fuel Cell analysis.

RETIRED

A. BrAC Testing: Subjects that present a poor field sobriety test resut and < 0.08 g/210L breath test the Toxicology section will evaluate biological fluids (urine or blood) for the presense of other intoxicant which maybe found.

B. General BrAC Unit Duties:

1) The Laboratory Re-certified all instrument sent out for service
2) Generated certificate upon re-certifying Draeger instrument
3) Generated all certificate for operators and instructors
4) File all officer's proficiency evaluations at toxicology lab BAC unit
5) Filing of all service records at toxicology lab BrAC unit
6) Respond to all Attorney request as a function of FOIA
7) Download and upload procedures for Draeger and Intox5000

C. More Specific Application Duties in BrAC unit:

a. Scheduling of Annual Training
   i. Recertification (2hrs) for Instructors
   See SOP 9
   ii. Provide initial Instructor training (7hrs)
   See SOP 8

b. Maintain the Draeger instrument
    i. Send out for maintenance as needed
    ii. Recertified instrument upon its refurn from Draeger Diagnostic.
    iii. Maintainence of instrument service records
    iv. Maintainence of the instrument service log

D. Repair of Draeger instrument:

a. Notify Toxicology Laboratory Breath Alcohol Section of problem and returning for service upon contacting forensic science examiner of the BrAC unit.

E. Instrument Validation and Certification:

See SOP 6

F. Uncertainty:

See SOP 6

G. Draeger Operation:

See SOP 8

H. Operation Issues

See SOP 8

I. Error Messages/ Test Aborted

See SOP 8

J. ALCOTEST 9510 UPDATING FIRMWARE/SOFTWARE

See SOP 2

P. ALCOTEST 9510 UPDATING FIRMWARE/SOFTWARE REMOTELY

(The process takes about a hour to complete).

See SOP 2

Q. ALCOTEST 9510 UPDATING FIRMWARE/SOFTWARE USING THUMB DRIVE

(The process takes about a twenty minutes to complete).

See SOP 2

R. ALCOTEST 9510 DOWNLOADING DATA REMOTELY

See SOP 2

S. ALCOTEST 9510 DOWNLOADING DATA USING THUMBE DRIVE

See SOP 2

RETIRED

T. INTOX 5000 DOWNLOAD
See SOP 3

U. Setting up modem # for downloading data from Intox at PD
See SOP 3

V. Downloading data from Intox at PD
See SOP 3

W. Downloading stored data
See SOP 3

X. Printing downloaded data
See SOP 3

Y. INTOX 5000 Modem Connection
See SOP 3

RETIRED

SOP 2 Download Data from Draeger Alcotest 9510 and retrieval of Data

A. **PURPOSE**:
Downloading of the Draeger instrument's data can be done locally or remotely. Data from the instrument can be purged and stored here at the Toxicology Laboratory prior to sending the instrument out for service where data can be lost. Another reason for Downloading the data is to demonstrate the use of the draeger such as the number of subjects tested, amount of change to refusals, blood, or urine. This data will be use to populate the data in which to be use for grant proposals in helping to support the BAC program.

B. **RESPONSIBILITY**:
All Forensic Science Examiners in the BAC section are responsible to follow the guidance of this SOP when utilizing a Alcotest 9510 for subject breath analysis.

C. **DEFINITIONS**:
Alcotest 9510
WinCE file
M16 file
MEASUREMENT SYSTEM FIRMWARE file
DATALOGGER
ZOOM box

D. **PROCEDURE**:

ALCOTEST 9510 UPDATING FIRMWARE/SOFTWARE

1.) Select: Shortcut to "SHORTCUT TO DIAGNOSTIC" icon

2.) A popup window appears "Shared Unitlist of 9510

3.) Select "NO"

4.) Selected "A9510"

5.) Now you are in the Alcotest 9510 module

   a. download data, change date/time on the instrument, and any instrument adjustment issues while at police department locations.

   b. download data, change date/time on the instrument, and any instrument adjustment issues while in Toxicology department of the Forensic Laboratory.

ALCOTEST 9510 UPDATING FIRMWARE/SOFTWARE REMOTELY

The process takes about a hour to complete.

1.) Downloaded Draeger database and saved

2.) WinCE – UPLOADED

RETIRED

3.) M16 – UPLOADED
4.) Went to “Data Logger” which showed the new version 4.9.21

ALCOTEST 9510 UPDATING FIRMWARE/SOFTWARE USING THUMB DRIVE
The process takes about a twenty minutes to complete.

1.) Downloaded Draeger database and save data
2.) Insert External drive (label) into USB port of the Draeger instrument
3.) Select “MENU”
4.) Select “MAINTENANCE”
5.) Select “UPDATE WinCE – UPLOAD (WinCE file 8321338_4921)
    a. Press “RUN”
    b. Press “OK”
6.) Select “UPDATE MEASUREMENT SYSTEM FIRMWARE– UPLOAD (firmware file 8321339_1_1.BIN.SIG)
    a. Press “RUN”
    b. Press “OK”
7.) Select “CONFIGURATION FILES (WinCE file 8321337 version 1.5AQ) – UPLOAD
    a. Press “RUN”
    b. Confirm and press “OK”
8.) The instrument

9.) Went to “Data Logger” which showed the new version 4.9.21

RETIRED

ALCOTEST 9510 DOWNLOADING DATA REMOTELY
1. Select “DATALOGGER”
2. Ensure your modem is connected to the “ZOOM” box connection to the CPU
3. Select “Police Department (Instrument serial number corresponds)”
4. Select “SELECT ” button
5. Select “DOWNLOAD”
6. Once download is complete “SAVE DATA”
    a. For example “Avon_114” (police dept and Instrument SN#)

ALCOTEST 9510 DOWNLOADING DATA USING THUMB DRIVE
1. Turn on Draeger instrument
2. Insert External drive (Thumb drive) into USB port of the Draeger instrument
3. Select “MENU” (lower left corner of the Draeger screen)
4. Select “SETTINGS ” (double click on selection)
5. Select “DATABASE MANAGEMENT” (double click on selection)
6. Select “COPY TO USB” button

7. Wait until finished (approximately 2 mins)
8. Popup will appear “DATA SUCCESSFULLY COPIED”
9. Select “OK”

ALCOTEST 9510 Modem Connection

“Z”
ZOOM box is the connection to the modem line when utilizing Draeger for Downloading

RETIRED

Printing downloaded data from Draeger (Alco Test 9510)

1. Go to “My Computer”
2. Data found in Program file
3. Select “Show other file”
4. Select “Draeger File”
5. Select “Diagnostic File”
6. Select “BIN File”
7. Select “Data Files”
8. Select “Data Logger File”
9. Select_Year where data is found (2014 file)
10. Select_Data and copy to a new excel sheet
11. Print after formatting Excell spread sheet with proper spacing

ALCOTEST 9510 DOWNLOADING DATA FROM THUMB DRIVE

1. The data records are saved in flat ASCII text in semi-colon delimited fields.

   Running Excel on your PC, you can import (File::Open) the file of interest.

2. From there, the "Text Import Wizard" in Excel opens. From there, choose the "Delimited" radio button and then press the "Next" button.
3. In the next screen it will ask what types of delimiters to use.
4. Select the checkbox for semicolon and then press the "Finish" button.
5. Now each datafield is in its own column structure. You can arrange the column widths so the data is visible. You can then save this file wherever you would like and in any format you wish.

RETIRED

DOWNLOADING THE DRAEGER INFORMATION STORED

** Instruments must be turned on for any downloading

1.) Open the computer and open "Shortcut to **Drager Diagnostics**"
2.) A prompt will ask. "A shared Unitlist of A9510 devices was found. Do you want to import this list to your system? Unitlist_A9510.ini (date)
3.) ****** **ALWAYS SELECT** ****"NO"**********
4.) Open the **"A9510"** tab
5.) Select (double click) **UNIT LIST** – here you can
    a. "add" an instrument – and list all the new information
    b. "edit" or make changes to Serial number, modem number, location of the instrument, etc.
    c. "delete" the instrument all together

For downloading instruments that are here in our lab

1.) Open UNIT LIST – scroll to the instrument (can sort by selecting the column)
2.) Before making changes, write down the **PD's modem #** on the "**Receipt / Return of the Draeger Alcotest**" Form
3.) Edit the modem # to Breath Alcohol Lab Modem # = **2034274613** → ok → close
4.) Plug in the phone line to the instrument on the side (area showing a phone)
5.) Make sure the plug into the computer is through the "Zoom Box" to the computer
6. Open DATALOGGER

For downloading instruments not on site and are at the PD's

1.) Open **UNIT LIST** – scroll to the instrument (can sort by selecting the column)
2.) Check to make sure the modem # is correct.
3.) Make sure the plug into the computer is through the "Zoom Box"
4.) Open **DATALOGGER**
5.) Select the serial # (highlight → select)
6.) Select "**DOWNLOAD**" (far left bottom corner)
    a. Databases 1-7 are checked off
    b. Once downloading can see the version # and make sure that it is up to date
7.) **SAVE** the data after it has been downloaded.
8.) The link is already set up → just save with "Town_Serial# Date" in that year's folder
9.) ERASE data only if you have saved it.

For downloading onto a USB – Use a Drager specific USB only with key

1.) Plug USB Drive into the side of the instrument
2.) Go into Menu → Settings → Database management
3.) Select under "actual databases" all the databases (one at a time) from the drop down box
4.) **COPY TO USB**
5.) If there are X's in the : # of records, max # of records, Next RecRefNo → there is nothing to download for that database

RETIRED

SOP 3 Download data for Intox 5000 and retrieving data

E. **PURPOSE**:

Downloading of the Intoxylizer 5000 instrument's data can be done locally or remotely. Data from the instrument can be purged and stored here at the Toxicology Laboratory prior to sending the instrument out for service where data can be lost. Another reason for Downloading the data is to demonstrate the use of the draeger such as the number of subjects tested, amount of change to refusals, blood, or urine. This data will be use to populate the data in which to be use for grant proposals in helping to support the BAC program.

F. **RESPONSIBILITY**:

All Forensic Science Examiners in the BAC section are responsible to follow the guidance of this SOP when utilizing a Intoxylizer 5000 for subject breath analysis.

G. **DEFINITIONS**:

Intoxylizer 5000
Cobra
Cobra software

H. **PROCEDURE**:

INTOXYLIZER 5000 DOWNLOAD
User Identity: ADMIN
Password: PASSWORD
Select: Shortcut to "COBRA"

Setting up modem # for downloading data from Intox at PD

1. Open Cobra software
2. Select "Set up"
3. Select "Instrument" on dropdown menu
4. Select "Locate" button (to select the instrument)
5. Click on ***Cobra*** icon (upper left corner)
6. Select "Next Window"
7. Select "EDIT" button
8. Enter "telephone #"
9. Save
10. Close

Downloading data from Intox at PD

1. Open Cobra software
2. Ensure modem is plugged in (see photo)
3. Select "communications" on dropdown menu
4. Select "Interactive"

RETIRED

5. Select “Instrument” (i.e., Manchester 68-011127)
6. Select “Connect” button (bottom left corner)
7. Select “Download” button
8. Data being down loaded appear in window on right side
9. Select “Clear” button
10. Select “Close” button (should be finished)

Downloading stored data

1. Open Cobra software
2. Select “Data” on dropdown menu
3. Select “Export Data”
4. Select Instrument number (e.g., 11-682 =>. Southington)
5. Select date range to be downloaded
6. Select items to be downloaded (Mark say’s “select all”)
7. Go to file format bottom left corner and dropdown menu
8. Select “Excel”
9. Give a filename (e.g., officer’s last name)
10. Select “SAVE”
11. Your are done

Printing downloaded data

12. Go to “My Computer”
13. Data found in Cobra file
14. Select the file
15. Print after formatting Excell spread sheet with proper spacing

RETIRED

INTOX 5000 Modem Connection

The connection to the modem line when utilizing Intox 5000 for Downloading

RETIRED

SOP 4 Breath Alcohol Instrument send for service, and disposition to PD

A. PURPOSE:

It will be necessary to send the Draeger instrument out for service to Draeger Diagnostic, Inc of Erving, Texas. Data from the instrument will be purged and stored here at the Toxicology Laboratory prior to sending the instrument out for service where data can be lost. Common issues are fuel cell replacement, error messages seen, The turn around time has been about two weeks. When instrument returns to the Toxicology laboratory BAC section the instrument is certified for use prior to returning to submitting police department.

B. RESPONSIBILITY:

All Forensic Science Examiners in the BAC section are responsible to follow the guidance of this SOP when utilizing a Alcotest 9510 for subject breath analysis.

C. DEFINITIONS:

Alcotest 9510 sent out for service

D. PROCEDURE:

Notify Toxicology Laboratory Breath Alcohol Section of problem and returning for service; contact Mark or Gerry

E. Draeger instrument in for service:

1. Only Bring the Draeger 9510
   a. DO NOT bring the regulator or base
   b. Only INSTRUMENT & COVER please
   c. Complete form “RECEIPT/RETURN OF THE DRAEGER ALCOTEST 9510” found at front desk

F. Maintain the Draeger instrument

Send out for maintenance as needed via FEDEX shipping instructions

G. Pickup of Draeger instrument after service complete:

1. Pick up instrument at front desk
2. Sign and date form attached
3. PLEASE LEAVE RECEIPT AT FRONT DESK
4. Instrument Certification
5. Will be attached to the instrument cover
6. Police Departments keep for their records

RETIRED

DRAEGER 'S TO BE SENT OUT - (TO TEXAS)

1.) Start "**Receipt / Return of the Draeger Alcotest**" Form – (hopefully the officer has attached with instrument already started with the top information)
   a. The contact info is important to obtain as well as issues
2.) Download the instruments data through DataLogger or onto a USB drive.
   a. Document that the information has been saved and where
   b. If unable to save the data – let Drager know on the below form to call you so you can set up a time to download at a future date with them.
   c. See Downloading Draeger instructions
3.) Start / Fill out "**Draeger Saftey Equipment Return Form**"
   a. Multiple instruments can be on 1 form
   b. Make a copy - put one in the box with the instrument(s) being sent
   c. Email a copy to Bret Everett @ Draeger.com
4.) Pack the Draeger into the box: **Just the instrument and cover**
   a. Place instrument in the plastic bag and attach the fitted foam sides
   b. Place all from above into the box
   c. Put a copy of the **"Draeger Saftey Equipment Return Form"** in with each instrument
   d. Tape with packing tape the box shut.
5.) Send the box out Via FEDEX
   a. See FEDEX Shipping Instructions

RETIRED

SOP 5 Shipping instrument to Texas via FedEx

RETIRED

A. **PURPOSE**:

Shipping Alcotest 9510 Draeger instrument to Draeger Diagnostic, Inc of Erving, Texas for servicing. The turn around time has been about two weeks. When instrument returns to the Toxicology laboratory BAC section the instrument is certified for use prior to returning to submitting police department.

B. **RESPONSIBILITY**:

All Forensic Science Examiners in the BAC section are responsible to follow the guidance of this SOP when utilizing a Alcotest 9510 for subject breath analysis

C. **DEFINITIONS**:

D. **PROCEDURE**:

Repackage by boxing the instrument with instruction as to possible problem

a. Send out for shipping instruction
    i. FEDEX shipping instructions
    ii. Log into www.Fedex.com
    iii. Username: toxlab278
    iv. Password: Shipping278
    v. Go to My shipment Profile and select “DRAEGER-MARA HERRERA”. This will auto-populate the fields
    vi. To complete shipment selest “SHIP”
    vii. Print shipping labe and attached to box for pickup by FedEx

FEDEX SHIPPING INSTRUCTIONS:

Log into: www.Fedex.com Tox Account#: 3438-5385-8

Create a Shipment by logging on
Username: **toxlab278** Password: **Shipping278**

1.) Create a Shipment
   a. Go into My Shipment **Profiles:** (drop down box)
   b. Select **"DRAEGER – MARA HERRERA"**
   c. This will auto-populate most other fields – it takes a moment to refresh
2.) #2 - Keep all the same
3.) (Questions needed to be filled in below) - Package and Shipment Details
   a. No. of Packages – select **#** with dropdown box
   b. Are packages identical? – Select **YES**
   c. Weight per package? -- Input **20** lbs
4.) Schedule a pickup – Billing Details
   a. [•] Select schedule a pickup
   b. [+] Edit Package information
   c. Select change **DATE** or **TIMES** as needed **NOTE – building is closed after 430pm
5.) Complete your shipment
   a. Select **SHIP** → check over all the information → select EDIT or **SHIP**
   b. Select All labels and receipt → PRINT → PRINT
6.) Attach FedEX label packet holder to the box
   a. Insert the label into the pouch and seal
7.) Bring to the front desk to be picked up.

RETIRED

SOP 6 Breathalyzer checkout, certification, pickup

E. **PURPOSE**:

Before Draeger instrument is returned to submitting agency it must be certified for use in CT by the Toxicology Laboratory. The instrument must demonstrate within acceptable limits of various dry gas concentration challenges. The instrument must also recognize an interference in a breath sample.

F. **RESPONSIBILITY**:

All Forensic Science Examiners in the BAC section are responsible to follow the guidance of this SOP when utilizing a Alcotest 9510 for subject breath analysis.

G. **DEFINITIONS**:

H. **PROCEDURE**:

1. Instruments are calibrated by manufacturer by Draeger Diagnostic
2. Instruments are recertified by Toxicology Lab upon its refurn from Draeger Diagnostic. A certificate is made and is returned with the submitting agency upon pickup of their instrument.
3. Maintain instrument service records
4. Maintain the instrument service log

Instrument Validation and Certification:

1. Use of certified Dry Gas Standards 0.02, 0.08, 0.25 concentrations and Methanol Galt to check for interfence.
2. Dry gas concentrations must be within +/- 5% and Methanol test should show interference and is considered validated and acceptable for use.
3. Complete the 'Checkout for Draeger Alcotest form
4. Attached Alcotest printout after dry gas and methanol analysis to the checkout form

Uncertainty: The overall uncertainty for the procedure ("expanded uncertainty") is calculated based on an evaluation of control performance (variance) and bias, documentation of uncertainty in materials used to prepare standards used in calibration associated with the performance of the procedure is +/- 5%.

Pickup of Draeger instrument after service complete:

1. Pick up instrument at front desk
2. Sign and date form attached

RETIRED

3. PLEASE LEAVE RECEIPT AT FRONT DESK
4. Instrument Certification
    a. Will be attached to the instrument cover
    b. Police Departments keep for their records

RETIRED

STATE OF CONNECTICUT

DEPARTMENT OF EMERGENCY SERVICES
AND PUBLIC PROTECTION
FORENSIC SCIENCE LABORATORY

DESPP
DIVISION OF SCIENTIFIC SERVICES

CHECKOUT OF THE DRAEGER ALCOTEST 9510

SERIAL NO. ARBD-_____

RETIRED

Instrument Mode	Ethanol Vapor (Grams/210 L)		Result 1	Result 2	Lot Number	Acceptable Range (IR-Result Grams/210 L)
ABA	Blank	N/A	0.0000			<0.0005
						5%
Cal Check	0.02%	TANK GUTH				0.0190 - 0.0210
Cal Check	0.08%	TANK				0.0760 - 0.0840
Cal Check	0.25%	TANK				0.2375 – 0.2625
Cal Check	0.30%	GUTH				0.2850 - 0.3150
Training Subject Test	Interferent** (Methanol Mix)		Interferent detected? Y / N			Print out -"Test Aborted Interferent Detected"

A blank, an interferent, a high, mid, and low concentration are run to check calibration.

Calibration and Function Test: **Pass** **Fail**

Analyst: _____ Date: _____

278 Colony Street, Meriden, Connecticut 06451
Phone (203) 427-4043 Fax (203) 427-4600
An Affirmative Action/Equal Opportunity Employer

STATE OF CONNECTICUT

DEPARTMENT OF
EMERGENCY SERVICES AND PUBLIC PROTECTION
DIVISION OF SCIENTIFIC SERVICES

Guy Vallaro, PhD.
Director

Robert H. Powers, Ph.D., DABFT
Deputy Director

RETIRED

Date: January 17, 2014

To: BERLIN POLICE DEPARTMENT

From: Robert H. Powers, Ph.D., Director
Controlled Substance/Toxicology Laboratory

Re: Recertification of Draeger 9510, Serial No. ARBD-0086

The Draeger 9510, Serial No. ARBD-0086 was checked and recertified on January 17, 2014 by the Toxicology Laboratory, Department of Emergency Services and Public Protection, 278 Colony Street, Meriden CT 06451.

.....
Gerald Hart
Forensic Science Examiner 1

Breath analyses instruments are examined and certified by the Department of Emergency Services and Public Protection prior to being placed in operation and after being repaired or recalibrated. There is no requirement for annual recertification.

SOP 7 Access software database usage

I. **PURPOSE**:
The Access software has multi-dimensional functions in the administration of the BAC unit important duties. The software allows for data entry of instructors and operators certifications as well as allowing the data entered to populate to produce officer's certificates. These certificates will be mail to the officers and their profieciency records maintain.
The software allows for keeping track of instrument maintainence. A certificate to certify the instrument use can then be prepared for the agency in which to maintain for their records.

J. **RESPONSIBILITY**:
All Forensic Science Examiners in the BAC section are responsible to follow the guidance of this SOP when utilizing a Alcotest 9510 for subject breath analysis.

K. **DEFINITIONS**:
Queries

L. **PROCEDURE**:
ACCESS DATABASE
Service Log Database

6.) Once entered select "Service Records"

7.) Make entries then close out

BAC Draeger Instructor/Operators Log Data base
Instructor

1. Select "Instructor Query (Qry)"

2. Enter data for each instructor certified after each recertification or new instructor class

3. Select "VIEW" in upper left corner

4. Select of enter "Log in date"

5. Select "update date" icon (red)

6. Data should match original query

7. Close "X" out and save

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8. Select form in use (FRM-IN USE_instructor BAT recertification) to populate certificate))

9. Print certificates

Operator

1. Select “Operator Breath Query (Qry)”

2. Enter data for each operator certified after each recertification class

3. Select “VIEW” in upper left corner

4. Select of enter “Log in date”

5. Select “update date” icon (red)

6. Data should match original query

7. Close “X” out and save

8. Select form in use (FRM-IN USE_ recertification operator BAT) to populate certificate)

9. Print certificates

RETIRED

SOP 8 New instructor training and certification class

PURPOSE:

Annual Certification of New Instructors are done at the Forensic Laboratory. Certification classes are (7hrs) presented using a power point style of presentation. Topics of Training should cover The Toxicology of alcohol as it relates to drunk driving, The laws in Connecticut as they relate to drunk driving in Connecticut and lastly, curent trends of BAC program in Connecticut, BAC, Instructor responsibility, our responsibility, communication with the agency, instrument usage and issues, to include instrument return/repair procedure.

RESPONSIBILITY:

All Forensic Science Examiners in the BAC section are responsible to follow the guidance of this SOP in the certification of New Instructors in breath analysis programs at their respective agency.

DEFINITIONS:

Ethanol (EtOH): Alcohol in beer, wine and spirits

DUI: Driving under the influence

BAC: Blood alcohol content

IR: Infrared

EC: Electrochemical Fuel Cell

Purging: The internal pump flushes the cuvette and breath hose with ambient air to clear alcohol in system.

Air Blank check: After ambiet air check the intensity of the IR energy is analyzed and stored as a reference.

Breath Test: The subject's breath is introduce through the mouth piece and the intensity of the IR and EC are analyzed..

Alveolar: means air expired from the deepest part of the lungs;

Blood alcohol content: means the grams of ethyl alcohol per one hundred (100) milliliters of blood expressed as percentage, or grams of alcohol per 210 liters of breath;

Direct breath alcohol test or direct breath alcohol testing: means the test of a sample of an individual's expired breath using an instrument designed for this purpose in order to determine the concentration of ethyl alcohol in the individual's blood

RETIRED

Instructor: means an individual trained and certified to make an analysis with a direct breath alcohol testing device or instrument and to train operators in the conduct of such test

Operator: means an individual trained and certified to make an analysis with a direct breath alcohol testing device or instrument certified or approved by the commissioner;

Laboratory: means any place or area in which any sample of blood, breath or urine is subjected to a chemical or instrumental analysis.

PROCEDURE:

The (7) hour new instructor certification traininig

Prepare handouts
Prepare sign in sheets
Set up lab top for powerpoint presentation
Setup Draeger instruments for the hands on portion of the class

The (7) hour new instructor certification traininig using powerpoint should include the following topics:

Toxicology of alcohol presentation

Connecticut laws governing DUI presentation

Topics of Training and Curent trends of BAC program in Connecticut

EtOH/Drug analysis (Blood and urine)

BAC serum conversion

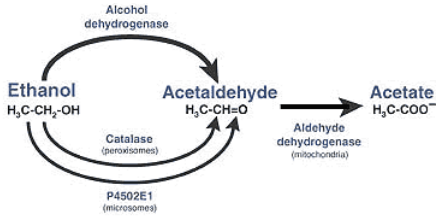
Instructor responsibility to their departments and the BAC program

Our responsibility to the departments and the BAC program

RETIRED

Toxicology of Alcohol

Metabolism of alcohol



Ethanol is a small two carbon alcohol that, due to its small size and alcoholic hydroxyl group is soluble in both aqueous and lipid environments. This allows ethanol to freely pass from bodily fluids into cells. Since the portal circulation from the gut passes first through the liver, the bulk of ingested alcohol is metabolized in the liver. The process of ethanol oxidation involves at least three distinct enzymatic pathways. The most significant pathway, responsible for the bulk of ethanol metabolism, is that initiated by

alcohol dehydrogenase, ADH. ADH is an NAD^+-requiring enzyme expressed at high concentrations in hepatocytes.

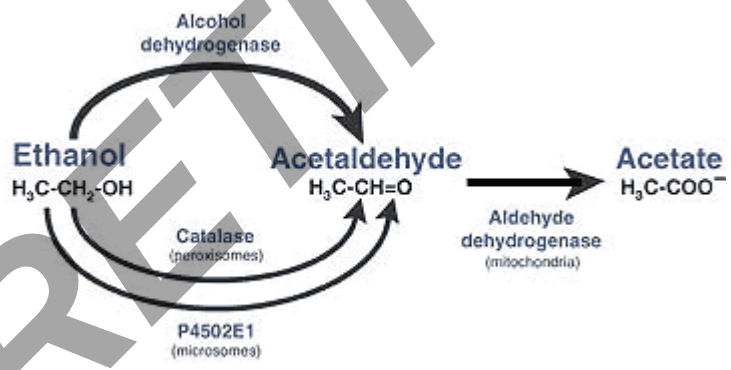
Metabolism of Ethanol: Ethanol is a small two carbon alcohol that, due to its small size and alcoholic hydroxyl group is soluble in both aqueous and lipid environments. This allows ethanol to freely pass from bodily fluids into cells. Since the portal circulation from the gut passes first through the liver, the bulk of ingested alcohol is metabolized in the liver. The process of ethanol oxidation involves at least three distinct enzymatic pathways. The most significant pathway, responsible for the bulk of ethanol metabolism, is that initiated by alcohol dehydrogenase, ADH. ADH is an NAD^+-requiring enzyme expressed at high concentrations in hepatocytes.

RETIRED

Acetate from EtOH Metabolism and Fatty Liver:

There are two primary ALDH genes in humans that are responsible for the oxidation of acetaldehyde generated during the oxidation of ethanol. Aldehyde Dehydrogenases

There are two primary ALDH genes in humans that are responsible for the oxidation of acetaldehyde generated during the oxidation of ethanol.



Basic Pharmacology of Ethanol

Ethyl alcohol (ethanol, ETOH) or just alcohol has been described as the "perfect" drug. It is soluble in water, the major constituent of all bodily fluids and tissues, not charged, a small molecule and not subject to changes in molecular structure as a result of changes in the acidity (pH) of the body fluids. Because ETOH is uncharged, it is also soluble in fatty substances (i.e., lipids) and passes easily through the lipid membrane barriers in the body (e.g., from the stomach into the blood or from the intestines into the blood).

Absorption

Absorption is the process by which alcohol is made available to the fluids of distribution of the body (e.g., blood, plasma, serum, aqueous humor, lymph, etc.). Approximately 80% of orally ingested ETOH is absorbed from the small intestines, and the remainder is absorbed from the stomach.

Distribution
Once a drug has been absorbed from the stomach and/or intestines (GI Tract) into the blood, it is circulated to some degree to all areas of the body to which there is blood flow. This is the process of distribution.
The police know that the absorption of alcohol from the GI tract into the blood and the distribution of alcohol from the blood into the brain (central nervous system, CNS) takes time.

Metabolism
Alcohol in the blood and tissues must be inactivated and excreted from the body. This process is initiated by altering the chemical structure of the alcohol in such a way as to promote its excretion. The transformation of the alcohol molecule into a chemically related substance that is more easily excreted from the body is called metabolism or detoxification.

Excretion
Excretion is the process by which a drug is eliminated from the body. In the case of ETOH, the kidney and lungs excrete only 5-10% of an absorbed dose of ethanol unchanged (unmetabolized). The rest must be metabolized prior to excretion.

http://www.doctorbenjamin.com/etoh/etoh.htm

Connecticut Laws governing the DUI program and the BAC instrument used in Connecticut are the Alco Test 9510 (Draeger) and the Intoxilyzer 5000.

Statues and regulations
Forensic Chemical Testing 14-227a-1b.

14-227a-8b. Operation and use of devices or instruments

14-227a-10b. Pg.16C Direct breath alcohol test
No person shall operate a direct breath alcohol test device or instrument unless such person has been certified by the commissioner and is employed by a law enforcement agency

14-227a-10b. Pg.16C Direct breath alcohol test
Certification of specific devices or instruments

14-227a-10b. 16C and 17C Direct breath alcohol test

- Sample collection
- (A) The expired breath sample shall be air that is alveolar in composition. The breath sample shall be collected only after the subject has been monitored for at least 15 minutes prior to the collection of each sample. During this period, the test subject shall not have ingested alcoholic beverages or food, regurgitated or smoked.

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(A) Operators shall follow the manufacturer's operating instructions for the device or instrument, unless the commissioner has accepted a modification of such instructions.

- (B) All agencies using a device or instrument shall make available for inspection by the commissioner all devices or instruments used by them, together with the current logbook associated with each such device or instrument. Such logbook shall include the identity of each operator using the device or instrument, the frequency with which the device or instrument has been checked for accuracy and the results of each subject's analysis and calibration.

Certification of operators and instructors

- (1) Certification of operators
- (A) General requirements
- An operator of a direct breath alcohol testing device or instrument shall meet the following requirements:

- Employment by a law enforcement agency or the department;

- Successful completion of at least four hours of training in the operation of the device or instrument to be used. Such training may be acquired by attending training courses offered by the department or by certified instructors; and

- Demonstration to the commissioner of the proper use and application of such device or instrument.

- (B) Proficiency instruction and review
(i)At any time after certification, the commissioner may require an operator to satisfactorily demonstrate proficiency in the use of such device or instrument.
- (ii)Each operator shall demonstrate to a certified instructor competence in the operation of a device or instrument at least once during the 12-month period following the last such demonstration. The results of each such review shall be reported to the commissioner.

- (2) Certification of instructors
- In order to be certified as an instructor in the use of a direct breath alcohol test device or instrument, the following requirements shall be met:
- (A) Employment by a law enforcement agency or the department;
- (B) Successful completion of at least seven hours of instruction approved by the commissioner on a designated device or instrument. Such instruction shall include the following:

- (i) The theory of the devices or instruments used in the analytical process that measures the alcohol content of the blood;
- (ii) Practical application and experience in the use of such devices or instruments; and

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- (iii) Presentations and discussions of the pharmacological and physiological effects of alcohol on the human body.

- (C) Proficiency instruction and review
- Each instructor shall attend an annual course of instruction conducted by the commissioner.

14-227a-10b. Pg.17C Direct breath alcohol test

- (e) Revocation of certification of operators and instructors
- (1)The commissioner may revoke a certification issued to an operator for the following reasons:

- (A) Failure to remain employed by a law enforcement agency or the department;
- (B) Misuse of the device or instrument or incompetence in the performance of tests; or
- (C) Failure to participate in proficiency review and testing or failure to properly perform tests during proficiency review and testing.

- The commissioner may revoke a certification issued to an instructor for the following reasons:
- (A) Failure to remain employed by a law enforcement agency or the department;
- (B) Failure to demonstrate knowledge of the device or instrument or testing procedures to the extent necessary to instruct operators; or
- (C) Failure to attend an annual course of instruction conducted by the commissioner.

RETIRED

Connecticut Statues

Sec. 14-227a. Operation while under the influence of liquor or drug or while having an elevated blood alcohol content. (a) Operation while under the influence or while having an elevated blood alcohol content.

- (1) while under the influence of intoxicating liquor or any drug or both, or
- (2) while such person has an elevated blood alcohol content.
    - For the purposes of this section, "elevated blood alcohol content" means a ratio of alcohol in the blood of such person that is eight-hundredths of one per cent or more of alcohol, by weight, …….

Sec. 14-227a. (b) Admissibility of chemical analysis

- **evidence respecting the amount of alcohol or drug in the defendant's blood or urine at the time of the alleged offense, as shown by a chemical analysis of the defendant's breath, blood or urine shall be admissible and competent provided:**
- **(1) The defendant was afforded a reasonable opportunity to telephone an attorney prior to the performance of the test and consented to the taking of the test upon which such analysis is made;**

Sec. 14-227a. (b) Admissibility of chemical analysis

General BAC Program what is current information for instructors to know

- (2) a true copy of the report of the test result was mailed to or personally delivered to the defendant within twenty-four hours or by the end of the next regular business day, after such result was known, whichever is later;
- (3) the test was performed by or at the direction of a police officer according to methods and with equipment approved by the Department of Public Safety and was performed in accordance with the regulations adopted under subsection (d) of this section;

(4) the device used for such test was checked for accuracy in accordance with the regulations adopted under subsection (d) of this section; (5) an additional chemical test of the same type was performed at least ten minutes after the initial test was performed or, if requested by the police officer for reasonable cause, an additional chemical test of a different type was performed to detect the presence of a drug or drugs other than or in addition to alcohol,

- (6) evidence is presented that the test was commenced within two hours of operation.

Sec. 14-227a. (d) Testing and analysis of blood, breath and urine.
Such regulations shall not require recertification of a police officer solely because such officer terminates such officer's employment with the law enforcement agency for which certification was originally issued and commences employment with another such agency.

Sec. 14-227a. (k) **Seizure and admissibility of medical records of injured operator.**

- (1) The blood sample was taken or the urine sample was provided for the diagnosis and treatment of such injury; (2) if a blood sample was taken, the blood sample was taken in accordance with the regulations adopted under subsection (d) of this section; . . . .

Such search warrant may also authorize the seizure of the medical records prepared by the hospital in connection with the diagnosis or treatment of such injury.

Sec. 14-227c. Blood or breath samples required following accidents resulting in death or serious physical injury.

a) As part of the investigation of any motor vehicle accident resulting in the death of a person, the Chief Medical Examiner, Deputy Chief Medical Examiner, an associate medical examiner, a pathologist as specified in section 19a-405, or an authorized assistant medical examiner, as the case may be, shall order that a blood sample be taken from the body of any operator or pedestrian who dies as a result of such accident.

Sec. 14-227c. Blood or breath samples required following accidents resulting in death or serious physical injury continued.

- (b) A blood or breath sample shall be obtained from any surviving operator whose motor vehicle is involved in an accident resulting in the serious physical injury, as defined in section 53a-3, or death of another person, if (1) a police officer has probable cause to believe that such operator operated such motor vehicle while under the influence of intoxicating liquor or any drug, or both, or (2) such operator has been charged with a motor vehicle violation in connection with such accident and a police officer has a reasonable and articulable suspicion that such operator

RETIRED

operated such motor vehicle while under the influence of intoxicating liquor or any drug, or both.

Sec. 14-227g. Operation by person under twenty-one years of age while blood alcohol content exceeds two-hundredths of one per cent. Procedures. Penalties.

- (a) No person who is less than twenty-one years of age shall operate a motor vehicle while the ratio of alcohol in the blood of such person is two-hundredths of one per cent or more of alcohol, by weight.
- (b) The fact that the operator of a motor vehicle appears to be sixteen years of age or over but under twenty-one years of age shall not constitute a reasonable and articulable suspicion that an offense has been or is being committed so as to justify an investigatory stop of such motor vehicle by a police officer.

Expectations of Intructors and Our Responsiblity:

Our Responsiblity

Provide initial Instructor training (7hrs)
Topics of the Training:
Toxicology of Alcohol to includes
EtOH/Drug analysis (Blood and urine) to include BAC serum conversion
DUI Connecticut Laws
Provide opportunity for hands on and more Q&A
Topics of Training and Curent trends of BAC program in Connecticut
Draeger Printout interpretation
Some reasons for External Standard failure
Case Study of something interesting that year

Instructor responsibility

Ensure good Communication with the toxicology laboratiory BrAC unit
Instrument return/repair procedure
Hands on and more Q&A

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Draeger Operation:

Two of the methods approved by State Statue Sec. 14-227a page 13 are Infrared and Fuel Cell analysis.

- Infrared Theory
  - EtOH molecules absorb energy at specific areas in the IR spectrum (9.5µm region of the IR spectrum).
- Electrochemical fuel cell
  - The fuel cell as a sensor can detect the presence of a chemical (EtOH)

Draeger Operation continue:

Breath Sequence warmup phase
Selfcheck
Warming up
Not Ready
Ready

BrAC Testing:
Subjects that present a poor field sobriety test resut and < 0.08 g/210L breath test the Toxicology section will evaluate biological fluids (urine or blood) for the presense of other intoxicant which maybe found.

Assist in legal matters
Court
Freedom of information Act Requests (FOIA)
DMV Per Se Hearing

Issues
Modem fails to connect
Fuel cells go bad
Loss of data
Too long for instrument to be "Ready"
# of copies set wrong
Opting Out – Refusal vs Terminate Test

Error Messages/ Test Aborted
External Standard Failure
Blowing not allowed
Alcohol ambient Air
Inhalation Detected
Interferent Detected
Mouth alcohol
Fuel Cell might be dying
Tank pressure might be too low

Turning Draeger instrument in for Repair:
Notify Toxicology Laboratory Breath Alcohol Section of problem and returning for service Contact Mark or Gerry

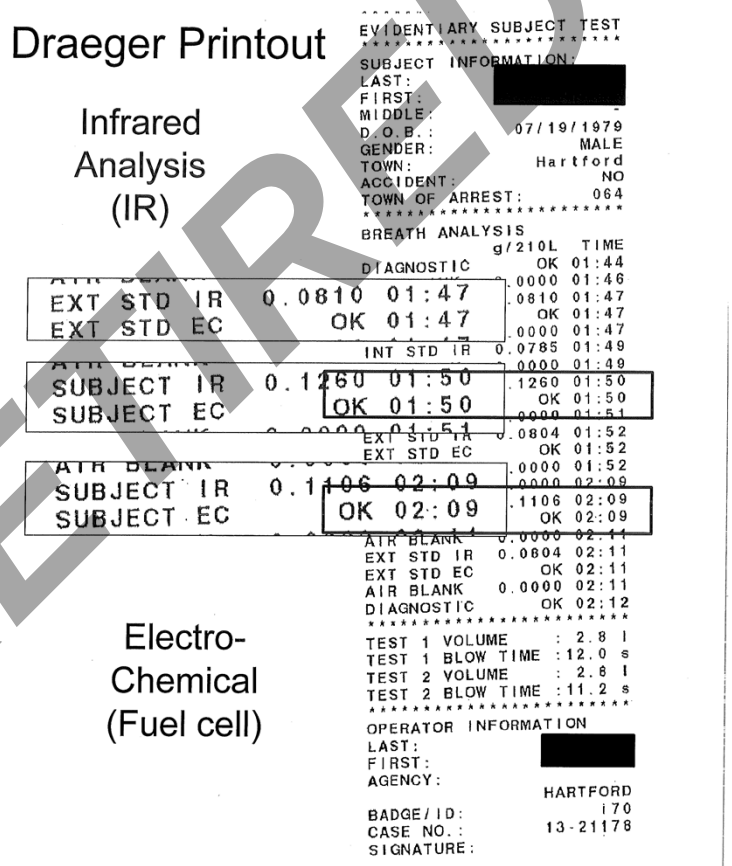
Only Bring the Draeger 9510
DO NOT bring the regulator or base
Only INSTRUMENT & COVER please
Please FILL-OUT form at front desk
Complete form "RECEIPT/RETURN of the Draeger 9510"

RETIRED

Pickup of Draeger instrument after repair:

Pick up instrument at front desk
Sign and date form attached
PLEASE LEAVE RECEIPT AT FRONT DESK
Instrument Certification
Will be attached to the instrument cover
Police Departments keep for their records

Draeger printout to demonstrate IR v fuel cell info



RETIRED

Page 1 exam example

Name and Rank:_____ Badge/ID:_____

Department/Troop:_____ Date:_____

Breath Analysis Instructor/ Operator Written Test

True False Questions

1. Data entries can be performed via the touch screen OR the Keyboard. _____
2. The breath hose has to be stored in its compartment to assure correct breath temperature. _____
3. The best way to abort or reset a test is to cycle the power switch. _____
4. When an air blank check fails, close all off ventilation in area and to ensure no fresh air and thus a clean air blank. _____
5. After "Subject Blow" prompt, there is one minute to deliver a sample. _____
6. A subject should be encouraged to blow until the grey bar goes all the way to the end of the indicator. _____
7. Keeping the 9510 powered up and on while the dust cover is on will cause overheating. _____
8. The message "Test aborted Interferent Detected" means that the instrument has detected narcotics. _____
9. When the 9510 flashes "Please Blow" for 3 minutes, the Draeger will automatically give a Refusal message on the screen. _____
10. The Alcotest 9510 should always be plugged into a surge suppressor when used at the departments. _____
11. There has to be at least 30 minutes between test #1 and #2. _____
12. If a message of "Mouth Alcohol" is displayed, you should wait 5 minutes and retest. _____
13. A defensible breath test should be "bracketed" by External STD. Cal checks. _____
14. Instructors are responsible for keeping records of the Operators they certify. _____
15. The Draeger uses a CAL GAS concentration of 0.08% for external std checks _____

RETIRED

Page 2 of exam example

16. What indicates that a breath sample is being delivered to the 9510?

17. Why do we want breath tests #1 and #2 to be of similar length and volume.

18. Acetone will cause the 9510 to give what message?

19. Why is the subject not allowed to hold or grasp the breath hose at any time?

20. What is pressed to "OPT OUT" of the test sequence?

21. How long after an arrest do you have to start the test?

22. If you see the message "MOUTH ALCOHOL", what should you do?

23. What "EXTERNAL STD FAILED" mean and what should you do?

RETIRED

SOP 9 Instructor Recertification class

M. **PURPOSE**:

Annual recertification of Instructors are done at the Forensic Laboratory. Recertification (2hrs) classes are presented using a power point style of presentation.. Topics of Training should cover curent trends of BAC program in Connecticut, BAC serum conversion, Instructor responsibility, our responsibility, communication with the agency, instrument usage and issues, instrument return/repair procedure.

N. **RESPONSIBILITY**:

All Forensic Science Examiners in the BAC section are responsible to follow the guidance of this SOP in the recertification of Instructors in breath analysis programs at their respective agency.

O. **DEFINITIONS**:

Infrared Theory

Electrochemical fuel cell theory

P. **PROCEDURE**:

The (2) hour Rescertification annual traininig

Prepare handouts
Prepare sign in sheets
Set up lab top for powerpoint presentation
Setup Draeger instruments for the hands on portion of the class

The (2) hour Rescertification annual traininig using powerpoint should include the following topics:

Topics of Training and Curent trends of BAC program in Connecticut

EtOH/Drug analysis (Blood and urine)

BAC serum conversion

Instructor responsibility

Our responsibility

Assist in legal matters

Court
Freedom of information Act Requests (FOIA)
DMV Per Se Hearing

Repair of Draeger instrument:

Notify Mark or Gerry of the Toxicology Laboratory Breath Alcohol Section of problem and returning for service

Dropping off Draeger instrument for service:

RETIRED

Only Bring the Draeger 9510

DO NOT bring the regulator or base!
Only INSTRUMENT & COVER please!
Please FILL-OUT form at front desk
Complete form “RECEIPT/RETURN OF THE DRAEGER ALCOTEST 9510”

Pickup Draeger instrument after service is complete:

Pick up instrument at front desk
Sign and date form attached
PLEASE LEAVE RECEIPT AT FRONT DESK
Instrument Certification

Will be attached to the instrument cover
Police Departments keep for their records

Communication (i.e., modem, email, contact persons at the agency)
Touch on the science of the technology involve with the Draeger

Infrared Theory

- EtOH molecules absorb energy at specific areas in the IR spectrum (9.5µm region of the IR spectrum).

Electrochemical fuel cell

- The fuel cell as a sensor can detect the presence of a chemical (EtOH)

Draeger Printout interpretation

External Standard failure
Case Study of something interesting that year
Hands on and Q&A

Maintain the Draeger instrument
Instrument return/repair procedure

Send out for maintenance as needed

Instrument Validation and Certification

- Instruments are calibrated by manufacturer by Draeger Diagnostic
- Instruments are recertified by Toxicology Lab upon its refurn from Draeger Diagnostic.
- A certificate is made and is returned with the submitting PD upon pickup of their instrument.

Maintain instrument service records

Maintain the instrument service log

RETIRED

1.0 Draeger Operation:

10.1 Breath Sequence warmup phase
10.2 Selfcheck
10.3 Warming up
10.4 Not Ready
10.5 Ready

2.0 Issues

12.1 Modem fails to connect
12.2 Fuel cells go bad
12.3 Loss of data
12.4 Too long for instrument to be "Ready"
12.5 # of copies set wrong
12.6 Opting Out – Refusal vs Terminate Test

3.0 Error Messages/ Test Aborted

External Standard Failure
Blowing not allowed
Alcohol ambient Air
Inhalation Detected
Interferent Detected
Mouth alcohol
Fuel Cell might be dying
Tank pressure might be too low
Readings are slightly higher
PSI of dry gas is <u>below 25 psi</u> (change tank)
Use correct concentration gas cylinder

RETIRED

DESPP
Division of Scientific Services

Recert Instructor
Thursday, June 12, 2014

NAME	AGENCY	Email	Phone	(√) INTOX 5000

DENIED

SOP 10 Administrative Duties

Q. **PURPOSE**:

The administrative duties are an intragral part of the BrAC section. This is the frontline in response to our stakeholders. Downloading of the Draeger and Intox 5000 instrument's data to be stored here at the Toxicology Laboratory. This data eill be further used in putting together grant proposals. The maintanence of Service records to includes instrument certification, scheduleling and teaching recertification classes as well as new instructor classes, producing certificates for each instructor upon their recertification as instructors. File management of hard copies of instrument certifications. The additional dutiy is to respond to Attorney request (FOI). Work directly agency to solve problems such as modem or email issues.

R. **RESPONSIBILITY**:

All Forensic Science Examiners in the BrAC section are responsible to follow the guidance of this SOP governing the administrative duties in this section.

S. **PROCEDURE**:

Administrative Duties in BAC unit:

Re-certifies all instrument sent out for service

- Generated instrument certificates upon re-certifying Draeger instrument
- Instrument certificates and all service records are kept on file in the Intox section
- Maintain instrument original documents of service records in the file cabinet
- Maintain the instrument service log which is done using Access software

Scheduling all Annual Training:

Provide initial Instructor training (7hrs)
Recertification (2hrs) for Instructors
Excel software:

- The use of the Excel software aid in scheduling Department's BAC instructors for annual recertification classes
- The use of the Excel software aid in monitoring Department's BAC instructors annual recertification

Generated all certificate for operators and instructors

- Each certificate is sign to validate certification
- Mail certifications to each officer via their respective agency

RETIRED

- Instructors and operators proficiency evaluations are kept on file in the Intox section

T. Assist in legal matters

Court

- Court subpoena are sometimes sent in which case will appear in court to testify in either civil or criminal matters as an expert witness

Freedom of information Act Requests (FOIA)

- Respond to all Attorney request as a function of FOIA

DMV Per Se Hearing

- Subpoena are sometimes sent in which to appear at DMV to testify as an expert witness in case involving DUI infraction.

Communication with Department Management:

- Modem telephone number keeping current and reliable for downloading data fand installing updates

- Email addresses keeping current and reliable

Data Management:

1. ALCOTEST 9510 UPDATING FIRMWARE/SOFTWARE
2. ALCOTEST 9510 UPDATING FIRMWARE/SOFTWARE REMOTELY
3. ALCOTEST 9510 UPDATING FIRMWARE/SOFTWARE USING THUMB DRIVE
4. ALCOTEST 9510 DOWNLOADING DATA REMOTELY
5. ALCOTEST 9510 DOWNLOADING DATA USING THUMBE DRIVE
6. INTOX 5000 DOWNLOAD
7. Downloading data from Intox at PD; see SOP 2 and SOP 3
8. Downloading stored data
9. Printing downloaded data

RETIRED

SOP 11 Draeger Program Contact Information

U. **PURPOSE**:
The following contacts will aid in the successful administration of the BAC program for the State of Connecticut..

V. **RESPONSIBILITY**:
All Forensic Science Examiners in the BAC section are responsible to follow the guidance of this SOP when the need arises to contact external sources related to the breath analysis program.

RETIRED

W. BrAC UNIT CONTACTS

Toxicology/Controlled Substances/Breath Alcohol

- Main Forensic Lab# 203-639-6400
- FAX# 203-427-4600

Mark Anderson
278 Colony St.
Meriden, CT 06451
mark.anderson@ct.gov
203-427-4038

Gerry Hart
278 Colony St.
Meriden, CT 06451
Gerry.hart@ct.gov
203-427-4044

Contact Information for Draeger Safety Diagnostic, Inc.
4040 W. Royal Lane, Suite 136
Irving, TX 75063
www.draeger.com
main# (866) 385-5900
modem# - 1-(972) 915-3904

Instrument Service Representative-Brett Everett
Office# (972) 929-1100
Cell# (817) 876-9855
Email# bret.everett@draeger.com

Software Engineer – Brian Shaffer
Office#: (970) 403-0282
Cell#: (970) 316-1261
Email: Brian.Shaffer@draeger.com

Fax#: ((970) 403-0314
Brian works off site in Colorado – leave message and he'll get back to you

Sales Representative – Don Pouliot
Office#: (973) 398-2117
Cell#: (973) 219-9520
Email: Don.Pouliot@draeger.com
Fax#: ((970) 403-0314

Mara Herrera
Office#: (866) 385-5900 ext 174
Direct line#: (972) 573-1752
Email: Mara.Herrera@draeger.com

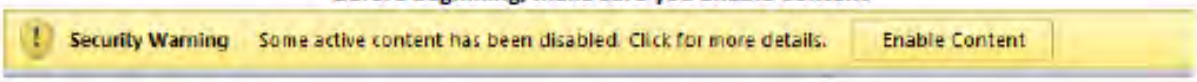
Central Equipment Co., Inc
Box 781
Mattaposett, MA 02739
Office# (508-5758
Fax# (508) 758-9758
www.central-equipment.net

RETIRED

To generate and email a certificate for Operators (submitted by an Agency Instructor)

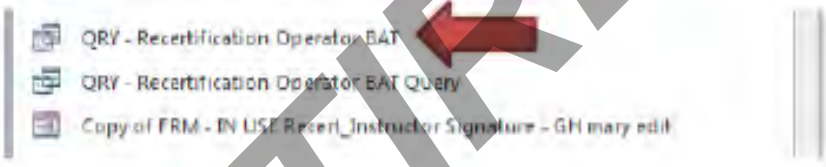
File Location: S:/TOXLAB Database/BAC Draeger Instructor_Operator Log.accdb

Before beginning, make sure you Enable Content

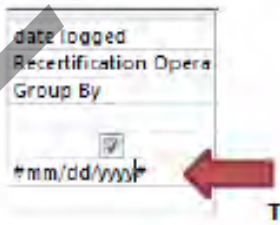


Step 1: Enter the data into the Recertification Operator BAT: Table. Only enter ONE email address in one record per Agency. (i.e. if there are 50 operators to enter, only enter the email address of the Instructor on the first record. This will prevent duplicate emails from being sent out.)

Step 2: (A) Open the query QRY – Recertification Operator BAT

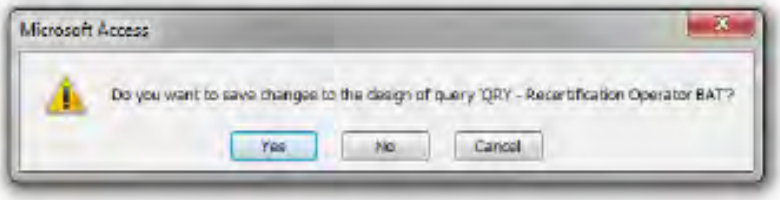


(B) Change to Design Mode and change the DATE LOGGED Date to Today's Date.



TODAY'S DATE

(C) Select Run and then close the query, and when it asks you whether you want to save the design of the query, select YES



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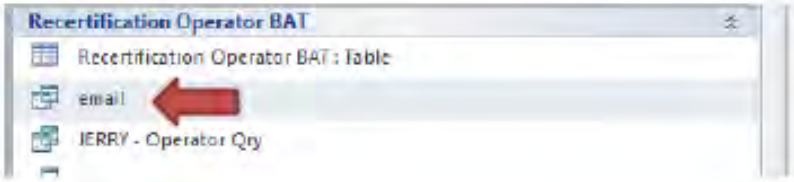
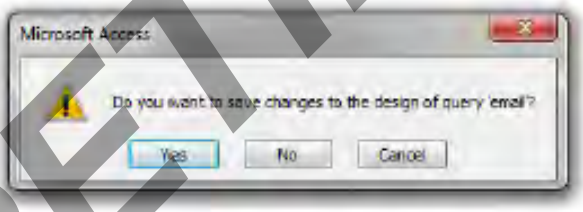
Step 3: (A) Open the query "email"



(B) Change to Design Mode and change the DATE LOGGED Date to Today's Date.

TODAY'S DATE

(C) Select Run and then close the query, and when it asks you whether you want to save the design of the query, select YES



Step 4: Open the Form CERT GENERATION (at the bottom of the list in Unrelated Objects)

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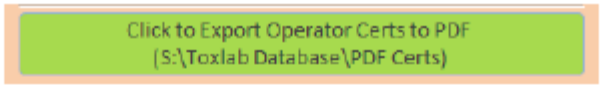
(A) Click on the Green "Click to Export Operator Certs to PDF" Button



(Your screen will start flashing as it generates each PDF)

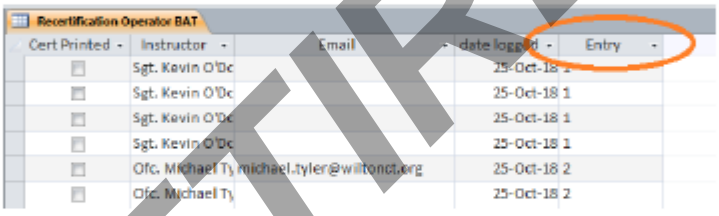
(B) THEN Click the Blue "Click to EMAIL Operator Certs" Button

(You will see email windows flash on your screen as they are sent)

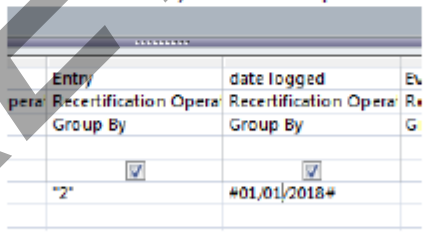
Step 5: You're Done! Confirm that all emails were sent by checking your Sent folder in Outlook.

TO MAKE MULTIPLE ENTRIES/EMAIL BATCHES

This method works once per day, since you're only sorting items by the date you logged it. If you get a list later in the day that you need to enter ASAP; simply enter a number in the "Entry" field in the Recertification Operator BAT: Table



and then when you perform the "Email" and "QRY – Recertification Operator BAT" queries, simply place the same number you entered into the "Entry" field into the "Entry" field in both queries



Once you hit Run, only the items that you entered with a number in the "Entry" field you selected will show up, and you can then follow the steps above to create PDFs and Email them.

Once these records have been entered, a pre-printed label is placed on the hard copy and the date/initials of the person who entered the names and the date/initials of the person who emailed the names is written on the label. The hard copies are then brought in to the Breath Analysis Lab for filing.

ENTERED INTO DRAGER DB
DATE: BY:

SENT DATE: BY:

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ASSOCIATION OF FIREARM & TOOL MARK EXAMINERS

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GLOSSARY
6th Edition

Version 6.120414

About the Glossary

GLOSSARY OF
THE ASSOCIATION OF FIREARM AND TOOL MARK EXAMINERS

Edited by
the AFTE Training and Standardization Committee

Version 6.120414

Sixth Edition - 2013
Fifth Edition – 2007
Fourth Edition - 2001
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Library of Congress Catalog Card Number
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Contributors

Association of Firearm and Tool Mark Examiners
Training and Standardization Committee

The first edition of this glossary of the Association of Firearm and Tool Mark Examiners (AFTE) was produced by the AFTE Standardization Committee in 1980. This committee was formed at the 1977 training conference at Virginia Beach, VA, in recognition of a need for a common frame of reference and clear definitions of the terms most frequently used in the discipline. This pioneering group consisted of the following individuals:

George R. Wilson, Chairman Metropolitan Police, D.C.
James Berglund, Member Michigan State Police
Don Gunnell, Member Chicago Police Department
Evan Hodge, Member Vermont State Police
Marshall K. Robinson, Member Waterbury Connecticut Police Department
John G. Ward, **Sr**., Member Virginia Division of Forensic Sciences

This committee eventually produced two additional editions of the AFTE Glossary in 1986 and 1994, with great success. The uniquely forensic perspective reflected in these three editions have been a great assistance to thousands of forensic firearms examiners and toolmark examiners for decades. As the original Standardization Committee realized, this type of reference work can never be regarded as complete. In any forensic discipline, innovations and developments rapidly enter the field constantly and must be addressed. New concepts and technological advances dictate the need for continuing consensus on terminology so that examiners may communicate uniformly with each other and those outside the discipline. Towards that end the AFTE President's Glossary Subcommittee was formed in order to explore the restructuring and re-formatting of the glossary in a key word searchable electronic medium adaptable to economical updating and distribution. This fourth edition on compact disc is the product of the subcommittee's work published with the approval of the AFTE Training and Standardization Committee. The Glossary Subcommittee members were:

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The Association of Firearm and Tool Mark Examiners would like to recognize the following individuals who made contributions to the Glossary over the decades. Their input is deeply appreciated. It constitutes a legacy to all examiners who follow.

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Revisions and Updates

It is anticipated and expected that this Glossary will be revised periodically. The Association of Firearm and Tool Mark Examiners Training and Standardization Committee will have the ongoing responsibility for this revision. If you see the necessity for changes or additions, it is requested that you contact the current Chairman of the Training and Standardization Committee in writing. Do not just suggest that a term needs inclusion or rewording: give a definition.

Version History

Version 4.020717

1. Corrected an omission in the definition, "**Theory of Identification as it Relates to Toolmarks**".
2. Corrected an omission in the definition, "**Range of Conclusions Possible When Comparing Toolmarks**".

Version 4.040106

1. Corrected an omission in the definition, "**Theory of Identification as it Relates to Toolmarks**" on pages 129 and 143
2. Corrected an omission in the definition, "**Range of Conclusions Possible When Comparing Toolmarks**" on pages 104 and 142.
3. Corrected an omission in the definition "**Subclass Characteristics**" on pages 127 and 143.
4. Corrected an omission in the definition "**Tool**" on page 144.

Version 5.020707

1. Definitions added from the glossary published by the Sporting Arms and Ammunition Manufacturers' Institute (SAAMI).
2. Excerpts of terms assembled by Luke Haag during the preparation of his book on shooting incident reconstruction and shared with the AFTE Glossary Committee.

Version 5.31109

1. Added new definitions and modified wording on definitions already present in all sections of the Glossary.

Version 5.41809

1. Added new definitions from SWGGUN Admissibility Resource Kit.

Version 6.101613

1. Updated **"Theory of Identification as it Relates to Toolmarks"** to reflect the changes made by the Committee for the Advancement of the Science of Firearm and Toolmark Identification.
2. Updated the definition for **Individual Characteristics.**
3. Updated Table 8 (previously Table 6) to include data for 40/10mm caliber and 50 caliber. Information for bore diameter was obtained from ANSI/SAAMI Z299.3-1993 available from SAAMI. Circumference and land plus groove impression widths were calculated from the bore diameter (as indicated previously in the table).
4. Updated Table 2 to include 0000 buck information.
5. Added metric measurements to tables.
6. Omitted both the **Computer Terminology** and **Fingernail** appendices.
7. Added terms, modified wording of definitions already present in the glossary, and removed outdated/repetitive terms.
8. Added term and definition for **Pattern Matching**.

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9. Added term and definition for **Practical Impossibility**.
10. Clarified wording of **Subclass Characteristics**.
11. Relocated definitions to their appropriate section or appendix.
12. Renamed **Section 3 – Gunshot Residue** to **Section 3 – Gunshot Residue and Gunpowder**.
13. Renamed **Useful Recipes** appendix to **Useful Reagents** and **Silencers** appendix to **Suppressor Terminology**.
14. Combined appendices **Machining Terms** and **Materials Terminology** and renamed to **Manufacturing and Machining Terminology**.
15. Definitions added to terms and graphics in the **Firearm Ignition Systems** appendix.
16. Updated formatting. The phrasing "refer to" suggests another term and definition that may be relevant to the term currently being viewed. The phrasing "also known as" suggests another name for the term currently being viewed; however, the "AKA" may not be defined elsewhere within the glossary.

Version 6.120414

1. Added term, definition, and images for **Bolt Seal** to the Locks and Keys Section.
2. Updated the image for the term **Slippage Marks**.
3. Added the term and definition for the terms **Folding Stock** and **Telescopic Stock**.
4. Other minor formatting with regard to font size and spacing.

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- Abbreviations
- Formulas
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Section 1 - Firearms Identification

Absorber, Recoil

Refer to **Recoil Absorber**.

Accelerator

(1) A device found in some semiautomatic and automatic firearms that, through mechanical advantage or spring energy, transfers kinetic energy from one part of the mechanism to another with a resultant speed increase of the action (e.g., Browning machine gun).

(2) A cartridge loaded with a subcaliber bullet in a caliber-sized sabot.

Accidental Discharge

The inadvertent discharge of a firearm as a result of some failure on the part of the firearm. Accidental discharge is often caused by a safety device that was broken, deficient, compromised, or missing from the firearm that, if present and operative, would have prevented the discharge.

Accidental Discharge Test

The examination of a firearm to determine if it is capable of firing without deliberate action on the part of the shooter (i.e., if it will fire without the trigger being pulled) and if the safety features of the firearm are operational.

Accuracy

In firearms using single projectiles at a given distance, accuracy is the measure of how close the fired shots are to the intended target.

Accuracy Life

An estimated, or empirically determined number of rounds that can be fired in a particular gun of a particular caliber, before it fails to meet a particular accuracy specification. Wide variations may occur due to caliber, ammunition characteristics, firing schedules, maintenance, and firearm design.

Accurize

Common term used in conjunction with firearms which have been subjected to special fitting and operations in the interest of improved accuracy.

Action

The working mechanism of a firearm. The combination of the receiver or frame, the breech bolt, and the other parts of the mechanism by which a firearm is loaded, fired, and unloaded.
May be broken down into action types as follows:

Automatic Action – A firearm design that feeds cartridges, fires, extracts, and ejects cartridge cases as long as the trigger is fully depressed and there are cartridges in the feed system. Actuation of the mechanism may be from an internal power source such as gas pressure or recoil, or external power source, such as electricity. Also known as fully automatic, full auto or **machine gun**.

Ballard Action – A type of dropping block, single shot action.

Blowback Action– A design found in semiautomatic and automatic firearms where the weight of the bolt, the inertia of some component, and/or a mechanical delay is the main locking force and no mechanical locking of the breech occurs. These firearms directly utilize the breech pressure exerted on the head of the cartridge case to actuate the mechanism. Refer to **Blowback, Delayed** and **Blowback, Simple**.

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Blow-Forward Action– A design for semiautomatic or automatic firearms wherein the breech block is stationary and the barrel moves forward by gas pressure to open and eject the cartridge and cycle the action.

Bolt Action – A firearm in which the breech closure is in line with the bore at all times, manually reciprocates to load, unload, and cock, and is locked in place by breech bolt lugs and engaging abutments, usually in the receiver. There are two principal types of bolt actions: the turn bolt and the straight pull.

Box Lock Action – A design in which the hammer and hammer spring(s) are located within the frame and the trigger assembly is located in the lower tang. Generally found on double barrel shotguns such as Parker, Fox, Stevens, Winchester Models 21 and 24, etc.

Break-Open Action – Refer to **Hinged Frame Action**.

Double Action – A handgun mechanism in which a single pull of the trigger first cocks and then releases the hammer or striker.

Dropping Block Action – Refer to **Falling Block Action**.

Falling Block Action – A single shot lever actuated mechanism in which the breechblock slides vertically (or nearly vertically) when the lever is moved to expose or lock the breech. Also known as dropping block action (e.g., Ruger No. 1).

Hinged Frame Action – A design wherein the barrel(s) is pivoted on the frame. When the action is open, the barrel(s) may pivot up, down, or sideways for loading or unloading. When the action is closed, the breech of the barrel(s) swings against the standing breech. Opening is normally accomplished by movement of a top-, side-, or under-lever.

Hybrid Action – A design that incorporates aspects of both single action and double action systems. For example, a pistol action which requires the slide to retract and set or partially cock the action, and the trigger to further cock and release the hammer or striker.

Lever Action – A design wherein the breech mechanism is cycled by an external lever generally below the receiver.

Martini Action – A hammerless, single shot rifle mechanism, lever actuated, in which the movement of the breechblock is entirely within the receiver and pivots at the rear.

Pump Action – Refer to **Slide Action**.

Revolver Action – A firearm, usually a handgun, with a cylinder having several chambers so arranged as to rotate around an axis. The firearm is discharged successively by the same firing mechanism. Refer to **Revolver**.

Rolling Block Action – A design in which a breechblock and hammer each rotate about their separate transverse pins in the receiver. The two members are swung rearward, away from the barrel breech to load the mechanism, or extract a cartridge case. To fire a cartridge, the breechblock is closed and locking is accomplished by the falling hammer engaging an abutment on the breechblock (e.g., Remington No. 4 and Winchester model 1887).

Semiautomatic Action – A repeating firearm that requires a separate pull of the trigger for each shot fired, and which uses the energy of discharge to perform a portion of the operating or firing cycle. Also known as an autoloader or self-loader.

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Sidelock Action – A design in which the firing mechanism is attached to a sideplate rather than being integral with the frame.

Single Action – An action requiring the manual cocking of the hammer or striker. Sufficient pressure on the trigger then releases the firing mechanism.

Single Shot Action – A firearm with no means in the mechanism for storing or loading more than a single cartridge housed in the chamber of the barrel.

Slide Action – An action which features a movable forearm that is manually actuated in a motion parallel to the barrel by the shooter. Forearm motion is transmitted to a breech bolt assembly which performs all the functions of the firing cycle assigned to it by the design. Also known as pump action.

Tip-Up Action – Refer to **Hinged Frame Action**.

Top Break Action – Refer to **Hinged Frame Action**.

Trap Door Action – An action in which a top hinged breechblock pivots up and forward to open. Locking on this action is accomplished by a cam located at the rear of the breechblock that fits into a mating recess (e.g., 1873 Springfield). Also known as a **cam lock.**

Under-Lever Action– Refer to **Hinged Frame Action**.

Achromatic Lens

A lens made up of a combination of glasses having different focal powers so that the image formed is free of unwanted colors.

Action Bar

A member or members designed to have considerable rigidity, which are used to connect and thus transmit the movement of the forearm or gas system to the breechblock. In many designs, the movement of the action bar(s) controls or actuates other parts of the mechanism.

Actuator

Part of the firing mechanism in certain automatic firearms which slides forward and back in preparing each cartridge to be fired. Component operated either manually or mechanically which transmits certain action or energy to other components which are dependent upon movement of the actuator in order to function. Also known as a trigger actuator.

Adapter

A device used to alter the use or function of a firearm. The most general use of an adapter permits using smaller caliber ammunition in a firearm designed for a larger caliber. May also be referred to as an **auxiliary cartridge**, **auxiliary chamber,** or **subcaliber device**.

Adapter, Grip

Refer to **Grip Adapter**.

Aim

The act of aligning the sights of a firearm on a target.

Aiming Point

A point on the target upon which the sights are aligned.

RETIRED

Air Gun

A gun that uses compressed air or gas (carbon dioxide) to propel a projectile. The majority of projectiles in the U.S. are 17 to 22 caliber and are either round or cup-shaped lead "pellets", however larger caliber air rifles are being manufactured (up to 50 caliber), which are used for hunting and competition. True air guns should not be confused with guns that use spring force to propel projectiles (commonly known as BB guns). Also known as an air rifle**,** pellet rifle, or pellet gun.

Air Resistance

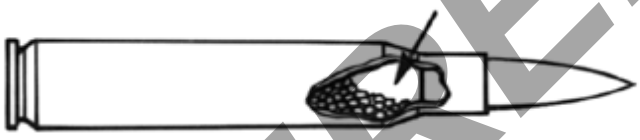
The force placed on an object by the atmosphere in opposition to forward motion. Also known as aerodynamic drag.

Air Rifle

Refer to **Air Gun**.

Air Space

The volume in a loaded cartridge not occupied by the propellant, bullet, wads, or shot. Also known as ullage.



RETIRED

Alloy

A mixture of two or more metals fused or melted together to form a new material. For example, brass is an alloy composed of copper and zinc.

Altitude Effect

The effect on velocity, and therefore projectile trajectory and shot pattern, caused by changes in atmospheric density due to altitude.

Ammunition

One or more loaded cartridges consisting of a primed cartridge case, propellant, and with or without one or more projectiles. Also referred to as **fixed ammunition** or live ammunition (slang term).

Ammunition, Ball

Refer to **Ball Ammunition**.

Ammunition Code Number

A code number and/or letter(s) usually found on the carton that identifies a particular quantity of ammunition for its manufacturer.

Ammunition Color Code

A method of distinguishing the various gauges of shotshells and types of metallic ammunition by color or plating.

Ammunition Lot

Typically refers to a single production run of ammunition and is designed with a code made up of numerical and/or alphabetical digits. Both a military and commercial ammunition term.

Ammunition, Match
Refer to **Match Ammunition**.

Ammunition, Metallic
Refer to **Metallic Ammunition**.

Ammunition, National Match
Refer to **National Match Ammunition**.

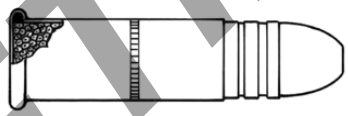
Ammunition, Reference
Refer to **Reference Ammunition**.

Ammunition, Small Arms
Refer to **Small Arms Ammunition**.

Ammunition Standards
(1) A collection and cataloging of both cartridges and ammunition components utilized by the firearm examiner; may include both unfired and fired ammunition components. Also known as **reference ammunition, fired standards,** or **known standards.**

(2) Ammunition used by test ranges to evaluate test barrels, ranges, and other velocity and pressure measuring equipment. May also be used as a control sample by which other characteristics are compared, such as accuracy, patterns, etc. Also known as **reference ammunition**.

Annular Rim
A circular rim at the rear of the cartridge containing a primer compound.



Annulus
The ring-like space between the top of the primer and the primer pocket or battery cup in the base of the cartridge.

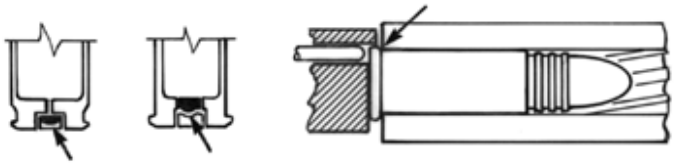
Antimony (Sb)
A metallic element used to alloy lead in order to increase hardness.

Antimony Sulfide (Sb_2S_3)
A component of most common priming mixtures that serves as a fuel.

Anvil
(1) In cartridge case with a boxer primer assembly, the anvil is an internal metal component against which the priming mixture is crushed by the firing pin blow.

(2) In a Berdan primed cartridge case, a metal component in the primer pocket against which the priming mixture is crushed by the firing pin blow.

(3) In rimfire firearms, the breech end of the chamber.



RETIRED

Anvil Marks

Microscopic marks impressed on the forward face of the rim of a rimfire cartridge case as it is forced against the breech end of the chamber by the firing pin. These marks are characteristic of the breech under the firing pin and have been used to identify a cartridge case to a specific firearm.

Armor Piercing Bullet

A bullet consisting of a hardened core or wholly composed of a substance other than lead or lead alloy.

Assault Rifle

A compact, select-fire, detachable box magazine-fed firearm, that utilizes a centerfire rifle cartridge and is designed primarily for military use.

Assault Weapon

Slang term to describe any firearm with a military appearance or large magazine capacity.

Autoloader

Refer to **Action – Semiautomatic Action**.

Automatic

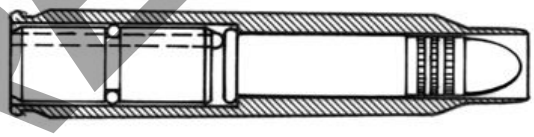
Refer to **Action –Automatic Action**.

Automatic Safety

Refer to **Safety, Automatic**.

Auxiliary Chamber

An adaptation to a firearm to enable the firing of smaller or lower-powered ammunition. Sometimes called an **adapter, auxiliary cartridge,** or **subcaliber device.**



Axial Engraving

Striations or contours on a fired bullet that run parallel or approximately parallel to the axis of the bullet. There are several possible types of axial engravings, including, but not limited to marks left by or due to: 1) the mouth of the cartridge case; 2) the chamber throat; 3) irregularities or burrs along the forward edge of a chamber in a revolver cylinder; 4) inconsistent rotation of a bullet as it moves down the bore; 5) misalignment of a revolver chamber with the axis of the bore (also known as forcing cone marks, misalignment marks, or out-of-time marks); and 6) the rifling, prior to engagement with and rotation of the bullet (also known as **slippage marks**, skid marks, or jump marks).

Axis

A real or an imaginary straight reference line passing through an object and about which the object turns or appears to turn.

RETIRED

Axis of Bore
A line through the center of the bore.

Axis Pin
Refer to **Center Pin**.

Backstop
A structure intended to safely stop a fired bullet or other projectile(s).

Backstrap
The exposed metal strip at the rear of a pistol or revolver grip.

Backthrust
The force exerted on the breechblock by the head of the cartridge case during propellant burning.

Ball Ammunition
A term generally used by the military for a cartridge with a full metal jacketed bullet or solid metal projectile. Refer to **Bullet – Full Metal Jacket Bullet**.

Ball Burnishing
The smoothing of the tops of the lands of a rifled barrel by the forced passage of a hardened steel ball of appropriate diameter. Also known as ballizing.

Ball Cartridge
Refer to **Ball Ammunition**.

Balled Shot
Refer to **Shot – Balled Shot**.

Ball, Frangible
Refer to **Bullet – Frangible Bullet**.

Ballistic Gelatin
A medium designed to simulate living soft tissue for use in wound ballistics studies of projectile behavior. The most common studies involve penetration depth and projectile deformation or expansion.

Ballistics
The science of projectiles in motion. Usually divided into three parts: 1) Interior Ballistics, which studies the projectile's movement inside the firearm; 2) Exterior Ballistics, which studies the projectile's movement between the muzzle and the target; and 3) Terminal Ballistics, which studies the effect of a projectile's impact at the target. A term often confused with Forensic Firearms Identification.

RETIRED

Ballistics, Exterior

The branch of ballistics which studies the motion of a projectile from the muzzle of a firearm to the time it impacts the target.

Ballistics, Interior

The branch of ballistics dealing with all aspects of the combustion phenomena occurring within a firearm, including pressure development and motion of the projectile along the bore of the firearm. Also known as internal ballistics.

Ballistic Soap

A glycerin–type soap specially designed to simulate muscle tissue for use in wound ballistics studies of projectile behavior. The most common studies involve penetration depth and projectile deformation or expansion.

Ballistics, Terminal

The branch of ballistics which deals with the effects of a projectile's impact at the target.

Ballistic Table

A table of factors relating to the flight of a given projectile. Usually includes, but is not limited to, velocity and energy at various distances from the muzzle.

Ball, Patched

Refer to **Patched Ball**.

Ball Screw

A pointed, spiral piece of metal secured at the end of a ramrod and used to remove a lead ball or patch from a muzzle loading gun. Also known as a ball or bullet puller.

Ball Seat

Refer to **Chamber Throat**.

Balloting (Bullet)

A bullet "ballots" when it moves through the bore of a firearm with a bumping, buffeting action. Balloting is a yawing motion of a bullet while traveling through the bore, resulting in incomplete, intermittent rifling impressions often extending onto the ogive of the bullet.

Band

Refer to **Bearing Surface**.

Barium Nitrate

A component of most priming mixtures which acts as an oxidizer of the particular fuel in such mixtures.

Barrel

That part of a firearm through which a projectile or shot charge travels under the impetus of powder gasses, compressed air, or other like means. A barrel may be rifled or smooth. The following are various barrel types:

Bulged Barrel – A barrel fired while containing an obstruction. The resultant excessive radial pressure causes a bulge in the barrel which may or may not be circumferential. Also known as a ringed barrel.

RETIRED

Damascus Barrel – A barrel formed by twisting or braiding together steel and iron wires or bars. The resulting cable is then wound around a mandrel and forged into a barrel tube. An obsolete barrel type. Also known as a laminated barrel.

Fluted Barrel – A barrel with longitudinal grooves cut into the outside surface for all or some portion of the overall barrel length.

Interchangeable Barrel – A barrel which may be readily installed on and removed from a particular action in the field.

Laminated Barrel – Refer to **Damascus Barrel**.

Pressure Barrel – Refer to **Test Barrel**.

Ringed Barrel – Refer to **Bulged Barrel**.

Rotating Barrel – A locking system in which the barrel, slide, and receiver are locked together by helically cut lugs. When the firearm is fired, the lugs cause the barrel to rotate, freeing the slide to move rearward (e.g., 9mm Steyr M12 pistol (Steyr-Hahn)).

Sleeved Barrel – A type of barrel construction which has an outer sleeve and an inner liner (e.g., Dan Wesson revolvers and Clerke 1st). Refer to **Barrel Liner** and **Sleeve**.

Test Barrel – A barrel produced and fitted with instrumentation to measure or evaluate breech or chamber pressure, velocity, and/or accuracy.

Barrel Arrangement

The relationship to each other, in which multiple barrel systems are positioned in forearms (e.g., over and under, side-by-side, etc.).

Barrel Assembly

A barrel of a firearm, either fixed or interchangeable, that has been fitted with necessary parts so that it may be assembled to the remainder of the firearm.

Barrel Band

A strip or strips of metal that encircle and hold the barrel and stock, forend, magazine, or other accessories together.

Barrel Blank

An unfinished barrel in any state of completion.

Barrel Broaching

Refer to **Barrel Manufacturing Techniques** in the **Manufacturing and Machining Terminology Section** of the appendix.

RETIRED

Barrel Catch

The catch on hinged frame firearms that locks the barrel in the closed position (e.g., Smith & Wesson New Departure, Beretta 950B and Stevens Tip Up pistols).

Barrel Channel

A groove in a stock or forend in which the barrel fits when the firearm is assembled.

Barrel Corrosion

Degradation of bore and chamber surface condition due to chemical reaction.

Barrel Diameter

The outside dimension of a barrel at any given point.

Barrel Erosion

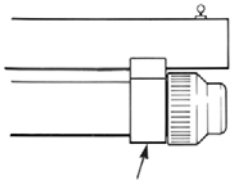
The wearing or physical deterioration of the bore of a firearm caused by hot powder gases or projectile passage.

Barrel Extension

A metal projection which extends rearward from the breech end of the barrel into which the breech locks while the firearm is in battery or firing position (e.g., Remington, Model 870).

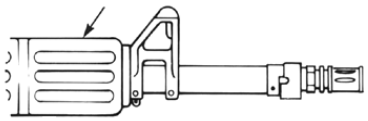
Barrel Guide

A ring shaped attachment on the barrel of many shotguns and rifles which encircles the magazine tube.



Barrel Jacket

A sleeve that surrounds the barrel. On certain firearms, the recoil spring is within the barrel jacket and is thus protected. Barrel jackets may be found on some machine guns to protect the shooter's hands from being burned by the hot barrel (e.g., Remington Model 8 and many sub-machine guns).



Barrel Latch

Refer to **Barrel Catch**.

RETIRED

Barrel Length

The distance between the muzzle end of the barrel and the face of the closed breechblock or bolt. On revolvers, it is the overall length of the barrel including the threaded portion within the frame. Barrel length normally should include compensators, flash hiders, etc., if permanently affixed.

Barrel Life

Refers to the total number of rounds that have been fired in a barrel or the total number of rounds fired in a barrel before it becomes unserviceable.

Barrel Liner

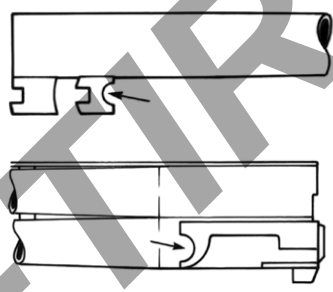
An insert of special material placed in a barrel to reduce bore erosion, renew an eroded bore, alter bore diameter, or to strengthen the barrel. Refer to **Barrel – Sleeved Barrel** and **Sleeve**.

Barrel Lock

Refer to **Barrel Catch**.

Barrel Lug

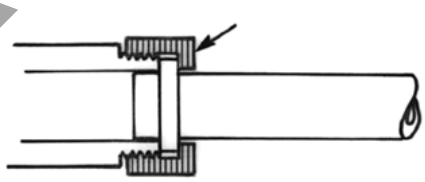
A general term for any projection extending at right angles to the barrel. One of several types of projections located on the underside of the barrel or barrels on hinged frame firearms which serves to secure the barrel to the action or receiver, is used for locking, and aids in barrel positioning in the frame. Also known as **lumps**.



RETIRED

Barrel Nut

A large nut which fits over the barrel, engages a shoulder on the barrel, and threads into the receiver to hold the barrel in place. Examples are on the Savage 110 and on the German MP40 sub-machine gun.



Barrel Obstruction

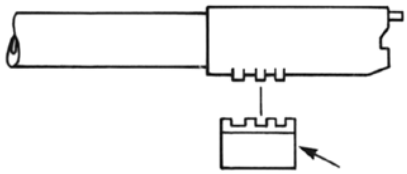
Any foreign object or lodged bullet in the bore of a barrel which prevents unhindered passage of projectile(s) when fired. Also known as a bore obstruction.

Barrel Reflector

Refer to **Borescope**.

Barrel Retainer

A grooved piece that mates with corresponding grooves on the barrels of certain firearms which serves to keep the barrels in the firearm (e.g., Czech Model P27 pistol).



Barrel Shank

The breech end of the barrel that fits into the action or receiver.

Barrel Step

An abrupt change in the exterior of a barrel's contour or diameter.

Barrel Straightening

The factory straightening or re-straightening of a barrel to remove bends induced during machining operations.

Barrel Swaging

The process of forming the interior and/or exterior shape of the barrel of a firearm by pneumatic or hydraulic hammering around a mandrel. Also known as **hammer forging**.

Barrel Threads

The screw threads found on the muzzle end or shank of the barrel that are used to screw attachments onto the muzzle end of the barrel or to screw the barrel into the frame or receiver of the firearm, respectively.

Barrel Time

The elapsed time from the contact of a firing pin with a cartridge primer to the emergence of the projectile(s) from the muzzle of the firearm.

Barrel Vent

Refer to **Gas Port**.

Barrel, Walking

A gun barrel that changes its center-of-impact point when heated by firing.

Barrel Weight

A separate weight attached to a regular barrel to change balance. A term for the size designation of barrel diameter (e.g., Winchester High Wall number 3 barrel).

Barrel Whip

The periodic movement of the barrel as the projectile passes through it.

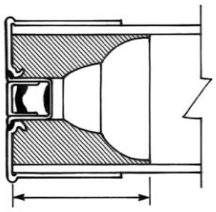
Base

(1) The rear portion of a bullet that is opposite the nose. Also known as the **heel**.
(2) That portion of a cartridge case which contains the primer (more commonly known as the **head**) or the filler material which constitutes the lowest or base wad in a shotshell.

RETIRED

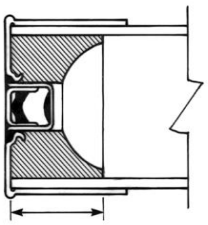
Base, High

Refers to the height of a relatively tall internal base wad within a shotshell.



Base, Low

Refers to the height of a relatively low internal base wad within a shotshell.

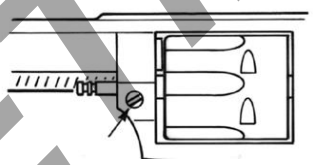


Base Pin

Refer to **Center Pin**.

Base Pin Screw

The screw that retains the base pin or cylinder pin. Found on revolvers like the early Colt "Frontier" revolvers.



Base, Split

Refer to **Split Base**.

Base Wad

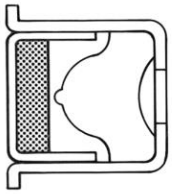
A cylindrical component that is assembled into the head of a shotshell.

Battery

The condition in a firearm action in which the bolt or breechblock is solidly secured in a fixed relationship with the chamber so as to resist being blown back by chamber pressure. Refer to **In Battery** and **Out of Battery**.

Battery Cup

A flanged metallic cup used in shotshell primer assemblies that provides a rigid support for the primer cup and anvil. Also known as a battery pocket.



RETIRED

BB

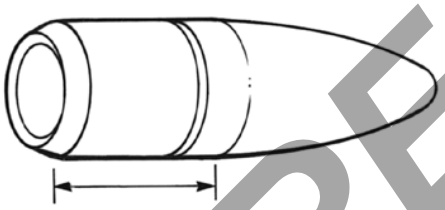
The designation of spherical shot having a diameter of .180" used in shotshell loads. The term BB is also used to designate steel or lead air gun shot of .175" diameter. Although the two definitions cause some confusion, they have co-existed for many years.

BB Cap

The abbreviation of bulleted breech cap. The original design in 1845 was a percussion cap without a well-defined head loaded with a 22 caliber ball. A rimfire cartridge designed for use in Flobert rifles for indoor use.

Bearing Surface

That portion of a bullet's outer surface that comes into direct contact with the interior surface of the barrel. Also known as the band.



Beavertail

(1) The forend or forearm of a shotgun or rifle that is made wider than a standard forend or forearm [21].

(2) The wider than normal version of the M1911A1 grip safety.

Bedding

Refers to the fit or the fitting of the parts of the barrel and receiver with the stock.

Belted Cartridge Case

Refer to **Cartridge – Belted Cartridge**.

Bench Block

Usually hardened steel or nylon with holes and channels cut into it that facilitate the disassembly of a firearm when used in conjunction with drifts and hammers.

Benchrest

A table specifically designed to eliminate as much human error as possible by supporting a rifle for competitive shooting or for sighting-in purposes.

Bent

A notch typically located in the hammer, in which the sear or trigger is held under tension of the mainspring until released by movement of the trigger. Also known as the **sear notch**.

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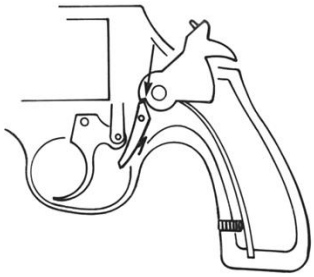


Berdan Primer

Refer to **Primer – Berdan Primer**.

Best Known Non-matching Agreement in Toolmarks

The greatest individual corresponding agreement between two toolmarks known to have been created by different tools that has either been personally observed by trained examiners or has been observed by others in the profession by rigorous studies.

Bird Shot

Refer to **Shot – Bird Shot**.

Black Powder

The earliest form of propellant, reputed to have been made by the Chinese or Hindus before the remote beginnings of history. First used for guns in the 13th century. It is a mechanical mixture of potassium or sodium nitrate ("saltpeter"), charcoal, and sulfur with the most common proportions being 75:15:10. For sporting arm use, various granulations are available. These are designated f_g, ff_g, fff_g, and $ffff_g$, largest to smallest, respectively [14].

Blank

A cartridge without a projectile designed to make noise. Blanks may also be used to launch rifle grenades.

Blish Lock

A delayed blowback breech lock designed by John Bell Blish, Commander, USNR in 1915. This system incorporated a phosphor bronze "H" shaped piece that rode in slots within the actuator and bolt on Thompson sub-machine guns. The purpose of this piece was to absorb the force produced by the gases, and due to the difference in angles, the piece was forced upwards and only slightly back. The theory was that the greater the force, the more the "H" piece jammed. The dissimilar metals were supposed to adhere while under pressure. At lower pressures, it moved freely to allow rearward movement of the bolt [15].

Block, Loading

Refer to **Loading Block**.

Block, Locking

Refer to **Locking Block**.

Blossoming

A deposit of white or yellow powder, usually forming around the juncture of a barrel and its receiver, on a newly blued firearm. It is caused by incomplete rinsing after bluing and can be removed by wiping off with a rag and then oiling the juncture. Also known as efflorescence.

Blowback

RETIRED

(1) In ammunition, a leakage of gas rearward between the case and the chamber wall from the mouth of the case.
(2) In firearms, a design found in semiautomatic and automatic firearms where the weight of the bolt, the inertia of some component, and/or a mechanical delay is the main locking force and no mechanical locking of the breech occurs. These firearms directly utilize the breech pressure exerted on the head of the cartridge case to actuate the mechanism. Refer to **Blowback, Delayed** and **Blowback, Simple**.

Blowback, Delayed

A system in which the breechblock is not locked but some mechanical delay is incorporated to ensure that the breechblock cannot move back with sufficient rapidity to allow the unsupported case to emerge from the chamber while the pressure is still high. Also known as retarded blowback.

Blowback, Simple

This system, in its simplest form, allows for a totally unlocked breech and relies simply on the mass of the breechblock and the strength of the return spring to prevent the cartridge case from emerging from the chamber during its period of peak pressure (e.g., 22 rimfire semiautomatic rifles or pistols).

Blow-by

The escaping of gases past the bullet, while the bullet is in the barrel. May also be used in reference to leakage of gas around a bullet fired through the baffles in a suppressor. Refer to **Blow-by** in the Suppressor Terminology section. Blow-by is often used erroneously as a slang term for blowback. Refer to **Gas Cutting**.

Blow Forward

An action design found in some semiautomatic pistols in which the breech is stationary and the barrel moves forward when fired to open the action and eject the fired cartridge case (e.g., Schwarzlose, model 1908, 32 Auto Pistol).

Blow, Light

Used to describe insufficient firing pin energy or protrusion. The result is erratic ignition or failure to ignite the primer.

Blown Pattern

A shot pattern, usually with a low percentage of pellets and of erratic distribution. Typically caused by pellets fired in a rifled barrel.

Blown Primer

Refer to **Primer – Blown Primer**.

Blue Pill

A slang term given to a cartridge loaded to specified pressures higher than service loads to test firearm barrels during manufacture, but before assembly. Also known as a provisional proof cartridge. Refer to **Cartridge – Proof Cartridge.**

Boattail Bullet

Refer to **Bullet - Boattail Bullet**.

Body (Case)

RETIRED

(1) The metallic portion of the cartridge case that contains the propellant, primer and projectile(s).
(2) The tubular section of a shotshell that contains the propellant, wads, and projectile(s).

Bolt

The locking and cartridge head support mechanism of a firearm that operates in line with the axis of the bore. Also known as the **breech bolt**. In revolvers, bolt is sometimes used to refer to the cylinder stop.

Bolt Action

Refer to **Action – Bolt Action**.

Bolt Body

The main part of the bolt that is typically tubular in shape, has locking lugs on it, and contains the firing pin, firing pin spring, extractor(s), and sometimes the ejector.

Bolt Catch

A device typically found on rifles which lock the bolt in the open position.

Bolt Face

That part of the breechblock or breech bolt which is against the head of the cartridge case or shotshell during feeding and firing. Also known as **breech face**.

Bolt Guides

Grooves or ridges on either the bolt or receiver intended to maintain alignment or prevent over rotation.

Bolt Handle

A protrusion from the bolt normally at right angles to the axis of the bolt which is used to actuate the mechanism manually.

Bolt Head

The forward end of the bolt embodying the bolt face/breech face.

Bolt Notch

One of the machined grooves on the circumference of a revolver cylinder that is engaged by the cylinder stop in order to ensure barrel and chamber alignment. Also known as **cylinder stop notch.**

Bolt Release

A device which allows the bolt to be removed from the firearm.

Bolt Sleeve

RETIRED

A component at the rear end of the bolt which guides the firing pin and supports the firing pin spring in bolt action rifles. Also known as bolt plug.

Bolt Stop

A device which retains the bolt in the firearm during normal operation.

Bolt Stroke

The distance a bolt travels from the "fully open" to the "fully closed" position.

Bolt Throw

The degrees of rotation required to open the bolt.

Bore

The interior of a barrel forward of the chamber.

Bore Axis

A theoretical line through the center of the bore.

Bore Brush

A brush usually having brass or plastic bristles that is used to clean deposits from the bore of a firearm.

Bore Casting

Pouring a special material that has exceptional dimensional stability into the bore or chamber of a firearm in order to make a cast for the study of its physical characteristics.

Bore Diameter

(1) In rifled barrels, it is the distance across the center of the barrel from the top of one land to the top of the opposite land.

(2) In shotguns or muskets, it is the distance across the center of the barrel forward of the chamber but before any restrictive choke or expanded muzzle.

Bore Erosion

The wear on the bore of a firearm caused by the action of the propellant powder and/or the friction of the projectile(s).

Bore Obstruction

Refer to **Barrel Obstruction**.

Borescope

An instrument usually consisting of a tube, mirror, light source, and lens used to examine the bore and/or chamber of a firearm.

Bore Slugging

The process of driving a piece of lead through the bore of a rifled barrel to determine the minimum bore and groove diameters.

Bottleneck Cartridge

Refer to **Cartridge – Bottleneck Cartridge**.

Bow Effect

RETIRED

The flow pattern of abrasive materials in soil, sod, and/or sand around the nose, ogive, and/or bearing surface of a bullet generated during penetration into and ricochet from such materials. This characteristic pattern is uniquely associated with ricochets from soil, sand, or sod that have yielded to the bullet's impact and allowed the bullet to enter into the substrate to some depth before departing the substrate. It is most noticeable on the ogive of the bullet, but may extend back along the bearing surface as well. This type of marking takes its name from the similarity of the flow pattern of water off the bow of a boat.

Boxer Primer

Refer to **Primer – Boxer Primer**.

Box Lock Action

Refer to **Action – Box Lock Action**.

Box Magazine

Refer to **Magazine – Box Magazine**.

Brass

(1) An alloy principally composed of copper and zinc in varying proportions, often used in the manufacture of cartridge cases, primer cups, and bullet jackets.

(2) A slang term sometimes used to refer to fired cartridge cases.

Brass, High

Refer to **Cup, High**.

Brass, Low

Refer to **Cup, Low**.

Brass Washed Bullet

Refer to **Bullet – Brass Washed Bullet**.

Breech

The part of a firearm at the rear of the bore into which ammunition components are inserted.

Breechblock

The block in breech-loading firearms that locks the rear of the barrel against the force of the charge and prevents gases from escaping; also provides support for the head of the cartridge case during firing.

Breech Bolt

A mechanism that operates in line with the axis of the bore which locks and unlocks the breech of a firearm; typically also functions to chamber a cartridge in semiautomatic and automatic firearms.

Breech Face

That part of the breechblock or breech bolt which locks against the rear of the chamber, and is against the head of the cartridge case or shotshell during firing.

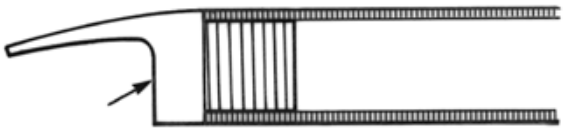
Breech Face Markings

A negative impression of the breech face of the firearm found on the head of the cartridge case and/or primer after firing. These impressed marks are caused by the pressures produced during firing.

Breech Plug

RETIRED

In muzzle loading firearms, the metal piece that is threaded into the breech end of the barrel to seal the breech and usually has an integral upper tang for securing to the stock [21].



Breech Pressure

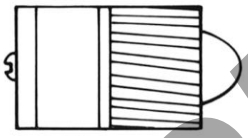
Refer to **Pressure – Chamber Pressure**.

Breech, Standing

Refer to **Standing Breech**.

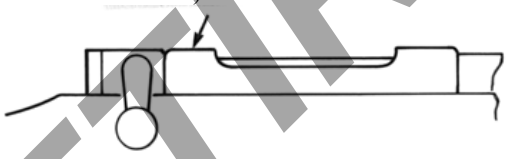
Brenneke Slug

A formed, rifled slug used in shotguns with a wad assembly either pressed into or attached to its base by a screw.



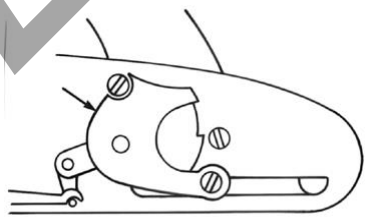
Bridge

A component of a firearm action which usually straddles other parts and acts as a guide or support (e.g., receiver bridge, Model 98 Mauser).



Bridle

A plate found in some firearm mechanisms that holds tumblers, sears, and pins in their proper relationships.



Brinell Hardness Test

A system of hardness measurements commonly used for measuring metals and alloys and to determine the Brinell hardness number (abbreviated BHN) of that metal or alloy.

Broach

Refer to the **Manufacturing Terminology Section**.

Brush Load

Refer to **Load – Scatter Load**.

Buck and Ball

(1) A cartridge with a round ball and shot.

RETIRED

(2) A load that was common for muzzleloaders.

Buckshot

Lead pellets ranging in size from 0.24" to 0.36" in diameter; normally loaded in shotshells.

Buffer

(1) In a firearm, any part intended to absorb shock and check recoil.
(2) In a shotshell, the plastic granular particles that some manufacturers use to prevent the deformation of the shot pellets in the shotshell and to protect the bore when the shot travels down the barrel.

Bulged Barrel

Refer to **Barrel – Bulged Barrel**.

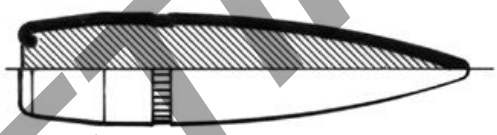
Bullet

A non-spherical projectile for use in a rifled barrel. The following are various bullet types and styles:

Armor Piercing Bullet – (1) A projectile consisting of a hardened core or wholly composed of a substance other than lead or lead alloy. (2) Any projectile manufactured, represented, or designed to be metal or armor piercing.

Bevel Base Bullet – A bullet possessing a beveled edge at its heel. This feature assists the seating of such bullets in the cartridge case at the time of manufacture or during a reloading process.

Boattail Bullet – A specific design of projectile having a tapered or a truncated conical base.



Brass Washed Bullet – A term used for lead projectiles with a thin brass colored coating. Sometimes referred to as golden bullets. This coating has been used on 22 caliber bullets.

Capped Bullet – Consists of a standard lead projectile having a harder metal jacket (gilding metal, copper, etc.) over the nose. Also known as a metal point bullet.

Cast Bullet – A projectile formed by pouring molten lead or lead alloy into a mold.

Coated Bullet – Lead alloy projectile having a thin metal alloy coating. Examples include Remington® Golden™ Bullet and Winchester Lubaloy®. Refer to **Copper Washed Bullet** and **Brass Washed Bullet**.

Conoidal Bullet – A cone shaped projectile. Also known as a conical bullet.

Copper Washed Bullet – A term used for lead projectiles with a thin copper colored coating. This finish is found extensively on 22 caliber bullets.

Disintegrating Bullet – Refer to **Frangible Bullet**.

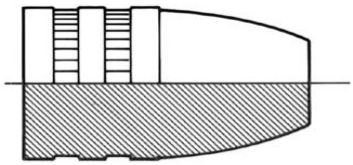
Dumdum Bullet – An obsolete term used to describe an expanding projectile. Derives its name from bullets manufactured at the British Arsenal in Dum Dum, India around 1900.

RETIRED

Expanding Bullet – A projectile designed to increase in diameter upon impact. Examples include hollow point and soft point bullets.

Exploding Bullet – A projectile containing an explosive material designed to detonate upon impact.

Flat-nosed Bullet – A projectile with a flattened tip at right angles to its axis. Refer to **Meplat**.

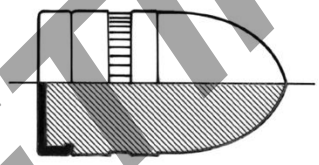


Frangible Bullet – A projectile designed to disintegrate upon impact with a hard surface in order to minimize ricochet. Sometimes referred to as a disintegrating bullet or gallery bullet.

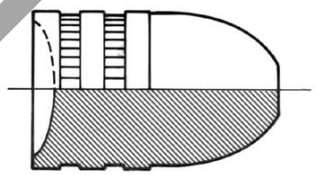
Full Metal Jacket Bullet – A projectile in which the bullet jacket encloses the entire bullet, with the usual exception of the base. Also called full jacketed, full patch, full metal case, metal cased, metal patched, and ball ammunition.

Gallery Bullet – Refer to **Frangible Bullet**.

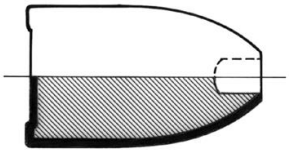
Gas Check Bullet – A lead alloy projectile with a copper or gilding metal cup pressed over the base. This metal cup is used to protect the base of the bullet from deformation due to the hot gases produced during firing.



Hollow Base Bullet – A projectile with a deep base cavity.



Hollow Point Bullet – A projectile with a cavity in the nose to facilitate expansion.



Incendiary Bullet – A projectile containing a chemical compound which ignites upon impact with the intended purpose of starting fires.

Inside Lubricated Bullet – A projectile having cannelures containing lubrication which are covered when seated in the cartridge case.

Jacketed Bullet – A projectile having an inner core typically enveloped by a metallic substance.

RETIRED

Jacketed Hollow Point Bullet – A bullet having a metal jacket enclosing a lead alloy core. The entire bullet is enclosed except for the nose, which has a cavity.

Lead Bullet – A projectile formed from a lead alloy.

Maxi-Ball® Bullet - An elongated, heavy weight muzzle loading bullet usually with a large cannelure to hold lubricant. The Maxi-Ball® (Thompson Center) is designed to be loaded and fired without a patch. Its major diameter, being slightly larger than bore diameter, causes engraving by the rifling upon loading.

Metal Cased Bullet – Refer to **Full Metal Jacket Bullet**.

Metal Cased Hollow Point Bullet – A projectile having a metal jacket enclosing a lead alloy core. The entire bullet is enclosed except for the cavity in the nose. Refer **Hollow Point Bullet**.

Minié Ball Bullet - A conical nosed lead bullet, slightly under bore diameter, incorporating a hollow base, designed to expand into the rifled bore upon firing for gas sealing purposes without the use of a patch.

Mushroomed Bullet – A projectile that has expanded upon impact to a mushroom-like shape.

Open Point Expanding Bullet – Refer to **Hollow Point Bullet**.

Outside Lubricated Bullet – A lead projectile lubricated on the surface not covered by the cartridge case.

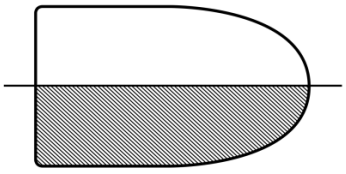
Partition Bullet – A projectile designed for controlled expansion that has a jacket divided into two chambers which encloses the forward and rear cores of the bullet. It is designed so that the first chamber expands and the rear chamber holds together for penetration.

Plated Bullet – A lead core or jacketed bullet that has been covered with a different metallic material. Electrolysis is the typical method used for plating bullets.

Pointed Bullet – A projectile designed with a pointed profile.

Round Ball Bullet – A spherical projectile, usually made of lead or lead alloy.

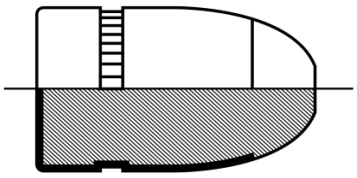
Round Nose Bullet – A projectile with a hemispherical nose.



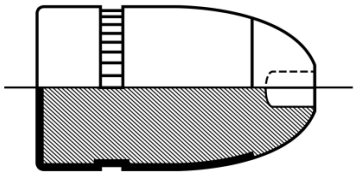
Saboted Bullet – A sub-caliber projectile centered in a lightweight carrier to permit firing the sub-caliber projectile in a larger bore firearm.

Semi-jacketed Bullet – A projectile with a partial jacket and exposed lead nose.

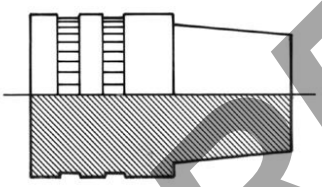
RETIRED



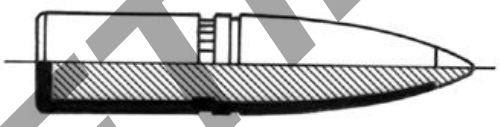
Semi-jacketed Hollow Point Bullet – A projectile with a partial jacket and exposed lead nose with a cavity.



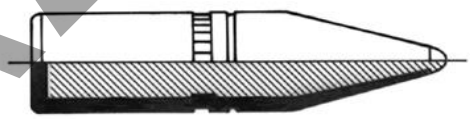
Semi-wadcutter Bullet – A projectile with a distinct, short truncated cone on a cylindrical body with sharp shoulders. Like the **wadcutter bullet**, it is intended to cut target paper cleanly but does not have the feeding issues that may be experienced with a wadcutter bullet.



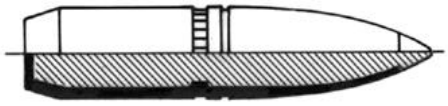
Soft Point Bullet – A design providing for exposure of a portion of the core at the nose of a jacketed projectile.



Spire Point Bullet – A projectile with a conical nose profile.



Spitzer Bullet – A projectile design having a sharp pointed nose, a long ogive, and sometimes a boattail base.



Steel Core Bullet – A jacketed projectile containing a core that is usually composed of mild steel. The steel core is frequently centered or secured inside the jacket with lead.

Steel Jacketed Bullet – A bullet with a jacket made of steel.

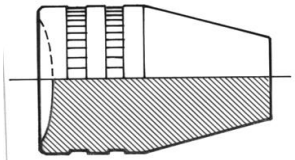
Swaged Bullet – A projectile that has been shaped by compressing and forming the bullet material in a die.

RETIRED

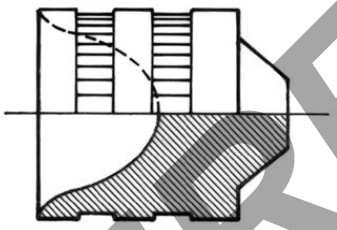
Total Metal Jacket Bullet – A projectile made by copper plating a lead core to create a jacket that encloses the entire bullet. The jacket is much thicker than cosmetic copper plating.

Tracer Bullet – A projectile that has a burning compound in its base which permits observation of its flight.

Truncated Cone Bullet – A design of flat-nosed projectile having a conical rather than rounded profile.



Wadcutter Bullet – A generally cylindrical projectile design having a sharp, shouldered nose intended to cut target paper cleanly to facilitate easy and accurate scoring.



Wax Bullet – A projectile made from paraffin and/or other wax preparations, usually used for short range indoor target shooting.

Wooden Bullet – A projectile made of hollow wood which will disintegrate in the bore or a short distance from the muzzle. Many European blanks are loaded with wooden bullets to facilitate feeding.

Bullet Blackening

The black discoloration of the bullet incidental to firing.

Bullet Casting

A process for making lead bullets by pouring molten metal into a mold.

Bullet Core

The inner portion of a jacketed bullet often made of lead.

Bullet Creep

The movement of a bullet out of the cartridge case as other cartridges are being fired. This phenomenon is due to the recoil of the firearm and the inertia of the bullet. Also known as bullet starting and popping.

Bullet Diameter

The maximum dimension across the largest cylindrical section of a bullet.

Bullet Energy

Refer to **Energy, Bullet.**

Bullet Engraving

RETIRED

Rifling impressions on the surface of a fired bullet.

Bullet Jacket
The envelope enclosing the core of a projectile that is typically of metallic construction.

Bullet Jump
The distance that a bullet must travel from its position at rest in the cartridge case to its initial engagement with the rifling of the barrel. Also known as free travel.

Bullet Jump Marks
Refer to **Axial Engraving** and **Slippage Marks**.

Bullet Mold
A split block of metal having one or more cavities into which molten lead or lead alloy is poured and allowed to harden to form bullets. This process can produce toolmarks on the bullet(s) being molded.

Bullet Penetration
The distance which a bullet travels in a target material.

Bullet Pull
The force required to pull a bullet from the case mouth. Also referred to as **neck tension**.

Bullet Puller
A tool used to remove a bullet from a cartridge. The most common bullet puller employs the inertia principle and is referred to as an inertia bullet puller.

Bullet Push
The force required to shorten a cartridge by pushing on the bullet.

Bullet Recovery System
Any method that will allow the recovery of a fired, undamaged bullet. Different systems are needed for various cartridges depending on bullet composition, jacket thickness, and velocity. Water tanks and cotton boxes are most commonly used.

Bullet Seating Marks
Refer to **Seating Lines**.

Bullet Shearing
The cutting of metal from a bullet due to a cylinder misalignment in a revolver. Also known as shaving.

Bullet Sizing
The process of forming a cast bullet to the proper size by forcing it through a lubricator/sizing tool. This process can produce toolmarks on the bullet.

Bullet Slippage
Refer to **Axial Engraving** and **Slippage Marks**.

Bullet Spin
The rotational motion imparted to a bullet by the rifling in the barrel.

RETIRED

Bullet Splash
The spatter and fragmentation of a bullet upon impacting a hard surface. Also known as lead splash.

Bullet Stabilization
The act of steadying a bullet in flight by use of the proper rifling twist and bullet velocity.

Bullet Stretch
Elongation of the bullet during firing.

Bullet Tipping
Refer to **Bullet Yaw**.

Bullet Trap
A means of safely stopping a bullet in flight when recovery is not the objective. Usually incorporates a steel plate set at an angle to deflect the bullet up or down into a pit or receptacle.

Bullet Upset
(1) In interior ballistics, it is the change of a bullet's form due to chamber pressure.
(2) In exterior ballistics, it is the expansion of a bullet upon impact with target.

Bullet Wobble
Refer to **Bullet Yaw**.

Bullet Yaw
An instability caused by the eccentricity or imbalance of the bullet in flight. Yaw is usually the greatest in the initial portion of its flight in the atmosphere after which the bullet "goes to sleep" and becomes fully spin-stabilized. Yaw may also occur after an elongated bullet strikes an object or when it enters media other than the atmosphere. Also known as bullet tipping or bullet wobble.

Bullseye
The aiming point in target shooting; usually the center of the target.

Burning Rate
A term used to describe the relative quickness of deflagration of a given powder as compared to a known standard. Burning rate is extremely important in determining a powder's suitability for a given cartridge.

Bushing
A removable lining used to reduce the effect of friction on moving parts or to decrease the diameter of a hole.

Butt
(1) In handguns, it is the bottom part of the grip.
(2) In long guns, it is the rear or shoulder end of the stock.

Button
Refer to the **Manufacturing Terminology Section**.

Butt Plate
A metal, rubber, or composition covering to reinforce and protect the shoulder end of a firearm stock.

RETIRED

Butt, Round
A revolver grip with a rounded shape.

Butt Stock
The rear end of a long gun which is normally placed against the shooter's shoulder.

Calcium Silicide
A component of some priming mixtures that serves as a fuel.

Caliber
(1) A term used to designate the specific cartridge for which a firearm is chambered.
(2) In firearms, caliber is the approximate diameter of the circle formed by the tops of the lands of a rifled barrel, typically expressed in hundredths of an inch (38 caliber) or millimeters (9mm caliber).
(3) In ammunition, caliber is a numerical term, without the decimal point, included in a cartridge name to indicate the nominal bullet diameter.

Calipers
A device consisting of two moveable legs or jaws used to measure diameter, thickness, or distance between two surfaces.

Cam Lock
A type of breech mechanism found on certain breechloaders, such as the 1873 Springfield. Locking on this action is accomplished by a cam located at the rear of the breechblock that fits into a mating recess. Refer to **Action – Trap Door Action**.

Cam, Locking
An incline, either helical or straight, to assist in locking the action of a breech mechanism.

Cannelure
A circumferential groove generally of a knurled or plain appearance on a bullet or cartridge case. Three uses of cannelures include crimping, lubrication, and identification.

Canting
The tipping or tilting of a gun to one side at the time it is fired.

Cap
(1) An obsolete term referring to a primer.
(2) Refer to **Percussion Cap**.

Cap and Ball
A phrase used to describe a muzzleloading firearm using the percussion cap ignition system.

Cape Gun
A double barreled shoulder arm with barrels side-by-side; one being smoothbore and the other being rifled.

Cap Flash
Refer to **Primer Flash**.

RETIRED

Capped Bullet

Refer to **Bullet – Capped Bullet**.

Cap, Percussion

Refer to **Percussion Cap**.

Carbine

A rifle of short length and light weight originally designed for mounted troops.

Card Wad

Refer to **Wad – Card Wad**.

Carrier

A transfer mechanism in some firearms that raises and positions the cartridge or shotshell for feeding into the chamber. Also known as a lifter or elevator..

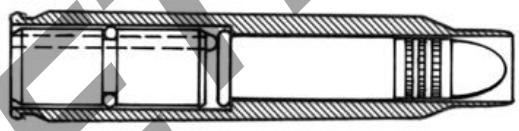
Carrying Strap

A simplified version of a sling used for carrying purposes only.

Cartridge

A single unit of ammunition consisting of the cartridge case, primer, propellant, and with or without one or more projectile(s). Also applies to a shotshell. The following are various types of cartridges:

Auxiliary Cartridge – An adaptation to a firearm that enables the firing of smaller or lower power ammunition. Sometimes called an **adapter**, **subcaliber device**, or **auxiliary chamber**.

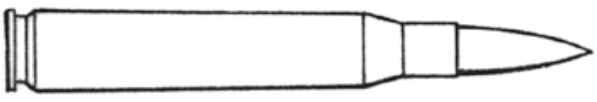


Ball Cartridge – A term generally used by the military for a cartridge with a full metal jacketed bullet or solid metal projectile. Also known as **Ball Ammunition**.

Belted Cartridge – A cartridge with a cartridge case design having an enlarged band or belt at the base ahead of the extractor groove. The belt acts as a case reinforcement and to headspace the cartridge. This construction is generally used on large capacity magnum cartridges.

Blank Cartridge – A cartridge loaded without a projectile that is designed to produce a loud noise.

Bottleneck Cartridge – A cartridge with a cartridge case having distinct angular shoulder stepping down to a smaller diameter at the neck position of the case.



RETIRED

Centerfire Cartridge – Any cartridge that has its primer central to the axis in the head of the cartridge case.

Dummy Cartridge – An inert cartridge which cannot be fired.

Duplex Cartridge – (1) A cartridge containing two projectiles with a single powder charge. (2) A cartridge containing a single projectile with two types of powder.

Express Cartridge – A cartridge that produces greater than standard velocity. The name was derived from cartridges developed in England around 1885.

Industrial Cartridge – Normally a blank cartridge used in various industrial applications, such as stud drivers, brake testing, and dog training devices. May be color coded to designate level of loading (light, medium, heavy, extra heavy, magnum).

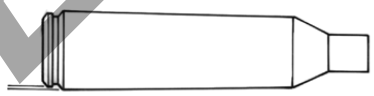
Magnum Cartridge – A term used to describe a rimfire or centerfire cartridge, or shotshell, that is larger, contains more shot, or produces higher velocities than standard cartridges or shotshells of a given caliber or gauge.

Metallic Cartridge – Ammunition having a metallic cartridge case.

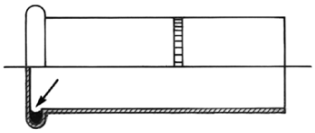
NATO Cartridge – A common designation for military cartridges produced under the specifications of the North Atlantic Treaty Organization (NATO). A common designation for 9mm and 7.62 mm NATO military cartridges.

Proof Cartridge
(1) Definitive – A cartridge loaded to specified pressures higher than service loads. Used only for testing assembled firearms or elements of firearms which contain the primary firing pressure.
(2) Provisional – A cartridge loaded to specified pressures higher than service loads to test firearm barrels during manufacture, but before assembly. Sometimes called a **blue pill**.

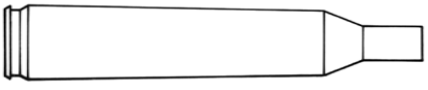
Rebated Cartridge – A centerfire cartridge having a cartridge case whose rim diameter is smaller than the diameter of the body of the case.



Rimfire Cartridge – A flange-headed cartridge containing the priming mixture inside the rim cavity.



Rimless Cartridge – A centerfire cartridge with a cartridge case whose case head is of approximately the same diameter as the body, having a groove forward of the head to provide an extraction surface.



Rimmed Cartridge – A cartridge with a cartridge case having a rimmed or flanged head that is larger in diameter than the body of the case. May be either rimfire or centerfire.

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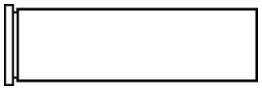
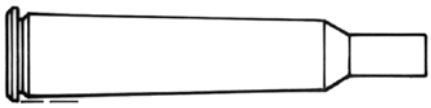


Semi-Rimmed Cartridge – A centerfire cartridge with a cartridge case having a case head only slightly larger in diameter than the body with an extractor groove just forward of the head.



Service Cartridge – Commercially loaded, sporting ammunition intended to meet the full service requirements of the targeted consumer. Typically military and/or departmental issued ammunition. Also known as service load or duty ammo.

Shot Cartridge – A centerfire or rimfire cartridge loaded with small diameter shot. Designed to be fired in firearms other than a shotgun (e.g., CCI 22LR shotshell).

Small Bore Cartridge – General term applied in the United States to 22 caliber rimfire cartridges. Normally used for target shooting and small game hunting.

Tapered Cartridge – A cartridge with a cartridge case in which the diameter is gradually reduced from head to shoulder or mouth.

Triplex Cartridge – A cartridge containing three projectiles.

Wildcat Cartridge – Any cartridge not standardized within the shooting industry or not standardized domestically within the Sporting Arms and Ammunition Manufacturers Institute (SAAMI). These cartridges typically arise from an individual altering existing cartridge cases to suit their own needs and are not mass produced [21].

RETIRED

Cartridge Block

A flat container having blind holes into which cartridges can be inserted in an upright position to be readily available to the shooter.

Cartridge Case

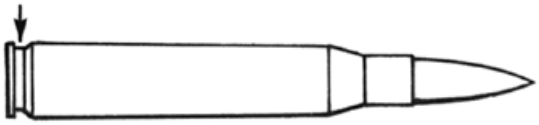
The container for all the other components which comprise a cartridge. Serves as a gas seal during the firing of a cartridge.

Cartridge Case Capacity

The amount by weight, in grains of water, of a particular type of powder that can be inserted in a cartridge case with the bullet fully seated, without compressing the powder charge.

Cartridge Case Extractor Groove

An annular groove cut in rimless, semi-rimmed, or belted cartridge cases forward of the head for the purpose of providing a surface that the extractor may grip to remove the case from the chamber.

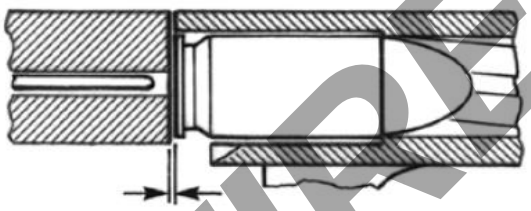


Cartridge Case Head

The base of the cartridge case which contains the primer.

Cartridge Case Head Clearance

The distance between the head of a fully seated cartridge and the face of the breech bolt when the action is locked. Commonly confused with headspace.



Cartridge Case Head Expansion

An enlargement of the head diameter upon firing.

Cartridge Case Head Separation

A 360 degree circumferential rupture of a cartridge case. Also known as cartridge case separation.

Cartridge Case Length

The dimension of a cartridge case from the face of the head to the mouth of the cartridge case.

Cartridge Case Life

An expression of the number of times a cartridge case can be reloaded and fired.

Cartridge Case Mouth

The opening in the cartridge case into which the projectile(s) is seated.

Cartridge Case Mouth Marks

Refer to **Seating Lines**.

Cartridge Case Neck

The reduced diameter, cylindrical portion of a cartridge case that extends from the top of the shoulder to the cartridge case mouth.

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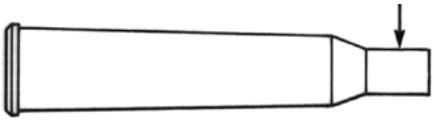


Cartridge Case Rupture

A separation in the side wall of a cartridge case. Also known as **cartridge case split**.

Cartridge Case Separation

Refer to **Cartridge Case Head Separation**.

Cartridge Case Sizing

The reduction in diameter of a cartridge case by forcing it into a die of smaller diameter than the case.

Cartridge Case Shoulder

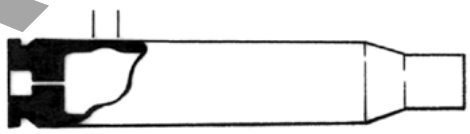
The section of a bottleneck cartridge case connecting the main body of the cartridge case and the smaller diameter neck.

Cartridge Case Split

A longitudinal rupture in the side wall of a cartridge case or shotshell. Also known as **cartridge case rupture**.

Cartridge Case Stretching

The elongation of the body of a cartridge case during firing.



Cartridge Case Trimmer

A tool used to shorten the necks of cartridge cases that have been elongated by repeated firing and reloading.

Cartridge Cook-off

Cook-off occurs when intense heat ignites the gunpowder inside a cartridge and causes it to discharge. This can occur during extreme overheating: (1) in a firearm chamber such as in machine guns, (2) due to fires, (3) without the operation of a firing mechanism.

Cartridge Designation

A term used to designate the specific cartridge for which a firearm is chambered.

Cartridge Designation, Metric

RETIRED

Cartridges that are identified by their nominal bullet diameter and cartridge case length, both of which are given in millimeters (e.g., 9x19mm, 7.62x39mm, 9x18mm, etc.).

Cartridge Guide

A firearm component which acts as a guide for the cartridge while it is being fed from the magazine to the chamber. Also known as cartridge lifter.

Cartridge Indicator

Refer to **Loaded Chamber Indicator**.

Cartridge Iris

A term used in industry to describe the circumferential discoloration around the neck or shoulder of brass cartridge cases as a result of the annealing process.

Cartridge Lifter

Refer to **Cartridge Guide**.

Cartridge Ramp

Refer to **Feed Ramp**.

Cartridge Stop

A mechanical device that is employed in firearms so that only one cartridge or shotgun shell will feed into the carrier, or lifter, with each cycle of the breech mechanism. Also known as a shell latch, **shell stop**, or **cut-off**.

Cartwheel Pattern

Refer to **Doughnut Pattern**.

Case

Refers to cartridge case. The term may be shortened through common usage.

Case Cannelure

One or more circumferential rings around a cartridge case typically used by manufacturers to denote a certain type of load or product line.

Case Capacity

Refer to **Cartridge Case Capacity**.

Case Fire-Forming

Refer to **Fire-Forming**.

Caseless Ammunition

Ammunition that has the propellant charge molded to the base of the bullet and not enclosed in any type of cartridge.

Case Life

Refer to **Cartridge Case Life**.

Case Mouth

Refer to **Cartridge Case Mouth**.

Case Separation

Refer to **Cartridge Case Head Separation**.

RETIRED

Case Split
Refer to **Cartridge Case Split**.

Case Stretching
Refer to **Cartridge Case Stretching**.

Case Taper
The gradual reduction in diameter of a cartridge case from head to shoulder or mouth.

Case Trimmer
Refer to **Cartridge Case Trimmer**.

Cast
The reproduction of a toolmark or the surface of a tool utilizing a molding material such as plaster of Paris, silicone rubber, etc. The cast of a toolmark is a positive case. The cast of the surface of a tool is a negative cast.

Cast Bullet
A bullet formed by pouring molten lead into a mold.

Casting
The process of pouring a liquid or suspension into a mold to produce an object of desired shape.

Cast-Off
The offset of the butt of a firearm to the right for a right-handed shooter with a dominant right eye to keep a straight line of focus on the target without having to tilt the head.

Cast-On
The offset of the butt of a firearm to the left for a left-handed shooter with a dominant left eye to keep a straight line of focus on the target without having to tilt the head.

CB Cap
A low velocity 22 caliber rimfire cartridge having a conical bullet (from which the name is derived) and no propellant other than the priming compound.

Centerfire Cartridge
Refer to **Cartridge – Centerfire Cartridge**.

Center of Impact
The center of a shot pattern or target made by a series of projectiles fired at the same aiming point.

Center Pin
The pin around which the cylinder of a revolver rotates. Also known as axis pin, base pin, and cylinder pin.

Chamber
The rear part of the barrel bore that has been formed to accept a specific cartridge or shotshell. In a revolver, the holes in the cylinder represent multiple chambers.

Chamber, Auxiliary

RETIRED

Refer to **Cartridge – Auxiliary Cartridge**.

Chamber Casting

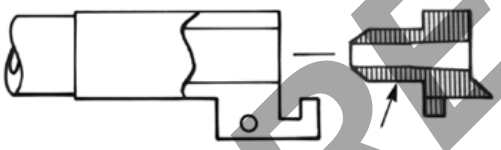
The process of making a cast of the chamber of a firearm to determine the cartridge for which the firearm is designed, or to better observe imperfections within that chamber.

Chamber Erosion

The wearing or physical deterioration of the chamber of a firearm caused by hot powder gases.

Chamber, Floating

A system in which a moveable chamber operates the mechanism of a firearm. For example, the Colt 22 caliber conversion unit for the 1911 Government model pistol.



Chamber, Fluted

A chamber that has grooves cut in the chamber wall to assist or retard cartridge case extraction (e.g., Russian Tokarev semiautomatic rifle and the Heckler and Koch model HK4 pistol).

Chambering

(1) The act of inserting a cartridge into the chamber.
(2) The reaming of a chamber in a barrel blank or cylinder.

Chamber Marks

Individual microscopic marks placed upon a cartridge case by the chamber wall as a result of any or all of the following: (1) chambering, (2) expansion during firing, and/or (3) extraction.

Chamber Pressure

Refer to **Pressure – Chamber Pressure**.

Chamber Reamer

A cutting tool used to form the chamber area of a barrel bore or revolver cylinder. Reaming is typically performed in two steps using a rough reamer and a finish reamer.

Chamber, Roughened

A chamber with walls that are deliberately roughened in a variety of ways to slow down or delay extraction (e.g., 30 Carbine caliber Kimball semiautomatic pistol).

Chamber Throat

That section of the bore of a rifled gun barrel located immediately ahead of the chamber in which the rifling is conically removed to provide clearance for the seated bullet. Also known as ball seat or leade. Refer to **Forcing Cone**.

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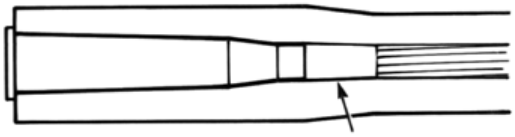

Charger

(1) A metal device which may hold five to ten cartridges used to rapidly load the magazine of a pistol or rifle. In some rifles, clip guides are machined into the receiver to guide the charger into the proper position for magazine loading. The empty charger may either be removed manually or ejected as the bolt closes. Also known as a **stripper clip**. Refer to **En Bloc Clip**.

(2) A term sometimes improperly used to describe removable magazines.

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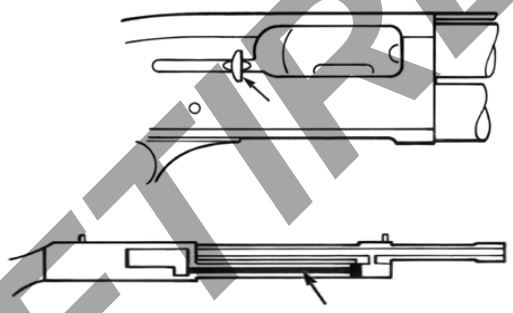
Charging Handle

Handle of semi or full automatic firearms used to cycle the firearm without firing. Also known as cocking handle, cocking knob, and operating handle.



Checkering

A diamond-like pattern in wood, plastic, or metal components of a firearm for improving grip or ornamentation.

Cheekpiece

A raised portion on the side of the buttstock of a long gun where the shooter rests his cheek when aiming.

Chilled Shot

Refer to **Shot – Hard Shot**.

Choke

An interior constriction at or near the muzzle end of a shotgun barrel bore for the purpose of controlling shot dispersion.

Choke, Jug

A type of shotgun choke wherein a slight recess is formed in the bore approximately one inch behind the muzzle. The recess causes the shot to gather before leaving the muzzle resulting in a denser pattern.

Choke Markings, European

Full Choke = *or +
Improved Modified = ** or ++
Modified = *** or +++
Improved Cylinder = **** or ++++
Cylinder = CL or +++++

Choke Markings, United States

Full Choke = *FC, Full* (greatest constriction)
Improved Modified = *Imp. Mod*. (less constriction than full)
Modified = *Mod*. (less constriction than improved modified)
Improved Cylinder = *IC*, *Imp. Cyl*. (less constriction than modified)
Skeet = *Sk* (less constriction than improved cylinder)
Cylinder Bore = *Cyl*. (least constriction or no constriction)
Some firearms' manufacturers in the United States also use the European system.

Choke, Swaged

A shotgun barrel interior constriction which has been formed at or near the muzzle by the use of dies or hammers.

Choke Tube

A replaceable insert in the muzzle of a shotgun barrel to provide the desired amount of choke.

Choke, Variable

An adjustable device attached to the muzzle of a shotgun that allows for control of shot patterns. Also known as adjustable choke.

Choke, Ventilated

A shotgun choke which is slotted for the release of powder gases.

Chronograph

An electronic instrument used to measure and record the velocities of projectiles.

Class Characteristics

Measurable features of a specimen which indicate a restricted group source. They result from design factors and are determined prior to manufacture.

Clip

(1) Refer to **En Bloc Clip**, **Stripper Clip**, and **Charger**.
(2) A misnomer for a detachable magazine.

Clip Guides

(1) Grooves cut in the receiver of certain firearms with fixed magazines to direct the stripper clip used to load the firearm into position (e.g., Mauser 98 rifle).
(2) Devices that can be attached to the top of removable magazines to allow stripper clips to be used to load the magazine (e.g., M-16/AR-15 type rifle).

Coated Bullet

Refer to **Bullet – Coated Bullet**.

Cock

(1) To place a firing mechanism (hammer, firing pin, or striker) in a position for firing.
(2) A firearm part that holds pyrite or flint; the predecessor of the hammer.

Cocking Handle

Refer to **Charging Handle**.

RETIRED

Cocking Indicator

Any device that indicates that a firearm's hammer or striker is cocked.

Cocking Knob

Refer to **Charging Handle**.

Cocking Lever

A mechanical means of cocking the hammer or striker of some firearms using a manually operated, external lever (e.g., Sauer Model 38H pistol).

Cocking Piece

(1) The end of a long firing pin or striker by which the firearm may be cocked manually.
(2) An internal portion of the cocking mechanism which holds the firing pin or striker in its cocked position against the sear.

Cocking Rod

A mechanical means of cocking the hammer of some firearms.

Cocking Stud

A small lug in the cocking mechanism of the firearm.

Coefficient of Form

A numerical term indicating the general profile of a projectile.

Comb

In a long gun, the ridge at the upper forward part of the buttstock just behind the wrist.

Combination Gun

A multiple barreled firearm designed to handle different sizes, calibers, or types of ammunition.

Comparison Microscope

Essentially two microscopes connected to an optical bridge which allows the viewer to observe two objects simultaneously with the same degree of magnification. This instrument can have a monocular or binocular eyepiece. May also be referred to as a comparison macroscope.

Comparison Projector

An instrument which will project a magnified image onto a screen.

Compensator

A device attached to or integral with the muzzle end of the barrel that uses propelling gases to reduce recoil. Also known as **muzzle brake**.

Concentric Fractures

Fractures or cracks in glass or other similar brittle or ceramic material that take a generally circular form around the bullet hole or impact site in such materials.

RETIRED

Coning Effect

The characteristic cone shape on the exit side of a projectile hole through a relatively brittle medium (e.g., glass, bone, etc.) caused by the spalling around the exit. Also known as **cratering**.

Conoidal Bullet

Refer to **Bullet – Conoidal Bullet**.

Consecutive Striae

Parallel, side by side, contour variations within a striated toolmark.

Consecutive Matching Striae (CMS)

Contour variations within two different striated toolmarks, which, when compared microscopically, line up with one another without unexplained dissimilarities.

Contour Variation

Variations in the elevations of the ridges and valleys in striated marks and of forms and shapes or depressions in impression marks.

Cook-Off

Refer to **Cartridge Cook-Off**.

Copper Clad Steel

A steel surface coated with copper by various methods, including alloying, welding, and cold-pressing.

Copper Jacketed Bullet

Refer to **Bullet – Copper Jacketed Bullet**.

Copper Units of Pressure

Relates to firearm discharge pressure measured by a copper crusher testing system. These values provide a means of testing and evaluating peak pressure generated during the discharge of a suitably modified firearm. Abbreviated: **C.U.P.**

Copper Washed Bullet

Refer to **Bullet –Copper Washed Bullet**.

Cordite

An early type of smokeless, double-base propellant, distinguished by its long, cord-like shape.

Core, Bullet

Refer to **Bullet Core**.

Crack Rule

The stopping of the propagation of one or more radial fractures during a projectile's impact in plate glass or other similar material by a fracture from a previous shot.

Crane

The part of a solid frame revolver on which the cylinder is swung out to the side to accomplish loading and ejecting. Also known as **yoke**.

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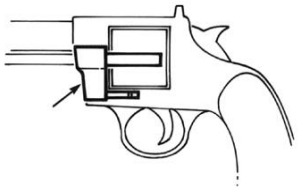


Cratering

The characteristic cone shape on the exit side of a projectile hole through a relatively brittle medium (e.g., glass, bone, etc.) caused by the spalling around the exit. Also known as **coning effect**.

Criminalistics

The application of scientific techniques in collecting and analyzing physical evidence in criminal cases.

Crimp

The inward shaping of the mouth of a cartridge case or shotshell to secure the projectile(s). Crimps are categorized as follows: roll, star or rose, fold, stab, semi-circular, or split ring and possibly others. Crimps may also be used to retain primers in primer pockets.

Crimped Primer

A primer cup which has been staked, stabbed, or otherwise crimped into the primer pocket. Commonly found on military cartridge cases.

Crimping Groove

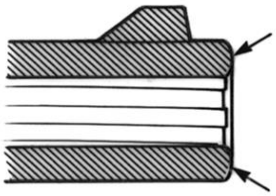
A circumferential groove generally of a knurled or plain appearance on a bullet or cartridge case. Three uses include securing bullets, lubrication, and identification.

Crossbolt

(1) A transverse operating type of lock used on some double barrel or break open firearms.
(2) A form of manual safety which operates transversely to prevent or permit firing of a firearm.

Crown

Any of various forms of muzzle treatment meant primarily to protect the rifling. Includes chamfering of the bore, counterbore, or radius. Also known as muzzle crown.



Crusher Gauge

A mechanical means of measuring and interpolating chamber pressure. This process is accomplished by subjecting a known copper or lead cylinder to the pressure developed during firing and noting the amount of compression of the cylinder that results. Also refer to **Radial Pressure Gauge**, **Lead Units of Pressure**, and **Copper Units of Pressure**.

Cup, High

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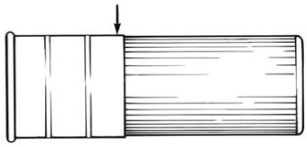
The length of the external metal cup on a shotshell; refers to a cup of high metal head construction. May be incorrectly referred to as high base.



Cup, Low

The length of the external metal cup on a shotshell; refers to a cup of low metal head construction. May be incorrectly referred to as low base.

Cupronickel

An alloy of copper with nickel.

Cup, Shot

Refer to **Shot Cup**.

Cup, Split

Refer to **Split Base**.

Cup Wad

Refer to **Wad – Cup Wad**.

Cut-Off

(1) A manually operated mechanical device which prevents cartridges from being fed from the magazine into the loading mechanism. This device can be used to convert an automatic, semiautomatic, or bolt action firearm to fire single shot.

(2) A mechanical device that is employed in firearms so that only one cartridge or shotgun shell will feed into the carrier, or lifter, with each cycle of the breech mechanism. Also known as **shell stop** or **cartridge stop**.

Cut-Out

(1) The portion of the slide or bolt which has been cast, formed, machined, or drilled out to permit the operation of the extractor.

(2) A hole or slot formed through the breech face of a firearm to permit the operation of the ejector.

Cyclic Rate

The rate at which a succession of movements repeats itself. In an automatic firearm, it is usually expressed in shots per minute that are theoretically possible to be fired, given an unlimited supply of ammunition.

Cycling Marks

A general term for those toolmarks imparted onto a cartridge, cartridge case, or shotshell that has been cycled through the action of a firearm. Examples include ejector marks, extractor marks,

RETIRED

feed ramp marks, magazine lip marks, and ejection port marks. Also known as feed marks or mechanism marks.

Cylinder

The rotating component of a firearm that contains the chambers.

Cylinder Alignment

The relationship of the axis of the chamber in a revolver cylinder to the axis of the bore. Refer to **Indexing**.

Cylinder Axle

The tubular piece upon which the cylinder rotates (e.g., Enfield revolver No. 2).

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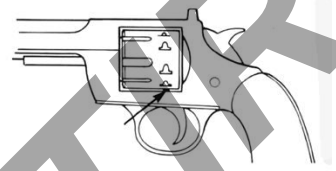
Cylinder Bolt

A device to stop cylinder rotation in proper alignment with the barrel. Also known as a **cylinder stop**.



Cylinder End-Play

The free longitudinal movement of the cylinder in the frame of a firearm. Also known as end shake.

Cylinder Flare

The circular gray-to-black deposit around the front margin of the chamber(s) of a revolver composed of gunshot residues deposited during the firing process. Also known as a **smoke ring**, halo, or simply flare.

Cylinder Frame

The portion of a single action revolver which houses the cylinder, hammer, and trigger group; distinguished from the grip frame (e.g., Ruger Single-Six revolver).

Cylinder Gap

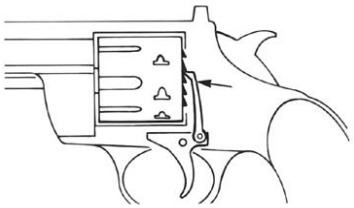
In a revolver, the maximum space between the cylinder and the barrel. This space is typically measured with the cylinder in the rearmost position. The cylinder gap is a source of high-energy gunshot residues with unique reconstructive value. Also known as cylinder-barrel gap.

Cylinder Latch

A device which permits the swinging out or release of the cylinder from the frame of a revolver. Also known as cylinder release latch or bolt.

Cylinder Pawl

The lever attached to the trigger that rotates a revolver cylinder. This action positions the next breech chamber in front of the barrel. Also known as the hand.

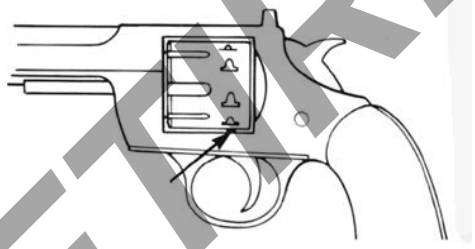


Cylinder Pin

Refer to **Center Pin**.

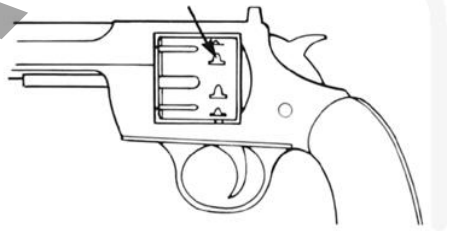
Cylinder Stop

A device to stop cylinder rotation in proper alignment with the barrel of a revolver. Also known as **cylinder bolt**.



Cylinder Stop Notch

One of the machined grooves on the circumference of a revolver cylinder that is engaged by the cylinder stop in order to ensure barrel and chamber alignment. Also known as **bolt notch**.



Damascus Barrel

Refer to **Barrel – Damascus Barrel**.

Decapper or Decapping Die

A tool used to remove primers from cartridge cases.

Deep Hole Drilling

A modern technique for barrel drilling involving rotation of the blank on a non-rotating bit, under high pressure lubrication. The drill bit itself is asymmetrical, cutting one side only. The hole is usually drilled about 5 thousands of an inch under the size of the bore diameter. A reamer is then

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used to bring the hole up in size, leaving a fine finish and a hole of uniform diameter from end to end.

Delayed Blowback

Refer to **Blowback, Delayed**.

Delayed Fire

Any delay in firing of an abnormal duration. This implies that firing does eventually occur. Also known as **Hangfire**.

Demi-Block

A type of double barrel construction in which the two barrels are fastened together at the breech by a vertical dovetail arrangement.

Derringer

The generic term applied to many variations of small one-, two- or even four-shot pistols, using both percussion caps and cartridges. The original designer, Henry Deringer, spelled his name "Deringer", not Derringer.

Detent

A spring loaded lock that engages a notch or indentation.

Detonation

An extremely rapid exothermic decomposition reaction which proceeds at a rate greater than the speed of sound within the reacting material (unlike deflagration). The normal mode of initiation is shock (such as a blasting cap or high level mechanical shock), or from initial combustion which, due to peculiarities of confinement or other circumstances accelerates to such a degree that a shock wave is formed. Behind the shock wave, is a reaction zone where material is converted to gaseous products at high temperature and pressure. The flow of the reaction is in the same direction as the propagation.

Discharge

To cause a firearm to fire.

Disconnector

A device intended to disengage the sear from the trigger. In a semiautomatic firearm it is intended to prevent full automatic firing. In a manually operated firearm, it is intended to prevent firing without pulling the trigger.

Disintegrating Bullet

Refer to **Bullet – Frangible Bullet**.

Distance Determination

The process of determining the distance from the firearm, usually the muzzle, to the target based upon patterns of gunpowder or gunshot residues deposited upon that target. Where multiple projectiles, such as shot, have been fired, the spread of those projectiles is also indicative of distance.

Dog, Carrier

A component in certain repeating action firearms which engages or is attached to the carrier or lifter and acts to control its motion. Also known as the lifter pawl.

Doll's Head

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Usually a round extension of the top of the barrel or rib of a shotgun which fits into a matching hole in the top of the standing breech.

Double Action

Refer to **Action – Double Action**.

Double-Barrel

Two barrels in a firearm mounted to one frame. May be vertically (over-under) or horizontally (side-by-side) aligned.

Doubling

Unintentional firing of a second shot that typically occurs through a malfunction of the firearm.

Doughnut Pattern

A pattern with a central area having no pellet hits. May also be referred to as a cartwheel pattern.

Downloading

An intentional reduction in propellant resulting in a cartridge that may produce less than standard chamber pressure while producing less than standard velocity for that specific cartridge.

Drag Mark

Refer to **Firing Pin Drag Mark** or **Slide Drag Mark**.

Dram Equivalent

The accepted method of correlating relative velocities of shotshells loaded with smokeless propellant to shotshells loaded with black powder. The reference black powder load chosen was a 3 dram charge of black powder with 1 $^{1}/_{8}$ oz. of shot and a velocity of 1200 fps. Therefore, a 3 dram equivalent load using smokeless powder would be with 1 $^{1}/_{8}$ oz. of shot having a velocity of 1200 fps or 1 $^{1}/_{4}$ oz. of shot and a velocity of 1165 fps. A 3 $^{1}/_{4}$ dram equivalent load might have 1 $^{1}/_{8}$ oz. of shot and a velocity of 1255 fps. Abbreviated Dram Equiv.

Draw Mark

A longitudinal scratch on a cartridge case caused by foreign material on either the draw punch or die during fabrication.

Drift

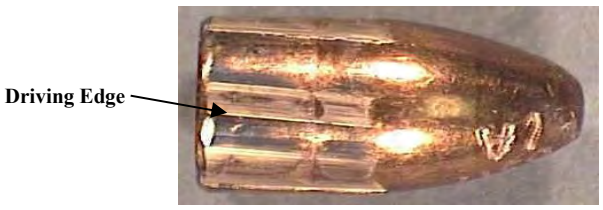
The deviation in flight of a bullet from the center line of the bore due to the gyrational spin of the bullet imparted by the rifling.

Drilling

A three-barreled gun in which a combination of smooth-bore and rifled barrels are used.

Driving Edge

The driving edge of a bullet fired from a gun with a right twist is the right edge of the land impression, or the left edge of the groove impression. The driving edge of a bullet fired from a gun with left twist is the left edge of the land impression or the right edge of the groove impression. Also known as leading edge when used in conjunction with the term following edge. Refer to **Trailing Edge**.



Photograph courtesy of Scott Doyle –http:// www.firearmsid.com

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Drop Fire

The discharge of a loaded firearm as a result of an impact after being dropped. This may be the consequence of a design shortcoming, a compromised safety system, or the failure of the handler to engage the appropriate safety device. Also refer to **Jar Off**.

Dropping Block Action

Refer to **Action – Dropping Block Action**.

Drop Shot

Refer to **Shot – Soft Shot**.

Dry Firing

The releasing of the firing pin on an unloaded chamber of a firearm.

Dumdum Bullet

Refer to **Bullet – Dumdum Bullet**.

Duplex Load

Refer to **Cartridge – Duplex Cartridge**.

Dust Cover

A cover over the magazine opening and/or ejection port of a firearm to prevent the entrance of foreign matter into the action.

Dust Shot

Refer to **Shot – Dust Shot**.

Effective Range

The maximum distance at which a projectile can be expected to be useful in its intended purpose.

Ejection

The process of expelling a cartridge, cartridge case, or shotshell from a firearm.

Ejection Pattern

The charting of where a particular firearm ejects fired cartridge cases or shotshells.

Ejection Port

An opening in the receiver or slide to allow for ejection of a cartridge, cartridge case, or shotshell.

Ejection Port Marks

An indentation or striated mark at one or more locations on a cartridge case (typically found on the sidewall) produced as the result of striking the ejection port during the ejection process. Such marks may be reproducible.

Ejector

A mechanical device of a firearm which expels a cartridge, cartridge case, or shotshell.

Ejector, Automatic

A device found on hinge-framed firearms which expels cartridges, cartridge cases, or shotshells when the action is opened.

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Ejector Housing

Refer to **Ejector Tube**.

Ejector Marks

Toolmarks produced by the ejector on the head of a cartridge case or shotshell.

Ejector Rod

A rod found on revolvers which is pushed rearward to facilitate extraction/ejection of cartridge cases or shotshells from a cylinder. This rod may be attached to the cylinder and provide simultaneous extraction/ejection of all cartridge cases, or housed in an ejector tube and individually extract/eject cartridge cases. Also known as rod ejector or extractor rod.

Ejector Tube

A tube attached to the underside of the barrel of some single action revolvers which contains and guides the ejector rod. Also known as the ejector housing (e.g., Colt Single Action Army).

Elevation

The term used to designate the vertical movement of an adjustable sight to cause the fired bullet to strike the point of aim at various ranges.

En Bloc Clip

A clip that holds cartridges either in a single column or in two staggered columns. The clip is inserted directly into the magazine well of certain firearms and is necessary for the firearm to function. The en bloc clip is ejected after the last cartridge is fired (e.g., U.S. M1 Garand rifles and Italian Carcano rifles and carbines).

Energy, Bullet

The capacity of a projectile to do work in flight. The units are typically expressed as foot-pounds, joules, or kilogram-meters. Also known as projectile energy.

Energy, Muzzle

The energy of a fired projectile(s) measured at the muzzle of a firearm.

Energy, Terminal

The energy of a projectile(s) measured at the point of impact.

Escutcheon

(1) A metal or plastic reinforcement around a hole.
(2) A metal part which displays a name or trademark.

Expanding Bullet

Refer to **Bullet – Expanding Bullet**.

Exploding Bullet

Refer to **Bullet – Exploding Bullet**.

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Explosion

The sudden release of a sufficient amount of energy to create a pressure wave. The energy to produce an explosion may come from a variety of sources including nuclear energy, pressure, or chemical reaction. Pressure related explosions include a rapid change in state (i.e., liquid to gas) or the over pressurization of a container (i.e., the failure of a gas cylinder). A chemical explosion is an event in which a quantity of matter is instantaneously converted to gaseous product with the generation of high temperature and pressure.

Explosive

Any substance that violently changes to a gaseous form with accompanying heat and pressure through a chemical reaction. A low explosive (e.g., smokeless powder) deflagrates relatively slowly whereas a high explosive (e.g., TNT) detonates almost instantaneously producing a shattering effect.

Express Cartridge

A cartridge that produces greater than standard velocity.

Exterior Ballistics

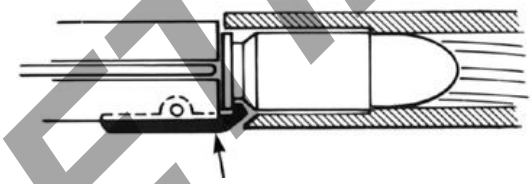
Refer to **Ballistics, Exterior**.

Extraction

The process of removing a cartridge, cartridge case, or shotshell from the chamber of a firearm.

Extractor

A component of a firearm which is designed to remove the cartridge, cartridge case, or shotshell from the chamber of the firearm.

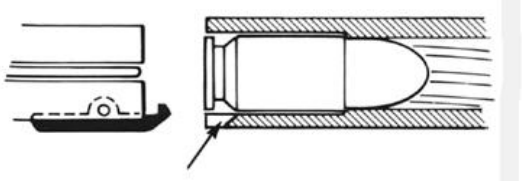


Extractor Cam

A disc shaped piece with a hook on its outer diameter which pivots on the joint pivot screw and impinges directly on the extractor post to push out the extractor when opening top break revolvers (e.g., Smith & Wesson New Departure).

Extractor Cut

Area cut in the rear of the chamber of a firearm that accommodates the extractor. Also known as **Cut-out**.



Extractor Groove

An annular groove cut in rimless, semi-rimmed, or belted cartridge cases forward of the head for the purpose of providing a surface that the extractor may grip to remove the case from the chamber.

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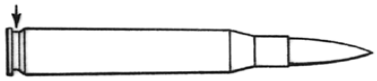
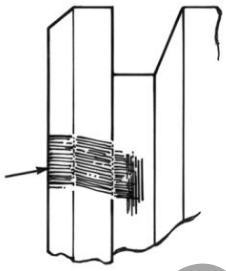


Extractor Hook

The hook on the forward end of the extractor which engages the extractor groove for removing a cartridge, cartridge case, or shotshell from the chamber of a firearm.

Extractor Mark

Impressed or striated toolmarks produced on a cartridge, cartridge case, or shotshell as a result of contact with the extractor. These marks are usually found on the outer edge or just ahead of the rim, the underside of the rim, or in the extractor groove.



Extreme Range

Refer to **Maximum Range**.

Evepiece

The lens or combination of lenses nearest the eye of the viewer in an optical instrument that is designed to further magnify the primary image of the objective. Also known as an **ocular**.

Facets (on bullets)

Multiple flat, squarish impressions on the nose and ogive of a bullet that has perforated previously shattered tempered glass. These facets are produced during the bullet's impact with the small, diced pieces of broken glass.

Fail To Fire

A failure of the firearm to discharge after the trigger has been pulled. Also refer to **Misfire**.

Failure To Feed

Refer to **Misfeed**.

Falling Block Action

Refer to **Action – Falling Block Action**.

Far Zero

The second point where the bullet path intersects with the line of sight, or where the point of aim and point of impact coincide.

Feed Marks

Refer to **Cycling Marks**.

Feed Ramp

A sloped surface located at the mouth of the chamber that serves to guide the cartridge as it is fed from the magazine into the chamber. Also known as cartridge ramp or feed throat cartridge guide.

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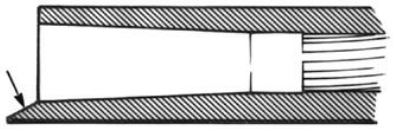

Feet Per Second

A unit of both speed and velocity of a projectile (abbreviated fps). The metric equivalent is meters per second (m/s).

Fiber Optics

Thin, transparent fibers of glass or plastic which transmit light throughout their length by internal reflections that are typically enclosed by an opaque material.

Filar Micrometer

A measuring device fitted to the eyepiece of an optical instrument for measuring small distances.

Filler Wad

Refer to **Wad – Filler Wad**.

Finger Lever

The operating lever on lever action firearms (e.g., Winchester Model 94).

Fire

To shoot or discharge a firearm.

Firearm

An assembly of a barrel and action from which a projectile(s) is propelled by products of combustion. The legal definition of a firearm may vary by jurisdiction.

Firearms Identification

A discipline of forensic science which has as its primary concern to determine if a bullet, cartridge case, or other ammunition component was fired by a particular firearm.

Firearms Reference Library

A collection of firearms maintained by a laboratory for the following reasons:

-To identify the make, model, and source of evidence firearms.

-To provide exemplar firearms for various testing purposes which otherwise compromise evidence firearms.

-To provide an exemplar resource for training new examiners or in developing new technology for the examination of firearms.

-To provide a source of firearms parts for the repair of evidence firearms for test firing purposes.

-To provide a resource for the identification of firearms parts recovered at crime scenes.

-To provide a resource for the location and style of firearms serial numbers.

Fired Standards

A collection and cataloging of test fired bullets, cartridge cases, and shotshells from known firearms. Also known as **known standards, ammunition standards, or reference ammunition**.

Fire-Forming

Fire-forming is the process of changing the original, external shape of a cartridge case to the shape of the chamber through the firing process. The caliber of the cartridge case must be dimensionally similar to the caliber of the firearm. This process can be engineered or unintentional depending on the circumstances.

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Firelapping

The process of shooting bullets coated with a fine abrasive substance in order to remove toolmarks from the barrel and increase the accuracy of the firearm.

Fire Train

A single term that describes the sequence of events in the discharge of a cartridge:
The detonation of the primer.
The flame from the primer detonation passing into the powder chamber via the flash hole(s).
The deflagration of the gunpowder and the subsequent production of gases.
The propelling of the projectile down the barrel by the above mentioned gases.

Firing Pin

That part of a firearm mechanism which strikes the primer or rim of a cartridge to initiate ignition in order to fire a cartridge or shotshell. Refer to **Hammer Nose** and **Striker**.

Firing Pin Aperture

The hole in the breech face of a firearm through which the firing pin protrudes.

Firing Pin Aperture Shear

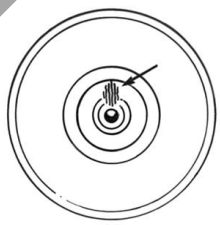
Striated marks caused by the rough edges of the firing pin aperture scraping the primer metal during unlocking of the breech. Refer to **Primer Flow-Back**.

Firing Pin Bushing

A removable collar or bushing in the breech face of certain firearms through which the firing pin passes. The bushing is often threaded and removable with a small pin spanner in order to change firing pins (e.g., Iver Johnson and Smith & Wesson top break revolvers). Also known as a **recoil plate**.

Firing Pin Drag Mark

The toolmark produced when a projecting firing pin comes into contact with a cartridge case or shotshell during the extraction/ejection cycle.



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Firing Pin Extension

A floating, often cylindrical, extension to the rear of the firing pin in some firearms which takes the direct impact of the hammer and transmits it to the firing pin (e.g., Astra Models 400 and 600 pistols).

Firing Pin Impression

The indentation of the primer of a centerfire cartridge case or in the rim of a rimfire cartridge case caused when it is struck by the firing pin. May also be referred to as a firing pin indent.

Firing Pin, Inertia

Refer to **Inertia Firing Pin**.

Firing Pin Protrusion

The distance the firing pin protrudes from the breech face when in its forward most position.

Firing Pin Retaining Plate

The removable plate at the rear of the slide on some pistols which holds in the firing pin. The rebounding firing pin has spring tension pushing it to the rear causing the rear of the firing pin to protrude through a hole in the retaining plate. This keeps the plate in position and allows the rear of the firing pin to be struck by the hammer (e.g., U.S. 1911 pistol).

Firing Pin Stop

A device to limit the forward motion of the firing pin thus preventing pierced primers.

Five-In-One Blank

A blank cartridge that was designed for use in firearms of different calibers. It can be used in caliber 38-40, 44-40, and 45 Colt revolvers and in caliber 38-40 and 44-40 rifles. Also known as a movie blank.

Fixed Ammunition

A cartridge or shotgun shell comprised of a case, primer, propellant powder, and projectile(s).

Flare

Refer to **Cylinder Flare** or **Smoke Ring**.

Flash Hole

(1) A hole(s) pierced or drilled through the web in the primer pocket of a metallic cartridge case. May also be known as a **Gas Vent** or G**as Port**.

(2) The hole in the end of a battery cup primer used in shotshells. May also be known as a **Gas Vent** or G**as Port**.

(3) The hole in a percussion nipple. May also be known as a **Gas Vent** or G**as Port**.

Flash Suppressant

A material that is added to the propellant for the purpose of reducing muzzle flash.

Flash Suppressor

A muzzle attachment designed to reduce muzzle flash. May also be referred to as a flash hider.

Flat-Nosed Bullet

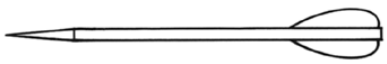
Refer to **Bullet – Flat-Nosed Bullet**.

Flats

Refer to **Water Table**.

Flechette

A small dart multiply loaded in some shotshells and rifle cartridges used by the military.



Flint Lock

A muzzleloading firearm ignition system wherein a piece of flint is secured to the hammer in such a manner as to strike steel upon hammer release. The interaction of the flint and steel causes

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sparks which ignite powder contained in the ignition system that subsequently ignites the main powder charge.

Floating Firing Pin

A type of firing pin which is unrestrained by a spring or other mechanical means.

Floor Plate

The bottom of a box magazine. The plate may be hinged, sliding, or immovable.

Fluted Barrel

Refer to **Barrel – Fluted Barrel**.

Fluting

(1) Grooves cut in the chamber wall of a firearm to assist or retard cartridge case extraction (e.g., Russian Tokarev semiautomatic rifle and the Heckler and Koch model HK4 pistol).
(2) Longitudinal grooves cut into the outside surface for all or some portion of the overall barrel length.
(3) A defect in a brass cartridge case usually in the form of a shallow indentation caused by excessive oil present in dies during manufacture, in resizing dies during handloading operations, or in a chamber during firing. Also known as **oil dent**.

Flyer

(1) A shot considerably outside the normal group on a target.
(2) A shot considerably outside the normal range with regards to velocity or pressure.

Follower

The part in a firearm or magazine which forces a cartridge or cartridges to move through spring action.

Following Edge

Refer to **Trailing Edge**.

Forcing Cone

(1) The tapered section at the front end of a shotgun chamber by which the diameter of the front end of the chamber is reduced to bore diameter.
(2) The tapered section towards the muzzle end of a shotgun barrel that gradually reduces in size from bore diameter to choke diameter.
(3) The tapered entrance to the bore at the breech end of a revolver barrel.

Forcing Cone Marks

Refer to **Axial Engraving**.

Forearm

The forward part of a two-piece stock. Refer to **Forend.**

Forearm, Anson Fastening

A means of attachment for the forearm of double barreled shotguns which utilizes a bolt that extends beyond the tip of the forearm. Pressure on the protruding stud or button allows removal of the forearm.

Forearm/Forend Iron

On some guns, an intermediate part that holds the wood against the barrel and frame and retains the latch mechanism.

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Forearm Tip Tenon

A transverse piece dovetailed into the underside of the barrels on certain rifles to accept screws from the sides to hold on the forearm tip (e.g., Marlin Model 39A).

Forend

The forward part of a one-piece stock [21]. Refer to **Forearm**.

Forend, Beavertail

The forward part of a stock, typically found on a shotgun, that is greater in width than standard forends. The beavertail provides a better grip for the forward hand, and protects the hand from hot barrels during fast shooting.

Forensic Science

The study and application of science to the purpose of the law.

Frame

In revolvers, pistols, and break-open guns, the basic unit of a firearm which houses the firing and breech mechanism and to which the barrel and grips are attached. Refer to **Receiver**.

Frame Lug

On some revolvers, the projection from the side of the frame located at the rear of the cylinder window, that prevents the cylinder from detaching from the crane/yolk when the cylinder is in the open position (e.g., Smith and Wesson Model 36 revolvers).

Frame, Solid

Refer to **Solid Frame**.

Frangible Projectile

Refer to **Bullet – Frangible Bullet**.

Freebore

A portion of the barrel ahead of the chamber throat in which the rifling has been cut away, leaving the bore smooth and without a taper [21]. Normally, use of the term freebore indicates the rifle has an unusually long throat, as is the case for most Weatherby chamberings [14].

Free Travel

Refer to **Bullet Jump**.

Friction Ring

A metallic ring surrounding the magazine tube to slow the rate of recoil allowing for safe ejection of the shotshell and subsequent feeding of the next cartridges (e.g., Browning Auto-5 shotgun).

Frizzen

The metal arm of a muzzleloader's lock that serves as a striking plate and guides sparks into the pan. When not in use, the frizzen also protects unfired priming power in the pan.

Front Strap, Grip

The part of the frame that forms the front of the grip of pistols and revolvers.

Full Auto

Refer to **Action – Automatic Action**.

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Full Metal Case Bullet

Refer to **Bullet – Full Metal Jacket Bullet**.

Full Metal Jacket Bullet

Refer to **Bullet – Full Metal Jacket Bullet**.

Full-Cock

The position of the hammer or striker when the firearm is ready to fire, excluding double action only firearms.

Fulminate of Mercury

A highly explosive/corrosive component of a priming compound [12].

Function Testing

The examination of a firearm concerning its mechanical condition and operation. It is usually performed to determine if all safety features are operable and/or if the firearm is capable of firing a cartridge. Refer to **Accidental Discharge Test**, **Jar Off**, **Drop Fire**, and **Safety Testing**.

Fused Shot

Refer to **Shot – Balled Shot**.

Fusing

(1) The balling of lead shot due to gas leakage.
(2) The melting of the core of a jacketed bullet.
(3) The melting of a lead alloy bullet.

Gain Twist Rifling

Rifling in which the rate of twist (pitch) of the lands and grooves increases as it approaches the muzzle.

Gallery Load

Refer to **Bullet – Frangible Bullet**.

Gas

In firearms, a product of the combustion of burning gunpowder. It is the rapidly expanding gases that move the projectile through the bore of the firearm.

Gas Check

Refer to **Bullet – Gas Check Bullet**.

Gas Cutting

An erosive effect in a firearm or on the base and/or bearing surface of a bullet caused by the high velocity and high temperature of propellant gases. Refer to **Blow-by**.

Gas Cylinder

(1) In gas operated firearms, the housing for the gas piston.
(2) A compressed gas container for use as ammunition in air guns.

Gas Operated

An automatic or semiautomatic firearm in which the propellant gases are used to unlock the breech bolt and to complete the cycle of extracting and ejecting. This action is usually performed in conjunction with a spring that returns the operating parts to battery (e.g., Remington Model 1100, US M1, US M14, US M16, and IMI Desert Eagle).

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Gas Piston

A cylindrical piece which is housed in the gas cylinder or gas tube of some gas operated firearms which is directly impinged upon by gas from the gas port in the barrel. It is this piston which is driven rearward that moves the breechblock and ejects the cartridge case and then feeds a cartridge into the chamber (e.g., US M1 and US M14).

Gas Port

An opening in the barrel or receiver of a firearm for several purposes: (1) to allow the vented gas to operate a mechanism, (2) to reduce recoil, (3) to provide an escape route for high-pressure gases should a case or primer rupture upon firing, reducing the amount of gas that might be directed back through the action into the shooter's face. May also be known as a **gas vent**. Also refer to **Gas Port** in the **Suppressor Terminology Section** of the appendix.

Gas Tube

(1) A cylindrical piece on gas operated firearms through which propellant gases are channeled to directly impinge on the bolt.
(2) A cylindrical piece on gas operated firearms which houses the gas piston.

Gas Vent

(1) Refer to **Gas Port** in reference to firearms.
(2) Any hole(s) pierced or drilled through the web in the primer pocket of a metallic cartridge case or the end of a battery cup primer of a shotshell. May also be known as a **Gas Port** or **Flash Hole**.
(3) The hole in a percussion nipple. May also be known as a **Gas Port** or **Flash Hole**.

Gauge

(1) A term used in the identification of a shotgun bore. The gauge is equal to the number of round lead balls of bore diameter that equal one pound. Thus 12 gauge is the diameter of a round lead ball weighing 1/12 pound.
(2) An instrument or device for measuring or testing a parameter such as a headspace gauge or trigger pull gauge.

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Gauge, Headspace

Refer to **Headspace Gauge**.

Gauge, Pressure

Refer to **Pressure Gauge**.

General Rifling Characteristics

The number, width, and direction of twist of the lands and grooves in a barrel of a given caliber firearm.

Gilding Metal

Metal alloys of 90% (Alloy No. 220) or 95 % (Alloy No. 210) copper and 10% or 5% zinc, respectively. Gilding metal is used extensively for the manufacture of bullet jackets. May also be known as commercial bronze.

Glass Bedding

The application of a mixture of fiberglass and resin between the action and/or barrel and stock.

Grain

(1) A unit of weight (avoirdupois). The grain unit is commonly used in American and English ammunition practice to measure the weight of components. There are 7,000 grains in a pound and 437.5 grains in one ounce.
(2) An individual kernel of black powder.

Grease Groove

A circumferential groove, or cannelure, on a bullet generally of a knurled or plain appearance which is designed to hold lubricant.

Greener Cross Bolt

A transverse square or cylindrical locking bar which is contained in the standing breech of some hinged frame firearms and which locks into the barrel extension.

Grip

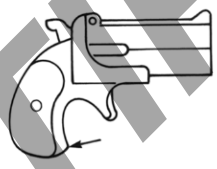
(1) In handguns, the handle.
(2) In long guns, the portion of the stock located behind the action which is normally grasped by the shooter's trigger hand. Also known as the **wrist**.

Grip Adapter

A device that alters the size or shape of the handle portion of a pistol or revolver. Generally used when a handgun is used for target practice.

Grip, Bird's Head

A handgun grip that comes to a point at the lower front making it somewhat resemble a bird's head.



RETIRED

Grip Frame

The metal structures that form the grip on certain handguns, especially where the grip is removable, such as Colt single action revolvers.

Grip Pin

A pin installed in the frame of some handguns to position the grips.

Grip, Pistol

Refer to **Pistol Grip**.

Grip Safety

An auxiliary locking device on the grip of some handguns which prevents firing until it is depressed, typically accomplished by grasping the grip correctly (e.g., US 1911 pistol).

Grip Screw Bushing

A small tubular piece threaded inside and outside that is installed on the frame of certain pistols into which the grip screws are threaded (e.g., US 1911 pistol).

Groove Diameter

The major diameter of a barrel which is the diameter of a circle circumscribing the bottom of the grooves in a rifled barrel.

Grooves

Depressed or cut channels in the bore of a firearm barrel to impart rotary motion to a projectile.

Group Measurement

The determination of the center distance between two bullet holes farthest apart in a target. This is referred to as the "group extreme spread." Other common measurements made are the extreme horizontal and vertical spread and the mean radius.

Guard Screw

A screw(s) that extends from the trigger guard into or through the stock.

Gunpowder

A variety of powders used in firearms as a propellant charge. A term commonly used when referring to cartridge and muzzle loading propellant. Refer to the **Gunshot Residue Section** for various types of gunpowder.

Gunshot Residue

The total residues resulting from the discharge of a firearm. It includes both propellant and primer residues, carbonaceous material plus metallic residues from projectiles, fouling, and any lubricant associated with the bullets. Refer to the **Gunshot Residue Section** for various types of residue.

Gusset

A term for the eight support brackets found in Remington plastic over-powder wads.

Gyroscopic Stability

The ability of a fired bullet to remain stable in flight due to its spin.

Half Cock

The position of the hammer of a firearm when about half retracted and held by the sear, intended to prevent release of the hammer without normal pull of the trigger.

Halo

Refer to **Cylinder Flare** or **Smoke Ring**.

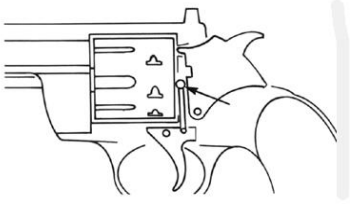
Hammer

A component of the firing mechanism which strikes the firing pin or primer.

Hammer Block

(1) A device which separates the firing pin from the hammer except when the trigger is pulled.

(2) A safety device which restricts hammer movement.



Hammer Fall

The travel of the hammer from the full cock position to the fully forward position.

Hammer Forging

Refer to **Rifling Methods – Hammer Forged Rifling** in the **Manufacturing Terminology Section** of the appendix.

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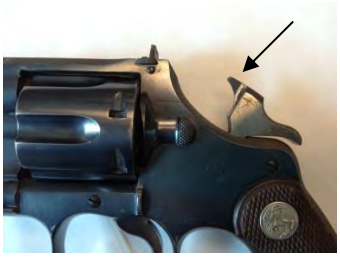
Hammerless

A firearm having no hammer or having a concealed hammer.

Hammer Nose

An extension on the front of some hammers which acts as the firing pin.



Hammer Notch

One or more grooves on the hammer which engages a firing or safety component such as the sear.

Hammer Rebound

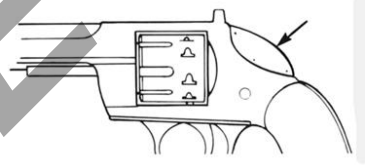
A safety feature of some firearms wherein spring action retracts the hammer after it has struck the firing pin.

Hammer Roll

A small roller installed at the bottom of the hammer which rides against the mainspring (e.g., Colt Single Action Army).

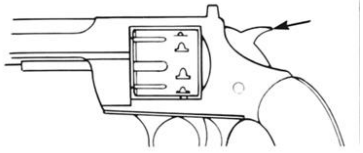
Hammer Shroud

A piece added to or a portion of the frame of a revolver to cover the sides of the hammer.



Hammer Spur

The knob or extension of an exposed hammer which acts as a cocking/decocking aid.



Hammer Strut

(1) A component part of the firing mechanism of some firearms which is connected between the hammer and trigger. This piece acts to move the hammer to the firing position when the trigger is pulled.

(2) A pivoting rod-like piece of metal attached to the hammer of certain firearms, which guides a coil mainspring to power a hammer (e.g., Colt US 1911 pistol).

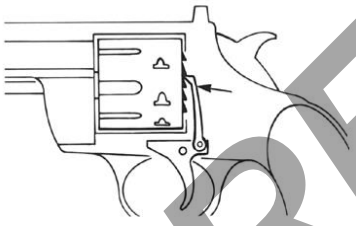
RETIRED

Hammer, Target

Refer to **Target Hammer**.

Hand

The lever attached to the trigger that rotates a revolver cylinder. This action positions the next breech chamber in front of the barrel. Also known as the cylinder pawl.



Handguard

A wooden, plastic, or metal forend/forearm that generally encircles the forward portion of the barrel to protect the shooter's hand from heat when firing.

Handgun

A firearm designed to be held and fired with one hand.

Handle, Operating

Refer to **Charging Handle**.

Handloading

Refer to **Reloading**.

Hand Slot

The opening in a revolver frame through which the hand protrudes to index the cylinder.

Hangfire

Any delay in firing of an abnormal duration. This implies that firing does eventually occur. Also known as **Delayed Fire**.

Hard Shot

Refer to **Shot – Hard Shot**.

Head

The base of the cartridge case which contains the primer and is the location of the headstamp.

Head Clearance

Refer to **Cartridge Case Head Clearance**.

Head, Rebated

RETIRED

Refer to **Cartridge – Rebated Cartridge**.

Head Separation

Refer to **Cartridge Case Head Separation**.

Headspace

The distance from the face of the closed breech of a firearm to the surface in the chamber on which the cartridge case seats.

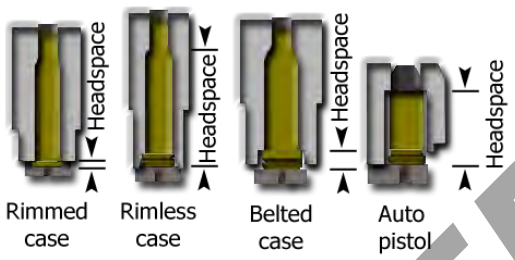


Image obtained from Reeds Target Shooting Club [22]

Headspace Gauge

A device used in a firearm to determine the distance between the breech face and the chamber surface on which the cartridge seats. Also known as a breeching plug.

Headstamp

Numerals, letters, and symbols (or a combination thereof) stamped into the head of a cartridge case or shotshell to identify the manufacturer, caliber, gauge, or other additional information.

Heel

(1) The part of a rifle or shotgun stock at the top of the butt end.
(2) The rear portion of a bullet, or its base.

Heel Cavity

A recess in the base of a bullet.

Helixometer

An instrument for inspecting the interior of a gun barrel and for measuring the rate of twist of the rifled bore of a firearm.

High Base

Refer to **Base, High**.

High Brass

Refer to **Cup, High**.

High Cup

Refer to **Cup, High**.

High Pressure Test Ammunition

Cartridges loaded to higher than normal pressures and used for proof testing only.

Hinged Frame

A firearm with a frame that is hinged to facilitate loading and ejection. Generally, the barrel(s) pivots downward.

RETIRED

Hinged Frame Action

Refer to **Action – Hinged Frame Action**.

Hinge Pin

A cylindrical part, in hinged frame guns, on which the barrel(s) pivots. Also called a joint pin.

Hold Open Latch

Refer to **Slide Stop**.

Hollow Base Bullet

Refer to **Bullet – Hollow Base Bullet**.

Hollow Point Bullet

Refer to **Bullet – Hollow Point Bullet**.

Holster Stock

A holster usually made of wood which attaches to the rear of the pistol grip of certain handguns and serves as a shoulder stock (e.g., Browning Hi-Power and Mauser "Broomhandle" pistols).

Hook Cut Rifling

Refer to **Rifling Methods** in the **Manufacturing Terminology Section** of the appendix.

Hull

A slang term for a cartridge or shotshell case.

Hyper-Obturation

Excessive swelling or upset of a bullet or cartridge case beyond the obturation required to make a seal for a normal firing event. Examples include expansion within an oversized barrel or chamber of a firearm (e.g., 9mm Luger cartridge fired in a 40 S&W chamber) or a high pressure event causing a bulged or flared bullet or cartridge case either inside or upon exit from the firearm's barrel or chamber (e.g., mushrooming of a hollow bullet base from a short barrel). Also refer to **Obturation**.

Ignition Barrel Time

The elapsed time from the contact of a firing pin with a cartridge primer to the emergence of the projectile(s) from the muzzle of the firearm.

Ignition Time

The time interval between the impact of the striker or firing pin on the primer, and a rise in pressure sufficient to start the bullet from its seated position in the cartridge case. The elapsed time from the moment of firing pin contact on the primer to the point on the x (time) axis equal to the point where the pressure-time curve indicates propellant burning has initiated [14].

Impact

The striking of one thing against another; collision (e.g., a bullet or projectile strike on a target).

Implode

To burst inward.

Impression

Refer to **Toolmark, Impressed**.

RETIRED

Impression Evidence

Objects or materials that have retained the characteristics of other objects or materials through physical contact (e.g., firing pin impressions, breech face impressions, footwear impressions, tire impressions) [2].

In Battery

A firearm is said to be "in battery" when the breeching mechanism is in the proper position for firing.

Incendiary Bullet

Refer to **Bullet – Incendiary Bullet**.

Indexing

(1) In firearms, the rotation and stopping of a revolver cylinder and the quality of alignment between the axis of the chamber and the axis of the bore.

(2) In Firearms Identification, the marking of specimens (bullets or cartridge cases) which were fired by the same firearm, orienting the features which show the alignment of corresponding detail (match). Also known as **orienting** or **phasing**.

(3) A method for marking cartridges and bullets to orient the position of test cartridges in a firearm. Also known as **orienting** or **phasing**.

Individual Characteristics

Marks produced by the random imperfections or irregularities of tool surfaces. These random imperfections or irregularities are produced incidental to manufacture and/or caused by use, corrosion, or damage. They are unique to that tool to the practical exclusion of all other tools.

Inertia

The tendency of an object at rest to remain at rest or of an object in motion to remain in motion in a straight line unless acted upon by an external force.

Inertia Block

(1) A device intended to function during recoil to set the fire control mechanism to fire a second barrel.

(2) A device intended to function during recoil to prevent doubling on some double barrel shotguns.

Inertia Bullet Puller

A tool used to remove the bullet from a cartridge by employing the inertia principle.

Inertia Firing Pin

A type of firing pin in which the forward movement is restrained until it receives the energy from a hammer blow. It is slightly recessed in the breech face before being struck by the hammer and is shorter in length than the housing in which it is contained. Upon hammer impact, the firing pin flies forward using only its own kinetic energy to strike and fire the primer [14] (e.g., Colt 1911 pistol).

Infrared

The part of the invisible spectrum of light that is contiguous to the red end of the visible light spectrum. The infrared spectrum of light comprises electromagnetic radiation of wavelengths from approximately 800 nm to 1mm.

RETIRED

Infrared Photography

A photographic technique which can be used in gunpowder pattern tests to facilitate the viewing of smoke patterns around a bullet hole on dark pieces of clothing.

Infrared Spectroscopy

An analytical method whereby a chemical compound is identified by passing infrared radiation through a thin layer of the material and measuring the pattern of absorption bands which are characteristic for the compound. Applications of infrared spectroscopy include the identification of the plastic components of shotshells and the identification of gunpowder.

Ingalls Tables

Ballistic tables computed by Captain James M. Ingalls that were first published in 1893.

Inside Lubricated Bullet

Refer to **Bullet – Inside Lubricated Bullet**.

Instrumental Velocity

Refer to **Velocity, Instrumental**.

Integral Base Wad

The base wad incorporated into the body of a plastic shotshell. May be referred to as a unibody shell.

Interchangeable Barrel

Refer to **Barrel – Interchangeable Barrel**.

Interior Ballistics

Refer to **Ballistics, Interior**.

Investment Casting

A metal forming technique that can produce complex shapes or parts with little or no additional finishing. In precision casting a ceramic mold is produced by surrounding or investing an expendable pattern with a refractory slurry. Once the slurry has dried, the mold is placed in a furnace or autoclave to melt and or vaporize the expendable pattern (made of wax, plastic, or frozen mercury). The mold is then filled with casting metal. After cooling, the shell is removed by hammering or through the use of a vibratory machine. Ruger and Thompson Center use this process. Also known as the lost wax process or precision casting.

Jacket

Refer to **Bullet Jacket**.

Jam

A colloquial term referring to the malfunction of a firearm that prevents the action from operating; may be caused by faulty parts, ammunition, improper maintenance, or improper use of the firearm.

Janka-Hardness Test

A system of rating the hardness of wood by the use of a 0.444 inch diameter ball. The test involves measuring (in pounds) the amount of force required to push one half the diameter of the ball into the wood. Also simply referred to as the janka test.

Jar Off

A condition in which a firearm may fire if dropped or jarred when the safety is off. This phenomenon occurs without the trigger being pulled. Testing for this condition involves placing

RETIRED

the safety in the "off" position and dropping the firearm from approximately waist height onto a hard surface to see if it will fire. Testing for this condition may also be referred to as drop testing. Also refer to **Drop Fire**.

Joint

A location where two or more parts come together.

Jump

The upward and rearward recoiling movement of a firearm when it is fired.

Jump Marks

Refer to **Axial Engraving** and **Slippage Marks**.

Keeper

A loop or clip made of leather, metal, or plastic used as a means of holding straps or cords, such as gun slings, together.

Kernel

An individual particle of any of the various forms of smokeless powder or the various granulations of black powder. Also known as a **granule**.

Keyhole

An oblong or oval hole that is produced by an unstable bullet striking the target at an oblique angle to the bullet's longitudinal axis.

Keyholed Bullet

A bullet that strikes or enters a medium in a yawed or destabilized orientation.

Known Non-matching Toolmarks

(1) Toolmarks known to have been made by different tools.
(2) Toolmarks produced by the same tool but deliberately placed in a non-matching position.

Known Standards

A collection and cataloging of both cartridges and ammunition components utilized by the firearm examiner; may include both unfired and fired ammunition components. Also known as **reference ammunition** or **fired standards**.

Killing Power

A non-scientific term for the lethality of a bullet.

Knuckle

(1) The front section of the water table upon which the barrels hinge.
(2) The front section of the action bar flats.

Knurling

(1) A series of ridges or diamonds impressed or cut into a surface to help prevent slipping.

RETIRED

(2) May be used to refer to cannelures on cartridge cases or bullets.

Laminated Barrel

Refer to **Barrel – Damascus Barrel**.

Laminated Cartridge Case

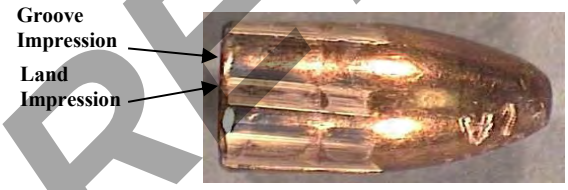
An area with a scaly appearance indicative of lamination caused by contamination of the brass.

Land

The raised portion between the grooves in a rifled barrel.

Land and Groove Impressions

Impressed areas on the bearing surface of a bullet caused by a bullet engaging with the rifling in the barrel of a firearm.



Photograph courtesy of Scott Doyle –http:// www.firearmsid.com

Lanyard

A strap or cord attached to a firearm (usually a handgun) to prevent loss of the firearm.

Lanyard Ring

A ring or loop on a firearm (typically a handgun) to which a lanyard may be attached.

Latch

A moveable device used to secure a part or piece in place relative to another. As applied to firearms, various types of latches are employed to hold barrels, cylinders, etc. in position during firing and to enable these parts to be moved for loading and unloading.

Lead Bullet

Refer to **Bullet – Lead Bullet**.

Leade (Lead)

Refer to **Chamber Throat**.

RETIRED

Leading

Refer to **Metal Fouling**.

Leading Edge

Refer to **Driving Edge**.

Lead-In Mark

A visible, thin, elongated deposition of bullet wipe transferred to a surface as a bullet first makes contact with that surface at a shallow incident angle. The lead-in mark is useful in establishing the direction of fire and travel of the projectile.

Lead Units of Pressure

A pressure value determined by means of lead crusher cylinders. Abbreviated: **L.U.P.**

Length, Overall

Refer to **Overall Length**.

Lever Action

Refer to **Action – Lever Action**.

Lever Lock

A device to hold the lever closed on lever action firearms.

Lifter

Refer to **Carrier**.

Light Hit

The result of insufficient firing pin energy or protrusion which causes erratic ignition or failure to detonate the priming compound. Also known as a light blow.

Link

A part that connects two other parts while permitting flexibility between the parts.

Link Assembly

A part or assembly which joins two parts together and transfers motion from one part to another (e.g., Winchester 1873 and US 1911 pistol).

Link Pin

A pin which secures together two moveable parts.

Live Ammunition

Refer to **Ammunition**.

Load

(1) The combination and quantity of components used to assemble a cartridge or shotshell.
(2) The act of putting ammunition into a firearm magazine or chamber.
The following are various types of loads encountered in ammunition:

Brush Load – Refer to **Scatter Load**.

Duplex Load – Refer to **Cartridge – Duplex Cartridge**.

RETIRED

Field Load – A shotshell loaded for hunting small game animals and birds.

Prescribed Load – The recommended combination of cartridge components as defined by SAAMI or the manufacturer for any cartridge or shotshell.

Proof Load – Refer to **Cartridge – Proof Cartridge**.

Scatter Load– A shotshell load designed to produce a wider spread and less dense pattern than a standard load at a close range in a choked gun. Also known as brush load, spreader load, and thicket load [21].

Service Load – Refer to **Cartridge – Service Cartridge**.

Squib Load – A cartridge or shotshell which produces substantially lower than normal projectile velocity and/or sound and which may result in the projectile and/or wads remaining in the bore. Also known as a squib or squib discharge.

Triplex Load – Refer to **Cartridge – Triplex Cartridge**.

Loaded Chamber Indicator

A mechanism on a firearm that reveals when a cartridge is chambered if the firearm is in battery. May take the form of a small rod, raised extractor, or other mechanism that protrudes or raises to indicated a loaded status. A small opening in the chamber can also function as a chamber indicator (e.g., Ruger LC9, Beretta PX4 Storm, Smith & Wesson Sigma Series, etc.).

Loading Block

A device containing a number of blind holes into which cases or completed ammunition are placed.

Loading Density

The percentage of the measured volume of propellant compared with the available cartridge case volume.

Loading Gate

(1) The hinged piece attached to the frame of revolvers that can be opened to permit loading of the chambers.

(2) A spring loaded cover for the loading port of a long gun.

Loading Ramp

A platform or cut in the bottom of the receiver of some single shot firearms that aids in guiding a cartridge into the chamber as the action in closed. This term is not synonymous with the feed ramp.

Lock

A general term referring to the total firing mechanism of a firearm. The action, either manual or automatic, of locking or supporting the bolt of a firearm immediately prior to firing.

Locked

The condition in a firearm action in which the bolt or breechblock is solidly secured in a fixed relationship with the chamber so as to resist being blown back by chamber pressure. Refer to **Battery**.

Lock Energy

The amount of energy delivered to the primer from the firing pin blow.

RETIRED

Lock Frame

A device incorporated into the mechanism of some firearms where the barrel and bolt assembly recoil upon firing. The lock frame acts to absorb the shock of the recoiling parts prior to the bolt unlocking and opening.

Locking Block

The component in a firearm designed so that when the action is closed, a block(s) slide into place securing the bolt in the locked position. Also known as locking bolt.

Locking Bolt

Refer to **Locking Block**.

Locking Cam

A type of lock found in certain breechloaders, such as the 1873 Springfield. Locking on this action is accomplished by a cam located at the rear of the breechblock that fits into a mating recess. This rifle is also referred to as a trap door action**.**

Locking Lugs

One or more projections from the breech bolt which serve to lock it into place when closed and may or may not be an integral part of the breech bolt. Also known as a bolt lug.

Lock Plate

A metal plate mounted on the stock of a firearm to provide for mounting of, or access to, the firing mechanism.

Lock, Rebounding

A type of firing mechanism wherein the hammer or striker retracts slightly to a rest position after causing ignition.

Lock-Time

The time interval between sear release and the firing pin striking the primer.

Long

A proper name given to some cartridges to differentiate them from others of the same caliber.

Long Gun

Any firearm fitted with a stock and designed to be used while held with both hands and supported by a shoulder. Also known as a shoulder arm.

Long Recoil

Refer to **Recoil Operation**.

Long Rifle

(1) Term originally used to refer to long barreled flintlock rifles.
(2) The name given to one type of a 22 caliber rimfire cartridge.

Lost Wax Process

Refer to **Investment Casting**.

Low Base

Refer to **Base, Low**.

RETIRED

Low Brass
Refer to **Cup, Low**.

Low Cup
Refer to **Cup, Low**.

Lubaloy®
A Winchester-Western trade name for a copper bullet coating and jacketing material. When plated on lead bullets, Lubaloy does not contain zinc. When used as jacketing on composite bullets, zinc is present.

Lubrication Groove
Refer to **Grease Groove**.

Lug, Barrel
Refer to **Barrel Lug**.

Lump
Refer to **Barrel Lug**.

Lump, Chopper
A projection on the underside of a barrel(s) which is integral with the barrel.

Lump, Hook of
A notch machined into a lump used to mate with the hinge pin.

Lumps, Bifurcated
A projection on the underside of a barrel(s) found mainly on expensive over/under shotguns. The bifurcated or divided lumps are mounted on each side of the lower barrel, as opposed to under it, which serves to reduce the overall height of the receiver-barrel assembly.

Machine Gun
A firearm design that feeds rifle cartridges, fires, extracts and ejects cartridge cases as long as the trigger is fully depressed and there are cartridges in the feed system. Actuation of the mechanism may be from an internal power source such as gas pressure or recoil, or external power source, such as electricity. Also called fully automatic or full auto. Refer to **Action – Automatic Action**.

Machine Rest
A mechanical device that will hold a firearm in a constant position for firing even when pressure is applied to the trigger. A machine rest is typically used for testing the accuracy of ammunition or the firearm itself.

Macroscopic
Visible to the unaided eye (or up to 10X magnification).

Magazine
(1) A secure storage place for gunpowder, ammunition, or explosives.
(2) A container for cartridges which has a spring and follower to feed those cartridges into the chamber of a firearm. The magazine may be detachable or an integral part of the firearm. The following are various types of magazines:

Blind Box Magazine – An integral magazine having a permanently closed bottom. Loading and unloading are accomplished through the same opening.

RETIRED

Box Magazine – A rectangular receptacle attached to or inserted into a firearm that holds cartridges stacked on top of one another ready for feeding into the chamber.

Detachable Magazine – Any of a large array of magazines which are removable from the firearm for reloading.

Drum Magazine – A drum shaped magazine in which the cartridges are arranged spirally around the central axis of the wind-up mechanism of the magazine. These were generally large-capacity magazines, being used most commonly on sub-machine guns (e.g., Thompson sub-machine gun, Luger, Russian PPSH and Degtyarov machine guns).

Rotary Magazine – A type of magazine in which the cartridges are arranged about a central rotating spindle or carrier. This type of magazine may be referred to as a Mannlicher magazine (e.g., Savage Model 99 and Mannlicher rifles).

Staggered Box Magazine – A box magazine having two zigzagged columns of cartridges. This increases the capacity without lengthening the magazine. Also known as a double stack magazine (e.g., Browning Hi-Power and S&W Model 59).

Tubular Magazine – A tube-shaped magazine in which the cartridges are arranged end-to-end. It may be either under the barrel or in the butt stock.

Magazine Catch

Refer to **Magazine Release**.

Magazine Cut-Off

Refer to **Cut-Off**.

Magazine Fed

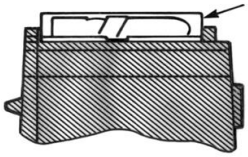
A repeating firearm in which the ammunition for subsequent firing is fed from a magazine.

Magazine Floorplate

The bottom of a magazine.

Magazine Follower

A spring actuated device to push cartridges in a magazine to the feeding position.



Magazine Latch

Refer to **Magazine Release**.

Magazine Lip Marks

Marks produced by the lips of a magazine as the cartridges are stripped from the magazine. These thin, often curvilinear, marks are located on the body and/or rim of a cartridge case at about the 10 o'clock and 2 o'clock positions.

Magazine Lock

Refer to **Magazine Release**.

Magazine Plug

RETIRED

(1) A part inserted in a magazine to reduce its capacity.
(2) A part in the end of a tubular magazine which closes the end and retains the spring.

Magazine Release
The device that retains or releases the magazine in a firearm. Also known as magazine catch, magazine latch, or magazine lock.

Magazine Ring
A ring fixed to the lower part of the barrel which serves to support the magazine tube.

Magazine Safety
A safety device on some semiautomatic handguns that prevents firing unless the magazine is fully inserted into the firearm.

Magazine Spring
The spring in a magazine that exerts tension against the follower.

Magazine Throat
A metallic insert found in some plastic magazines which aligns the next cartridge to be fed into the chamber (e.g., Ruger 10/22 carbine).

Magazine Well
That opening in a firearm which receives a detachable magazine.

Magnifying Power
The ratio of apparent size of an object viewed through an optical instrument to its actual size, e.g., 4X. This means the object would appear 4 times larger than it actually is.

Magnum
(1) Any cartridge or shotshell, that is larger, contains more shot, or produces higher velocities than standard cartridges or shotshells of a given caliber or gauge.
(2) Firearms that are designed to fire magnum cartridges or shotshells.

Mainspring
The mechanical energy storage device which operates the striker or hammer of a firearm.

Mainspring Guide
A rod-like device that keeps the mainspring from bending or twisting.

Mainspring Housing
A channel in which the mainspring rides (e.g., US 1911 pistol).

Malfunction
The failure of a firearm to function properly. Malfunctions can be caused by the firearm, ammunition, and/or human factors.

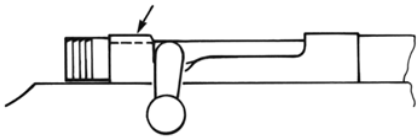
Mandrel
A metal rod or bar used as a core around which metal, wire, etc. is cast, molded, forged, or shaped.

Mannlicher Magazine
Refer to **Magazine – Rotary Magazine**.

Mannlicher Type Bolt

RETIRED

A bolt action rifle design in which the receiver bridge has a gap at the top to permit passage of the bolt handle. In this type of action, the bolt handle also serves as a locking lug (e.g., Mannlicher-Schoenauer and Italian Carcano rifles and carbines).



Manometer

An instrument for measuring gas and vapor pressure.

Martini Action

Refer to **Action – Martini Action**.

Match Ammunition

Ammunition made specifically for match target shooting. It is produced with special controls to assure maximum uniformity of cartridge performance.

Matchlock

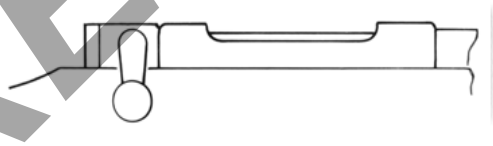
A device for igniting gunpowder developed in the fifteenth century, a major advance in the manufacture of small arms. The matchlock was the first mechanical firing device. It consisted of an S-shaped arm, called a serpentine, that held a match, and a trigger device that lowered the serpentine so that the lighted match would fire the priming powder in the pan attached to the side of the barrel.

Matte Finish

A dull, non-reflecting surface.

Mauser Type Bolt

A bolt action rifle design in which the bolt handle is all the way to the rear and the action cocks when opening or closing the bolt; the bolt handle does not pass through the receiver bridge.



Maxi-Ball®

Refer to **Bullet – Maxi-Ball® Bullet**.

Maximum Charge

The greatest charge weight, in grains, of a particular propellant that may be used with specified ammunition components without exceeding the safe, maximum, allowable pressure limit for the specific cartridge or shotshell being loaded.

Maximum Range

The greatest distance a projectile can travel when fired at the optimum angle of elevation of the barrel. Also known as extreme range.

Mean Radius

A method used to measure ammunition and/or firearm accuracy capability. To determine the mean radius, the center of the group is located and the distance to the center of each shot from the group center is measured and recorded. The sum of these measurements divided by the number of shots is the mean radius.

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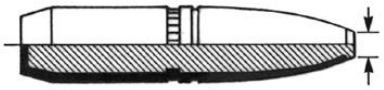
Mechanism Marks

Refer to **Cycling Marks**.

Meplat

A term describing the flat, measurable portion of a bullet, specifically the tip's diameter.



Metal Cased Bullet

Refer to **Bullet – Full Metal Jacket Bullet**.

Metal Cased, Hollow Point Bullet

Refer to **Bullet – Jacketed Hollow Point Bullet**.

Metal Fouling

The accumulation of lead or bullet jacket material in the bore of a firearm from the passage of projectiles. Also known as leading.

Metallic Ammunition

A generic term for rimfire and centerfire ammunition which have metallic cases.

Metal Patched Bullet

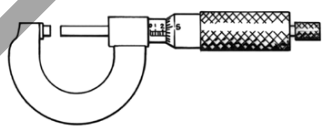
Refer to **Bullet – Full Metal Jacket Bullet**.

Microfractology

The microscopic study and comparison of fracture surfaces. Light or scanning electron microscopes are often used.

Micrometer

An instrument for making precise measurements that has a spindle moved by a finely threaded screw. It may be used in conjunction with a comparison microscope.



Micrometer, Eyepiece

A microscope eyepiece (ocular) with a permanently mounted micrometer scale engraved on the glass and permanently mounted in the plane of the diaphragm.

Micrometer, Stage

A glass microscope slide bearing a scale in the center of the slide that is 1 or 2 mm long, subdivided into tenths and hundredths of a millimeter or tenths, hundredths, and thousandths of an inch.

Microscope

An optical instrument consisting of a combination of lenses which allows the operator to view a magnified image of a small object(s).

Microscopic Comparison

A general term for the comparison of two or more items under a microscope. Also refer to **Comparison Microscope**.

RETIRED

Microscopic Marks

Striae, patterns of minute lines or grooves, or impressions in an object which are generally smaller than the unaided eye can distinguish. In firearm and toolmark identification, these marks are characteristic of the object which produced them and are the basis for identification.

Mid-Range

(1) A term that defines a specific point in the trajectory of a projectile that is half the distance between the firearm and the target.
(2) A reduced velocity, centerfire cartridge used principally in target shooting.

Minié Ball

Refer to **Bullet – Minié Ball Bullet**.

Minute of Angle (M.O.A.)

An angular measurement method used to describe accuracy capability. A minute of angle is one sixtieth of a degree. It subtends 1.047 inches at 100 yards, which for practical shooting purposes is considered to be one inch. A minute of angle group, therefore, equals one inch at 100 yards, two inches at 200 yards, etc. At 8" for example, it is approximately .002".

Misalignment Marks

Refer to **Axial Engraving**.

Misfeed

Any malfunction during the feeding cycle of a repeating firearm resulting in the failure of a cartridge or shotshell to enter the chamber completely. Also known as failure to feed.

RETIRED

Misfire

A failure of the priming mixture to be initiated after the primer has been struck an adequate blow by a firing pin, or the failure of the initiated primer to ignite the powder.

Mold Line

The residual line on an object, such as a cast bullet, resulting from the flow of metal into the juncture where the two mold halves come together.

Monoblock

The solid section at the breech end of some multibarrel guns into which the barrels are inserted.

Monocular

A word referring to a single eye (i.e., monocular eyepiece).

Mounting Stage

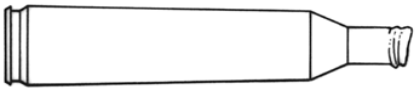
A platform on a microscope on which the object is positioned for viewing.

Mouth

The open end of a cartridge case or shotshell, from which the projectile(s) is expelled in firing.

Mouth Pull-Down

If a bullet is incorrectly inserted into the mouth of a cartridge case, it may deform the case mouth rearward and downward, causing a defect.



Movie Blank

Refer to **Five-in-one-blank**.

Mushroom/Mushrooming

A descriptive term for a soft point, hollow point, or special type of bullet point that expanded upon impact with a target, as designed. Bullets of this design are meant to increase sectional diameter with a minimum weight loss and release of all of the bullet's kinetic energy within the target, resulting in a mushroom shape.

Musket

A firearm with long barrel and forend or forearm extending nearly to the muzzle.

Musket Cap

The ignition source for most military muzzle loading rifles of the Civil War era, usually consisting of a copper alloy cup containing the priming mix. They are larger than percussion caps and typically incorporate continuous or segmented flanges (wings) at the cup mouth for ease of handling.

Muzzle

The end of a firearm barrel from which the projectile emerges.

Muzzle Blast

Noise that occurs during the discharge of a firearm as a result of rapid expansion of gases leaving the muzzle.

Muzzle Booster

A device that can be affixed to the muzzle end of a firearm barrel. When the firearm is fired, the gases following the bullet down the bore expand into the chamber of the device and exert a backward force on the barrel face, accelerating the rearward movement of the barrel and the breech locked to it. Some primary applications of this principle are machine guns, pistol suppressors, and blank firing adaptors. Also known as a recoil intensifier.

Muzzle Brake

A device attached to or integral with the muzzle end of the barrel that uses propelling gases to reduce recoil. Also known as a **compensator**.

Muzzle Cap

A cover on the muzzle end of a barrel to keep out foreign matter.

Muzzle Crown

Refer to **Crown**.

Muzzle Energy

Refer to **Energy, Muzzle**.

Muzzle Flash

The illumination that occurs during firing which is the result of the expanding gases from the burning propellant particles emerging from the barrel behind the projectile and uniting with oxygen in the air.

RETIRED

Muzzle Imprint

A general term describing the marks produced by the muzzle, front sight, magazine tube, spring housing, etc., and caused by the contact discharge of a firearm.

Muzzle Loader

Any firearm which is loaded with gunpowder and projectile(s) through the muzzle end of the bore or through the front end of a cylinder in the case of a muzzle loading revolver.

Muzzle Velocity

Refer to **Velocity, Muzzle**.

Muzzle Wave

The air that is compressed and moves out spherically from the muzzle of a firearm after firing a projectile.

National Match Ammunition

Ammunition produced with special care for the National Matches (at Camp Perry, Ohio) by appropriate government or commercial manufacturing facilities. Cartridges are usually, but not always, head-stamped **"NM"** for identification purposes.

NATO Cartridge

Refer to **Cartridge – NATO Cartridge**.

Neck

Refer to **Cartridge Case Neck**.

Neck Clearance

The dimensional difference between the diameter of the neck of a loaded cartridge case and the chamber.

Neck Crack

A longitudinal defect in a cartridge case neck that may occur with repeated reloading. It is a result of the metal stretching during firing, followed by diameter reduction during resizing in a die (cold working). Neck cracks may also occur due to the aging of the brass in cartridges that have been stored for many years, particularly if in a corrosive atmosphere. Also refer to **Split Neck**.

Necking Down

The use of case forming dies to reduce both outside and inside diameter of a cartridge case neck.

Neck Radius

The curved surface between the neck and the shoulder of a cartridge case.

Neck Sizing

The operation performed by reloaders to reduce or restore the original neck diameter of a fired cartridge.

Neck, Split

Refer to **Split Neck**.

Neck Tension

RETIRED

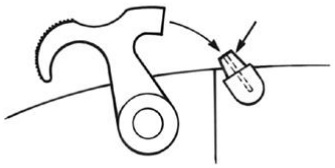
The circumferential stress that the case neck exerts on the seated bullet, as a result of the fit provided by inside diameter of the case neck and the outside diameter of the bullet.

Neck Thickness

Average thickness of the wall of a cartridge case surrounding the bullet.

Nipple

A hollow, cone shaped part of a percussion firearm upon which a percussion cap is placed. When fired, the flame, or flash, from the percussion cap passes through a hole in the nipple and into the chamber to ignite the powder charge.



Nipple Pick

A pointed instrument used to clean the orifice of nipples on percussion firearms.

Nipple Wrench

A tool used to remove or replace the percussion cap nipple on percussion firearms.

Nitro Wad

Refer to **Wad – Nitro Wad**.

Nominal Charge

A typical charge weight of a specific powder for a specific combination of components.

Non-Corrosive Primer

Refer to **Primer – Non-Corrosive Primer**.

Non-Mercuric Primer

Refer to **Primer – Non-Mercuric Primer**.

Nose

The point or tip of a bullet.

Notch Sight

An open rear sight having either a "V," "U," or square shaped cut on its upper edge.

Nutcracker Tool

Refer to **Tong Tool**.

N-Wave

The wave generated at the base or heel of a bullet when that bullet's velocity exceeds the speed of sound.

Nyclad® Bullet

An inert nylon jacketing or coating on a lead bullet; a Smith and Wesson original trade name which was purchased by Federal Cartridge Corporation.

RETIRED

Objective
That lens or lenses in an optical instrument which form the image of an object.

Oblique Angle
Any angle other than perpendicular or parallel.

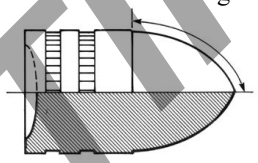
Obliterated
A term usually referring to serial numbers which are no longer decipherable.

Obturation
The sealing of a bore and chamber by pressure. During the firing process, pressure swells the cartridge case against the chamber walls which minimizes the rearward flow of gases between the case and the chamber wall. The same pressure, applied to the base of the projectile, causes it to swell or upset, filling and sealing the bore [14]. Also refer to **Hyper-obturation**.

Ocular
The lens or combination of lenses nearest the eye of the viewer in an optical instrument that is designed to further magnify the primary image of the objective. Also known as an **eyepiece**.

Offset Mount
A mount for telescopes or iron sights affixed to the gun in such a manner that the line of sight is to the side and above the receiver or barrel.

Ogive
The curved portion of the bullet forward of the bearing surface.



Ogive, Secant
A projectile nose with the curvature not tangent to the cylindrical bearing portion.

Oil Dent
A defect in a brass cartridge case, usually in the form of a shallow indentation caused by excessive oil present in dies during manufacture, in resizing dies during handloading operations, or in a chamber during firing. Also known as **fluting**.

Oil Proof
The treatment of a cartridge case to minimize the entry of oil or water.

Open Bolt System
A system of automatic or semiautomatic firing wherein the bolt remains in the rearward position after each shot or when firing is stopped. Generally weapons of this system have a fixed firing pin. Theoretically, the open bolt system allows for greater cooling to prevent cookoff and a simplified action (e.g., Thompson, Ingram/MAC M-10, and US M3 sub-machineguns). The Winchester Model 55, 22 caliber single shot rifle also fires from an open bolt. Also known as open-breech action.

Open Case Ammunition File
A file containing bullets and/or cartridge cases recovered from unsolved crimes wherein a firearm was discharged but not recovered. Also known by a variety of terms in various laboratories and

RETIRED

agencies which maintain them, (e.g., open case bullet file, open case file, open shooting file, or unsolved case file).

Open Point

Refer to **Bullet – Hollow Point Bullet**.

Open Sight

A rear sight having a notch through which the front sight is aligned for aiming.

Operating Handle

Refer to **Charging Handle**.

Operating Lever

That part operated by the hand to open and close the breech of a lever action firearm.

Operating Rod

A long rod extending from the gas piston which may be attached to a charging handle.

Operating Spring

Refer to **Recoil Spring**.

Optics

Informally, it is a general term for the components of an optical system. More precisely, the term refers to the scientific study of light and vision.

Orange Peel

A rough textured finish similar to the skin of an orange, generally referring to certain stock finishes.

Orienting

The aligning of two bullets, which were fired from the same barrel, on the comparison microscope so that the land and groove impressions on those bullets which were produced by the same lands. and grooves in the barrel are opposite each other. The term can also refer to the alignment of cartridge cases or other toolmark-bearing surfaces, so that directionality of marks made by the same tool are consistent on both stage of the comparison microscope. Sometimes called **phasing** or **indexing**.

Orifice

A small hole or vent, such as the gas port in the barrel of a gas operated firearm or in the receiver or bolt body of a manually operated firearm.

Origin of the Trajectory

The location of the center of the bore of a firearm at that specific point in time when the bullet exits the barrel.

Out of Battery

A firearm is said to be “out of battery” when the breeching mechanism is not in proper position for firing.

Out of Battery Discharge

A discharge that takes place when the firearm’s locking mechanism is not fully closed. Unlike a slam-fire, an out of battery firing is normally the result of the shooter intentionally pulling the

RETIRED

trigger. Upon firing, the unsupported case may rupture and vent gasses back into the action. This is a very hazardous situation for the shooter, and can damage or destroy the firearm. Also known as premature firing.

Out-Of-Time Marks

Refer to **Axial Engraving.**

Outside Lubricated Bullet

A lead bullet lubricated on the surface not covered by the cartridge case.

Overall Length

(1) For a firearm, it is the dimension measured parallel to the axis of the bore from the muzzle to a line at a right angle to the axis and tangent to the rearmost point of the butt plate or grip.

(2) For ammunition, it is the greatest dimension of a loaded cartridge. For centerfire and rimfire cartridges, it is the measurement from the face of the head to the tip of the bullet. For shotshells or blanks, it is the measurement from the face of the head to the crimp. Overall length does not refer to the uncrimped length of a shotshell.

Over And Under

A firearm with two barrels, one placed over the other.

Over-Bore

A shotgun whose barrel bore diameter is greater than the SAAMI maximum for that gauge.

Overbore Capacity

Characteristic attributed to a cartridge with too much volume for efficient powder combustion in relationship to bore size.

Overpowder Wad

Refer to **Wad – Overpowder Wad**.

Overshot Wad

Refer to **Wad – Overshot Wad**.

Overtravel

The distance a part travels after a particular event occurs in the normal operation of the firearm (e.g., the rearward motion of the trigger after the sear or hammer release or the rearward motion of a bolt beyond ejection, etc.).

Palm Rest

An adjustable support for a target rifle extending downward from the forearm.

Paper Disc

A small circular piece of treated paper cut and pressed into the primer cup in contact with the priming mixture. Also known as foil.

Paper Shell

Shotshells which are constructed with a body made from paper tubing.

Parabellum

RETIRED

From the Latin "for war." This term is typically associated with the 9mm Parabellum (9mm Luger) cartridge.

Paradox Rifling

Refer to **Rifling, Paradox**.

Parkerizing

A non-reflecting, rust-resistant finish used on metal surfaces of some firearms. Also known as phosphatizing or phosphate coating.

Partition Bullet

Refer to **Bullet – Partition Bullet**.

Patch

(1) A piece of paper or cloth wrapped around a ball or lead bullet to prevent leading of the barrel and to improve the gas seal.
(2) A piece of cloth used with a rod to clean the bore of a firearm.

Patched Ball

(1) In modern cased ammunition, the term refers to a full metal jacketed bullet (FMJ).
(2) A round or conical lead projectile that utilizes cloth, paper, or other material which acts as a gas seal or a guide for the projectile. An early type of fixed ammunition.

Pattern

The distribution of shot fired from a shotgun. Among firearms manufacturers, pattern is generally measured as a percentage of pellets striking a 30-inch circle at 40 yards. Some 410 bore or skeet guns are measured with a 30-inch circle at 25 yards. In Firearm Identification, the term is used to describe the distribution of either shotshell pellets or gunpowder particulate.

Pattern, Blown

Refer to **Blown Pattern**.

Pattern, Doughnut

Refer to **Doughnut Pattern**.

Pattern Matching

The act of visually comparing the surface contours of two or more striated or impressed toolmarks for corresponding and/or differentiating features.

Pawl

A pivoted or hinged part used to cause unidirectional motion over a definite distance or angle of rotation. Also known as a dog or **hand**.

Pellet

(1) The common name for the spherical projectiles loaded into shotshells. Also known as shot.
(2) A nonspherical projectile used in some air rifles and air pistols.

Pellet, Cup

A cylindrical cup-shaped pellet used in air rifles and pistols.

Pellet, Skirted

A pellet designed for use in air or CO_2 rifles, pistols, or revolvers with a round or flat nose and a flared base. Also known as a waisted pellet.

RETIRED

Pen Gun

A small caliber firearm shaped like a pen or pencil. These firearms may be converted smooth bore tear gas guns or have a rifled barrel.

Penetration

The depth that a bullet or shot pellet will travel into a target medium.

Percussion

A means of ignition of a propellant charge by mechanical blow against the primer or percussion cap.

Percussion Cap

A small, generally cylindrical metallic cup containing a priming compound used to ignite the powder charge in muzzle loading firearms (placed on the nipple of percussion firearms).

Percussion Composition

A combination of chemical ingredients that will explode when struck sharply with sufficient energy.

Phantom Safety

A situation where the handler incorrectly senses or believes that a manually operated safety system has been engaged. This claim or theory usually arises with traditional single action revolvers and postulates that the trigger sear was perched rather than seated in the safety or quarter-cock notch of the hammer.

RETIRED

Phasing

(1) In Firearm and Toolmark Identification, the marking of specimens (bullets, cartridge cases, or toolmarks) which were fired by the same firearm or created by the same tool, orienting the features which show the alignment of corresponding detail (match). Also known as **orienting** or **indexing**.

(2) A method for marking cartridges and bullets to orient the position of test cartridges in a firearm. Also known as **orienting** or **indexing**.

Photomicrograph

A photograph taken through a microscope.

Physical Match

The examination of two or more objects through physical, optical, or photographic means, where the examiner determines whether the objects were either one entity or were once held or bonded together in a unique arrangement.

Piezoelectric Gauge

A method of measuring pressure through the use of a quartz or tourmaline crystal. The quartz and tourmaline crystals will develop electric charges of opposite signs at the ends of what are called electric axes when subjected to pressure. The magnitude of the charges developed is directly proportional to the amount of pressure. Thus the output of the crystal is recorded against time and a pressure curve graph is obtained. Also known as a **pressure transducer**.

Pinch Point

In painted metal surfaces, a small area of surviving paint that was pinched between the initial contact point of a low incident angle bullet and the painted metal surface. The pinch point establishes the entry side of an impact or ricochet mark and thereby the bullet's direction of travel.

Pistol

A handgun in which the chamber is integral with the barrel.

Pistol, Air

A handgun with the same principle of operation as an air gun. Also known as a pellet pistol.

Pistol, Automatic

A common but improperly used term applied to autoloading pistols in use today. Most current "automatic" pistols are semiautomatic in action only.

Pistol Grip

On shoulder firearms, that part of the stock behind the trigger, shaped similarly to the grip of a pistol, to afford a better grasp.

Pistol Grip Adapter

An accessory made to attach to the front of a revolver grip to afford a better grasp.

Piston

Refer to **Gas Piston**.

Piston Ring

A sharp edged ring mounted on the piston of a gas operated firearm which makes a close seal between the piston and cylinder wall, and by scraping action reduces the accumulation of carbon in the cylinder.

Pit

A scar on a metal surface usually the result of extensive rusting.

Pitch

(1) In barrel rifling, the distance a bullet must travel in the bore to make one revolution.
(2) In grips, the angle that the front of the handgun grip makes with the line of sight.
(3) In shotguns, pitch is an expression used to indicate the relationship of the bore to the plane of the buttplate (pad). It is found by extending a line across the butt and drawing at right angles to this line an additional line through the highest point on the receiver or frame and measuring the distance from an extension of this line to a point at the base of the front sight bead. The pitch is said to be down if the described line is above the front sight and up if below. The pitch is normally down.
(4) A component used in making clay targets.

Pitch, Rifling

Refer to **Rifling Pitch**.

Plated Bullet

Refer to **Bullet – Plated Bullet**.

Plinking

RETIRED

Informal shooting, not following any organized rules of competition or at any designated distance. Plinking is shooting just for fun.

Point Blank Range

The range to which a shooter can obtain a hit in the vital zone of a particular target or game animal, while the sights are correctly aligned on the target.

Point of Aim

The place or point on a target which intersects the straight line generated by the alignment of the front and rear sight of a firearm.

Point of Impact

The point at which a projectile hits a target.

Polygonal Rifling

Refer to **Rifling, Polygonal**.

Port

(1) An opening in a receiver to allow loading or ejection.
(2) An opening in the wall of a barrel or bolt body to allow gas to escape, operate a mechanism, or reduce sensible recoil.

Port, Loading

The opening in a receiver where a cartridge may be placed in the firearm either directly into the chamber or the magazine.

Port Pressure

Applies only to gas operated firearms. The amount of pressure remaining in the bore as the bullet passes the gas port. If port pressures are too high, damage can result from the violent cycling of the action. It is important to understand that this can occur even when chamber pressures are within acceptable limits. Port pressure can be controlled by proper powder selection.

Post Sight

A front sight with flat sides and top.

Powder

Gunpowder; a commonly used term for the propellant in a cartridge or shotshell. Refer to the **Gunshot Residue and Gunpowder Section** for various types of gunpowder.

Powder Charge

The amount of gunpowder by weight in a cartridge or shotshell.

Powder Scale

A balance or weighing instrument for accurately weighing powder charges or other ammunition components.

Power Actuated Tool

A tool/ammunition system for fastening devices used in construction. The ammunition for these systems are known as power device cartridges and industrial cartridges. Abbreviated PAT.

Practical Impossibility

RETIRED

A phrase, which currently cannot be expressed in mathematical terms, that describes an event that has an extremely small probability of occurring in theory, but which empirical testing and experience has shown will not occur. In the context of firearm and toolmark identification, "practical impossibility" means that based on 1) extensive empirical research and validation studies, and 2) the cumulative results of training and casework examinations that have either been performed, peer reviewed, or published in peer-reviewed forensic journals, no firearms or tools other than those identified in any particular case will be found that produce marks exhibiting sufficient agreement for identification.

Precision Casting

Refer to **Investment Casting**.

Premature Firing

Refer to **Out of Battery Discharge**.

Prescribed Load

Refer to **Load – Prescribed Load**.

Pressure

The force developed in a firearm by the expanding gases generated by the combustion of the propellant. The following are various types of pressure associated with the discharge of a firearm:

Average Pressure – The arithmetic mean of a number of cartridges tested for pressure.

Chamber Pressure - The pressure in a firearm generated by the expanding propellant gases after ignition. Normally measured by means of piezoelectric transducers or crusher gauges. Also known as breech pressure or barrel pressure.

Peak Pressure – The highest value that the chamber pressure reaches during the burning of propellant.

Residual Pressure – The pressure level that remains in the cartridge case or shotshell within the firearm's chamber and in the bore immediately after the projectile leaves the muzzle of the firearm.

RETIRED

Pressure Barrel

Refer to **Barrel – Test Barrel**.

Pressure Curve

A graphical relationship between gas pressures in the chamber verses the time it takes the gas to exit the barrel when a cartridge is fired.

Pressure Determination

The act of measuring the pressure generated during the firing process in cartridges or shotshells contained in the chamber of a test barrel.

Pressure Estimation

A procedure for establishing pressure of a cartridge by visual observation or fired cartridge case measurements. Not an accurate method for determining pressure.

Pressure Gauge

A piston and crusher system or a piezoelectric transducer system used to measure internal chamber and/or barrel pressure in a firearm or test device. Refer to **Crusher Gauge** and **Piezoelectric Gauge**.

Pressure Transducer

A device which generates an electrical charge that is proportional to the pressure applied to its crystal element. Refer to **Piezoelectric Gauge**.

Pretravel

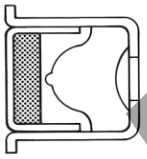
Refer to **Trigger Take-Up**.

Primer

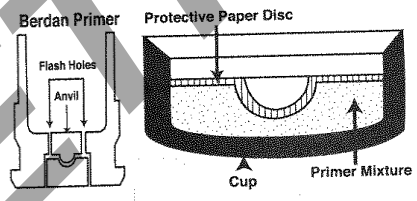
The ignition component of a cartridge. The following are various types of primers:

American Primer – Refer to **Boxer Primer**.

Battery Cup Primer – A type of primer using a flanged metallic cup as a holder for the primer components.

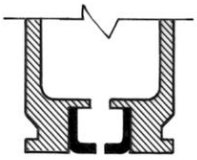


Berdan Primer – An ignition component consisting of a cup, explosive mixture, and covering foil. The anvil is an integral part of the cartridge case head in the bottom of the primer pocket. Generally two flash holes are drilled or pierced through the bottom of the primer pocket into the propellant cavity of the case. This system was designed by Col. Hiram Berdan. Although widely used throughout the world, this system has never been popular in the U.S., due largely to the difficulty in reloading Berdan cases.



Images obtained from Lyman 49th Edition Reloading Handbook [13]

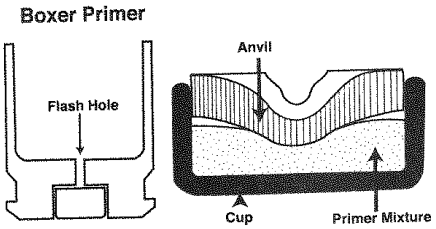
Blanked Primer – A fired primer cup in which the firing pin impression has been punched out by internal gas pressure. A potentially dangerous situation, normally indicating excessively high pressures, but is not unique to this cause.



Blown Primer – A primer that is separated completely from the cartridge or shotshell after firing due to severe expansion of the primer pocket and head.

Boxer Primer – An ignition component consisting of a cup, explosive mixture, anvil, and covering foil disc which together form the completed primer ready for assembly into the primer pocket of a cartridge case. A central flash hole is pierced through the bottom of the primer pocket into the propellant cavity of the case. Used in modern commercial centerfire ammunition made in Canada and the US.

RETIRED



Images obtained from Lyman 49th Edition Reloading Handbook [13]

Centerfire Primer – A primer system (Boxer, Berdan, or battery cup) which is assembled central to the axis of the head of the cartridge case and which is actuated by a blow to the center of its axis.

Corrosive Primer – A priming mixture that contained compounds of chlorine and oxygen, generally used in military ammunition made before 1952. The residues are hygroscopic (retain moisture) and therefore promote bore rusting.

Drop Test Primer – A method of determining the sensitivity of primed cases. The primed cases are held in a specified die and subjected to a range of specified firing pin blows imparted by a freely falling ball.

Dropped Primer – A primer that is separated completely from the cartridge or shotshell after firing without obvious distortion of the primer pocket and head. Also refer to **Blown Primer**.

Electric Primer – A primer which is ignited by electricity instead of impact from a firing pin. Ex. Remington Model 700 ETRONX Bolt Action Rifle uses cartridges with an electric primer (R-P 22-250 REM).

Flattened Primer – 1) A condition in which the normally round perimeter of a fired primer cup is squared off due to internal pressure. 2) A primer cup configuration in which the normally domed crown is flattened to improve sensitivity.

High Primer – A primer that has not been fully seated in the primer pocket and extends slightly above the head of the case. High primers can result in slam fires, particularly in semi-automatic firearms.

Loose Primer – A primer which does not fit properly in the primer pocket of a cartridge case or shotshell.

Non-Corrosive Primer – A primer which does not contain chemical compounds that could produce corrosion or rust in gun barrels.

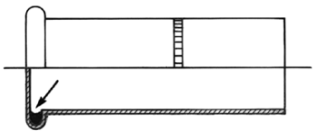
Non-Mercuric Primer – A primer which contains no fulminate of mercury or other mercuric compound in its priming mixture. A mercuric primer may or may not be corrosive, depending on whether or not it contains potassium chlorate.

Pierced Primer – A primer that has been pierced by the firing pin during the firing process. This allows gas to flow back into the action, and can be injurious to the shooter.

Protruding Primer – A primer that has been pushed out of, or protrudes from, the primer pocket of a cartridge or shotshell after firing.

RETIRED

Rimfire Primer – A primer system in which the priming mixture is found in the circumferential rim cavity.



Ruptured Primer – Refer to **Pierced Primer**.

Primer Cratering

Refer to **Primer Flow-Back**.

Primer Cup

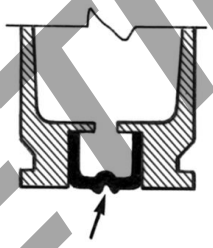
A metal cup (usually made of brass or copper) designed to contain priming mixture. The primer cup fits in the primer pocket or battery pocket of a cartridge or shotshell, respectively.

Primer Flash

The high temperature illumination produced by the extremely hot gases which result from the rapid build-up of pressure and temperature when the priming mixture detonates.

Primer Flow-Back

The extrusion of the primer into the firing pin aperture. This phenomenon can produce identifiable marks. Also refer to **Firing Pin Aperture Shear**.



RETIRED

Primer Leak

The escape of gas between the primer cup and head of the cartridge case or shotshell.

Primer Pellet

The explosive component of a primer.

Primer Pocket

A cylindrical cavity formed in the head of a centerfire cartridge case or shotshell for the insertion of a primer or battery cup primer assembly.

Primer Seating

The insertion of a centerfire primer or battery cup in the head of a cartridge case or shotshell. Properly seated, it should be flush or below the face of the head.

Primer Setback

The condition when a primer, or battery cup primer assembly, moves partially out of its proper location in the primer pocket of a cartridge or shotshell during firing.

Primer Shearing

Refer to **Firing Pin Aperture Shear**.

Primer Tube

A tube for holding primers in a handloading press.

Priming Mixture

A combination of explosive and/or pyrotechnic chemical ingredients which explode or deflagrate from the impact of a firing pin. The priming mixture is pressed into a cup or spun into the rim cavity of a rimfire cartridge.

Print-Through

A condition where the impressions of the lands and/or grooves in a jacketed bullet print through the jacket and can be seen on the separated lead core. May also be known as pseudo rifling.

Projectile

An object propelled by external force and continuing in motion by its own inertia (e.g., a bullet propelled from a firearm by the force of rapidly burning gases or other means). Refer to **Bullet** for various types of projectiles.

Projectile Rotation

The spinning motion that is imparted to a projectile due to engagement with the rifling in the barrel of a firearm, as it is driven down the barrel. The rate of rotation is dependent upon the rate of twist of the rifling and the velocity of the projectile. The barrel twist (left or right) determines the direction of the rotation.

Proof Load

Refer to **Load – Proof Load**.

Proof Mark

A stamp applied to a firearm after it has passed a proof test.

Proof Test

The firing of a deliberately overloaded cartridge to test the strength of a firearm barrel and/or action. Also refer to **Cartridge – Proof Cartridge**.

Propellant

In a firearm, the chemical composition that generates gas when ignited by the primer. The gas propels the projectile(s). Also known as gunpowder. Refer to the **Gunshot Residue and Gunpowder Section** for various types of gunpowder.

Provisional Proof Cartridge (Historical)

Refer to **Cartridge – Proof Cartridge**.

Pump Action

Refer to **Action – Slide Action**.

Pumpkin Ball

A round lead ball used in shotshells for hunting purposes, replaced by rifled slugs.

Push Button Safety

Refer to **Safety, Manually Operated**.

Quadrant Elevation

RETIRED

A military term for the elevation of the muzzle of the firearm above the horizontal plane, usually expressed in mils.

Quantitative Consecutive Matching Striae

A numerical tabulation of CMS runs. Typically, the number of CMS is designated by a number, followed by the letter x (e.g., 2x, 3x, etc.).

Quantitative Consecutive Matching Striae Identification Criteria

A numerical standard used when making a quantitative assessment of CMS in a comparison of a test striated toolmark with a questioned striated toolmark. The amount of CMS is compared to an empirically determined numerical threshold which is greater than the best known non-matching (KNM) quantitative CMS value. When the best KNM value is exceeded, a positive toolmark identification can be made with confidence.

Quick Detachable Swivel

A two part sling swivel which has a stud that is attached to the stock or barrel and a bow portion which is mounted on a spring plunger arrangement. The plunger passes through a hole in the stud for attachment of the bow to the firearm. They are sometimes called Q.D. swivels, and allow for quick mounting and dismounting of a sling from a firearm.

Quill

A tubular projection upon which fits the cylinder of certain hinged frame revolvers (e.g., Iver Johnson Model 66 revolver). Also refer to **Center Pin**.

Radial Fractures

The fractures or cracks that radiate out from an impact site in non-crystalline materials such as glass, ceramics, bone and certain plastics.

Radial Pressure Gauge

A British term denoting a gauge to measure cartridge-developed pressure through the sidewall of a cartridge case or shotshell tube as opposed to measuring the pressure transmitted through the head.

Radius, Neck

Refer to **Neck Radius**.

Radius, Shoulder

Refer to **Shoulder Radius**.

Ramrod

A rod used to seat a load in muzzle-loading firearms.

Range

(1) An area equipped for testing firearms and ammunition.
(2) The horizontal distance between the firearm and the target.

Range, Effective

Refer to **Effective Range**.

Range, Maximum

Refer to **Maximum Range**.

Range of Conclusions Possible When Comparing Toolmarks

RETIRED

The examiner is encouraged to report the objective observations that support the findings of toolmark examinations. The examiner should be conservative when reporting the significance of these observations.

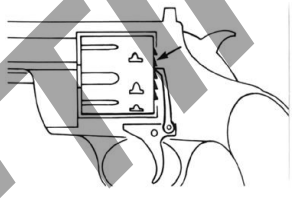
Identification:
Agreement of all discernible class characteristics and sufficient agreement of a combination of individual characteristics where the extent of agreement exceeds that which can occur in the comparison of toolmarks made by different tools and is consistent with the agreement demonstrated by toolmarks known to have been produced by the same tool.

Inconclusive:
A. Agreement of all discernible class characteristics and some agreement of individual characteristics, but insufficient for an identification.
B. Agreement of all discernible class characteristics without agreement or disagreement of individual characteristics due to an absence, insufficiency, or lack of reproducibility.
C. Agreement of all discernable class characteristics and disagreement of individual characteristics, but insufficient for an elimination.

Elimination:
Significant disagreement of discernible class characteristics and/or individual characteristics.

Unsuitable:
Unsuitable for examination.

Ratchet
A notched wheel on the rear of a revolver cylinder which causes the cylinder to rotate when force is applied by a lever called a hand.



Rate of Twist
Refer to **Rifling Pitch**.

Reamer
One of many spiral or straight-fluted, multi-edged cutting tools used to size and shape a hole.

Rebarrel
The replacing of a barrel with another barrel.

Rebated Head
Refer to **Cartridge – Rebated Cartridge**.

Rebated Rim
Refer to **Cartridge – Rebated Cartridge**.

Rebounding Hammer
A type of firing mechanism wherein the hammer automatically retracts slightly to a rest position after causing ignition.

Rebound Lever
A lever found in Colt revolvers which is actuated by the mainspring and serves to control the motion of the bolt (cylinder stop) and trigger.

RETIRED

Rebound Slide

(1) A reciprocating device found in some double action revolvers which moves the hammer into a position in which there is no contact of either hammer and firing pin or firing pin and primer. It can also be used to position a safety device between the hammer and firing pin or primer in such revolvers.

(2) A spring loaded metal block which directly impinges on the trigger in Smith & Wesson revolvers. The rebound slide causes the trigger to return to its forward position and rebounds the hammer. It is the primary safety.

Receiver

The basic unit of a firearm which houses the firing and breech mechanism and to which the barrel and stock are assembled. In revolver, pistols, and break-open firearms, it is called the frame.

Receiver Axle

The large pin which secures the rear toggle link to the breech bolt on Luger pistols.

Receiver Bridge

That part of the receiver on bolt action rifles that arches over the rear of the bolt hole. Also refer to **Split Bridge**.

Receiver Ring

That part of the receiver on bolt action rifles into which the barrel is fitted.

Receiver, Universal

A heavy duty mechanical device into which chambered barrels are secured for testing ammunition.

Rechamber

The cutting of a new chamber in a barrel to accommodate a cartridge of the same bore diameter.

Recoil

The rearward movement of a firearm resulting from firing.

Recoil Absorber

Any device that reduces the perceived recoil of a firearm.

RETIRED

Recoil Arm

A spring loaded arm which reaches up into the slide on certain semiautomatic pistols to absorb the recoil of the slide while moving rearward after firing (e.g., Webley and Scott pistol).

Recoil Energy

The force of the firearm's movement opposite to the direction of the bullet, usually expressed in foot-pounds (ft-lbs) or joules (J).

Recoil Intensifier

Refer to **Muzzle Booster**.

Recoil Lug

A block or plate on the bottom of the receiver and/or barrel to help absorb some of the recoil to the stock and prevent splitting of same.

Recoil Operated

An automatic or semiautomatic type firearm in which the force of recoil is used to unlock the breech bolt and then to complete the cycle of extracting, ejecting, and reloading.

Recoil Operation

Short Recoil: A firearm mechanism (action) in which the breechblock remains locked to the barrel only while the pressure is high. This involves a barrel travel of only about ½ inch. The device locking the breechblock to the barrel is then released and the two components separate. The barrel may remain stationary and await the return of the breechblock, but in most modern designs, the barrel has its own spring and goes forward into battery.

Long recoil: A system in which the bolt and barrel recoil a greater distance than the length of the unfired cartridge. The breechblock is then held to the rear while the barrel is driven forward by its own spring. When the barrel is fully forward it trips the catch, releasing the breechblock, which then feeds the next cartridge into the chamber.

Recoil Pad

A butt plate, usually of rubber, to reduce the sensible recoil of shoulder firearms.

Recoil Pendulum

A device for measuring free recoil energy in which a firearm is suspended from fixed points so as to allow it to swing freely while the barrel remains horizontal.

Recoil Plate

A removable collar or bushing in the breech face of certain firearms through which the firing pin passes. The bushing is often threaded and removable with a small pin spanner in order to change firing pins (e.g., Iver Johnson and Smith & Wesson top break revolvers). Also known as a **firing pin bushing**.

Recoil Shield

On a revolver, a lateral extension of the standing breech to each side, to prevent fired or unfired cartridges from coming out of the chambers and to protect the otherwise exposed primers of unfired cartridges.

RETIRED

Recoil Spring

The spring which returns a semiautomatic or automatic firearm to battery. Also known as an operating spring, retracting spring, or closing spring.

Recoil Spring Guide

A rod shaped device that fits inside of a recoil spring to guide and keep the spring from kinking.

Recoil Spring Plug

A tubular device, closed on one end that fits over a recoil spring at one end and rides against the barrel bushing. It is found on certain semiautomatic pistols and facilitates disassembly (e.g., US 1911 pistol).

Recoil Tables

Tables that list recoil velocity and recoil energy of specific cartridges fired in specific firearms.

Reconstruction

The determination of the sequence of two or more events in a particular incident utilizing information derived from the physical evidence.

Reduced Charge

A less than nominal powder charge.

Reference Ammunition

(1) A collection and cataloging of both cartridges and ammunition components utilized by the firearm examiner; may include both unfired and fired ammunition components. Also known as **ammunition standards, known standards, or fired standards.**

(2) Ammunition used in test ranges to evaluate test barrels, ranges, and other velocity and pressure measuring equipment. May also be used as a control sample by which other characteristics are compared, such as accuracy, patterns, etc.

Regulating Barrels

The adjusting of the relationship of the axes of multi-barreled firearms so as to make the points of impact coincide.

Relief Engraving

The carving of raised scenes that produces three-dimensional figures.

Reload

(1) A cartridge which has been reassembled with a new primer, powder, projectile(s), and/or other components.

(2) To create a cartridge by loading new components into a previously fired cartridge case.

Reloading

The process of manually reassembling a fired cartridge case with a new primer, propellant, and bullet or wads and shot. Also known as handloading.

Reloading Components

Primers, propellant powder, bullets, shot, wads, and cartridge cases.

Reloading Data

Refer to **Reloading Tables**.

Reloading Dies

Tools which hold and/or reform cartridge cases or shotshells during a reloading operation.

Reloading Press

A mechanical device for hand loading cartridges or shotshells.

Reloading Tables

A compilation of data suggesting combinations of various components suitable for reloading a given caliber or gauge. These data are usually referred to as reloading data.

Remaining Velocity

Refer to **Velocity, Remaining**.

Repeater

Any firearm equipped to hold more than one shot without reloading.

RETIRED

Residual Pressure

Refer to **Pressure – Residual Pressure**.

Resizing

In the reloading process, the reduction in diameter of a fired cartridge case to unfired dimensions by forcing it into a die of smaller size than the fired case.

Resizing Die

A tool used to form a cartridge case or bullet to proper dimensions. Also known as a sizing die.

Resizing, Full Length

The operation of completely reforming a fired cartridge case to approximately its original dimensions.

Resizing, Neck

The operation performed by reloaders to reduce only the neck diameter of a fired cartridge case.

Resolution

In microscopy, the ability to distinguish details. Also refer to **Resolving Power**.

Resolving Power

The ability of an optical system to render two closely spaced points as distinct. The resolving power of a lens is usually measured by its ability to distinguish parallel lines of equal width equidistantly spaced by an amount equal to the width of each line and is stated in terms of lines per millimeter of the image.

Rest

A device to support a firearm during firing.

Rest, Mann "V"

A type of machine rest that was designed by Dr. Franklin W. Mann. Commonly called a V Block. A heavy Mann barrel, suitably chambered for the cartridge to be tested, is used with the rest.

Restock

The replacement of one stock with another.

Retarded Blowback

Refer to **Blowback, Delayed**.

Reticle

(1) Markings in a microscope eyepiece used to establish location or scale. Commonly used to measure rifling.

(2) The aiming reference seen when looking through a telescopic sight. It may consist of straight or tapered lines, dots, or other marks used to determine the point-of-aim, size of, or range to the target.

Retracting Spring

Refer to **Recoil Spring**.

Retractor

Part used to move a breech bolt to rear.

Revolver

RETIRED

A firearm, usually a handgun, with a cylinder having several chambers so arranged as to rotate around an axis. The firearm is discharged successively by the same firing mechanism.

Revolver, Double Action

A type of revolver in which the rotation of the cylinder, cocking and firing are performed by a single pull of the trigger. Most double action revolvers can also be fired in the single action mode by manually cocking the hammer.

Revolver, Single Action

A type of revolver in which the hammer must be cocked manually to rotate the cylinder for each shot. The firearm is then discharged by a pull of the trigger. The process is repeated for each shot.

Rib

A raised surface used as a sighting plane that may either be solid or ventilated.

Rib Extension

The protrusion of a rib beyond the muzzle end of a barrel.

Rib Marks

A series of raised and depressed features on the edges of radial and concentric fractures in bullet-struck glass and other comparable materials that, in the case of radial fractures start at right angles to the backside (exit) surface of the fracture and turn toward the source of breaking force. Rib marks on concentric fractures start at right angles to the front side (entry) surface.

Rib, Solid

A solid raised surface above a barrel or barrels which functions as a sighting plane.

Rib, Ventilated

A raised sighting surface which is separated from the barrel by means of posts that allow air to circulate around it. Its purpose is to eliminate heat waves in the line of sight. Also known as a bridge rib.

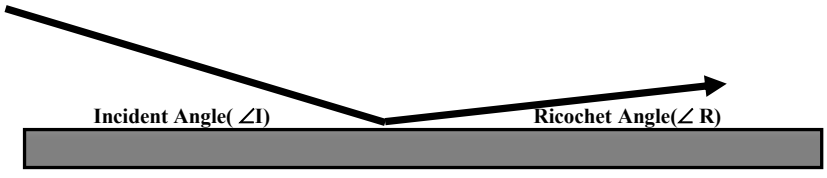
RETIRED

Ricochet

The continued flight of a rebounded projectile and/or major projectile fragments after a low angle impact with a surface or object.

Ricochet Angle

This angle is defined by the path taken by the ricocheted projectile (or major projectile fragments) as it departs the impacted surface.



Rifle

A firearm with a rifled bore designed to be fired from the shoulder.

Rifle, Automatic

A fully automatic shoulder firearm that starts firing when the trigger is pulled and continues until the trigger is released, a malfunction occurs, or ammunition is exhausted. The term should not be used in conjunction with semiautomatic firearms.

Rifle, Benchrest

A rifle designed for optimum accuracy while being shot from the shoulder and supported by a specifically designed table (rest).

Rifled Slug

A single projectile intended for use in smoothbore shotguns designed with spiral grooves for increased accuracy and a hollow base.

Rifle, Long

Refer to **Long Rifle**.

Rifle, Varmint

A sporting rifle designed for long range small game hunting which features a heavy barrel and fires high velocity, flat trajectory bullets.

Rifling

Helical grooves cut or impressed into the bore of a firearm barrel to impart rotary motion to a projectile when fired.

Rifling Broach

A tool having a series of cutting edges of slightly increasing height which is used to cut the spiral grooves in a rifled barrel, usually in a single pass.

Rifling, Gain Twist

A form of rifling where the twist rate (pitch) increases towards the muzzle.

Rifling Head

The end of a hook or cut rifling tool which holds the cutter and deepens the groove with subsequent passes.

Rifling Marks

Rifling impressions on the surface of a fired bullet. Also known as bullet engraving.

Rifling Pitch

The angle at which the rifling is cut in relationship to the axis of the bore. It is usually stated as the number of inches required for one revolution, for example one turn in 12 inches. Also known as rate of twist.

Rifling Twist

The direction (right or left) and rate at which the rifling of the firearm turns within the bore. This is normally expressed as the distance required for the rifling (and projectile) to make one complete revolution. Depending on the origin of the firearm, this may be written in inches or in millimeters (e.g., 1 turn in 12 inches or 1 turn in 305 mm).

Rifling, Paradox

An obsolete barrel design in which the major length of the barrel is smooth and the last few inches are rifled.

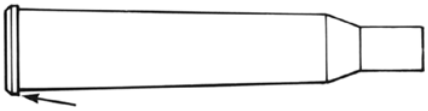
RETIRED

Rifling, Polygonal

Lands and grooves having a rounded profile instead of the traditional rectangular profile. Polygonal rifling is often seen in hammer forged barrels.

Rim

The flanged portion of the head of a rimfire cartridge, certain types of centerfire rifle and revolver cartridges, and shotshells. The flanged portion is usually larger in diameter than the cartridge or shotshell body diameter and provides a projecting lip for the extractor to engage. In a rimfire cartridge, the rim provides a cavity into which the priming mixture is placed.



Rim, Cracked

A radial rupture of the head and rim of a shotshell or cartridge case.

Rimfire

A flange-headed cartridge containing the priming mixture inside the rim cavity. Often used to refer to 22 caliber ammunition, the most common current rimfire caliber.

Rimless Case

Refer to **Cartridge – Rimless Cartridge**.

Rimmed Case

Refer to **Cartridge – Rimmed Cartridge**.

Rim Seat

A counterbore in the rear end of a chamber or bolt face to support the head of a rimmed cartridge.

Rim, Split

A circumferential rupture of the rim of a shotshell or cartridge case.

Ringed Barrel

Refer to **Barrel – Bulged Barrel**.

Riot Gun

A shotgun with a short barrel designed for riot control or guard duty.

Rod Ejector

Refer to **Ejector Rod**.

Rolled Crimp

RETIRED

The closure of the mouth of a shotshell by inverting the mouth of the tube over a top wad or slug, or a crimp to hold a bullet in a cartridge.

Rolling Block Action

Refer to **Action – Rolling Block Action**.

Rotary Magazine

Refer to **Magazine – Rotary Magazine**.

Rotating Barrel

Refer to **Barrel – Rotating Barrel**.

Round

A military term for a cartridge.

Round Ball Projectile

Refer to **Bullet – Round Ball Bullet**.

Round Butt

Grip of a revolver with a rounded shape.

Round Nose Bullet

Refer to **Bullet – Round Nose Bullet**.

Rule of 17

An equation that can be applied to American, numerical shot sizes to determine the average shot diameter in hundredths of an inch. Refer to the **Rule of 17** in the **Formula Section** of the appendix.

Runaway (Gun)

A malfunctioning automatic firearm that fires multiple shots after trigger pressure is released.

Runovers/Runover Checkering

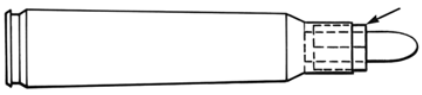
Checkering or engraving lines that extend beyond the border.

Rupture

A separation in the side wall of a cartridge case.

Sabot

(1) A lightweight carrier in which a subcaliber projectile(s) is centered to permit firing in a larger bore firearm.



(2) An enclosure to facilitate the firing of shot usually in a rifled barrel.

Saddle Ring

A ring usually attached to the receiver of a firearm designed to be carried by a horseback rider.

Safe

An incorrect, generic term for safety.

RETIRED

Safety, Automatic

A locking device on some firearms designed to return to the "on" (safe) position when the action of the firearm is opened.

Safety (Handling)

Precautions taken by firearms users to help provide protection against accidental or unintentional discharge.

Safety, Magazine

Refer to **Magazine Safety**.

Safety, Manually Operated

Cross Bolt – A type of firearm safety operated by lateral force on a button usually located in the trigger guard. Also known as a push button safety.

Grip – An auxiliary locking device in the grip of some handguns which prevents firing until it is depressed (e.g., US 1911 pistol).

Half Cock – This so-called safety is a sear engagement which holds the hammer back away from the firing pin.

Lever – A type of firearm safety operated by the movement of a pivoted lever (e.g., Luger pistol).

Sliding Safety – A safety mechanism on a firearm that is operated by a sliding motion.

Tang – A safety mounted on the upper receiver tang of a firearm.

Thumb – A safety on a firearm located as to be operated conveniently by the thumb of the trigger hand.

Trigger Lock – A form of firearm safety which blocks trigger movement. The trigger lock is designed to be engaged while the firearm is in storage and it should not be applied to a loaded firearm. A trigger lock is not a typical part of a firearm.

Wing – A safety found on bolt action rifles which is usually mounted at the rear of the bolt assembly. The safety pivots up and down at right angles to the bore line in a manner of a bird's wing.

RETIRED

Safety Mechanism

A device on a firearm intended to help provide protection against accidental discharge under normal usage when properly engaged. Such a mechanism is considered "on" when the position of the safety device is set in a manner to provide protection against accidental discharge under normal usage. Such a mechanism is considered "off" when it is set to allow the firearm to be discharged by a normal pull of the trigger. A manual safety is one that must be manually engaged and subsequently disengaged to permit normal firing. An automatic safety is one that goes to the "on" position when the action of the gun is opened. A passive safety is in place (or "on") until the trigger is pulled. An example would be the transfer bar system in some revolvers.

Safety, Passive

A safety that is in place (or "on") until the trigger is pulled.

Safety Testing

The examination of a firearm to determine if it is capable of accidental fire (i.e., if it will fire without the trigger being pulled) and if the safety features of the firearm are operational. Also known as an **Accidental Discharge Test**. Refer to **Function Testing**, **Jar Off**, and **Drop Fire**.

Scatter Load

Refer to **Load – Scatter Load**.

Schnabel

A German term for a hook-shaped knob at the forearm tip of a stock.

Schuetzen Buttplate

An extremely concave metal butt plate with a rearward extension usually at the bottom used mostly on target rifles for offhand (standing) shooting.

Scrape Cut

Refer to **Rifling Methods – Scrape Rifling** in the **Manufacturing Terminology Section** of the appendix.

Screw Plug

Screw used to fill holes for sight or telescope (sight) mounting when not in use. Also used to fill access holes to internal pins. Also known as a dummy screw.

Screw, Tang

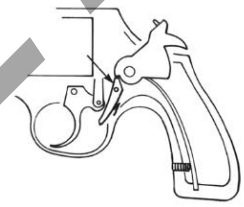
The screw or screws passing through either one or both tangs by which the stock or trigger guard is attached to the receiver or frame.

Screws, Stockmakers Hand

Extra-long screws used by stockmakers during the inletting operation to maintain alignment of the trigger guard and other auxiliary items with the action. Also known as inletting screws or guide screws.

Sear

A part which retains the hammer or striker in the cocked position until the trigger is pulled.



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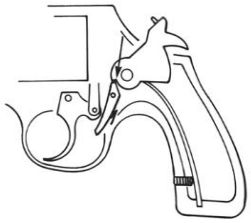
Sear Notch

A notch typically located in the hammer, in which the sear or trigger is held under tension of the mainspring until released by movement of the trigger. Also known as the **bent.**



Sear Spring

A spring which puts pressure on the sear of a firearm to return the sear to its normal position upon release of the trigger.

Season Cracking

A term used for stress-corrosion cracking that involves metallic cartridge cases or shotshell cups. The condition results from the combination of both residual stress and specific corrosive agents.

Seating Depth

The longitudinal position of a bullet or primer in a cartridge case or a wad in a shotshell.

Seating Lines

The circumferential striae which are parallel to the axis of the projectile, generated on the surface of the bullet by the cartridge case. Also known as bullet seating marks or cartridge case mouth marks.

Secondary Explosive Effect (S.E.E.)

A condition that can occur when slow-burning tubular powders are used at greatly reduced charge weights in large capacity bottle necked cartridges. Rather than burning in a normal fashion, the powder detonates, as though it were a severe overload. Also known as a high pressure excursion.

Secondary Projectile

Any object which, due to the energy imparted by the primary projectile(s), becomes a missile. Examples include buttons, glass chips, splinters of wood, or bone.

Sectional Density

The ratio of the weight of a bullet in pounds to the square root of its diameter in inches.

Selective Fire

The capability of some automatic firearms to fire in either the automatic or semi-automatic mode at the firer's discretion. These firearms normally have a switch or selector lever to facilitate the operator's choice.

Selector

(1) In a double barreled firearm, a device to allow the shooter to choose which barrel is to be fired by the first pull of the trigger.

(2) A lever which enables the shooter to choose the type of fire –automatic, burst, or semiautomatic, high or low rate of automatic fire.

Self-Loader

Refer to **Action – Semiautomatic Action**.

Semiautomatic Action

Refer to **Action – Semiautomatic Action**.

Serial Number

A number applied to a firearm for identification purposes. The Gun Control Act of 1968 requires all firearms manufactured after 1968 to bear a unique serial number.

Service Ammunition

Refer to **Cartridge – Service Cartridge**.

Service Loads

Refer to **Cartridge – Service Cartridge**.

Setting

The arming of a set-trigger mechanism of a firearm so equipped.

RETIRED

Shaving
Refer to **Bullet Shearing**.

Sheath Trigger
Refer to **Trigger – Sheath Trigger**.

Shell Catcher
A device for catching fired cartridge cases. May also be commercially known as Brass Catcher.

Shell Holder
An attachment used in handloading to hold a cartridge case.

Shell Latch
Refer to **Shell Stop**.

Shell Stop
A mechanical device often seen in pump action and autoloading shotguns that prevent more than one shell from feeding into the carrier, or lifter, with each cycle of the breech mechanism. Also known as **cartridge stop**, **cut-off**, or shell latch.

Shell, Unibody
Refer to **Shotshell, One Piece**.

Shocking Power
The ability of a projectile to dissipate its kinetic energy effectively into a target.

Shock Wave
The disturbance of air surrounding and behind the bullet caused by a compression of the air column directly in front of the bullet.

Shoe
(1) An adapter fastened to a trigger to widen the surface.
(2) An insert in the forearm of a side-by-side double barreled shotgun by which the forearm is attached to the barrels. Also known as forend iron or forend plate.

Shooting Glasses
Eye protection and/or sight improvement specifically designed for use when shooting firearms.

Shoot-Off
The separation of the mouth end of a shotshell from the body when it is fired.

Short
A cartridge designation that generally has a shorter cartridge case than others in the same caliber (e.g., 22 short versus 22 long or 22 long rifle).

Short Recoil
Refer to **Recoil Operation**.

Short Round
A cartridge in which the bullet is seated below the specified minimum length.

Shot

RETIRED

Generally, spherical pellets used in loading shotshells or cartridges. Shot can be found in many compositions such as lead, steel, bismuth, tungsten-polymer, tin, zinc, etc. The following are various types of shot:

Balled Shot – Two or more shot pellets joined together during the process of manufacturing or during firing. Usually caused by hot propellant gases leaking past the wadding and fusing the shot while the shot is still in the barrel. Also known as fused shot.

Bird Shot - A general term used to indicate any shot smaller than buckshot.

Chilled Shot – Refer to **Hard Shot**.

Coated Shot – Refer to **Plated Shot**.

Drop Shot – Refer to **Soft Shot**.

Dust Shot – Lead shot of small size (0.040in/1.016mm or smaller in diameter) normally used in making shot cartridges for small caliber ammunition.

Fused Shot – Refer to **Balled Shot**.

Hard Shot – Lead shot containing more than 0.5% of an alloying metal such as antimony, to increase its hardness. Also known as chilled shot.

Lead Shot – Generally, spherically shaped pellets compared of lead used in loading shotshells or cartridges. Also refer to **Hard Shot** and **Soft Shot**.
Plated Shot – Lead shot covered with a thin layer of a different metallic material to increase shot hardness and to reduce in-bore shot deformation. Typically copper and nickel are used to plate shot.

Soft Shot
(1) Lead shot manufactured by the process of pouring molten lead through colander and allowing the droplets to free-fall into water in a shot tower.
(2) Lead shot containing less than 0.5% alloying metal. Also known as drop shot.

Steel Shot – Soft steel pellets made specifically for use in shotshells.

RETIRED

Shot Bridging

A wedging action of shot pellets in a tube causing a stoppage of flow in a shotshell loading or reloading operation.

Shot Cartridge

A centerfire or rimfire cartridge loaded with small diameter shot. Ex. CCI 38/357 Shotshells

Shot Collar

A plastic or paper insert surrounding the shot charge in a shotshell which aids in keeping the shot compact, reducing the shot pattern. The collar also assists with cutting down flattening or deformation of shot/pellets during barrel travel. Also known as a shot sleeve.

Shot Column

The length of the shot load in a shotshell.

Shot Cup

Various designs of cup-shaped plastic inserts in shotshells which hold shot pellets and reduce their deformation during barrel travel.

Shotgun

A long gun designed to shoot from the shoulder, typically having a smooth bore and designed to fire shotshells.

Shotgun, Double Barrel

A shotgun with two barrels adjacent to each other in the same plane. If arranged vertically, it is typically called an "over and under" shotgun. If arranged horizontally, it is typically called a "side-by-side" shotgun.

Shot Protector Wad

Refer to **Wad – Shot Protector Wad**.

Shotshell

A unit of ammunition that may contain a single projectile or multiple projectiles/pellets. Generally shotshells are designed to be fired from shotguns.

Shotshell, One Piece

(1) A shotshell component having the body and base wad as a single unit with a metallic cup. Sometimes called a unibody shell.

(2) A complete round of ammunition having the body and base wad as a single unit without a head of a different material.

Shotshell, Plastic

A complete round of ammunition having a plastic body, a base wad that may or may not be a single unit, and a metallic head.

Shot Size

A numerical or letter(s) designation indicating the average diameter of a pellet. The Rule of 17 is an equation that can be applied to American, numerical shot sizes to determine the average shot diameter in hundredths of an inch. Refer to the **Rule of 17** in the **Formula Section** of the appendix.

Shot Spread

The diameter of a shot pattern.

Shot String

(1) The characteristic elongation of a column of shot in flight.

(2) The distance between the leading and trailing pellets of a shot charge in flight.

Shoulder

RETIRED

(1) The act of placing a shotgun or a rifle to a shooter's shoulder to align the sights and fire at a target.
(2) The sloping portion of a metallic cartridge case that connects the neck and the body of a bottleneck cartridge.
(3) The square, or angular step between two diameters on a barrel, pin, stud, or other part commonly used in firearms.

Shoulder Arm

Refer to **Long Gun**.

Shoulder Radius

The curved surface between the body and the shoulder of a cartridge case.

Sidearm

A pistol or revolver.

Side-By-Side

A firearm with two barrels arranged adjacently in the horizontal plane.

Side Hammer

A firearm with the hammer located externally on the side of the frame as contrasted to an internally pivoted hammer.

Side Lock

A firearm design in which the firing mechanism is attached to a detachable side plate rather than being integral with the frame.

Side Pin

Refer to **Side Screw**.

Sideplate

A removable plate in the frame or receiver of a firearm that allows access to internal parts or upon which some internal parts are mounted.

Side Screw

Screw used to attach side-locks to frame and/or stock.

RETIRED

Sight

Any of a variety of structures or devices, mechanical or optical, designed to assist in aiming a firearm. Refer to the **Sight and Scope Terminology Section** of the appendix for terms related to sights.

Sighting-Shot

Shot(s) fired to determine point of impact.

Silencer

A colloquial term used to describe a device attached to the barrel of a firearm designed to reduce the noise of discharge. Such a device is more accurately identified as a sound suppressor. Refer to the **Suppressor Terminology Section** of the appendix for additional related terms.

Single Action

Refer to **Action – Single Action**.

Single Shot

Refer to **Action – Single Shot Action**.

Single Trigger

A trigger mechanism for multiple barrel firearms wherein repeated pulls of one trigger fires the barrels successively.

Sizing

The reduction in diameter of a cartridge case or bullet by forcing it into a die of smaller diameter than the cartridge case or bullet, respectively.

Sizing Die

Refer to **Resizing Die**.

Sizing, Full Length

Refer to **Resizing, Full Length**.

Sizing, Neck

Refer to **Resizing, Neck**.

Skeet Gun

A shotgun with an open choke specifically designed for skeet shooting or close range hunting.

Skeet Shooting

A shotgun target sport in which shooters move around a semi-circle and fire at clay targets thrown at specified angles from a high and a low "house", each containing a target trap.

Skeleton Butt Plate

A metal buttplate forming only a border for the butt.

Skeleton Stock

A buttstock which is usually metallic without material in the center so that the weight is reduced.

Skid Marks

Refer to **Axial Engraving** and **Slippage Marks**.

Skirted Pellet

Refer to **Pellet, Skirted**.

Skive

A small slit or cut in the ogive portion of a jacketed bullet for the purpose of improving expansion.

Slam Fire

The accidental discharge of a firearm upon closing of the action which may be due to one of the following:
- A firing pin that has stuck and failed to retract.
- A primer that is either inadequately seated or overly sensitive.
- A weak or broken firing pin retaining spring which fails to overcome the inertia of motion imparted to the firing pin during closure, thereby allowing the firing pin to strike the primer with sufficient force to cause discharge.

RETIRED

- A firearm with inadequate headspace.

Slave Action

A special substitute action used for testing a barrel or barrels.

Slave Pin

A pin used during assembly of a firearm to facilitate the process and later replaced by a permanent pin or screw.

Sleeve

(1) An insert of special material placed in a barrel to reduce bore erosion, renew an eroded bore, alter bore diameter, or to strengthen the barrel.

(2) A tube surrounding the receiver of a target rifle to improve rigidity. Refer to **Barrel Liner** and **Barrel – Sleeved Barrel**.

Sleeved Barrel

Refer to **Barrel – Sleeved Barrel**.

Slide

A member attached to and reciprocating with the breechblock.

Slide Action

Refer to **Action – Slide Action**.

Slide Drag Mark

A toolmark that occurs in a semiautomatic pistol when there is a cartridge at the top of the magazine and the slide is retracted. The lug on the underside of the slide drags across the 12 o'clock position on the cartridge case leaving a striated mark. This mark cannot occur when a cartridge is manually inserted in the chamber and the slide is released or to the first cartridge when a loaded magazine is inserted in the firearm with the slide locked back.

Slide Lock

Refer to **Slide Stop**.

Slide Lock Safety

A lever mechanism found on some semiautomatic handguns which serves as a slide lock and a safety mechanism (e.g., Colt 32 and 380 pistols).

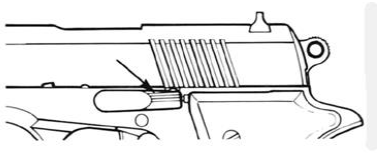
RETIRED

Slide Scuff Mark

A toolmark that occurs in a semiautomatic pistol when there is a cartridge at the top of the seated magazine and the retracted slide, either upon release or forward movement, impacts the 12 o'clock edge of the cartridge head producing a small, indented mark at this location. This mark also occurs during the normal chambering of a cartridge during the firing process. This mark cannot occur when a cartridge is manually inserted in the chamber and the slide is released.

Slide Stop

A latch found on some firearms which holds the slide open. It may be either manually operated or automatically activated by an empty magazine after the last cartridge is fired. Also known as hold open latch or slide lock.



Sliding Safety

A manual safety mechanism on a firearm that is operated by a sliding motion.

Sling

A strap fastened to a firearm to assist in carrying or to steady it during firing.

Sling Swivel

A metallic loop to which the sling is attached.

Sling, Target

A special form of strap to aid target shooting.

Slippage Marks

Slippage marks are typically produced by revolvers and have the appearance of widening of the land impressions at their beginning point. Also known as skid marks or jump marks. Refer to **Axial Engraving**.

Slot Blank

A dovetail-shaped metal part to occupy an unused dovetail slot.

Slotted Tip

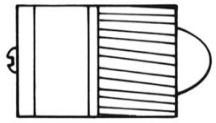
A cleaning rod tip with a slot to hold a cleaning patch.

Slug

(1) A single projectile for shotshells.
(2) A slang term for a fired bullet.

Slug, Brenneke

A formed, rifled slug having either a wad assembly attached to its base by a screw or a pressed on plastic wad that is intended for use in shotguns.



Slugging (Bore)

Refer to **Bore Slugging**.

Slug, Rifled

A single projectile with spiral grooves and a hollow base that is intended for use in shotguns. The theory behind this design is that after leaving the muzzle of the gun, the slug will rotate and thus reach its target much more accurately.

Small Arms and Light Weapons

RETIRED

Man-portable weapons made or modified to military specifications for use as lethal instruments of war that expel a shot, bullet, or projectile by action of an explosive. Small arms are broadly categorized as those weapons intended for use by individual members of armed or security forces. They include handguns, rifles, carbines, sub-machine guns, and light machine guns. Light weapons are broadly categorized as those weapons designed for use by two or three members of armed or security forces serving as a crew, although some may be used by a single person. They include heavy machine guns, hand-held under-barrel and mounted grenade launchers, portable anti-aircraft guns, portable anti-tank guns, recoilless rifles, man-portable launchers of missile and rocket systems, and mortars.

Small-Bore

In the United States, any firearm or ammunition of the rimfire type with a lead alloy bullet not over 0.23 inches (5.8 mm) in diameter.

Small-of-the-Stock

That portion of the stock between the rear of the action and the comb which has the smallest circumference.

Smoking

A technique using the smoke from a burning magnesium strip to plate or cover a bullet or surface with toolmarks to alter its reflective properties.

Smooth Bore

A firearm with an unrifled bore.

Snap Cap

A protective device to permit dry firing without damage to the firing pin.

Snap-Fire

The discharge of a loaded firearm through the inadvertent release of the hammer or striker from a partially retracted position. Such discharge may be the consequence of a compromised safety system, the lack of a safety system for such an event, or the failure on the part of the handler to engage the appropriate safety system.

Soft Point Projectile

Refer to **Bullet – Soft Point Bullet**.

Soft Shot

Refer to **Shot – Soft Shot**.

Solid Frame

(1) In long guns, one in which the barrel and receiver are not designed for quick disassembly.

(2) In handguns, a frame that is not hinged.

Solid Rib

Refer to **Rib, Solid**.

Spall

A crater formed from chipped or fragmented material as a result of projectile impact in brittle or frangible materials such as concrete, cinder blocks, sheetrock, etc. It more often describes such a crater on the exit side of a bullet impact site, but the term has also been used to describe an impact crater in otherwise hard materials.

RETIRED

Speed of Rotation (Spin)
The rate of spin of a projectile fired from a rifled barrel. Refer to the **Formula Section** of the appendix.

Speed of Sound
The velocity at which sound travels through air. The velocity can be affected by conditions such as air temperature, altitude, barometric pressure, and relative humidity, but at standard sea level conditions is approximately 1115 feet per second.

Spent Projectile
A fired projectile which has lost all of its kinetic energy.

Spire Point Projectile
Refer to **Bullet – Spire Point Bullet**.

Spitzer Bullet
Refer to **Bullet – Spitzer Bullet**.

Split Base
A longitudinal split in the sidewall of the brass or steel cup assembled on a plastic or paper shotshell.

Split Bridge
A receiver bridge that is cut to allow the passage of the bolt handle.

Split Case
A longitudinal rupture in the wall of a cartridge case or shotshell.

Split Cup
Refer to **Split Base**.

Split Neck
A longitudinal rupture in the neck of a metallic cartridge case.

Split Rim
A circumferential rupture of the rim of a cartridge or shotshell.

Split Shoulder
A longitudinal rupture in the side wall of the shoulder of a bottleneck cartridge case.

Spool Magazine
Refer to **Magazine – Rotary Magazine**.

Sporterize
The act or process of converting a military firearm to a sporting firearm, usually by removing unneeded accessories and altering the barrel and/or stock.

Sporting Gun
A firearm intended primarily for sport and recreation.

RETIRED

Spotting Scope

A telescope used to view hits on a distant target without leaving the firing line (approximately 30X).

Spreader Load

Refer to **Load – Scatter Load**.

Spread, Extreme

The distance between the centers of the two shots which are the farthest apart in a group of shots on a target.

Sprue

(1) The opening through which metal or plastic can be poured into a mold.
(2) The waste piece that is cast in the opening.

Sprue Cutter

The portion of the mold which removes the sprue in the casting process.

Sprue Cutter Mark

The toolmark left on the base of a cast bullet which results from cutting off the sprue.

Spur Hammer

The knob or extension on an exposed hammer which acts as a cocking or decocking aid.

Spur Trigger

A trigger which has a minimal projection from the action and is not usually surrounded by a trigger guard. Also known as a sheath trigger.

Square Butt

Handle of a revolver with a flat or squared end.

Squeeze Bore

A barrel design in which fired bullets having a spool-like body are squeezed to a smaller diameter in passing from the breech to the muzzle. The idea of this firearm was to present a large cross-sectional area of the projectile to the propellant gases and to present a small cross-sectional area to the atmosphere in order to reduce air resistance and thus increase the muzzle velocity of the projectile. Also known as a Gerlich type gun, a taper bore gun, or a reducing bore gun.

Squib Load

Refer to **Load – Squib Load**.

Standard Velocity

An industry term for rimfire ammunition loaded to a velocity level below high velocity ammunition of that type.

Standing Breech

The part of the frame of a revolver or break-open firearm which supports the head of the cartridge when it is fired.

Star Crimp

A type of closure of the mouth of a metallic case or shotshell in which the sidewalls are folded in a star-shaped pattern. Also called rose crimp or a pie crimp.

RETIRED

Star Gauge

An instrument used to measure the inside diameter of the bore of a firearm.

Starter Pistol

A pistol or revolver designed to fire blanks which may be used to sound the start of sporting events. These firearms are designed so they cannot fire bullets.

Steady Pin

A projection at the end of a mainspring which fits into the lock plate of a side lock action gun.

Steel Core Bullet

Refer to **Bullet – Steel Core Bullet**.

Steel Jacketed Bullet

Refer to **Bullet – Steel Jacketed Bullet**.

Steel Penetrator

A hardened steel component within a jacketed bullet that is designed to improve penetration in "hard" targets.

Stem (Stemming)

A failure of a cartridge to feed, in which the cartridge jams against the top or bottom of the chamber.

Step Base Bullet

A jacketed bullet having a small, flat recess or 'step' in its base.

Stereomicroscope

An optical instrument which provides three dimensional viewing of an object through paired objectives and eyepieces. Some models share a common, main objective.

Stippling

(1) In reference to firearms, it is the roughing of wood or metal with a pointed tool. It is normally performed to provide a gripping or decorative surface. Also known as matting.

(2) In reference to gunshot residue, refer to **Powder Stippling** in the **Gunshot Wound Terminology Section** of the appendix.

Stirrup

A component with divided legs to apply force equally. It is mainly used in trigger assemblies where it is necessary to straddle another component.

Stock

The wood or plastic component(s) to which the metal parts of a firearm are attached to enable the shooter to hold the firearm. The following are various types of stocks:

Benchrest Stock – Generally, a large and heavy stock used exclusively for benchrest shooting.

Blank Stock – A rough sawed piece of wood having the approximate external outline of a stock prior to final shaping.

Folding Stock – A type of collapsible stock where the buttstock collapses or pivots along a

RETIRED

hinge point *behind the action** to change the overall length of a firearm. (**example: German MP40*).

Free Rifle Stock – A target rifle stock used for position shooting matches. The word "free" refers to the fact that there are no restrictions on its configuration or weight.

Half Stock – A rifle stock with a forend which extends approximately to the midpoint of the barrel.

Laminated Stock – A stock which is made from two or more pieces of wood which have been glued together longitudinally.

Mannlicher Stock – A full length rifle stock which extends from the butt to the muzzle.

Monte Carlo Stock – A stock with a raised comb to bring the eye in alignment with the sight.

Offset Stock – A curved buttstock for use by a shooter who wishes to use the left eye when shooting right handed or vice versa. Also known as a cross-over stock.

One Piece Stock – A full length stock made from a single piece of wood. Includes both the butt and forend.

Pistol Grip Stock – A stock or buttstock having a downward extension behind the trigger guard somewhat resembling the grip of a pistol. Often found on target rifles.

Rough Inletted Stock – Refer to **Semi-Inletted Stock**.

Semi-Inletted Stock – A firearm stock that has been shaped partially so that the barreled action fits into the wood and requires additional hand fitting to achieve a proper fit. Also known as a rough inletted stock.

Semi-Finished Stock – The term for a stock which has been rough shaped and partially or completely inletted.

Small of the Stock – That portion of the stock between the rear of the action and the comb which has the smallest circumference.

Straight Stock – A stock with no pistol grip or one with less than the normal amount of drop.

Target Stock – Any stock specifically made for firearms designed for target shooting.

Telescopic Stock – A type of collapsible stock where the buttstock rides along an inner tube and can be locked into positions to change the overall length of a firearm.

Three-Quarters Stock – A rifle stock that has an abnormally long forend that does not reach the muzzle.

Thumbhole Stock – Any stock having a contoured hole in the grip area to accommodate the thumb of the trigger hand.

Two-Piece Stock – A stock consisting of two separate parts – the buttstock and forearm.

Whelan Stock – A rifle stock which features a forward sloping comb and cheekpiece.

Stock Bolt

RETIRED

A bolt which passes through a buttstock lengthwise to secure it to the receiver or frame.

Stock Dimensions

General stock dimensions consist of the following: *cast, drop at comb, drop at Monte Carlo, drop at heel, girth, length of pull* and *pitch.*

Cast – The lateral displacement of the centerline of the buttplate (pad) from the centerline of the bore. For a right-handed shooter, when the centerline of the buttplate is to the left of the bore, it is expressed as cast-on and to the right as cast-off. The opposite is true for left-handed shooters. Applies to both rifles and shotguns.

Drop – The vertical distance from the line of sight to the comb, Monte Carlo, or heel of the stock. For rifles, it is measured as a straight line drawn from the top of the front sight through the top surface of the open rear sight adjacent to the notch. The drops for target rifles are usually measured from the centerline of the bore. For shotguns, it is measured as a straight line drawn from the base of the front bead sight across the highest point on the frame or receiver.

Girth – The smallest circumferential dimension at the pistol grip. Applies to both rifles and shotguns.

Length of Pull – The distance from the center of the trigger to the center of the buttplate or recoil pad. Applies to both rifles and shotguns.

Length of Stock – The greatest dimension of the stock material. Applies to both rifles and shotguns.

Pitch – An expression used to indicate the relationship of the bore to the plane of the buttplate (pad). It is found by extending a line across the butt and drawing at right angles to this line an additional line through the highest point on the receiver or frame and measuring the distance from an extension of this line to a point at the base of the front sight bead. The pitch is said to be down if the described line is above the front sight and up if below. The pitch is normally down. Applies to shotguns, as pitch is not typically given for a rifle.

Stock Screw

A screw used for attaching the stock to the receiver or frame of a firearm. More than one may be used per firearm.

Stock Warpage

The twisting and bending of a wood stock due to moisture absorption.

Stop, Hand

An attachment beneath the forend or forearm of a target rifle to restrict the forward movement of the hand. Often used to attach a target sling.

Stoppage

This term is used when a firearm stops firing due to a malfunction of either the gun mechanism or ammunition. This term is normally used in connection with automatic firearms, machine guns, etc.

Stove-Pipe

A failure to eject in which the cartridge case is caught in the ejection port by the forward motion of the bolt or slide.

RETIRED

Straight Pull

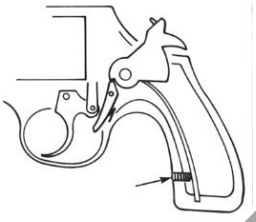
A bolt action firearm in which the bolt need not be rotated to open or close the action but is reciprocated by a straight backward and forward motion of the shooter's hand.

Strain Gauge

An electronic device to measure stress and strain in mechanical devices.

Strain Screw

A screw which bears against a leaf spring and will change the tension of the spring by its movement in or out.



Stray

Refer to **Flyer**.

Striae/Striations

Refer to **Toolmark, Striated**.

Striker

A rod-like firing pin or a separate component which impinges on the firing pin.

Striker Fire

A method of firing which involves the striker being held by a spring until released by the sear.

Striking Velocity

Refer to **Velocity, Striking**.

Stripper Clip

A metal device which may hold five to ten cartridges used to rapidly load the magazine of a pistol or rifle. In some rifles, clip guides are machined into the receiver to direct the clip into the proper position for magazine loading. The empty stripper clip may either be removed manually or ejected as the bolt closes. Also known as a **charger**.

Stripping

(1) The act of disassembling a firearm.
(2) The act of transferring cartridges from a loaded stripper clip to a magazine, or a magazine to a chamber.
(3) The failure of a bullet to engage the rifling when traversing down the barrel.

Strut

Refer to **Hammer Strut**.

Stud Trigger

A button-type trigger, the end of which is pressed to fire the firearm.

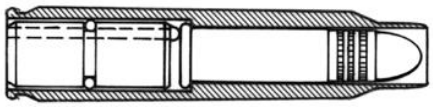
Subcaliber Ammunition

RETIRED

A cartridge that is smaller than standard for the firearm in which it is used, utilizing a subcaliber device.

Subcaliber Device

An adaptation to a firearm to enable the firing of smaller or lower-powered ammunition. Also known as an **adapter**, **auxiliary cartridge** or **auxiliary chamber**.



Subcaliber Tube

A tube which is placed in the bore of a firearm to enable the firing of smaller or lower-powered ammunition.

Subclass Characteristics

Features that may be produced during manufacture that are consistent among items fabricated by the same tool in the same approximate state of wear. These features are not determined prior to manufacture and are more restrictive than class characteristics.

Sub-Machine Gun

An automatic or selective fire firearm chambered for a pistol cartridge. These firearms are normally compact, and intended to be used at close combat ranges.

Suigi Finish

A wood finish which is achieved by scorching to give a darkened and grainy appearance.

Swage

To form metal under pressure. Swaging is normally performed in a press, using a punch or die.

Swaged Bullet

A bullet that has been shaped by pressing and forming the bullet material in a die.

Swaged Choke

Refer to **Choke, Swaged**.

Swaged Rifling

Refer to **Rifling Methods – Hammer Forged Rifling** in the **Manufacturing Terminology Section** of the appendix.

Swivel

Metallic, elongated loops attached to rifles or shotguns through which a sling may be fastened.

Swivel Bow

That part of a swivel which is in the form of an elongated loop and to which a sling or carrying strap is passed through or otherwise attached.

Swivel, Quick Detachable

A two part sling swivel which has a stud that is attached to the stock or barrel and a bow portion which is mounted on a spring plunger arrangement. The plunger passes through a hole in the stud

RETIRED

for attachment of the bow to the firearm. They are sometimes called Q.D. Swivels, and allow for quick mounting and dismounting of a sling from a firearm.

Sympathetic Firing

The simultaneous firing of two or more cartridges chambered in the cylinder of a revolver, one of which is in battery.

Take Down

(1) To disassemble a firearm for cleaning, repair, or transportation. Also known as stripping or field stripping.

(2) A type of firearm designed for ease of disassembly and transportation.

Takedown Latch

Refer to **Takedown Lever**.

Takedown Lever

A catch device found on many firearms to facilitate disassembly (e.g., Sauer 38H and Mauser HSc pistols). Also known as a takedown latch.

Takedown Screw

A screw found in many firearms to facilitate disassembly.

Tang

A rearward projecting tongue on a receiver or frame to which the buttstock is attached. Some parts of the operating mechanism of a firearm may also pass through the tang. A firearm may have an upper tang, a lower tang, or both.

Tang Safety

A type of safety in which the external control component is mounted on the upper receiver tang of a firearm.

Tang Screw

The screw or screws passing through either one or both tangs by which the stock or trigger guard is attached to the receiver or frame.

Tarage Table

A mathematical table which correlates the amount of compression of a copper or lead crusher to the chamber pressure testing firearm.

Target, Clay

A circular, domed, frangible disc used as an aerial target for shotgun shooting games. Originally formed out of clay, modern "clay" targets are generally made from a formulation of pitch and limestone. Dimensions and weights are regulated by skeet and trap shooter's associations. Also called clay bird or clay pigeon.

Target Hammer

A firearm hammer having a wider spur designed for convenient and rapid cocking.

Targeting

The act of shooting a firearm to align sights. Also known as sighting in.

Target Rifle

RETIRED

Any rifle designed and equipped for match or target shooting.

Target Shooting

The act of shooting at inanimate objects. "Formal" target shooting is done at specified distances at targets designated for scoring. "Informal" target shooting is done at varying distances at impromptu targets for practice. This informal target shooting is also known as plinking.

Target Sling

A special form of strap to aid target shooting.

Target Trigger

(1) A trigger which provides a large bearing surface for the trigger finger.
(2) A trigger mechanism which provides for adjustment of such characteristics as pull force, travel, or overtravel.
(3) A relatively light, crisp trigger designed to assist in accurate shooting.

Tattooing

Refer to **Powder Tattooing** in the **Gunshot Wound Terminology Section** of the appendix.

T-Bolt

(1) A patented reciprocation and locking system for use on a rimfire rifle.
(2) Sometimes used to describe straight pull bolt action rifles with a horizontally protruding straight bolt handle.

Telescope

A sight containing optical elements which magnify or enlarge the target.

Telescope Mount

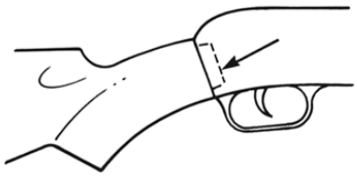
A device to hold a telescope on a firearm. Frequently abbreviated as scope mount.

Temperature of Ignition

The lowest temperature to which the surface of material must be raised for the combustion of the material to become self-sustaining.

Tenon

(1) A projecting wood component of a firearm that fits into the receiver or frame.
(2) The portion of the rimfire bullet which fits into the brass case; from bullet base to bottom of band. Also known as the heel.



Terminal Ballistics

Refer to **Ballistics, Terminal**.

Terminal Energy

Refer to **Energy, Terminal**.

Terminal Velocity

RETIRED

Refer to **Velocity, Terminal**.

Test Barrel

Refer to **Barrel – Test Barrel**.

Test Bullet

A bullet fired into a bullet recovery system in a laboratory for comparison or analysis.

Test Cartridge Case

A cartridge case obtained while test firing a firearm in a laboratory to be used for comparison or analysis.

Test Fire

To discharge a firearm in a laboratory or controlled setting in order to obtain representative bullets and cartridge cases for comparison or analysis, to determine functionality of the firearm, or to produce gunshot residue or shot patterns at known distances.

Theory of Identification as it Relates to Toolmarks

1. The theory of identification as it pertains to the comparison of toolmarks enables opinions of common origin to be made when the unique surface contours of two toolmarks are in "sufficient agreement."
2. This "sufficient agreement" is related to the significant duplication of random toolmarks as evidenced by the correspondence of a pattern or combination of patterns of surface contours. Significance is determined by the comparative examination of two or more sets of surface contour patterns comprised of individual peaks, ridges and furrows. Specifically, the relative height or depth, width, curvature and spatial relationship of the individual peaks, ridges and furrows within one set of surface contours are defined and compared to the corresponding features in the second set of surface contours. Agreement is significant when the agreement in individual characteristics exceeds the best agreement demonstrated between toolmarks known to have been produced by different tools and is consistent with agreement demonstrated by toolmarks known to have been produced by the same tool. The statement that "sufficient agreement" exists between two toolmarks means that the agreement of individual characteristics is of a quantity and quality that the likelihood another tool could have made the mark is so remote as to be considered a practical impossibility.
3. Currently the interpretation of individualization/identification is subjective in nature, founded on scientific principles and based on the examiner's training and experience.

RETIRED

Thicket Load

Refer to **Load – Scatter Load**.

Throat

Refer to **Chamber Throat**.

Through Bolt

A long bolt extending through the shoulder stock and threaded into the frame.

Thumb Rest

A ledge in the grip area of a rifle or handgun on which to rest the thumb of the trigger hand.

Thumb Safety

A type of manual safety in which the external control component is located for convenient operation by the thumb of the trigger hand.

Time of Flight
The total elapsed time that a projectile requires to travel a specific distance from the muzzle.

Timing
An industry term referring to the mechanical process of rotating the cylinder of a revolver.

Tipping
The flight characteristics of a projectile resulting in its entering the target at an angle to its trajectory characterized by an oblong or oval hole in a target. Also known as keyhole.

Tip-up Pistol
(1) A pistol that is hinged near the rear sight that allows the barrel to tip up at the muzzle, exposing the chamber(s) for loading or unloading (e.g., Smith & Wesson Model 1).
(2) A pistol that is hinged near the muzzle that allows the barrel to tip up at the breech, exposing the chamber for loading or unloading (e.g., Beretta model 21 Bobcat).

Toe
The bottom (lower) end of a buttplate and adjacent portion of the stock on a shoulder firearm.

Tong Tool
An unmounted, portable handloading tool using a "nutcracker" or plier action for handloading centerfire cartridges. Also known as a nutcracker tool.

Toolmark, Impressed
Contour variations on the surface of an object caused by a combination of force and motion where the motion of the tool is approximately perpendicular to the plane being marked. The class characteristics (shape) can indicate the type of tool used to produce the mark. These marks may contain class, subclass, and/or individual characteristics of the tool producing the marks. Also known as compression marks.

Toolmark, Striated
Contour variations, generally microscopic, on the surface of an object caused by a combination of force and motion where the motion of the tool is approximately parallel to the plane being marked. Friction marks, abrasion marks, and scratch marks are terms commonly used when referring to striated marks. These marks may contain class, subclass, and/or individual characteristics of the tool producing the marks.

Top Break
Any firearm on which the barrel or barrels are allowed to tip down at the muzzle, exposing the chamber(s) for loading or unloading.

Top Extension
A projection which extends behind the breech end of the barrels on some break open firearms. When the firearm is closed, it fits into a corresponding recess slot in the frame and is used for locking purposes.

Top Lever
The lever located on top of the breech end of hinged frame firearms. When pushed to one side, the lever will unlock the barrel(s) from the frame and allow it to be moved. Also known as top snap.

Top Snap
Refer to **Top Lever**.

RETIRED

Top Strap

That portion of a solid frame revolver which passes over the cylinder.

Top Wad

Refer to **Overshot Wad**.

Trace Evidence

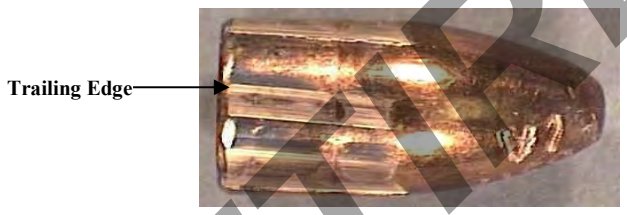
Physical evidence of a microscopic or submicroscopic size which, due to its small size, is deposited on or transferred to one or more objects without being manifestly apparent at the time of transfer or deposition.

Tracer Bullet

A bullet that has a burning compound in its base which permits observation of its flight.

Trailing Edge

The trailing edge of a bullet fired from a gun with a right twist is the left edge of the land impression, or the right edge of the groove impression. The trailing edge of a bullet fired from a gun with left twist is the right edge of the land impression or the left edge of the groove impression. Also known as following edge when used in conjunction with the term leading edge. Refer to **Driving Edge**.



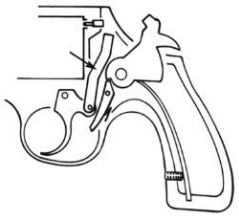
Photograph courtesy of Scott Doyle –http:// www.firearmsid.com

Transducer, Piezoelectric Pressure

A device which generates an electrical charge that is proportional to the pressure applied to its crystal element. This device may be used by ammunition and firearm manufacturers in proof testing barrels and ammunition.

Transfer Bar

An intermediary piece of metal which usually reciprocates with the trigger or hammer of a revolver to transfer the energy of the hammer to the firing pin. When the transfer bar is not in battery with the firing pin, the hammer cannot touch the firing pin (e.g., Ruger New Model single action and double action revolvers).



Trap Door Action

Refer to **Action – Trap Door Action**.

Trap Door Buttplate

A hinged closure plate in the butt end of a firearm. Opening of the trap door exposes a recess in the stock which can be used for storage.

RETIRED

Trap, Electrical

An electro-mechanical device for throwing clay targets.

Trap Gun

A shotgun specifically designed for the game of trap shooting.

Trap, Hand

A small, hand operated device for throwing clay targets.

Trap, Mechanical

A mechanically operated device for throwing clay targets.

Trap Shooting

A shotgun target sport in which clay targets are thrown away from the shooter by a reciprocating trap located forward of the firing line. May also be known as trapshooting.

Trigger

That part of a firearm mechanism that is moved manually to cause the firearm to discharge. The following are various types of triggers:

Adjustable Trigger – Any trigger mechanism that has adjustable features.

Crisp Trigger – A trigger that releases the firing mechanism cleanly, suddenly, and unpredictably as pressure is applied. Also known as a clean break trigger [25].

Double Trigger – A term used for firearms having two barrels and a separate trigger for the discharge of each barrel. This term does not apply to firearms with double set triggers.

Double Pull Trigger –A two stage trigger pull wherein slack must be taken up before the resistance of the sear is encountered (e.g., Mauser Model 98.) Also known as a two-stage trigger.

Double Set Trigger – An arrangement of two triggers in which the actuation of one trigger presets the second, resulting in a lighter trigger pull of the second trigger [25].

Folding Trigger – Refer to **Hinged Trigger**.

Hair Trigger – (1) A slang term for a trigger requiring very low force to actuate.
(2) The light pull of a second trigger in a double set trigger mechanism.

Hinged Trigger – A trigger which is hinged to fold forward.

Inertia Trigger – A firearm having two barrels and a single selective trigger wherein the inertia resulting from the recoil forces of firing one barrel causes the trigger sear to switch to the unfired barrel.

Non-Selective Single Trigger – A single trigger on double-barrel guns which fires the barrels in a fixed sequence.

Release Trigger – An unconventional mechanism in which the firearm is fired by the release of rather than the pull of the trigger. Pulling the trigger sets the sear, which cocks the hammer (or striker). Releasing the trigger allows the hammer (or striker) to discharge the firearm.

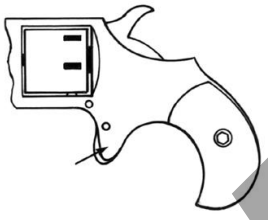
RETIRED

Ring Trigger – A ring-shaped trigger.

Selective Single Trigger – An arrangement on double-barrel firearms having a single trigger which enables the shooter to choose the barrel to fire first. The mechanism will then subsequently switch to the unfired barrel, which can be fired with a second pull of the trigger.

Set Trigger – A trigger that can be engaged with the sear so that a very light pull will release the trigger. This is performed by means of levers or small bars. May be either a single set or double set trigger [25].

Sheath Trigger – A trigger that has a minimal projection from the frame and is not usually surrounded by a trigger guard. Also known as a spur trigger.



Single-Double Trigger – A type of trigger mechanism found on some double-barrel shotguns consisting of two triggers. The front trigger functions as a conventional non-selective single trigger, while the rear trigger will fire only the barrel with the tighter choke.

Single Trigger – A trigger mechanism for multiple barrel firearms wherein repeated pulls of one trigger fires the barrels successively.

Single Set Trigger – A trigger system that permits setting the trigger by means of lever or bars, thus engaging it with the sear so that a very light trigger pull fires the gun. The trigger is typically set by pushing it forward until the engagement is felt and heard. Firing is accomplished by pulling the trigger rearward [25].

Single Stage Trigger – (1) A trigger mechanism in which the trigger travel is relatively short and there is no significant, perceptible change in pull force from the beginning of movement up to the point of firing. (2) A trigger which releases with a single pull.

Spur Trigger – Refer to **Sheath Trigger**.

Target Trigger – (1) A trigger mechanism which provides for adjustment of such characteristics as pull force, travel, or overtravel. (2) A relatively light, crisp trigger designed to assist in accurate shooting. (3) A trigger which provides a large bearing surface for the trigger finger.

Thumb Trigger – A trigger mechanism actuated by pressing the thumb of the trigger hand on a button located directly behind the bolt.

Twin-Single Trigger – A non-selective, double trigger arrangement used on some double-barrel firearms. The barrels are fired sequentially with each pull of one or both triggers.

Trigger Bar

A connecting piece between the trigger and the sear.

Trigger Creep

The perceptible movement of the trigger after takeup or pretravel prior to sear release. Refer to **Trigger Takeup**.

RETIRED

Trigger Group

A number of components within the firearm assembly that work together to release the hammer/striker when the trigger is pulled. Also known as a trigger assembly or trigger mechanism.

Trigger Guard

A rigid loop which partially surrounds the trigger to prevent damage or an accidental discharge.

Trigger Lock

Refer to **Safety, Manually Operated**.

Trigger Overtravel

Refer to **Overtravel**.

Trigger Plate

(1) A metal plate fitted to the lower part of some frames through which the trigger(s) pass.
(2) A component attached to the frame or receiver of some firearms which supports the major components of the firing mechanism and may have the trigger guard as an integral part.

Trigger Pull

The amount of force which must be applied to the trigger of a firearm to cause sear release. It is measured by hanging weights or an instrument touching the trigger at a point where the trigger finger would normally rest. The force applied during measurement is approximately parallel to the bore axis.

Trigger Puller

An instrument used to accurately measure the trigger pull of a firearm. Examples include standard weights, spring gauges, and mechanical/digital devices. Also known as a trigger tester.

Trigger Shoe

An accessory which is attached to the trigger to give a larger, non-slip bearing surface. Also refer to **Target Trigger**.

Trigger Slack

Refer to **Trigger Takeup**.

Trigger Stop

(1) A device to prevent overtravel of the trigger.
(2) A device to prevent certain firearms from being fired until the finger lever is closed.

Trigger Stud

A button-type trigger mechanism in which the end of which is pressed to fire the firearm.

Trigger Takeup

The initial distance the trigger moves prior to sear movement. Also known as pretravel and trigger slack.

Trip

A part of the mechanism of some firearms which is released by the action of the trigger.

RETIRED

Try-Gun

A shoulder firearm with an adjustable stock that can be customized to the shooter's physical characteristics.

Tube

The cylindrical body of a shotshell.

Tumbling

The end-over-end rotation of an unstable projectile in flight. May also be referred to as bullet tipping, keyholing, or yaw.

Twelve-Twenty Burst

A dangerous situation where a 20-gauge shotshell is inserted into the chamber of a 12-gauge shotgun followed by a 12-gauge shotshell. The 20-gauge shotshell represents a serious obstruction at the forcing cone when the 12-gauge shotshell is discharged. This obstruction will produce a 'ringing' of the barrel in this area at a minimum or a bursting of the barrel in this area.

Twist

The direction of turn of the rifling helix, such as left hand or right hand twist.

Twist Rate

The rate at which the rifling of the firearm turns within the bore. This is normally expressed as the distance required for the rifling (and projectile) to make one complete revolution. Depending on the origin of the firearm, this may be written in inches or in millimeters (e.g., 1 turn in 12 inches or 1 turn in 305mm).

Ullage

Refer to **Air Space**.

Under Lever

A lever found on some hinged frame guns which is located on the bottom of the receiver. When the lever is moved to the unlocked position, it allows the barrel(s) to pivot for loading or unloading.

Underbolt

The moveable lock of some break-open firearms. It typically engages the barrel lug (underlug) and is actuated by the top lever.

Underlocking Lug

The downward projecting lug(s) at the breech end of a hinged frame firearm used for locking and barrel positioning in the frame.

Universal Receiver

Refer to **Receiver, Universal**.

Unload

(1) The complete removal of all ammunition from a firearm and/or magazine.
(2) The disassembly of a cartridge or shotshell.

Unserviceable

A firearm which is no longer fit for firing for any reason.

RETIRED

Variable Choke

Refer to **Choke, Variable**.

Varmint Rifle

A sporting rifle designed for long range small game hunting. Typically made with a heavier barrel and of a small caliber for high velocity projectiles with a flat trajectory.

Vector Ammunition

A unique line of illuminating projectiles manufactured by the Hornady company that utilized an igniter composition followed by a fine zirconium wire centered in the lead core of these open-based pistol bullets. These special cartridges are no longer available.

Velocity

The speed of a projectile at a given point along its trajectory.

Velocity, Instrumental

The velocity of a projectile(s) that is recorded by suitable instrumentation located at a predetermined distance from the muzzle of a test barrel or a firearm. In reference to determining instrumental velocity using a chronograph, the instrumental velocity is the average velocity between the two screens, as measured by the instrument.

Velocity, Muzzle

The velocity of a projectile as it exits the muzzle of a firearm.

Velocity, Remaining

The velocity of a projectile at any point of its trajectory.

Velocity, Striking

The velocity of a projectile upon impact.

Velocity, Summit

The velocity of a projectile at the highest point of the trajectory.

Velocity, Terminal

(1) In popular use, refers to the striking velocity.

(2) In small arms ballistics, it is the constant speed of a projectile after air drag and gravitation pull have reached the point where the two forces cancel each other's effect on the projectile [25].

Vented Test Barrel

A vented barrel used by ammunition manufacturers to provide ballistic data of revolver ammunition. The barrel takes into account cylinder gap, barrel length, powder position, production tolerances, and allows for reasonable wear and tear.

Ventilated Choke

Refer to **Choke, Ventilated**.

Ventilated Rib

Refer to **Rib, Ventilated**.

Vernier Caliper

A measuring instrument having a fixed jaw, a sliding jaw, and a point that slides along an attached scale.

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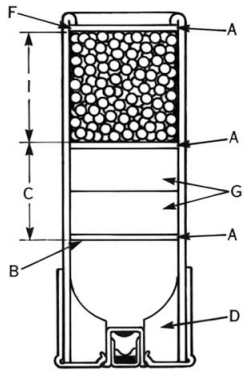
Wad

A felt, paper, cardboard, or plastic component used in a shotshell for various purposes. The following are various types of wads. Refer to illustration below for many of the wad definitions.

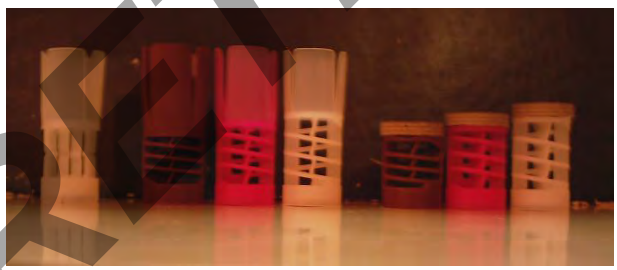


Base Wad – A cylindrical component that is assembled into the head end of a shotshell. Refer to letter D in the illustration above.

Card Wad – A thin card-like disc placed over powder or shot in shotshells. Also refer to overshot wad, undershot wad, and overpowder wad. Refer to letter A in the illustration above.

Column Wad – The wads between propellant and projectiles in a shotshell. Refer to letter C in the illustration above.

Combination Wad – A one piece plastic wad which combines the function of some or all of the following: shot protector, filler wad, and overpowder wad.



Cup Wad – A powder and shot separator of very shallow cup design. When loaded with lips down, the wad acts to help seal powder gases and to protect the rear of the shot column.

Filler Wad – Wads of various shapes (typically circular) and various thicknesses used to adjust the volume of the contents of a shotshell. These disks may be constructed of various materials such as paper, cardboard, fiber, or plastic. Refer to letter G in the illustration above.

Nitro Wad – An unlubricated, overpowder wad used with smokeless powder that is constructed of cardboard or felt. Also known as a nitro card wad or **overpowder wad**.

Overshot Wad – The closure disc over the top of the shot column held in place by a rolled crimp. Refer to letter F in the illustration above. Also known as a top wad.

Overpowder Wad – A plastic or fiber spacing disk positioned immediately over the powder charge in a shotshell to separate the propellant from other components within the shotshell. Refer to letter B in the illustration above.

RETIRED

Shot Protector Wad – Various designs of shot cups made of plastic and designed to reduce pellet deformation during barrel travel. Also known as a **shot cup**.

Top Wad – Refer to **Overshot Wad**.

Undershot Wad – A type of card wad placed directly under the shot within the shotshell.

Wadcutter

Refer to **Bullet – Wadcutter Bullet**.

Wad Pressure

The force applied to a wad column as it is sealed firmly against the propellant.

Wadcutter, Semi-

Refer to **Bullet – Semi-wadcutter Bullet**.

Walking Barrel

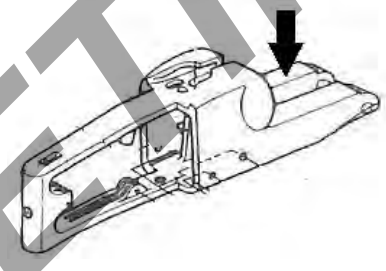
Refer to **Barrel, Walking**.

Warming Shots

Preliminary shots performed before firing for velocity, pressure, accuracy, etc.

Water Table

The flat portion of the frame on break-open firearms which extends forward from and is approximately at right angles to the standing breech face. This is the surface on which barrel flats rest when the gun is closed. May also be referred to as action bar flats.



Photograph obtained from http://www.gun-parts.com[1]

Web

(1) The solid portion of a brass centerfire cartridge case between the inside of the case at the head end, and the bottom of the primer pocket.
(2) This word also refers to the smallest dimension of a smokeless powder granule.

Wedge

A wedge shape device found on many percussion, and a few modern firearms. It holds the barrel assembly to the frame or stock (e.g., Colt 1851 Navy and Steyr Model 1912 pistol).

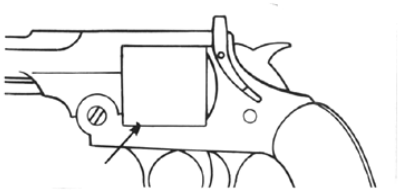
Wildcat Cartridge

Refer to **Cartridge – Wildcat Cartridge**.

Window

That area in the frame of a revolver into which the cylinder fits.

RETIRED



Wing Safety

Refer to **Safety, Manually Operated**.

Witness Mark

A line on each of two mating parts which must be an extension of each other when assembled to indicate proper alignment. Also known as a draw line or an index mark.

Witness Panel

Any one of a variety of materials such as thin cardstock or poster board positioned and mounted in such a way so as to "witness" or record the position and orientation of a perforating projectile(s). Cardstock witness panels are also used to record pellet patterns from shotgun discharges at selected ranges. The patterns of gunshot residue deposits are also recorded on witness panels of selected materials for this purpose.

Work Hardened

A change in the grain structure of a metal as a result of repeatedly stressing it. In cartridge cases, work hardening most frequently occurs in and around the neck area, from the stresses of repeated firings and resizing. This causes brittleness and leads to cracking and splitting of the case.

Wood's Metal

An alloy consisting of 50% bismuth, 25% lead, 12.5% tin, and 12.5% cadmium. It melts at approximately 150 degrees Fahrenheit (65 degrees Celsius) and can be used for making castings of chambers, barrels, etc.

Wound Ballistics

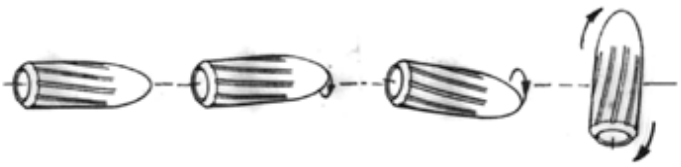
A special case in terminal ballistics dealing with the behavior of projectiles in tissue and tissue simulants. It includes bullet performance, penetration characteristics, and velocity loss as a consequence of the perforation of tissue and tissue simulants.

Wrist

The portion of a rifle or shotgun stock located behind the action which is normally grasped by the shooter's trigger hand.

Yaw

The angle between the longitudinal axis of a projectile and the line of the projectile's trajectory. Yaw is usually considered to exist before a bullet achieves full gyroscopic stability.



Yaw Card

A material such as cardstock that is selected to faithfully record the outline and orientation of a perforating projectile. Also refer to **Witness Panel**.

Yoke

RETIRED

The part of a solid frame revolver on which the cylinder is swung out to the side to accomplish loading and ejecting. Also known as a **crane**.

Zero

The adjustment of a firearm's sights in order to obtain impact at a desired point in relation to a specific point of aim at a given range.

Zwilling

European term for a double barreled shoulder arm with one rifled and one smooth-bored barrel.

RETIRED

Section 2 - Toolmarks

Abrasion Marks
Refer to **Toolmark, Striated**.

Bunter
The die which produces the headstamp on rimfire cartridge cases or the headstamp and primer pocket on centerfire cartridge cases.

Cam
A rotating or sliding piece (such as an eccentric wheel or a cylinder with an irregular shape) in a mechanical linkage used to give motion or locking action to another part.

Cast
The reproduction of a toolmark or the surface of a tool utilizing a molding material such as plaster of Paris, silicone rubber, etc. The cast of a toolmark is a positive cast. The cast of the surface of a tool is a negative cast.

Casting
The process of pouring a liquid or suspension into a mold to produce an object of desired shape.

Class Characteristics
Measurable features of a specimen which indicate a restricted group source. They result from design factors, and are therefore determined prior to manufacture.

Die
Any of various tools used to impart a specific shape to a given material.

Draw Marks
Refer to **Extrusion Marks**.

Drift Punch
A tool used for removing pins and for aligning holes.

Extrusion Marks
Longitudinal striations occurring on an object which was produced by being forced through a die or draw punch during fabrication. They are commonly found on wire and cartridge cases. Also known as draw marks.

Fracture Match
The examination of two or more objects either through physical, optical, or photographic means which permits one to conclude whether the objects were either one entity or were held or bonded together in a unique arrangement. Also known as a physical match.

Friction Marks
Refer to **Toolmark, Striated**.

Identifiable Striae
Striations in the evidence mark which can be identified with reproduced striations in the test marks. Refer to **Toolmark, Striated**.

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Impression

Refer to **Toolmark, Impressed**.

Index Mark

A line on each of two mating parts which must be an extension of each other when assembled to indicate proper alignment. Also known as draw mark, draw line, or witness mark.

Individual Characteristics

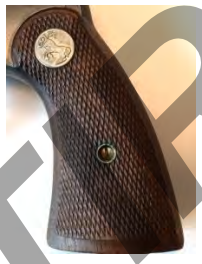
Marks produced by the random imperfections or irregularities of tool surfaces. These random imperfections or irregularities are produced incidental to manufacture and/or caused by use, corrosion, or damage. They are unique to that tool to the practical exclusion of all other tools.

Jig

A mechanical device that holds the correct position relationship between a piece of work and a tool or two pieces of work.

Knurling

A series of regular ridges or diamonds cut or impressed into a surface to help prevent slipping. Used on tool handles, jaws, etc.



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Mandrel

A metal rod or bar used as a core around which metal, wire, etc., is cast, molded, forged, or shaped.

Opposed Blade Cutting Tool

Refer to **Tool – Pinching** and **Tool – Shearing**.

Photomicrograph

A photograph taken through a microscope.

Physical Match

Refer to **Fracture Match**.

Practical Impossibility

A phrase, which currently cannot be expressed in mathematical terms, that describes an event that has an extremely small probability of occurring in theory, but which empirical testing and experience has shown will not occur. In the context of firearm and toolmark identification, "practical impossibility" means that based on 1) extensive empirical research and validation studies, and 2) the cumulative results of training and casework examinations that have either been performed, peer reviewed, or published in peer-reviewed forensic journals, no firearms or tools other than those identified in any particular case will be found that produce marks exhibiting sufficient agreement for identification.

Range of Conclusions Possible When Comparing Toolmarks

The examiner is encouraged to report the objective observations that support the findings of toolmark examinations. The examiner should be conservative when reporting the significance of these observations.

Identification:

Agreement of all discernible class characteristics and sufficient agreement of a combination of individual characteristics where the extent of agreement exceeds that which can occur in the comparison of toolmarks made by different tools and is consistent with the agreement demonstrated by toolmarks known to have been produced by the same tool.

Inconclusive:

A. Agreement of all discernible class characteristics and some agreement of individual characteristics, but insufficient for an identification.
B. Agreement of all discernible class characteristics without agreement or disagreement of individual characteristics due to an absence, insufficiency, or lack of reproducibility.
C. Agreement of all discernable class characteristics and disagreement of individual characteristics, but insufficient for an elimination.

Elimination:

Significant disagreement of discernible class characteristics and/or individual characteristics.

Unsuitable:

Unsuitable for examination.

Scratch Marks

Refer to **Toolmark, Striated**.

Serration

Narrow parallel grooves cut into a surface to: 1) provide a gripping surface (e.g., the gripping surface of a slide or rough surface of a firearm hammer) or 2) to break up light reflections (e.g., front sight).

Striae/Striations

Refer to **Toolmark, Striated**.

Subclass Characteristics

Features that may be produced during manufacture that are consistent among items fabricated by the same tool in the same approximate state of wear. These features are not determined prior to manufacture and are more restrictive than class characteristics.

Test Mark

A striated or impressed toolmark produced by the suspect tool which is to be used in making a comparison with the evidence mark.

Theory of Identification as it Relates to Toolmarks

1. The theory of identification as it pertains to the comparison of toolmarks enables opinions of common origin to be made when the unique surface contours of two toolmarks are in "sufficient agreement."
2. This "sufficient agreement" is related to the significant duplication of random toolmarks as evidenced by the correspondence of a pattern or combination of patterns of surface contours. Significance is determined by the comparative examination of two or more sets of surface contour patterns comprised of individual peaks, ridges and furrows. Specifically, the relative height or

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depth, width, curvature and spatial relationship of the individual peaks, ridges and furrows within one set of surface contours are defined and compared to the corresponding features in the second set of surface contours. Agreement is significant when the agreement in individual characteristics exceeds the best agreement demonstrated between toolmarks known to have been produced by different tools and is consistent with agreement demonstrated by toolmarks known to have been produced by the same tool. The statement that "sufficient agreement" exists between two toolmarks means that the agreement of individual characteristics is of a quantity and quality that the likelihood another tool could have made the mark is so remote as to be considered a practical impossibility.

3. Currently the interpretation of individualization/identification is subjective in nature, founded on scientific principles and based on the examiner's training and experience.

Tool

An object used to gain mechanical advantage. Also thought of as the harder of two objects which, when brought into contact with each other, results in the softer object being marked by the harder object. May be broken down into tool types as follows:

Chopping – a single blade tool accompanied by compression forces (e.g., axes, butcher cleaver).

Compression – tool designed to impress or compact by pressure or striking (e.g., hammers, die stamps).

Crimp – tool with a pair of interactive blades on the same plane (i.e., opposing) that are designed to bend, crease, or press together (e.g., wire crimps, bank seal press).

Prying – a bladed tool, either squared or tapered, usually designed to pry or perform a special function (e.g., tire iron, screwdriver, pry bar).

Gripping – tool with a pair of interactive jaws on the same plane (i.e., opposing) that abut each other and is designed to grasp or squeeze (e.g., vise grips, pipe wrenches).

Pinching – a tool with sharpened opposing jaws which cut with a pinch-pinching action. The various designs include the following:

Center Cut – a design in which the apex of the blades' sharpened bevels are centered on one another and cut as they meet along the edges. The cutting edges are not intended to pass one another (e.g., diagonal cutting pliers, bolt cutters, pliers with side cutters).

Clipper Cut – a design in which the blades have a single bevel and cut as the edges meet. The cutting edges are not intended to pass one another (e.g., flush cutters, diagonal cutting pliers, linesman pliers, wire stripper, bolt cutters).

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Anvil and Blade – a design in which there is only one cutting blade with a bevel. The opposing jaw has a surface that acts as an anvil for the cutting blade. The apex of the cutting edge may stop on (center cut design), stop in (clipper cut design), or pass the anvil (shear design).

Anvil and Center Cut blade

Anvil and Clipper Cut blade

Anvil and Shears blade

Sawing – a multiple-bladed tool on the same line or plane used to remove material in a continuous or reciprocating action (e.g., hand saws, circular saw).

Shearing – opposed jaw cutting tool with two interactive working edges where one cuts and the other acts as an anvil. On tools with a pair of designed cutting blades, these blades are on adjacent planes and pass by each other. Each blade of the shear has only one sharpened bevel leading to the apex of the cutting edge (e.g., scissors, tin snips).

Slicing – tool with at least one sharp (tapered) blade designed to cut by moving the blade in the direction of the cut by applying force on the backside (opposite) of the working edges (e.g., knives, razors).

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Toolmark Identification

Toolmark identification is a discipline of forensic science which has as its primary concern to determine if a toolmark was produced by a particular tool.

Toolmark, Impressed

Contour variations on the surface of an object caused by a combination of force and motion where the motion of the tool is approximately perpendicular to the plane being marked. The class characteristics (shape) can indicate the type of tool used to produce the mark. These marks may contain class, subclass, and/or individual characteristics of the tool producing the marks. Also known as compression marks.

Toolmark, Striated

Contour variations, generally microscopic, on the surface of an object caused by a combination of force and motion where the motion of the tool is approximately parallel to the plane being marked. Friction marks, abrasion marks, and scratch marks are terms commonly used when referring to striated marks. These marks may contain class, subclass, and/or individual characteristics of the tool producing the marks.

Witness Mark

Refer to **Index Mark**.

Section 3 - Gunshot Residue and Gunpowder

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Acetic Acid

An organic acid used in the Modified Griess test for gunpowder residue detection. Acetic acid forms nitrous acid in reacting with nitrites in gunpowder residues. The nitrous acid reacts with the other constituents in the reagent to form an azo-dye.

Acetone

An organic solvent that may be used to dissolve gunpowder.

Alpha Naphthylamine (1-Naphthylamine)

A chemical reagent used in the Griess (Walker) test for gunpowder residues. This reagent supplies the amine needed for the formulation of the azo-dye showing the presence of nitrites. (Caution: This reagent has been found to be carcinogenic in concentrations above one percent.)

Alpha-naphthol

A chemical reagent combined with sulfanilic acid used to treat desensitized photographic paper for sensitivity to nitrite compounds used in the Modified Griess test.

Antimony

A brittle, metallic element with the chemical symbol Sb. It is frequently used to harden lead by alloying the two metals. When used in the manufacture of lead shot it is said to be "chilled."

Antimony Sulfide

A component of most priming mixtures that serves as the fuel.

Atomic Absorption (AA)

A method of qualitative and quantitative elemental analysis wherein the element which is being analyzed must be dissociated from its chemical bonds after which the atoms of this element will absorb radiation of an energy specific for that element. The amount of energy absorbed is proportional to the concentration of that element in the specimen. The main application in forensic science is to analyze cotton swabs for elemental components of primer mixtures when testing hands or other objects for gunshot residues; however, this method has nearly become obsolete due to the use of the scanning electron microscope and x-ray analysis of primer residues.

Azo Dye

A nitrogen bearing dye that is utilized by the Modified Griess test to allow the visualization of nitrite patterns produced from gunpowder residues. The dye is a bright orange/red color.

Ballistite

A type of smokeless powder made of nitrocellulose dissolved in nitroglycerin.

Barium Nitrate

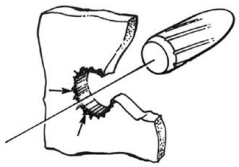
A component of most priming mixtures which acts an oxidizer.

Brisance

A term describing the shattering power of high explosives.

Bullet Wipe

The discolored area on the immediate periphery of a bullet hole, caused by the transfer of residues from the bearing surface of the bullet. These dark gray to black residues typically contain carbon, lead, bullet material, and possibly other constituents such as bullet lubricant and primer residues. Bullet wipe may occur at any range of fire. Also known as burnishing or leaded edge.



C-Acid

2-Naphthylamine-4, 8 disulfonic acid. Chemical reagent proposed by Joseph T. Walker for use with desensitized photographic paper in spatial detection of gunpowder residues.

Calcium Silicide

A component of some priming mixtures that serves as the fuel.

Cap

(1) An obsolete term referring to a primer.
(2) Refer to **Percussion Cap**.

Charge

The amount, by weight, of a component of a cartridge (e.g., priming weight, propellant weight, shot weight).

Charge, Maximum

A charge weight, in grains, of a particular propellant that may be used with other specified ammunition components without exceeding the safe, maximum, allowable pressure limit for the specific cartridge being loaded.

Charge, Nominal

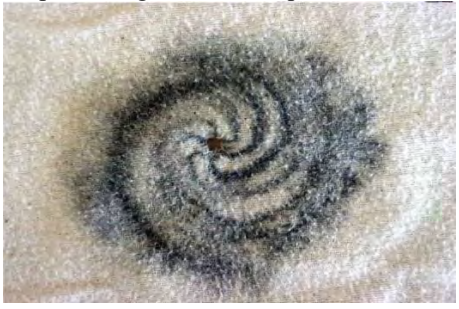
A typical charge weight of a specific powder for a specific combination of components.

Charge, Reduced

A less than nominal powder charge.

Comet Tails

A distinct pattern of particulate lead which may contain one or more separate fan-shaped (semi-circular) swirls around the axis of the bullet hole. Lead particles originate from the core of jacketed bullets and are expelled by centrifugal force when fired. Also known as the vortex effect. The photograph below is a complete 6-right comet tail pattern on cloth.



Photograph from Thompson, E. and Haag, L., "Comet Tail/Vortex Effect", AFTE Journal, Vol. 41, No. 3, pp. 257-267.

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Cylinder Flare

The circular gray-to-black deposit around the front margin of the chamber(s) of a revolver composed of gunshot residues deposited during the firing process. Also known as a **smoke ring**, halo, or simply flare.

Deflagration

A rapid combustion reaction which is propagated at a sub-sonic rate by heat transfer into the reacting material. This reaction is accompanied by a vigorous evolution of heat and flame. The flow of reaction products is in the direction opposite that of the combustion propagation (unlike detonation). Deflagration is usually dependent upon having fuel and oxidizing agent in very close contact, either from having the fuel as a finely divided mixture with the oxidant (e.g., black powder), or by combining the two in the same chemical compound or mixture (e.g., nitrocellulose propellants). Deflagration exhibits a dependence upon the surrounding gas pressure.

Dermal Nitrate Test

Refer to **Diphenylamine Test.**

Detonate

To explode with sudden violence. A low explosive (e.g., black or smokeless powder) will deflagrate whereas a high explosive (e.g., TNT) will detonate violently by a sudden chemical change resulting in a brisant explosion.

Dibutylphthalate

An organic compound used as a coating on some gunpowder to retard the burning rate.

Diethyldiphenylurea (ethyl centralite)

An organic compound used as a coating on some gunpowder to retard the burning rate.

Dinitrotoluene (DNT)

An explosive used as a coating on gunpowder to retard the burning rate and to act as a moisture proofing agent.

Diphenylamine

(1) Chemical reagent used in solution with sulfuric acid and acetic acid in the dermal nitrate/diphenylamine test.

(2) Diphenylamine is also used in smokeless powder as a stabilizer to inhibit decomposition.

Diphenylamine Test

An obsolete and unreliable test to determine if a person has fired a firearm; however, a variation of this test may still be used as a presumptive test for identification of gunpowder. The test consists of treating paraffin casts taken of the person's hands with diphenylamine which is a reagent that reacts positively with nitrate residue. Also known as the dermal nitrate test or paraffin test.

Dithiooxamide Test (DTO)

A chemically specific color-producing test for the detection of copper-bearing material, such as bullet wipe.

Ethyl Centralite

An organic compound used as a coating on some gunpowder to retard the burning rate.

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Explosion

The sudden and rapid expansion of gases from a confined space, accompanied by extremely high temperatures, violent shock (pressure), and loud noise. There are three types of explosions: mechanical, chemical (i.e. powders), and nuclear.

Fire Train

A single term that describes the sequence of events in the discharge of a cartridge:
(1) The detonation of the primer.
(2) The flame from the primer detonation passing into the powder chamber via the flash hole(s).
(3) The deflagration of the gunpowder and the subsequent production of gases.
(4) The propelling of the projectile down the barrel by the above mentioned gases.

Flare

Refer to **Cylinder Flare** or **Smoke Ring**.

Flash Hole

1. A hole(s) pierced or drilled through the web in the primer pocket of a metallic cartridge case.
2. The hole in the end of a battery cup primer used in shotshells.
3. The hole in a percussion nipple.

Flash Inhibitor

A material that is added to propellant for the purpose of reducing muzzle flash.

Fouling

Refer to **Metal Fouling**.

Fulminate of Mercury

Refer to **Mercury Fulminate**.

Gas Chromatography/Mass Spectrometry (GCMS)

A quantitative and qualitative method for the separation and identification of organic materials in complex mixtures or solutions. This method can be used to analyze material to determine if it is gunpowder. This application is limited by the availability of enough of the suspected gunpowder for adequate examination.

Granule

An individual particle of any of the various forms of gunpowder. Also known as a **kernel**.

Graphite

A soft form of carbon, used as a lubricant and as coating for some propellant powders.

Griess Test

A chemical test for the detection of nitrites used to develop patterns of gunpowder residues (nitrites) around bullet holes.

Gun Cotton

Nitrated cellulose (either cotton linters, wood pulp, or a mixture of the two) which is used for the manufacture of smokeless propellant (single base, double base, and triple base gunpowder). Contains 13% or more nitrogen [8]. Also known by its chemical name, **nitrocellulose**.

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Gunpowder

Any of various powders used as the propellant in a cartridge or shotshell. The following are varieties of gunpowder:

Ball Powder - A generally spherical type of smokeless powder. Ball powder is often further modified by a flattening process into forms known as flattened ball, rolled ball, and cracked ball. Ball powder is sometimes found in an elongated form which is called irregular ball.

Black Powder – The earliest form of propellant, reputed to have been made by the Chinese or Hindus. First used in firearms in the 14th century. It is a mechanical mixture of potassium nitrate or sodium nitrate (saltpeter), charcoal, and sulfur in approximately the following proportion: 75% potassium nitrate, 15% charcoal, and 10% sulfur. For sporting arms use, various granulations are available. These are designated Fg, FFg, FFFg, and FFFFg, largest to smallest, respectively.

Blank Powder – An extremely fast burning powder used in blank cartridges.

Canister Powder – Refer to **Reloading Powder**.

Cordite – A double base smokeless powder made of gun cotton, nitroglycerin, and mineral jelly that is extruded in a tubular or cord-like shape.

Digressive Burning Powder – A powder in which the surface area decreases while burning, thereby generating gas at a decreasing rate (e.g., non-perforated powders).

Disk Powder – An extruded form of smokeless powder cut into thin, circular disks that may have a central perforation to modify its burning characteristics. Such propellants are most commonly used in pistol and shotgun ammunition and may be of either single or double base formulation. Also known as perforated or unperforated disk-flake powder depending on whether the central hole is present.

Double Base Powder – A propellant composed of colloided nitrocellulose and nitroglycerin as its base as opposed to single base powder which has colloided nitrocellulose only as its base material. The percentage of nitroglycerin can be as low as 3% and as high as 39%.

Flake Powder – A type of smokeless propellant in the form of thin disks or cut squares.

Improved Military Rifle Powder (IMR) – A single base, tubular, smokeless propellant.

Irregular Flake Powder – A type of smokeless powder whose final form has been so highly modified by the manufacturer to achieve the desired performance characteristics that none of the morphology of the original particles has survived. The resultant particles have no consistently measurable dimensions such as length or diameter.

Lamel Powder – A type of smokeless propellant in which the individual particles are in the shape of thin square, diamond and/or parallelogram-shaped flakes. In some samples, the shape and dimensions of the particles are closely controlled while others may show considerable variation. This type of powder is typically found in European small arms ammunition and is also available as an imported canister powder (e.g., Alcan 5, 7, and 8).

Lot Powder – A homogeneous blend of powder having defined chemical and physical properties, as well as performance characteristics.

Neutral Burning Powder – A type of gunpowder which is designed to burn at a fixed rate until consumed (e.g., perforated powders).

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Nitrocellulose Powder – A smokeless propellant for ammunition whose principal ingredient is colloided nitrocellulose. The nitrogen content of the nitrocellulose is usually greater than 13%. Also known as single base powder.

Nonhygroscopic Powder – Smokeless powder which resists water absorption.

Priming Powder – Fine grain black powder used to ignite the propellant charge in flintlock and some other early firearms.

Progressive Burning Powder – A smokeless propellant in which the burning rate is controlled by physical and/or chemical means. Physically speaking, as the burning area of particles increases the rate of burning increases.

Reloading Powder – Propellant offered to individual consumers for loading small arms ammunition. Also known as canister powder.

Semi-Smokeless Powder – An obsolete propellant; a combination of black and smokeless powder.

Single Base Powder – Refer to **Nitrocellulose Powder.**

Smokeless Powder - A propellant containing mainly nitrocellulose (single base) or both nitrocellulose and nitroglycerin (double base). There are triple base powders as well, but they are not used in reloading powders in the United States. Smokeless powder comes in several forms, such as tubular, ball, and flake.

Triple Base Powder – A propellant composed of nitrocellulose, nitroglycerin, and nitroguanidine. Generally used in large caliber military ammunition.

Tubular Powder – An extruded form of smokeless powder formed by forcing the dough-like nitrocellulose composition through a die of specific dimensions and cutting it into particles of specific length. The length normally equals or exceeds the diameter. Most, but not all, examples will have a central perforation and will possess fairly uniform dimensions of length and diameter. Some extruded tubular powder has more than one perforation running its length. The IMR canister powders are all typical examples. Some non-United States specimens, however, may have their ends cut on a bias and therefore permit further distinction.

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Gunpowder Pattern

The spatial distribution of gunpowder residues deposited upon a surface. The pattern can be used to determine the distance between the muzzle of the firearm and the target at the time the shot that created the pattern was fired.

Gunshot Residues

The total residues resulting from the discharge of a firearm. It includes both gunpowder and primer residues, carbonaceous material, metallic residues from projectiles, fouling, and any lubricant associated with the projectiles. Abbreviated GSR.

H-Acid

Alpha-naphthylamine and sulfanilic acid. Chemical reagent proposed by Joseph T. Walker for use with desensitized photographic paper in the spatial detection of gunpowder residues.

Hydrochloric Acid

A chemical reagent used in the sodium rhodizonate test for lead and other primer residues (HCl).

Inhibitor

A chemical used to retard or stop an undesirable reaction. In the manufacture of powder propellants it is used to retard the burning rate. Refer to **dibutylphthalate**, **diethyldiphenylurea**, and **dinitrotoluene**.

Kernel

An individual particle of any of the various forms of gunpowder. Also known as a **granule**.

Lacquer

A material used to seal the primer and/or bullet in the cartridge case. It is used as a waterproofing agent.

Lead Azide

An explosive chemical compound used in priming mixtures designed to explode when heated or shocked. It is a more efficient detonating agent than mercury fulminate and it does not decompose on long continued storage [8].

Lead Styphnate (Trinitroresorcinate)

An impact sensitive initial detonating agent that is used in common priming mixtures. Also known as trinitroresorcinate, it is one source of particulate and vaporous lead in gunshot residue and bullet wipe.

Lead Snowstorm

A pattern of white specks on the dark background of an x-ray of a body part. It occurs when bullet fragments block the x-rays on the x-ray film. This phenomenon is usually associated with fragmentation resulting from the penetration of a high velocity jacketed bullet [9].

Marshall's Reagent (N-(1-Naphthyl)-ethylenediamine Dihydrochloride)

A chemical reagent used to form the azo-dye in the standard Griess Test for gunshot residues.

Mercury Bath

A process using mercury for the removal of lead residue from a barrel.

Mercury Fulminate

(1) A highly explosive component of a primer mixture designed to explode when crushed [8].

(2) A highly corrosive, toxic, and unstable component of a primary compound which has been replaced by more efficient chemicals (e.g., Lead Styphnate).

Metal Fouling

The accumulation of lead or bullet jacket material in the bore of a firearm from the passage of projectiles. Metal fouling may be the result of galling of the jacket materials and/or vaporization of lead at the relatively hot chamber end of the bore and deposition near the relatively cool muzzle end of the bore. These residual deposits can change the character of the identifiable striations imparted to the projectile from one shot to another. Also known as leading.

Modified Griess Test

A chemically specific, chromophoric (color-producing) test for the presence of nitrites. This version of the original Griess test uses alpha naphthol in place of Marshall's reagent.

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Muzzle Blast

Noise that occurs during the discharge of a firearm as a result of the rapid expansion of gases leaving the muzzle.

Muzzle Flash

Illumination that occurs during the discharge of a firearm as a result of the rapid expansion of gases leaving the muzzle.

2-NN

2-nitroso-1-naphthol: a colorimetric reagent for copper residues in suspected bullet holes and bullet impact sites.

N-(1-Naphthyl)-Ethylenediamine Dihydrochloride

Refer to **Marshall's Reagent**.

Neutron Activation Analysis (NAA)

A nuclear method of qualitative and quantitative elemental analysis. One if its applications is to analyze cotton swabs for elemental components of primer mixtures when testing hands or other objects for gunshot residues. This method is practically obsolete due to the use of the scanning electron microscope and x-ray analysis of primer residues.

Niter

Refer to **Potassium Nitrate**.

Nitrates

A chemical component of black and smokeless gunpowder.

Nitrite

A byproduct of the combustion of black and smokeless powder.

Nitrocellulose

The principal ingredient of single base, double base, and triple base propellants. Also known as cellulose nitrate, cellulose hexanitrate, and **gun cotton**.

Nitroglycerin

Manufactured by treating glycerin with a nitrating mixture of nitric and sulfuric acid. Nitroglycerin is a thick, clear to yellow-brown liquid that is an extremely powerful and shock-sensitive high explosive. Nitroglycerin is mixed with nitrocellulose smokeless powder formulations to make double base propellants. Also known as glyceryl trinitrate.

Nitroguanidine

An explosive with an especially low burning temperature, primarily used in firearm propellant charges to reduce barrel wear. A portion of nitrocellulose and nitroglycerin is replaced by nitroguanidine to make triple base powders.

Noncorrosive

A term applied to primers that contain no chemical compounds that produce corrosion or rust in firearms.

Paraffin Test

Refer to **Diphenylamine Test**.

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Percussion Cap

A small, generally cylindrical metallic cup containing a priming compound used to ignite the powder charge in muzzle loading firearms (placed on the nipple of percussion firearms).

PETN (Pentaerythrite tetranitrate)

A high explosive used as a component of some priming mixtures.

Potassium Nitrate

A crystalline compound used in the manufacture of explosives and pyrotechnics. Also known as niter or saltpeter.

Powder

Refer to **Gunpowder**.

Powder Bridging

The wedging action of powder in the feed tube of a cartridge loading device causing stoppage of normal flow.

Powder Burn

(1) The charring or singeing of a surface from contact with hot gunpowder particles.
(2) A small sidewall rupture, or pinhole, in a paper or plastic shotshell body that is caused by hot powder gases burning completely through the body wall [9].

Powder Burning Rate

The speed with which a propellant burns. It is affected by both physical and chemical characteristics, as well as the conditions under which it is burned.

Powder Charge

The amount of gunpowder by weight in a cartridge or shotshell.

Powder Fouling

Gunpowder residue left in firearms after firing.

Powder Measure

A device for measuring and dispensing a preselected amount of gunpowder volumetrically.

Powder Mill

(1) A manufacturing facility that produces gunpowder.
(2) The machinery that produces gunpowder to the required geometry.

Powder Deterioration

The chemical decomposition of modern smokeless propellant. It typically occurs over a long period of time but may be accelerated with improper storage conditions.

Powder Patterning

The orderly process of preparing gunpowder patterns at selected standoff distances on some form of witness panel material with a specific firearm and ammunition combination.

Powder Scale

A balance or weighing instrument for accurately weighing powder charges.

RETIRED

Primer

The ignition component of a cartridge. Refer to the **Firearms Identification Section** for various types of primers.

Primer Residues

The components of gunshot residue which contain elements which were originally part of the primer mixture of a cartridge. The scanning electron microscope (SEM) with energy dispersive X-Ray are used to analyze for some of the elements in primer residue, such as antimony, barium, and lead. Other methods that have been used for analyzing the elements of primer residue are neutron activation analysis (NAA) and atomic absorption (AA).

Pyrodex®

A Hodgdon trade name for a black powder substitute with similar burning characteristics that is safer and produces less fouling. Pyrodex® comes in several granulations for use in percussion revolvers, small and large caliber rifles, and shotguns.

RDX (Cyclonite, cyclotrimethylenetrinitramine)

A high explosive used as a component of some priming mixtures.

Saltpeter

Refer to **Potassium Nitrate**.

Scanning Electron Microscope (SEM) and X-Ray Analysis (SEM-EDX)

A system employing a fine electron beam that sweeps systematically over a specimen. The resulting secondary electrons and X-rays are analyzed to provide high magnification and nondestructive elemental analysis of the surface of the specimen. This type of analysis has the ability to identify gunshot residue more specifically than is possible by the use of atomic absorption or neutron activation analysis.

Shot Pattern

1. The distribution of shot fired from a shotgun. Generally measured as a percentage of pellets striking in a 30" circle at 40 yards to determine the degree of choke.
2. The test firing of a shotgun for a muzzle-to-target distance determination.

Smoke

The carbonaceous byproduct of burned gunpowder. Also refer to **Smoke Residue**.

Smoke Residue

The portion of gunpowder residue which consists essentially of carbon and leaves a black deposit on a target surface when the muzzle of the firearm is very close to the target. Also known as sooting and smudging.

Smoke Ring

The circular gray-to-black deposit around the front margin of the chamber or chambers of a revolver composed of gunshot residues deposited during the discharge process. Also known as a halo, **cylinder flare**, or simply flare.

Sodium Bitartrate

A chemical combined with tartaric acid (or some other acid) to create a buffer in the Sodium Rhodizonate test. This buffer is used to alter the pH of the lead Rhodizonate thereby changing the color of the compound.

RETIRED

Sodium Rhodizonate

A chemical reagent used in the sodium rhodizonate test to convert the lead to lead rhodizonate. Also known as SoRho.

Sodium Rhodizonate Test

A chemically specific, chromophoric (color-producing) test for the presence of lead in gunshot residues.

Soft X-Ray

Low energy X-rays used to detect the presence of lead fouling and particles of metal in clothing [11].

Stippling

Refer to **Powder Stippling** in the **Gunshot Wound Terminology Section** of the appendix.

Sulfanilic Acid

A chemical reagent combined with Marshall's reagent or alpha-naphthol used to treat desensitized photographic paper for sensitivity to nitrite compounds used in the Griess test or modified Griess test, respectively.

Tartaric Acid

A chemical used in the sodium rhodizonate test to alter the pH of the lead rhodizonate, thereby changing the color of the compound.

Tartrate Buffer

An aqueous solution of sodium bitartrate and tartaric acid formulated to provide a 2.8 pH environment for the sodium rhodizonate test reagent.

Tattooing

Refer to **Powder Tattooing** in the **Gunshot Wound Terminology Section** of the appendix.

Temperature of Ignition

The minimum temperature to which a substance must be heated before it will combust.

Tetracene (1-guanyl-4-nitrosoaminoguanyltetrazene)

A primary explosive used as a sensitizer or aid in the ignition process in priming mixtures. Tetracene is sensitive to friction, shock, and flame [8].

Trinitrotoluene (TNT)

A powerful, high explosive produced from toluene, sulfuric acid, and nitric acid. It is a stable explosive and is relatively insensitive to shock [8].

Trace Metal Detection Test (TMDT)

A test for metal ions which may be transferred from a metal object, such as a firearm to the hand. The hand is sprayed with a reagent such as 8-hydroxyquinoline and then viewed with ultraviolet light. If metal ions are present fluorescence will occur.

Vortex Effect

Refer to **Comet Tails**.

RETIRED

Walker Test

The original chemical test for the detection of nitrites and their distribution on targets. It is nicknamed after Joseph T. Walker who developed the technique of using desensitized photographic paper as the background for the formulation of the azo dye which is specific for nitrites. Refer to **H-Acid**, **C-Acid**, **Griess Test**, and **Modified Griess Test**.

X-Ray

Electromagnetic radiation of shorter wavelength than visible light that is capable of penetrating solids.

RETIRED

Serial Number Restoration

Chemical Etching

Refer to **Serial Number Restoration Methods**.

Etch

To produce a corrosive action on material such as metal or glass.

Electrolytic

Refer to **Serial Number Restoration Methods**.

Ferrous Materials

From the Latin word "ferrum" meaning iron, an alloy containing a significant amount of iron. Ferrous metals are magnetic [23].

Hidden Serial Number

An additional manufacturer's applied serial number not in plain view.

Magnetic Particle Inspection

Refer to **Serial Number Restoration Methods**.

Non-ferrous

Any metal in which the main component is not iron. Non-ferrous metals are not magnetic [23].

Obliterate

(1) To erase, wipe-out or remove as being legible.
(2) To make undecipherable or imperceptible.
(3) To deface, destroy, or alter.
(4) To make illegible.

Methods of Obliteration [20]

Drilled – The serial number is drilled out by using various types and sizes of drill bits and milling bits.

Engraved – A power-assisted pointed engraving tool is used to obliterate or disfigure the stamped or engraved depression of the serial number.

Ground (course, smooth, concave) – The stamped or engraved depression of the serial number and surrounding area is ground or sanded to a common plane or concave.

Peened or punching – A series of manually applied impact depressions administered repetitively with a hammer or similar tool over and around the serial number causing a flattening of the surrounding metal to render the number unreadable.

Scratched (pointed tool, broad tip tool) – A broad tip or pointed tool is used to repeatedly scratch the stamped or engraved depression of the serial number and the surrounding area until the number is unreadable.

Overstamping or Overpunching – To add characters to an existing number to cover up or obliterate the original number.

Welding or Other Heating Processes – Heating has the effect of normalizing or annealing metal and thus destroying the disturbed metal evidence [26].

RETIRED

Rusting – A naturally occurring process that can result in the unintentional obliteration of a serial number [26].

Reapplication of Original Finish – The original finish is reapplied in an effort to enhance the appearance of the firearm or to conceal an obliterated serial number [26].

Personal Marking

Any unique designation placed on an object by an owner, purchaser, or user for identification or proof of ownership.

Plastic Deformation

Permanent change in the shape or size of a solid body without fracture resulting from the application of a sustained stress beyond the elastic limit. May also be referred to as permanent deformation.

Serial Number

Numbers and/or letters applied to a firearm for identification purposes. The serial number may appear on the slide, chamber, or other areas of the firearm but must be conspicuously located on the frame or receiver. All firearms are required by law under the Gun Control Act to have a serial number if manufactured after 1968.

Serial Number (manufacturer) Applications

Roll Stamped
Impressed
Pin Stamped
Dot Matrix
Laser Etched

Serial Number Restoration

The application of scientific techniques used for the retrieval, recovery, and/or revisualization of the manufacturer's identifier [20].

Serial Number Restoration Methods

Polishing – The process of creating a smooth and shiny surface either by a chemical reaction or by an abrasive action in which the surface is worn down by friction.

Light and Alternate Light Sources – A non-destructive process of directing light at different angles onto an obliterated serial number to be able to observe that number. Light sources can include: natural light, indoor lighting, light magnifier, and other light sources.

Chemical Etching – Restoration technique utilizing the oxidation properties of chemicals on both ferrous and non-ferrous materials. Refer to **Ferrous Materials** and **Non-ferrous Materials**.

Electrolytic Chemical Etching – A restoration technique utilizing a direct current source to facilitate dissolution of metal. This technique can dissolve non-ferrous materials too quickly; best used on ferrous materials. Also known as electrochemical processing.

Magnetic Particle Inspection – Magnetic particle inspection is a nondestructive test that uses magnetic fields and small magnetic particles (iron filings) to detect defects or flaws in components.

RETIRED

Serial Number Restoration Conclusions

Full Restoration – All characters of the obliterated serial number were restored.
Partial Restoration – Some characters of the obliterated serial number were restored.
No Restoration – Restoration attempts were unsuccessful.

Specular Reflection

The reflective properties of light that allow one to distinguish between metal that has been cold worked and metal that has not. This is a result of non-uniform etching of cold-worked metal versus the uniform etching of non cold-worked metal.

RETIRED

Shooting Scene Reconstruction

Angle of Deflection

The angle formed between the path of the departing projectile subsequent to an impact and the pre-impact path of the projectile's flight.

Angle of Departure

The angle formed between a horizontal line and the centerline of the bore at the moment the projectile leaves the muzzle of the firearm.

Angle of Elevation

The vertical angle formed between the target and the axis of the bore.

Angle of Fall

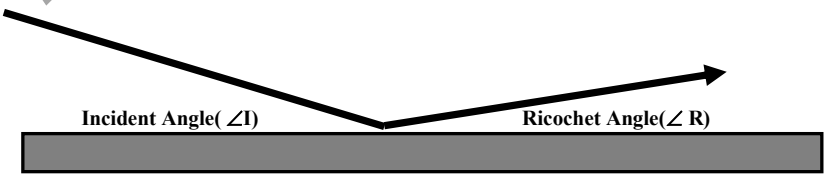
In shooting scenes, this term is used to describe the arrival angle relative to the horizontal plane of a bullet descending from a long range flight. This angle is the same as the vertical angle component of any bullet path at a shooting scene.

Angle Finder

A device that is designed to measure or display vertical and/or azimuth angles.

Angle of Incidence

In ricochet events, this is the intercept angle (∠I) described by the pre-impact path of the projectile and the plane of the impact surface at the impact site when viewed in profile.



Angle of Ricochet

This angle is defined by the path taken by the ricocheted projectile as it departs the impacted surface with one additional qualification- the plane of the impact site is that surface prior to bullet impact even though in some situations the bullet is departing a much modified surface (e.g., water, sheet metal, soil).

RETIRED

Azimuth Angle

An angle or bearing lying in the horizontal plane usually described on the basis of compass directions or with north, south, east, or west descriptors. In shooting reconstruction, an arbitrary north-south or east-west reference line may be chosen as a reference for azimuth angles related to that line.

Ballistic Coefficient

The ratio of the sectional density of the projectile to its coefficient of form (sometimes referred to as its form factor). It is an index of the manner in which a particular projectile decelerates in free flight due to resistance or drag of the atmosphere in which it is traveling.

Ballistic Curve

The curved path of a projectile from muzzle to target.

Bullet Drift

The lateral deviation in a bullet's flight through the atmosphere due to rotational effects. A bullet fired from a right twist firearm will drift right, and a bullet fired form a left twist firearm will drift left. This effect is only noticeable and significant in long range fire (such as 1000 yards).

Bullet Drop

The vertical distance a bullet has fallen, under the influence of gravity, at any point in its flight path. The distance is measured from a point on its path to the straight line from the axis of the bore to target.

Bullet Path

(1) The vertical distance, normally expressed in inches, above or below a firearm's line of sight.

(2) The arched path of a projectile from the muzzle to the target. Also known as trajectory.

Chisum Trail

An elongated transference of bullet metal at the departure end of low incident angle ricochet marks on smooth, flat, unyielding surfaces. This asymmetrical elongated transference will be on the left side of ricochet marks for bullets fired from left twist firearms, and on the right side for bullets from right twist firearms. It is caused by the right or left edge of a flattened bullet remaining in contact with the surface after the main body of the bullet has lifted off the surface.

Photograph obtained from Shooting Incident Reconstruction [14]

RETIRED

Critical Angle

The angle of incident or intercept of impact at and above which the particular projectile at a given impact velocity no longer ricochets from the impacted surface.

Deflection

As differentiated from ricochet: a deviation in the projectile's normal path through the atmosphere as a consequence of an impact with some object. This term is further refined for two types of impact events in a projectile's normal flight path.

As a consequence of ricochet: is used to describe any lateral component of the ricocheted projectile's departure path relative to the plane of the impacted surface as viewed from the shooter's position and with the plane of the surface normalized to a horizontal attitude.
The angle formed between the path of the departing projectile subsequent to impact and the pre-impact plane of the projectile's path.

As a consequence of perforating or striking an object: the term deflection is used to describe deviations in any direction from the projectile's normal flight path as a consequence of perforating or striking an object rather than rebounding off of surfaces. For example, a bullet may be deflected by passage through a tree branch, a windshield, or a panel of sheet metal. These are not ricochet events. Since such deflection can occur in any direction (up, down, right, or left), the clock position of such deflection is used to describe this form of deflection. As viewed from the shooter's position (or position directly behind the projectile at impact), 12 o'clock will be taken as straight up relative to the horizontal plane at the location of the event, 3 o'clock to the right, 9 o'clock to the left, and so forth.

Doppler Radar

A continuous-wave radar used chiefly to make precise speed measurements. It works on the basis of the Doppler effect, which is a change in observed wave frequency caused by motion. By measuring the difference in frequency, Doppler radar determines the speed of the object or projectile observed.

Event

A single occurrence, action, or happening.

Inclinometer

A device for measuring or displaying the angle of a surface relative to the horizontal or vertical plane.

Lagtime

The difference in time between the sound of the arrival of a bullet (sound of impact or sound of passage) at a specific downrange location and the arrival of the sound of the shot at that same location. This time interval is useful in calculating the range of fire when the approximate muzzle velocity and ballistic coefficient of the bullet are known.

Laminated Glass

Layers of glass bonded to a plastic material. This bonding is a safety feature that, upon impact, serves to hold the pieces of broken glass in place and is commonly seen in automobile windshields. May also be referred to as safety glass.

Line of Departure

The direction in which a projectile is moving when it leaves the muzzle of a firearm; tangent to the trajectory at the muzzle.

RETIRED

Line of Sight

The straight line drawn from the shooter's eye, passing through the sights of a firearm and extending from the firearm to the target or point of aim.

Plumb Line

A simple device to indicate vertical direction made by suspending a small mass of lead or other heavy material on a string line and allowing it to hang freely and still.

Reconstruction

The determination of the sequence of two or more events in a particular incident utilizing information derived from the physical evidence, data from the analysis of physical evidence, recognized physical laws and/or inferences drawn from experimentation related to the incident under investigation.

Reenactment

The demonstration of a reconstruction through the use of live actors or animation.

Trajectory

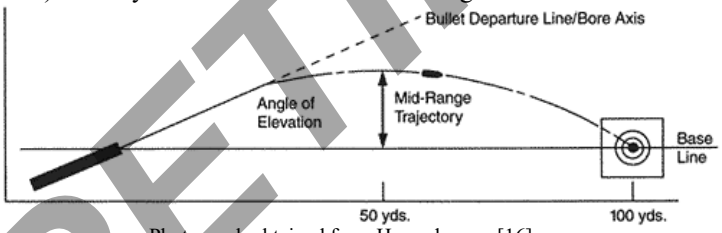
The arched path of a projectile from the muzzle to the target.

Trajectory, Flat

A relative term for minimal arching of a projectile in flight.

Trajectory, Mid-Range

The distance measured in inches that a projectile travels above the line of sight (base line in the diagram below) halfway between the firearm and a target.



Photograph obtained from Hornady.com [16]

Trajectory, Origin of

The center of the bore where a projectile exits the barrel of a firearm.

Trajectory Rod

A straight probe constructed of inert, brightly colored materials specifically designed for tracking and illustrating the nominal path of a projectile through one or more materials. Trajectory rods often have centering cones.

Trajectory Table

A computed table describing the downrange trajectory of a projectile(s).

Vertical Angle

In shooting scene reconstruction, it is the vertical component of a projectile's reconstructed flight path. This angle is given a negative sign if the path followed by the projectile is downward and a positive sign if the path is upward. A flight path that parallels a level surface has a vertical angle of 0.0^0.

Visual Aid

Any device used to demonstrate any aspect of a reconstruction or reenactment.

RETIRED

Sight and Scope Terminology

Action Mounting Sight

Refer to **Aperture Sight**.

Adjustable Sight

A rear sight that is adjustable for windage, elevation, or both. May also refer to adjustable front sights that are sometimes used on target firearms.

Aperture Sight

A type of iron sight. The rear sight consists of a small disk with a center hole through which the shooter views the front sight and the target. The front sight may be a post, bead, or another aperture. Light entering the center hole of the sight frames the front sight, which enables the eye to focus more easily than in could when sighting through open sights, and thus facilitates the centering of the front sight on a target. Also known as orthoptic sight, peep sight, aperture back sight, or receiver sight (if mounted to the receiver). May be categorized according to position on a firearm as follows:

Action mounting – A conventional aperture sight which mounts on the action of a firearm and may vary in design with firearms of different purposes.
Bolt mounting – An aperture sight which mounts to the bolt sleeve or cocking piece of bolt action firearms.
Tang mounting – An aperture sight which mounts to the tang of a firearm.

Back Sight

A metal or plastic structure, usually notch-like in appearance, located at the top rear portion of a firearm and used in aiming at a target. The conventional style consists of a V-shaped or U-shaped blade into which has been cut a square, U-shaped, or V-shaped notch. When a firearm is in line with a target the front sight is seen to lie in the center of the rear sight notch. Also called a **rear sight**.

Barleycorn Sight

Refer to **Pyramid Sight**.

Bead Sights

The small, cylindrical (bead-like) top portion of some forms of front sights (typically found on shotguns).

Big Game Scope

A telescopic sight with characteristically low magnification (2x to 3.5x), universal focus, a large field of view, an adjustable reticule, and long eye relief. Used for sighting and hunting large animals often in the context of safaris. This design is generally weatherproof, short, and compact for portability, low-mounted, and sturdy enough in construction to withstand the recoil from larger caliber ammunition. Compare to **Target Scope** and **Small Game Scope**.

Bolt Mounting Sight

Refer to **Aperture Sight**.

Bore Sighting

Determining the position of a telescopic sight in which it is in alignment with or parallel to the bore of a firearm.

RETIRED

Buckhorn Sight

A simple, rugged sight used on many hunting rifles with a rear sight blade that has a nearly closed V-shaped notch, often with a small circular or U-shaped cutout at the bottom. Sight alignment consists of placing the front post bead squarely in the bottom of the cutout in the notch.

Bullet Drop

The vertical distance a bullet has fallen, under the influence of gravity, at any point in its flight path. The distance is measured from a point on its path to the straight line from the axis of the bore to target.

Canting

The act of tilting or angling a firearm so as to slant the sights to one side.

Click Adjustments

Screws or other devices set in the turret of a telescopic sight which permit changes in elevation and windage. Each $^1/_4$ inch turn of a screw corresponds to a $^1/_4$ minute angle adjustment and produces an audible sound, or "click," which facilitates alterations made in low lighting. Refer to **Turret**.

Collimation

The alignment of the optical components of a telescopic sight such that the longitudinal optical axis of the scope is perpendicular to the lenses and passes through the center of each.

Collimator

A small scope with a crosshair graph in its focal plane used to align a telescopic sight with the bore of a firearm.

Condenser Lens

A component of a telescopic sight, located behind the objective, which collects light from the source and uniformly directs it through the projecting lens (the eyepiece of the scope).

Crawling the Stock

The act of positioning the head too close to a telescopic sight.

Crosshairs

The aiming reference observed when looking through a telescopic sight. A reticle establishes a frame of reference for the measurement of distance or the position of objects viewed through the lens. May consist of intersecting or parallel lines, lines etched into glass, a post(s), a pinhead dot, or any combination of wires, dots, and post of varying sizes and thicknesses. Also known as a fixed reticle**,** reticule**,** or graticule**.**

Dot Point Aiming Sight

A sight which consists of a tube or single lens through which the target is viewed. Inside the sight, a small light-emitting diode (LED) is reflected off the lens surface and into the shooter's view. The sight can then be adjusted to bring the dot onto the point of bullet impact. Since the dot is superimposed over the target, there is infinite eye relief, no magnification of the target image, and no parallax. The sight can be used with both eyes open. Also known as red dot scope, electronic dot sight, and reflex sight.

RETIRED

Dovetail

A mechanism by which rings and scope mounts attach to each other, and by which some sights attach to the barrels of a firearm. A dovetail consists of an angled base similar to the bottom portion of a triangle which slides into a slightly larger angled base.

Dust Covers

Protective covers, usually made of plastic, rubber, or leather, which fit over the front and rear lenses of a scope. May be connected to each other by a band which facilitates easy removal. Also known as a lens cover or lens cap.

Electronic Dot Sight

Refer to **Dot Point Aiming Sight**.

Elevation

The height of a target above the ground.

Erector Lens

A component of a telescopic sight, located between the reticle and the eyepiece lens, which consists of two lenses which cause a transmitted image to invert and reverse, thus producing a vertically-oriented final image.

Exit Pupil

The point in the ocular portion of a telescopic sight at which all beams of incoming light are equivalent in diameter and at which the shooter's eye receives transmitted images.

Express Sight

A type of open sight (typically a rear sight, but may also be a front sight) mounted on one base, with folding sight leaves to be used for fast sight acquisition at varying ranges or elevations.

Eye Distance

The distance between a shooter's eye and the ocular lens of a telescopic sight required for an optimum field of view (FOV). Distance usually exceeds three inches. Also known as eye relief.

Eye Relief

Refer to **Eye Distance**.

Eyepiece

The lens of a telescopic sight that the shooter looks through, consisting of a pair of lenses, which transmits images to the eye. Also known as the ocular lens.

Eyepoint

(1) The specific location of the exit pupil.
(2) The location at which the eye should be placed when utilizing a telescopic sight.

Field of View

The widest dimension visible through a telescopic sight, measured in feet seen at a range of 100 yards. Field of View (FOV) decreases as the magnification power of a scope increases.

Fixed Power Scope

A telescopic sight in which images are magnified at one specific setting.

Fixed Reticle

An immovable reticle which remains in the center of a field of view and cannot be adjusted.

RETIRED

Fixed Sights
Metallic sights that are not readily adjustable. However, most fixed sights can be regulated with tools to change the point of impact.

Fogging
The condensation of moisture trapped within a scope on lens' surfaces. Fogging prevents clear transmission of visual images.

Fogproofing
A means to prevent the fogging of a scope by replacing the air inside with nitrogen and hermetically sealing the device with compressed O rings, thus creating an airtight seal.

Folding Sight
A rear sight that can be folded down.

Fore Sight
Refer to **Front Sight**.

Front Sight
Any form of sighting device at or near the muzzle of a firearm.

Front Sight Hood
A cover to protect the front sight from damage.

Globe Front Sight
A form of front sight typically used on target rifles. The sight is generally cylindrical in shape and arranged to accept various inserts containing either rings or posts.

Graticule
Refer to **Crosshairs**.

Hooded Sight
A front sight that is provided with a cover to shade it from direct light.

Iron Sight
Any front or rear sight not containing optical magnifying elements. It may be fixed or adjustable. Also known as a metallic sight.

Laser Sight
A unit consisting of a battery-powered portable laser usually mounted below the barrel, which generates a laser beam that can be projected onto a target.

Lee Dot
A type of reticle in which the intersection of the crosshairs is marked by a dot.

Lens Cap
Refer to **Dust Cover**.

Lens Cover
Refer to **Dust Cover**.

RETIRED

Machined Ring
A scope ring fashioned from a top metal hemisphere and a lower metal hemisphere connected on both sides by bolts or screws. Uniformly surrounds scope and remains fixed during recoil.

Metallic Sight
Refer to **Iron Sight**.

Micrometer Sight
A sight with a mechanism for adjusting windage and elevation settings controlled by calibrated knobs, usually with detents to control setting intervals.

Middle Sight
A second, smaller bead sight near the middle of the barrel or barrels of some shotguns.

Minute of Angle (MOA)
An angular measurement method used to describe accuracy capability. It is equivalent to 1/60 of a degree of arc, or 1.047 inches at 100 yards.

Multiple Leaf Sight
A type of open, rear sight generally having more than one folding leaves.

Night Sight
Iron sights into which glass capsules coated with a phosphor and containing the radioactive gas tritium have been inserted. As the tritium emits electrons, the phosphor produces visible light, perceived as a glow, useful for sighting at night or in low-light conditions. Green is the color of the phosphor most commonly used, although in some designs the front sight is given a different color than the rear sights to allow a shooter to distinguish between front and rear sights and thus aim more easily. Also called self-luminous iron sights.

Notch Sight
An open rear sight having either a “V”, “U”, or square-shaped cut on its upper edge.

Objective
The lens at the front of a telescopic sight which collects light from an object viewed through the instrument and forms an image of it.

Ocular Lens
Refer to **Eyepiece**.

Open Sight
A rear sight having a notch through which the front sight is aligned for aiming.

Optical Sight
A telescopic sight (or scope) in which two or more lenses are focused to create an image.

Optimum Magnification
The best possible enlargement of an image seen through a scope, obtained when the exit pupil is equal in diameter to the pupil of a shooter’s eye.

Orthoptic Sight
Refer to **Aperture Sight**.

RETIRED

Orthoptics
Perforated opaque discs, used in conjunction with or in place of a lens, which sharpen a shooter's view of the sights and target.

Paine Sight
A system used on pistols or revolvers that has a rear sight with a flat top and a U-shaped notch and a bead-topped front sight.

Parallax
The apparent movement of objects within the field of view in relation to the reticle. It occurs when the eye is moved to either side of the objective of a stationary scope and is caused by the improper alignment of a reticle and the optical center of a scope. If parallax is not corrected, the result is an optical illusion [27].

Patridge Sight
Refer to **Post-and-Notch Sight**.

Peep
The hole on an aperture or peep sight through which the shooter looks to align a front sight with a target.

Peep Sight
Popular term for **aperture sights**.

Post-and-Notch Sight
A sighting system that consists of a vertical rear sighting blade with a square notch cut in the center teamed with a front post with parallel sides and a flat top.

Pivot Mount
A design in which a hinge permits positioning of the scope rings on either the top of the barrel or off to the side such that the scope or the iron sights may be used.

Pyramid Sight
A type of front sight of triangular appearance. Also known as a barleycorn sight.

Ramp Sight
A front sight which is mounted atop a ramp.

Rear Sight
Any metallic sight used in conjunction with a metallic front sight located anywhere between the shooter's eye and the front sight.

Receiver Sight
Any rear sight fitted to the receiver of a firearm, but usually refers to an aperture or peep sight.

Red Dot Scope
Refer to **Dot Point Aiming Sight**.

Reflex Sight
Refer to **Dot Point Aiming Sight**.

RETIRED

Resolution
The clarity or freedom from distortion of an image transmitted through a scope.

Reticle
Refer to **Crosshairs**.

Reticule
Refer to **Crosshairs**.

Ring
Refer to **Scope Ring**.

Scope
An optical sight consisting of a series of lenses and a reticle which magnify a target and increase a shooter's accuracy in aiming; a terrestrial telescope equipped with a reticle that is superimposed on the field of view and automatically focuses on the image.

Scope Hood
An extension piece attached to the objective on a telescopic sight which protects the lens from damage and weather and which prevents the entry of excess light, thereby increasing the clarity of the transmitted image. May also be known as sun shade.

Scope Mount
A metal base or mechanical device which fastens a scope and the scope rings holding it to the barrel, action, or receiver of a firearm. Types of scope mounts include **Pivot Mounts**, **See Through Mounts**, **Side Mounts**, and **Top Mounts**.

Scope Ring
A metal band which encircles a scope and attaches it to a scope mount. Also known simply as a ring. Scope rings may be either a machine ring or a stamped ring.

Scope Sight
A sight containing optical elements which magnify or enlarge the target. Also known as a telescopic sight.

See Through Mount
A design in which conventional scope rings mounted above elliptical rings allow simultaneous use of the scope and the iron sights.

Self-Luminous Iron Sights
Refer to **Night Sights**.

Side Mount
A design in which the scope is not attached directly over the bore but is offset to one side of the barrel.

Sight
Any of a variety of structures or devices, mechanical or optical, designed to assist in aiming a firearm at a target.

Sight Adjustment
The movement of a sight to change the point of impact.

RETIRED

Sight Aperture
The hole in the disc of an aperture sight.

Sight Base
The part of a sight that is usually permanently attached to the firearm.

Sight Bead
A beadlike spherical structure or raised dot located on the foremost portion of a gun's barrel and used in aiming at a target.

Photograph obtained from Brownells.com[3]

Sight Blade
A thin, flat metal post used as the front sight on some firearms.

Sight Cover
A protective metallic cover fastened about a sight to guard it from being moved out of adjustment by jars or blows and from direct sunlight while aiming. Also known as a sight hood or front sight hood.

Sight Disc
That part of an aperture (peep) sight that contains the hole. May have either a fixed orifice or contain an iris diaphragm to vary its size.

Sight Elevation
The height to which a rear sight is set to zero-in the firearm for any specific range.

Sight Extension
A device that increases the distance between the sights.

Sight Hood
Refer to **Front Sight Hood**.

Sighting-In
The procedure of adjusting the sights so as to bring the point of impact to coincide with the point of aim.

Sight Inserts
Metal or plastic discs with either apertures or posts for use in globe front sights.

Sight Leaf
The vertical portion of a metallic rear sight containing the notch.

Sight Picture
The visual image observed by the shooter when the firearm sights are properly aligned on the point-of-aim.

RETIRED

Sight Radius
The distance between the rear sight and the front sight on a firearm.

Sight Tube
A metal tube that contains the rear sight.

Sight Ramp
A sloping rear surface which serves to elevate the sighting device.

Six O'Clock Aim
A sighting position in which the bullseye of a target is fixed just above the top of the front sight. Also known as six o'clock hold.

Small Game Scope
A telescopic sight characterized by a relatively fine reticle and attributes intermediate between big game scopes and target scopes, including magnification (4x to 8x), field of view, and eye relief. Used for sighting and shooting small animals. Also known as a varmint scope. Compare to **Big Game Scope** and **Target Scope.**

Stamped Ring
A scope ring consisting of a metal band which surrounds a scope and clamps to or folds in on itself by means of a single bolt or screw. May not fit evenly around a scope and may loosen or detach during recoil.

Sun Shade
Refer to **Scope Hood**.

Tang Mounting Sight
Refer to **Aperture Sight**.

Tang Sight
Any sight mounted on the upper tang of a firearm.

Tangent Sight
A type of rear sight in which the blade is adjusted for elevation correction by sliding along a curved cam(s). Typically read in yards of range.

Target Scope
A telescopic sight with characteristically high magnification (10x to 20x), a small field of view, a fine reticle for precision aiming, and short eye relief. Used for specific, usually stationary sighting and shooting. This design is high-mounted, long, and of variable weigh and must be refocused at different ranges. Compare with **Big Game Scope** and **Small Game Scope**.

Telescopic Sight
Refer to **Scope Sight**.

Top Mount
A conventional design in which the scope is fastened directly over the barrel of the firearm.

Turret
A protrusion in the center section of the shaft of a telescopic sight which contains mechanisms of adjustment for windage and elevation.

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Variable Power Scope

A telescopic sight in which the magnification of images can be increased or decreased, usually within the range of 3x to 9x.

Varmint Scope

Refer to **Small Game Scope**.

Vernier Scale

On scopes, a short auxiliary scale utilized for reading fractional measurements, such as those measurements to hundredths of an inch and smaller, when adjusting for elevation and windage.

Vernier Sight

A long-range sight with a mechanism for adjusting windage and/or elevation settings, as read on a short scale in conjunction with graduations on a longer, linearly calibrated scale.

Windage

Lateral correction of a firearm's sights to compensate for the projectile's deflection by wind or drift.

Windage Adjustment

The transverse movement of a sight to compensate for the horizontal displacement of a bullet or bullets from the aiming point.

Windage Knob

The knob on some iron and telescopic sights which is turned in either direction to adjust the horizontal setting of the notch, aperture, or reticle of the sight.

Windage Micrometer

A micrometer type device found on some iron and telescopic sights which allows the shooter to make precision adjustments to the horizontal setting.

Windage Scale

A scale used in adjusting a sight to the laterally deviant trajectory of a bullet caused by wind.

Zero-In

Refer to **Sighting-In**.

Zeroing

Aligning a gun's sights such that, at a given range, a fired bullet crosses the line of sight.

RETIRED

Suppressor Terminology

Ablative

A coolant that is used in wet suppressor systems. Examples include water, gel, or other liquid materials.

Absorbent

Material used to slow and cool gases emitted during the discharge of a firearm, thus dampening or reducing sound.

(1) Sound absorbing materials: aluminum eyelets, screen, corrugated cardboard, and fiberglass.

(2) Gas trapping materials: metal (aluminum or copper screen), cork, leather, and plastic [7].

Action Noise

Refer to **Mechanical Action Noise**.

ANSI Standard (American National Standards Institute)

A type of sound field standard used when measuring sound in a diffuse field. Also refer to **Diffuse Field**.

Artificial Environment

The technique/technology of using an ablative or coolant material within a suppressor to reduce the noise of a gunshot. Energy is consumed by the transfer of heat and phase change of the material that would otherwise contribute to the noise produced.

Baffle Spacers

Materials used to separate the baffles in a silencer in order to trap expanding gases. Also known as spacers.

Baffles

Components of a suppressor - usually discs, washers, wafers, or some other material. These components can be made of metal, rubber, cork, leather, or plastic and can be conical, flat, or spiral in shape. The following are various types of baffles. Also refer to **Baffling System**.

Back Baffles – A type of baffling system in which disc-like baffles permit gases to escape both backwards and forwards from an expansion chamber.

K Baffle – Integrated flat baffle with a conical spacer that resembles the letter "K" when sectioned. This is one of the most efficient baffle designs combined with various cuts, scoops, and ports and is most prevalent in low to medium pressure calibers (rimfire and pistol). The K-baffle generates high turbulence (spread the gas exit curve) and provides a large surface area for gas cooling. It is used primarily in rimfire and pistol caliber cartridges [7].

M Baffle – A simple conical baffle with an integrated spacer that resembles the letter "M" when sectioned. This is physically one of the strongest designs. Variations include cuts, holes, or slots in the conical part to increase turbulence [7].

Monocore Baffle (unitized core) – A design where the baffle structures and spacers for the entire baffle stack are fabricated from a single bar of metal. It is also encountered as a weldment from sheet metal components. While not as efficient as a well-designed stack of discrete components, it lends itself to easy fabrication by CNC milling, casting, or spot-welding and is easier to clean [7].

RETIRED

Slant Baffle (Z) – A design using flat baffles at a non-perpendicular angle to the bore axis. With its associated spacer, in section it resembles the letter "Z". A mechanically strong design of moderate efficiency, it is encountered with spacers also cut at angles or as a casting with the slanted baffle inside a conventional spacer [7].

Baffle Stack

The complete collection of baffles that make up the interior of a suppressor.

Baffle Strike

The impact of a bullet with the interior of a suppressor. This can be due to an unstable bullet or an improperly mounted suppressor. This can damage the suppressor, produce toolmarks on a fired bullet, and cause a fired bullet to yaw in flight.

Baffling System

A series of baffles designed to restrict the passage of gas generated during the discharge of a firearm.

Ballistic Crack

Refer to **Sonic Boom**.

Barrel End Support

A structure which aligns the trajectory of the bullet in both the barrel of the firearm and the suppressor.

Bleed Holes

Refer to **Gas Port**.

Bleeding

The escape of gases through bleed holes drilled in the barrel of a firearm which permit gases to escape into a suppressor.

Blow-by

In reference to sound suppressors, blow-by is the leakage of gas around a bullet fired through the baffles in a suppressor. It is generated by the difference in diameter between the bullet and holes in the baffles, thus permitting the gas to precede the bullet out of the suppressor. Refer to **Blow-by** in the **Firearms Identification Section**.

Can

A device which reduces muzzle blast by decreasing the velocity of escaping gases but maintains a bullet's high velocity. Also known as a **suppressor**, muzzle suppressor, noise suppressor, sound suppressor.

Chamber (of a suppressor)

1) **Entrance Chamber** – In some suppressors, the first chamber may contain absorbent materials. The entrance chamber is the most rearward and the first which the projectile encounters. Typically, this chamber should trap the bulk of the gases and allow a significant pressure reduction.
2) **Middle Chamber** – The middle chamber, if present, often contains a series of baffles. The purpose of the middle chamber is to trap the gases between baffles, to cool the gases, and to delay gas exit. Usually the boundary of the middle chamber is considered to be the most rearward baffle (or partition) to the most forward baffle.
3) **Exit Chamber** – In some suppressors, a last chamber leading to an end cap which blocks and deflects blow-by gases. The exit chamber, if present, is at the far end of the suppressor and is usually an empty space. It is the area between the last baffle of the middle chamber and the front end cap.

RETIRED

Coolant
Refer to **Ablative**.

Decibel
The primary unit of sound measurement used to quantify both sound pressure level and sound power level [4]. Abbreviated dB. Also refer to **Sound Pressure Level**.

Decibel Meter
A calibrated instrument used to measure sound pressure level in decibels. Used for testing the efficiency of suppressors.

Diffuse Field
A sound field in which the sound pressure level is the same everywhere and the flow of energy is equally probable in all directions [4].

Diffuse Sound
Sound that is completely random in phase or sound that appears to have no single source.

End Cap
The sealed forward portion of a suppressor through which the bullet and escaping gases pass.

End Wipe
The baffle closest to the end cap which contains a hole smaller in diameter than the bullet passing through it.

Expansion Round
Space within a suppressor which allows escaping gases to slow, diffuse, and cool.

First Round Flash
Muzzle flash generated by secondary burning of residue gases and propellants due to oxygen present in a suppressor when the first shot is fired.

First Round Pop
The elevated sound produced by the first shot fired in the suppressor that does not contain any ablative or coolant.

Free Field
An environment in which sound does not reflect from any surface. Sound is radiated into space from a source uniformly in all directions. The sound pressure produced by the source is the same in every direction at equal distance from the point source.

Gas Port
1) Holes drilled in the barrel of a firearm which permit gases to escape into a suppressor, thereby decreasing ballistic pressure and reducing the velocity of a bullet to a subsonic speed. Also known as bleed holes, porting, and port holes.
2) A hole in a baffle other than the one intended for the bullet to pass through that directs gas within the suppressor.

Housing
Refer to **Suppressor Tube**.

IEC (International Electrochemical Commission) Standard
A type of sound field standard used when measuring free fields of sound. Refer to **Free Field**.

RETIRED

Jet Noise
Sound created by the turbulence, rotation of air, and reverberation within a suppressor.

K-factor
A correction factor which is added to measured values in some sound meters.

Leq
Equivalent continuous sound pressure level, or Leq, is the constant noise level that would result in the same total sound energy being produced over a given period. This measurement is not an average sound level.

Mechanical Action Noise
Noise produced by mechanical movements within a firearm, such as trigger pull, movement of the bolt or slide, and hammer drop during firing. Also known as action noise.

Microphone
An electroacoustic transducer which converts the energy in sound waves to electrical energy which can be more easily displayed and analyzed using a sound level meter. Refer to **Oscilloscope**, **Sound Level Meter**, and **Transducer**.

Muffler
Refer to **Suppressor**.

Muzzle Blast
Noise occurring during the discharge of a firearm as a result of the rapid expansion of gases leaving the muzzle.

Muzzle Suppressor
Refer to **Suppressor**.

Noise Suppressor
Refer to **Suppressor**.

Oscilloscope
An electronic instrument used to show the relationship between time and the amount of sound produced or the relationship between two other variables.

Outer Tubing
Refer to **Suppressor Tube**.

Port Holes
Refer to **Gas Port**.

Porting
Refer to **Gas Port**.

Primer Initiation Pulse
Sound produced by the leakage of some propellant gases backwards around the cartridge case during firing.

Report
A combination of sounds produced by the discharge of a firearm, including but not limited to mechanical noise, sonic crack, and muzzle blast.

RETIRED

Silencer

(1) A device attached to or integral with the barrel of a firearm that is designed to reduce the noise of discharge. The term silencer is a misnomer as this device does not silence sound, it can only suppress it. The designer of the first sound suppressor was Maxim Hiram, who coined the phrase "silencer." Refer to **Suppressor**.

(2) Defined by the Federal Firearms Regulations Reference Guide as any device for silencing, muffling, or diminishing the report of a portable firearm, including any combination of parts, designed or redesigned, and intended for use in assembling or fabricating a firearm silencer or firearm muffler, and any part intended only for use in such assembly or fabrication.

Silencing Tube

Portion of a silencer which encapsulates all components of the silencing unit and which contains and controls the expansion of escaping gases. Also known as housing and outer tubing.

Sonic Boom

Sound made by the shock waves of a bullet traveling faster than the speed of sound. Also known as sonic crack or ballistic crack.

Sonic Crack

Refer to **Sonic Boom**.

Sound Exposure Level (SEL)

A sound level that is maintained for a one second interval and is equivalent to the total acoustic energy generated by the measured noise.

Sound Level Meter

An instrument used to measure sound or noise. The microphone (transducer) within the sound level meter picks up the small variations in pressure that are the sound waves and, after passing it through amplifiers, filters, etc., provides the user with a reading in decibels.

Sound Moderator

Refer to **Suppressor**.

Sound Pressure Level (SPL)

The measure of variation in atmospheric pressure resulting in a sound level to which the ear responds. Measured by a sound level meter in decibels. In suppressors, sound pressure level is reduced by increasing the volume and decreasing the temperature of expanding gases, in accordance with the Ideal Gas Law (PV = nRT), where P is the sound pressure level.

Sound Reflection

The return of a sound wave from a surface. Sound is reflected much as light is reflected, with the angle of incidence equaling the angle of reflection.

Spacers

Refer to **Baffle Spacers**.

Subsonic

An object traveling slower than the speed of sound (<1128 ft/s or <344 m/s at 68°F/20°C).

Suppressor

A device which reduces muzzle blast by decreasing the velocity of escaping gases but maintains a bullet's high velocity. The following are various forms of suppressors:

RETIRED

Auxiliary Suppressor – A suppressor which can be removed from the firearm. Contrast with **Integral Suppressor**.

Baffle Suppressor – A suppressor containing metal baffles which typically does not mark the bullet fired through it. It typically does not produce forensic evidence on the bullet which could allow determination of the type of suppressor used. Contrast with **Wipe Suppressor**.

Barrel Suppressor – A suppressor which surrounds the barrel of a weapon.

Disposable Suppressor – A suppressor which decreases in effectiveness after each use and must be cleaned or replaced frequently.

Eccentric Suppressor – Refer to **Offset Suppressor**.

Homemade Suppressor – An improvised suppressor fashioned from readily-available materials.

Integral Suppressor – A suppressor which is designed to be a component part of a firearm and generally cannot be removed. Contrast with **Auxiliary Suppressor**.

Internal Barrel Suppressor – A suppressor housed inside the barrel of a firearm which is fired using subcaliber ammunition. Also known as an internal suppressor.

Offset Suppressor – A suppressor in which the trajectory of the bullet is offset from the center of the suppressor so as to permit use of the original sights on the firearm. Also known as an eccentric or maxim suppressor.

Wet Suppressor – A suppressor which lowers sound pressure level (SPL) by means of the evaporation of a liquid by the hot propellant gases. Refer to **Ablative** and **Wet Can**.

Wipe Suppressor – A suppressor containing discs with holes smaller in diameter than the bullet passing through them. Results in markings on the bullet which could be utilized in forensic examination to determine the type of suppressor used but may deform original markings produced by the passage of the bullet down the barrel. Contrast with **Baffle Suppressor**.

Suppressor Tube

Portion of a suppressor which encapsulates all components of the silencing unit and which contains and controls the expansion of escaping gases. Also known as housing and outer tubing.

Threaded Collar

The region of an auxiliary suppressor used for attachment to the muzzle of a firearm.

Transducer

A device, such as a microphone, which transforms energy from one form to another.

Uncorking Pressure (Uncorking Noise)

The pressure (noise) produced at the muzzle end of a firearm or suppressor at the moment the bullet exits. Also referred to as **muzzle blast**.

Wet Can

Slang for a suppressor which lowers sound pressure level (SPL) by means of the evaporation of a liquid by the hot propellant gases.

RETIRED

Manufacturing and Machining Terminology

Abrasive

A material that penetrates and cuts another material that is softer than itself.

Abrasive Machining

A process that uses an abrasive in wheel or belt form to remove material from a work piece [28].

Adhesion

The process of joining materials by using an agent that sticks to both work pieces [28].

Age Hardening

Increasing the hardness of a malleable metal by a low temperature heat treatment that causes precipitation of components. Also known as precipitation hardening.

Agglomerate

Small particles bonded together into an integrated mass.

Alloy

A mixture of two or more metals fused or melted together to form a new material. For example, brass is an alloy composed of copper and zinc.

Anneal

To soften a metal piece and remove internal stresses by heating to its critical temperature and allowing it to cool slowly.

Anode

The positive pole or electrode of an electrolytic cell [24, 30].

Anodizing

A process for applying an oxide coating to aluminum. This process is performed electrolytically in an acid solution with equipment similar to that used for electroplating [28].

Barrel Manufacturing Techniques

Drilling – The first operation in producing a gun barrel is deep hole gun barrel drilling. The three major components for a deep hole gun barrel drill are:

- the driver which holds the drill in the machine;
- the shank which connects the driver to the tip; and
- the tip, typically made of solid carbide, which performs the cutting.

There is a hole running down the center of the drill to supply coolant oil while drilling. When drilling, the work turns at between 5000-7000 RPM (revolutions per minute), and the drill feeds into the work at between 1 ¾ and 3 IPM (inches per minute). Both the feed rate and RPM are dependent upon the drill diameter. As the drill feeds into the work, coolant which is being pumped down the center of the drill flushes the chips from the barrel into the bed of the machine. This procedure does not produce a surface smooth enough for a finished bore, nor does it hold the required diameter tolerances throughout the gun barrel. None of the toolmarks generated by the barrel drilling will remain in the finished barrel, if the barrel is subjected to the reaming process.

Reaming – The operation that typically follows barrel drilling is reaming. Reaming is done to smooth the surface left by the drill and to produce a bore that has a true round diameter. The cutting tool usually has four straight blades with a flute between each blade. The cutting edge is

RETIRED

on the leading edge of the blade. In normal operation, the reamer, which is attached to a hollow shank through which coolant is pumped, turns and is pushed or pulled through the barrel. Toolmarks generated appear as annular rings when viewed through the barrel.

Rifling – Once the barrel is reamed, it is ready to be rifled. Rifling is the method of creating spiral grooves within the bore of the barrel. Refer to **Rifling Methods.**

Lead Lapping – Lead lapping is an operation that is performed on barrels to improve the surface finish and also to help make the size throughout the barrel more uniform. To produce the lead lap, molten lead is poured into the end of the barrel. The lead forms to the inside size and configuration of the barrel, and after hardening the lead lap is removed. Lapping grit is applied to the lead slug before the slug is reinserted into the barrel. The lapping grit creates marks that run parallel to the rifling. The amount of material removed is approximately .0001" in 10 minutes.

Upset Forging – Forming of a gun barrel blank by hammering a steel bar to increase its diameter at one end for the purpose of providing a shank section.

Billet

A bar of solid round or square metal that has been forged, hot rolled, cast, or extruded [6].

Blanking

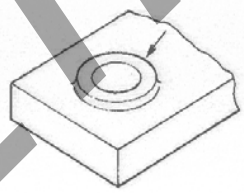
A stamping operation in which a die is used to shear or cut a desired shape from flat sheets or strips of metal. This is the first step in the process of making a cartridge case [28].

Bore

To enlarge a hole with a boring tool as in a lathe or boring mill [18].

Boss

A projection, usually cylindrical, on a machine part in which a pin or shaft is to be supported.



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Broaching

A manufacturing process for machining flat, round, and contoured surfaces. A toothed cutting tool known as a broach is pushed or pulled across the work piece, with each tooth removing only a small portion of material.

Button

A tool made from solid carbide used in barrel rifling that is larger than the reamed bore. There are three surfaces to the button tool. The straight cylindrical portion of the tool is the pilot and aligns the tool to the bore. The next portion of the button tool is larger in diameter and it is the portion of the tool that forces the grooves into the barrel. This section is ground to the exact reverse of the finished rifling pattern, i.e., the land on the button produces the groove in the barrel. The last diameter area of the button tool is the bore section of the tool, which passes over the top of the land, smoothing it and maintaining a true round bore. Any toolmarks that are in the barrel before rifling with the button will still be in the barrel after the rifling, although they will be smoothed considerably. Refer to **Rifling Methods – Button Rifling**.

Brittleness

The quality of a metal or material to fracture without undergoing appreciable plastic deformation [6].

Bunting
The process of forming the head of a cartridge case by flattening it [12].

Burnishing
Smoothing metal or plastic surfaces by friction, pressure, and polishing medium [6].

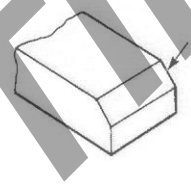
Carburizing
The process of chemically combining carbon and a metal, by mizing and heating fine powders, to form a metallic compound such as carbide [23].

Case Hardening
A process of surface-hardening alloys so that the surface layer or case is made substantially harder than the interior or core. Case hardening is used to improve wear resistance properties of parts. Typical case hardening processes are **carburizing**, **cyaniding**, and **nitriding**.

Casting
The process of pouring a liquid or suspension into a mold to produce an object of desired shape [28].

Cathode
The electrode which receives electrons from an external circuit.

Chamfer
1) To bevel a sharp external edge.
2) A beveled edge.



Chase
1) To cut threads in a lathe.
2) To groove; indent.

Chatter
The vibration between the manufacturing tool and the work piece sufficient enough to cause irregular toolmarks on the finished surface [23].

Chip
The metal removed during certain machining processes such as turning, drilling, boring, and shaping.

Chipbreaker
A small step or groove located at the top of a cutting tool to break-up chip formations [28].

Chip Formation
In metal cutting operations, shapes are formed by removing small pieces of material from the work piece in the form of chips. Chip formation and the quality of the cutting edge can be controlled with speed, angle of cutting edge, feed rate, and other variables.

RETIRED

Chip Types

Three basic types of chips exist and are defined below:
Continuous – During the formation of continuous chips, plastic deformation occurs, but the metal does not fracture. Instead, it is forced to flow over the face of the tool (like a ribbon), where it is hardened by the high pressure.
Discontinuous – Cutting a less ductile material prevents it from being able to flow, causing it to fracture. This causes the chip to break into particles known as a discontinuous chip.
Segmented – When a heavy feed rate is used in chip removal, the high stress creates internal fractures which move to the outside and cold weld together from the high pressure. This causes segmented chips.

Coin

To stamp and form a metal piece in one operation, usually with a surface design.

Cold Drawing

The process of finishing metal bars by drawing them through a die while they are cold [23].

Cold-Working

A process that involves changing the shape of a metal object by bending, shaping, rolling, or forming. As the metal is shaped, internal stresses develop which act to harden the part. The process is typically carried out at room temperature, below the recrystallization point [23].

Compacting

The forming of an object from powder by the compression of the powder which is generally confined in a die or mold [23]. This process is performed prior to **sintering**.

Component (design)

The individual parts of a machine or similar engineering design.

Computer Numerical Control (CNC)

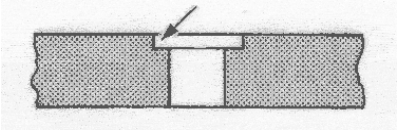
A system in which a program is used to precisely position tools and/or work pieces and to carry out the sequence of operations needed to produce a part [28].

Coolant

A cutting fluid used to cool the tool and work piece that is usually water based [23]. Refer to **Cutting Fluid**.

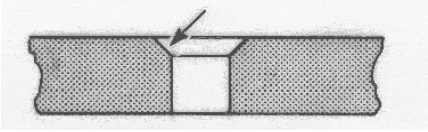
Counterbore

1) The cylindrical enlargement of the end of a drilled or bored hole.
2) A cutting tool for counterboring.
3) To enlarge a hole to a given depth.



Countersink

1) To form a depression (hole) to fit the conical head of a screw or the thickness of a plate so that the face will be even with the surface.
2) A conical tool for countersinking.



RETIRED

Crystal

A three dimensional repetitive pattern of atoms, ions, or molecules, usually synonymous with grain and crystallite in metals [6].

Crystallization

A crystal formation taking definite positions in a crystal lattice. Observed when a molten metal or material solidifies [6].

Cup

The second step in the manufacturing of a cartridge case. The blanked disc is formed into a cup by a cupping die pushing down on the center of the blanked disc [12].

Cutting Fluid

Three basic types of cutting fluids are used in the manufacturing process: water soluble oils, straight cutting oils, and chemical fluids. The following are the various functions of metal cutting fluids:

(1) To cool the cutting tool as a primary function, and to cool the work piece;
(2) To reduce the heat generated by friction through lubrication;
(3) To provide anti-weld properties in order to prevent welding of the chip to the tool; and
(4) To wash away the chips [19].

Cyaniding

Introducing a ferrous metal to molten cyanide salt, which is then held at a suitable temperature, creating surface hardening followed by quenching [6]. This allows the part to have high hardness and good wear resistance [28].

Die

1) A steel device that will form, punch, or bend a work piece when used with a matching die, tool, or punch in a press.
2) The component on a machine which gives the work piece its shape (ex. Extrusion dies, injection molding dies, etc.).
3) A tool for cutting external threads.

Die Casting

A casting process in which molten metal is forced under pressure into a permanent mold.

Die Stamping

The use of a die to impress (stamp) a piece of metal.

Draw

1) To stretch, elongate, or distort metal by pulling through a die.
2) To temper steel by gradual or intermittent quenching.

Drawing

A step in the manufacturing of a cartridge case. The process involves a punch and a series of dies to lengthen the walls of the cartridge case from the cup stage.

Drill

1) A revolving tool used for cutting (twist drill) cylindrical holes in a work piece.
2) To sink a hole with a drill.

RETIRED

Drop Forging
A process by which metal is formed in a die, usually done by impact.

Ductility
The property of a material to be deformed or stretched permanently without fracture, while under tension [23].

Elastic Limit
Stress that a material will tolerate before permanent deformation occurs [6].

Elasticity
Nonpermanent deformation.

Electrical Discharge Machining (EDM)
An electrical process using a spark gap to remove material. This is accomplished by rapidly recurring discharges between two electrodes separated by a dielectric liquid causing the metal to be removed. This method removes metal from both the work piece and the tool simultaneously.

Electrochemical Machining (ECM)
A process which employs a machining tool (cathode) that is driven through the barrel, an electrolyte that is pumped through the gap between the cathode and the barrel, and current that is applied to the cathode, thus dissolving the material in the barrel. This method removes metal only from the work piece.

Electrolysis
Production of chemical reactions by the passage of an electrical current through an electrolyte.

Electrolyte
(1) An electrically conductive fluid or medium in which the flow of current is accompanied by movement of matter. Most often an aqueous solution of acids, bases, or salts, but also includes many other media, such as fused salts, ionized gases, some solids, etc.
(2) A substance that is capable of forming a conducting liquid medium when dissolved or melting.

Electroplating
The formation of an adherent metallic coating upon a base metal by passing an electric current from an anode through an electrolyte to the cathode.

Face
1) To machine a flat surface perpendicular to the axis of rotation on a lathe.
2) That part of a component in contact with a mating component.

Filing
A finishing technique, usually performed using a hand tool or file, where machining may be difficult. The file has multipoint cutters (teeth) that when reciprocated over a surface remove a small amount of surface material.

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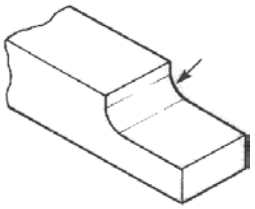
Fillet

A rounded filling of the internal angle between two surfaces.



Flame Hardening

A process of hardening a ferrous metal by heating it above its transformation range by means of a flame, and then quenching as required [6].

Flange

A projecting rim or edge for fastening or stiffening which can have bolts drilled to connect to the matching part.

Forge

To shape metal either by pressure or impact.

Forming

A method of working sheet metal into useful shapes by pressing and bending [28].

Grain

In reference to metal, a single crystal consisting of parallel rows of atoms called a space lattice [23].

Grinding

An operating that removes material by rotating an abrasive wheel or belt against the work piece [28]; a type of **abrasive machining**.

Hard-drawn

Cold worked to high hardness by drawing.

Hardenability

The ability to develop maximum hardness.

Hardening

Any process that increases the hardness of a metal. Hardness can be attained using several methods. Refer to **Age Hardening**, **Case Hardening**, **Flame Hardening**, **Induction Hardening**, and **Quench Hardening**.

Heat Treatment

The heating, soaking, quenching, and tempering of a ferrous metal or alloy will produce the desired transformations and structure. Heat treatment affects characteristics such as ductility, hardness, malleability, and strength [6].

Honing

A finishing process which uses abrasives to smooth and straighten holes [23].

RETIRED

Hot-working
The use of heat to form or shape metal at a temperature high enough to avoid strain hardening. The lower limit of temperature for this process is the recrystallization temperature [6].

Hydrogen Embrittlement
The reduction in ductility of a metal when hydrogen is allowed to be absorbed. The inclusion of hydrogen is often a result of the plating process [6].

Induction Hardening
Hardening a ferrous alloy by heating it above the transformation range using electrical induction created with a coil, quenching the material, and then tempering the material [6].

Knurl
To roughen or indent a smooth, round surface (such as a knob or handle) to aid in griping.

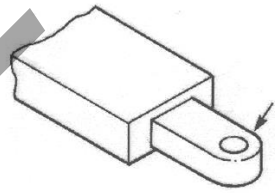
Lapping
The process of polishing a metal surface with a fine abrasive substance.

Lathe
Machine tool for turning, facing, boring, and other similar operations [23].

Low-alloy steels
Steels containing up to 10% alloying elements.

Lubricant
A substance capable of reducing friction, heat, and wear.

Lug
A handle or part that projects like an ear off of a larger piece which is typically rectangular in cross section.



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Malleability
The ability of a material to deform under compressive stress.

Mechanical Plating
The formation of an adherent, metallic coating upon a base metal by impingement of solid particles of the coating metal.

Mechanical Properties
Those properties associated with stress and strain.

Mechanical Working
Rolling, hammering, drawing, machining, etc., to change a material's shape, properties, or structures [6].

Mesh
The screen size for particle measurement.

Metal Injection Molding

A manufacturing process which involves combining fine metal powders with a plastic binders which allow metal to be injected into a mold using equipment similar to standard plastic injection molding machines. A process often used to manufacture small, complex, metal parts.

Mill

To machine with rotating toothed cutters on a milling machine.

Neck Annealing

A heating process in cartridge case manufacture applied to the neck of the cartridge case to relieve internal stress caused by cold working.

Nitriding

A case hardening technique in which a ferrous alloy is heated in an atmosphere of ammonia or in contact with a nitrogenous material to produce surface hardness by absorption of nitrogen. Quenching is not necessary in this process [28].

Normalizing

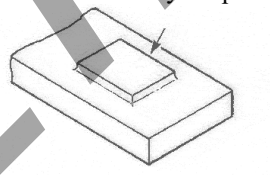
A process in which ferrous alloys are heated above a critical temperature range and cooled slowly in still air at room temperature. This process is performed to relieve stress that may have developed during machining, welding, or forming operations [28].

Oxidation

Chemical formation of a compound whereby oxygen forms an oxide. Exposure of a heated metal to atmosphere usually results in oxidation on the surfaces in the form of discoloration [6].

Pad

A shallow projection. Distinguished from boss by shape or size.



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Passivation

Changing the active surface of a metal to a much less reactive state using chemical processing. Passivation cleans the surface of the minute particles of steel chips from the cutting tools and prevents corrosion. Stainless steel resists rusting due in part to a thin invisible film oxide that covers the surface and resists corrosion [6].

Peeling

The detachment or partial detachment of an electro-deposited coating from a basis metal or undercoat.

Peen

1) To stretch, rivet, or clinch over by strokes with the peen of a hammer.
2) The rounded end of the head of a ball peen hammer.

Pickling

Chemically removing oxides and scale from metals by chemical or electrochemical reaction [6].

Plane

To machine work on a planer having a fixed tool with a reciprocating head.

Planish

To finish sheet metal by hammering with polished face hammers.

Plastic Deformation

Permanent deformation that occurs when a metal is stressed beyond its elastic limit resulting in the displacement of atoms (or molecules) to new lattice sites.

Powder Metallurgy

Forming parts out of powdered metal by compacting the powder into a die or mold under great pressure and then heating it. Also refer to **Sintering**.

Profile

To machine an outline with a rotary cutter, usually controlled by a master cam or die.

Punch

To perforate by pressing a non-rotating tool through the work.

Quenching

A rapid cooling of heated metal by contact with fluids or gases to impart hardness or softness depending on the material [6, 29].

Ream

To finish a drilled or punched hole very accurately with a rotating fluted tool, called a reamer, of the required diameter.

Recrystallization

The heating and cooling of a metal through its critical temperature causes crystal structures to change from one structure to another [6].

Recrystallization Temperature

The minimum temperature required to complete a change in crystal structure during a specified time [6].

Residual Stresses

Internal stresses of a metal that result from non-uniform plastic deformation. Cold working from forming or machining and quick temperature changes can cause residual stresses [6].

Resilience

The ability of a material to return to the original shape after removal from work stress [6].

Rifling Methods

Cut Rifling –The oldest method of rifling is the single point or hook rifling method. With this method, a cutter is adjusted to make a cut of approximately .0005" depth and is then pulled through to create each groove in the barrel. After making the cut in each groove, the cutter is adjusted to cut .0005" deeper and then pulled through each groove again with the process repeated until the desired depth of the groove is obtained. With this method, the annular rings produced by the reaming are completely removed by cutting the groove, but left on the top of the land.

Hook Rifling – A cut rifling method that employs a cutting tool which has a hook shape and cuts only one groove at a time.

RETIRED

Scrape Rifling – A cut rifling method that employs a cutting tool which cuts two opposing grooves simultaneously.

Broach Rifling – As barrel manufacturing techniques improved, the cut rifling method was replaced with broach rifling. The broach is made up of a single cutting edge or a series of individual cutting edges. Each cutting edge is called a "button." Like cut rifling, all trace of annular rings are removed in the groove but left on top of the land. A bore broach may be used to give the same type of finish on the top of the lands as the groove broach gives to the groove.

Single Broach Rifling – Rifling is created by a non-adjustable tool that cuts all of the grooves simultaneously, and is used in a series of increasing dimensions until the desired groove depth is achieved.

Gang Broach Rifling– A tool having a series of cutting edges (buttons) of slightly increasing height is used to cut spiral grooves in a barrel. All grooves are cut with a single pass of the broach. Each button is the equivalent of one set of passes through the barrel with the cutter at a set depth using cut rifling. The buttons on the broach grow in diameter approximately .0005" with each proceeding button, which is the same amount the cut rifling tool is raised after making a cut in each groove. The broaching tool pulled through the barrel one time with multiple buttons, each comprised of a series of cutters, is the equivalent of making multiple passes through the barrel with cut rifling. The images below are of a gang broach.

Photographs courtesy of Scott Doyle –http:// www.firearmsid.com

Button Rifling – After broach rifling, the button rifling method was developed. This was a radical departure from both cut rifling and broach rifling in that rather than cutting the grooves into the barrel, they were pressed into the barrel. This tool employed is made from solid carbide, is larger than the reamed bore, and is either pulled or pushed through the bore, depending upon the type of machinery used. Any toolmarks that are in the barrel before rifling with the button will still be in the barrel after the rifling, although they will be smoothed considerably. When the carbide button was first introduced it was described as a swaging process or swaged rifling. Refer to **Button**. The image below is of a button.

Photograph courtesy of Scott Doyle – http:// www.firearmsid.com

Electrical Discharge Machining (EDM) Rifling – An electrical process to produce rifling grooves in barrels using a spark gap to remove material. Refer to **Electrical Discharge Machining**.

Electrochemical Machining (ECM) Rifling – An electrochemical process used to produce rifling grooves in barrels. Refer to **Electrochemical Machining**.

RETIRED

Hammer Forged Rifling – The formation of spiral grooves in the bore of a rifle barrel by means of inserting a mandrel with a rifling configuration into the barrel blank. The outer surface of the barrel is machine hammered, forcing the barrel material down against the mandrel causing the inner surface of the barrel to take on the shape of the mandrel. Also known as swage rifling.

Rigidity

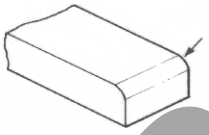
The property of resisting elastic deformation.

Rockwell Hardness

A measure of the hardness of a material. The depth of the indentation into the material in question caused by either a steel ball or a specially designed diamond cone penetrator under a prescribed load is the basis for the test [28].

Round

A rounded exterior corner between two surfaces. Compare to **Chamfer** and **Fillet.**



Rupture Stress

True stress at the time of fracture.

Sandblast

To clean castings or forgings by means of sand driven through a nozzle by compressed air.

Sanding

A finishing process that utilizes an abrasive material to smooth or polish.

Sawing

A process where a narrow slit is cut into the work piece by a tool containing a series of narrowly spaced teeth called a saw blade [23].

Scale

A form of oxide from iron that forms on the outside of hot steel [6].

Shape

To machine with a shaper.

Shaper

A machining tool different from a planer in that the work is stationary and the tool reciprocates.

Shear

To cut sheet or bar metal between two blades which pass immediately adjacent to each other.

Shim

A thin spacer of sheet metal used for adjusting.

Shim Stock

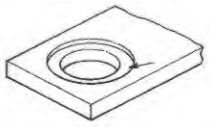
Thin sheets of metal of precise thickness, usually measured in thousands of an inch, from which shims are produced.

RETIRED

Sintering
The operation of heating a powder compact so that it shrinks and fuses to a near void-free condition [23].

Spin
To shape sheet metal by forcing it against a form as the sheet metal revolves.

Spot-Face
To finish a round spot on a rough surface, usually around a drilled hole, to give a good sheet to a screw or bolt head.



Strain Hardening
Hardness and strength increases caused by plastic deformation at temperatures below the recrystallization range. Also refer to **Work Hardening** [6].

Stress
Force per unit area.

Stress Rupture
The sudden and complete failure of a material held under a definite constant load for a given period of time at a specific temperature.

Supercooling
Cooling a liquid or gas below the freezing point without it becoming a solid.

Swage
To shape metal by hammering or pressure with the aid of a form or anvil called a swage block.

Sweat
To join metal pieces by clamping together, with solder between, and applying heat.

Tack Weld
To join at the edge by welding in short, intermittent sections. The armored face plates of some safe doors are tack welded to the body of the door.

Tap
To cut threads in a hole with a rotating tool, called a tap, having fluted threads to give cutting edges.

Tarnish
Dulling, staining, or discoloration of metals due to superficial corrosion.

Temper
To make hardened steel less brittle by controlled heating and cooling.

Tensile Strength
The maximum amount of force, stress, or tension in a material before failure occurs [6, 24].

RETIRED

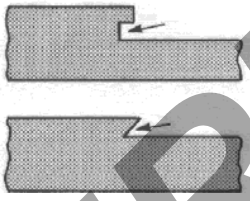
Threading
A turning operation that uses a single point tool performed on a lathe or machining centers [23].

Tumble
To clean, smooth, and polish metal parts by placing them in a rotating barrel or drum with abrasive materials.

Turning
Machining operation in which the work is rotated against a single point tool [23].

Ultrasonic Machining
A machining process in which a range of sound waves propel an abrasive for use as a metal-cutting tool [28].

Undercut
1) To cut, leaving an overhanging edge.
2) A cut having inwardly sloping sides.



Upset
To forge a larger diameter or shoulder on a bar.

Viscosity
The measure of the resistance of a fluid being deformed by stress.

Welding
A joining operation involving the melting of the joined metals.

Work Hardening
Increased resistance to deformation as a result of cold working. As the metal is shaped, internal stresses develop which act to harden the part. Heat created by high cutting speeds or friction can also work harden a material by acting like a catalyst to produce higher hardness levels in the work piece [6, 24]. Also refer to **Strain Hardening**.

Yield Point
The point of stress or strain at which a material fractures [28].

Yield Strength
Maximum resistance to elastic deformation. Yield strength is measured just prior to the point before permanent deformation occurs.

RETIRED

Knife Terminology

Actuator Button

The button which releases the blade on an automatic opening knife.

Back

The flat, unsharpened side of the blade opposite the edge. Refer to the K**nife Illustrations**.

Back Spring

The spring which provides tension upon opening and closing of a folding knife. Also known as the butt spring, head spring, and double end spring (in slip-joint folders).

Bevel

The sloping areas which fall from the spine or thickest section of the blade toward the edge.

Blade Shapes

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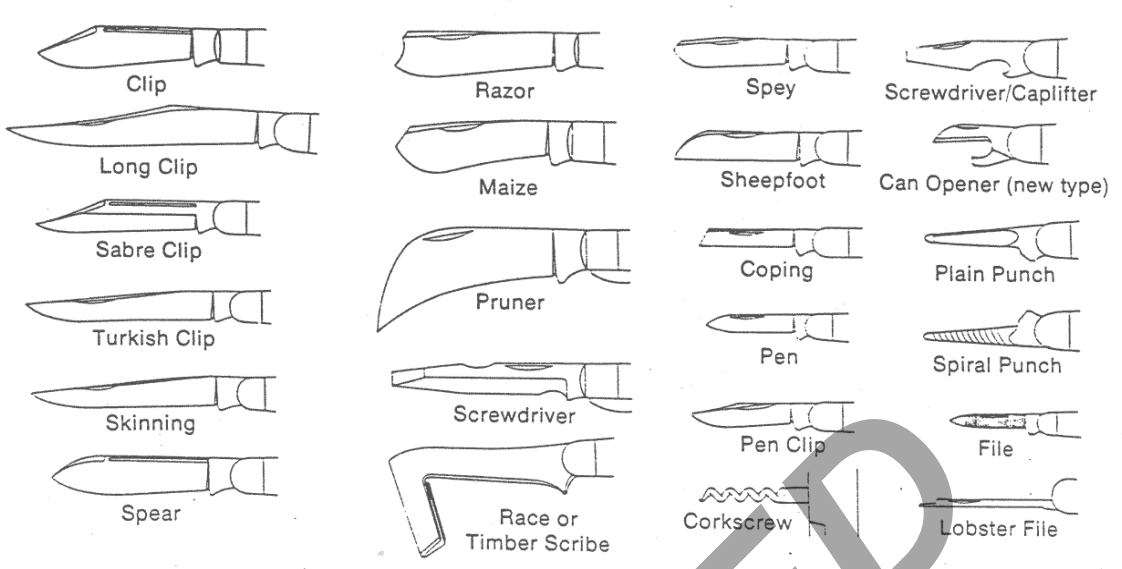

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Bolster

A piece that joins the handle to the blade and increases the strength to the knife. May also be found on the end of the handle to further increase blade and handle strength.

Butt Cap

A simple and typically ornamental piece of metal applied and mounted to the butt of a knife handle for strength, for a surface to apply pressure or light impact, for a surface to engrave or embellish, or to prevent wear and splitting of the handle material (wood, horn, or bone).

Choil

The area between the cutting edge and the tang.

Crink

A slight bend at the tang of a multi-bladed knife which permits the blades to miss one another and close properly.

Crossguard or Guard

A piece of metal affixed to the handle to protect the users' hands from an opponent's blade. Refer to the **Knife Illustrations**.

Dagger

A short weapon for stabbing that is usually considered to be double edged.

Edge

The cutting (sharpened) side of the blade.

Escutcheon

A small piece of metal on the handle which can be used for engraving information or decoration. Also known as the shield.

False Edge

A section on the back of the blade which is sharpened a short distance from the point. Refer to the K**nife Illustrations**.

Ferrule

A metal portion normally strengthening the junction between the grip and the guard. Refer to the K**nife I**llustrations.

Finger Grooves

Notches or grooves in a knife handle which provide a secure, comfortable holding surface.

Finger Stalls

The adjoined loops or rings through which the fingers pass when the knife is held for combat. A feature typically found on knuckle-duster pattern knives.

Fixed Blade

Any knife in which the blade does not fold or retract into the handle (e.g., hunting and survival knives).

Fly Spring

The spring which opens the blade of an automatic opening knife (switchblade).

Folding Knife

Any knife in which the blade pivots into the handle.

Fuller

A groove designed to lighten yet strengthen the blade. Also known as a blood groove.

Grind

The method used to form a particular type of blade. There are three basic types of grinds.

(1) **Convex Grind** – In cross section, the sides leading to the edge are rounded outward (convex). This grind is best in applications where heavy materials like wood need to be cut with a great deal of force.

(2) **Flat ("V") Grind –** In cross section, the blade forms a "V" with straight sides. A compromise of convex and hollow grinds. May also be known as a straight grind.

(3) **Hollow Grind** – In cross section, the blade forms a concave shape with the sides hollowed out along the edge. Best suited for softer materials. Straight razors and many hunting knives are hollow grind.

Grip

The part of the hilt that is normally held in the hand. May be a single piece of material or a two-piece construction riveted or bolted to the tang. Refer to the **Knife Illustrations**.

Hilt

The handle of a sword or dagger. A single-edged modern knife does not have a hilt, it has a handle.

Kick

On a folding knife, the projection on the front edge of the tang on which the blade rests in the closed position. Prevents the edge from hitting the spring.

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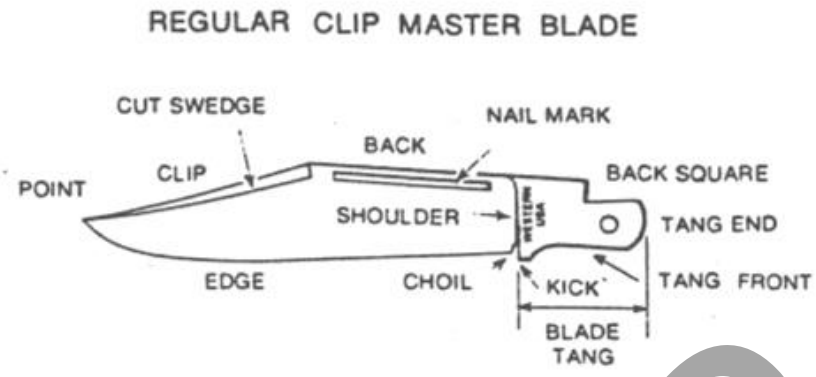
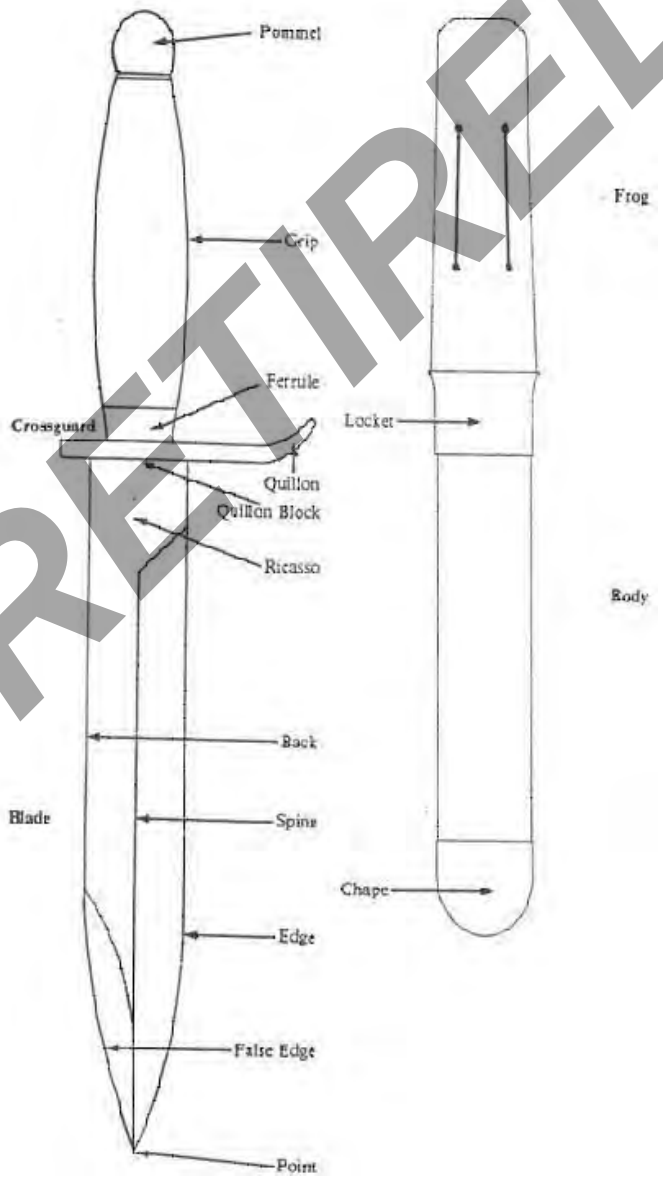
Knife Illustrations





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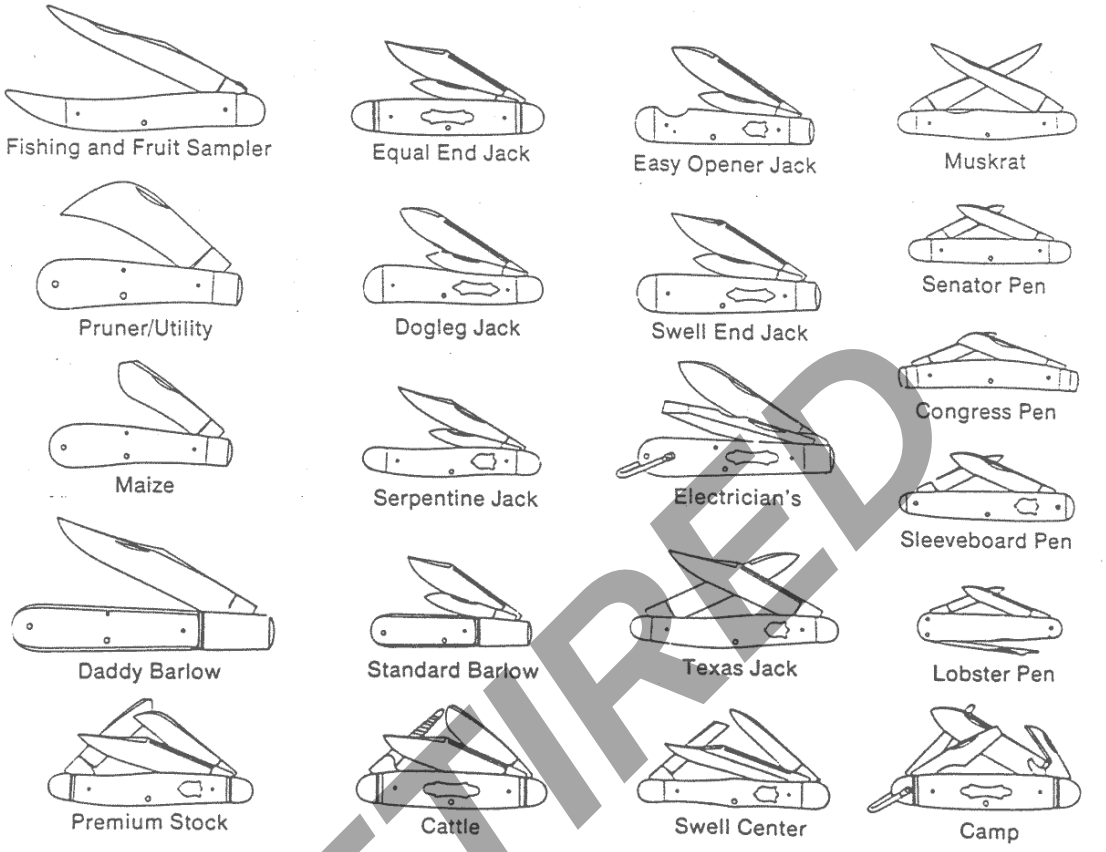
Folding Knife Patterns



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Liner-Lock
A folding knife in which an internal liner locks the blade into the open position.

Lockback Folder
A folding knife in which the back spring locks the blade into the open position.

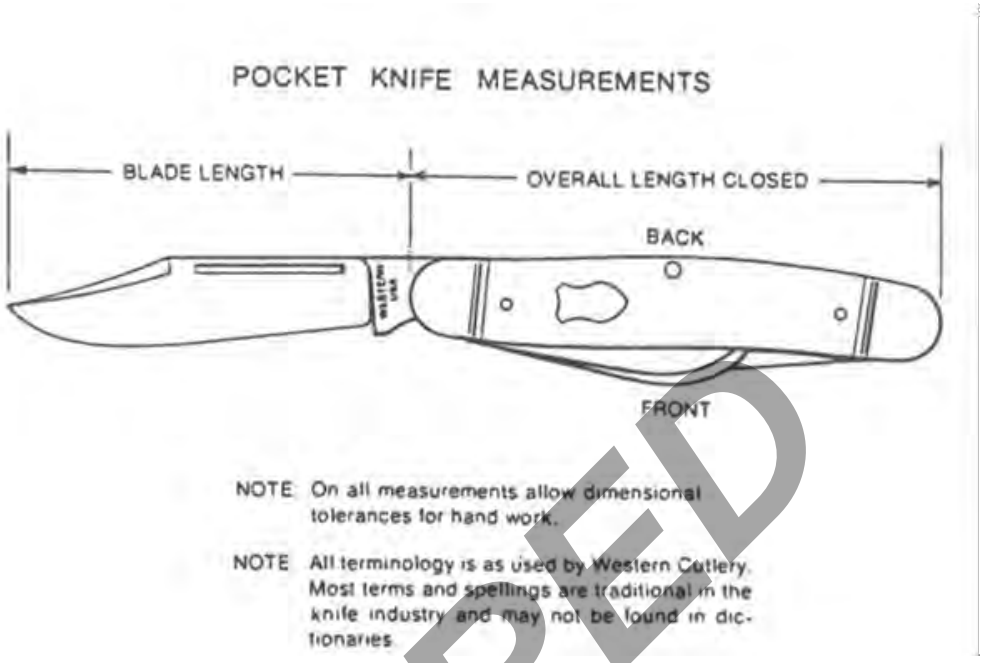
Mark Side
Side of the blade with the nail mark.

Nail Mark
A thumbnail groove cut into the blade so it can be opened easily. Also known as a nail nick.

Obverse Side
Front or display side of the knife. Usually has the manufacturer's mark and shield. Also known as the **Mark Side**.

Pile Side
The reverse side of the blade.

Pocket Knife Measurements



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Point

The tip of the blade. Refer to the **Knife Illustrations**.

Pommel

On a knife, dagger, or sword, it is the ornamental globular ball that terminates the handle at the end. On modern knives, it is the tapped and threaded component that mechanically secures the handle to the threaded tang or rod in a hidden tang knife. Refer to the **Knife Illustrations**.

Poniard

A dagger with a slender triangular or square blade.

Quillion

The projections that keep the hand from sliding forward onto the cutting edge, or backwards off the butt of the knife handle. May also be spelled Quillon. Refer to the **Knife Illustrations**.

Ricasso

A flat section of the blade between the guard and the beginnings of the grind bevel. It is commonly preferred for the maker's mark. Refer to the **Knife Illustrations**.

Scabbard or Sheath

Device in which the knife is carried that may be comprised of the following components:

1. **Body** – The main portion of the scabbard.
2. **Chape –** The reinforced tip of the scabbard body.
3. **Frog** – A belt loop that acts as a seating for the scabbard body.
4. **Locket** – A reinforced section at the throat of the scabbard.
5. **Throat –** The opening in the scabbard in which the blade is inserted.

Refer to the **Knife Illustrations**.

Scales

Pieces of handle material which are attached to the sides of full tang knives to form the handle.

Shoulders

The upper portion of the ricasso which marks the dividing line between the blade and the tang.

Side Opening Knife

A knife in which the blade swings outward from the handle. May be of a manual or automatic opening design.

Side-Lock Folder

A folding knife in which a locking blade mechanism on the side of the handle (usually on the bolster) locks the blade into the open position. A button on the side releases the blade.

Slip-Joint Folder

A folding knife in which the blade does not lock into the open position. The blade is held open only by spring tension on the back spring.

Spine

A high, centrally placed ridge running the length of the blade from the ricasso to the tip. Refer to the **Knife Illustrations**.

Stiletto

A slender dagger with a blade that is thick in proportion to its breadth.

Straight-Line Knife

An automatic knife in which the blade springs straight out of the handle when activated.

Swedge/Swage

The beveled portion of the blade between the spine and the false edge. Refer to the **Knife Illustrations**.

Tang

The metal portion which projects into the handle of fixed blade knives.

RETIRED

Gunshot Wound Terminology

Abrasion Ring

A zone of mechanical abrasion involving part or the entire margin of a bullet entry wound. As a projectile pushes its way inward, there is a period of time during which tissues that are going to constitute the margins of the entry wound are in contact with the front and/or bearing surfaces of the projectile. After the bullet enters and the tissues rebound, the scraped or abraded zone, which is due to mechanical scraping, can be seen immediately adjacent to the defect through which the bullet entered. When asymmetrical, it can sometimes help to determine the angle at which the projectile entered. Abrasion margins tend to be narrower and harder to see in skin which is supported by underlying bone because it is not as easily indented. Also known as an abrasion margin, abrasion collar, and abraded edge.

Abrasions

The result of skin contacting something with sufficient force to rub away part of the skin surface. Also known as an abraded injury, grazes, scrapes, and brush abrasions.

Anatomic Position

The frame of reference for the body is known as the standard anatomic position, which is standing at attention with hands open and palms to the front, thumbs outward. This is the position pathologists should be using when describing wound locations. For example, a wound described as being at the lateral aspect of the wrist should be near the base of the thumb - not the little finger.

Anterior Axillary Line

An imaginary line that runs vertically down the skin of the trunk from the front of the armpit.

Antero-Lateral

To the front and away from the midline of the body.

Antero-Medial

To the front and towards the midline of the body.

Antero-Posterior

From front to back. Often abbreviated as AP and used in reference to x-ray films. An AP chest x-ray is one in which the x-ray film holder is behind the chest and x-ray tube where the ray originates is in front. Thus the x-ray travels from the front to back in traveling from the tube through the chest to the film.

Avulsion

A forceful separation or detachment; tearing away of a body part.

Beveling of Bone

When bullets pass through bone they usually produce a chip which is beveled or cone shaped. Like a classic cone chip in glass, the entry side is sharp edged and the exit side larger and sloping. The chip is thrown and the dimensions of the cone get bigger in the direction in which the bullet traveled. Bone is extremely useful for confirming the direction of travel within the body. Most of the time, direction can be determined at a glance when beveling is involved. It is most commonly used in determining bullet direction in shots through the skull. There are some places in the body where it is less informative, primarily where the bone is too thin to permit the reading of the direction of the chipping, for instance in the long bones of the hands and feet. Beveling may also be seen in teeth, dentures, finger nails, and in items on and about a victim when shot.

RETIRED

Billiard Ball Effect

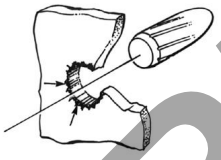
The divergence of pellets from the axis of the wound channel caused by collisions between pellets in a shot string as they move into and through tissue or organs. The resultant scatter of pellets can give the appearance of a distant shot when viewed in X-ray films [14].

Bullet Embolus

Anything that remains separate from the blood and travels in the bloodstream is called an embolus (air bubbles, blood clots, etc.). When a bullet shot into the body comes to rest inside the heart of a large blood vessel, it can be carried to other parts of the body by the blood flow. When this happens, it is known as a bullet embolus.

Bullet Wipe

The discolored area on the immediate periphery of a bullet hole, caused by the transfer of residues from the bearing surface of the bullet. These dark gray to black residues typically contain carbon, lead, bullet material, and possibly other constituents such as bullet lubricant and primer residues. Bullet wipe may occur at any range of fire. Also known as burnishing, leaded edge, gray collar, gray rim, or gray ring.



Charring

Searing of edges of an entry bullet hole is due to the momentary heating of the tissue by hot gasses when the gun is very close to or in contact with the body at the time of discharge. Also known as scorching or searing.

Contusions

Contusions result from blunt forces distorting the soft tissues to an extent sufficient to result in disruption and leakage of blood vessels. Escape of blood into tissues is what produces the discoloration. The amount of blood which escapes from the vessels will depend on features such as their size and the pressure within them, the ability to clot, and the space available for blood to fill. Also known as bruising.

Costal Margin

The lower edge of the rib cage.

Distal

Direction away from the origin or point of reference. In a blood vessel, distal is the direction away from the heart. In the spinal cord or a nerve, distal is the direction away from the brain.

Dorsal

Pertaining to the back of the torso.

Ecchymosis

A purple patch in the skin caused by extravagation of blood (akin to a bruise). A “black and blue” spot.

Exit Wound

The typical exit wound has sharp, well defined edges, free from abrasion. This reflects the mechanism of production which is that the bullet bursts or pushes its way out of the skin. Thus exit wounds may often be slit-like, stellate (star-shaped), "T" shaped, "L" shaped, or crescentric. Also known as outshoot.

RETIRED

Frontal Plane
An imaginary plane that divides the body into a front half and a back half. Also known as a coronal plane.

Hematoma
A localized mass of blood that is relatively or completely confined within an organ, tissue, space or potential space. It may be totally or partially clotted.

Hemothorax
A collection of blood in the pleural space, which is a potential space between the surface of the lung and the wall of the chest. Ordinarily, the surface of the lung is in contact with the chest wall (which is why it is called a "potential" space). A hemothorax occurs when a lung is injured and blood leaks in between the lung and chest wall.

Inferior
Pertains to the lower half of a body.

Intermediate Target
Refers to something located between the firearm and the target at the time of shooting. While clothing is occasionally regarded in this way, the term most often refers to objects not usually associated with a person's body. Also known as interposed target or interposed object.

Lead Snowstorm
A pattern of white specks on the dark background of an x-ray of a body part. It occurs when bullet fragments block the x-rays on the x-ray film. This phenomenon is usually associated with fragmentation resulting from the penetration of a high velocity jacketed bullet.

Lumbar
Lower back, extends from the chest to the pelvis. There are five lumbar vertebrae.

Medial
Toward the midline of the body.

Median Plane
An imaginary plane that extends longitudinally through the midline of the body and divides it into right and left.

Metal Stippling
When there is significant misalignment in revolvers, bullets may be "shaved", resulting in metal fragments exiting from the cylinder gap or following the bullet down the barrel. These fragments may contact the skin and/or clothing.

Muzzle Imprint
A general term describing the marks produced by the muzzle, front sight, magazine tube, spring housing, etc., and caused by the contact discharge of a firearm. Also known as muzzle abrasion.

Penetrating Injury
Caused by an object entering but not exiting the body.

Perforating Injury
Caused by an object passing through the body.

RETIRED

Petechiae

Round purplish-red spots of blood, pinpoint to pinhead in size, that appear on the skin as a result of bleeding into layers of the skin.

Posterior

Pertaining to the backside of a body.

Posterior Axillary Line

An imaginary line that runs vertically down the skin of the trunk from the back of the armpit.

Powder Burns

Burning grains of black powder from the muzzle of a firearm deposit on skin and clothing, where they smolder, producing actual burns on skin. This term should be reserved for those occasions on which a black powder or replica black powder firearm is used [9].

Powder Stippling

(1) Small hemorrhagic marks on the skin produced by the impact of gunpowder particles. Also used when referring to any small pits or defects in objects impacted by unburned and partially burned gunpowder particles. To be accurate from a medical point of view, the term stippling should be used when the marks are made on the surface of the skin by the grains without penetration. The term tattooing should be used when the particles penetrate the skin.

(2) On inanimate objects, it is the creation of small pits or defects caused by the impact of unburned and partially burned gunpowder particles.

Powder Tattooing

The embedding of unburned and partially burned gunpowder particles in the skin or other tissue with accompanying hemorrhagic marks associated with living tissue. The term almost always applies to skin because skin is the surface, apart from clothing, through which bullets commonly enter the body. To be accurate from a medical point of view, the term tattooing should be used when the particles penetrate the skin. The term stippling should be used when the marks are made on the surface of the skin by the grains without penetration.

Proximal

Direction toward the origin or point of reference. In a blood vessel, proximal is the direction toward the heart. In the spinal cord or a nerve, proximal is the direction toward the brain.

Punctate

Point-like, referring to points or dots.

Puncture

A hole or wound made with an instrument or object.

Radial

(1) Related to or situated near the radius or thumb side of the hand or forearm.

(2) Developing uniformly around a central axis.

Range of Fire

The distance between the muzzle of the firearm and the target. Also known as muzzle to target distance.

RETIRED

Range of Fire Classifications

These classifications are used by pathologists to describe the range of fire for muzzle to target distance determinations via the body and/or clothing (target):

Contact – The muzzle of the firearm is held against the surface of the body/target at the time of discharge. Contact wounds/shot may be hard, loose, angled, or incomplete.

Hard Contact – The muzzle is pressed firmly against the skin/target; the edges of the entrance wounds may be seared by the hot gases and blackened by soot.

Loose Contact – The muzzle is pressed lightly against the skin/target; there is a gap of soot around the edges of the wound, resulting in soot deposited in a band around the entrance.

Angled Contact – The barrel is held at an acute angle to the skin/target so that the entire muzzle is not in contact with the skin/target. The result is an eccentrically arranged pattern of soot.

Incomplete Contact – The muzzle is held against the skin/target, but because the surface is not completely flat, there is a gap between the muzzle and skin/target (e.g., knuckles on a hand).

Near Contact – The muzzle of the firearm is not in contact with the skin/target, being held a short distance away. The distance is so small, that the powder grains emerging from the muzzle do not have a chance to disperse and mark the skin/target. The zone of soot on the skin/target is widened.

Intermediate – 1) The muzzle of the firearm is held away from the body at the time of discharge yet is close enough that the powder grains expelled from the muzzle along with the bullet produce "powder tattooing" or "stippling" on the skin. 2) The range at which a firearm and ammunition will deposit visible gunpowder particles on a target.

Distant – The only marks on the skin/target are those produced by the mechanical action of the bullet during perforation or penetration.

Sagittal Plane

Any plane parallel to the median plane. Unlike the median plane, the sagittal plane does not have to be in the middle of the body.

Scaphoid

Concave, hollowed out, boat-like.

Shored Exit

If the skin is supported by a firm object or surface at the moment a bullet exits, the skin may be squeezed between the exiting bullet and the supporting surface, giving rise to so-called pseudo or false abrasion (abrasion ring) which may be mistaken for true inward-pushing frictional abrasion of an entry wound. Common supporting objects include, but are not limited to, car seats, chairs, mattresses, bedding, clothing, articles in pockets, floors, walls, and doors. Supported exit wounds often have both exit and entry characteristics and can therefore be mistaken for entrance wounds. Shored exits can also occur from tight supportive garments, such as brassieres and belts. Indications of a shored exit include the zone of abrasion being too large, too wide, irregular, or lopsided. The weave of material imprinted within or near the edges of the abraded zone may also be observed. Also known as a supported exit.

Stellate

Star-shaped wound. Contact wounds in regions of the body where only a thin layer of skin and tissue overlies the bone usually have a stellate appearance (ex. Head). This wound is due to the effects of the expansion of gas at discharge. Where a thin layer of skin overlies bone, these gases expand between the skin and outer table of the skull, lifting up and ballooning/bursting out of the skin; thus tearing the skin in a star shape.

RETIRED

Stellation

A star shaped gunshot wound characteristic which shows radial splitting from the bullet hole. Stellation is usually found in contact wounds. However, it may also be observed in intermediate or distant shots into skin over bony prominences or curved areas of bone covered by a thin layer of tightly stretched skin. Also known as stellate tearing.

Stippling

Refer to **Powder Stippling**.

Superior

Situated above or directed upward.

Tattooing

Refer to **Powder Tattooing**.

Terminal Ballistics

Refer to **Ballistics, Terminal** in the **Firearms Identification Section**.

Tissue Delamination

The traumatic separation of layers of skin caused by the high pressure gases of a firearm discharge in contact or near contact wounds. It usually occurs over heavy bone.

Track

(1) Detectable evidence that something has passed through.
(2) The course along which something has moved. Also referred to as a projectile's path. Not to be confused with a **tract**.

Tract

A series of body parts collectively serving some combined anatomical purpose.

Ventral

Pertaining to the belly or front part of the torso.

Wound Ballistics

The scientific study of the motions and effects of penetrating projectiles in tissue. It is a branch of terminal ballistics.

RETIRED

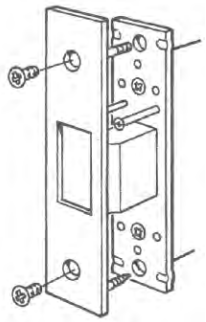
Lock and Key Terminology [17]

Active Leaf

The single door of a pair on which the active or locking hardware is mounted. Also known as the active door.

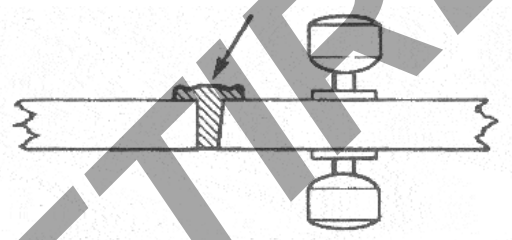
Armored Face Plate

A face plate that conceals all lock and/or cylinder mounting screws.



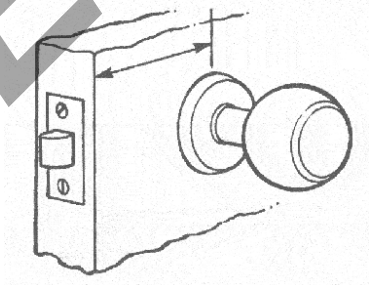
Astragal

A molding to seal the opening between a pair of doors.



Backset

The distance between the center of a cross-bore and the bolt edge of a door or drawer.



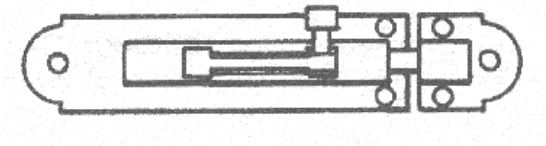
Ball Friction Catch

A push and pull fastening device installed on the edge of a door to hold the door in the closed position.

RETIRED

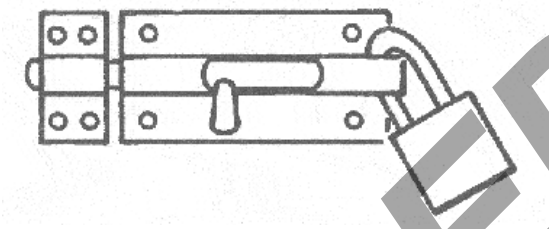
Barrel Bolt

A surface mounted slide bolt which has a cylindrical shape.



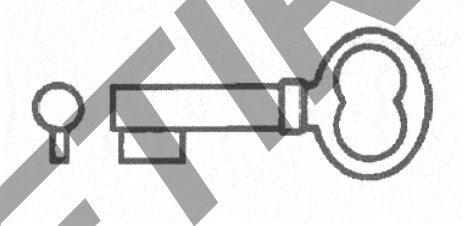
Barrel Bolt, Locking Type

A barrel bolt that may be locked with a padlock. The bolt may be locked in the locked or unlocked position.



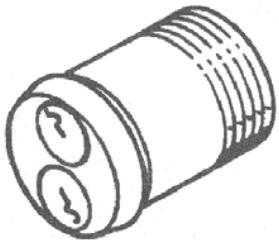
Barrel Key

A key with a round post that has a hole in the end which fits over a pin in the lock. Commonly for trunks, cabinets, suit cases, and handcuffs.



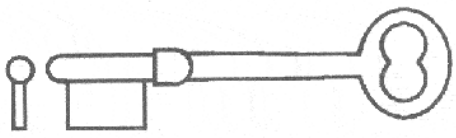
Bicentric Cylinder

A cylinder which has two independent plugs, usually with different keyways. Both plugs are operable from the same face of the cylinder. It is designed for use in extensive master key systems.



Bit Key

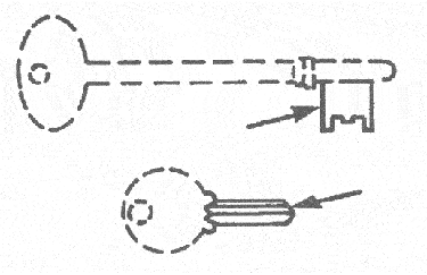
A key with a blade (or bit) projecting from a round shank. The key may possess one or more blades/bits. This key is similar to the barrel key, but has a solid rather than a hollow shank. (The old type skeleton key is a bit key.)



RETIRED

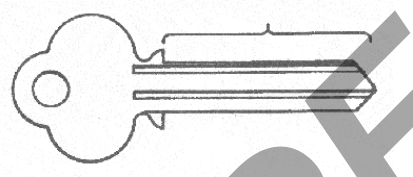
Bit

1) The part of a key which serves as the blade, usually for use in a warded or lever tumbler lock.
2) To cut a key.



Blade

The portion of a key which may contain the cuts and/or millings.



Blank

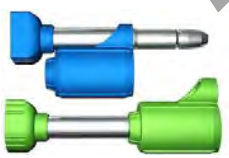
An uncut key or the unfinished key as it comes from the manufacturer before any tumbler cuts have been made.

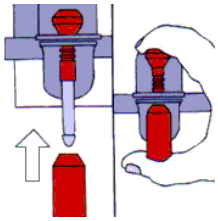
Bolt

Any moveable projection which blocks the movement of one object relative to another.

Bolt Seal

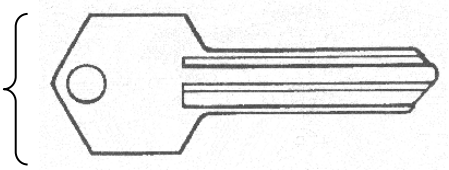
Bolt seals are typically used to secure the doors of shipping containers, trucks and trailers. Bolt seals are generally closed by hand and opened with the use of bolt cutters.





Bow

The handle or head of a key.



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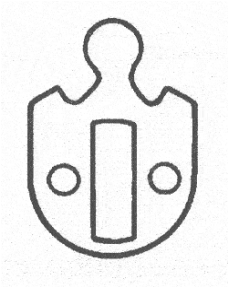
Cam

1) A lock or cylinder component which transfers the rotational motion of a key or cylinder plug to the bolt.
2) The bolt of a cam lock.

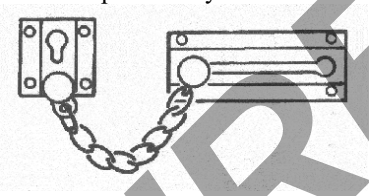


Case

The housing for the bolt and locking mechanism of a lock.

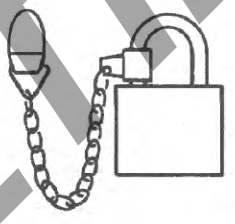
Chain Door Guard

A device which allows a door to be opened only a short distance.



Chain Padlock

The general term used for any type of padlock that has a chain permanently attached to it.

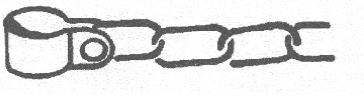


Change Key

1) A key which operates only one cylinder or one group of keyed alike cylinders in a keying system.
2) Any device that is used to mechanically or electronically allow resetting of certain key or combination locks.

Clevis

A metal link for attaching a chain to a padlock.



Code

An arrangement of numbers or letters by which a key can be cut to operate a lock without first having a pattern or the original key. The code can be for either the key or the lock.

Combination

The group of numbers which represents the bitting of a key and/or the tumblers of a lock or cylinder.

Combination Lock

RETIRED

Any lock which requires the setting in a proper sequence of certain predetermined numbers or characters in order for it to unlock (e.g., safes and combination padlocks).

Combination Pins

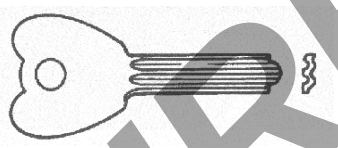
Metal pins which are used in setting combinations in pin tumbler cylinders. They are varied in length and tapered at one end, enabling them to sit into the cuts made in key blanks. When the proper key is inserted, the combination pins level off at the cylinder plug shearline allowing the plug to turn easily and activate the lock.

Compression Coil Spring

A spring made of wire coiled into a helix. A spring of this kind is used as a bolt spring and usually fits over a rod or lies in a channel formed by various parts within the lock case.

Corrugated Key

A key made of sheet metal in which corrugations are pressed length-wise in the bit.



Cremone Bolt

A surface mounted, top and bottom locking, deadbolt mechanism operated by a central handle.

RETIRED

Cylinder

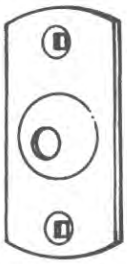
A complete operating unit which usually consists of the plug shell, tumblers, springs, plug retainer, a cam/tailpiece or other actuating device, and all other necessary operating parts.

Cylinder Collar

A plate or ring installed under the head of a cylinder to improve appearance and/or security. Collars can be either free wheeling or rigid.

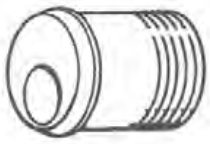
Cylinder Guard

A device used to protect the cylinder of a lock, making it less vulnerable to an attack.



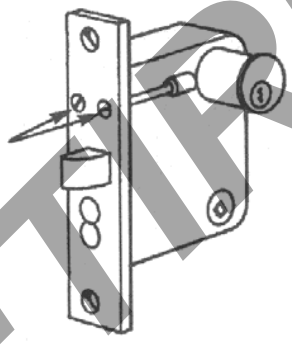
Cylinder Housing

The external case of a lock cylinder that houses the plug. Also known as the housing or cylinder shell.



Cylinder Set Screw

The screw which secures the mortise cylinder into place after it has been placed in the mortise lock.



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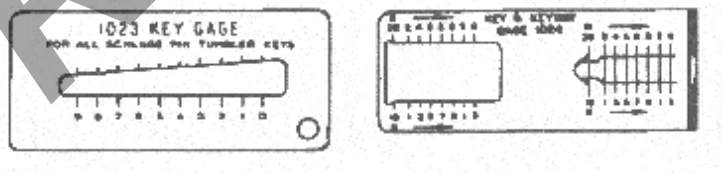
Decoder Gauge

A measuring device which helps determine the combination of a lock or cylinder without removing the tumblers.



Disc Tumbler

1) A flat tumbler which must be drawn into the cylinder plug by the proper key so that none of its extremities extends into the shell.
2) A flat, usually rectangular tumbler with a gate which must be aligned with a sidebar by the proper key.

Dog

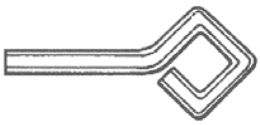
1) To place an exit device in the holdback mode.
2) In marine applications, the latch for a hatch.

Dogging Device

In an exit device, a mechanism used to temporarily disable the latch from locking.

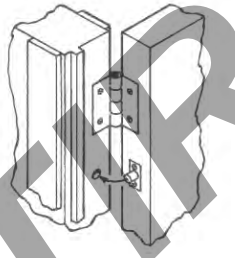
Dogging Key

A key or tool used to dog an exit device.



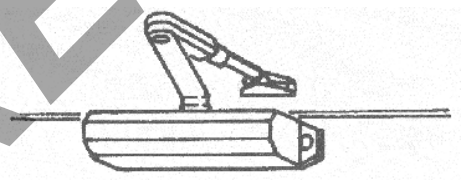
Dogging Pin

Pin(s) placed on the inside surface of the hinge stile with the hole(s) in the adjacent door frame. If the hinge pin(s) is removed, the dogging pins will prevent the door from being removed.



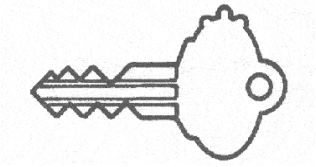
Door Check

A device used to control the action of a door by use of either hydraulic pressure, high tension spring, electrical devices, or air pressure. Also called a door closer.



Double Bitted Key

A key having cuts on two sides to activate the tumblers of a lock.



Double Throw Bolt

A deadbolt which has two distinct extended positions, both of which are deadlocked.

Escutcheon

A surface mounted trim which enhances the appearance and/or security of a lock installation.

Flat Key

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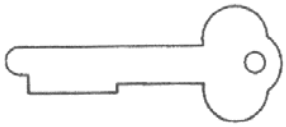
A key usually made of sheet steel without grooves or corrugations. This type of key is commonly used for some safety deposit boxes, lockers, and strong cabinets.

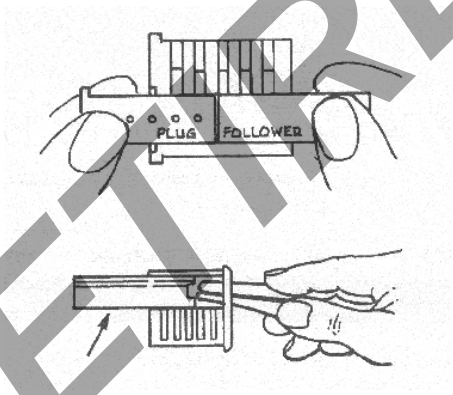


Flush Bolt

A non-keyed deadbolt designed so that when installed within the door, it is flush with the surface of the door.

Follower

A locksmith's tool which holds the driver pins and the springs in the cylinder housing while the plug is being removed.

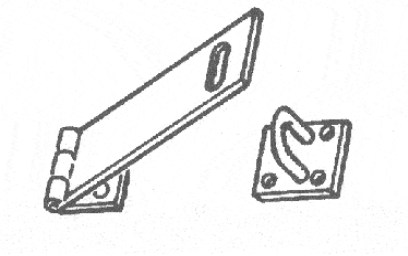


Foot Bolt

A bolt affixed to the bottom portion of the door which locks into the floor and may be operated either by hand or foot.

Hasp

A fastening device consisting of a staple and a slotted hinged plate.



Hasp with Self-Contained Lock

A hasp with a padlock type of lock attached. The bolt may be locked in the locked or unlocked position.

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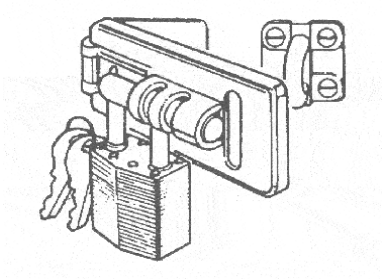
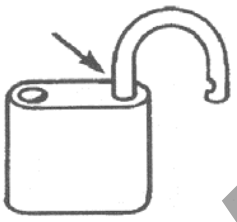


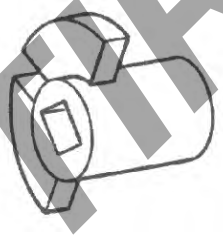
Heel of a Padlock

The end of the shackle on a padlock which is not removed from the case when unlocked. Also refer to **Toe of a Padlock**.



Hub

The part of a lock or latch which is turned by the knob or handle to withdraw the bolt or latch.



Hub Spring

The spring that returns the hub to its normal position after being turned.

Individual Key

A key for an individual cylinder.

Jimmy Resistant Rim Lock

An industry term for the lock type that has a movement in the bolt to resist prying the door away from the jamb to bypass the bolt. The bolt, in the locked position, is surrounded by a sectional hollow strike.

Key

A properly combinated device which is, or most closely resembles, the device specifically intended by the lock manufacturer to operate the corresponding lock.

Key Changes

The different combinations that are available or that can be used in a specific cylinder.

Key Cuts

The portion of the key blade which remains after being cut and which aligns the tumbler.

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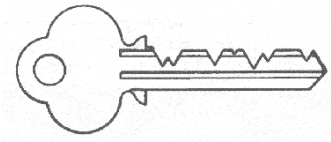


Keyed Alike

Of or pertaining to two or more locks or cylinders which have or are to have the same combination. They may or may not be part of a keying system.

Keyed Different

Of or pertaining to a group of locks or cylinders, each of which is or is to be combinated differently from the others. They may or may not be part of a keying system.

Keyhole

The opening through which a non-cylinder key must pass to enter a lock.

RETIRED

Keying

Any specification for how a cylinder or group of cylinders are or are to be combinated in order to control access.

Keyway

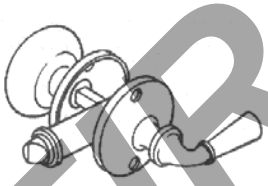
1) The opening in a lock or cylinder which is shaped to accept key bit or blade of a proper configuration.
2) The exact cross sectional configuration of a keyway as viewed from the front. It is not necessarily the same as the key section.

Key Wear

A phenomenon that occurs as a result of the continued use of a key. It is observed as marks on the area of the combination pin which comes into contact with a key. Also known as key tracking.

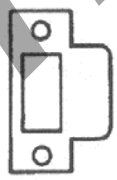
Latch

A device that secures but has no locking functions.



Lip of a Strike

The projecting part of a strike plate which guides the springs bolt to the latch point.



Lock

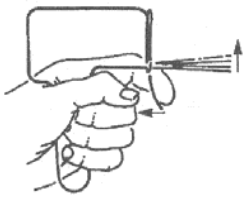
Any device which prevents access or use by requiring special knowledge or equipment.

Lock Picking

The process of operating a lock into a locked or unlocked condition by means other than the specifically planned key or keys.

Lock Picking Gun

A tool utilizing a tempered steel needle (pick) to strike the combination pins sharply. This force is transferred to the drive pins causing them to rise and fall in the chambers, momentarily creating a shearline. Pressure from the tension wrench will then turn the plug to the open position.



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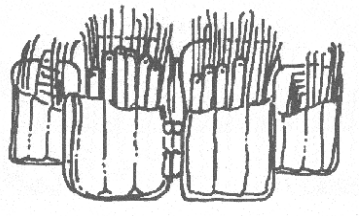
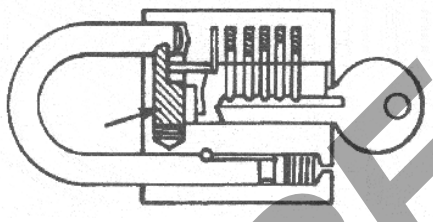
Lock Picks

Tools or instruments made for the purpose of operating a lock into a locked or unlocked condition by means other than the specifically planned key.



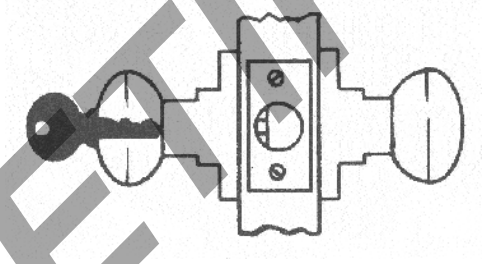
Locking Dog of a Padlock

That part of the padlock mechanism which engages the shackle and holds it in the locked position.



Lock-in-Knob

A lockset having the cylinder(s) contained within the knob.



Lock-in-Knob Cylinder

Cylinder used in lock-in-knob locks. This type of cylinder may also be found in tubular lock sets.

Locksmith, Forensic

A court-qualified physical security specialist.

Loiding

The action of slipping or shimming a spring bolt with a piece of celluloid or other thin, flexible material.

Master Disc

A special disc tumbler with multiple gates to receive a sidebar.

Master Key

1) A key which operates all the master keyed locks or cylinders in a group, each lock or cylinder usually operated by its own change key.
2) To combinate a group of locks or cylinders such that each is operated by its own change key as well as by a master key for the entire group.
3) An automotive key which operates all or most locks on a vehicle where there is also a valet key employed.

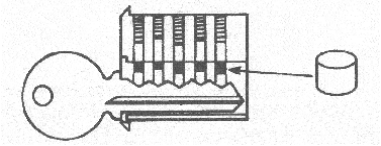
RETIRED

Master Keyed

1) Of or pertaining to a cylinder or group of cylinders which are or are to be combinated so that all may be operated by their own change key(s) and by additional key(s) known as master key.
2) A warded lock with warding that has been designed to allow different hierarchies of keys to operate.

Master Pin(s)

1) Usually a cylindrical shaped tumbler, flat on both ends, placed between the top and bottom pin to create an additional shear line.
2) A pin tumbler with multiple gates to accept a sidebar.

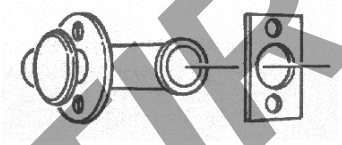


Mortise

1) A cavity made to receive a lock or other hardware.
2) The act of making a cavity to receive a lock or other hardware.

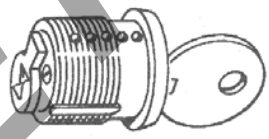
Mortise Bolt

A locking bolt installed within the door.



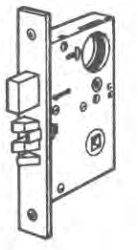
Mortise Cylinder

A threaded cylinder typically used in mortise locks of American manufacture.



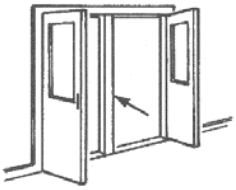
Mortise Lock

A lock installed in a hole cut in the edge of a door.



Mullion

A movable or fixed center post used on double door openings, usually for locking purposes.



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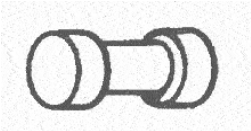
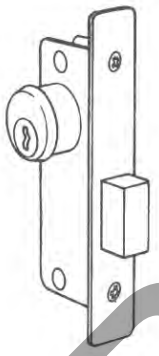
Mushroom Pin

A pin tumbler, usually a top pin, which resembles a mushroom. It is typically used to increase pick resistance.



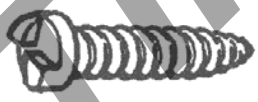
Narrow Stile Lock

Mortise type lock used in aluminum narrow stile door(s).



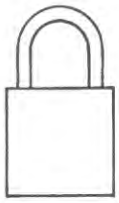
One-Way Screw

A screw specifically designed to resist being removed, once installed.



Padlock

A detachable and portable lock with a hinged shackle. Normally used with a hasp shackle which locks into its case.



Padlock, Laminated

A padlock, in which the body consists of a number of flat plates, which are riveted together. Holes in the plates provide spaces for the mechanism and the ends of the shackle.

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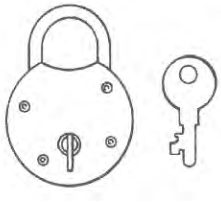
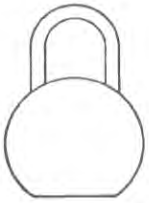
Padlock, Pressed

A padlock wherein the case is pressed into the shape from sheet metal and then riveted together.



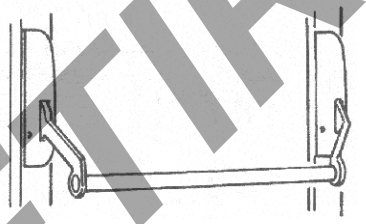
Padlock, Solid Body

A padlock in which the body is a solid piece of metal with the areas for the shackle and lock cylinder milled out.



Panic Bar

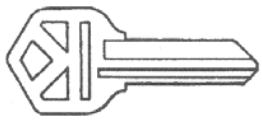
A horizontal bar attached to the inside surface of a door. The door can be opened by pushing on the bar which will retract the latches usually found on the top and bottom of the door. Also known as an exit device.



Paracentric

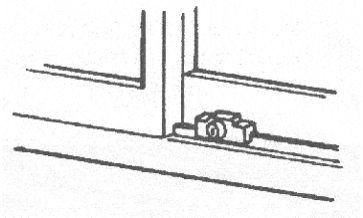
(1) Of or pertaining to a keyway with one or more wards on each side projecting beyond the vertical center line of the keyway to hinder picking.

(2) Of or pertaining to a key blank made to enter such a keyway.



Patio Sliding Door Lock

A lock designed to secure a sliding patio door by locking the track or rails.



Pin Tumbler

Usually a cylindrical shaped tumbler. Three types are normally used: bottom pin, master pin, and top pin.

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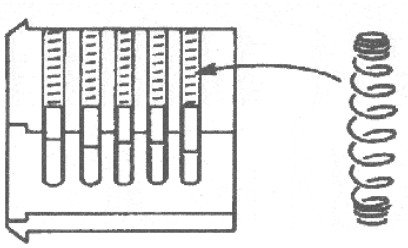
Pin Tumbler Springs

Small coil compression springs which are used in cylinders made with pin tumbler mechanisms and are found above the driver pins.

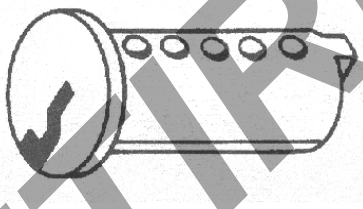


Plug

The part of a cylinder which contains the keyway, with tumbler chambers usually corresponding to those in the cylinder shell.

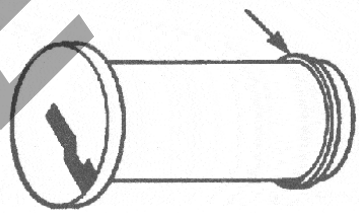
Plug, Cylinder

The round core of a lock cylinder which receives the key and rotates when the key is turned, activating the bolt or spring bolt.



Plug Retainer

The part often fixed to the rear of the plug in a lock cylinder to retain or hold the plug firmly in the cylinder.



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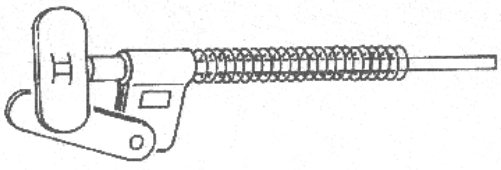
Plug Spinner

Tool designed to spin the plug past the locked position in the event the plug was picked in the wrong direction.



Rail

The horizontal construction member located on the top and bottom of a door.

Rail, Bottom

The lowermost portion of a door between the lock stile and hinge stile.

Rail, Top

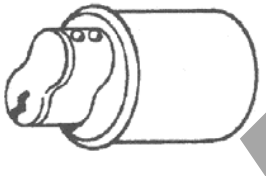
The uppermost portion of a door between the lock stile and hinge stile.

Removable Core

One whose design limits its installation to one or more specific types of housing or shell from a given manufacturer.

Removable Cylinder

A cylinder which can be removed from a locking device by a key and/or tool.

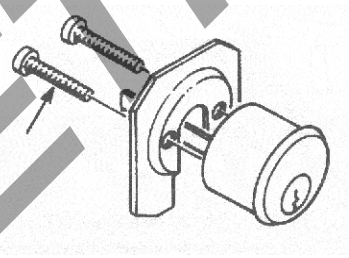


Restricted Keyway

A special keyway restricted to such applications as U.S. mailboxes, telephone company locks, etc., which must be requested specifically from the manufacturer.

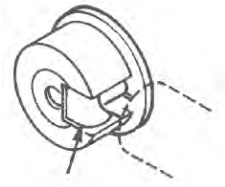
Retaining Screws, Rim Cylinder

Screws used to mount a rim cylinder on the surface of the door.



Retractor

The part of a lock in the knob assembly which retracts the springs bolt when the knob is turned.



Reversible Lock

A lock which may be used in the opposite direction by reversing the spring bolt.

Rim Cylinder

A cylinder used with a rim lock.

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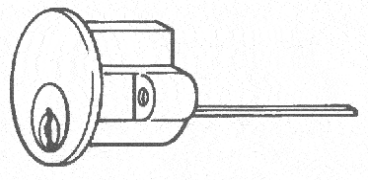
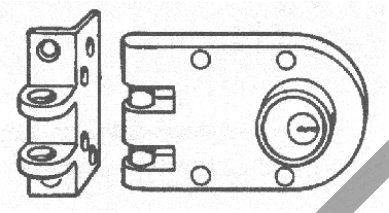


Rim Latch

A latch made for mounting on the inside surface of the door.

Rim Lock

A lock made for mounting on the inside surface of the door.

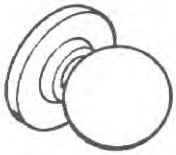


Rollback

The part activated by the knob or key to transfer motion to the retractor.

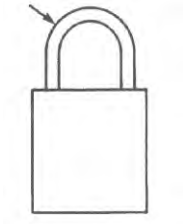
Rose

The part of a lock used as an ornamental or bearing surface for a knob, normally placed against the surface of the door. They are often used to conceal an enlarged hole through the door, and are used as a spacer for longer lock-in-knob locks on narrow doors.



Shackle

The hinged or sliding part of a padlock that does the fastening.



Shearline

The area between the housing and plug, which is normally obstructed by the tumblers until the insertion of the correct key.

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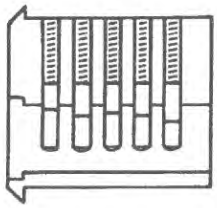
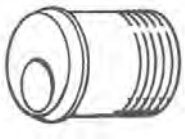


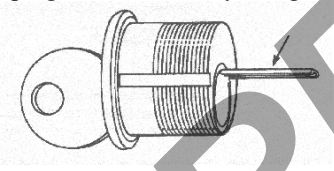
Shell

The external case of a lock cylinder that houses the plug. Sometimes referred to as the housing.



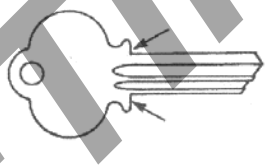
Shim

1) A thin piece of material used to unlock the cylinder plug from the shell by separating the pin tumblers at the shear line, one at a time.
2) To unlock a cylinder plug from its shell by using a shim.



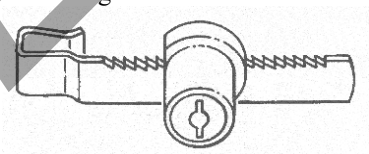
Shoulder

One or two projections on a key between the bow and the blade that is used as a reference point for bit spacing.



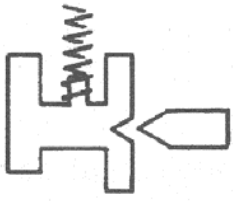
Showcase Lock

A lock designed to secure showcase glass doors.



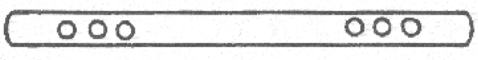
Side Bar Tumbler

A tumbler with a gate in its side.



Spindle

That part of a lockset, usually square in shape, which is fitted in the handle(s) and passed through the hub to engage the handle or knob when turned to operate the bolt.



Split Hub

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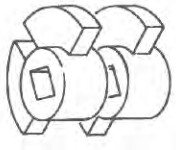
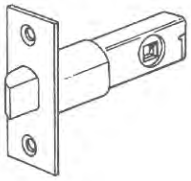
A hub made in two parts that enables the inside handle or knob to turn one part of the hub and withdraw the bolt while the other part of the hub and the outside handle or knob are locked.



Spring Bolt

The locking component of a lock. The spring bolt automatically retracts upon contact with the lip of the strike and then extends into the hole of the strike, securing the door.



Spring Bolt with Anti-Shim Device

A small device, normally adjacent to the spring bolt which prevents the spring bolt from being depressed or forced with a flat object (loided) when the door is closed.

Staple

That part of a hasp that receives the shackle of a padlock.

Stile

The vertical members of a paneled door. The stile containing the lock is called the lock stile while the stile containing the hinge is called the hinge stile.

Stop of a Key

The part of a key from which all cuts are indexed and which determines how far the key enters the keyway.

Strike

Metal plates installed on or in a door jamb that receive the bolt or spring bolt.

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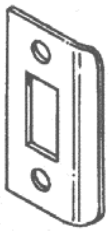


Strike Box

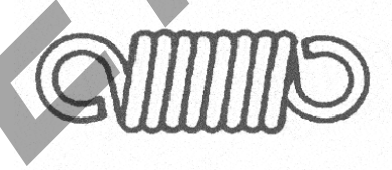
A strike plate with a box to enclose the lock bolt or spring bolt when in a locked position.

Tail Piece

An actuator attached to the rear of the cylinder, parallel to the plug that is typically used on rim, key-in-knob, or special application cylinders.

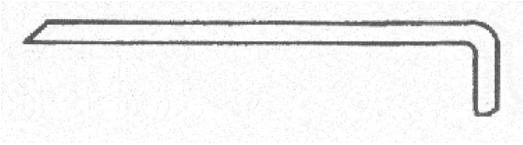
Tension Coil Spring

A spring made of a wire coiled into a helix with the coils in close contact and an eye at each end to enable the spring to be stretched as the bolt or other parts move.



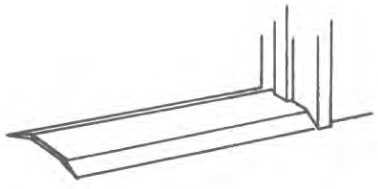
Tension Wrench

An instrument used for picking a lock to apply pressure or tension on the cylinder plug while lining up the tumblers with the shear line. This enables the plug to turn and subsequently, the lock to open.



Threshold

The wood or metal plate forming the bottom of a doorway.



Throw

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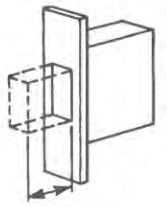
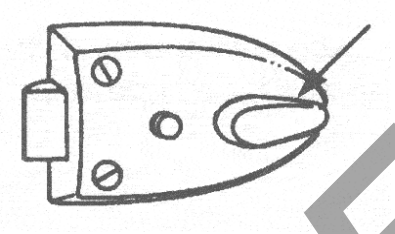
The distance a bolt or spring bolt travels. When the bolt or spring bolt is moved from the open position to the locked position (extended), it is said to have been thrown.



Thumb Turn

A component part of a lock designed to extend or retract a bolt or spring bolt without the turn of a key which is gripped between the thumb and finger.

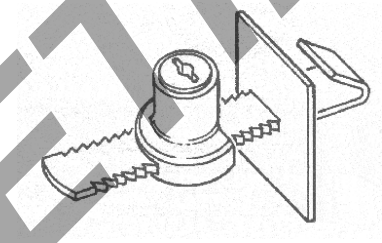


Toe of a Padlock

The end of the shackle on a padlock that extends from the case. Also refer to **Heel of a Padlock**.

Travel Lock

A small, portable auxiliary lock designed for traveling. It will prevent a door from being opened from the outside even with a key. This lock can only be put in place when the occupant is in the room.

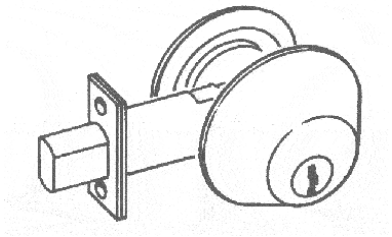


Trim

A plate, either protective or ornamental, which contains openings for any and all of the controlling members of the lock, such as the knob, handle, cylinder, and keyhole.

Tubular Lock

A lock having a simple tubular construction consisting of a bolt, spring bolt, or spring bolt with an anti-shim device. It may have a cylinder on both sides or a thumb turn on the inside.



Tubular Key

A key with a tubular blade. The key cuts are made into the end of the blade, around its circumference.

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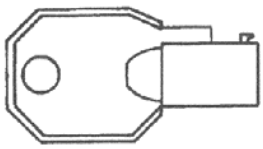
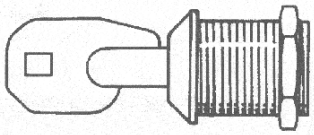


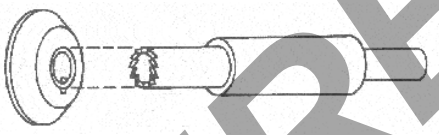
Tubular Key Cylinder

A cylinder in which the pins are arranged radially and is operated by a tubular key.



Tubular Lock Drill

A rotary drill designed to cut away the pins to allow the lock to be opened.

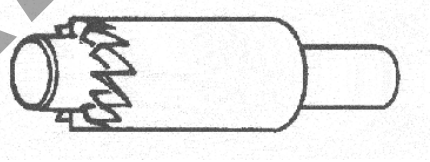


Tubular Lock Pick

A tool designed to pick tubular lock cylinders.

Tubular Lock Saw

A rotary saw designed to cut away the shoulder over the pins to allow the pins to be removed and the lock opened.

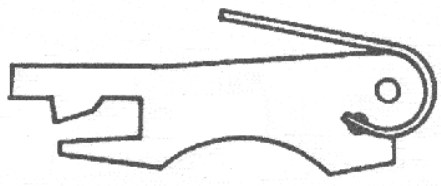


Tumbler

A moveable obstruction of varying size and configuration in a lock or cylinder which make direct contact with the key or another tumbler and prevents an incorrect key or torque device from activating the lock or other mechanism.

Tumbler Lever

A flat tumbler unit, usually made of brass or steel, used to determine combinations in lever locks.



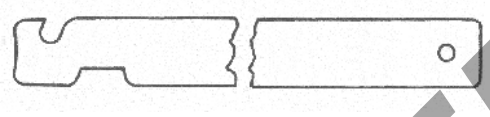
RETIRED

Unit Lock

A lock made to be installed in a cutout in the door without any disassembly or reassembly required.

Vehicle Opening Tool (Slim Jim)

A locksmithing tool designed to slip between the door and the window from the outside of the vehicle. It will open the door by hooking or pushing the connecting rod or the lock cam.

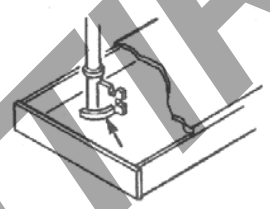


Wafer Tumbler

A double acting, spring loaded flat plate designed to slide in slots which run through the diameter of the cylinder plug. Wafer tumblers require a double bitted key.

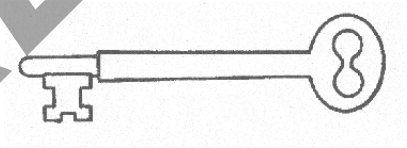
Ward

Any obstruction which prevents a blank key or a key without the proper cut from entering or turning a lock.



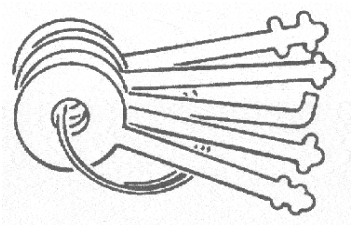
Warded Key

A key used in a ward lock which will bypass obstructions in the keyway and lock case.



Warded Padlock Pick Set

A set typically consisting of five key-type picks designed to open warded padlocks.



Ward-Lever System

A lock containing both wards and levers.

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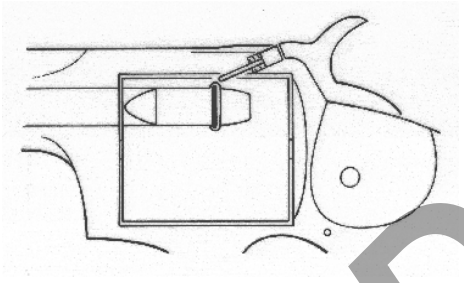
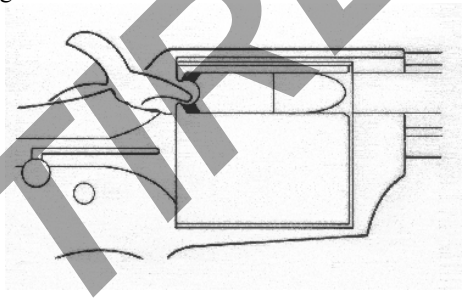
Firearm Ignition Systems

Annular Rim



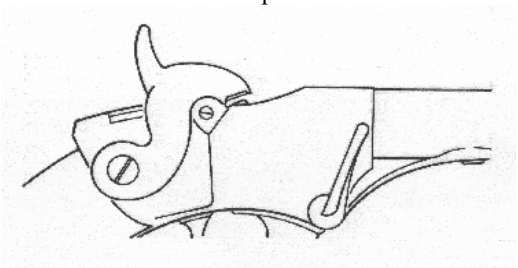
Cupfire System

A rimfire cartridge used in early revolvers designed to be loaded from the front of the cylinder. The fulminate was distributed around the concave base of the cartridge. The hammer of the firearm struck the inside of the base of the cartridge.



Disc Primer

This system held a tiny fulminate detonator disc in its pan.



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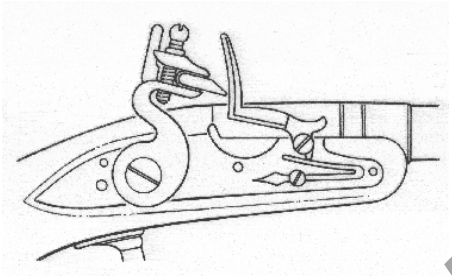
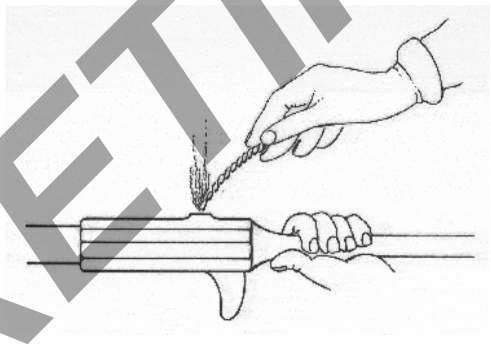
Flintlock

A muzzle loading firearm ignition system comprised of a cock with jaws that gripped a piece of flint, a frizzen, and a priming pan. The frizzen was a steel plate near the priming pan that intercepted the falling, spring-driven cock when the trigger was pulled. When the flint struck the frizzen, the resulting sparks would ignite the priming powder and subsequently ignite the main powder charge.



Hand Cannon

The earliest hand cannon was developed in the fifteenth century. For firing, the hand cannon could be held in two hands while a helper applied the means of ignition. These could range from smoldering wood or coal, red-hot iron rods, to slow-burning matches. Alternatively, the hand cannon could be placed on a rest and held by one hand while the gunner applied the means of ignition himself. Projectiles used in these weapons were varied, with many utilizing a variety of different ammunition. Rocks or pebbles found on the ground could be fired from hand cannons. More sophisticated ammunition, such iron or stone in the shape of balls, and arrows could also be used.



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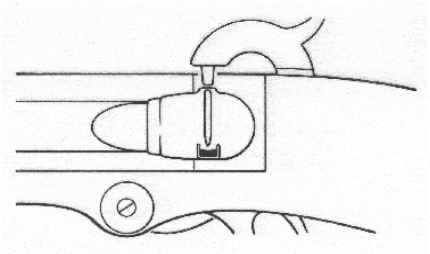
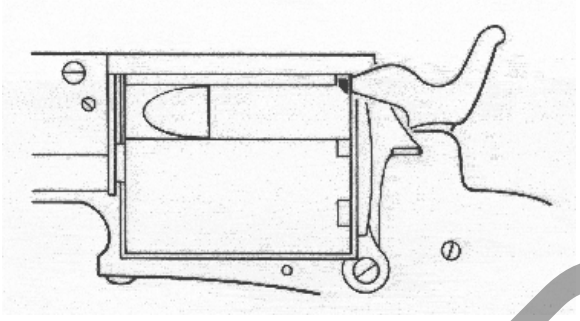
Inside Pinfire System

A form of ignition system that utilized very rare cartridges. The cartridges were ignited by the impact of the hammer on the wall of the cartridge case. Within the cartridge case was an internal mechanism of a rod or pin and priming compound.



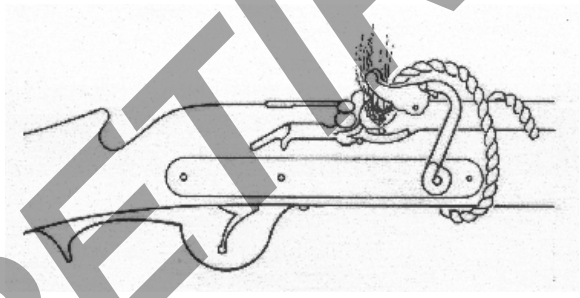
Lip Fire System

A form of rimfire cartridge patented by Ethan Allen (1860) for use in his line of guns. A small fulminate-filled lip projects from the base rather than a full circumferential rim as found on common rimfire. These were made in 25, 32, 36, and 44 calibers.



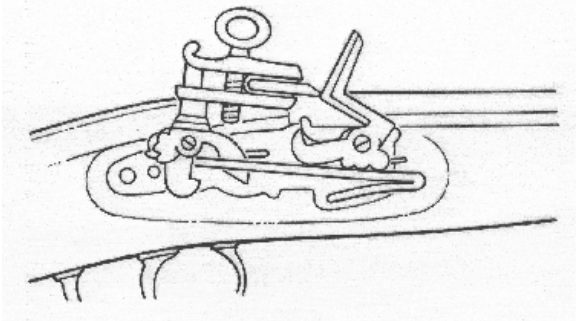
Matchlock

The classic European matchlock gun held a burning slow match in a clamp at the end of a small curved lever known as the serpentine. Upon the pulling of a lever (or in later models a trigger) protruding from the bottom of the gun and connected to the serpentine, the clamp dropped down, lowering the smoldering match into the flash pan and igniting the priming powder. The flash from the primer travelled through the touch hole igniting the main charge of propellant in the gun barrel. On release of the lever or trigger, the spring-loaded serpentine would move in reverse to clear the pan.



Miquelet

An early form of flintlock in which the frizzen covered the priming pan to hold the powder in place. When the trigger was pulled, the flint struck the face of the frizzen, knocked it forward, and exposed to the powder in the pan to the falling sparks. The miquelet has both Spanish and Italian versions. The main difference between the two is where and how the mainspring pushed on the cock foot. The miquelet's spring was carried on the outside of the firearm so that it could be easily repaired.



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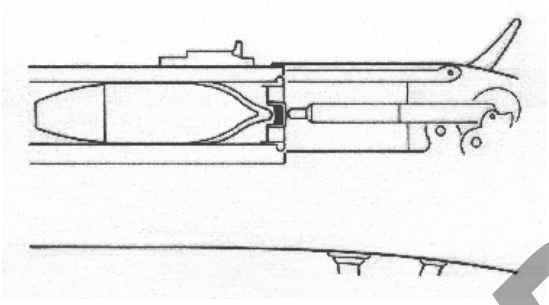
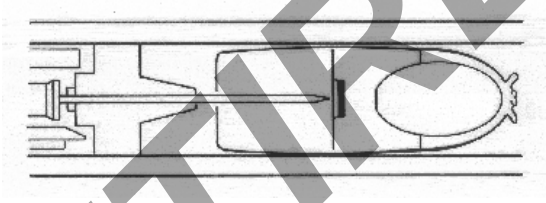
Morse

Early, self-contained cartridges produced by George Morse in the mid-1800s for breech-loading firearms. The cartridges were metallic, reloadable, and were produced in a variety of types and calibers. The most well-known version of the cartridge used a rubber base ring to retain the percussion cap primer and to provide a gas seal.



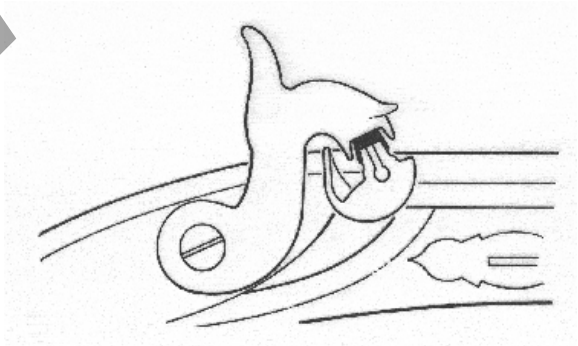
Needle Fire System

A firing system developed by Nikolaus von Dreyse of Prussia. The system can be considered the ancestor of every bolt action rifle since it was the first to utilize a bolt system of closure. The cartridge used was a paper cylinder containing a bullet with a percussion cap in its base and powder charge. The long, needle-like firing pin passed through the bolt. When the trigger was pulled, the needle passed through the paper cartridge and struck the percussion cap, firing the round.



Percussion Lock System

This ignition system employs the use of a hammer, a hollow bolt or vent leading to the chamber, and a fulminating mixture as a primer. Several types of this ignition system exist. One version used the percussion cap containing the fulminating mixture. The cap was placed over the open end of the tubular vent, known as the nipple, and when the hammer fell it crushed the mixture between the cap and the mouth of the tube. The flash of the exploding mixture then passed through the tube into the chamber, igniting the gunpowder.



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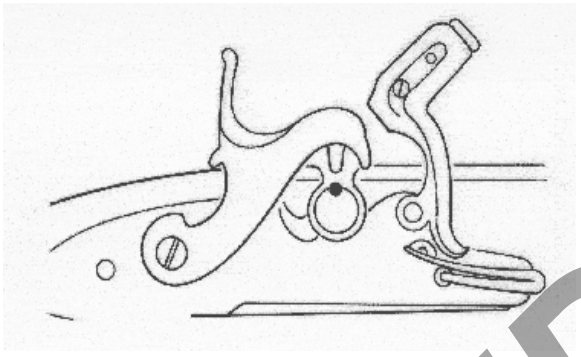
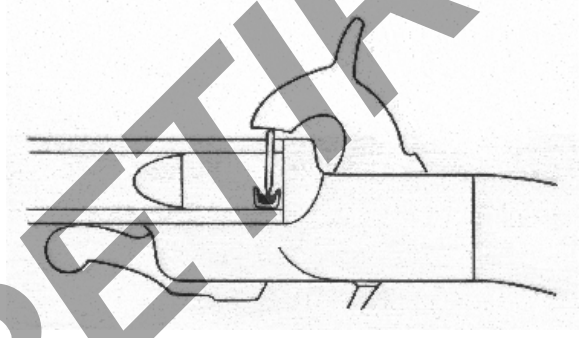
Pill Lock System

A type of percussion lock system that utilized fulminate of mercury formed into a small ball. The "pill" was placed into the drum/touch hole, and when it was struck by the hammer it exploded, igniting the main powder charge.



Pinfire System

An ignition system that is similar to the inside pinfire. The metal or cardboard cartridges used with this system had a small hole in the side through which a metal pin passed. The tip of this rod rested on a small pocket of fulminate that was placed on the sidewall of the case. When the hammer fell and struck the pin, it would detonate the fulminate and ignite the powder charge. The firearms using these cartridges had a small slot in the breech through which the pin could project.



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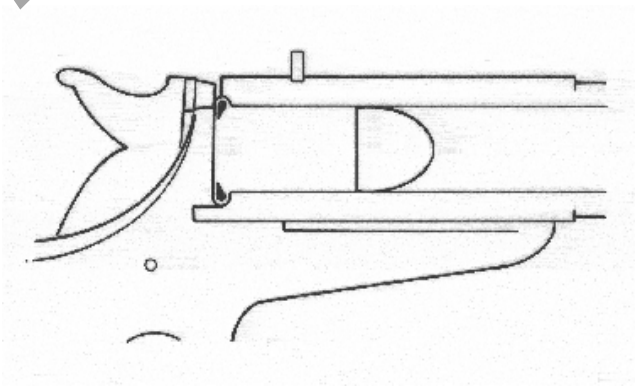
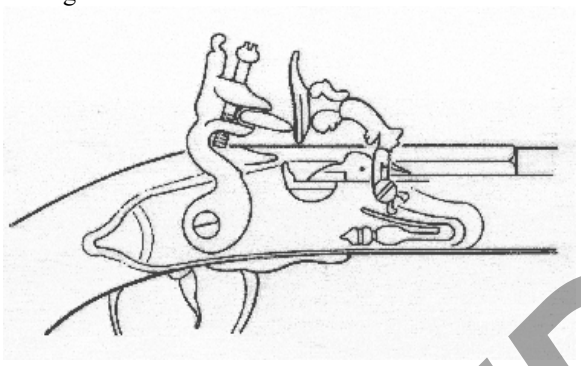
Rimfire

An ignition system which uses a metal-cased cartridge with priming compound distributed into the rim of the cartridge case. When the trigger is pulled, the firing pin crushes the priming compound within the rim and detonates the primer.



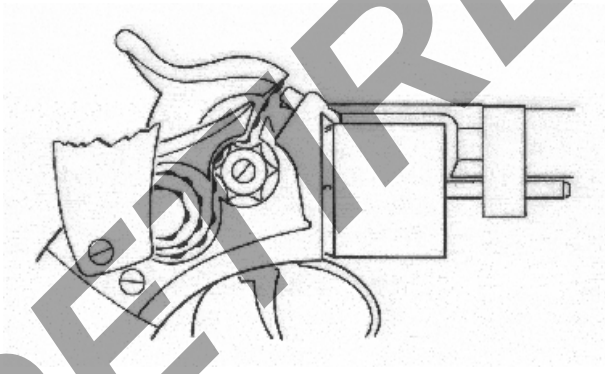
Snaphaunce

A Dutch form of flintlock very similar to the Spanish miquelet. In this ignition system, the frizzen acted only to strike sparks. The pan cover was a separate component with a rod linked to the cock. When the cock moved forward, it pushed the pan cover open, exposing the powder. The spring of this firearm was internal, protecting it from damage.



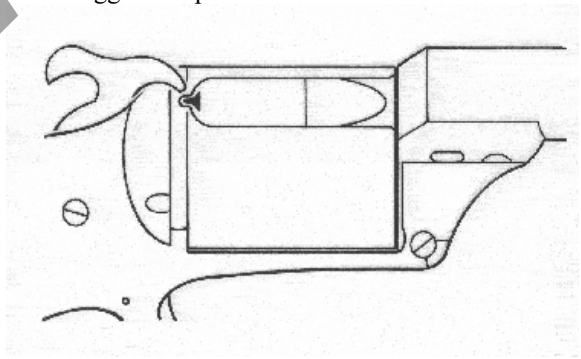
Tape Primer System

A type of percussion lock system. The tape is a double layer of paper or linen with pellets of percussion mixture at specific intervals. A wheel linked to the hammer mechanism feeds one primer to the top of the nipple when the hammer is cocked.



Teat Fire System

This ignition system utilized cartridges which contained the primer in a small, rounded "teat" at the base of the cartridge. The teat protruded through a tiny opening in the rear of the cylinder of the revolver, allowing the hammer to strike it when the trigger was pulled.



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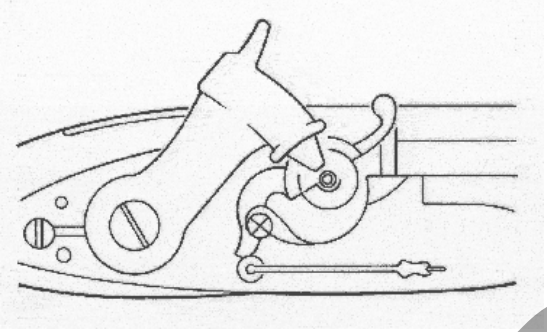
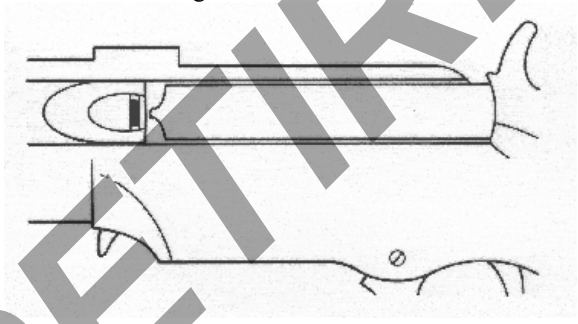
Tube Lock

A type of percussion lock system that employed a hammer. When the hammer fell, it struck and crushed a small tube filled with fulminate of mercury. The resulting explosion created a flash to ignite the powder in the breech.



Volcanic Ignition System

The Volcanic cartridge was basically a Minié ball with its base cavity filled with propellant. A paper disc holding a fulminate pellet closed off the back of the cartridge. The ignition system was similar in concept to the needle fire system, but it did not require the long, easily damaged firing pin. The cartridges were underpowered and the gas seal was inefficient, but the Volcanic repeating rifle could hold a large quantity of ammunition in its under-barrel tubular magazine.



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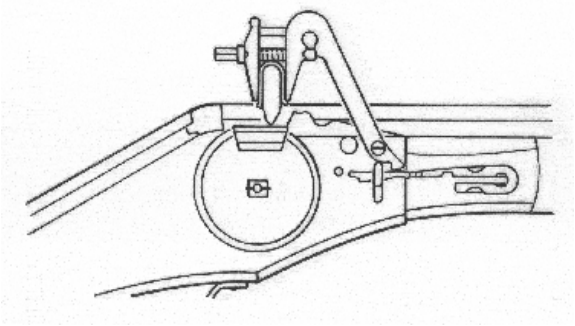
Wheel Lock

This ignition system had a wheel with a serrated or roughened edge that rotated behind, and somewhat into, the powder pan. Iron pyrites were held by the jaw of a hinged arm, or cock. When the trigger was pulled, two actions occurred. The wheel began to spin forward, and the cock was released, creating sparks as the iron pyrites struck the wheel. The sparks fell into the powder pan, and the resulting flame traveled to the main powder charge via the vent.



Section 13 - Appendices

Abbreviations

5 R L = G	Means 5 lands and grooves, Right hand twist. L=G – lands equals grooves
ACP	Automatic colt pistol; used with caliber designations such as 25 ACP, 32 ACP, 380 ACP and 45 ACP
AP	Armor piercing
API	Armor piercing incendiary
bbl	Barrel
bfm	Breech face marks
BC	Ballistic coefficient
BHN	Brinell Hardness Number
BP	Black powder
BT	Boattail
chem	Chemical examination or test
CMS	Consecutive matching striae Case mouth seal, color identification
CN	Cupro Nickel, bullet jacket
CNCS	Cupro Nickel Clad Steel, bullet jacket
Cu	Copper
CUP	Copper units of pressure
CWS	Copper washed steel, case finish
DC	Dual core
ejt	Ejector
exam	Examined or examination
ext	Extractor
f	Function
FMC	Full metal case
FMJ	Full metal jacket, also known as **FULL PATCH**
FP	Firing pin
FPI	Firing pin impression
FSLC	Fired since last cleaned
G or GIMP	Groove impression
GM	Gilding metal, bullet jacket
GMCS	Gilding metal clad steel, bullet jacket
GRC	General rifling characteristics
Griess	Griess test for nitrates
GSR	Gunshot residue
HB	Heavy ball, round-nose bullet
HE	High explosive
HP	Hollow point
HPB	Heavy pointed ball, boattail bullet
HPT	High pressure test
I	Incendiary
I.D. or IDENT	Identification
IP	Inside primed

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JHP	Jacketed hollow point
JSP	Jacketed soft point
KNM	Known non-match
L or LIMP	Land impression
LPB	Light pointed ball – flat based bullet
LRN	Lead round nose bullet type
LS	Lacquered steel, case finish
MA	Mouth annulus, color identification. Also see case mouth seal
MC	Metal cased
mfg	Manufacture
Mic or Micro	Microscopic
Na Rho	Sodium Rhodizonate test
NC	No conclusion
NI or Nonident	Nonidentification (could not have fired the specimen)
P	Pointed
PA	Primer annulus, color identification
Pb	Lead
PPK	Walther's ***Pistole, Polizei, Kriminal***. A small handgun designed for the German "criminal police" or plainclothes detective
RF	Rimfire
RN	Round nose
SN	Soft nose
SoRho	Sodium Rhodizonate test
SP	Soft point
SWC	Semi-wadcutter
SXT	Supreme Expansion Technology, a Winchester Trademark
T	Tracer
TC	Truncated cone
Tests = Item X	Means that the evidence, item X, can be identified as having been fired in the firearm being examined
Tests = Tests	Means test cases and/or bullets can be identified as having been fired in the same firearm
TMJ	Total metal jacket
UMC	Union Metallic Cartridge Co.
WC	Wad cutter
Wt	Weight

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Formulas

Bullet Energy

The general formula for kinetic energy (E) is:

$$E = \frac{MV^2}{2}$$

Where M is mass expressed by $\frac{\text{pounds}}{32.17}$ or $\frac{\text{grains}}{7000(32.17)}$

And velocity is expressed in feet/second.

$$E = \frac{\text{bullet weight in grains (velocity)}^2}{450{,}380}$$

Therefore, bullet energy, at any point on its trajectory where its velocity is known, can be determined by the formula:

$$E = \frac{85(502)(502)}{450{,}380} = 47.6 \text{ foot-pounds}$$

Example: Given an 85 grain 32 S & W caliber bullet at a velocity of 502 feet/second

Rate of Spin (Bullet Rotational Velocity)

Given:

T = Rifting twist in inches per turn (360°)
Mv = Muzzle velocity in feet per second (fps)
R = Revolutions per minute (rpm)

Then:

$$R = \frac{12 \text{ (revolutions per foot)} \times M_v \times 60 \text{ (seconds per minute)}}{T}$$

Or

$$R = \frac{720\, M_v}{T}$$

Example: A loading which produces a Mv of 2700 fps from a barrel rifled one turn in 10 inches.

$$R = \frac{720(2700)}{10} = 194{,}400 \text{ rpm}$$

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Rate of Twist

(From angle of rifling impressions on bullet)

Given:

a = angle formed between rifling impression edge and longitudinal axis of bullet
d = bore diameter
1 = number of inches per turn of rifling

Then:

$$\tan a = \frac{\Pi d}{T}$$

and

$$t = \frac{\Pi d}{\tan a}$$

Recoil Energy

E = 1/2 MV2

Given:

E = Recoil Energy in ft. lbs
M = Mass of firearm
g = 32.17 = standard gravity in ft/sec
V = Recoil velocity of firearm in ft/sec
Vp = Average effective velocity of the powder **
Wf = Weight of firearm in pounds
Wb = Weight of bullet in grains
Vm = Muzzle velocity of bullet in ft/sec
Wp = Weight of powder in grains
7000 is the conversion to pounds from grains (1lb = 7000 grains)

Where M = (Wf /g)

And V = [(Wb + 1.75Wp)Vm] / [Wf(7000)]

so

E = 1/2 {Wf / 32.17} {[(Wb + 1.75Wp)Vm] / [Wf(7000)]}squared.

**The value for Vp will vary depending on the firearm. The average effective velocity of the powder may be approximately 1 to 2 times the muzzle velocity of the bullet with higher numbers being suitable for high pressure loads in short barrels and lower numbers being suitable for shotguns with maximum length barrels. Revolvers ~1.5, rifles (32" barrel) ~1.5, rifles (24" barrel) ~1.75 (Per Hatchers Notebook). Be cognizant of the type of firearm and the average effective velocity of powder for that type of firearm when calculating Recoil Energy.

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Rule of 17

The theoretical diameter of shot is calculated as follows:

Diameter of shot = 17 – shot size

Where all measurements are expressed in hundredths of an inch.

Example (No. 6 Shot):
Diameter of shot = 17 – 6 = 11 (or 0.11 inches in diameter)

NOTE: This technique does not apply for buckshot.

Calculation of Number of Lands/Grooves (damaged bullets)

Number of lands and grooves = C / LWD + GWD

Given:

C = Bullet Circumference
LWD = Land Width Dimension (average)
GWD = Groove Width Dimension (average)
d = Bullet diameter (measured or estimated caliber)
$\prod = 3.1416$

Where: $C = d \prod$

Example (applied to a 9mm Luger caliber bullet with the following measurements):

Estimated d = 0.355”
LWD = 0.069”
GWD = 0.115”

$C = (0.355) \prod = 1.115$

Number of lands and grooves = 1.115 / 0.069 + 0.115 = 1.115 / 0.184 = 6.06 (or 6 lands and grooves)

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Useful Reagents

Diphenylamine

0.3 grams diphenylamine
20 mL concentrated sulfuric acid
10 mL glacial acetic acid

Dissolve the diphenylamine in the sulfuric acid, then pour the mixture into the acetic acid.

Lead Solvent

10 mL glacial acetic acid
2 mL 30% hydrogen peroxide
70 mL distilled water

Caution: When using to remove lead from copper bullet jackets, care must be taken to remove jacket from solution before it acts on the jacket material itself.

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Distance Determination Reagents (Common)

Modified Griess Test for Nitrites

Modified Griess Test Reagent

0.5 g Sulfanilic acid
100 mL distilled water
100 mL Methanol
0.28 g Alpha-naphthol

Dissolve 0.5g sulfanilic acid in 100 mL of distilled water. Dissolve 0.28g alpha-naphthol in 100 mL of Methanol. Mix the two solutions – prepare only that amount needed for immediate use.

Acetic Acid (15% solution)

85 mL distilled water
15 mL Glacial Acetic acid

Nitrite Test Swabs/Strips

0.6 g Sodium nitrite
100 mL distilled water

Dissolve 0.6 g sodium nitrite in 100 mL distilled water. Soak cotton swabs or strips of filter paper in solution and air dry.

Sodium Rhodizonate Test for Lead

Sodium Rhodizonate Solution

Saturated solution of Sodium rhodizonate and distilled water.

Potassium Chloride Buffer

1) 0.75 g potassium chloride dissolved in 50 mL distilled water.
2) Dilute 5.0 mL of concentrated hydrochloric acid in 295.0 mL of distilled water.
3) Combine 25.0 mL of (1) with 67 mL of (2) to make a buffer with pH 1.0.

Dithiooxamide (DTO) Test for Copper

Dithiooxamide Solution

0.2 g of dithiooxamide dissolved in 100 mL of 100% ethanol.

Ammonium Hydroxide Solution

Dilute concentrated ammonium hydroxide with an equal volume of distilled water.

Serial Number Restoration Reagents (Common)

10% Sodium Hydroxide

10 g sodium hydroxide
90 mL distilled water

25% Nitric Acid

25 mL nitric acid
75 mL distilled water

Phosphoric Acid/Nitric Acid (Knowles Reagent)

98 mL 85% phosphoric acid
2 mL concentrated Nitric acid

or

50 mL concentrated phosphoric acid
3 mL concentrated nitric acid

Ferric Chloride

25 g ferric chloride
100 mL distilled water

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Acidic Ferric Chloride

25 g ferric chloride
25 mL hydrochloric acid
100 mL distilled water

Fry's Reagent

90 g cupric chloride
120 mL hydrochloric acid
100 mL distilled water

Turner's Reagent

2.5 g cupric chloride
40 mL hydrochloric acid
25 mL ethyl alcohol
30 mL distilled water

Davis' Reagent

5 g cupric chloride
50 mL hydrochloric acid
50 mL distilled water

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Tables

Table 1 - Shot Sizes And Weights

All values are approximate – Grain weights are calculated based on number of pellets per ounce (1 oz = 437.5 grains).

No.	Approx. Dia.		Lead (may vary based on antimony content – ½% to 6%)		Steel		Bismuth		Tung./Iron		Tung. Polymer		Tung. Matrix	
	in.	mm	no./oz.	grains	no./oz.	grains	no./oz.	grains	no./oz.	grains	no./oz.	grains	no./oz.	grains
12	.05	1.27	2360-2457	.18-.19	3363	.13	2723	.16	2591	.17	2360	.19	2542	.17
11	.06	1.52	1366-1422	.31-.32	1946	.22	1576	.28	1500	.29	1366	.32	1471	.30
10	.07	1.78	860-895	.49-.51	1226	.36	992	.44	944	.46	860	.51	926	.47
9	.08	2.03	576-599	.73-.76	821	.53	664	.66	632	.69	576	.76	620	.71
8 1/2	.085	2.16	480-500	.88-.91	685	.64	554	.79	527	.83	480	.91	517	.85
8	.09	2.29	404-421	1.03-1.08	577	.76	466	.94	444	.99	404	1.08	435	1.01
7 1/2	.095	2.41	344-358	1.22-1.27	490	.89	397	1.10	378	1.16	344	1.27	370	1.18
7	.10	2.54	295-307	1.43-1.48	420	1.04	340	1.29	324	1.35	295	1.48	318	1.38
6	.11	2.79	221-230	1.90-1.98	316	1.38	255	1.72	243	1.80	221	1.98	238	1.84
5	.12	3.05	170-177	2.47-2.57	243	1.80	196	2.23	187	2.34	170	2.57	183	2.39
4	.13	3.3	134-139	3.15-3.26	191	2.29	155	2.82	147	2.98	134	3.26	144	3.04
3	.14	3.56	107-111	3.94-4.09	153	2.86	123	3.56	117	3.74	107	4.09	115	3.80
2	.15	3.81	87-91	4.81-5.03	125	3.50	100	4.38	96	4.56	87	5.03	94	4.65
1	.16	4.06	72-74	5.91-6.08	103	4.25	83	5.27	79	5.53	72	6.08	78	5.61
B	.17	4.32	60-62	7.06-7.29	86	5.09	69	6.34	66	6.63	60	7.29	65	6.73
air rifle	.175	4.45	55-57	7.68-7.95	78	5.61	63	6.94	60	7.29	55	7.95	59	7.42
BB	.18	4.57	50-52	8.41-8.75	72	6.08	58	7.54	55	7.95	50	8.75	54	8.10
BBB	.19	4.83	43-44	9.94-10.17	61	7.17	50	8.75	47	9.31	43	10.17	46	9.51
T	.20	5.08	36-38	11.51-12.15	53	8.25	42	10.42	40	10.94	36	12.15	39	11.22
TT	.21	5.33	31-33	13.26-14.11	45	9.72	36	12.15	34	12.87	31	14.11	33	13.26
TTT/F	.22	5.59	27-28	15.63-16.20	39	11.22	31	14.11	30	14.58	27	16.20	29	15.09
TTTT/FF	.23	5.84	24-25	17.5-18.23	35	12.5	28	15.63	26	16.83	24	18.23	26	16.83

Brussard, M.E. and Wormley Jr., S.L., NRA Firearms Sourcebook, National Rifle Association, Fairfax, Virginia, 2006, p. 387.

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Table 2 - Lead Buck Shot Information

No.	Approximate Diameter		approx. no. per pound (may vary based on antimony content – ½% to 6%)	Gr.	mg
	in.	mm.			
4	.24	6.10	338-352	19.9-20.7	1289-1342
3	.25	6.35	299-311	22.5-23.4	1458-1517
2	.27	6.86	238-248	28.2-29.4	1829-1906
1	.30	7.62	173-180	38.9-40.5	2520-2622
0	.32	8.13	143-149	47-49	3044-3172
00	.33	8.38	130-135	51.9-53.9	3360-3489
000	.36	9.14	100-104	67.3-70	4361-4536
0000	.38	9.65	90-96	72.9-77.8	4725-5040

Brussard, M., Ammo Encyclopedia, Blue Book Publications, Minneapolis, MN, 2008, p.732.

Table 3 - Nominal Shot Pattern Diameters (Inches/cm)

Choke	Amount of Constriction (inches/mm)	Common Name	Range (in yards/m)						
			10/9.1	15/13.7	20/18.3	25/22.9	30/27.4	35/32	40/36.6
Cylinder bore	none	cylinder bore	19/48.3	26/66.0	32/81.3	38/96.5	44/111.8	51/129.5	57/144.8
Quarter choke	.010/.254	improved cylinder	15/38.1	20/50.8	26/66.0	32/81.3	38/96.5	44/111.8	51/129.5
Half choke	.020/.508	modified	12/30.5	16/40.6	20/50.8	26/66.0	32/81.3	38/96.5	46/116.8
Three-quarter choke	.030/.762	improved-modified	10/25.4	14/35.6	18/45.7	23/58.4	2973.7	35/88.9	43/109.2
Full choke	.040/1.016	full choke	9/22.9	12/30.5	16/40.3	21/53.3	26/66.0	32/81.3	40/101.6

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Table 4 - Rifled Slug Characteristics

Gauge	Bore Diam. (inches/mm)	Brand	Outside Diam. (inches/mm)	Average Weight		# of Grooves and Angle
				Grams	Grains	
12	.729/18.517	Federal	.690/17.526	28.0	435	14 @ 15º
12	.729/18.517	Remington	.690/17.526	28.0	440	14 @ 9º
12	.729/18.517	Winchester	.725/17.526	28.0	437	14 @ 15º
16	.662/16.815	Federal	.635/16.129	23.0	355	12 @ 15º
16	.662/16.815	Remington	.655/16.637	23.5	365	14 @ 9º
16	.662/16.815	Winchester	.625/15.875	22.5	345	12 @ 15º
20	.615/15.621	Federal	.600/15.240	19.0	290	15 @ 15º
20	.615/15.621	Remington	.605/15.367	19.0	292	13 @ 9º
20	.615/15.621	Winchester	.611/15.519	21.0	328	15 @ 15º

Table 5 - Maximum Ranges for the Various Sizes of Shot (calculated from Journee's formula)

Shot Size	**Diameter** (inches)	**Diameter** (mm)	**Maximum Range** (yards)	**Maximum Range** (meters)	**Shot Size**	**Diameter** (inches)	**Diameter** (mm)	**Maximum Range** (yards)	**Maximum Range** (meters)
12	.05	1.27	110	101	BB	.18	4.57	396	362
11	.06	1.52	132	121	BBB	.19	4.83	418	382
10	.07	1.78	154	141	T	.20	5.08	440	402
9	.08	2.03	176	161	TT	.21	5.33	462	422
8.5	.085	2.16	187	171	TTT/F	.22	5.59	484	443
8	.09	2.29	198	181	TTTT/FF	.23	5.84	506	463
7.5	.095	2.41	209	191	#4 Buck	.24	6.10	528	483
6	.11	2.79	242	221	#3 Buck	.25	6.35	550	503
5	.12	3.05	264	241	#2 Buck	.27	6.86	594	543
4	.13	3.30	286	262	#1 Buck	.30	7.62	660	604
3	.14	3.56	3.556	282	#0 Buck	.32	8.13	704	644
2	.15	3.81	330	302	#00 Buck	.33	8.38	726	664
1	.16	4.06	352	322	#000 Buck	.36	9.14	792	724
B	.17	4.32	374	342	#0000 Buck	.38	9.65	836	764
Air rifle	.175	4.45	385	352	Rifled Slugs	All Gauges		Approx. 1500	Approx. 1372

Journee's formula gives the maximum range, in yards, as the product of the shot diameter, in inches, times 2200. 1 yard = 0.9144 meters

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Table 6 – Shotgun Bore Diameters

Gauge	**Diameter (inches)**	**Diameter (mm)**
10	.775	19.7
12	.729	18.5
14	.693	17.6
16	.662	16.8
20	.615	15.6
24	.579	14.7
28	.550	14.0
32	.526	13.4
36	.506	12.85
410 bore	.410	10.4

Table 7 - Calculated Weights and Corresponding Diameters of Lead Balls

Diameter (Inches)	Diameter (mm)	Grains	Diameter (Inches)	Diameter (mm)	Grains	Diameter (Inches)	Diameter (mm)	Grains
0.2	5.080	12.0107	0.385	9.779	85.6762	0.57	14.478	278.037
0.205	5.207	12.9342	0.39	9.906	89.0578	0.575	14.605	285.418
0.21	5.334	13.9039	0.395	10.033	92.5272	0.58	14.732	292.929
0.215	5.461	14.9029	0.4	10.16	96.0855	0.585	14.859	300.57
0.22	5.588	15.9862	0.405	10.287	99.734	0.59	14.986	308.343
0.225	5.715	17.1012	0.41	10.414	103.474	0.595	15.113	316.249
0.23	5.842	18.2668	0.415	10.541	107.306	0.6	15.240	324.289
0.235	5.969	19.4842	0.42	10.668	111.231	0.605	15.367	332.464
0.24	6.096	20.7545	0.425	10.795	115.251	0.61	15.494	340.775
0.245	6.223	22.0789	0.43	10.922	119.367	0.615	15.621	349.223
0.25	6.350	23.4584	0.435	11.049	123.579	0.62	15.748	357.81
0.255	6.477	24.8942	0.44	11.176	127.89	0.625	15.875	366.537
0.26	6.604	26.3875	0.445	11.303	132.299	0.63	16.002	375.405
0.265	6.731	27.9393	0.45	11.430	136.908	0.635	16.129	384.414
0.27	6.858	29.5508	0.455	11.557	141.42	0.64	16.256	393.566
0.275	6.985	31.2231	0.46	11.684	146.134	0.645	16.383	402.863
0.28	7.112	32.9574	0.465	11.811	150.951	0.65	16.510	412.304
0.285	7.239	34.7547	0.47	11.938	155.873	0.655	16.637	421.893
0.29	7.336	36.6161	0.475	12.065	160.901	0.66	16.764	431.628
0.295	7.493	38.5429	0.48	12.192	166.036	0.665	16.891	441.512
0.3	7.620	40.5361	0.485	12.319	171.279	0.67	17.018	451.546
0.305	7.747	42.5969	0.49	12.446	176.631	0.675	17.145	461.731
0.31	7.874	44.7263	0.495	12.573	182.093	0.68	17.272	472.068
0.315	8.001	49.9256	0.5	12.700	187.667	0.685	17.399	482.558
0.32	8.128	49.1958	0.505	12.827	193.354	0.69	17.526	493.202
0.325	8.255	51.5381	0.51	12.954	199.154	0.695	17.653	504.002
0.33	8.382	59.9536	0.515	13.081	205.069	0.7	17.780	514.958
0.335	8.509	56.4433	0.52	13.208	211.1	0.705	17.907	526.072
0.34	8.636	59.0086	0.525	13.335	217.248	0.71	18.034	537.345
0.345	8.763	61.6503	0.53	13.462	223.514	0.715	18.161	548.777
0.35	8.890	64.3698	0.535	13.589	229.9	0.72	18.288	560.371
0.355	9.017	67.1681	0.54	13.716	236.406	0.725	18.415	572.126
0.36	9.144	70.0464	0.545	13.843	243.034	0.73	18.542	584.045
0.366	9.296	73.0057	0.55	13.97	249.785	0.735	18.669	596.128
0.37	9.398	76.0472	0.555	14.097	256.659	0.74	18.796	608.377
0.375	9.525	79.1721	0.56	14.224	263.659	0.745	18.923	620.793
0.38	9.652	82.3814	0.565	14.351	270.784	0.75	19.05	633.376

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Table 8 - Land plus groove impression widths for the various small arms calibers -- calculated from the bore diameter

(All measurements are in .000 inch)

Bore Diameter	168	200	218	237	244	251	255	270	277	300	303	307	311	313	347	390	400	417	444	450	488	Number of Grooves
Circumference	528	628	685	745	767	789	801	848	870	942	952	964	977	983	1.090	1.225	1.257	1.310	1.395	1.414	1.533	
	264	314	343	372	384	395	400	424	435	471	476	482	489	492	545	613	629	655	698	707	767	2
	176	209	228	248	256	263	267	283	290	314	317	321	326	328	363	408	419	437	465	471	511	3
	132	157	171	186	192	197	200	212	218	236	238	241	244	246	273	306	314	328	349	354	383	4
	106	126	137	149	153	158	160	170	174	188	190	193	195	197	218	245	251	262	279	283	307	5
	088	105	114	124	128	132	134	141	145	157	159	161	163	164	182	204	209	218	232	236	256	6
	075	090	098	106	110	113	114	121	124	135	136	138	140	140	156	175	180	187	199	202	219	7
	066	079	086	093	096	099	100	106	109	118	119	121	122	123	136	153	157	164	174	177	192	8
	059	070	076	083	085	088	089	094	097	105	106	107	109	109	121	136	140	146	155	157	170	9
	053	063	068	075	077	079	080	085	087	094	095	096	098	098	109	123	126	131	139	141	153	10
	044	052	057	062	064	066	067	071	073	079	079	080	081	082	091	102	105	109	116	118	128	12
	033	039	043	047	048	049	050	053	054	059	060	060	061	061	068	077	079	082	087	088	096	16
	026	031	034	037	038	039	040	042	044	047	048	048	049	049	055	061	063	066	070	071	077	20
	024	029	031	034	035	036	036	039	040	043	043	044	044	045	050	056	057	060	063	064	070	22
Nominal Calibers	17	5mm	22 5.56 mm	243 6mm	25 Auto 6.35 mm	25 rifles	6.5 mm	270	280 7mm	30 32 Auto 7.62 7.65 mm	some 32 Autos 303 Brit 7.7 mm	32 revs	8mm	32 rifles	357 38 380 9mm	40 10mm	41	44	45 Auto 45 revs	45 rifles	50	

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Recall/Safety Warning List for Firearms and Ammunition

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USE OF THE AFTE RECALL/SAFETY WARNING LIST
FOR FIREARMS & AMMUNITION

The *AFTE Firearms and Ammunition Recall/Safety Warning List* is provided as an easy way to access information about firearm and ammunition that are the subject of a manufacturers' recall or a report of a safety issue to promote the health and safety of Forensic Firearms Examiners because manufacturers' literature or product information rarely is submitted to the laboratory with evidence.

The list is divided into two sections – Firearms and Ammunition. Additionally it contains two different types of notices – Recalls and Safety Warnings. The Recall is a manufactured generated notification that there is an issue related to functionality, health and/or safety. The Safety Warning is a notification from a forensic examiner that they had an issue relating to functionality, health and/or safety when examining a firearm or ammunition.

Each firearm and ammunition recall and safety warning notices includes the source of these notices, an explanation of the potential problem and a manufacturer point of contact, when available. All notices are organized by manufacturer in the Table of Contents. However, the notices are organized by date within the document. This will allow future notices to be added to the end of the document without needing to reprint everything.

The table of contents is arranged to make it easy to check a submitted firearm or ammunition for a determination of whether or not it is the subject of a recall or warning that may pose a potential danger to laboratory personnel prior to examination.

When an examiner determines, from the spreadsheet, that evidence in their case is the subject of a recall/safety warning they can go to the specific page listed, read and copy the notice for inclusion in the case file.

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Please report corrections and additional recalls/safety warnings to:

WWW.AFTE.ORG

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Make	Caliber	Type	Lot Number/ Headstamp	Bullet/ Projectiles	Index No.	Date	Pg
American Eagle	45 Auto	H	Lot # 38X628 thru 38X765, 38T491 thru 38T414		AE45A, AE45N1, AE45A250	2011-02	38
BlackGun Ammo	5.56mm	R	Lot# MJC13B581-004	62gr re-manufactured		2013-09	52
Brenneke	12 GA 3"	S	Lot# 1.0.01.07.08, 1.02.07.08, 1.03.07.08	Magnum rifled slug		2010-04	34
CBC	7.62x51 mm	R	HS: CBC 7.62 75			1975	1
CBC	7.62x51 mm	R	“reengastada” on box			1975	1
CCI/Federal	17 HMR	R				2009-08	32
CCI/Speer	25 Auto	H	J25Gxx toJ30Gxx, KxxGxx,LxxGxx,MxxGxx, AxxHxx, BxxHxx, CzzHxx	Gold Dot		2002-04	21
CCI/Speer	32 Auto	H	J25Gxx toJ30Gxx, KxxGxx,LxxGxx,MxxGxx, AxxHxx, BxxHxx, CzzHxx	Gold Dot		2002-04	21
CCI/Speer	380 Auto	H	J25Gxx toJ30Gxx, KxxGxx,LxxGxx,MxxGxx, AxxHxx, BxxHxx, CzzHxx	Gold Dot		2002-04	21
CCI/Speer	40 S&W	H	J25Gxx toJ30Gxx, KxxGxx,LxxGxx,MxxGxx, AxxHxx, BxxHxx, CzzHxx	Gold Dot		2002-04	21
CCI/Speer	9mm Luger	H	J25Gxx toJ30Gxx, KxxGxx,LxxGxx,MxxGxx, AxxHxx, BxxHxx, CzzHxx	Gold Dot		2002-04	21
CCI/Speer	9mm Luger+P	H	J25Gxx toJ30Gxx, KxxGxx,LxxGxx,MxxGxx, AxxHxx, BxxHxx, CzzHxx	Gold Dot		2002-04	21
Champion	45 Auto	H	Lot # 38X628 thru 38X765, 38T491 thru 38T414		WM5233	2011-02	38
Federal	12 GA 3”	S	4V-01QX425	Black Cloud Snow Goose	PWB143-2	2010-10	35
Federal	243 Win	R	Lot# 5A OR 5B - 4592, 4593, 4594, 4595, 4598 (gold boxes only)	85 gr BTHP	P24D	1986-05	9
Federal	243 Win	R	Lot# ends in 5T561 thru 5T566	80 gr SP Power-Shok	243AS	2011-09	41
Federal	30-30 Win	R	Lot# Final Four Digits: -5289 thru -6285			1976-11	2
Federal	40 S&W	H	Lot# A27T3, A27T4	9mm in 40 S&W Box	WM5223	2012-03	43

RETIRED

Make	Caliber	Type	Lot Number/ Headstamp	Bullet/ Projectiles	Index No.	Date	Pg
Federal	45 Auto	H	Lot # 38X628 thru 38X765, 38T491 thru 38T414		C45C, C45D	2011-02	38
Federal	45 Long Colt	H	Lot# 8J191 thru 8J199	Semi-WC HP	C45LCA	2004	23
Fiocchi	22-250 Rem	R	Lot# 001, 002, 003, 004	55gr PSP, 55gr V Max Polymer Tip	22250B, 22250HVD	2012-09	46
Fiocchi	243 Win	R	Lot# 006, 007, 008	70gr PSP	243SPB	2012-09	46
Fiocchi	243 Win	R	Lot# 004, 005	95gr SST POLY TIP BT	243HSB	2012-09	46
Fiocchi	243 Win	R	Lot# 001, 002, 003, 004	100gr INTERLOCK BT	243SPD	2012-09	46
Fiocchi	9mm Luger	H	Lot# 4509222 thru 4509230, 4510241 thru 4510259, 4510261, 4511263 thru 4511272, 4511276	115 gr FMJ		2006	27
Frontier	30-30 Win	R	Lot# 6-38-76-9, 6-42-76-9, 9-68-76-9, 9-71-76-9	150 gr, 170 gr		1976-11	3
G2R	45 R.I.P.	H	Manuf between 7/1/2014-11/30/2014			2014-12	57
Gold Medal	45 Auto	H	Lot # 38X628 thru 38X765, 38T491 thru 38T414		GM45B	2011-02	38
Hi-Shok	45 Auto	H	Lot # 38X628 thru 38X765, 38T491 thru 38T414		45C, 45D	2011-02	38
Hiawatha	30-30 Win	R	Lot# Final Four Digits: -5289 thru -6285			1976-11	2
Hirtenberger	9x19mm	H	HS: HP 90 L7A1 ⊕, HP 91 L7A1 ⊕, HP 92 L7A1 ⊕	Submachine Gun Load		1996-11	18
Hodgon	Smoke-less Powder	P	Lot# 103080221	1 lb LIL' GUN		2003-07	22
Hornady	30-06 Sprg	R	Lot# 3101052	165gr SST	81153	2010-11	36
Hornady	30-30 Win	R	Lot# 3060134		82730	2007-02	28
Hornady	470 Nitro Express	R	Lot# 3140935	500gr DGX	8263	2014-11	56
Hornady	500 S&W	H	Lot# 3101327, 3110256, 3110683, 3110695, 3110945, 3111388, 3111885	300gr FTX custom	9249	2012-02	42
Hornady	9mm Luger+P	H	Lot# 3141635	135gr	90226	2014-07	55
Magtech	45 Auto	H	Lot# L-602			1992-08	14

RETIRED

Make	Caliber	Type	Lot Number/ Headstamp	Bullet/ Projectiles	Index No.	Date	Pg
PMC	22 Long Rifle	H, R				2004-05	24
PMC	22 Magnum	H, R				2004-05	24
Remington	12 GA 2 3/4"	S	Lot # AJ?J522 or 523 or 532, BJ?J522 or 523 or 532, PAJ?J522 or 523 or 532, PBJ?B522 or 523 or 532, AK?B522 or 523 or 532, BK?B522 or 523 or 532, PAK?B522 or 523 or 532, PBK?B522 or 523 or 532	00 Buckshot 9 Pellets, 3 ¾ dr. eq.		1989-04	12
Remington	12 GA 2 3/4"	S	Lot# AT01H218	1 oz.HP rifled slug	SP12RS	1983-06	7
Remington	12 GA 2 3/4"	S	Lot# N27YC525, N29YA525, P02TA525, P03TA525, P06TA525, N28YC525, N29YA525, P02TB525, P03TB525, P08UB899, N28YB525, N29YC525, P02TC525, P05TB525	00 Buckshot, 8 Pellet	Product: RR128B00; Item: 20645	2012-12	49
Remington	17 HMR	R				2009-08	31
Remington	22 Hornet	H, R	Lot# J29EAI, J29EBI, J30EAI, J30EBI, K01JDI	45 gr PSP		2010-11	37
Remington	22 Long Rifle	H, R	Lot# S22BB1237, S22BC1237	22 Thunderbolt	TB-22A	2013-07	51
Remington	223 Rem	R	Lot# H03RAI, H04RDI, H16NAI or H17NDI	62 gr HP		2011-03	39
Remington	243 Win	R	Lot# U06DC4605, U07DA4605, U07DB4605 & U07DC4605	100 gr PSP	R243W3	1993-03	15
Remington	270 Win	R	Lot# N16SA24L, N16SA24R, N16SB24L, N16SB24R, N13SC24L, N13SC24R	150gr SP		2012-09	44
Remington	338 Lapua Magnum	R	Lot# L13SA29L, L13SA29R, L13SB29L, OR L13SB29R	250gr Scenar		2012-09	47
Remington	38 Special	H	Lot# P28A, P29A			1981-05	6
Remington	38 Special	H	Code begins with C and fourth character is A, T, or H	158 gr LEAD	R38S5	1987-01	10
Remington	38 Special+P	H	Code begins with C and fourth character is A, T, or H	158 gr HP	R38S12	1987-01	10
Remington	38 Special+P	H	Code begins with C and fourth character is A, T, or H	158 gr lead	R38S8	1987-01	10
Remington	38 Special+P	H	Code begins with C and fourth character is A, T, or H	95 gr JHP	R38S1	1987-01	10

RETIRED

Make	Caliber	Type	Lot Number/ Headstamp	Bullet/ Projectiles	Index No.	Date	Pg
Remington	38 Special+P	H	Code begins with C and fourth character is A, T, or H	110 gr JHP	R38S10	1987-01	10
Remington	38 Special+P	H	Code begins with C and fourth character is A, T, or H	125 gr JHP	R38S2	1987-01	10
Remington	38 Special+P	H	Lot# P24UA18R	125gr semi-jacketed HP	L38S2B	2012-11	48
Remington	6mm Rem	R	Code # First Letter S, T, or U	80gr PSP & PWR LOKT HP	R6MM1 R6MM2	1983-12	8
Remington	7mm Express Rem	R	Lot# M021, M031, M061, M071, M081, M091, M131, M20F thru M29F, M010 thru M110	150 gr PSP Core Lokt	R7M061	1980-10	5
Remington	9mm Luger	H	Code begins with C and fourth character is A, T, or H	115 gr JHP	R9MM1	1987-01	10
Remington	9mm Luger	H	Code begins with C and fourth character is A, T, or H	124 gr FMJ	R9MM2	1987-01	10
Revelation	30-30 Win	R	Lot# Final Four Digits: -5289 thru -6285			1976-11	2
Rottweil	12 GA	S	Batch# fourth digit X, Y, or Z Batch# 52VW4, 53KA3	Rifled slug		2007-08	30
RWS	40 S&W	H	Lot# TMP12C300202	115gr frangible NT		2013-11	53
Speer	25 Auto	H	Lot# J25Gxx to J30Gxx, KxxGxx, LxxGxx, MxxGxx, AxxHxx, BxxHxx, CxxHxx			2002-04	21
Speer	32 Auto	H	Lot# J25Gxx to J30Gxx, KxxGxx, LxxGxx, MxxGxx, AxxHxx, BxxHxx, CxxHxx			2002-04	21
Speer	380 Auto	H	Lot# J25Gxx to J30Gxx, KxxGxx, LxxGxx, MxxGxx, AxxHxx, BxxHxx, CxxHxx			2002-04	21
Speer	9mm Luger, 9mm Luger+P	H	Lot# J25Gxx to J30Gxx, KxxGxx, LxxGxx, MxxGxx, AxxHxx, BxxHxx, CxxHxx			2002-04	21
Speer	40 S&W	H	Lot# J25Gxx to J30Gxx, KxxGxx, LxxGxx, MxxGxx, AxxHxx, BxxHxx, CxxHxx			2002-04	21
Wards	30-30 Win	R	Lot# Final Four Digits: -5289 thru -6285			1976-11	2
Winchester	12 GA 3"	S	Lot# 49X3SG29	1 3/8 oz. # 2 Steel Super X Drylock	XSM1232	2001-12	20
Winchester	17 HMR	R				2009-08	33
Winchester	22 Long Rifle	H, R	Lot# EK72	40gr lead hp	W22SUB	1992-07	13

RETIRED

Make	Caliber	Type	Lot Number/ Headstamp	Bullet/ Projectiles	Index No.	Date	Pg
Winchester	22 Long Rifle	H, R	Code # LF03, LF12, LF22, LF32, LF41, LF42, LF52, LF62, LF81, LF82, LF91, LF92		WW22LR	1977-01	4
Winchester	22 Long Rifle	H, R	Lot# 3RA42L, 1RH71L, 1RH81L, 2RH81L, 3RH81L		WW22LR	2001-09	19
Winchester	22 Long Rifle	H, R	Lot# containing XN, YA, YB, or YC		XPERT22 or WW22LR	2007-04	29
Winchester	22 Long Rifle	H, R	Lot# GD42L & GD52L	40gr Black Cu plated RN	S22LRT	2014-01	54
Winchester	22 Magnum	H, R	Lot# (1st three characters) 1WN & 1XA	45 gr Dynapoint	USA22M	2006-06	26
Winchester	223 Rem	R	Lot# ending in DK01, DK11, DK21, DK31, DK41, DK51, DM70, DM80, DM90, DM01, DM11 or DM21	64 gr Power-Point	RA223R2	2011-06	40
Winchester	30-06 Sprg	R	Lot# 137HF22	180 gr Power-Point SP (Super X)	X30064	1995	16
Winchester	40 S&W	H	4 character "Day Code" (2 letters & 2 numbers); all Q4355 with day codes "F…" & "EN.."	180gr Bonded	Q4355	2012-08	45
Winchester	5.56mm (223 Rem)	R	Lot# WCC10M106-004	62gr Pene	ZGQ3308	2013-02	50
Winchester	7mm Rem Mag	R	Lot# last four VL82, VL92, or VM10	150 gr Ballistic Silvertip		2005-05	25
Winchester	9mm Luger	H	Lot# Containing BC, BD, BE, or BF	115 gr STHP		1988-10	11
Winchester	9mm Luger	H	Lot # 68GF92, 87GF92, & 016GF92	Subsonic	XSUB9MM	1995-08	17

RETIRED

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RETIRED

RECALL

Company: CBC
Caliber: 7.62x51mm
Lot #: Headstamp "CBC 7.62 75"
Source: Company Notice, 1975

CBC, 7.62MM X 51MM CALIBER CARTRIDGES

It has come to our attention that CBC 7.62mm x 51mm cartridges from lots dated 1975 may pose a serious safety risk. The specific cartridges in question bear the headstamp "CBC 7.62 75".

We hereby provide written notice to you that a potential hazard may exist with respect to outdated CBC 7.62mm x 51mm cartridges. There is a reported risk that such cartridges may cause excess pressure upon discharge and could result in serious bodily injury or damage.

Under no circumstances should these cartridges be used, given to anyone, or sold.

There have been isolated reports of rifles bursting as a result of excessive pressure in what appear to be these outdated CBC cartridges. An independent testing lab reports that it detected a peak pressure that they estimated to be in excess of 130,000 CUP in one out of a batch of 20 outdated CBC 7.62mm x 51mm cartridges dated 1975 that it tested.

CBC 7.62mm x 51mm cartridges are manufactured solely for military use in several nations around the world. CBC has not exported or sold any of these cartridges to or in the United States. These particular cartridges are not intended for civilian use under any circumstances.

In addition, a warning is issued for any CBC 7.62mm x 51mm cartridges from any year that bear a label on the packing material with the Spanish word "reengastada". That label was not affixed by CBC, and its application to ammunition is not yet clear.

Anyone who has, or believes they have, CBC 7.62mm x 51mm cartridges from lots dated 1975, or CBC 7.62mm x 51mm cartridges from any year with the word "reengastada" on the packing label should immediately contact the dealer from whom they bought the rounds, and call the following toll-free phone number:

(800) 742-1094
CBC/Brazil

RETIRED

RECALL

Company: Federal, Revelation, Wards, Hiawatha
Caliber: 30-30 Winchester
Lot #: final four numbers -5289 through -6285
Source: Unknown Newspaper Notice, November 1976

FEDERAL, 30-30 WINCHESTER CALIBER

REVELATION, 30-30 WINCHESTER CALIBER

WARDS, 30-30 WINCHESTER CALIBER

HIAWATHA, 30-30 WINCHESTER CALIBER

RETIRED

Federal is recalling several lots of cartridges and empty unprimed cartridge cases due to a structural weakness that can cause a case head separation. This can result in a jammed rifle and possible injury from escaping gas.

Cartridges and unprimed cartridge cases being recalled are identified by **LOT NUMBERS** with the final four numbers **-5289 through -6285**. The code appears on the back of each box. **Example: 3A-6032**..

Boxes have these brand names Federal, Revelation, Wards or Hiawatha.

If you have boxes with these codes call this toll-free number: (800) 328-7525 or write:

Customer Service
Federal Cartridge Corporation
Anoka, Minnesota 55303

This notice is limited to 30-30 cartridges and cartridge cases with the lot numbers described.

RECALL

Company: Frontier
Caliber: 30-30 Winchester
Lot #: 6-38-76-9, 6-42-76-9, 9-68-76-9, 9-71-76-9
Source: Guns & Ammo, Jan 1977, pg 17;
Unknown Newspaper Notice, Nov 1976

FRONTIER, 30-30 WINCHESTER CALIBER

RETIRED

Frontier Cartridge Company is recalling 30-30 Winchester caliber cartridges in 150 and 170-grain bullet weights from the following **LOT NUMBERS**:

6-38-76-9

6-42-76-9

9-68-76-9

9-71-76-9

The lot number is located on the inside end flap of each box. Return this ammo to your dealer for replacement or write:

Frontier Cartridge Co. Inc.
P.O. Box 1848
Grand Island, Nebraska 68801

RECALL

Company: Winchester
Caliber: 22 Long Rifle
Lot #: LF03, LF12, LF22, LF32, LF41, LF42, LF52, LF62, LF81, LF91, LF92
Source: Guns & Ammo, Jan 1977, page 12;
Guns, April 1977

WINCHESTER, 22 LONG RIFLE CALIBER, WILDCAT

RETIRED

Recall of 22 Rimfire Wildcat Brand Ammunition

Code Numbers LF03, LF12, LF22, LF32, LF41, LF42, LF52, LF62, LF81, LF91, LF92

We are recalling cartridges from 12 lots of Wildcat brand 22 long rifle rimfire ammunition due to excessive pressure in some of the cartridges, which can make them potentially hazardous to shoot. Use of this ammunition can cause damage to 22 caliber firearms and could cause personal injury.

The ammunition being recalled is identified by codes that contain LF03, LF12, LF22 and all "LF" codes followed by a two-digit number greater than 22. The number 1, 2, or 3 may precede the "LF" in the code, and one or more letters may follow the two-digit number.

(A typical code on the box reads "1LF12PY".)

This ammunition can be identified by the Code Numbers shown above which are printed on the inside tuck flap of each 50 round box and on the upper right hand corner of the end panel of 5000 round cases.

If you have Wildcat brand 22 rimfire ammunition with any of these identifying numbers, please return it to your dealer for replacement. Do not mail the ammunition to us as it is against postal regulations.

This notice is limited to the Code Numbers shown above. All other Wildcat Ammunition is not included in this recall.

Winchester-Western

RECALL

Company: Remington
Caliber: 7mm Remington Express
Lot #: M2OF through M29F and M010 through M11O
Source: AFTE Journal, Oct 1980, Vol 12, Num 4:103;
AFTE Journal, Jan 1982, Vol 14, Num 1:6;
American Rifleman, Oct 1980, pg 65

REMINGTON, 7MM REMINGTON EXPRESS CALIBER

RETIRED

Remington Arms Co., Inc., has announced the recall of defective 7 mm Express Remington cartridges which were manufactured in September and October of 1979.

Remington said the defective cartridges may produce higher than normal pressures which could damage some firearms, and which, under some circumstances, might cause personal injury. The company also recommended that fired cases from the ammunition not be reloaded because of possible adverse effects.

The 7 mm Express Remington cartridges covered by the recall have the following markings on the packages:
Caliber: 7 mm Express Remington
Bullet: 150 gr. Pointed Soft Point "Core Lokt"
Index No.: R7M061 (on outside of end flap)
Lot Nos.: M2OF through M29F and M010 through M11O (on inside of end flap).

Remington said it recalled several other lots of 7 mm Express Remington cartridges in September 1980, because of a similar problem.

The **LOT NUMBERS**, which were manufactured in August 1979, were: **M021, M031, M061, M071, NI081, M091, and M131**

The company said it is informing wholesalers and dealers to withhold from sale all 7 mm Express Remington cartridges in their inventories which bear lot numbers from both recalls. Remington representatives will make arrangements for return of the cartridges.

Consumers who have purchased 7 mm Express Remington ammunition since October 1979, bearing any of the above lot numbers, should return the unused cartridges and fired cases for free replacement. They should be sent collect via United Parcel Service to:
Remington Arms Company, Inc. Attention: J.H. Chisnall
939 Barnum Avenue P.O. Box #1939 Bridgeport, Conn. 06601

RECALL

Company: Remington
Caliber: 38 Special
Lot #: P28A & P29A
Source: AFTE Journal, July 1981, Vol 13, Num 3:6;
Associated Press Report, May 13, 1981

REMINGTON, 38 SPECIAL CALIBER

Remington Arms Co. has recalled two Lots of .38 special caliber ammunition manufactured in July 1980, that company officials said could malfunction when fired. The cartridges may separate and leave part of the case obstructing the barrel, the firm said.

Remington spokesman E.A. McCawley, Jr. said the cartridges can be identified by the lot numbers printed on the inside of the boxes' end flap.

LOT NUMBERS P28A & P29A

The recall involves a total of 270,000 cartridges, he said.

RETIRED

RECALL

Company: Remington
Caliber: 12 Gauge
Lot #: AT01H218
Source: Unknown Notice, June 5, 1983;
AFTE Journal, July 1983, Vol 15, Num 3:16

REMINGTON, 12 GAUGE, 1 OZ. SLUGS

A recall of defective deer slugs has been announced by the Remington Arms Co. A news release said a small quantity of shells do not contain enough powder and the slugs could lodge in the barrel, possibly resulting in gun damage and danger to shooters.

The rifled slugs being recalled are from **LOT NUMBER AT01H218** and are in boxes marked on end flaps:

"Remington SP12RS-5PK, Slugger 12-gauge 2 3/4"

"1 oz. HPRS, 5 Hollow Point Rifled Slugs,"

and on inside of end flaps, "AT01H218".

Persons owning such shotshells should send them via United Parcel Service to:

Attention:

Remington Arms Co.
R.H. Potter
939 Barnum Avenue
Box 1939
Bridgeport, Connecticut 06601

RETIRED

RECALL

Company: Remington
Caliber: 6mm Remington
Lot #: first letter is S, T, or U
Source: American Rifleman, Dec 1983, pg 57

REMINGTON, 6MM REMINGTON CALIBER

Remington Arms Co., Inc., has announced the recall of defective 6 mm Remington cartridges which were loaded with 80 grain hollow point “Power Lokt” and 80 grain pointed soft-point bullets. in September and October of 1979. Abnormal pressure growth over those recorded when the ammunition was loaded in 1981 and 1982. Firearms damage has occurred and use of this ammunition could present a hazard to the shooter.

The 6 mm Remington cartridges covered by the recall have the following markings on the packages:

Caliber: 6 mm Remington
Bullet: 80 gr. Pointed Soft Point or 80 gr. Hollow Point "Power Lokt"
Index No.: R6MM1 or R6MM2 (on lower right corner of end flap)
Code Nos.: If first letter is S, T, or U (on the left side of end flap).

The company said it is informing wholesalers and dealers to withhold from sale all 6 mm Remington cartridges in their inventories which bear code numbers. Remington representatives will make arrangements for return of the cartridges.

Consumers who have purchased 6 mm Remington ammunition in boxes bearing any of the above code numbers, should return the unused cartridges and fired cases for free replacement. They should be sent collect via United Parcel Service to:

Remington Arms Company, Inc.
Attention: G.T. Porter
Interstate Rt. 40 & Remington Rd.
Lonoke, AR72086

RETIRED

RECALL

Company: Federal
Caliber: 243 Winchester
Lot #: 5A-4592, 5B-4594, 5B-4592, 5A-4595, 5B-4593, 5A-4598, 5A-4594, 5B-4598
Source: American Rifleman, July 1986, pg 61;
California Dept. of Justice Firearms Safety Note 86-3;
Guns & Ammo, June 1986, pg 12;
Shooting Industry, May 1986, pg 18

RETIRED

FEDERAL, 243 WINCHESTER CALIBER, PREMIUM

Federal Cartridge Company is recalling ten lots of Premium brand caliber 243 Winchester cartridges. Certain cartridges in these lots have been found to produce higher than normal pressures that could cause damage to some firearms and personal injury. Do not shoot this ammunition.

These cartridges are ***packaged only in gold-colored boxes*** and not in red boxes.

These cartridges may be identified by the following markings on the outside of both package end flaps:

Federal Premium
243 Winchester
85 grain No. P24D
Boat-Tail Hollow Point Bullet

On the back panel of the box, there is a white rectangular area in which the lot number is printed.Only the following **LOT NUMBERS** are being recalled:

5A-4592	**5B-4592**	**5A-4593**	**5B-4594**	**5A-4595**
5B-4595	**5B-4593**	**5A-4594**	**5A-4598**	**5B-4598**

Persons having any Federal Premium brand 243 Winchester cartridges from these lots are instructed to return all of these cartridges to:
Federal Cartridge Company
900 Ehein Drive
Anoka, Minnesota 55303
Attn: Ronald L. Ives

Ship via United Parcel Service "Collect". Do not use United States Mail; it is illegal to send ammunition through the U.S. Postal Service! Include your name and mailing address so that we may send a refund check to you.
Federal Cartridge Company Minneapolis, MN 55402

RECALL

Company: Remington
Caliber: 38 Special, 38 Special+P, 9mm Luger
Lot #: beginning with the letter C and with the letters A, T, or H as the fourth character
Source: AFTE Journal, Jan 1987, Vol 19, Number 1:7; California Dept. of Justice Firearms Safety Note 86-5

REMINGTON, 38 SPECIAL CALIBER, 38 SPECIAL +P CALIBER, 9 MM LUGER CALIBER

RETIRED

Remington has recalled some .38 Special & 9mm Luger caliber ammunition. Certain lots may have insensitive primers, which could result in a failure to fire. Some of this ammunition may have been purchased by officers for use in off-duty weapons. This ammunition is being recalled at the expense of the Remington Arms Company, Inc.

Look for the following product specifications on the end flap of the box:

.38 Special (+P) 95 JHP R38S1 .38 Special (+P) 110 JHP R38S10

.38 Special (+P) 125 JHP R38S2 .38 Special (+P) 158 HP R38S12

.38 Special (+P) 158 lead R38S8 .38 Special 158 lead R38S5

9mm Luger 115 JHP R9MM1 9mm Luger 124 FMJ R9MM2

If you have any of the above cartridges, look for the identification code printed on the inside end flap.

Only ammunition with identification codes beginning with the letter C and with the letters A, T, or H as the fourth character is being recalled. Example - C03T132441 or C03H16213.

If you have any of these cartridges, do not use them. For replacement at no charge, call collect:

Bill Smith
Remington Arms Company, Inc.
Remington Road
Lonoke, Arkansas
(501) 676-3161 ext. 840

RECALL

Company: Winchester
Caliber: 9mm Luger
Lot #: BC, BD, BE, OR BF
Source: Company Notice, Oct 25, 1988;
California Dept. of Justice Firearms Safety Note 88-5

WINCHESTER, 9MM LUGER CALIBER, SILVERTIP

RETIRED

Olin Corporation, Winchester Division is recalling certain lots of its 9MM Luger (Parabellum) 115 gr. Silvertip Hollow Point (9MM STHP 115 gr.) ammunition.

Through extensive evaluation, Winchester has determined that certain lots of its 9MM STHP gr. ammunition may contain cartridges with low powder charge weights. Our evaluation determined an approximate frequency of low powder weight cartridges of 1 out of 20,000 rounds. Low powder weight ammunition may fire improperly, leaving a bullet in the barrel of the gun, and rendering the gun inoperable.

DO NOT USE WINCHESTER 9MM LUGER (PARABELLUM) 115 GR. SILVERTIP HOLLOW POINT AMMUNITION THAT HAS LOT NUMBERS CONTAINING THE FOLLOWING REFERENCES: BC, BD, BE, OR BF.

The ammunition lot number is stamped in the inside flap of the ammunition box. To determine if your ammunition is subject to this notice, review the lot number and if it contains the letter reference identified above, return it to Winchester for replacement. (An example of a lot number subject to this notice is 29BF 02.) Lot numbers containing letter references other than BC, BD, BE or BF are not subject to this recall and should not be returned to Winchester.

Winchester will replace any 9MM STHP 115 gr. ammunition effected by this recall at no cost to you. Please send the ammunition to the following address, freight collect:

Olin Corporation
Winchester Division
427 N. Shamrock St.
East Alton, IL 62024
Attn: Art Ambrosi, Manager, Product Services

If you have questions, please call Art Ambrosi at the following telephone numbers:
(800) 576-0015 (except Illinois, Alaska and Hawaii) (800) 258-2833 (collect for Illinois, Alaska and Hawaii)

We apologize for this inconvenience. There will be no charge for the replacement of the ammunition and you will be reimbursed for shipping costs.

Sincerely,
C.E. Becker Director, Marketing Olin Corporation

RECALL

Company: Remington
Caliber: 12 Gauge
Lot #: AJ (any #) J522 or 523 or 532, BJ (any #) J522 or 523 or 532, PAJ (any #) J522 or 523 or 532, PBJ (any #) J522 or 523 or 532, AK (any #) B522 or 523 or 532, BK (any #) B522 or 523 or 532, PAK (any #) B522 or 523 or 532, PBK (any #) B522 or 523 or 532
Source: Law Enforcement Technology, April 1989; page 21

RETIRED

REMINGTON, 12 GAUGE, 00 BUCKSHOT, 9 PELLET

The Remington Arms Company is recalling a quantity of **12gauge, 2-3/4 inch, 9-pellet, 00 buckshot shells produced during 1988**.

A limited number of these shotshells were found to have been manufactured without powder. Using a powderless shell would result in a failure of the shell to fire properly and could possibly result in a wad being lodged in the barrel. In law enforcement applications, this could impair an officer's ability to return fire.

While the total number of affected shotshells is small, Remington asks that you withdraw from use any of their 2-3/4 inch, non-magnum, 00 buckshot shotshells if they were purchased since June 1988. Then, call Remington at either of the following toll-free numbers to determine if your shotshells are in an affected lot. *In USA call 1-800-654-2459. In Canada: call 1-800-654-5401.*

When you call, you'll need the product code Identification and lot number, both of which appear on the packaging. The product code identification is located on both end-flaps of the 5-shotshell box and on both end-labels of the 250-shotshell package. This identification should read:

Remington, 00 Buckshot, 12 Gauge, 2-3/4 inch, 3-3/4-Dr. Eq., 9 pellets

The lot number locations are as follows:
On 5-pack boxes: on the inside, facing part of the top flap
On 250-shell cases: at the lower, right-hand corner of both end-labels.

The lot number will be an 8 or 9-digit combination of letters and numbers; for example: AJ21J532.

Only the following **LOT NUMBERS** are being recalled:

AJ *(any #) J522 or 523 or 532*	***BJ (any #) J522 or 523 or 532***	***PAJ (any #) J522 or 523 or 532***
PBJ (any #) J522 or 523 or 532	***AK (any #) B522 or 523 or 532***	***BK (any #) B522 or 523 or 532***
PAK (any #) B522 or 523 or 532	***PBK (any #) B522 or 523 or 532***	

If your shotshells are in the affected lots, you will be given instructions on how return the ammunition for quick replacement. Remember, only a limited quantity of standard, non-magnum, 2-3/4 inch, 12 gauge, 00 buckshot, 9 pellet loads are affected.

RECALL

Company: Winchester
Caliber: 22 Long Rifle
Lot #: EK72
Source: Guns & Ammo, July 1992; page 83; American Hunter, May 1992; AFTE Journal, July 1992, Vol 24, Num 3:253

WINCHESTER, 22 LONG RIFLE CALIBER, SUBSONIC, LOT NUMBER EK72

RETIRED

The Olin Corporation, through its Winchester Division, is recalling a lot of 22 Caliber Subsonic Ammunition.

Through extensive evaluation, Winchester has determined that one lot of 22 Caliber Subsonic Ammunition manufactured in its Australian plant may contain cartridges with double powder charges. Our evaluation determined that approximately 1,024 double powder charge weight cartridges may have been produced out of 440,000 rounds manufactured in **LOT NUMBER EK72**. Double powder charge weight ammunition may cause firearm damage, rendering the firearm inoperable, and subject the shooter to a risk of personal injury when fired.

DO NOT USE WINCHESTER .22 CALIBER SUBSONIC AMMUNITION THAT HAS LOT NUMBER EK72.

The ammunition lot number is stamped (embossed into the cardboard) on the inside flap of the ammunition box. To determine if you ammunition is subject to this notice, review the lot number and if it contains **LOT NUMBER EK72**, return it to Winchester at the address below for replacement. Other lot numbers are not subject to this recall and should not be returned to Winchester.

Olin Corporation
Winchester Division
427 North Shamrock Street
East Alton, Illinois 62024
Attention: Product Service Department
.22 Caliber Subsonic Recall

Winchester will send you a coupon good for replacement ammunition from your local Winchester dealer.

If you have any questions concerning this .22 Caliber Subsonic Ammunition Recall, please write to the address above or call: (800) 526-0015 (except Alaska, Hawaii and Canada) (800) 258-2833 (for Alaska, Hawaii and Canada

We apologize for any inconvenience. WINCHESTER

RECALL

Company: Magtech
Caliber: 45 Auto
Lot #: L-602
Source: AFTE Journal, Apr 1993, Vol 25, Num 2:151; Gun World, Sept 1992, pg 36; Shooting Industry, Aug 1992, pg 11

MAGTECH, 45 AUTO CALIBER

RETIRED

Magtech Recreational Products, Inc., has recently received several reports of a "squib", or low-powder load, in Magtech brand .45 ACP ammo. Each of these reports has been traced to ammunition manufactured in **LOT NUMBER L-602.**

If a squib is shot, the bullet might lodge in the barrel of the firearm. If another cartridge is then loaded and fired, there is a serious risk that bodily injury or damage to the firearm could occur.

Anyone with Magtech brand .45 ACP **LOT NUMBER L-602** is requested to telephone the Magtech office at (800) 466-7191 for disposition and replacement instructions.

DO NOT, under any circumstances, FIRE OR SELL THIS AMMUNITION.

The **LOT NUMBER** is located on the inside flap of each individual carton of Magtech ammo. It will appear similar to this: **AR 1114 D *L-602* 16.553.**

The L-602 is the lot number; all other numbers refer to the actual day of packaging

Magtech Recreational Products Inc.
5030 Paradise Road
Suite A104
Las Vegas, NV 89119
(702) 736-2043

RECALL

Company: Remington
Caliber: 243 Winchester
Lot #: U06DC4605, U07DA4605, U07DB4605, & U07DC4605
Source: American Rifleman, March 1993, pg 12;
Guns & Ammo, March 1993, pg 9

REMINGTON, 243 WINCHESTER CALIBER

RETIRED

The Remington Arms Company is recalling a quantity of .243 Winchester, 100 gr., PSP, centerfire ammunition, index # R243W3. This action was taken because a limited number of these cartridges were loaded with an insufficient powder charge. This will result in failure to fire properly and could possibly leave a bullet lodged in the barrel. This creates an obstruction that may be dangerous if another cartridge is fired.

While the total number of cartridges is small, any shooters who have purchased Remington .243 Winchester, 100 gr., PSP, centerfire ammunition, index #R243W3 since July 1992, are requested by Remington not to use them. They are requested to contact Remington first by calling the toll-free number:

(800) 634-2459 (USA)

(800) 634-5401 (Canada)

Remington personnel will assist them in determining if they possess affected cartridges by product description and lot number. The lot numbers of the affected product are located on the inside left flap of the 20-pack cardboard box and on the label of the 500 cartridge package.

The LOT NUMBERS are: U06DC4605, U07DA4605, U07DB4605, & U07DC4605

No other product is involved but the one mentioned above.

This ammunition should NOT be fired.

If you have affected product, replacement or reimbursement will be made directly to you. Please call our toll-free numbers listed above for directions.

Remington Dupont

RECALL

Company: Winchester
Caliber: 30-06 Springfield
Lot #: 137HF22
Source: American Rifleman, July 1995, pg 62

WINCHESTER, 30-06 SPRINGFIELD CALIBER, SUPER X

RETIRED

Olin Corporation, through its Winchester Division, is recalling one (1) lot of its Super-X7 30-06 Springfield, 180 Gr. Power-Point (S.P.) ammunition (Symbol Number X30064).

Through extensive evaluation, Winchester has determined that a small number of cartridges of **LOT NUMBER 137HF22** Super-X7 30-06 Springfield, 180 gr. Power-Point Ammunition may contain low powder charges. These low powder charges can cause a squib, or weak shot, which could result in a bullet remaining in the barrel after firing, obstructing the barrel. Firing a second shot into the obstructed barrel will cause firearm damage, and will subject the shooter and bystanders to a risk of personal injury.

DO NOT USE WINCHESTER SUPER-X 30-06 SPRINGFIELD, 180 GR. POWER-POINT LOT NUMBER 137HF22.

The ammunition lot number is stamped on the inside of the flap of the ammunition box.

To determine if your ammunition is subject to this notice, review the lot number. If it contains **LOT NUMBER 137HF22**, return the ammunition to Winchester at this address:

Olin Corporation
Winchester Division
427 North Shamrock Street
East Alton, Illinois 62024-1191
Attention: Product Service Department, 30-06 Springfield Recall

Winchester will send you coupons good for replacement ammunition from your local Winchester dealer.

This notice applies only to Lot 137HF22. Other lot numbers are not subject to this recall, and should not be returned to Winchester.

If you have any questions concerning this Ammunition Recall, please call: (800) 852-5734 (except Alaska, Hawaii and Canada) or (618) 258-2670 or write to the address above.

We apologize for this inconvenience.

RECALL

Company: Winchester
Caliber: 9mm Luger
Lot #: 68GF92, 87GF92, and 016GF92
Source: Company Notice, Aug 9, 1995

WINCHESTER, 9MM LUGER CALIBER, 147 GRAIN SUBSONIC

Through extensive evaluation, Winchester and the Maryland State Police have determined that a small number of rounds of **LOT NUMBERS 68GF92, 87GF92, and 016GF92** of Winchester 9MM Luger Subsonic ammunition (XSUB9MM) may contain long cartridge cases. These long cartridge cases can interfere with the firing mechanism, and can cause firearms to jam ("lock-up").

The lot number is stamped on the inside of the flap of the ammunition box.

Winchester will replace, free of charge, any ammunition from **LOT NUMBERS 68GF92, 87GF92 and 016GF92** of Winchester 9mm Luger Subsonic ammunition. To obtain replacement ammunition, call Winchester Product Service at 800-852-5734.

This notice applies only to **LOT NUMBERS 68GF92, 87GF92 and 016GF92** of Winchester 9mm Luger Subsonic ammunition. Other lot numbers are not affected by this notice.

Winchester apologizes for this inconvenience, and thanks to the Maryland State Police for bringing this matter to our attention.

RETIRED

WARNING

Company: Hirtenberger AG
Caliber: 9x19mm
Lot #: L7A1
Source: BATF Bulletin, Nov 7, 1996;
American Rifleman, Jan 1997, pg 6;
IAA Journal, Jan/Feb 1997, pg 4

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9 X 19 MM Warning

Austrian ammunition maker Hirtenberger AG has put the word out concerning a quantity of its 9x19 mm ammunition that is "unique for use in any handgun." According to a November 7 BATF Industry News release. "The ammunition was loaded to produce pressures far in excess of that intended for use in handguns...This ammunition should not be fired." The ammunition was produced for the British Ministry of Defense from 1990 through 1992 for use in submachine guns "under adverse conditions" and carries the "L7A1" designation. While BATF is unaware of this ammunition being imported into the U.S., the maker advises that up to 12 million rounds were sold recently on the world surplus market.

The ammunition covered by this warning can be identified by the following headstamps:

12 o'clock position: HP

3 o'clock position: 90, 91, or 92

6 o'clock position: L7A1

9 o'clock position: a cross within a circle

For additional information contact BATF at (202) 927-8320.

RETIRED

RECALL

Company: Winchester
Caliber: 22 Long Rifle
Lot #: 3RA42L, 1RH71L, 1RH81L, 2RH81L and 3RH81L
Source: Company Notice, Sept 18, 2001;
American Rifleman, Nov 2001, pg 71;
Shotgun News, Oct 10, 2001; Vol 55, Num 29:11;
Albany Times Union Newspaper, Oct 11, 2001; page C-8;
IAA Journal, Issue 423, Jan/Feb, 2002:37

RETIRED

WINCHESTER, 22 LONG RIFLE CALIBER, WILDCAT

Recall of 22 Rimfire Wildcat Brand Ammunition
Lot Numbers: 3RA42L, 1RH71L, 1RH81L, 2RH81L and 3RH81L

Olin Corporation, through its Winchester Division, is recalling five (5) lots of its WILDCAT 22 Long Rifle High Velocity Rimfire ammunition (Symbol Number WW22LR).

Through extensive evaluation Winchester has determined the above five (5) lots of WILDCAT 22 ammunition may contain double powder charges. Double powder charge weight ammunition may cause firearm damage, rendering the firearm inoperable, and subject the shooter to a risk of personal injury when fired.

DO NOT USE WINCHESTER WILDCAT 22 LONG RIFLE HIGH VELOCITY RIMFIRE AMMUNITION that has Lot Numbers 3RA42L, 1RH71L, 1RH81L, 2RH81L and 3RH81L.

The ammunition Lot Number is imprinted (stamped without ink) on the outside of the right tuck flap of the 50-round box.. IF your ammunition contains these Lot Numbers, return the ammunition to Winchester via United Parcel Service (UPS). Securely pack the ammunition into a corrugated cardboard box, write “CARTRIDGES, SMALL ARMS ORM-D” on the outside, and ship to:

Olin Corporation – Winchester Division
Tile Warehouse
Powder Mill Road, Gate 4A
East Alton,IL 62024
Attention: 22 Caliber Recall

Winchester will send you coupons good for replacement ammunition from your local Winchester dealer. If you have any questions concerning this WILDCAT 22 Long Rifle High Velocity Rimfire ammunition recall please call toll-free 866-423-5224 (U.S. & Canada), write to the above address, or visit our website @ **www.winchester.com**

RECALL

Company: Winchester
Caliber: 12 Gauge
Lot #: 49X3SG29
Source: Company Notice, Dec 12, 2001;
International Ammunition Assoc. Journal, Issue 423, Jan/Feb 2002:37;
North American Hunter, Feb/Mar

WINCHESTER, **12 GAUGE, 3 INCH**1 3/8 OZ. OF #2 STEEL SHOT, SUPER X DRYLOCK SUPER STEEL

Winchester is recalling one lot of Winchester Super X Drylock Super Steel 12 gauge, 3 inch, 1 3/8 oz. of #2 steel. Lot Number 49X3SG29 only. Olin has determined that these shells are loaded with the incorrect propellant.

The recalled LOT NUMBER 49X3SG29 is located on the front lip of the inside top tuck flap of the box and the product symbol number is (XSM1232).

Olin Corporation – Winchester Division
Tile Warehouse
Powder Mill Road, Gate 4A
East Alton, IL 62024

If you have any questions concerning this ammunition recall please call toll-free 866-423-5224 (U.S. & Canada), write to the above address, or visit our website @ **www.winchester.com**

RETIRED

RECALL

Company: CCI/Speer
Caliber: 25 Auto, 32 Auto, 380 Auto, 9mm Luger, 9mm Luger+P, 40 S&W
Lot #: J25Gxx to J30Gxx, KxxGxx, LxxGxx, MxxGxx, AxxHxx, BxxHxx, CxxHxx
Source: Company Notice, April, 2002

CCI/SPEER GOLD DOT

AMMUNITION PRODUCED BETWEEN 9/25/01 & 3/31/02 IN 25 AUTO, 32 AUTO, 380 AUTO, 9MM LUGER, 9MM LUGER +P, 40 S&W CALIBERS

You should discontinue use of the subject ammunition due to an intermittent, low frequency occurrence of primer misfires.

Speer Gold Dot 25 Auto, 32 Auto, 380 Auto, 9MM Luger, 9MM Luger +P and 40 S&W ammunition produced between 9/25/01 and 3/31/02 should be returned to the factory for a no charge replacement. Replacement ammunition will incorporate an "A" mark in the primer cup for identification purposes.

SPEER GOLD DOT AMMUNITION RECALL LOT #'S

Letters in Lot Code indicate month and year of production. Recall period is from September 25th, 2001 to March 31st, 2002. Lot #'s with the sequence of letters shown below are part of the recall.

2001
J25Gxx to J30Gxx
KxxGxx
LxxGxx
MxxGxx

2002
AxxHxx
BxxHxx
CxxHxx

RETIRED

CCI/Speer (866) 288-7436 or (208) 746-2351
2299 Snake River Avenue
Lewiston, ID 83501

RECALL

Company: Hodgon Powder Co
Caliber: Lil' Gun Smokeless Powder
Lot #: 103080221
Source: Shotgun Sports, July 2003; page 8

HODGDON POWDER COMPANY, LIL' GUN SMOKELESS, LOT # 103080221

Hodgdon Powder Company, Inc. has determined a small number of 1 lb. containers of Hodgdon's LIL' GUN smokeless powder may create excessive pressures in firearms when loaded in cartridges and/or shotshells using standard loading data. Such a condition, if present, may cause personal injury to the user and/or bystanders and damage to the firearm. Users should immediately cease using this product and return it to Hodgdon Powder Co.

RETURN OF-PRODUCT AND REPLACEMENT

All persons in possession of Lot# 103080221 of Hodgdon's LIL' GUN l 1 lb. Containers of smokeless powder should immediately call (913) 362-9455 or (800) 622-4366 (toll free) for instructions from Hodgdon's Customer Service Department as to the return of this product.

RETIRED

RECALL

Company: Federal
Caliber: 45 Long Colt
Lot #: 8J191, 8J192, 8J193, 8J194, 8J195, 8J196, 8J197, 8J198, 8J199
Source: Shooting Industry, April 2004, pg 12;
Shot Business, April 2004, pg 7;
Federal Website

FEDERAL, 45 LONG COLT CALIBER, SEMI WADCUTTER HOLLOW-POINT

Federal Cartridge Company is recalling several lots of 45 Long Colt semi-wadcutter hollow-point ammunition - product number C45LCA in the Classic 20-round box. According to Federal officials, rounds from the suspect lot numbers may exhibit excessive chamber pressures, and use of the ammunition may result in firearms damage and/or personal injury. Company officials warn shooters not to use the lot numbers below.

Only the following LOT NUMBERS are being recalled:

8J191 8J192 8J193
8J194 8J195 8J196
8J197 8J198 8J199

These lot numbers can be found on the back of the carton or on the inside of the end flap.

For those possessing the ammunition, or for more information concerning this recall, contact Federal's Product Service Department at 1 (800) 831-0850 or 1 (800) 322-2342.

Federal Cartridge is guaranteeing shipping charges and replacement product.

Additional information can also be found on-line at www.federal-premium.com.

RETIRED

WARNING

Company: PMC
Caliber: 22 Long Rifle and 22 Magnum
Lot #: any
Source: North American Arms Company Notice, May 17, 2004

PMC 22 LR AND 22 MAGNUM CARTRIDGES ARE SUBJECT TO INADVERTANT DOUBLE DISCHARGE WHEN LOADED AND FIRED IN NORTH AMERICAN ARMS REVOLVERS

This Produce Performance Advisory Bulletin is issued due to the potential of an **INADEVERTANT, DOUBLE DISCHARGE** (two rounds simultaneously firing. One aligned with the barrel and the other out of battery). This notice is for the benefit of NAA's customers, owners and users of its 22 caliber revolvers. NAA has recently learned that the use of PMC 22 caliber ammunition (Magnum and LR) may affect the performance of its revolvers.

At this time, NAA recommends that its customers, owners and users of NAA 22 revolvers **DO NOT USE ANY PMC 22 CALIBER** ammunition (Magnum and/or LR) in any model NAA revolvers.

If you have any questions concerning this Product Performance Advisory Bulletin, please contact NAA at (800) 821-5783 or write to North American Arms, 2150 South 950 East, Provo, Utah 846066285, Attention General Manager.

RETIRED

RECALL

Company: Winchester
Caliber: 7mm Remington Magnum
Lot #: last four characters VL82, VL92, AND VM10
Source: Company Notice, Winchester Website 2005

WINCHESTER, 7 MM REMINGTON MAGNUM, SUPREME LOAD, 150 GRAIN BALLISTIC SILVERTIP

Olin Corporation is recalling three (3) lots of its Supreme 7 mm Remington Magnum 150 grain Ballistic Silvertip centerfire rifle cartridges due to incorrect propellant loading. This ammunition may cause firearm damage, rendering the firearm inoperable, and subject the shooter or bystanders to a risk of personal injury when fired.

LOT NUMBERS (with the last four characters):

VL82, VL92, AND VM10

This ammunition should be returned to Winchester via United Parcel Service (UPS). Winchester will send you coupons good for replacement ammunition from your local Winchester dealer.

If you have any questions concerning this Supreme Ballistic Silvertip ammunition recall, please call toll-free 866-423-5224 or visit our website at www.winchester.com

RETIRED

RECALL

Company: Winchester
Caliber: 22 Magnum
Lot #: first three characters 1WN and 1XA
Source: Company Notice June 2006

WINCHESTER BRAND, 22 MAGNUM CALIBER, 45 GRAIN DYNAPOINT RIMFIRE CARTRIDGES

Olin Corporation, through its Winchester Division, is recalling several lots of Dynapoint Magnum 22 Win. Mag. 45 grain, Dynapoint rimfire ammunition (Symbol Number USA22M).

Lot Numbers (first three characters): 1WN and 1XA

Note: the ammunition Lot Number is ink stamped inside the right tuck flap of the 50-round carton.

For questions, or to make comments or suggestions, call toll-free 866423-5224.

Winchester Ammunition
427 N. Shamrock Street
East Alton, IL 62024
618258-2000
http://www.olin.com/

RETIRED

RECALL

Company: Fiocchi
Caliber: 9mm Luger
Lot #: 4509222 thru 4509230, 4510241 thru 4510259, 4510261, 4511263 thru 4511272, 4511276
Source: Website: http://www.fiocchiusa.com/recall_pr.php, 2006

FIOCCHI BRAND, 9MM LUGER CALIBER, 115 GRAIN FMJ CARTRIDGES

FIOCCHI HAS DETERMINED THAT THE LOTS LISTED BELOW MAY CONTAIN DEFECTIVE PROPELLANT CAUSING SQUIB LOADS. THIS COULD RESULT IN DAMAGE TO THE FIREARM AND SUBJECT THE SHOOTER OR BYSTANDER TO RISK OF INJURY.

Fiocchi of America, Inc. is recalling several lots of 9mm Luger, 115 grain FMJ ammunition that was imported from Fiocchi Munizioni, Italy.

These lots of ammunition have been determined to contain defective propellant. The propellant in this ammunition may cause squib loads which could cause firearm damage, and subject the shooter or bystander to a risk of personal injury when fired.

4509222	4510230	4510248	4510256	4511266
4509223	4510241	4510249	4510257	4511267
4509224	4510242	4510250	4510258	4511268
4509225	4510243	4510251	4510259	4511269
4509226	4510244	4510252	4510261	4511270
4509227	4510245	4510253	4511263	4511271
4509228	4510246	4510254	4511264	4511272
4509229	4510247	4510255	4511265	4511276

The ammunition lot numbers are stamped inside the box tuck flap.

Fiocchi of America, Inc.
Attn: Recall
6930 N. Fremont Road
Ozark, MO 65721
800721-26666 or 800 595-6020

RETIRED

RECALL

Company: Hornady
Caliber: 30-30 Winchester
Lot #: 3060134
Source: Company Notice Feb 17, 2007

HORNADY 30-30 WINCHESTER CALIBER

Homady Mfg Company is recalling one (1) lot of 30-30 Win. Homady Leverevolution ™ Ammunition. This lot was shipped between the dates of 3-29-06 and 4-7-06.

Item number 82730, **Lot number 3060134**

Homady Mfg Company ballisticians have determined that some cartridges from Lot # 3060134 may be under loaded. An under loaded cartridge may result in a bullet remaining in the barrel after firing, causing an obstruction. Firing a second shot with a barrel obstruction from the first shot, will cause firearm damage and may cause personal injury to the shooter and bystanders.

The lot number can be found printed on the underside of the box label.

THIS NOTICE ONLY APPLIES TO **LOT #3060134.**

If you own this Lot # or have any questions regarding this recall, please call 800-338-1242. Homady Mfg Company will make all arrangements associated with this return and replacement of the product.

Any other lot numbers or item numbers are not subject to this recall and require no action.

Thank you for your attention. We apologize for this inconvenience.

RETIRED

RECALL

Company: Winchester
Caliber: 22 Long Rifle
Lot #: containing Letters XN, VA, YB or YC
Source: Company Notice Apr 18, 2007

WINCHESTER® 22 LONG RIFLE, WILDCAT® & XPERT® 22

Olin Corporation, through its Winchester Division, is recalling several lots of its WILDCAT® 22 (Symbol Number WW22LR) and XPERT® 22 (Symbol Number XPERT22) 22 Long Rifle rimfire ammunition.

Lot Numbers containing Letters: XN, VA, YB or YC

Through extensive evaluation Winchester has determined the above lots ofWfLDCAT® 22 and XPERT® 22 ammunition may contain double powder charges. Double powder charge weight ammunition may cause firearm damage, rendering the firearm inoperable, and subject the shooter to a risk of personal injury and/or death when fired.

DO NOT USE WINCHESTER® WILDCAT® 22 RIMFIRE AMMUNITION WITH LOT NUMBERS CONTAINING LETTERS XN, YA, VB or YC. The ammunition Lot Number is imprinted (stamped without ink) on the outside of tile right tuck flap of the 50-round box, as indicated here:

To determine if your ammunition is subject to this notice, review the Lot Number. If the Lot Number contains the letters XN, YA, YB or YC return the ammunition to Winchester via United Parcel Service (UPS). Securely pack the ammunition into a corrugated cardboard box, write "CARTRIDGES, SMALL ARMS ORM-D" on the outside, and ship to:
Olin Corporation - Winchester Division
Tile Warehouse Attention: 22LR Recall
Powder Mill Road, Gate 4A
East Alton, IL 62024

Please include your name, address, and phone number. Winchester will send you coupons good for replacement ammunition from your local Winchester dealer. **This notice applies only to WILDCAT® 22 and XPERT® 22 rimfire ammunition with Lot Numbers containing the letters XN, YA, YB or YC.** Other Symbol Numbers or Lot Numbers are not subject to this recall and should not be returned to Winchester.

If you have any questions concerning this recall please call toll-free 866-423-5224 (U.S. & Canada), write to the above address, or visit our website at www.winchester.com.

We apologize for this inconvenience.WINCHESTER

RETIRED

RECALL

Company: Rottweil
Caliber: Rifled Slug
Lot #: X, Y or Z as the fourth digit & batch numbers 52 VW 4 & 53 KA 3
Source: Shot Business Magazine Aug/Sept 2007:55;
American Rifleman, Oct 2007:119;
Guns and Ammo, Nov 2007

ROTTWEIL, BRENNEKE, RIFLED SLUG AMMUNITION

DO NOT USE RIFLED SLUGS

A limited number of rifled slugs may fail to clear the barrel completely upon firing. Although this is unlikely to occur, a follow-up shot could result in catastrophic failure of the barrel, and cause property damage or severe personal injury.

The company is undertaking a program to identify and recover all affected rifled slugs. As a precaution, we will have the product returned at no charge, and refund the purchase price.

This program applies only to rifled slugs with **batch numbers** bearing the letters **X, Y or Z** as the **fourth digit & batch numbers 52 VW 4 & 53 KA 3**, no other rifled slugs are involved.

We ask that you call the toll-free number for the Rifled Slug Refund Program at: 1-866-646-4767.

RETIRED

RECALL

Company: Remington
Caliber: 17 HMR
Lot #: any
Source: Company Notice, Aug 18, 2009;
Gun Week, Sept 15, 2009, pg 6

REMINGTON, 17 HORNADY MAGNUM RIMFIRE CALIBER CARTRIDGES

AMMUNITION NOT SUITABLE FOR USE IN SEMI AUTOMATIC FIREARMS

RETIRED

Remington has been notified by its supplier of 17 HMR ammunition that 17 HMR ammunition is not suitable for use in semi-automatic firearms. The use of this ammunition in a semi-automatic firearm could result in property damage or serious personal injury.

If you have a semi-automatic firearm chambered for 17HMR ammunition, immediately discontinue use of Remington 17 HMR ammunition. If you have any Remington 17 HMR ammunition that you wish to return to Remington contact the Remington Consumer Service number below. Do not return the ammunition to the dealer. Remington will provide you with a $10.00 coupon for each complete box of 50 rounds of Remington branded 17 HMR ammunition you return to Remington. This coupon will be good for the purchase of any Remington ammunition at your local dealer.

In light of the ammunition manufacturer's notice, it is very important that you immediately stop using your Remington Model 597 17HMR semi-automatic rifle. If you own a Remington Model 597 17 HMR semi-automatic rifle and wish to return it to Remington please contact the below Remington Consumer Service Number. In return for your Remington Model 597 17 HMR synthetic stock semi-automatic rifle, Remington will provide you a coupon valued at $200.00 good for the purchase of a replacement Remington firearm. If you have a laminate stock Remington Model 597 17 HMR semi-automatic rifle, Remington will provide you a coupon valued at $250.00 good for the purchase of a replacement Remington firearm. Remington will also reimburse you for the actual postage to return your Model 597 17 HMR semi-automatic rifle to Remington.

Please allow up to 6 weeks after Remington receives your Model 597 17 HMR semi-automatic rifle or your Remington branded 17 HMR ammunition for the appropriate coupons to arrive. Instructions for redemption of the coupons will be contained on the coupon.

For any consumer questions or instructions on how to return of your Model 597 17 HMR semiautomatic rifle or your Remington branded 17 HMR ammunition, please contact the Remington Consumer Service Department at 1-800-243-9700, Prompt #3.

We apologize for any inconvenience this may cause.

WARNING

Company: CCI, Federal
Caliber: 17 HMR
Lot #: any
Source: Company Notice, Aug 20, 2009

CCI /FEDERAL, 17 HORNADY MAGNUM RIMFIRE CALIBER AMMUNITION

AMMUNITION NOT SUITABLE FOR USE IN SEMI AUTOMATIC FIREARMS

Use of the 17HMR ammunition in semi-automatic firearms may result in serious personal injury or property damage.

17HMR ammunition is manufactured in accordance with standards issued by the Sporting Arms and Ammunition Manufacturers' Institute (SAAMI). Subsequent to the design and introduction of 17HMR ammunition to the market, 17HMR semi-automatic firearms were introduced by various firearm manufacturers. Those firearms have proven to be incompatible with 17HMR ammunition. 17HMR ammunition is suitable for use in quality firearms on good condition that are NOT semiautomatic.

Accordingly, do not use 7HMR ammunition in semi-automatic firearms. Only use 17HMR in modem firearms in good condition marked for 17HMR cartridges.

Please contact your gun manufacturer. For ammunition questions contact CCI Technical Service at 1-866-286-7436 or Federal Premium Product Services at 1-800-831-0850.

RETIRED

WARNING

Company: Winchester
Caliber: 17 HMR
Lot #: any
Source: Company Notice, Aug 21, 2009

WINCHESTER, SUPREME OR SUPER-X 17 HORNADY MAGNUM RIMFIRE CALIBER RIMFIRE AMMUNITION

AMMUNITION NOT SUITABLE FOR USE IN SEMI AUTOMATIC FIREARMS

Use of the 17HMR ammunition in semi-automatic firearms may result in serious personal injury or property damage.

17HMR ammunition is manufactured in accordance with standards issued by the Sporting Arms and Ammunition Manufacturers' Institute (SAAMI). Subsequent to the design and introduction of 17HMR ammunition to the market, 17HMR semi-automatic firearms were introduced by various firearm manufacturers. Those firearms have proven to be incompatible with 17HMR ammunition. 17HMR ammunition is suitable for use in quality firearms on good condition that are NOT semiautomatic.

If you have any questions concerning this warning notice please contact your firearms' manufacturer or call Winchester toll-free at 1-866-423-5224.

RETIRED

RECALL

Company: Brenneke
Caliber: 12 Gauge
Lot #: 1.0.01.07.08, 1.0.02.07.08, 1.0.03.07.08
Source: Company Website Apr 14, 2010:
www.brenneke- munition.de/cms/startnorthamerica.html

BRENNEKE, BLACK MAGIC MAGNUM RIFLED SLUG AMMUNITION

RETIRED

Brenneke of America, L.P., has announced a recall of certain lots of its Black Magic Magnum 12gauge 3" (76mm) cartridges. **CUSTOMERS SHOULD NOT USE THE CARTRIDGES BECAUSE THEY POSE A RISK OF SERIOUS PERSONAL INJURY.**

The recall applies only to cartridges from the following lot numbers, which are printed on the inside of the top box flap:

1.0.01.07.08 1.0.02.07.08 1.0.03.07.08

If you do not have the box to determine whether your cartridges are from the recalled lots, please call the number below and Brenneke will help make the, determination. The recall does not apply to other lot numbers of this cartridge or to any other Brenneke ammunition, including the Black Magic Short Magnum 12 gauge 2 ¾ " (70mm) cartridge.

Brenneke of America, L.P. has determined that a limited number of the cartridges from these lots may contain low- or no-powder charges that may result in the slug remaining in the barrel. Firing a second shot into the obstructed barrel may cause firearm damage and may subject the shooter and bystanders to a risk of serious injury.

Remedy**: Customers SHOULD NOT USE the cartridges** and should not keep them where they could be used by others. Customers should immediately **contact Brenneke at 1-877-684-0477**, between 8 am and 4 pm central time, Monday to Friday, to make arrangements for the return and replacement of the cartridges. Customers may also call that number with any other questions about the recall.

RECALL

Company: Federal
Caliber: 12 GA
Lot #: 4V-01QX425
Source: Company Notice, Oct 21, 2010

BLACK CLOUD PRODUCT WARNING

FEDERAL PREMIUM AMMUNITION
900 BOB EHLEN DRIVE
ANOKA, MN 55303
PHONE 763.323.2300
Toll Free: 1-800-322-2342

One lot of Federal Premium® Black Cloud® SNOW GOOSE 12 gauge 3" 1 1/8 oz ammunition (product number PWB143-2) may contain incorrect propellant. The lot number is 4V-01QX425.

Use of product from this lot may result in firearm damage and/or personal injury.

DO NOT USE LOT NUMBER: 4V-01QX425

The lot number can be found on the bottom of individual boxes or on the side panel of the 10 box case.

THIS WARNING APPLIES ONLY TO THE LOT LISTED ABOVE.

If you possess ammunition from this lot number or have questions concerning this warning, please contact us at 1-800-831-0850 or 1-800-322-2342 and ask for Product Service. Federal Premium will provide replacement product and will cover the cost of returning the affected product.

We apologize for any inconvenience this may cause.

RETIRED

RECALL

Company: Hornady
Caliber: 30-06 Springfield
Lot #: 3101052
Source: Company Notice, Nov 2010

HORNADY, 30-06 SPRINGFIELD CALIBER, 165 GRAIN SST HORNADY SUPERFORMANCE AMMUNITION

ITEM 81153, LOT # 3101052

RETIRED

Hornady Manufacturing Company is recalling one (1) lot of 30-06 Springfield caliber, 165 grain SST Hornady Superformance ammunition. Hornady ballisticians have determined that some cartridges from Lot # 3101052 may exhibit excessive chamber pressures. Use of this product may result in firearm damage and/or personal injury.

If you own this Lot # or have any questions regarding this recall, please call 800-338-1242. Hornady will make all arrangements associated with this return and replacement of the product.

RECALL

Company: Remington
Caliber: 22 Hornet
Lot #: J29EAI, J29EBI, J30EAI, J30EBI, or K01JDI
Source: Company Website and Notice, Nov 11, 2010

REMINGTON, 22 HORNET CALIBER, 45 GRAIN POINTED SOFT POINT AMMUNITION

Do Not Use Remington 22 Hornet 45 GR PSP Ammunition
Lot Numbers J29EAI, J29EBI, J30EAI, J30EBI, or K01JDI

Remington has determined that five lots of its 22 hornet 45 grain pointed soft point ammunition may have improper powder charge weights. These lots may result in a malfunction of the cartridge when the firearm is discharged. The malfunction may result in a bullet being lodged in the barrel creating an obstruction. If another round is fired with an obstruction in the barrel, a barrel burst may occur. A barrel burst could result in property damage, serious personal injury or death.

To identify if you have one of these Lots of ammunition:

- If you have a case of 22 Hornet 45 Gr PSP ammunition the Lot Numbers is stenciled on the outside of the case; and
- If you have a box of 22 Hornet 45 Gr PSP ammunition the Lot Number is stamped on the inside flap of the box.

RETIRED

RECALL

Company: Federal, American Eagle, Champion, Gold Medal, Hi-Shok
Caliber: 45 Auto
Lot #: 38X628 thru 38X765, 38T409 thru 38T414
Source: Company Notice, Feb 7, 2011

FEDERAL & AMERICAN EAGLE, 45 AUTO CALIBER

Certain lots of recently manufactured 45 Auto ammunition may contain an incorrect propellant charge. Use of product from these lots may result in firearm damage and possible serious injury.

DO NOT USE PRODUCT FROM THE FOLLOWING LOTS:

38X628 through 38X765
38T409 through 38T414

If you have in your possession any 45 Auto with the following brand names and part numbers, check to see if your ammunition package contains the above lots:

American Eagle (AE45A, AE45Nl, or AE45A250)
ChampionTM (WM5233)
GoldMedal (GM45B)
Hi-Shok (45C, 45D)
Federal Personal Defense (C45C, C45D)

THIS WARN1NG APPLIES ONLY TO THE LOTS LISTED ABOVE.

If you possess ammunition from any of these lots, or have questions concerning this warning, please call at 1-800-831-0850 or 1-800-322-2342 and ask for Product Service. Federal will provide replacement product and will cover the cost of returning the affected product.

RETIRED

WARNING

Company: Remington
Caliber: 223 Remington
Lot #: H03RAI, H04RDI, H16NAI OR H17NDI
Source: Company Notice, March 14, 2011

REMINGTON, 223 REMINTON CALIBER, 62 GRAIN HOLLOW POINT AMMUNITION

DO NOT USE LOT NOS. H03RAI, H04RDI, H16NAI OR H17NDI

RETIRED

Remington has determined that four (4) Lot Numbers of its .223 Remington 62 gr Hollow Point (Match) Ammunition may have been improperly loaded. The four (4) Lot Numbers are identified above. Improper loading may cause a malfunction of the cartridge when the firearm is fired resulting in higher than normal pressures. This malfunction-may result in damage to the firearm; serious personal injury or death.

To identify if you have one of these Lots of ammunition:
* If you have a **case** of .223 Remington 62 gr Hollow Point (Match) Ammunition the Lot Number is stenciled on the outside of the case; and,
* If you have a **box** of .223 Remington 62 gr Hollow Point (Match) Ammunition the Lot Number is stamped on the inside flap of the box.

If you have any of this .223 Remington 62 gr Hollow Point (Match) Ammunition, as identified above, immediately discontinue use of this ammunition and contact Remington at the below telephone number. Remington will arrange for the return shipment of your ammunition and upon receipt will send you replacement ammunition at no cost to you. If you are unsure whether or not you have one or more of these Lots of ammunition or if you have mixed boxes of ammunition; please immediately discontinue the use of the ammunition and contact Remington at the below telephone number - we will replace this ammunition for you.

For any consumer questions or instructions on how to return of your .223 Remington 62 gr Hollow Point (Match) Ammunition with one of the following **Lot Numbers H03RAI, H04RDI, H16NAI OR H17NDI**, please contact the Remington Consumer Service Department at 1-800-243-9700, Prompt #4.

The only Remington .223 Remington 62 gr Hollow Point (Match) Ammunition affected by this recall has one of the following **Lot Numbers H03RAI, H04RDI, H16NAI OR H17NDI** . No other Lots or ammunition is affected.

We apologize for any inconvenience this may cause.

RECALL

Company: Winchester
Caliber: 223 Remington
Lot #: last four characters DK01, DK11, DK21, DK31, DK41, DK51, DM70, DM80, DM90, DM01, DM11, or DM21
Source: Company Notice, June 1, 2011

WINCHESTER, RANGER, 223 REMINGTON CALIBER, 64 GRAIN POWER POINT AMMUNITION

RETIRED

Olin Corporation, through its Winchester Division, is recalling twelve (12) lots of its RANGER® 223 Remington 64 Grain Power-Point® (PP) centerfire rifle ammunition (Symbol Number RA223R2).

Lot Numbers (last four characters): DM70, DM80, DM90, DM01, DM11, and DM21
Lot Numbers (last four characters): DK01, DK11, DK21, DK31, DK41, and DK51

Through extensive evaluation Winchester has determined the above lots of RANGER® Law Enforcement ammunition may contain incorrect propellant. Incorrect propellant in this ammunition may cause firearm damage, rendering the firearm inoperable, and subject the shooter or bystanders to a risk of serious personal injury when fired.

DO NOT USE WINCHESTER® RANGER® 223 REMINGTON 64 GRAIN POWERPOINT® AMMUNITION THAT HAS A LOT NUMBER ENDING IN DK01, DK11, DK21, DK31, DK41, DK51, DM70, DM80, DM90, DM01, DM11, or DM21.

The ammunition Lot Number is ink stamped inside the right tuck flap of the 20-round carton.

To determine if your ammunition is subject to this notice, review the Lot Number. If the last four characters of the Lot Number are **DK01, DK11, DK21, DK31, DK41, DK51, DM70, DM80, DM90, DM01, DM11, or DM21 immediately discontinue use** and contact Winchester toll-free at 866-423-5224 to arrange for replacement ammunition and free UPS pick-up of the recalled ammunition.

This notice applies only to RANGER® 223 Remington 64 Grain Power-Point® centerfire rifle ammunition with lot numbers ending in DK01, DK11, DK21, DK31, DK41, DK51, DM70, DM80, DM90, DM01, DM11, or DM21. Other Symbol Numbers or Lot Numbers are not subject to this recall.

If you have any questions concerning this RANGER® Law Enforcement ammunition recall please call toll-free 866-423-5224, write to Winchester (600 Powder Mill Road, East Alton, IL 62024 Attn: RA223R2 Recall), or visit our website at www.winchester.com.

We apologize for this inconvenience.

WINCHESTER

RECALL

Company: Federal
Caliber: 243 Winchester
Lot #: ends in 5T561 thru 5T566
Source: Company Notice, September 6, 2011

POWER-SHOK™ .243 Win PRODUCT WARNING

Several lots of Federal Power-Shok™ .243 Win 80-grain soft point ammunition (product number 243AS) may contain incorrect propellant. Use of product from this lot may result in firearm damage and/or personal injury.

DO NOT USE LOT NUMBERS ENDING IN:
5T561, 5T562, 5T563, 5T564, 5T565 and 5T566

The lot number can be found on the bottom of individual boxes or on the side panel of the 10 box case.

THIS WARNING APPLIES ONLY TO THE LOTS LISTED ABOVE.

If you possess ammunition from this lot number, or have questions concerning this warning, please contact us at 1-800-831-0850 or 1-800-322-2342 and ask for Product Service. Federal Premium will provide replacement product and will cover the cost of returning the affected product.

We apologize for any inconvenience this may cause.

RETIRED

RECALL

Company: Hornady
Caliber: 500 S&W
Lot #: 3101327, 3110256, 3110683, 3110695, 3110945, 3111388, or 3111885
Source: Company Website and Notice, Feb 2012;
AFTE Website, June 15, 2012

HORNADY, 500 SMITH & WESSON CALIBER, 300 FTX CUSTOM PISTOL AMMUNITION

Lot Numbers 3101327, 3110256, 3110683, 3110695, 3110945, 3111388, or 3111885
ITEM # 9249

Hornady Manufacturing Company ballisticians have determined that some cartridges from Lot #'s 3101327, 3110256, 3110683, 3110695, 3110945, 3111388, 3111885, may exhibit excessive chamber pressures. Use of this product may result in firearm damage and/or personal injury.

DO NOT USE HORNADYCUSTOM PISTOL AMMUNITION
ITEM # 9249, 500 S&W CALIBER, 300 GRAIN FROM THE ABOVE LISTED LOT NUMBERS.

If you own any one of these Lot #'s or have any questions regarding this recall, please call (800) 338-1242.

RETIRED

WARNING

Company: Federal/Champion
Caliber: 40 S&W
Lot #: A27T3 or A27T4
Source: Company Notice, March 9, 2012

FEDERAL/CHAMPION, 40 S&W CALIBER AMMUNITION PACKAGING MIX

9 MM AMMUNITION MAY HAVE BEEN LOADED INTO 40 S&W BOXES

Federal has identified (2) lots of Federal Champion ammunition with boxes labeled 40 S&W that may contain 9 mm ammunition. If you have 40 S&W 50 round box with Lot Number A27T3 or A27T4. DO NOT USE 9 mm ammunition in 40 caliber firearms may result in firearm damage and/or personal injury.

RETIRED

RECALL

Company: Remington
Caliber: 270 Winchester
Lot #: N16SA24L, N165SA24R, N16SB24L, N16SB24R, N13SC24L, N13SC24R
Source: Company Website and Notice, Sept 18, 2012;
AFTE Website, May 19, 2012

REMINGTON, 270 WINCHESTER CALIBER, 150 GRAIN SOFTPOINT AMMUNITION WITH LOT #'S N16SA24L, N165SA24R, N16SB24L, N16SB24R, N13SC24L, N13SC24R

Remington has determined that six (6) Lots of its 270 Winchester, 150 grain soft point ammunition may have been improperly loaded. Improper loading may cause a malfunction of the cartridge when the firearm is fired resulting in higher than normal pressures. This malfunction may result in damage to the firearm, serious personal injury or death.

For any consumer questions or instructions on how to return your Remington 270 Winchester 150 grain soft point ammunition with one of the above listed Lot #'s, please contact the Remington Consumer Service Department at 1-800- 243-9700, Prompt #4.

RETIRED

RECALL

Company: Winchester
Caliber: 40 S&W
Lot #: All Q4355 with day codes beginning with the letter F and any Q4355 with the two letter combination of EN
Source: Company Notice, August 2, 2012;
AFTE Website, August 3, 2012

WINCHESTER, 40 S&W RANGER, 180 GRAIN CALIBER CARTRIDGES

Some Winchester, 40 S&W Ranger, 180 grain, bonded service ammunition cartridge cases were produced WITHOUT A FLASH HOLE. This condition completely eliminates the ability of this service cartridge to fire – this is a silent killer in that no visual inspection can detect this manufacturing defect and it would only be known at the time it is attempted to be fired.

Affected ammunition will have a four character "day code", two letters and two numbers, i.e. FA42. All Q4355 with day codes beginning with the letter F and any Q4355 with the two letter combination of EN are included in this recall.

Address any requests, questions, or concerns regarding the information on this INFOALERT to William Urban, CBP Intelligence Officer, (410)962-4415, TAUBALTIMORE@cbp.dhs.gov

FOR OFFICIAL USE ONLY – LAW ENFORCEMENT SENSITIVE (LES)

NOT AUTHORIZED FOR PUBLIC DISCLOSURE

RETIRED

RECALL

Company: Fiocchi
Caliber: 22-250 Rem, 243 Winchester
Lot #: 001, 002, 003, 004, 005, 006, 007, 008
Source: Company Notice, Sept 2012

PRODUCT WARNING AND RECALL NOTICE FOR FIOCCHI 22-250 REM AND 243 WIN AMMUNITION

Effective September 17, 2012, Fiocchi of America is recalling various lots of 22-250 Rem. and 243 Win. centerfire rifle ammunition. No other Fiocchi products are included in this recall and only the following items and lot numbers are affected. The following ammunition products are affected by this recall:

Fiocchi Item Number	Caliber	Bullet Weight/Type	Lot Ending Numbers
22250B	22-250 Rem.	55 Pointed Soft Point	001, 002, 003, 004
22250HVD	22-250 Rem.	55 V Max Polymer Tip	001, 002, 003, 004
243SPB	243 Win.	70 Pointed Soft Point	006, 007, 008
243HSB	243 Win.	95 SST Polymer Tip Boat Tail	004, 005
243SPD	243 Win.	100 Interlock Boat Tail	001, 002, 003, 004

RETIRED

Through extensive evaluation Fiocchi has determined that the above lots of 22-250 and 243 may have been loaded with multiple bullets that can cause excessive pressure. This ammunition with multiple bullets may cause firearm damage rendering the firearm inoperable, and subject the shooter or bystanders to a risk of personal injury when fired.

DO NOT USE THE ABOVE LISTED FIOCCHI 22-250 REM. OR 243 WIN. AMMUNITION OF SPECIFIED LOT NUMBERS.

The ammunition lot number is black ink stamped inside one of the box end tuck flaps that you would open to remove the 20-round carrier.

To determine if your ammunition is subject to this notice, review the **ITEM** and **LOT NUMBER.** If the item number **AND** last three digits of the lot number match the above listed products, please return the ammunition to **FIOCCHI of America** via United Parcel Service (UPS) or FedEx Ground. Securely pack the ammo in a strong fiberboard box with packing materials to make it "snug" in the box. You will need an "LQ" label to ship the ammunition. To get the "LQ" label, visit this web address **HERE** or you can call the factory customer service directly at **417-449-1039** or email **service@fiocchiusa.com** prior to shipping. Affix the "LQ" label to the package with packing tape ensuring that all sides are adhered to the carton and ship to: **FIOCCHI OF AMERICA, INC. ATTN: Recall 6930 N. FREMONT RD. OZARK, MO 65721**

Fiocchi will provide replacement product upon receipt of your return. Please include your name, street address, phone number (required for return shipping), and email address with your return. Ammunition cannot be shipped to post office boxes.

This notice only applies to above listed Fiocchi 22-250 Rem. and 243 Win. ammunition with the above lot numbers. Other products or lot numbers are not subject to this recall and should not be returned to Fiocchi.

If you have any questions concerning this ammunition recall, please call customer service directly at **417-449-1039,** write to the above address, email **service@fiocchiusa.com** or visit our website.

RECALL

Company: Remington
Caliber: 338 Lapua Magnum
Lot #: L13SA29L, L13SA29R, L13SB29L, L13SB29R
Source: Company Notice Sept 19, 2012

REMINGTON, 338 LAPUA MAGNUM CALIBER, 250 GRAIN SCENAR MATCH AMMUNITION

Remington has determined the following lots may have been improperly loaded. Improper loading may cause a malfunction may result in damage to the firearm, serious personal injury or death.

Lot Numbers L13SA29L, L13SA29R, L13SB29L, L13SB29R

If you have any of the ammunition identified above, immediately discontinue use and contact Remington at 1-800-243-9700, Prompt #3. We will arrange for return shipment and upon receipt, will send replacement ammunition at no cost.

RETIRED

RECALL

Company: Remington
Caliber: 38 Special+P
Lot #: P24UA18R
Source: Company Notice, Nov 2012

Remington/UMC 38 Special +P Packaging Mix

CONSUMER NOTICE

RETIRED

Remington has determined that one specific lot code of the 100 round box of 38 SPECIAL+P 125 GRAIN SEMI-JACKETED HOLLOW POINT (L38S2B) ammunition, may contain 357 Magnum ammunition. This packaging mix is specific to lot number: P24UA18R only. DO NOT USE. Usage of 357 Magnum ammunition in 38 Special firearms could result in firearm damage and personal injury.

DO NOT USE Remington/UMC 38 Special +P 125 Gr. semi-jacketed hollow point Ammunition with Lot Number: P24UA18R

To identify if you have one of these Lots of ammunition:

If you have a case of 38 Special +P 125 Gr. Semi-Jacketed Ammunition the Lot Number is stenciled on the outside of the case; and,
If you have a box of 38 Special +P 125 Gr. Semi-Jacketed Ammunition the Lot Number is stamped on the inside flap of the box.
If you have any of this Remington/UMC 38 Special +P 125 Gr. Semi-Jacketed Ammunition, as identified above, immediately discontinue use of this ammunition and contact Remington at the below telephone number. Remington will arrange for the return shipment of your ammunition and upon receipt will send you replacement ammunition at no cost to you.

For any consumer questions or instructions on how to return of your Remington/UMC 38 Special +P 125 Gr. Semi-Jacketed Ammunition with Lot Number P24UA18R, please contact the Remington Consumer Service Department at: 1-800-243-9700, Prompt #4. We apologize for any inconvenience this may cause.

http://www.remington.com/pages/news-and-resources/safety-center/safety-warning-RemingtonUMC38-Special.aspx

RECALL

Company: Remington
Caliber: 12 Gauge, 2 ¾”
Lot #: N27YC525, N29YA525, P02TA525, P03TA525, P06TA525, N28YC525, N29YB525, P02TB525, P03TB525, P08UB899, N28YB525, N29YC525, P02TC525, P05TB525
Source: Company Notice December 7, 2012

REMINGTON, 12 GAUGE, 2 ¾”, 00 BUCKSHOT, 8 PELLET LAW ENFORCEMENT REDUCED RECOIL AMMUNITION

Remington has determined the following lots may have been improper propellant charge weights. Improper loading may cause a malfunction may result in damage to the firearm, serious personal injury or death.

Lot Numbers

N27YC525, N29YA525, P02TA525, P03TA525, P06TA525, N28YC525, N29YB525, P02TB525, P03TB525, P08UB899, N28YB525, N29YC525, P02TC525, P05TB525

For cases, the lot number is stenciled on the outside of the case; and for boxes, the lot number is stamped on the inside flap of the box. Product Code; RR128B00 Item Code: 20645

If you have any of the ammunition identified above, immediately discontinue use and contact Remington at 1-800-243-9700, Prompt #3. We will arrange for return shipment and upon receipt, will send replacement ammunition at no cost.

RETIRED

RECALL

Company: Winchester
Caliber: 5.56mm (223 Remington)
Lot #: WCC10M106-004
Source: Company Notice Feb 4, 2013;
AFTE Website Feb 13, 2013

WINCHESTER® 5.56 MM (223 REMINGTON) M855 62 GRAIN PENE CENTERFIRE RIFLE AMMUNITION

RETIRED

Olin Corporation, through its Winchester Division, is recalling one (1) lot of 5.56 MM M855 62 Grain Pene centerfire rifle ammunition.

Symbol Number: ZGQ3308
Lot Numbers: WCC10M106-004

Through extensive evaluation Winchester has determined the above lot of 5.56mm M855 ammunition may contain incorrect propellant. Incorrect propellant in this ammunition may subject the shooter or bystanders to a risk of serious personal injury and/or death, cause firearm damage, rendering the firearm inoperable.

DO NOT USE WINCHESTER® SYMBOL NUMBER ZGQ3308 LOT NUMBER WCC10M106-004 5.56MM M855 62 GRAIN PENE AMMUNITION. The ammunition Symbol Number Lot Number are ink stamped on the outside of the 900-round shipping container, and on the outside of the 30-round carton.

To determine if your ammunition is subject to this notice, review the Symbol Number and Lot Number. If it is Symbol Number ZGQ3308 and Lot Number WCC10M106-004

IMMEDIATELT DISCONTINUE USE

and contact Winchester toll-free at 866-423-5224 for free UPS pick-up of the recalled ammunition. Upon receipt of your recalled ammunition, Winchester will ship replacement ammunition directly to you.

This notice applies only to Symbol Number ZGQ3308 with Lot Numbers WCC10M106-004. Other Symbol Numbers or Lot Numbers are not subject to this recall.

If you have any questions concerning this 5.56MM m855 ammunition recall call toll-free 866-423-5224, write to Winchester (600 Powder Mill Road, East Alton, IL 62024 Attention: 5.56MM M855 Recall), or visit our website at www.winchester.com

We apologize for this inconvenience.

RECALL

Company: Remington
Caliber: 22 Long Rifle
Lot #: S22BB1237, S22BC1237
Source: Company Notice, July 31, 2013

Consumer Product Notification
Remington Rimfire: 22 Thunderbolt® TB-22A 22 Long Rifle Ammunition

RETIRED

Remington has determined that two specific lot codes of Remington 22 Thunderbolt® ammunition may contain rounds with an insufficient crimp on the bullet. This issue is specific only to lot numbers: S22BB1237 and S22BC1237. Handling or loading this 22 Thunderbolt® ammunition could result in the separation of the bullet from the shell casing. In certain situations, this occurrence could render the firearm inoperable.

The lot numbers are located on the interior of the tuck flap of an individual box or along the exterior of a shipping case.

If you have recently purchased a box, brick, or case of Remington 22 Thunderbolt® ammunition with the specific lot numbers: S22BB1237 or S22BC1237, Remington will exchange this ammunition at no charge. For questions, or to arrange for exchange and replacement of this 22 Thunderbolt® ammunition, please contact the Remington Customer Service staff at: 1-800-243-9700, Prompt #3.

Remington would like to apologize for any inconveniences or concerns that this issue may have created for our customers. We thank you for your continued support of Remington firearms and ammunition products.

RECALL

Company: BlackGun Ammo
Caliber: 5.56mm
Lot #: MJC13B581-004
Source: Company Notice, Sept 30, 2013

BlackGun Ammo RECALL Lot 004 556/62Grain 5.56mm 62gr Remanufactured Ammunition

BlackGun Ammo LLC is recalling one (1) lot of 5.56mm 62gr Remanufactured Ammunition. This lot was shipped approximately between the dates of 2-1-13 and 3-1-13.

BlackGun Ammo LLC ballisticians, after extensive evaluation, have determined that some cartridges from Lot # MJC13B581-004 may exhibit dropped primers and excessive chamber pressures. Use of this product may result in firearm damage and or personal injury.

DO NOT USE BLACKGUN AMMO 5.56mm 62gr Remanufactured Lot # MJC13B581-004. The lot number can be found printed on a label on the 50 round tray.

THIS NOTICE ONLY APPLIES TO LOT # MJC13B581-004.

If you own this Lot # or have any questions regarding this recall, please call 866.855.2842 or write to BGA, PO Box 191, Blue Springs, MO 64013 or email info@blackgunindustries.com. BlackGun Ammo LLC will make all arrangements associated with this return and replacement of the product.

OTHER LOT NUMBERS OR ITEM NUMBERS ARE NOT SUBJECT TO THIS RECALL AND REQUIRE NO ACTION.

Thank you for your attention. We apologize for this inconvenience.
BlackGun Ammo, LLC

RETIRED

WARNING

Company: RWS
Caliber: 40 S&W
Lot #: TMP12C300202
Source: AFTE Website, November 1, 2013

RWS, 40 S&W CALIBER, 115 GRAIN COPPER-MATRIX NTF AMMUNITION

LOT NUMBER TMP12C300202 WITH RUAG PRIMERS

The training officer of the Scottsdale, AZ Police Department reports three (3) damaged Glock handguns as a result of what is believed to be defective RWS, 40 S&W Caliber, Copper-Matrix NTF (Totally Lead Free Non-Toxic 115 Grain Frangible Training Ammunition loaded with RUAG primers.

The department experienced one (1) Glock handgun had a bulged barrel, two (2) others catastrophic failed. The damage in the two (2) catastrophic failures were almost identical. Additionally, there were a high percentage of rounds that failed to fire and an overall inconsistency in ammo pressures when firing shot to shot was noted.

RETIRED

RECALL

Company: Winchester
Caliber: 22 Long Rifle
Lot #: GD42L, GD52L
Source: Company Notice Jan 26, 2014;
AFTE Website Mar 7, 2014

WINCHESTER® 22 LONG RIFLE RIMFIRE AMMUNITION

Olin Corporation, through its Winchester Division, is recalling two (2) lots of M*22, 22 Long Rifle 40 Grain Black Copper Plated Round Nose rimfire ammunition.

Symbol Number: S22LRT
Lot Numbers: GD42L & GD52L

Winchester has determined the above lots of 22 Long Rifle rimfire ammunition may contain double powder charges. Ammunition with double powder charge may subject the shooter or bystanders to a risk of serious personal injury and/or death, cause firearm damage, rendering the firearm inoperable.

DO NOT USE WINCHESTER® M 22, 22 Long Rifle RIMFIRE AMMUNITION WITH LOT NUMBERS GD42L or GD52L. The ammunition Lot Number is imprinted (stamped without ink) on the left flap of the 500-round carton. The 1000 round intermediate carton does not have a Lot Number.

To determine if your ammunition is subject to this notice, review the Symbol Number and Lot Number. If the Symbol Number S22LRT with Lot Number containing GD42L or GD52L

IMMEDIATELT DISCONTINUE USE

and contact Winchester toll-free at 866-423-5224 or visit: http://www.winchesster.com/Product-Service/Pages/Contact-Usaspx(/Product-Service/Pages/Contact-Us.aspx) for free UPS pick-up of the recalled ammunition.

This notice applies only to Symbol Number S22LRT with Lot Numbers GD42L and GD52L. Other Symbol Numbers or Lot Numbers are not subject to this recall.

If you have any questions concerning this 22 Long Rifle rimfire ammunition recall call toll-free 866-423-5224, write to Winchester (600 Powder Mill Road, East Alton, IL 62024 Attention: S22LRT Recall), or contact Winchester Product-Service/Pages/Contact-Usaspx(/Product-Service/Pages/Contact-Us.aspx) online.

We apologize for this inconvenience.

RETIRED

RECALL

Company: Hornady
Caliber: 9mm Luger+P
Lot #: 3141635
Source: Company Notice July 2014

Product Warning and Recall Notice: 90226

July 2014

Hornady Mfg Company is recalling one (1) lot of 9mm 135gr +P Hornady Critical Duty™ Ammunition, Item#90226. This lot was shipped between the dates of 6-5-14 and 7-16-14.

Item number 90226 LOT # 3141635

Hornady Mfg Company ballisticians have determined that some cartridges from Lot # 3141635 may exhibit light or no powder charges. Use of this product may result in firearm damage and or personal injury.

DO NOT USE HORNADY CRITICAL DUTY 9MM 135GR. +P, LOT #3141635.

The lot number can be found printed on the underside of the box label.

This Notice Only Applies to Lot #3141635.

If you own this Lot # or have any questions regarding this recall, please call 800-338-1242. Hornady Mfg Company will make all arrangements associated with this return and replacement of the product.

ANY OTHER LOT NUMBERS OR ITEM NUMBERS ARE NOT SUBJECT TO THIS RECALL AND REQUIRE NO ACTION.

Thank you for your attention. We apologize for this inconvenience.

RETIRED

RECALL

Company: Hornady
Caliber: 470 Nitro Express
Lot #: 3140935
Source: Company Notice November 2014

Product Warning and Recall Notice: Item No. 8263

RETIRED

Hornady Mfg Company is recalling one (1) lot of 470 NE 500 gr DGX Rifle Ammunition, Item #8263. This lot was shipped between the dates of 4-14-14 and 11-21-14.

ITEM NUMBER 8263 LOT # 3140935

Hornady Mfg Company ballisticians have determined that some cartridges from Lot # 3140935 may exhibit light or no powder charges. Use of this product may result in firearm damage and or personal injury.

DO NOT USE HORNADY 470 NE 500 GR DGX AMMUNITION, LOT #3140935.

The lot number can be found printed on the underside of the box label.

THIS NOTICE ONLY APPLIES TO LOT #3140935.

If you own this Lot # or have any questions regarding this recall, please call 800-338-1242. Hornady Mfg Company will make all arrangements associated with this return and replacement of the product.

ANY OTHER LOT NUMBERS OR ITEM NUMBERS ARE NOT SUBJECT TO THIS RECALL AND REQUIRE NO ACTION.

Thank you for your attention. We apologize for this inconvenience.

RECALL

Company: G2R
Caliber: 45 Auto
Lot #: Manuf between 7/1/2014-11/30/2014
Source: Company Notice December 2014

Product Warning and Limited Recall Notice

RETIRED

.45 ACP Radically Invasive Projectile (R.I.P.™) Manufactured in July – Nov 2014 for use in unsupported chambers in certain Glock .45 ACP firearms.

G2 Research, Inc., has determined that some cartridges for .45 ACP R.I.P. ™ that were manufactured between July 1, 2014 to November 30, 2014 may exhibit excessive chamber pressures when utilized in unsupported chambers of certain Glock .45 ACP firearms. Use of this product in Glock .45 ACP firearms may result in firearm damage and or personal injury. This does not affect any other .45 ACP firearms.

DO NOT USE G2 REASEARCH INC., .45 ACP R.I.P. ™ AMMUNITION: Lots Manufactured between 7/1/2014 and 11/20/2014 in Glock .45 ACP firearms: The lot manufactured date can be found printed on the inside of the box flap. The UPC code can be found on the side of the box.

THIS NOTICE ONLY APPLIES TO .45 ACP R.I.P. ™ MANUFACTURED BETWEEN JULY 3014 AND NOVEMBER 2014 WITH UPC CODE 63552 0002 AND USED IN .45 ACP GLOCK FIREARMS.

If you have a Glock chambered in .45 ACP, please call 678-533-2033 Ext 4 or contact us through our website www.g2rammo.com for a free replacement of your .45 ACP R.I.P. rounds.

ANY OTHER LOTS OF .45 ACP R.I.P. ™ ARE NOT SUBJECT TO THES RECALL AND REQUIRE NO ACTION.

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RETIRED

Make	Model	Type	Recall/ Warning	Date	Page
Action Arms / Uzi	A	Carbine	W	1984	24
American Firearms	Stainless Steel Derringer	Pistol	W	1973	4
Arminius	HW3	Revolver	W	1993	68
Astra	Cub	Pistol	R	1984	27
Astra	M2000	Pistol	R	1984	27
Bauer	Automatic	Pistol	W	1982	19
Benelli	M1 Super 90	Shotgun	W	1990	52
Beretta	NEOS	Pistol	R	2010	142
Bersa	86	Pistol	W	2002	113
Bersa	Firestorm (grips LG-442)	Grips	R	2009	136
Bersa	Thunder (grips LG-442)	Grips	R	2009	136
Blackwater	GSR 1911	Pistol	R	2014	154
Blaser	Camex Blaser	Rifle	R	1990	50
Blaser	R93	Rifle	R	2002	112
Blaser	SR830	Rifle	R	1990	50
Blaser	SR850	Rifle	R	1990	50
Blaser	Ultimate	Rifle	R	1990	50
Browning	81L BLR	Rifle	R	1991	60
Browning	A-500	Shotgun	R	1988	38
Browning	A-Bolt	Rifle	R	1990	53
Browning	A-Bolt II Stainless	Rifle	R	2001	101
Browning	Belgium	Shotgun	R	1987	37
Browning	Buck Mark	Box	R	1997	80
Browning / FN	Hi-Power	Pistol	W	1996	78
Bryco Arms	38	Pistol	W	2001	103
Bryco Arms	58	Pistol	W	2004	117
Bryco Arms	59	Pistol	W	1999	92
Bryco Arms	Jennings Nine	Pistol	W	1999	91
Bryco Arms	Jennings Nine	Pistol	W	2000	98
Bushmaster	ACR	Rifle	R	2010	143
Calico	M-100	Pistol	W	1998	86
Calico	M-100P	Pistol	W	1998	86
Calico	M-950	Rifle	W	1997	82
Caracal	C	Pistol	R	2013	149
Chinese / Navy Arms	SKS	Rifle	R	1993	65
Cobray / Jersey Arms	Avenger Commando	Pistol	R	1990	47
Colt	(all 380 Auto)	Pistol	R	1988	41
Colt	1911 WWI Replica	Pistol	R	2009	135
Colt	1918 WWI Replica	Pistol	R	2009	135
Colt	All American	Pistol	R	1995	74
Colt	CLR3064SRBL	Rifle	R	2000	96

RETIRED

Make	Model	Type	Recall/ Warning	Date	Page
Colt	Combat Elite	Pistol	R	2009	135
Colt	Commander	Pistol	W	1977	7
Colt	Defender	Pistol	R	2009	135
Colt	Junior Colt	Pistol	R	1984	26
Colt	New Agent	Pistol	R	2009	135
Colt	Talo Night Defender	Pistol	R	2009	135
Concorde	M16	Rifle	W	1985	31
Concorde	M16R	Rifle	W	1985	31
Concorde	M20	Rifle	W	1985	31
Connecticut Valley Arms	In-Line Muzzleloaders	Rifle	R	1997	81
C.O.P.	COP	Pistol	W	1982	18
Crimson Trace	Lasergrips LG-442 for Bersa Thunder and Firestorm	Grips	R	2009	136
Crimson Trace	Lasergrips LG-432 for Sig Sauer P230 and P232	Grips	R	2010	140
Crosman	3100	Air Rifle	R	1978	9
CZ	52	Pistol	W	1996	75
CZ	52	Pistol	R	2000	99
Daisy	Legacy 2203	Rifle	R	1993	64
Daisy	Legacy 2213	Rifle	R	1993	64
Davis Industries	DM-22	Derringer	W	1990	51
EAA Corp	Witness	Pistol	R	2008	128
Erma / Excam	RX22	Pistol	W	1985	29
Excam / Erma	RX22	Pistol	W	1985	29
FAL / Williams Arms Co	Williams Alumalite	Rifle	W	2001	102
FIE Corp / Franchi	LAW 12	Shotgun	R	1989	44
FIE Corp / Franchi	SAS 12	Shotgun	R	1989	44
FIE Corp / Franchi	SPAS 12	Shotgun	R	1989	44
FIE Corp / Tanfoglio	Titan	Pistol	W	1998	89
FN	FS2000	Carbine	R	2006	121
FN / Browning	Hi-Power	Pistol	W	1996	78
Franchi / FIE Corp	LAW 12	Shotgun	R	1989	44
Franchi / FIE Corp	SAS 12	Shotgun	R	1989	44
Franchi / FIE Corp	SPAS 12	Shotgun	R	1989	44
Glock	17	Pistol	W	1999	90
Glock	19	Pistol	W	1990	49
Glock	19	Pistol	W	1993	66
Glock	19	Pistol	W	1999	90
Glock	22	Pistol	W	2006	124
Glock	26	Pistol	W	2000	94
Glock	27	Pistol	W	2000	94

RETIRED

Make	Model	Type	Recall/ Warning	Date	Page
Glock	35	Magazine	W	2005	119
Glock	All Models	Pistol	W	2002	107
Glock	G-17 (Gen 4)	Pistol	R	2011	146
Glock	G-17T (Gen 4)	Pistol	R	2011	146
Glock	G-19 (Gen 4)	Pistol	R	2011	146
Glock	G-21 (Gen 4)	Pistol	R	2011	146
Glock	G-22 (Gen 4)	Pistol	R	2011	146
Glock	G-23 (Gen 4)	Pistol	R	2011	146
Glock	G-31 (Gen 4)	Pistol	R	2011	146
Glock	G-32 (Gen 4)	Pistol	R	2011	146
Glock	G-34 (Gen 4)	Pistol	R	2011	146
Glock	G-35 (Gen 4)	Pistol	R	2011	146
Glock	G-37 (Gen 4)	Pistol	R	2011	146
Golden West	781DL	Rifle	R	1982	20
Hiawatha	587	Rifle	R	1982	20
High Standard / JC Higgins	10	Shotgun	R	1998	85
HJS Industry	Frontier Four	Derringer	W	1996	77
Howa Machinery	1500	Rifle	R	2001	106
Howa Machinery	1550	Rifle	R	2001	106
Howa Machinery	1700 LS	Rifle	R	2001	106
IMI	Galil	Rifle	R	1985	32
Interarms / Howa Machinery	1500	Rifle	R	2001	106
Interarms / Howa Machinery	1550	Rifle	R	2001	106
Interarms / Howa Machinery	1700LS	Rifle	R	2001	106
Interarms / Star	M43 Firestar	Pistol	W	1991	59
Interdynamic of America	KG-99	Pistol	W	1984	25
Intratec	CAT-9	Pistol	R	1994	73
Intratec	TEC-22 Scorpion	Pistol	R	1990	56
Intratec	TEC-9	Pistol	W	1984	28
Intratec	TEC-9	Pistol	W	2002	108
Iver Johnson	Safety Hammer 1st Model	Revolver	W	1991	62
Iver Johnson	Safety Hammer 2nd Model	Revolver	W	1991	62
Iver Johnson	Safety Hammerless 1st Model	Revolver	W	1991	62
Iver Johnson	Safety Hammerless 2nd Model	Revolver	W	1991	62

RETIRED

Make	Model	Type	Recall/ Warning	Date	Page
JC Higgins / High Standard	10	Shotgun	R	1998	85
Jennings Firearms	J.A. Nine	Pistol	W	2015	158
Jennings Firearms	J-22	Pistol	W	2001	104
Jersey Arms / Cobray	Avenger Commando	Pistol	R	1990	47
Kahr Arms	PM9	Pistol	R	2005	118
Kassnar Imports	M16	Rifle	W	1985	31
Kassnar Imports	M16R	Rifle	W	1985	31
Kassnar Imports	M20	Rifle	W	1985	31
Knight	Revolution	Rifle	R	2009	133
Knight	Revolution II	Rifle	R	2009	133
Liberty	21	Revolver	W	1972	1
Llama	IX-A	Pistol	W	1992	63
Llama	XI	Pistol	R	1985	30
Llama	XI-B	Pistol	W	1992	63
Lorcin	L25	Pistol	W	1998	84
Lorcin	L380	Pistol	W	2001	105
Magnum Research	Galil	Rifle	R	1985	32
Magnum Research	Magnumlite	Rifle	W	2009	134
Magtech	7022	Rifle	W	1998	87
Mossberg	500	Shotgun	W	2005	120
Mossberg	600	Shotgun	W	2005	120
Mossberg	695	Shotgun	R	2000	97
Mossberg / Howa Machinery	1500	Rifle	R	2001	106
Mossberg / Howa Machinery	1550	Rifle	R	2001	106
Mossberg / Howa Machinery	1700 LS	Rifle	R	2001	106
Navy Arms / Chinese	SKS	Rifle	R	1993	65
Norinco	Dragunov	Rifle	R	2002	111
North American Arms	Revolvers	Revolver	W	2004	115
Phoenix Arms	Raven	Pistol	W	1998	83
Raven Arms	P-25	Pistol	W	1977	8
Remington	40-X	Rifle	R	2002	109
Remington	40-X	Rifle	R	2010	144
Remington	40-XB	Rifle	W	1988	42
Remington	40-XC	Rifle	W	1988	42
Remington	597	Rifle	W	2009	137
Remington	600	Rifle	W	1979	13
Remington	600	Rifle	R	2002	109

RETIRED

Make	Model	Type	Recall/ Warning	Date	Page
Remington	660	Rifle	W	1979	13
Remington	660	Rifle	R	2002	109
Remington	700	Rifle	W	1988	42
Remington	700	Rifle	R	1990	48
Remington	700	Rifle	R	2002	109
Remington	700	Rifle	R	2010	144
Remington	700	Rifle	R	2014	153
Remington	710	Rifle	R	2002	110
Remington	721	Rifle	R	2002	109
Remington	722	Rifle	R	2002	109
Remington	887	Shotgun	R	2014	157
Remington	Mohawk 600	Rifle	W	1979	13
Remington	Seven	Rifle	W	1988	42
Remington	Seven	Rifle	R	2014	153
Remington	Sportsman 78	Rifle	W	1988	42
Remington	Versa Max	Shotgun	R	2010	145
Remington	XP-100	Pistol	R	1978	11
Remington	XP-100 Target	Pistol	R	2002	109
Remington Arms	1911	Shotgun	W	1973	3
Revelation	135	Rifle	R	1982	20
Revelation	160	Rifle	R	1982	20
RG Industries	RG 26	Pistol	W	1973	2
Romanian / Tokarev	TT33	Pistol	R	2010	138
Ruger	"Old Model" Bearcat	Revolver	R	1980	16
Ruger	"Old Model" Blackhawk	Revolver	R	1980	16
Ruger	"Old Model" Single-Six	Revolver	R	1980	16
Ruger	LCP	Pistol	R	2008	130
Ruger	M-77	Rifle	R	1985	35
Ruger	Mark I	Pistol	W	1980	14
Ruger	Mark II	Pistol	R	1985	33
Ruger	Mini-14 Stainless Steel	Rifle	R	1981	17
Ruger	P85	Pistol	W	1990	58
Ruger	Police Service Six	Revolver	W	1983	23
Ruger	SR9	Pistol	R	2008	127
Ruger	Standard	Pistol	W	1980	14
Savage	110E	Rifle	W	1994	70
Savage	110E Series K	Rifle	W	1994	69
Savage	170C	Rifle	W	1983	22
Sears	200	Shotgun	W	2014	155
Sears	300	Shotgun	W	2014	155
Sears	2200	Rifle	R	1982	20

RETIRED

Make	Model	Type	Recall/ Warning	Date	Page
Sears	583.13 thru 583.22	Shotgun	R	1998	85
Sig Sauer	P220	Pistol	W	1993	67
Sig Sauer	P225	Pistol	W	1993	67
Sig Sauer	P226	Pistol	W	1993	67
Sig Sauer	P228	Pistol	W	1993	67
Sig Sauer	P229	Pistol	W	1993	67
Sig Sauer	P230	Pistol	W	1993	67
Sig Sauer	P238	Pistol	R	2010	141
Sig Sauer	P230 (Lasergrips LG-432)	Grips	R	2010	140
Sig Sauer	P232 (Lasergrips LG-432)	Grips	R	2010	140
Sig Sauer	Replacement Grips	Grips	W	1989	43
Smith & Wesson	14	Revolver	W	1978	10
Smith & Wesson	15	Revolver	W	1978	10
Smith & Wesson	16	Revolver	W	1978	10
Smith & Wesson	17	Revolver	W	1978	10
Smith & Wesson	18	Revolver	W	1978	10
Smith & Wesson	19	Revolver	W	1978	10
Smith & Wesson	22-A	Pistol	R	2009	132
Smith & Wesson	39	Pistol	W	1980	15
Smith & Wesson	48	Revolver	W	1978	10
Smith & Wesson	53	Revolver	W	1978	10
Smith & Wesson	66	Revolver	W	1978	10
Smith & Wesson	67	Revolver	W	1978	10
Smith & Wesson	329	Revolver	R	2006	122
Smith & Wesson	439	Pistol	W	1986	36
Smith & Wesson	459	Pistol	W	1986	36
Smith & Wesson	460PC	Revolver	R	2006	123
Smith & Wesson	469	Pistol	W	1986	36
Smith & Wesson	539	Pistol	W	1986	36
Smith & Wesson	559	Pistol	W	1986	36
Smith & Wesson	581	Revolver	W	1987	39
Smith & Wesson	581-1	Revolver	W	1987	39
Smith & Wesson	586	Revolver	W	1987	39
Smith & Wesson	586-1	Revolver	W	1987	39
Smith & Wesson	639	Pistol	W	1986	36
Smith & Wesson	645	Magazine	W	1989	45
Smith & Wesson	659	Pistol	W	1986	36
Smith & Wesson	659	Pistol	W	1994	71
Smith & Wesson	669	Pistol	W	1986	36
Smith & Wesson	681	Revolver	W	1987	39
Smith & Wesson	681-1	Revolver	W	1987	39

RETIRED

Make	Model	Type	Recall/ Warning	Date	Page
Smith & Wesson	686	Revolver	W	1987	39
Smith & Wesson	686-1	Revolver	W	1987	39
Smith & Wesson	686CS-1	Revolver	W	1987	39
Smith & Wesson	745	Magazine	W	1989	45
Smith & Wesson	916T	Shotgun	R	1978	12
Smith & Wesson	1006	Pistol	W	1990	54
Smith & Wesson	1026	Pistol	W	1991	61
Smith & Wesson	1066	Pistol	W	1990	54
Smith & Wesson	1076	Pistol	W	1991	61
Smith & Wesson / Howa Machinery	1500	Rifle	R	2001	106
Smith & Wesson / Howa Machinery	1550	Rifle	R	2001	106
Smith & Wesson / Howa Machinery	1700LS	Rifle	R	2001	106
Smith & Wesson	3904	Pistol	W	1990	54
Smith & Wesson	3906	Pistol	W	1990	54
Smith & Wesson	3913	Pistol	W	1990	54
Smith & Wesson	3913LS	Pistol	W	1990	54
Smith & Wesson	3914	Pistol	W	1990	54
Smith & Wesson	3914LS	Pistol	W	1990	54
Smith & Wesson	4006TSW	Pistol	W	2007	125
Smith & Wesson	4506	Magazine	W	1989	45
Smith & Wesson	4506	Pistol	W	1990	54
Smith & Wesson	4516	Magazine	W	1989	45
Smith & Wesson	4516	Pistol	W	1990	54
Smith & Wesson	4516-1	Pistol	W	1990	54
Smith & Wesson	4526	Pistol	W	1991	61
Smith & Wesson	4536	Pistol	W	1991	61
Smith & Wesson	4576	Pistol	W	1991	61
Smith & Wesson	5903	Pistol	W	1990	54
Smith & Wesson	5904	Pistol	W	1990	54
Smith & Wesson	5906	Pistol	W	1990	54
Smith & Wesson	5924	Pistol	W	1991	61
Smith & Wesson	5926	Pistol	W	1991	61
Smith & Wesson	5946	Pistol	W	1990	54
Smith & Wesson	6904	Pistol	W	1990	54
Smith & Wesson	6906	Pistol	W	1990	54
Smith & Wesson	6924	Pistol	W	1991	61
Smith & Wesson	6926	Pistol	W	1991	61
Smith & Wesson	6946	Pistol	W	1990	54

RETIRED

Make	Model	Type	Recall/ Warning	Date	Page
Smith & Wesson	I-Bolt	Rifle	R	2008	126
Smith & Wesson	I-Bolt	Rifle	R	2008	129
Smith & Wesson	M&P Shield	Pistol	R	2013	148
Smith & Wesson / Walther	PK 380	Pistol	R	2012	147
Smith & Wesson / Walther	PPK	Pistol	R	2009	131
Smith & Wesson / Walther	PPK/S	Pistol	R	2009	131
Smith & Wesson	Sigma	Pistol	W	1998	88
Smith & Wesson	SW 1911	Pistol	R	2003	114
Springfield	187	Rifle	R	1982	20
Springfield	187TS	Rifle	R	1982	20
Springfield Armory	3.3 XD-S	Pistol	R	2013	150
Springfield Armory	Beretta BM-59	Rifle	W	1987	40
Springfield Armory	M1 Garand	Rifle	W	1987	40
Springfield Armory	M1A	Rifle	W	1987	40
Squires Bingham	M16	Rifle	W	1985	31
Squires Bingham	M16R	Rifle	W	1985	31
Squires Bingham	M20	Rifle	W	1985	31
Star / Interarms	M43 Firestar	Pistol	W	1991	59
Stevens	80	Rifle	R	1982	20
Stevens	88	Rifle	R	1982	20
Stevens	887	Rifle	R	1982	20
Stevens / Savage	110E Series K	Rifle	W	1994	69
Steyr -Mannlicher	All Centerfire Bolt Action Double Trigger	Rifle	R	1996	79
Tactilite	T-1 Conversion Kit for AR-15	Rifle	R	2010	139
Tanfoglio / FIE Corp	Titan	Pistol	W	1998	89
Taurus	74	Revolver	R	1977	6
Taurus	80	Revolver	R	1977	6
Taurus	82	Revolver	R	1977	6
Taurus	84	Revolver	R	1977	6
Taurus	86	Revolver	R	1977	6
Taurus	94	Revolver	R	1977	6
Taurus	96	Revolver	R	1977	6
Taurus	24/7 DC	Pistol	R	2013	151
Taurus	PT145 Pro Millennium	Pistol	R	2013	152
Taurus	PT92	Grips	W	1990	57
Taurus	PT99	Grips	W	1990	57
Ted Williams	100	Rifle	W	2014	156

RETIRED

Make	Model	Type	Recall/ Warning	Date	Page
Ted Williams	200	Shotgun	W	2014	155
Ted Williams	300	Shotgun	W	2014	155
Thompson Center	Renegade	Rifle	W	1983	21
Tikka	Sako Stainless Steel	Rifle	R	2004	116
Tokarev	TT33	Pistol	R	2010	138
US Repeating Arms	70	Rifle	W	2000	93
Uzi / Action Arms	A	Carbine	W	1984	24
Vektor	CP1	Pistol	R	2000	95
Vektor	CP1N	Pistol	R	2000	95
Walther	P38	Pistol	W	1985	34
Walther	PK 380	Pistol	R	2012	147
Walther	PP	Pistol	W	1996	76
Walther	PPK	Pistol	R	2009	131
Walther	PPK/S	Pistol	R	2009	131
Weatherby	SA-08	Shotgun	R	2015	159
Weatherby	Vanguard	Rifle	R	2001	100
Westpoint	487T	Rifle	R	1982	20
Williams Arms Co / FAL	Williams Alumalite	Rifle	W	2001	102
Winchester	54	Rifle	W	2014	156
Winchester	55 Sears	Rifle	W	2014	156
Winchester	64	Rifle	W	2014	156
Winchester	64A	Rifle	W	2014	156
Winchester	70	Rifle	W	2000	93
Winchester	94	Rifle	W	2014	156
Winchester	100	Rifle	W	1990	55
Winchester	120	Shotgun	W	2014	155
Winchester	140	Shotgun	W	2014	155
Winchester	490	Rifle	R	1976	5
Winchester	1001	Shotgun	R	1994	72
Winchester	1200	Shotgun	W	2014	155
Winchester	1300	Shotgun	W	2014	155
Winchester	1400	Shotgun	W	2014	155
Winchester	1500	Shotgun	W	2014	155
Winchester	1894	Rifle	W	2014	156
Winchester	2200	Shotgun	W	2014	155
Winchester	2400	Shotgun	W	2014	155
Winchester	9422	Rifle	W	2014	156
Winchester	9422M	Rifle	W	2014	156
Winchester	Canadian Centennial ‘67	Rifle	W	2014	156
Winchester	Centennial ‘66	Rifle	W	2014	156
Winchester	SXP	Shotgun	R	2015	160

RETIRED

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RETIRED

WARNING

Make: Liberty
Model: 21
Caliber: 22 caliber
Source: AFTE Journal, July 1972, Vol 4, Num 3:24

LIBERTY, MODEL 21, 22 CALIBER, REVOLVER

This make and model revolver may have the potential for **"SYMPATHETIC", MULTIPLE CHAMBER, DISCHARGE** and may **DISCHARGE WHEN DROPPED.**

This make and model revolver may have the potential to **DISCHARGE WHEN DROPPED** and may fire sympathetically causing multiple cartridges to discharge if dropped.

RETIRED

WARNING

Make: RG Industries
Model: RG26
Caliber: 25 Auto
Source: AFTE Journal, 1973; Volume 5, Number 1:25-26

RG INDUSTRIES, MODEL RG26, 25 AUTO CALIBER, SEMIAUTOMATIC PISTOL

The RG Industries, 25 Auto caliber, model RG26, pistol may potentially **MISFIRE** and/or **UNINTENTIONALLY DISCHARGE**. With the magazine loaded. The first five rounds failed to fire. No firing pin impressions were apparent in the primers of the first three rounds but the fourth and fifth rounds contained faint firing pin impressions. Examination of the underside of the slide revealed that the metal ridges bordering the firing pin canal were worn down. As soon as the magazine was moved a slight distance, the weapon discharged, even though there was no finger near trigger. The top round in the magazine was examined. It interfered with the forward motion of the firing pin.

RG Industries
2485 N.W. 20th Street, S.E.
Miami, FL 33124

RETIRED

WARNING

Make: Remington Arms
Model: 1911
Caliber:
Source: AFTE Journal, 1973, Vol 5, Num 3:21

REMINGTON ARMS, MODEL 1911, SHOTGUNS

Older model Remington 1911 Shotguns may have the potential for **UNINTENTIONAL DISCHARGE**.

There should be a fiber washer located in the rear hump of the receiver which prohibits the bolt assembly from coming back to the rear of the receiver. This washer has an opening in the center to provide clearance for the rear of the firing pin which is in the center of the bolt assembly which protrudes approximately 1/8 of an inch beyond the rear. When the washer is removed or missing and the bolt is jammed into the rear of the receiver the shotgun could unintentionally discharge.

Remington Arms
14 Hoefler Avenue
Illion, NY 13357

RETIRED

WARNING

Make: American Firearms Mfg.
Model: Stainless Steel O/U Derringer
Caliber: 38 Special
Source: AFTE Journal, Oct 1973, Vol 5, Num 5:36

AMERICAN FIREARMS MFG. CO., MODEL STAINLESS STEEL O/U DERRINGER, 38 SPECIAL CALIBER, PISTOL

During laboratory examination of one of this model derringer with serial number 24158, **CARTRIDGES IN BOTH CHAMBERS DISCHARGED SIMULTANEOUSLY,** when the firing pin selector was indexed to fire the cartridge in the top chamber. This dual discharge was also produced with primed cartridge cases.

RETIRED

RECALL

Make: Winchester
Model: 490
Caliber: 22 caliber
Source: AFTE Journal, Mar 1976, Vol 8, Num 1:28

WINCHESTER, MODEL 490, .22 CALIBER, SEMIAUTOMATIC RIFLES

These rifles are the subject of unintentional discharge problems. Redesigned parts are available by contacting Winchester.

Winchester Firearms (801) 876-3440
U.S. Repeating Arms Company, Inc.
275 Winchester Avenue,
Morgan, UT 84050

RETIRED

RECALL

Make: Taurus
Model: 74, 80, 82, 84, 86, 94, 96
Caliber: 32 S&W, 38 Special, 22 Long Rifle
Source: Shooting Industry, June 1977;
AFTE Journal, April, 1978, Vol 10, Num 2:33-38

TAURUS, MODEL 74, 32 SMITH & WESSON CALIBER, REVOLVER
MODEL 80, 82, 84 & 86, 38 SPECIAL CALIBER, REVOLVER
MODEL 94 & 96, 22 LONG RIFLE CALIBER, REVOLVER

The listed Taurus revolvers, when dropped from a height of three feet on the hammer may discharge.

Revolvers	**Serial Number BELOW the following:**
Model 74, Blue	701,219
Model 74, Nickel	699,118
Model 80, 3" Blue	1,000,225 (not including 977,537 through 977,582)
Model 80, 3" Nickel	977,656, and 987,259 to 987,358
Model 80, 4" Blue	1,035,178
Model 80, 4" Nickel	1,001,684
Model 82, 3" Blue	996,964
Model 82, 3" Nickel	1,014,120
Model 82, 4" Blue	1,025,816
Model 82, 4" Nickel	1,034,375
Model 84, Blue	1,026,497
Model 84, Nickel	1,009,613 (also 1,009,614 through1,009,655 & 1,009,626 through 1,009,655)
Model 86	1,011,470
Model 94, Blue	94,170
Model 94, Nickel	94,206
Model 96	97,336

RETIRED

WARNING

Make: Colt
Model: Commander
Caliber: 45 Auto
Source: AFTE Journal, July 1977, Vol 9, Num 2:179-180

COLT, MODEL COMMANDER, 45 AUTO CALIBER, SEMIAUTOMATIC PISTOL

This make and model pistol may have the potential for **UNINTENTIONAL DISCHARGE.**

When the hammer is lowered to the half cock position while a live cartridge is in the chamber, the sear can come to rest on the edge of the half cock safety notch. In this position, when the pistol is jarred, the hammer can slip off the tip of the sear. This will allow the hammer to fall causing an unintentional discharge of the pistol.

RETIRED

WARNING

Make: Raven Arms
Model: P-25
Caliber: 25 Auto
Source: AFTE Journal, July 1977, Vol 30, Num 2:59-61

RAVEN ARMS, MODEL P 25, 25 AUTO CALIBER, PISTOL

This make and model pistol, equipped with a button type safety may have the potential for **UNINTENTIAL DISCHARGE**.

Produced from 1970 through 1977 pistols with a button type slide safety on the left side of the frame in a cut-out portion of the upper part of the grip have a design feature problem. This safety is moved to the FIRE POSITION when the slide is activated.

Shooters may not be aware that the safety feature is off without manually moving the safety button.

RETIRED

RECALL

Make: Crosman
Model: 3100
Caliber:
Source: AFTE Journal, Oct, 1990, Vol 22, Num 4:452;
American Rifleman, June 1978, pg 50;
Consumer Reports, Nov 1990, pg 767

CROSMAN, MODEL 3100, AIR RIFLE

This make and model Air Rifle may have the potential for **UNINTENTIONAL DISCHARGE.**

The Crosman, model 3100, Air Rifle is being recalled. The notice states: "When cocked, rifle could fire unexpectedly without the trigger being pulled, even with the safety engaged."

Crosman Airguns (800) 836-3100 or (716) 657-6161
Routes 5 & 20
East Bloomfield, NY 14443

RETIRED

WARNING

Make: Smith & Wesson
Model: 14, 15, 16, 17, 18, 19, 48, 53, 66, 67
Caliber: 38 Special, 32 S&W Long, 22 Long Rifle, 357 Magnum, 22 caliber
Source: Crime Control Digest, Oct 9, 1978, pg 3

SMITH & WESSON, MODEL 14, 15, 16, 17, 18, 19, 48, 53, 66, & 67, REVOLVERS

Smith & Wesson recommends that the trigger stop be removed from all medium-size frame revolvers with target sights that are used for law enforcement or defense purposes.

When the K-Target revolver was redesigned in 1946, a trigger stop was provided to limit overtravel of the trigger when firing single action. Its removal does not otherwise affect normal functioning of the revolver.

Under certain conditions of use, however, a possibility exists that the trigger stop may loosen and rotate in such a way as to render the revolver inoperative. This is limited to the models **14, 15, 16, 17, 18, 19, 48, 53, 66, 67 revolvers with target sights.**

A redesigned trigger stop that eliminates even the remote possibility of interfering with the operation of the revolver is presently available.

The serious single-action target shooter may have a new design trigger stop installed by shipping his unloaded revolver, transportation prepaid, to his nearest Smith & Wesson Factory Service Center. Modification will be made and the revolver returned free of charge.

Agencies with a number of revolvers should contact Smith & Wesson to schedule service. Parts and instructions for armorers are also available free of charge. Contact:

F.J. Longtin
Service Manager
Smith & Wesson
2100 Roosevelt Avenue
Springfield, MA 01101

RETIRED

RECALL

Make: Remington
Model: XP-100
Caliber:
Source: Company Notice Oct 25, 1978;
Virginia Ledger-Star Oct 26, 1978;
AFTE Journal, Jan 1979, Vol 11, Num 1:19;
American Firearms Industry, Jan 1989, pg 30;
California Department Of Justice Firearms Safety Note 89-1

REMINGTON, MODEL XP-100, PISTOLS

Remington Arms Model XP-100 bolt action pistols manufactured before February 1975 and between January 1, 1987 and October 4, 1988 have been withdrawn from sale temporarily for correction of trigger assembly mechanisms.

This action was taken because a limited number of pistols produced during that time period may have an improperly installed part in the trigger assembly mechanism. Although it is unlikely, the affected part could inadvertently allow the pistol to fire when the bolt is closed or when the safety is released.

The company is now undertaking a program to identify and recover all pistols made and sold during this period. As a precaution, Remington will, at no charge, correct the affected trigger assembly part in all pistols that could possibly be involved.

All Remington customers (whole-salers and dealers) and individual consumers are being notified, and it is expected that this program will quickly identify owners of the affected pistols.

Please remember that this trigger assembly correction program applies only to a limited number of Model XP-100 pistols and not to any other Remington gun models.

If you have purchased a Remington model XP-100 pistol before February of 1975 or between January 1, 1987 and October 4, 1988, **DO NOT LOAD IT**.

We ask that you call our XP-100 Correction Program at (800) 634-2459 (in Canada 800 634-5401) with the serial number of your pistol. From that number we can tell you immediately if yours is one of the possibly affected pistols, and if it is, how you can arrange for free correction of the trigger assembly.

RETIRED

RECALL

Make: Smith & Wesson
Model: 916-T
Caliber:
Source: American Rifleman, Sept 1979, pg 74;
American Rifleman, Feb 1979, pg 85;
Crime Control Digest, Nov 20, 1978, pg 6

SMITH & WESSON, MODEL 916-T SHOTGUN

Smith & Wesson said last week it was recalling all barrels for a 12-gauge, interchangeable barrel shotgun because they are defective and potentially hazardous.

"Under certain conditions the barrels rupture, but no one has been injured. None of the barrels exploded," said company spokesman William Clede.

"We learned about the problem from reports in the field," he said.

Owners of the 916T Model, which has been manufactured for several years, should not fire the takedown type shotgun with its existing barrels, he said. Barrels should be removed from guns and shipped to the factory in Springfield for replacement.

The company, a major national gun manufacturer, declined to say immediately how many guns were covered by the recall order.

The company will replace barrels for its 916T Model with new barrels designed not to rupture, Clede said. The recall does not cover the Model 916 shotgun, which has a fixed barrel.

The newly designed replacement barrels for the 916T will be identified by the letter "O" on the top of the barrel near the breech end, Clede said.

Contact:

F.J. Longtin
Service Manager
Smith & Wesson
2100 Roosevelt Avenue
Springfield, MA 01101

RETIRED

WARNING

Make: Remington
Model: 600, 660, Mohawk 600
Caliber: all
Source: AFTE Journal, January 1979, Vol 11, Num 1:19;
Shooting Times, Jan 1993, pg 9

REMINGTON, MODEL 600, 660, MOHAWK 600, RIFLES

RETIRED

The Remington Arms Company, Inc. announced today that under certain unusual circumstances on some of its centerfire bolt-action rifles, the safety selector and trigger could be manipulated in such a way that subsequently moving the selector to the fire position could result in accidental discharge. Remington firearms involved are **Model 600, 660, and Mohawk 600 rifles** manufactured prior to February 1975.

The difficulty can be corrected by installation of a new trigger assembly. In view of the potential safety hazard, the Company is recalling all of these guns produced prior to February 1975 for inspection and modification as required. Efforts are being made to contact owners of these guns. Individuals who have Model 600, 660 and Mohawk 600 rifles involved in the recall should write to:

Remington Arms Company, Inc.
Bridgeport, CT 06602

Or call:

800-241-8444 (all states), GA residents call 800-282-1333.

Customers should give the operator the model and serial numbers of their gun when calling. Serial numbers involved in the recall are as follows:

Model	600	#0001 to 131,552
Model	660	#0001 to 131,552 #6,200,000 to 6,899,999
Model	Mohawk 600	#6,200,000 to 6,899,999

WARNING

Make: Ruger
Model: Standard, Mark I
Caliber: 22 Long Rifle
Source: AFTE Journal, April 1980, Vol 12, Num 2:16

RUGER, MODEL STANDARD & MARK I, 22 LONG RIFLE CALIBER, SEMIAUTOMATIC PISTOL

The examination revealed that the pistol could be fired by means other than pulling the trigger. Striking the front of the trigger guard with a plastic mallet or dropping the pistol on its muzzle may cause an **UNINTENTIONAL DISCHARGE**.

Sturm, Ruger & Company, Inc.
Lacey Place
Southport, CT 06490

RETIRED

WARNING

Make: Smith & Wesson
Model: 39
Caliber: 9mm Luger
Source: AFTE Journal, July 1980, Vol 12, Num 3:48-50

SMITH & WESSON, MODEL 39, 9MM LUGER CALIBER SEMIAUTOMATIC PISTOL

An accidental discharge may occur when manually unloading a pistol if there is an obstruction of the ejection port.

When semiautomatic pistols are manually unloaded:

1. Engage the safety in the "safe" position.
2. Remove the magazine.
3. Point the muzzle in a safe direction.
4. Retract the slide without obstructing the ejection port.

If the primer of a cartridge comes in contact with internal parts, such as the tip of the ejector, an unintentional discharge may result. This can occur because the cartridge strikes an object which prevents ejection from the port during the extraction/ejection process.

RETIRED

RECALL

Make: Ruger
Model: Old Model Single-Six, Blackhawk, Bearcat
Caliber: all
Source: Company Notice 1980 & 1982;
American Rifleman, Nov 1980, pg 9;
American Rifleman, Feb 1982, pg 65;
Handgun, Feb 1999, pg 37

RUGER, "Old Model" (pre-1973) SINGLE-SIX, BLACKHAWK, & BEARCAT, REVOLVERS

The patented Ruger Conversion Kit is an entirely new operating system for these old revolvers**. It can help prevent accidental discharges caused by a drop or blow to the hammer if the user has failed to take the basic safety precaution of keeping the hammer down on an empty chamber.** That's very important!

This mechanism can be factory installed without any further alteration. The frame and other major parts will not be affected by this Conversion. The value of the gun will not be impaired, and we will return your original parts for collector's purposes.

To receive a free factory safety conversion, write to us at:

Sturm, Ruger and Company, Inc.
Lacey Place
Department KC
Southport, CT 06490

We will provide you with a shipping container and instructions. You only pay initial shipping to our factory. We will cover all other charges including return shipping costs. Please write to us without delay if you have one of these guns, and tell your friends about the availability of this kit. Remember that the safest way to carry any older single-action revolver, regardless of manufacturer, is with the hammer down on an empty chamber.

If your revolver has the words "New Model" on the frame, this offer is not applicable. Please write to us at "Department C" for full details.

RETIRED

RECALL

Make: Ruger
Model: Stainless Steel Mini-14
Caliber:
Source: Company Notice 1981;
AFTE Journal, July 1981, Vol 13, Num 3:6;
American Rifleman, Nov 1981, pg 66

RUGER, MODEL STAINLESS STEEL MINI-14 RIFLES

Ruger is recalling all stainless steel Mini-14 rifles with serial numbers below 182-51929 with only one proofmark on the receiver.

We have examined a total of three stainless steel Mini-14 rifles that have been returned from users with cracked receivers. In every instance, this situation was traced to a combination of two factors:

1. The receivers exhibited excessive hardness; and
2. The rifles were fired either with an obstruction in the bore or with faulty ammunition creating extremely excessive pressures.

Not all stainless steel Mini-14 rifles have an excessively hard receiver. Every Mini-14 ever shipped, like other Ruger firearms, has been proof tested. We cannot tell which ones are too hard by serial number alone. Therefore, we are recalling all of our stainless steel Mini-14 rifles below serial number 182-51929 bearing only one proofmark on the receiver for inspection, testing, and re-heat treatment, if necessary. Most rifles will require only a Rockwell test and will be returned after testing. Rifles tested will be given an additional proofmark. Only some rifles will require re-heat treatment.

Remove the buttstock from your rifle, and remove all custom accessories. The remainder of the rifle is all that we need. Securely package and insure your barreled action, bolt, trigger housing group, and handguard (a shipping carton is available upon request), and ship it UPS AOD to:

Sturm, Ruger & Co., Inc.
Guild Road - Dept. 14
Newport, New Hampshire 03773
(603) 863-3300 ext. Mini-14

RETIRED

WARNING

Make: C.O.P.
Model: COP
Caliber: 357 Magnum
Source: AFTE Journal, Jan 1982, Vol 14, Num 1:44-47;
AFTE Journal, Oct 1983, Vol 15, Num 4:18

C.O.P., MODEL "COP", 357 MAGNUM CALIBER, 4 BARRELED PISTOL

This make and model pistol may have the potential for **"SYMPATHETIC", MULTIPLE CHAMBER, DISCHARGE** and may **DISCHARGE WHEN DROPPED.**

The C.O.P. pistol design did not incorporate a **Drop Safety** until late 1982 or early 1983. Pistols produced before this time-frame may be subject to discharge if dropped. At approximately the same time an internal design change was incorporated to prevent the retaining plate from allowing a second firing pin from coming in contact with a live cartridge not under the hammer.

Bellmore Johnson Tool Company
445 Putnam Avenue
Hamden, CT

RETIRED

WARNING

Make: Bauer
Model: Automatic
Caliber: 25 Auto
Source: AFTE Journal, Jan 1982; Vol 14, Num 1:62

BAUER, MODEL AUTOMATIC, 25 AUTO CALIBER, SEMIAUTOMATIC PISTOL

The Bauer, 25 Auto caliber, model Automatic, pistol may **DISCHARGE WHEN DROPPED**. A case was submitted to a laboratory where a ruptured cartridge case was identified as having been discharged while the slide was partially opened. Examination revealed when the pistol was dropped on the butt, the momentum allowed the slide to partially open as the firing pin was jarred off the sear as the pistol hit the ground.

Bauer/Fraser Firearms
34750 Klein Avenue
Fraser, MI 48026

RETIRED

RECALL

Make: Springfield; Stevens; Golden West; Hiawatha; Revelation; Sears; Westpoint
Model: 187, 187TS; 80, 88, 887; 781DL; 587; 125, 160; 2200; 487T
Caliber:
Source: AFTE Journal, Jan 1982; Vol 14, Num 1:6;
California Department of Justice Firearms Safety Note 86-6;
Unknown Publication, pg 31

RETIRED

.22 CALIBER SEMIAUTOMATIC RIFLES

Springfield Models 187 and 187TS
Stevens Models 80, 88 and 887
Golden West Model 781DL
Hiawatha Model 587
Revelation Models 135 and 160
Sears Model 2200
Westpoint Model 487T

To the owners of the .22 caliber semiautomatic rifles listed above:
Recently we learned that there is a remote possibility that these rifles might fire while on "SAFE". To our knowledge, no one has been injured as a result of this condition. However, to avoid the possibility of an accident, we are recalling these rifles for inspection and correction of this condition, if necessary.

If you have one of the models listed above, and if its serial number is:
B256621 or higher
C000001 or higher
B000001 or higher

Unload the rifle, package it carefully, and return it via UPS to:

Stevens Rifle Inspection Program (413) 562-7764
Springdale Road
Westfield, MA 01085

Your rifle will be inspected, adjusted and returned at no charge to you.

WARNING

Make: Thompson Center
Model: Renegade
Caliber:
Source: AFTE Journal, April 1983, Vol 15, Num 2:15-16

THOMPSON CENTER, MODEL RENEGADE, RIFLES

These rifles have the potential for an accidental discharge if the hammer is not in the safety notch position.

Rifles manufactured prior to the mid-1970's may accidentally discharge. When the hammer is pulled back, prior to reaching the full cock position, a second click is heard. After the second click and prior to reaching full cock, the hammer can be released and will fall, bypassing the safety notch and firing the rifle.

RETIRED

WARNING

Make: Savage
Model: 170C
Caliber: 30-30 Winchester
Source: AFTE Journal, Oct 1983, Vol 15, Num 4:5

SAVAGE, MODEL 170C, 30-30 WINCHESTER CALIBER, RIFLE

The firing pin nose may extend out of the breech bolt because the spring was not functioning properly. The firing pin spring can be damaged by debris such as a large piece of brass being lodged in it's coils. This situation causes the binding and may result in an unintentional discharge.

Savage Arms (413) 562-7001
100 Springdale Road,
Westfield, MA 01085

RETIRED

WARNING

Make: Ruger
Model: Police Service-Six
Caliber: 38 Special
Source: AFTE Journal, Oct 1983, Vol 15, Num 4:56-59

RUGER, MODEL POLICE SERVICE-SIX, 38 SPECIAL CALIBER, REVOLVER

After opening the cylinder in normal fashion by pressing on the cylinder release button, the hammer, which is locked down as part of its safe design, can be unlocked and cocked by applying thumbnail pressure to the forward edge of the cylinder release button. When the cylinder is then closed, the hammer drops discharging the firearm as the trigger moves forward. The transfer bar is defeated without touching the trigger.

Sturm, Ruger & Company, Inc.
Lacey Place
Southport, CT 06490

RETIRED

WARNING

Make: Uzi by Action Arms
Model: A
Caliber: 9mm Luger
Source: AFTE Journal, July 1984, Vol 16, Num 3:19

ACTION ARMS LTD. - UZI MODEL A, 9MM LUGER CALIBER, SEMIAUTOMATIC CARBINE

The Action Arms Ltd. - UZI model A carbine differs from many other firearms in that under some circumstances, it can be fired with the bolt open or partially open by pulling the trigger.

Therefore, users should be careful to keep fingers clear of the trigger whenever:
- Extracting a live cartridge from the chamber.
- Checking an obstruction lodged in the chamber.
- Looking into the ejection port area.
- Opening or closing the bolt whenever there is a cartridge in the chamber or magazine.

Action Arms LTD. (215) 744-3400
4567 Bermuda
Philadelphia, PA 19124

RETIRED

WARNING

Make: Interdynamic of America
Model: KG-99
Caliber: 9mm Luger
Source: AFTE Journal, July 1984, Vol 16, Num 3:24;
AFTE Journal, April 1985, Vol 17, Num 2:88;
California Department of Justice Firearms Safety Note 86-4;
Department of Treasury, BATF Notice, Dec 28, 1984

INTERDYNAMIC OF AMERICA, MODEL KG-99, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

During a recent investigation of an accidental discharge involving a 9mm KG-99 pistol manufactured by Interdynamic of America, a major safety defect was discovered. This condition can result in serious injury to the firearm examiner or nearby individuals. Please disseminate this information to concerned examiners.

The design of the KG-99 allows for the protrusion of the firing pin into the bolt face during extraction. This presents no problem during actual firing. Should a live cartridge be cycled through the action, there is a 10 to 20 percent chance of a discharge occurring outside of the chamber area.

While pulling the bolt to the rear, the extractor holds the cartridge (live) in position. When the bolt has traveled to the rear approximately 1 inch, the firing pin then protrudes into the bolt face under significant spring tension. The firing pin's impact with the primer is sufficient to indent the primer cup or sometimes ignite the primer compound. Metal fragments and gas resulting from the case rupture can produce serious injury to the examiner or those in close proximity.

During test firing, eye and hearing protection should always be used. While test firing the pistol, all ammunition should be expended before attempting to make the firearm safe. Manual functioning should employ test (dummy) cartridges. Attempt to maintain a position opposite the ejection port when extracting live cartridges.

RETIRED

RECALL

Make: Colt
Model: Junior Colt
Caliber: 25 Auto
Source: Law Enforcement Technology, Dec 1984, pg 29

COLT, MODEL JUNIOR COLT, 25 AUTO CALIBER, SEMIAUTOMATIC PISTOL

It has come to our attention that some of the small pocket automatic pistols marketed by Colt in 25 ACP between 1957 and 1973 (these have serial numbers ending in CC or beginning with OD) are susceptible to *accidental discharge* if improperly carried with a round in the chamber and dropped or otherwise carelessly handled. This is because of the type of firing mechanism in these pistols. Some of these pistols were marked "Junior Colt/Cal. 25," others "Made in Spain for Colt," and still others " Colt Automatic/Cal. 25."

COLT WILL MODIFY THE FIRING MECHANISM OF THESE PISTOLS FREE OF CHARGE. This modification will substantially reduce the possibility of accidental discharge.

If you own one of these pistols, please notify Colt in writing (see below) but do not return the pistol at this time. You will be given further details and instructions as to when and how to ship your firearm to Colt. We will replace the firing pin and the firing pin spring, and return the gun as promptly as possible.

Please do not delay in writing to us. Meanwhile, carry your pistol only with an empty chamber. Please send a postcard or note giving your name, address and the serial number of your pistol to:

Colt Industries, Inc.
Firearms Division
P.O. Box 1868
Department RC 25
Hartford, CT 06101

RETIRED

RECALL

Make: Astra
Model: CUB, M2000
Caliber: 25 Auto
Source: American Rifleman, Dec 1984 pg 26;
American Rifleman, Sept 1985 pg 18;
California Department of Justice Firearms Safety Note 86-8

ASTRA, MODEL CUB/M2000, 6.35MM/25 CALIBER, SEMIAUTOMATIC PISTOL MANUFACTURED 1954 TO 1968

If you own a small, pocket type, 25 caliber semiautomatic pistol marketed by Astra between 1954 and 1968, please take note. **IT MAY BE SUSCEPTIBLE TO ACCIDENTAL DISCHARGE.** Recent studies show that if the gun is dropped or otherwise carelessly handled with a round in the chamber, it may go off -- this can be due in part to the type of firing mechanism that was used in those days.

ASTRA WILL UPDATE YOUR PISTOL FREE OF CHARGE. In order to substantially reduce the possibility of an accidental discharge, Astra will update your pistol with its current firing mechanism components. Just send your pistol -- pre-paid -- via UPS, along with your name and address to:

Walker Arms Company, Inc.
Hwy 80 West, Rt. 2 Box 73
Selma, AL 36701
(205) 872-6231

or

Walker Arms Company, Inc.
127 N. Main Street
Joplin, Missouri 64801
(417) 781-6440

Your pistol will be serviced and returned to you as soon as possible, ALL FREE OF CHARGE. It is very important that this adjustment be made. So be sure that the chamber is empty before you carry it. And be sure you send us your pistol today!

RETIRED

WARNING

Make: Intratec
Model: Tec-9
Caliber: 9mm Luger
Source: Department of Treasury, BATF Notice, Dec 28, 1984

INTRATEC, MODEL TEC-9, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

During a recent investigation of an accidental discharge involving a 9mm TEC-9 pistol manufactured by Intratec, a major safety defect was discovered. This condition can result in serious injury to the firearm examiner or nearby individuals. Please disseminate this information to concerned examiners.

The design of the TEC-9 allows for the protrusion of the firing pin into the bolt face during extraction. This presents no problem during actual firing. Should a live cartridge be cycled through the action, there is a 10 to 20 percent chance of a discharge occurring outside of the chamber area.

While pulling the bolt to the rear, the extractor holds the cartridge (live) in position. When the bolt has traveled to the rear approximately 1 inch, the firing pin then protrudes into the bolt face under significant spring tension. The firing pin's impact with the primer is sufficient to indent the primer cup or sometimes ignite the primer compound. Metal fragments and gas resulting from the case rupture can produce serious injury to the examiner or those in close proximity.

During test firing, eye and hearing protection should always be used. While test firing the pistol, all ammunition should be expended before attempting to make the firearm safe. Manual functioning should employ test (dummy) cartridges. Attempt to maintain a position opposite the ejection port when extracting live cartridges.

RETIRED

WARNING

Make: Erma/Excam
Model: RX22
Caliber: 22 caliber
Source: AFTE Journal, April 1985, Vol 17, Num 2:56-57

ERMA/EXCAM, MODEL RX22, 22 CALIBER, SEMIAUTOMATIC PISTOL

The Erma/Excam, 22 caliber, model RX22, pistol may **SLAM FIRE**. With the magazine loaded and the safety in the on position, the slide is pulled to rear and released to load a cartridge into the chamber of the pistol. As the slide moves forward, feeding a cartridge into that chamber, the pistol may discharge. Examination of cartridge cases have revealed the absence of a firing pin impression. Whenever loading a firearm the muzzle should be pointed in a safe direction.

Excam Inc.
4480 E. 11 Avenue
P.O. Box 3483
Hialeah, FL 33013

RETIRED

RECALL

Make: Llama
Model: XI
Caliber: 9mm Luger
Source: AFTE Journal, April 1985, Vol 17, Num 2:80

LLAMA, MODEL XI, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

The disconnector of pistols having the below serial numbers may malfunction and permit a cartridge to be fired before the slide is fully forward and action closed. This condition may cause a cartridge case to rupture resulting in potential injury to the shooter.

Stoeger and Llama request that the pistol be forwarded to the repair facility immediately for correction. Should you have any questions, please call 800-831-0722 or 201-440-2700. See Serial Number list below:

685351
836482 thru 836552
841264
841296
841304
842938 thru 843142
848171 thru 848180
848219
851287
887687
888014 thru 888083
888093 thru 888112
890302 thru 890401
892669
895194 thru 895295
895317
895373
895513
895823
896137
897094 thru 897447
903393 thru 903592
905412 thru 905623
914271
914474 thru 914480
915811 thru 915911
943151 thru 943641
945893 thru 947292
949774 thru 950253
950824 thru 951383
951606 thru 951609
951660 thru 951680
951700 thru 951746
951765 thru 951784
951920 thru 951960
952004 thru 952113
953606 thru 953804
953806 thru 953854
953856 thru 954099
954916 thru 955015
956016 thru 956039
957965 thru 957999
958001 thru 958040
958043
959844 thru 959853
962397
963022
963344 thru 963699
963725 thru 965584
969180 thru 969219
970287
970323
970368
970375
970255 thru 970414
971855 thru 971976
972335 thru 972579
974814
975042 thru 975048
975080 thru 975335
977842 thru 977859
978144 thru 978306
978450 thru 978521
979317 thru 979339
980003 thru 980079
981430 thru 981669
982840 thru 982999
983870 thru 984069
984400 thru 984599
984930 thru 985429
988020 thru 988060
988061 thru 988117
988840 thru 989379
990140 thru 990239
991174 thru 991264
991346 thru 991366

RETIRED

WARNING

Make: Kassnar, Concorde, Squires Bingham
Model: M-16, M-20, M-16R
Caliber: 22 Long Rifle
Source: Guns & Ammo, July 1985, pg 25

KASSNAR/CONCORDE/SQUIRES BINGHAM, MODEL M-16, M-20, & M-16R, 22 LONG RIFLE CALIBER, SEMIAUTOMATIC RIFLES

RETIRED

It has come to our attention that a limited number of .22 LR semiautomatic rifles, marketed in recent months by Kassnar Imports, Inc. under the "Concorde" trademark name or bearing the label Squires Bingham, may have been assembled incorrectly, which could cause the gun to function improperly.

We are at this time initiating a recall of these guns bearing the following serial numbers:

M-16 and M-16R Models:

A176626 – A176645 A280266 – A285557	A288576 – A291958 A292264 – A293313	A293744 – A295143

M-20 Models

A168233 to A171357	A215026 to A216550	A216581 to A216980

Do not return your gun at this time. Please write to us with your name, address, phone number and gun serial number to:

Kassnar Imports, Inc.
Dept. SB
P.O. Box 6097
Harrisburg, PA 17112

When we receive your information, we will send you a service number and additional instructions for returning your gun. We recommend that you stop using your gun until our service department has inspected it and made any necessary repairs.

RECALL

Make: IMI, Magnum Research
Model: Galil
Caliber: 308 Winchester
Source: AFTE Journal, Oct 1985, Vol 17, Num 4:3;
AFTE Journal, July 1985, Vol 17, Num 3:62

GALIL/IMI, 308 WINCHESTER CALIBER, RIFLE

RETIRED

Magnum Research, Inc. as importer of the Galil Rifle System has been advised by Israel Military Industries (IMI) that an improved bolt assembly has been developed by IMI and is now available for the .308 caliber Galil rifles. This new bolt assembly is intended to prevent primer dimpling and to decrease the probability of **accidental discharge, which can result in damage to the gun and possibly serious personal injury.**

Therefore, for safety purposes, please discontinue using your .308 caliber Galil; remove your current bolt assembly and return the bolt assembly as soon as possible to Magnum Research Inc. at the address below via UPS.

We will promptly provide you with a new bolt assembly at no charge together with a free sight adjustment tool to compensate you for any inconvenience. The new bolt assembly will have an engraved number matching your current serial number followed by a "-1".

We have been advised by Israel Military Industries that failure to comply with this recall may adversely affect your rights if any damage or injury results from continued use of your Galil, including, but not limited to, rights you may have under express and implied warranties on your Galil rifle.

Magnum Research, Inc. (612) 574-1868
7110 University Avenue NE
Minneapolis, MN 55432

RECALL

Make: Ruger
Model: Mark II
Caliber: 22 Long Rifle
Source: Shooting Times, Sept 1985, pg 96;
Guns, Oct 1985, pg 18;
American Rifleman, Aug 1985, pg 2;
California Department of Justice Firearms Safety Note 86-7

RUGER, MODEL MARK II, 22 LONG RIFLE CALIBER, SEMIAUTOMATIC PISTOLS

RETIRED

This important notice pertains only to the following semiautomatic pistols:

Ruger Mark II Standard - Automatic Pistol (4 3/4 inch barrel, blued only) Serial Number Range 210-92816 to 210-94772.

Ruger Mark II Pistols - Standard, Target and Bull Barrel models, (blued only) Serial Number Range 211-28500 to 211-40000.

Two pistols in the above serial number range have been discovered to fire if the trigger is pulled when the safety is on "S" and then the safety is moved to the "F" position.

To determine if this can happen, we urge all owners of these pistols to perform the following test:

1. After ascertaining that the pistol is completely unloaded, retract the bolt, let it return fully forward, and place the safety in the "S" position.
2. With the muzzle pointed in a safe direction, pull the trigger.
3. Then place the safety in the "F" position.
4. Pull the trigger. You should hear a click as the hammer falls in a properly functioning pistol.

If you do not hear a click, the pistol can fire simply by moving the safety to the "F" position. Do Not Use the Pistol. Ship it at once via U.P.S. to:
Sturm Ruger & Company, Inc.
Department MK II
Lacey Place
Southport, Connecticut 06490

We will repair the pistol and return it to you at no charge.

WARNING

Make: Walther
Model: P-38
Caliber: 9mm Luger
Source: California Department of Justice Safety Note 86-1;
Bureau of Alcohol, Tobacco & Firearms, National Broadcast Sept 85;
Florida Department of Law Enforcement Bulletin, Feb 1986;
New York State Police Academy Training Bulletin, Num 86-1, May 86

RETIRED

WALTHER, MODEL P-38, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOLS CONVERTED TO FULLY-AUTOMATIC FIRING

Officers should exercise extreme caution with any Walther P38 type pistol. Law enforcement agencies at the Federal, State and local levels plus branches of the U.S. and Canadian military service have encountered Walther P38s having a potentially hazardous conversion.

The conversion consists of a modified firing pin, which is activated by placing the safety in the safe position. When the safety is placed in this position, the weapon will immediately fire without the trigger being pulled if a round is in the chamber. If the safety is engaged with the slide open the weapon will fire in the full automatic mode as soon as the slide is closed. The full automatic is activated by the safety alone. The conversion can be accomplished on post-war P38s made by Walther or Manurhin, manufactured since April 1940. Full automatic fire may be accomplished by changing the thumb safety lever.

There is no field test, which can be performed to determine if a P38 has this conversion installed and there are no visible modifications. Consequently, any Walther P38 type pistols encountered should be handled in the following manner:

1. Do not touch the trigger or safety
2. Remove the magazine
3. Place the safety in the fire position
4. Open the slide and ensure that the chamber is empty

RECALL

Make: Ruger
Model: M-77
Caliber:
Source: American Rifleman, Nov 1985, pg 10;
Shooting Times, Dec 1985, pg 19;
Guns, Jan 1986, pg 21;
California Department of Justice Firearms Safety Note 86-9

RUGER, MODEL M-77, BOLT ACTION RIFLES

The trigger overtravel adjustment setscrew in a few of these rifles may not be securely tightened and may move too readily. This change in original adjustment can, in extreme cases, either cause the rifle to fire unexpectedly (with the safety "off") or cause the rifle to not fire at all. This may occur suddenly, without warning.

Current production rifles have this setscrew permanently secured. These rifles have a "T" inscribed on the underside of the bolt handle. This condition cannot occur in any of our other firearms, such as the 77/22, 10/22, No. 1, No. 3, or the .44 Carbine, as their trigger mechanisms differ.

For your safety, we ask that all owners of M-77 rifles (except those with a "T" underneath the bolt handle) contact us.

You will receive a new locking screw that will replace your present overtravel adjustment set screw and detailed instructions for quick, easy installation. There is no charge for this service and replacing the screw will have no adverse effect on trigger pull.

Do not use your M-77 rifle until you have received and installed the replacement screw.

RETIRED

WARNING

Make: Smith & Wesson
Model: 439, 459, 469, 539, 559, 639, 659, 669
Caliber: 9mm Luger
Source: AFTE Journal, July 1986, Vol 18, Num 3:68;
AFTE Journal, Jan 1987, Vol 19, Num 1:36;
American Rifleman, July 1986, pg 12;
Shooting Times, Aug 1986, pg 23;
California Department of Justice Firearms Safety Note 86-2

SMITH & WESSON, MODEL 439, 459, 469, 539, 559, 639, 659 & 669, 9MM CALIBER, SEMIAUTOMATIC PISTOLS

This warning is issued for **Smith & Wesson 9mm Semiautomatic Pistols** which were shipped from the factory between May 1, 1983 and February 26, 1986. Included are Models **439, 459, 469, 539, 559, 639, 659 and 669** but only with serial numbers in the following ranges:

A745000 - A865000	TAA0001 - TAL9999

The pistols within the serial number ranges listed above must be inspected for function from the half-cock notch. The safety mechanism will function in a perfectly satisfactory manner in many of these pistols, as well as in all pistols with serial numbers outside these ranges and in all pistols shipped from the factory after February 26, 1986.

Every one of these models bearing a serial number within the ranges listed above should be carefully inspected by the owner for this special condition. To determine if your pistol has this condition, do the following:

1. Point the muzzle in a safe direction
2. Completely unload the pistol. Check both the magazine and chamber to assure they are empty of live rounds.
3. With your finger off the trigger, operate the manual safety with the unloaded pistol fully cocked and fully uncocked so that you can recognize how the manual safety looks and feels when it is fully in the safe position. The hammer will fall from the fully cocked position into the "at rest" position when the manual safety is moved down toward a safe position. When you are thoroughly familiar with placing the manual safety fully in the safe position, place it up in the fire position.
4. With the manual safety in the fire position, place the hammer in the half-cock notch:

   For the Model 439, 459, 539, 559, 639 and 659, put the hammer of your pistol in the half-cock notch by pulling the hammer to the rear slowly until you hear the first click and then releasing the hammer so that it catches between the full cock and the "at rest" position.

   For the Model 469 and 669, put the hammer of your pistol in the half-cock notch by inserting an empty magazine and slowly pulling the trigger until you hear the first click and then releasing the trigger so that the hammer catches between the full cock and the "at rest" position.

5. With the hammer in the half-cock notch, push the manual safety down toward the safe position. If you can push the manual safety fully into the safe position and the hammer falls into the "at rest" position, your pistol does not require modification. If you **cannot** push the manual safety fully into the safe position or the hammer **does not** fall into the "at rest" position, **your pistol is being recalled** for modification free of charge.

Recalled pistols should be sent promptly to a Smith & Wesson Warranty Service Center for modification. Call 800-633-0164 for the name and location of the one nearest you or for answers to nay questions you may have. Law enforcement departments who have pistols, which exhibit this condition, should contact the Smith & Wesson Service Department to arrange for modifying the pistols.

RETIRED

RECALL

Make: Browning
Model: Belguim
Caliber:
Source: Guns & Ammo, March 1987, pg 22;
California Department of Justice Firearms Safety Note 87-1

BROWNING, MODEL BELGUIM OVER and UNDER, SHOTGUNS

A certain quantity of Belgian-made, single trigger, over and under shotguns and over and under Express Rifles contains a selector-safety that can be moved to the left or right while the selector-safety is in the forward, off-safe, fire position. It is sometimes possible for these specific guns to accidentally discharge upon opening the gun following a certain sequence of rather unnatural manipulations of the selector-safety.

This warning does NOT concern Browning Citori over and under shotguns.

If you possess a single trigger, Belgian-made, over and under of any gauge or caliber, regardless of when you may believe it was produced, you are urged to perform the below inspection sequence:

1. Point the muzzle in a safe direction.
2. Open the gun to make certain it is unloaded.
3. Move the selector-safety to one side and then fully forward to the OFF SAFE (fire) position.
4. With the selector-safety fully forward, attempt to move the selector-safety either straight to the left or to the right (barrel selection).

WARNING: If your selector-safety can be moved to the left or right while in the OFF SAFE (fire) position, you are urged to have your gun promptly modified by an authorized Browning Service Center or by Browning's Arnold Service Department.

For return instructions and a special expedite label please write to: Browning Service Center, Department B-25, 3005 Arnold Tenbrook Road, Arnold, Missouri 63010-9406, giving your name, address, phone number, firearm description and serial number. (The serial number is located under the top (opening) lever.) Should you prefer to phone for return instructions, call toll free 1-800-237-3995. Missouri residents call collect at (314) 287-6800.

We apologize for any inconvenience and will modify and return your gun in the shortest time possible. There will be no charge for this service, and we will reimburse you for your postage.

RETIRED

RECALL

Make: Browning
Model: A-500
Caliber:
Source: American Rifleman, August 1988, pg 80;
California Department of Justice Firearms Safety Note 88-4

BROWNING, MODEL A-500, SEMIAUTOMATIC SHOTGUN

RETIRED

Browning is recalling the trigger assemblies from all Browning A-500 shotguns. This is a new semiautomatic shotgun, which Browning introduced in 1987.

During extensive shooting tests under laboratory conditions, Browning encountered a parts breakage in the trigger assembly on the A-500. This failure could cause an unsafe condition if it were to occur.

As a precaution, Browning is replacing all trigger assemblies on all A-500 shotguns sold since its introduction. All A-500 shotguns, which contain the new replacement trigger assembly, can be identified by the letter H stamped on the bottom front of the trigger guard plate.

Therefore, if you own a Browning A-500 shotgun which does not have the letter H stamped on the bottom forward part of the trigger guard plate, your trigger assembly must be recalled.

DO NOT LOAD YOUR A-500 UNTIL A NEW TRIGGER ASSEMBLY IS INSTALLED.

Please call our A-500 Recall Department at 1 (800) 322-4626. Missouri residents call collect at (314) 287-6800.

You will receive a special A-500 recall kit to expedite this modification of your A-500 shotgun.

We apologize for this inconvenience. There will be no charge for the replacement trigger assembly and you will be reimbursed for all your shipping costs.

WARNING

Make: Smith & Wesson
Model: 581, 586, 681, 686, 581-1, 586-1, 681-1, 686-1, 686CS-1
Caliber: 357 Magnum
Source: Shotgun News Nov 1, 1989;
Guns & Ammo, Dec 1987, pg 6

SMITH & WESSON, MODEL 581, 586, 681, 686, or 581-1, 586-1, 681-1, 686-1 & 686CS-1, REVOLVERS

Reports have been received from the field where the combination of a **SMITH & WESSON L-FRAME 357 MAGNUM REVOLVER** and some .357 Magnum ammunition has resulted in unacceptable cylinder binding. L-frame revolvers bearing model numbers:

581, 586, 681, 686, or 581-1, 586-1, 681-1, 686-1, 686CS-1

Cylinder binding can cause a failure to fire. Mishandling a revolver while freeing the cylinder can result in accidental discharge.

Cylinder binding can result from a number of causes, including characteristics of an individual revolver or the use of ammunition, which does not conform to industry pressure specifications or is particularly fast burning. Recent developments in ammunition manufacture emphasize the production of .357 Magnum ammunition with increased velocity and greater primer sensitivity.

Although there have been very few reported incidents of cylinder binding, in view of our concern for our customer's safety and the reliability of Smith & Wesson products in all circumstances, we issue the following warning:

In a situation where a failure to fire can be critical - such as law enforcement or personal protection - do not use .357 Magnum ammunition with an L-frame revolver bearing model numbers 581, 586, 681, 686 or 581-1, 586-1, 681-1, 686-1, 686CS-1 without an "M" over the model number until you have had the revolver modified.

Those who need to use their L-frame revolver under these conditions prior to modification can safely fire .38 Special caliber ammunition.

Smith & Wesson has developed a modification to improve existing L-frame revolvers. This improvement enables them to fire all .357 Magnum ammunition, without cylinder binding. Shipments of L-frame revolvers from our factory after August 21, 1987 already include this improvement.

You can check if your revolver includes this improvement by looking at the left side of the frame when the cylinder is fully open. If your revolver has been stamped either with a "2" or higher number after the basic three-digit model number or with an "M" above the model number, your revolver includes this improvement and does not need modification. If your revolver bears the model number 581, 586, 681, 686, or 581-1, 586-1, 681-1, 686-1, 686CS-1 without an "M" over the model number, it does not include this improvement and your should have your revolver modified.

Smith & Wesson will modify your L-frame revolver free of charge to eliminate the possibility of cylinder binding with .357 Magnum ammunition. Law enforcement agencies wishing to arrange for modification of L-frame revolvers should call 800-458-8469 between 9 A.M. and 6 P.M. Eastern time (MA residents call 413-734-8244). Other users should send their revolvers to a Smith & Wesson Warranty Service Center, specifying "L-frame improvement program" and enclosing their name and return address.

One of the modifications to improve the L-frame revolver is the installation of a new hammer nose. This obsoletes all old L-frame hammer noses (part numbers 4702 and 7513) and all old L-frame hammer assemblies (part numbers 3366, 3378, 3380, 3382, 3391, 4722, 4723, 4726 and 4728) in field parts inventories. Superseded parts should never be fitted into a modified revolver as this may result in malfunction. It is essential for safety that you return these obsolete L-frame hammer noses and obsolete L-frame hammer assemblies for a free exchange to:

Smith & Wesson Service Department 2100 Roosevelt Avenue Springfield, MA 01101

Do not return L-frame hammer-nose bushings from your spare parts inventory inasmuch as they are useable in J, N and K-frame revolvers, which are not included in this Product Warning.

We regret any inconvenience this may cause. Smith & Wesson's first concern is the safety of its products and the protection of its customers.

RETIRED

WARNING

Make: Springfield Armory
Model: M1A, M1 Garand, Beretta BM-59
Caliber: 7.62x51mm
Source: Guns & Ammo, Dec 1987; pg 91;
Guns & Ammo, Mar 1988, pg 14;
Shooting Times, Jan 1988, pg 67;
California Department of Justice Firearms Safety Note 88-1

GARAND, MODEL M1As / M1A4s, M1 and BERETTA MODEL BM-59s, RIFLES

If you own an M14 type rifle, an M1 Garand type rifle, or Beretta BM-59 rifle, a certain quantity could contain safeties that can be disengaged by applying an abnormal amount of force to the trigger, thus overriding the safety. The guns will not fire when the safety is overridden in this manner, but they will fire when the trigger is released and pulled again under normal pressure.

If you possess a Springfield Armory, Inc. M1A, M1 Garand, or Beretta BM-59, you are urged to perform the following inspection procedure. (Note: before beginning, be sure to unload rifle and point muzzle in a safe direction.)

1. Begin test procedure with safety in normal "off" position.
2. Engage the safety.
3. Using one or two hands, pull the trigger very hard, and then release it. Repeat this procedure several times.
4. If at any time during this test procedure you detect any forward movement in the safety, remove only the trigger group and return it to Springfield Armory, Inc.

WARNING: If during the above test procedure the safety moves forward on your rifle, you are urged to remove only the trigger group and return it immediately to Springfield Armory using either of the following procedures:

A. Write to Springfield Armory requesting special mailing label, shipping container, and return instructions. Write to:

Springfield Armory, Inc. RE-1 420 West Main Street Geneseo, IL 61254
B. Or, call Springfield Armory on this toll-free number and request return instructions: 1-800-223-5708. Illinois residents call collect: (309) 944-5631.

M1A Bolts

Some M1A bolts manufactured by Springfield Armory, Inc. might not function reliably. If you have a Springfield Armory M1A bolt with any of the following markings, contact Springfield Armory, Inc. as outlined in steps A or B above for replacement.

The bolts in question are marked as follows:

a. No numerical or alphabetical characteristics on either top or back of bolt (completely unmarked).
b. Any bolt with any numerical or alphabetical markings at all on the back of the bolt.
c. Any bolt with the top marked "7790185" and with "SA RRR" centered below that number.
d. Any bolt with the top marked "790185" and with "SA" centered below that number.

Springfield Armory, Inc. regrets any inconvenience caused by the return of any firearm affected by this notice. Repairs will be completed in the shortest possible time, and all service, parts, and shipping costs will be the responsibility of Springfield Armory, Inc.

RETIRED

RECALL

Make: Colt
Model:
Caliber: 380 Auto
Source: Law Enforcement Technology, Jan/Feb 1988, pg 7;
Law Enforcement Technology, Mar/Apr 1988, pg 72;
Guns & Ammo, April 1988, pg 24;
Outdoor Life, June 1988, pg 96;
American Rifleman, Mar 1988, pg 12;
California Department of Justice Firearms Safety Note 88-2

COLT, 380 AUTO CALIBER, SEMIAUTOMATIC PISTOL

RETIRED

We are incorporating a safety improvement on all Colt 380 semiautomatic pistols that we are now manufacturing. We are offering this same opportunity to all owners of Colt 380 semi-automatic pistols free of charge.

It has come to our attention that the sear spring may be bent in some Colt 380 autos. Where this occurs there is a possibility that the sear spring will become unseated from the base of the receiver which could result in accidental discharge.

To hold the sear spring in place, Colt will send you a 380 Auto improvement kit, which includes a spring clip and instructions for attaching the clip to the sear spring. Once the spring clip is installed the sear spring cannot come loose. This is a simple but effective safety modification.

This important message applies to all Colt 380 autos in the current series first introduced in 1982. If a spring clip has already been installed, it will appear in the magazine well as a yellow piece of metal attached to the sear spring.

To obtain the spring clip we ask that all owners of Colt 380 semiautomatic pistols contact us now by calling our special toll-free number below, available Monday through Friday between 8 AM and 5 PM, Eastern Time. Colt will send you a spring clip and complete instructions for quick, easy installation. There is no charge for this service. In the meantime we urge that you do not use your Colt 380 auto until you make this change.

Colt endeavors to manufacture firearms of the highest quality. We also continue to serve our motto, A tradition of safety.

CALL TOLL FREE 1-800-533-0380

WARNING

Make: Remington
Model: 700, Seven, 40-XB, 40-XC, Sportsman 78
Caliber:
Source: Guns & Ammo, June 1988, pg 89;
Rifle, July-Aug 1988, Vol 22, Num 4;
California Department of Justice Firearms Safety Note 88-3

REMINGTON, MODEL 700, SEVEN, 40-XB, 40-XC, & SPORTSMAN 78

Remington Arms centerfire rifle Models 700, Seven, 40-XB, 40-XC and Sportsman 78 manufactured between July 29 and December 11, 1987, have been withdrawn from sale temporarily for replacement if trigger assembly mechanisms.

This action was taken because a limited number of rifles produced during that period may have an improperly manufactured part in the trigger assembly mechanism. Although it is unlikely, the defective part could break and cause the rifle to fire accidentally.

Remington Arms has launched a program to identify and recover all rifles made and sold during this period, and as a precaution, will replace the trigger assembly on every affected rifle without charge to the owner.

All Remington trade customers and individual rifle owners are being notified, and it is expected that this program will quickly identify owners of the affected rifles.

This notice applies only to those bolt action models listed. No other Remington firearms are involved.

If you have purchased one of these rifles since July 29, 1987, do not load it.

We ask that you call our Trigger Assembly Replacement Program at 1-800-634-2459 with the model and serial number of your rifle. From that number, we can tell you immediately if yours is one of the affected rifles, and if it is, how you can arrange for a free replacement of the trigger assembly.

RETIRED

WARNING

Make: Sig Sauer – Replacement Grips
Model: all
Caliber: all
Source: Law Enforcement Technology, July/Aug 1989;
AFTE Journal, Oct 1989, Vol 21, Num 4:656;
California Department of Justice Firearms Safety Note 89-2

RETIRED

SIG SAUER PISTOL REPLACEMENT GRIPS

Pistols May Malfunction if Grips are Replaced

Police officers who have replaced the original grips on their Sig Sauer pistols are being advised that some replacement grips may cause the pistol to malfunction. The National Institute of Justice's Technology Assessment Program has learned that at least one police officer who replaced the grips on his Sig Sauer pistol has experienced malfunctions. While the pistol performed fine at the time the grips were replaced, it soon began to malfunction with further firing. The National Institute of Justice (NIJ) is concerned that other police officers who have replaced the grips on their Sig Sauer pistols may be unaware of the problem and might experience the first malfunction during a life-threatening situation.

Sigarms, Inc., which distributes the pistols, responded to NIJ's concern in the following way:

"...concerning the use of 'aftermarket' grips on Sig Sauer pistols, we wish to advise the following:

The grip plates on our pistols serve a dual purpose:

1. conventional covers of the metal frame to properly 'fit' the hand, and
2. to hold in place & protect the various springs, levers, & pins that are assembled to the frame.

The factory grip plates are of rigid 'plastic' material designed to accomplish these two purposes.

It is most important that any 'aftermarket' grip be rigid, particularly in the areas immediately adjacent to these components. It is most important that the grip be rigid at the hammer pin area to prevent this pin from 'walking' as otherwise this pin could move and cause the firearm to become inoperable.

The 'aftermarket' grips of the 'soft' type should be constructed with some form of rigid insert in the area of the hammer pin to prevent any flexing of the material that would allow the pin to move, Further, care should be taken in the design of such grip that 'squeezing' of the grips would not interfere with the operation or movement of springs or levers in the frame.

Anyone who has replaced the original grips on a Sig Sauer pistol is encouraged to verify the suitability of the grips by contacting Sigarms, Inc., 470 Spring Park Place, Unit 900, Herndon, VA 22070, 703-481-6660.

While NIJ is not aware of similar problems arising from the use of replacement grips on handguns manufactured by other companies, police officers are encouraged to check with the manufacturer of a handgun before installing replacement grips.

RECALL

Make: Franchi by F.I.E Corp
Model: SPAS 12, SAS 12, LAW 12
Caliber: 5.56mm
Source: AFTE Journal, July 1990, Vol 22, Num 1:87;
AFTE Journal, July 1991, Vol 23, Num 3:801;
Guns & Ammo, Oct 1989, pg 22;
Guns & Ammo, Dec 1989, pg 94;
American Rifleman, Oct 1989;
American Rifleman, July 1991, pg 4;
California Department of Justice Firearms Safety Note 89-3

FRANCHI, MODEL SPAS 12, SAS 12 and LAW 12, SHOTGUN

Your Shotgun May Fire When You Take It "Off Safe"!!!

A limited number of SPAS-12, SAS-12 and LAW-12 shotguns, formerly imported by the bankrupt F.I.E. Corporation, may accidentally fire when the "CARRY SAFETY" (the large rotating safety lever located on the right front side of the trigger guard) is rotated from the "ON SAFE" to the "OFF SAFE" position.

Franchi urges all owners to immediately cease all use of these shotguns. Carefully unload the chamber and magazine, with the muzzle pointed in a safe direction, and return the shotgun to American Arms, Inc.

American Arms, Inc., the Franchi factory authorized service center, will replace the potentially defective rotating lever safety system with a new push button cross bolt safety system. The service work, replacement safety system, and shipping are FREE OF CHARGE.

Send your shotgun carefully packed and insured via the United Parcel Service Company to:

American Arms, Inc.
Franchi Recall
715 E. Armour Rd.
N. Kansas City, MO 64116

If you require additional information or have any questions on this recall, call (816)
474-3161.

RETIRED

WARNING

Make: Smith & Wesson
Model: 4506, 4516, 645, 745
Caliber: 45 Auto
Source: AFTE Journal, April 1990, Vol 22, Num 2:227;
American Rifleman, Jan 1990, pg 4;
American Rifleman, Feb 1990, pg 4;
Guns & Ammo, Jan 1990, pg 93;
Guns & Ammo, Feb 1990, pg 7;
Shooting Industry, Jan 1990, pg 18;
American Firearms Industry, Dec 1989, pg 13;
California Department of Justice Firearms Safety Note 89-4

RETIRED

SMITH & WESSON, MODEL 4506, 4516, 645 & 745, 45 AUTO CALIBER, SEMIAUTOMATIC PISTOL MAGAZINES

It has come to our attention that a small number of users have experienced cartridge-feeding problems with Smith & Wesson magazines for .45 ACP pistols. This can result in a failure to feed or jamming, and thereby **a failure to fire**. The cause of this problem is the magazine.

This recall applies to two types of MAGAZINES ONLY: Magazines manufactured by Smith & Wesson for Pistol Model number 4516.

Smith & Wesson requests that you inspect your caliber .45 ACP magazine to determine if it is subject to this recall. To determine if your magazine is affected, follow these simple inspection procedures:

A. Inspection Procedure for Magazines for Model 4506, 645, or 745 Pistols.

1. Remove the magazine from the firearm following the procedure set forth in the instruction manual. Before proceeding further, you **must ensure** that the pistol and magazine are unloaded.
2. Examine the butt plate of the magazine. If the butt plate is not molded plastic with a crescent-shaped cutout molded into the bottom near the front edge, then the magazine **is not** affected by this recall. If the butt plate is molded plastic and contains a crescent-shaped cutout molded into the bottom near the front edge, proceed directly to the next step.
3. Carefully inspect each broad flat side of the magazine near the plastic butt plate. If a punch mark is present above the butt plate on either side of the magazine, your magazine **is not** affected by this recall.
4. If, however, a punch mark is not present on the right side at the bottom, your magazine must be replaced.

B. Inspection Procedure for Magazines for Model 4516 and 4516-I Pistols.
Any magazines shipped by Smith & Wesson with model 4516-I pistols are already inscribed for use in that particular model and are NOT subject to this recall.

1. Remove the magazine from the firearm following the procedures set forth in the instruction manual. Before proceeding further, you must ensure that the pistol and magazine are unloaded.
2. Inspect each broad flat side of the magazine. If neither side is inscribed "For S&W M4516 only" or "For S&W M4516-I only"; **your magazine must be replaced**.

To ensure reliable functioning of your Model 4516 or 4516-I pistol, the model number marked on a 7-round .45 ACP magazine must be identical to all digits of the model number stamped on your pistol. Do not use a Smith & Wesson magazine marked "M4516 only" in a pistol stamped "MOD 4516".

WARNING: DO NOT USE ONE OF THESE RECALLED MAGAZINES IN YOUR PISTOL FOR A SITUATION WHERE A FAILURE TO FIRE CAN BE CRITICAL, SUCH AS FOR LAW ENFORCEMENT OR PERSONAL PROTECTION. RETURN AFFECTED MAGAZINES TO SMITH & WESSON FOR FREE REPLACEMENT.

To receive a free replacement magazine under this recall, send the magazine to be replaced directly to:
Smith & Wesson Attention: Magazine Exchange 2100 Roosevelt Avenue
Springfield, MA 01102

Or call toll-free 800-458-8469 between 8 AM and 5 PM EST. You will need to provide the customer service representative with the model and serial numbers of your pistol. We ask that all affected magazines be returned to Smith & Wesson in trade for the new magazines.

This toll-free number can also be called if you have any questions concerning this recall.

We regret any inconvenience this may cause; however, this action was dictated by our concern for the customer's safety and the reliability of Smith & Wesson products in all circumstances.

RETIRED

RECALL

Make: Cobray / Jersey Arms Works
Model: Avenger Commando
Caliber: 45 Auto
Source: AFTE Journal, Jan 1990, Vol 22, Num 1:43-46

COBRAY/JERSEY ARMS WORKS, MODEL AVENGER COMMANDO, 45 AUTO CALIBER, SEMIAUTOMATIC PISTOL

RETIRED

This make and model pistol may have the potential for **UNINTENTIONAL DISCHARGE**.

When the safety is on and the bolt is opened vigorously, the bolt can remain open with the sear NOT engaged. Internally, the bottom of the sear and the slide have been forced into hard contact causing enough friction to hold the bolt in the open position. If this occurs and the pistol is struck by another object, unintentional discharge could occur.

RECALL

Make: Remington
Model: 700
Caliber: 17 Remington
Source: AFTE Journal, April 1990, Vol 22, Num 2:227;
Shooting Times, Mar 1990, pg 47;
American Firearms Industry, Feb 1990, pg 19;
American Rifleman, Jan 1990, pg 2;
American Rifleman, Feb 1990, pg 10;
California Department of Justice Firearms Safety Note 90-1

REMINGTON, MODEL 700, 17 REMINGTON CALIBER, RIFLE

The Remington Arms Company is recalling all Model 700 bolt-action centerfire rifles in .17 Remington caliber. The company is taking this action because some of these .17 caliber barrels could develop cracks and splits. A barrel split could result in serious personal injury.

Shooters who purchased Model 700s after January 1, 1981 are requested by Remington not to use them. They are requested to contact Remington by calling its toll-free numbers in the U.S.: (800) 634-2459 or in Canada: (800) 634-5401. The customer should present the serial number of his rifle to Remington personnel to determine if he owns an affected rifle. All affected rifles returned to Remington under the recall will have barrels replaced at no charge to the owners.

No other Model 700 rifles in other calibers or any other Remington autoloading or pump-action centerfire rifles, rimfire rifles, or shotguns are included in this recall.

RETIRED

WARNING

Make: Glock
Model: 19
Caliber: 9mm Luger
Source: AFTE Journal, April 1990, Vol 22, Num 2:227

GLOCK, MODEL 19, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL WITH SERIAL NUMBER PREFIXS AN,BP,BR, BV, BW, BX, DL, DM, DN, DP, DR, DS, DT, DV, DW, DX, DY, & DZ.

This pistol may have the potential of the action to malfunction as followings:

1. The slide locking back on a full magazine
2. Failure to lock into battery because the barrel lug drags on the slide lock
3. The magazine follower tips or sticks in the magazine tube

Glock, Inc. (770) 432-1202
6000 Highlands Parkway
Smyrna, GA 30082

RETIRED

RECALL

Make: Blaser
Model: Ulitmate, SR830, SR850, Camex Blaser
Caliber: all
Source: AFTE Journal, Jan 1991, Vol 23, Num 1:507;
Guns & Ammo, July 1990, pg 32-32

BLASER,

MODEL ULTIMATE, BOLT ACTION RIFLE - ALL CALIBERS

MODEL SR 830, BOLT ACTION RIFLES - ALL CALIBERS

MODEL SR 850, BOLT ACTION RIFLES - ALL CALIBERS

MODEL CAMEX BLASER, BOLT ACTION RIFLES - ALL CALIBERS

The Blaser, bolt action rifle - models ULTIMATE, SR 830, SR 850 and CAMEX BLASER are the subject of a recall due to firing pins breaking. These rifles may have the potential for **UNINTENTIONAL DISCHARGE** while the bolt is closing.

Rifles with a new improved firing pin assembly will have a red dot on the right hand bolt rail.

Blaser Jagdwaffen (817) 335-1634
Dept. GA
1320 Lake Street
Ft. Worth, TX 76102

RETIRED

WARNING

Make: Davis Industries
Model: DM-22
Caliber: 22 Magnum
Source: AFTE Journal, July 1990, Volume 22, Number 3:310-314

DAVIS INDUSTRIES DERRINGERS

Davis Industries double barrel derringers Model DM-22, in 22 Magnum caliber with a cross bolt safety have been known to wear the shoulder of the hammer allowing the hammer to seat close to or on the firing pins when the safety is in the “on” position. The firing pins are free floating and do not have springs in them to prevent forward movement. This situation creates the potential for accidental discharge.

RETIRED

WARNING

Make: Benelli
Model: M1 Super 90
Caliber: 12 GA
Source: AFTE Journal, July 1990, Vol 22, Num 3:315-317;
AFTE Journal, Oct 1991, Vol 23, Num 4:908-909

BENELLI, MODEL M1 SUPER 90, 12 GAUGE, SEMIAUTOMATIC SHOTGUN

The Benelli, 12 gauge, model M1 Super 90, shotgun may have the potential for **UNINTENTIONAL DISCHARGE**. This semiautomatic shotgun has discharged while loading due to a protruding firing pin. This shotgun discharged a shotshell with the action partially open.

Benelli USA Corp. (301) 286-6981
17603 Indian Head Highway
Accokeek, MD 20607-2501

RETIRED

RECALL

Make: Browning
Model: A-Bolt Rifles
Caliber: 22 WMR
Source: Shooting Times, Sept 1990, pg 88;
AFTE Journal, July 1990, Vol22, Num 3:329;
Guns & Ammo, Aug 1990

BROWNING, A-BOLT RIFLES, 22 MAGNUM RIMFIRE CALIBER

THIS RECALL IS FOR 22 MAGNUM RIMFIRE RIFLES ONLY (22 WMR caliber). 22 Long Rifle Rimfire A-Bolt models are not included in this recall.

During our continual and extensive shooting tests under laboratory conditions, we encountered a parts tolerance variation, which could cause an unsafe condition. This is due to the possibility that some guns may fire when the bolt is being closed.

DO NOT LOAD OR SHOOT YOUR 22 MAGNUM RIMFIRE A-BOLT RIFLE until it has been sent to us and the problem corrected. Failure to follow these instructions could result in injury or death to yourself or others.

If your rifle has the following inscription on the right side of the barrel, your rifle is included in this recall:

A-BOLT 22 MAGNUM 22 WIN MAG CALIBER ONLY
In all cases, **the entire gun must be returned to Browning** to make the correction.

Please call our 22 Magnum Recall Department at 1-800-727-4312.

When we receive your call we will verify that you own one of the recalled rifles, and you will be sent a special 22 Magnum Recall Kit to expedite the return and repair of your rifle.

We apologize for this inconvenience. There will be no charge for the repair of your rifle and all shipping costs will be prepaid or quickly reimbursed.

RETIRED

WARNING

Make: Smith & Wesson
Model: 3904, 3906, 3913, 3913LS, 3914, 3914LS, 1006, 4506, 4516, 4516-1, 5903, 5904, 5906, 5946, 6904, 6906, 6946
Caliber: 9mm Luger, 10mm Auto, 45 Auto
Source: American Rifleman, Aug 1990, pg 20;
AFTE Journal, Oct 1990, Vol 22, Num 4:452;
Petersen's Handguns, Oct 1990, pg 25;
American Rifleman, Oct 1991;
Guns & Ammo, Sept 1990, pg 108

SMITH & WESSON, MODEL 3904, 3906, 3913, 3913LS, 3914, 3914LS, 1006, 1066, 4506, 4516, 4516-1, 5903, 5904, 5906, 5946, 6904, 6906 & 6946, SEMIAUTOMATIC PISTOLS

RETIRED

Smith & Wesson has shipped over 300,000 Third generation Pistols. We have had a very small number or reports where a dropped pistol resulted in a broken grip resulting in a failure to fire by releasing mainspring tension. Resultant testing and review have shown that a pistol dropped at a specific angle can cause a fracture of the grip.

As a result of these occurrences and our testing, we have made a change in the grip material to a thermoplastic alloy of increased durability. This new material, which is now being used on all current production models, can be identified by a dimple on the bottom of the grip, next to the large opening. Additionally, the blue product box has a green dot sticker on the label end.

We believe it unlikely that you will experience any problem with your original grips. However, in order to safeguard our customers from the possibility of such a malfunction, we will make available, free of charge, an easily installed replacement grip. Please inspect your pistol and if you have a model listed below that does not have a small dimple next to the large opening, please write us including your model and serial number or call 800-338-2466. If you prefer, you can send your pistol to a Smith & Wesson Warranty Station where we will replace the grip for you.

Models Affected:

3904, 3906, 3913, 3913LS, 3914, 3914LS, 1006, 1066, 4506, 4516, 4516-1, 5903, 5904, 5906, 5946, 6904, 6906 & 6946

Smith & Wesson's development process is a continuing one. We are constantly striving both to develop new products and improve existing products. We are committed to building the safest, most reliable, finest performing firearms possible.

WARNING

Make: Winchester
Model: 100
Caliber:
Source: AFTE Journal, Oct 1990, Vol 22, Num 4:452

WINCHESTER, MODEL 100, RIFLES AND CARBINES

An accidental discharge may occur BEFORE the action is locked.

These rifles and carbines may have firing pins that can break due to metal fatigue. When lodged in the breech face they can cause discharge before the action is closed.

Winchester Firearms Notice (800) 852-5734
Attn: Product Service Department
P.O. Box 10
Cottage Hills, IL 62018

RETIRED

RECALL

Make: Intratec
Model: Tec-22 “Scorpion”
Caliber: 22 caliber
Source: Guns & Ammo, Oct 1990, pg 144

INTRATEC, MODEL TEC-22 “SCORPION”, 22 CALIBER SEMIAUTOMATIC PISTOL

To all owners of TEC-22s from serial # 100 to # 20,033 and # 27,001 to # 27,222. A flawed part used in the manufacture of the pistol may cause the gun to function as a fully automatic pistol. Already modified guns within the serial number range can be checked by:

Opening the cover and looking by the front/side, under the cover axis screw.

If there is an indentation with an "R"affixed inside of it (check both sides) then your gun has been modified. If not, send your TEC-22 to Intratec for factory authorized installation free of charge.

For return instructions, call: (305) 232-1821 or send your TEC-22, carefully packaged and insured to:
Intratec
12405 S.W. 130th Street
Miami, FL 33186

RETIRED

WARNING

Make: Uncle Mike's Grips for Taurus
Model: PT92, PT99
Caliber: 9mm Luger
Source: Shooting Times, Dec 1990, pg 77;
Gunweek, Nov 2, 1990

"UNCLE MIKE'S" BRAND GRIPS for TAURUS, MODEL PT92 & PT99, 9MM CALIBER, SEMIAUTOMATIC PISTOLS

RETIRED

Michaels of Oregon is recalling its "Uncle Mike's" Custom Grade Molded Handgun Grips. Set No. 59507, for Taurus PT92 ad PT 99 semi-automatic 9mm pistols. It has come to our attention that the trigger bars of some of these pistols can come away from the frame when the pistols are equipped with our grips. A pistol in this condition could accidentally discharge, causing possible injury or death to the shooter or bystander.

To ensure the safety of our customers and protect them from the possibility of such a malfunction, we are making available replacement grips. The new grips can be identified by the two cylindrical nubs located on the inside top of the right grip and the "A" stamped between them. Please inspect your grips and if you do not have the new grips, mail us your old ones for replacement to the address below. There is no charge for the exchange and we will reimburse you for postage costs.

"Uncle Mike's" is committed to giving its customers high quality, safe products. We apologize for this inconvenience to our customers.

Uncle Mike's
P.O. Box 13010
Portland, OR 97213
(503) 255-5890

WARNING

Make: Ruger
Model: P-85
Caliber: 9mm Luger
Source: American Rifleman, Dec 1990, pg 17;
Guns, March 1991, pg 72;
Guns, Dec 1991, pg 65;
AFTE Journal, Jan 1991, Vol 23, Num 1:507

RUGER, MODEL P-85, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOLS

RETIRED

We have recently learned of a broken firing pin in a P85 automatic pistol, which caused the pistol to fire as a result of decocking. This is the first report of this nature in over 200,000 pistols delivered to customers since 1987. No injury occurred because the shooter kept the pistol pointed in a safe direction during decocking.

Nevertheless, we regard this incident as important because it reveals a potential danger, which we are prepared to eliminate in all P85 pistols now in use. This modification is very simple and does not alter the handling or appearance of this pistol, but it must be installed at the factory.

Accordingly, we urgently request that all owners of P85 pistols contact us immediately to obtain any further information they may desire, and to arrange for return of their P85 pistols to the factory for modification. Please contact us at:
Sturm, Ruger & Co., Inc.
Department S
Ruger Road
Prescott, AZ 86301

Or call us for P85 inquiries only at 800-424-1886. Please have your pistol serial number available when contacting us.

We will schedule your gun for factory installation of a new safety/decock system free of charge. It will prevent this type of accidental firing even in the rare event of firing pin breakage. This free safety modification applies only to pistols rollmarked "P85" on the slide. Pistols, which are rollmarked “MKII”, will have these modifications as part of their original manufacture, and are not subject to this modification.

We are also taking this opportunity to remind shooters of the most common and basic firearms safety rule: Always Keep The Pistol Pointed In A Safe Direction! This is particularly important when loading, unloading, or decocking any pistol.

WARNING

Make: Star
Model: M43 Firestar
Caliber: 9mm Luger
Source: Guns, June 1991, pg 66;
American Rifleman, May 1991, pg 21

STAR, MODEL M43 FIRESTAR, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

INTERARMS has learned of occasional firing pin breakage in the Star M43 FIRESTAR 9MM pistols within the limited serial number range 1,953,001 to 1,958,000 ONLY. Such breakage does not pose any direct hazard to the shooter; however, the pistol is rendered inoperable.

WARNING! This can be critical when used for self protection or law enforcement.

STAR and INTERARMS are committed to providing our customers with high quality firearms of the utmost reliability. We regret any temporary inconvenience.

INTERARMS (703) 548-1400
10 Prince Street
Alexandria, VA 22314

RETIRED

RECALL

Make: Browning
Model: 81L BLR
Caliber:
Source: Shooting Industry, July 1991, pg 10;
Shooting Times, Aug 1991, pg 8;
Shooting Times, Oct 1991, pg 106;
AFTE Journal, July 1991, Vol 23, Num 3:802;
American Rifleman, July 1991, pg 14

BROWNING, MODEL 81L BLR, LONG ACTION, RIFLES

Browning has identified a potential safety hazard on its BLR Long Action, and is recalling all of these rifles for repair. This recall does not include the Short Action BLR Rifle.

Long Action BLR owners should NOT load or shoot their rifles until they have been returned to Browning and the problem has been corrected. The problem is easily corrected but the affected rifles must be sent to Browning for the correction to be made.

The rifles in question have the following inscription on the right side of the barrel: Model 81L BLR followed by one of these calibers: CALIBER 270 WIN., 30-06, or 7MM REM. MAG.

To arrange shipping and service, call Browning's service facility at (800) 727-4312. Browning pays the freight and provides the container.

RETIRED

WARNING

Make: Smith & Wesson
Model: 1026, 1076, 4526, 4536, 4576, 5924, 5926, 6924, 6926
Caliber: 9mm Luger, 10mm Auto, 45 Auto
Source: Guns & Ammo, Oct 1991, pg 112;
Shooting Times, Nov 1991, pg 95;
American Handgunner, Nov/Dec 1991, pg 128;
AFTE Journal, Oct 1991, Vol 23, Num 4:939;
American Rifleman, Oct 1991

SMITH & WESSON, MODEL 1026, 1076, 4526, 4536, 4576, 5924, 5926, 6924 and 6926 SEMIAUTOMATIC PISTOLS

RETIRED

Models affected have frame mounted decocking levers. Other model Smith & Wesson pistols are not affected by this advisory.

We have determined that it is possible for a Smith & Wesson Third Generation 9mm, 10mm and .45 ACP pistol with a frame-mounted decocking lever to malfunction so as to make the pistol inoperable.

In order to safeguard our customers from the possibility of such a malfunction, Smith & Wesson will inspect and, if necessary, modify these pistols free of charge. Owners of Smith & Wesson 9mm, 10mm and .45 ACP pistols featuring a frame-mounted decocking lever with model numbers listed above, and which do not have two dots stamped on the frame underneath the decocking lever (when the lever is depressed) should send their pistols and accompanying magazines to Smith & Wesson for a no-charge inspection.

Please send your pistol and magazines to: Smith & Wesson
2100 Roosevelt Avenue
Springfield, MA 01102-2208
Attn: Service Department-DL

If you have any questions regarding this product advisory, please call 800-331-0852 ext. 211.

While we have your pistol, our armorers will stamp your pistol with two dots under the decocking lever, and give your pistol an ultrasonic cleaning and full lubrication (a $25.00 value) at no charge.

We apologize for this inconvenience. Smith & Wesson remains committed to building the safest, most reliable and finest-performing firearms possible.

WARNING

Make: Iver Johnson
Model: Safety Hammer & Hammerless First and Second
Caliber: 22 caliber, 32 caliber, 38 caliber
Source: IVER JOHNSON ARMS & CYCLE WORKS HANDGUNS 1871 - 1978, Copyright 1991 Blacksmith Corp. Chino Valley, AZ, by W. E. Goforth 1st Edition:22

IVER JOHNSON, SAFETY HAMMER & HAMMERLESS, FIRST & SECOND MODEL, REVOLVER

RETIRED

The First and Second Models were strong and dependable revolvers, but – by today's standard – **they can only be considered safe with black powder loads**.

The First Model was produced from 1894 to 1896 and Second Model 1897 to 1908 for black powder loads. The Third Model produced from 1909 to 1941 was strengthened enough so that it was now safe to use with the new smokeless powder.

Below are the serial number ranges and frame sizes for the First and Second Models:

22 Hammerless Small Frime	22 Hammer Small Frame	32 Hammerless Small Frame	32 Hammer Small Frame
00001 - 100000	00001 - 100000	00001 - 100000 B00001 - B100000 H00001 - H100000 L00001 - L100000 O00001 - O100000 U00001 - U100000	00001 - 100000 A00001 - A100000 E00001 - E100000 F00001 - F100000 K00001 - K100000 N00001 - N100000 P00001 - P100000 S00001 - S100000

38 & 32 Hammerless Large Frame	38 & 32 Hammer Large Frame
00001 - 100000 D00001 - D100000 J00001 - J100000 R00001 - R100000 T00001 - T100000	00001 - 100000 C00001 - C100000 G00001 - G100000 I00001 - I100000 M00001 - M100000 Q00001 - Q100000 T00001 - T100000

WARNING

Make: Llama
Model: XI-B, IX-A
Caliber: 9mm Luger, 45 Auto
Source: AFTE Journal, Apr 1992, Vol 24, Num 2:197;
American Rifleman, Apr 1992, pg 16;
Guns & Ammo, May 1992, pg 72;
Guns & Ammo, June 1992, pg 27;
Handgunning, May/June 1992

LLAMA,

MODEL XI-B, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

MODEL IX-A, 45 AUTO CALIBER, SEMIAUTOMATIC PISTOLS

Serial Numbers: 848184 to 848223 A 07876 to A 07915, A 08422 to A 08661, A11788 to A 99999, B 00001 to B 09512

Some of these pistols may have a firing pin which extends past the breech face if the hammer is in the full down position against the rear of the slide. If the chamber is loaded and the gun is dropped with the hammer in position, the gun can fire.

A round should not be chambered in a Llama single action semiautomatic pistol until you are ready to shoot. If the gun must be carried or handled with a round chambered, the safety should be engaged. The gun should not be carried or handled when a round is chambered with the hammer down and the safety off. No gun should be dropped.

In order to minimize the potential for accidental discharge if these rules are violated, Stoeger Industries is inspecting those guns which may have the above-described firing pin condition. We will modify any gun with this firing pin condition, free of charge.

Owners of guns within the serial numbers listed above should call Stoeger's customer service department at (800) 631-0722. We regret any inconvenience. We remain committed to providing our customers with safe, reliable, accurate and affordable firearms.

Have your serial number ready. We will tell you if your gun is affected by this program. We will give you instructions for sending the gun to Stoeger. We will reimburse you for UPS or parcel post shipping.
Stoeger Industries
55 Ruta Court
S. Hackensack, NJ 07606

RETIRED

RECALL

Make: Daisy
Model: Legacy 2203 and 2213
Caliber: 22 caliber
Source: Guns & Ammo, Mar 1993, pg 27;
American Rifleman, Feb 1993, pg 86

DAISY, MODEL LEGACY 2203 and 2213, 22 CALIBER, SEMIAUTOMATIC RIFLES

Daisy Manufacturing Company, Inc. is voluntarily recalling all Legacy Model 2203 and 2213, semiautomatic .22 caliber rifles for retrofitting.

It has been determined that the sear or disconnect may break on the Legacy semiautomatic rifles which may permit the gun to discharge more than one round with a single pull of the trigger. Because of this condition, Daisy has elected to recall all Legacy 2203 and 2213 semiautomatic rifles and replace the sear and disconnect components. The rifle will be repaired and returned at no charge to the owners.

This recall applies only to Daisy Legacy semiautomatic .22 caliber rifles Models 2203 and 2213, all with the serial number beginning with the letters AC. Owners of these rifles should contact Daisy at 1-800-354-8841 for information on returning their gun(s).

Daisy Manufacturing Company, Inc. (800) 643-3458
2111 South Eighth Street
Rogers, AR 72756

RETIRED

RECALL

Make: Chinese
Model: SKS
Caliber: 7.62x39mm
Source: American Rifleman, March 1993, pg 14

CHINESE, MODEL SKS, SEMIAUTOMATIC RIFLES, IMPORTED BY NAVY ARMS

We hereby provide written notice to you that some Chinese SKS semiautomatic rifles may pose a serious risk of bodily injury or death.

Some of these rifles may have a manufacturing defect of the engaging surfaces in the trigger-hammer-sear assembly, which can cause intermittent failure of the sear to hold the hammer in a cocked position. This condition can result in the unexpected firing as a round is chambered, without pulling the trigger, or if the weapon is jarred or dropped. This condition may also result in sudden and unexpected automatic fire and loss of control of the firing weapon.

Navy Arms Company strongly recommends that owners of Chinese SKS rifles have their weapons inspected for this defect by a competent and qualified gunsmith before using the weapon.

If you own a Chinese SKS semiautomatic rifle from Navy Arms Company and believe your weapon needs a new sear, mail your sear with the serial number of your SKS to us at the address below, and include a self-addressed envelope. We will send you a free replacement sear.

When firing any semiautomatic weapon for the first time, always follow these safety procedures:

Load two rounds into the magazine, and in a stable shooting position, with the muzzle pointed down range, pull the charging handle (or slide) back and let it fly forward to chamber the first round. Do not ease the carrier or the slide into battery. If the weapon fires, cease firing immediately. If no firing occurs, aim the weapon down range and pull the trigger. If both rounds fire with one pull of the trigger, cease firing immediately. Take your weapon to the gunsmith or the place of purchase for repair.

The safety procedures identified above are from the Navy Arms Company Shooting Maintenance Manual distributed to its SKS purchasers. For a free copy of the manual, please send a self-addressed, stamped business-size envelope with the serial number of your SKS. Remember, always follow all firearms safety practices.

Navy Arms Company, Inc.
689 Bergen Boulevard
Ridgefield, NJ 07657

RETIRED

WARNING

Make: Glock
Model: 19
Caliber: 9mm Luger
Source: AFTE Journal, July 1993, Vol 25, Num 3:206-208

GLOCK, MODEL 19, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

This make and model pistol may have the potential for an **UNINTENTIONAL DISCHARGE** of a cartridge with the action open.

The Glock, model 19 pistol slide has a bottom protrusion, whose function is to push cartridges from the top of the magazine into the chamber . When the pistol is jammed in a double feed situation, the slide protrusion can come in contact with the primer of the jammed cartridge and cause **UNINTENTIONAL DISCHARGE** while the action is open.

Glock, Inc. (770) 432-1202
6000 Highlands Parkway
Smyrna, GA 30082

RETIRED

WARNING

Make: Sig Sauer
Model: P220, P225, P226, P228, P229, P230
Caliber:
Source: Handguns, July 1993, pg 61;
Law Enforcement Technology, Aug 1993, pg 20

SIGARMS, MODEL P220, P225, P226, P228, P229 & P230, SEMIAUTOMATIC PISTOLS

All owners and users of SIG-Sauer pistols are reminded to use the decocking lever to decock their pistols. This is the only way to safely lower the hammer from the cocked position and prevent an accidental discharge caused by thumb-slipping or dropping the pistol.

The warning applies to all pistols with decocking levers. The model numbers include:

P220, P225, P226, P228, P229 & P230

The only positive way to safely lower the hammer is by use of the decocking lever.

Hammers should never be lowered by manually lowering the hammer by pulling the trigger. Manually lowering the hammer is dangerous in itself and prevents the full application of the pistol's safety features.

The decocking lever is the only proper method of lowering the hammer and assuring that the hammer rests in the intercept notch. The intercept notch prevents the hammer from striking the firing pin, whether from slipping manually or accidentally dropping the pistol.

DO NOT thumb the hammer down. The consequence can be serious injury or death. Only and always use the decocking lever!

RETIRED

WARNING

Make: Arminius
Model: HW3
Caliber: 32 S&W
Source: AFTE Journal, Oct 1993, Vol 25, Num 4:286-291

ARMINIUS, MODEL HW3, 32 SMITH & WESSON CALIBER, REVOLVER

The Arminius, 32 Smith & Wesson caliber, model HW 3, revolver has the potential for **UNINTENTIONAL DISCHARGE**. The firing pin may protrude through the breech face. Revolvers of this model were found to lack a firing pin spring. Additionally, a device that, in theory, should serve the purpose of a hammerblock did not function as such consistently.

RETIRED

WARNING

Make: Savage
Model: 110E Series K
Caliber: 243 Winchester, 270 Winchester, 30-06 Springfield
Source: AFTE Journal, January 1994, Vol 26, Num 1:2-3

STEVENS/SAVAGE, MODEL 110E SERIES K,
.243 WINCHESTER, .270 WINCHESTER, .30-06 SPRINGFIELD CALIBER, RIFLES

These rifles did not fire with its safety engaged. However, when the safety is "off" these rifles will fire when dropped either on the muzzle or with their barrel in a horizontal position.

Savage Arms (413) 562-7001
100 Springdale Road
Westfield, MA 01085

RETIRED

WARNING

Make: Savage
Model: 110E
Caliber: 30-06 Springfield
Source: AFTE Journal, Jan 1994, Vol 26, Num 1:2-3

WARNING

SAVAGE, MODEL 110E, .30-06 SPRINGFIELD CALIBER, RIFLE

The rifle did not fire with its safety engaged. However, with the safety "off" it fired when struck on top of the receiver bridge or grip area of the buttstock from underneath, regardless of the whether the rifle is in an horiztonal, vertical or upside down position.

Savage Arms (413) 568-7001
100 Springdale Road
Westfield, MA 01085

RETIRED

WARNING

Make: Smith & Wesson
Model: 659
Caliber: 9mm Luger
Source: AFTE Journal, Jan 1994, Vol 26, Num 1:18-20

SMITH & WESSON, MODEL 659, 9MM LUGER CALIBER SEMIAUTOMATIC PISTOL

An accidental discharge may occur when manually unloading a pistol if there is an obstruction of the ejection port.

When semiautomatic pistols are manually unloaded:

1. Engage the safety in the "safe" position.
2. Remove the magazine.
3. Point the muzzle in a safe direction.
4. Retract the slide without obstructing the ejection port.

If the primer of a cartridge comes in contact with internal parts of the pistol, such as the tip of the ejector, an unintentional discharge may result. This can occur because the cartridge strikes an object, which prevents ejection from the port during the extraction/ejection process.

RETIRED

RECALL

Make: Winchester
Model: 1001
Caliber:
Source: Guns & Ammo, Jan 1995, pg 76;
Shooting Times, Jan 1995, pg 8;
Shooting Industry, Nov 1994

WINCHESTER, MODEL 1001, SHOTGUNS

U.S. Repeating Arms Co. has announced an immediate recall of all Winchester Model 1001 over/under shotguns sold since the introduction in November 1992. No other Winchester shotguns, past or present, are involved in this recall.

U.S. Repeating Arms Co. officials report that continual and extensive shooting tests have revealed a safety problem in some guns when subjected to higher than normal pressures. Screening and testing of all Model 1001 shotguns are required. Consequently, U.S. Repeating Arms Co. warns all Model 1001 owners to immediately discontinue any further shooting of its 1001 shotguns regardless of the type of ammunition used.

To return a shotgun, call U.S. Repeating Arms' Model 1001 Recall Department immediately at 800-945-5372. Arrangements for returning the shotguns will be made at the time. Special shipping boxes preaddressed shipping labels, and easy instructions will be provided. All shipping costs will be prepaid or quickly reimbursed by U.S. Repeating Arms Co.

RETIRED

RECALL

Make: Intratec
Model: CAT-9
Caliber: 9mm Luger
Source: American Gunsmith, 1994

INTRATEC, MODEL CAT-9, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

These pistols bearing serial numbers 00001 to 003122 are being recalled due to improper brazing on the slides which can cause the pistol to jam in an open position so that no more shots can be fired. The CAT 9 was introduced in late 1992 however, production delays kept the gun from hitting the market until 1994. For further information contact;

Intratec, Inc.
Repair per Recall (CAT 9)
12405 S.W. 130 Street
Miami, FL 33186

RETIRED

RECALL

Make: Colt
Model: All American
Caliber: 9mm Luger
Source: Law Enforcement Technology, April 1995, pg 67;
Guns, May 1995, pg 69

COLT, MODEL ALL AMERICAN, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

RETIRED

It has come to Colt's attention that All-American double action pistols with serial numbers Letter A prefix or suffix
RA01001 – RA01052 RK00001 – RK03000
RM01001 – RM01005 PF01005 – PF24248

MAY ACCIDENTLY DISCHARGE IF STRUCK OR DROPPED

A blow to the rear of the slide while a round is in the chamber can cause the pistol to discharge. The firing mechanism of these pistols will be modified free of charge, and thereby reduce the possibility of accidental discharge resulting from a blow.

MEANWHILE, ALL OWNERS AND SHOOTERS OF THESE PISTOLS ARE CAUTIONED:

1. Carry this pistol ONLY with the chamber empty.
2. Never chamber a round until immediately before you are ready to fire.
3. Always control your pistol so as to prevent its being dropped or struck a blow, especially with a round in the chamber.

If you own one or more of these pistols write to Colt and give them your name, address and pistol serial number of each All-American pistol you own. Send all correspondence to:

Colt Manufacturing Company, Inc.
P.O. Box 1868 Dept. RC2000
Hartford, CT 06144-1868

Please DO NOT SEND YOUR PISTOLS TO COLT at this time. For further information you may call us toll-free at 1-800-426-8001.

WARNING

Make: CZ
Model: 52
Caliber: 7.62mm Tokarev
Source: Czech CZ-52 Pistol Manual, 1996

CZECH, MODEL 1952, 7.62 TOKAREV CALIBER, SEMIAUTOMATIC PISTOL

This make and model pistol may be prone to **FIRING PIN BREAKAGE.**

Owners manual states, "If pistol is subjected to repeated DRY FIRING the model 52 firing pin will break."

Firing Pin Enterprises
P.O. Box 80696
Phoenix, AZ 85060-0696

RETIRED

WARNING

Make: Walther
Model: PP
Caliber: 32 Auto
Source: AFTE Journal, Jan 1996, Vol 28, Num 1:48-54

WALTHER, MODEL PP, 32 AUTO CALIBER, SEMIAUTOMATIC PISTOL

FULL AUTO MODIFICATION

These pistols are subject to unintentional and uncontrollable full automatic fire **if the hammer block (automatic safety) plunger and spring, along with the hammer release (de-cocking lever) are removed. When the slide is drawn to the rear and released, the acceleration of the slide by the recoil spring, and the sudden stop as the slide goes into battery causes the hammer to fall. This results in the pistol discharging in a full automatic mode.**

Carl Walther GmbH Sportwaffen
Karlstrasse 33,
D-89073 Ulm, Germany

RETIRED

WARNING

Make: H.J.S. Industries
Model: Frontier Four
Caliber: 22 Long Rifle
Source: AFTE Journal, Jan 1996, Vol 28, Num 1:55-56

H.J.S. INDUSTRIES, MODEL FRONTIER FOUR, 22 LR CALIBER, DERRINGER

H.J.S. Industries Inc. produces a reproduction of the Sharps four barrel 22 caliber derringer named the "Frontier Four Derringer" which may **UNINTENTIONAL DISCHARGE**. The fixed rectangular firing pin projects from a circular hammer which rotates 1/4 turn each time the firearm is cocked for single action firing. When the chamber under the firing pin nose is empty, a live cartridge, NOT under the firing pin nose, may discharge because it is struck by the circular edge of the hammer.

H. J. S. Industries (800) 453-2767
2965 E. 123th Street #C
P.O. Box 3711
Brownsville, TX 78520

RETIRED

WARNING

Make: FN/Browning
Model: Hi-Power
Caliber: 9mm Luger
Source: AFTE Journal, Oct 1996, Vol 28, Num 4:233-240

FN/BROWNING, MODEL HI-POWER, 9MM LUGER CALIBER, PISTOL

This make and model pistol may have the potential for **UNINTENTIONAL DISCHARGE** as the slide finishes closing without pulling the trigger.

With the manual thumb safety on safe, magazine inserted, and the firearm loaded and cocked, pulling the trigger will usually not discharge the firearm. However, pulling the trigger after performing the above operations will cause the trigger lever to contact the forward end of the sear lever. The sear lever then rotates about its pin, causing the rear end of the sear lever to push down on the sear, but not enough for disengagement from the hammer since the safety physically blocks its path. When the trigger is released the sear does not return to its full seated position at the cocking notch.

The force now needed to disengage the sear from the hammer is less, due to the new position of the sear. Theoretically, the trigger pull is lowered and a jar to the firearm could disengage the sear from the hammer, possibly bypassing the half cock safety. The loading of a live round into the chamber and letting the slide close was enough force to cause a discharge without the trigger being pulled.

RETIRED

Browning (801) 876-2711 or (800) 727-4312.
1 Browning Place
Morgan, UT 84050

WARNING

Make: Steyr-Mannlicher
Model: Bolt-action rifles with double triggers
Caliber: all
Source: Guns, Nov 1996, pg 74

STEYR-MANNLICHER, BOLT-ACTION, RIFLES

RETIRED

Steyr-Mannlicher AG is recalling some Steyr-Mannlicher centerfire bolt-action rifles with double set triggers in the serial number range 92,000 through 150,000. This recall applies to all Steyr-Mannlicher centerfire rifles with double triggers regardless of model designation and does not apply to any Mannlicher Shoenauer rifles, nor to any other Steyr-Mannlicher products.

This action is taken because some rifles manufactured in the serial number range noted above were assembled with trigger housings (not trigger guards) which may crack or corrode. Steyr-Mannlicher has discovered that some trigger housing alloy supplied by a raw material source is below-grade, resulting in the possibility of cracking and corrosion. Improper maintenance may increase the risk. Cracking, corrosion, or any other failure of the trigger housing could result in unintended firing of the rifle. Serious personal injury or death could result from an unintentional discharge if other safety rules are violated.

This possibly dangerous condition may not be discovered by ordinary inspection.

IF YOU OWN OR KNOW THE WHEREABOUTS OF A STEYR-MANNLICHER CENTERFIRE RIFLE WITH DOUBLE SET TRIGGERS WITHIN THE SERIAL NUMBER RANGE FROM 92,000 THROUGH 150,000, DO NOT LOAD, FIRE OR OTHERWISE USE THE RIFLE.

We ask that you call our toll-free number at 888-467-8397 with the model and serial number of your rifle, or contact GSI, Box 129, Trussville, AL 35173. From that serial number, we can verify the status of the rifle.

Our distributor in the United States, GSI, at no charge will repair all affected rifles returned to Steyr-Mannlicher under this recall to the owners. Shipping costs and freight costs will also be borne by Steyr-Mannlicher.

IF YOU HAVE ANY DOUBTS, CALL 1-888-467-8397.

RECALL

Make: Browning
Model: Box for Buck Mark
Caliber: 22 cailber
Source: Guns & Ammo, July 1997, pg 36;
Handguns, July 1997, pg 20;
Shooting Times, Aug 1997, pg 57

BROWNING, MODEL BUCK MARK, 22 CALIBER, PISTOL BOX

Browning is recalling all Buck Mark 22 Pistol boxes sold between January of 1992 to present.

THIS RECALL IS FOR THE BOX ONLY. DO NOT SEND YOUR BUCK MARK PISTOL. RETURN ONLY THE BOX FOR REPLACEMENT.

DO NOT PLACE YOUR BUCK MARK 22 PISTOL IN A BOX TO BE RECALLED.

NEVER PLACE ANY LOADED GUN IN ANY CASE. THIS IS A BASIC RULE OF SAFETY.

Do not use the box until it has been replaced by Browning. Failure to follow these instructions could result in injury or death to yourself or others.

In all cases, only the original Buck Mark box is to be returned. Please call our office and inform us which box you own.

When we receive your call we will verify and record which box you own. If you have a box to be recalled, you will be sent a special Buck Mark Box Recall Kit to expedite the return and replacement of your Buck Mark box.

We apologize for this inconvenience. There will be no charge for the replacement and all shipping costs will be prepaid by Browning.

RETIRED

RECALL

Make: Connecticut Valley Arms
Model: In-Line Muzzleloading Rifles
Caliber: all
Source: Connecticut Valley Arms Recall Notice, Aug 1997;
Shooting Industry, Nov 1997, pg 10;
Connecticut Valley Arms Recall Notice, Aug 2002;
North American Hunter, Aug 2002, pg 100;
North American Hunter, April/May 2003

CONNECTICUT VALLEY ARMS, IN-LINE MUZZLELOADING RIFLES MANUFACTURED IN 1995 & 1996.

These make and model rifles may create **EXTREMELY DANGEROUS CONDITIONS** and a **POTENTIAL FOR SERIOUS INJURY** when firing the rifle.

The recall is for barrel replacement of the affected rifles. To identify the rifles involved in the recall read the serial number on the barrel opposite the side of the bolt. The last two digits are 95 or 96.

Connecticut Valley Arms (800) 482-3470 8:30 AM to 4:30 PM EST or
5988 Peachtree Corners E. (770) 449-4687
Norcross, GA 30091

RETIRED

WARNING

Make: Calico
Model: M-950
Caliber: 9mm Luger
Source: AFTE Journal, Vol 29 Num 3, Summer, 1997:316

CALICO INDUSTRIES, MODEL M-950, 9MM LUGER CALIBER SEMI-AUTOMATIC PISTOL

These pistols may create an **EXTREMELY DANGEROUS CONDITION** and a **POTENTIAL FOR SERIOUS INJURY** by firing without pulling the trigger.

During the testing of a Full Auto Conversion of a Calico, M-950 Pistol by a forensic firearms examiner it was noted that alteration of internal parts had taken place. Testing of laboratory reference firearms that had not been altered revealed a condition where these model pistols will fire with a pull of the trigger.

In the unaltered pistols, during the charging sequence (cocking the striker to chambering a cartridge) there existed a point of demarcation in bolt travel where a unique situation occurs. At that point the bolt is retracted far enough to the rear to strip a cartridge from the magazine, but not far enough to lock the striker and sear. If the charging handle is released, the bolt will accelerate forward under spring tension chambering and firing a cartridge.

RETIRED

WARNING

Make: Phoenix Arms
Model: Raven
Caliber: 25 Auto
Source: AFTE Journal, Spring 1998, Vol 30, Num 2:352-353

PHOENIX ARMS, MODEL RAVEN, 25 AUTO CALIBER, SEMIAUTOMATIC PISTOL

This make and model pistol may have the potential for **UNINTENTIONAL DISCHARGE.**

With the pistol charged (cartridge in the chamber) and the safety "on", if you apply pressure to the trigger, when you release the safety the pistol may fire without touching the trigger.

Examination of the internal parts reveals the sear tip was damaged or altered making it shorter than normal. Due to the damage/alteration of the sear, there is insufficient/improper engagement in the area of contact between the sear and striker. In this condition when the safety is disengaged, the force of the compressed striker spring overcomes the force of the sear spring. This situation lowers the sear allowing the striker to release, firing the pistol.

Phoenix Arms (909) 947-4843
1420 South Archibald Avenue
Ontario, CA 91761

RETIRED

WARNING

Make: Lorcin
Model: L25
Caliber: 25 Auto
Source: Northeast Regional Firearms & Toolmark Examiners Meeting; Connecticut State Police Laboratory, April 15, 1998

LORCIN, MODEL L 25, 25 AUTO CALIBER, SEMIAUTOMATIC PISTOL

This pistol was the subject to **MECHANICAL PARTS FAILURE**.

The Lorcin, 25 Auto caliber, model L25, pistol may have a breechface failure causing the slide to separate from the frame of the pistol as a result of discharge. This model of Lorcin pistol was submitted in a case to the Connecticut State Police Lab. During examination of the pistol, the slide separated from the frame when fired. Inspection of the slide revealed the breechface was blown back into the firing pin channel.
It appeared to the examiner that very little metal was left, after the breech was machined, to support a cartridge when fired.

Lorcin Engineering (909) 360-1406
3830 Wacker Drive
Mira Loma, CA 91752

RETIRED

RECALL

Make: High Standard / J.C. Higgins, Sears
Model: 10; 583.13-583.22
Caliber: 12 GA
Source: American Rifleman, April 1998, pg 24

HIGH STANDARD/J.C. HIGGINS, MODEL 10, 12 GAUGE BOLT ACTION SHOTGUN AND SEARS MODEL NUMBERS: 583.13, 583.14, 583.15, 583.16, 583.17, 583.18, 583.19, 583.20, 583.21 AND 583.22

The model of shotgun may be subject to a **BOLT FAILURE.** When this occurs the bolt will dislodge from the action, striking the shooter causing serious injury. These shotguns were manufactured for and sold by Sears, Roebuck & Co. during the 1950's.

Call (800) 817-9165 for identification verification. Instructions for the return of the bolt and refund of $160.00 will be provided.

RETIRED

WARNING

Make: Calico
Model: M-100/M-100P
Caliber: 22 Long Rifle
Source: AFTE Journal, Summer 1998, Vol 30, Num 3:527-530

CALICO, MODEL M-100 RIFLE/M-100P PISTOL, 22 LONG RIFLE CALIBER

These make and model rifles/pistols may have the potential for **"SLAM FIRE" UNINTENTIONAL DISCHARGE**.

The Calico M-100 and M-100P will consistently **SLAM FIRE** when live cartridges are manually loaded directly into the chamber and the bolt is allowed to go forward under its own spring tension. At no time during this process does the trigger need to be pulled for discharge. These **SLAM FIRES** occur with the safety in the **ON** or **OFF** positions.

The **SLAM FIRES** described only occur when a cartridge is placed directly into the chamber and the bolt released forward under its own spring tension.

Calico Light Weapons Systems (702) 358-6000
1489 Greg Street
Sparks, NV 89431

RETIRED

WARNING

Make: Magtech
Model: 7022
Caliber: 22 Long Rifle
Source: Company Notice, Sept 1998

MAGTECH COMPANY. - MODEL 7022, 22 LONG RIFLE CALIBER, SEMIAUTOMATIC RIFLES

Approximately 20,000 of these rifles were produced with a nylon "recoil buffer." A number of these rifles have malfunctioned, which are attributed to the failure of the recoil buffers. The part should have been made of Delrin instead of Nylon. The recoil buffer in these rifles can fracture and jam the action prevent the cycling of the action and firing.

MAGTECH
837 Boston Post Road #12
Madison, CT 06443
(203) 245-8983

RETIRED

WARNING

Make: Smith & Wesson
Model: Sigma
Caliber: 40 S&W
Source: Glendora, CA PD, Teletype Message, Dec 14, 1998

SMITH & WESSON, MODEL SIGMA, 40 S&W CALIBER SEMIAUTOMATIC PISTOL

Three department issue SMITH & WESSON, MODEL SIGMA, 40 CALIBER PISTOLS EXPERIENCED INTERNAL PART FAILURES PREVENTING THE PISTOLS FROM FIRING.

These pistols had trigger return springs, slide stop springs or sear driver pins break. When these parts fail the pistol becomes inoperable.

Sgt. Ernie Didier (626) 914-8250
Glendora Police Department
150 S. Glendora Avenue
Glendora, CA 91741

RETIRED

WARNING

Make: F.I.E. Corp/ Tanfoglio Giuseppe
Model: Titan
Caliber: 25 Auto
Source: Orange County Register, Mar 1999;
Jackson v. F.I.E. Corp., et al, U.S. District Ct., Eastern District of LA, New Orleans, LA, # 95-2389 S (12/17/98)

FIREARMS IMPORT AND EXPORT (F.I.E.) CORP./TANFOGLIO GIUSEPPE, MODEL TITAN, .25 CALIBER, SEMIAUTOMATIC PISTOL

The F.I.E. Corp/ TANFOGLIO GIUSEPPE., Model Titan .25 caliber semiautomatic pistol has the potential for **ACCIDENTAL DISCHARGE.**

The owner of one of these pistols was paralyzed when the pistol allegedly fell on its "uncocked" hammer causing discharge, striking him in the neck.

During a subsequent civil suit against the manufacturer and the importer, the U.S. District Court awarded the gun owner $10.8 million stating, "Gun manufacturers have an obligation to reasonably anticipate that owners of guns, will, at some point, unexpectedly drop them. The manufacture of the pistol with a defective firing pin made it virtually certain that the gun would fire if dropped or bumped on its hammer."

RETIRED

WARNING

Make: Glock
Model: 17, 19
Caliber: 9mm Luger
Source: Arizona Dept of Public Safety Officer Safety Bulletin, Feb 1999;
Rocky Mountain Information Network Bulletin, April 1999;
Virginia Criminal Intelligence Center, The Validator, May 1999

GLOCK, MODEL 17 & 19, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

CONVERSION TO FULLY-AUTOMATIC FIRING

RETIRED

It has been determined that gang members in California and Florida have been converting Glock pistols to have **FULLY-AUTOMATIC** firing capability. In some regions of California, police are treating any Glock they encounter as a machine gun until proven otherwise. It would be wise to assume that gangs in other localities have also made this conversion.

The conversion from standard to fully-automatic is fast and simple, requiring no technical expertise. The conversion part involved is referred to as the slide cover plate. The original Glock slide cover plate is made of polymer but the bootleg piece is made of steel. The conversion is accomplished merely by swapping one piece for the other. (A "real pro" can make the switch in 15 seconds.)

On a modified Glock the polymer edge of the trigger housing mechanism, which sits just forward of the cover plate, has to be partially cut away. It is apparent without having to remove the slide that a significant amount of material has been cut away, leaving a large void visible through the notch.

Finding a Glock modified in this manner should be considered a clear indication that the weapon has been altered to function in a fully-automatic mode.

WARNING

Make: Bryco Arms
Model: Jennings Nine
Caliber: 9mm Luger
Source: AFTE Journal, Summer 1999, Vol 31, Num 3:379-381

BRYCO ARMS, MODEL JENNINGS NINE, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

The Bryco Arms, model Jennings Nine, 9mm Luger caliber, semiautomatic pistol is subject to accidental discharge. The pistol **MAGAZINE SAFETY** is subject to **FAILURE** when the trigger is pulled and the magazine release button is depressed simultaneously. When this occurs the pistol will fire even though the magazine has been removed from the pistol.

Update - Jan 2015, AFTE Contributor

Jimenez Arms Model J.A. Nine, 9mm Luger caliber semiautomatic pistol is subject to the same accidental discharge as the Bryco Arms, Model Jennings Nine.

RETIRED

WARNING

Make: Bryco Arms
Model: 59
Caliber: 9mm Luger
Source: AFTE Journal, Summer 1999, Vol 31, Num 3:379-381

BRYCO ARMS, MODEL 59, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

The Bryco Arms, model 59, 9mm Luger caliber, semiautomatic pistol is subject to misfeeding and jamming during the loading of cartridges into the chamber from the magazine. If the primer of a cartridge comes in contact with internal parts, such as the tip of the ejector, an unintentional discharge may result. This occurs while the port is open and without the trigger being pulled. This situation is extremely dangerous and could result in serious injury.

RETIRED

WARNING

Make: Winchester / U.S. Repeating Arms
Model: 70
Caliber: 270 Winchester, 30-06 Springfield, 7mm Remington Magnum, 300 Winchester Magnum
Source: Company Notice, Jan 2000

WINCHESTER/U.S. REPEATING ARMS COMPANY - MODEL 70, BOLT ACTION RIFLES

CALIBERS: 270 Winchester
30-06 Springfield
7mm Remington Magnum
300 Winchester Magnum

RETIRED

The Winchester model 70 bolt action rifles in 270 Winchester, 30-06 Springfield, 7mm Remington Magnum and 300 Winchester Magnum with serial numbers greater than G2235000 with BLACK COMPOSITE/SYNTHETIC STOCKS may have a potential safety problem in the front sling swivel stud. There is a possibility that under heavy weight the swivel stud can pull loose from the composite stock, causing the rifle to fall from the shoulder, which is a dangerous condition.

Sellers and owners of the affected model 70 rifles are being warned to immediately discontinue any further use until the front swivel stud has been replaced. Many of the affected rifles were sold at Wal-Mart Discount Stores.

WARNING

Make: Glock
Model: 26, 27
Caliber: 9mm Luger, 40 S&W
Source: Company Notice, Feb 21, 2000

GLOCK - MODEL 26, 9MM CALIBER, SEMIAUTOMATIC PISTOL
SERIAL NUMBER PREFIX DGU, DHR & DKU
MODEL 27, 40 S&W CALIBER SEMIAUTOMATIC PISTOL
SERIAL NUMBER PREFIX DGD, DGV, DHS, DHT,
DKV, DKW, & DKX

Mr. Don Bulver, warranty department, of Glock, Inc. indicated the recoil springs produced before September 1999 may shear off on some of these pistols due to over hardness of the support tube.

Replacement springs will be distinguished by a vertical mark from the center on the front polymer portion of outer ring of the guide rod assembly, which will appear to be a mold line.

Glock (770) 432-1202
6000 Highlands Parkway
Smyrna, GA 30082

RETIRED

RECALL

Make: Vektor
Model: CP1, CP1N
Caliber: 9mm Luger
Source: American Firearms, Nov/Dec 2000, Vol 28, Num 11:12;
American Handgunner, Jan/Feb 2001, Vol 29, Num 1:114;
American Rifleman, Oct 2000, pg 25;
Shooting Industry, Sept 2000, pg 26;
Company Notice June 24, 2002

VEKTOR - MODEL CP1 AND CP1N, 9MM CALIBER, SEMIAUTOMATIC PISTOL

The Vektor CP1 pistol may accidentally fire when the loaded firearm is dropped or bumped. DO NOT LOAD OR USE THIS PISTOL. To render this pistol safe remove the magazine from the firearm, ensure no cartridge is in the chamber, engage the safety and remove the slide from the frame. No authorized solution to make this pistol safe is currently available. For further information contact:

Vektor, a division of Denel
P.O. Box 5445
Pretoria, 0001
Republic of South Africa

P: 011-27 (12) 620-2616
F: 011-27 (12) 664-1905

RETIRED

RECALL

Make: Colt
Model: CLR3064SRBL
Caliber: 30-06 Springfield
Source: American Firearms, Nov/Dec 2000, Vol 28, Num 11:12;
American Handgunner, Jan/Feb 2001, pg 114;
Colt Website, Sept 1, 2000

COLT - MODEL CLR3064SRBL, 30-06 SPRINGFIELD CALIBER, LIGHT RIFLES

In some rifles, the firing pin may fail to cock, and thus rest on the primer of a chambered cartridge making an accidental drop and discharge possible. Colt is requesting all gun dealers return this model immediately. Colt further states other calibers of the Light Rifle are not affected.

COLT
(800) 962-2658

RETIRED

RECALL

Make: Mossberg
Model: 695
Caliber: 12 GA
Source: American Firearms, Nov/Dec 2000, Vol 28, Num 11:12;
Guns & Weapons for Law Enforcement, Feb 2001, Vol 13, Num 2:60;
Guns & Weapons for Law Enforcement, April 2001, Vol 13, Num 3:25;
O.F. Mossberg & Sons, Inc. Website, Oct 2000

MOSSBERG, MODEL 695, 12 GAUGE, BOLT ACTION SHOTGUNS,
SERIAL NUMBERS M000101 THRU M015304

The Mossberg model 695 bolt action shotguns produced between 1995 and 1996 with serial numbers M000101 to M015304 have been recalled. These shotguns may discharge when closing the bolt during the loading of a live cartridge into the chamber. These firearms should not be loaded or used due to the possibility of accidental discharge.

O.F. Mossberg & Sons, Inc. (888) 920-0486
7 Grasso Avenue
North Haven, CT 06473

RETIRED

WARNING

Make: Bryco Arms
Model: Jennings Nine
Caliber: 9mm Luger
Source: AFTE Journal, Spring 2001, Vol 33, Num 2:145-147;
Illinois State Police Laboratory, Springfield - Notice, Dec 7, 2000

BRYCO ARMS, MODEL JENNINGS NINE, 9MM LUGER CALIBER SEMIAUTOMATIC PISTOL

These pistols may create an **EXTREMELY DANGEROUS CONDITION** and a **POTENTIAL FOR SERIOUS INJURY** by firing without pulling the trigger.

During the testing of a Bryco Arms, Jennings Nine pistol by a forensic firearms examiner it was noted that it would fire upon release of the thumb safety and spontaneously fire in a FULL AUTO MODE on an inconsistent basis. When loaded with the manual thumb safety in the "safe" position, if the trigger of the submitted firearm has been pulled stiffly a few times, the firearm will discharge when the thumb safety is moved to the "fire" position.

Disassembly of the pistol revealed some wear/damage to the sear, which allows slight downward movement when the trigger is pulled. It appears that due to the wear/damage the sear/striker engagement is reduced allowing the striker to override the sear after the thumb safety is released.

MANUALLY UNLOADING THIS PISTOL MAY BE VERY DANGEROUS SINCE IT COULD DISCHARGE DURING THIS PROCEDURE.

RETIRED

RECALL

Make: Czech
Model: 52
Caliber: 7.62mm Tokarev
Source: Shotgun News, Unknown Issue 2000

CZECH, MODEL 52, 7.62MM TOKAREV CALIBER, SEMIAUTOMATIC PISTOL

Some CZ52 pistols (Cal. 7.62 Tokarev) distributed by Century International Arms, identified with the marking "CAI ST.ALB VT" may have a defective hammer drop safety. If your pistol does not have an inspector's mark "Z" on the left rear side of the trigger guard; your safety must be inspected to ensure proper operation in order to avoid possible grave bodily injury and/or property damage.

You must not use the specific pistol until it has been properly inspected to ensure it is working properly.

We will notify all of our customers via certified U.S. mail with instructions for returning their pistols.

We will inspect, repair and return your pistol(s) at no cost to you.

Thank you for your cooperation.

Century International Arms (800) 527-1252
Dept. CZ52R
P.O. Box 714
St. Albans, VT 05478

RETIRED

RECALL

Make: Weatherby
Model: Vanguard
Caliber:
Source: Company Notice May 25, 2001;
Shooting Sports Industry, July/Aug 2001, Vol 19, Num 4:83;
American Rifleman, Sept 2001, pg 94;
SHOT Business, July/Aug 2002, pg 9

WEATHERBY INC., MODEL VANGUARD BOLT ACTION, RIFLES

RETIRED

SERIAL # 00001 - 03810; V0001 - V80966; VX00001 - VX44065; VS0001 - VS23699; VL00001 – VL46984; W0001 – W0099; NV0001 – NV0099

If you have a Weatherby, model Vanguard bolt action rifle with a serial number from one of the serial numbers referred to above: **USE OF THIS RIFLE MAY RESULT IN AN ACCIDENT AND SEVERE INJURY. STOP USE OF THIS RIFLE IMMEDIATELY** and visit the Weatherby website at www.weatherby.com or call Weatherby at 1 (800) 227-2018 to receive a Bolt Return Kit.

Weatherby Inc. of Atascadero, California, is performing a **safety upgrade** on all Vanguard by Weatherby brand rifles manufactured between the years 1970 to 1993. The affected rifles bear serial numbers from one of the following series on the receiver:

00001 – 03810	V00001 - V80966	VX00001 - VX44065
VS00001 - VS23699	VL00001 - VL46984	W001 - W0099
NV001 - NV0099		

The safety upgrade will prevent misassembly of the bolt, which might occur on the affected rifles. Such misassembly might contribute to a hazardous condition in which the rifle can be accidentally discharged without the bolt being fully engaged, causing severe injury. Only the bolt is being upgraded. Bolts will be returned to the owners following the upgrade.

Weatherby (800) 227-2018 Ext. 109
3100 El Camino Real
Atascadero, California 93422

RECALL

Make: Browning
Model: A-Bolt II Stainless
Caliber: 300 Remington Ultra Magnum
Source: Company Notice September, 2001

BROWNING, MODEL A-BOLT II STAINLESS, 300 REMINGTON ULTRA MAGNUM CALIBER RIFLES

No attempt should be made to load or shoot a recalled Browning rifle.

Browning, model A-Bolt II Stainless, 300 Remington Ultra Magnum caliber Rifles with a ten digit serial number beginning with the number 0 are being recalled. Continual and extensive shooting tests have revealed a potential safety problem in this particular model of the A-Bolt rifle. Browning warns all owners of the A-Bolt II Stainless 300 Remington Ultra Magnum rifles to immediately discontinue any further shooting of these rifles and return the entire rifle as soon as possible to Browning at the address listed below.

Browning 1 (800) 727-4312
Attn: Dept. A-Bolt II
One Browning Place
Arnold, Missouri 63010

To verify if the rifle in question had been returned to the factory for the recall please call (800) 333-3288 and Browning will provide assistance.

RETIRED

WARNING

Make: FAL by Williams Arms Co
Model: Williams Alumalite
Caliber: .308/7.62mm
Source: Company Notice Oct 2001 www.dsarms.com/safety_warning.cfm

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FAL .308/7.62MM CALIBER ALUMINUM RIFLE RECEIVERS MANUFACTURED BY WILLIAMS ARMS CO.

On October 2, 2001 testing of the FAL "Williams Alumalite" receivers was terminated when a proof firing caused receivers to blow-up into multiple fragments after less than 200 shots of a scheduled 1,000 shot test.

These receivers were experimental NOT production parts.

For additional information contact:

DSA, Inc.
P.O. Box 370
Barrington, Il 60011
P: 847.277.7258
F: 847.277.7259
dsarms@earthlink.net

RETIRED

WARNING

Make: Bryco Arms
Model: 38
Caliber: 380 Auto
Source: AFTE Journal, Winter, 2001, Vol 33, Num 1:48-49

BRYCO ARMS, MODEL 38, 380 AUTO CALIBER SEMIAUTOMATIC PISTOL

These pistols may create an **EXTREMELY DANGEROUS CONDITION** and a **POTENTIAL FOR SERIOUS INJURY** by firing when dropped.

This model pistol trigger/disconnector bar and sear level may have too small a gap between them such that a sharp movement of the slide rearward could cause the weapon to discharge without the trigger being pulled.

The discharge is created by the moving slide impacting the disconnector and driving the trigger bar/disconnector rearward into the cam lever before it can pivot downward and out of alignment.

RETIRED

WARNING

Make: Jennings
Model: J-22
Caliber: 22 Long Rifle
Source: AFTE Journal, Winter, 2001, Vol 33, Num 1:48-49

JENNINGS FIREARMS, MODEL J-22, 22 LONG RIFLE CALIBER SEMIAUTOMATIC PISTOL

These pistols may create an **EXTREMELY DANGEROUS CONDITION** and a **POTENTIAL FOR SERIOUS INJURY** by firing when dropped.

This model pistol trigger/disconnector bar and sear level may have too small a gap between them such that a sharp movement of the slide rearward could cause the weapon to discharge without the trigger being pulled.

The discharge is created by the moving slide impacting the disconnector and driving the trigger bar/disconnector rearward into the cam lever before it can pivot downward and out of alignment.

RETIRED

WARNING

Make: Lorcin
Model: L380
Caliber: 380 Auto
Source: AFTE Journal, Winter, 2001, Vol 33, Num 1:48-49

LORCIN, MODEL L380, 380 AUTO CALIBER SEMIAUTOMATIC PISTOL

These pistols may create an **EXTREMELY DANGEROUS CONDITION** and a **POTENTIAL FOR SERIOUS INJURY** by firing when dropped.

This model pistol trigger/disconnector bar and sear level may have too small a gap between them such that a sharp movement of the slide rearward could cause the weapon to discharge without the trigger being pulled.

The discharge is created by the moving slide impacting the disconnector and driving the trigger bar/disconnector rearward into the cam lever before it can pivot downward and out of alignment.

RETIRED

RECALL

Make: Howa Machinery by Smith & Wesson, OF Mossberg & Sons, and Interarms
Model: 1500, 1550, 1700LS
Caliber:
Source: American Rifleman, December 2001, pg 20;
North American Hunter, Dec/Jan 2002, pg 8

HOWA MACHINERY LTD., MODEL 1500, 1550, 1700LS SERIES RIFLES distributed by SMITH & WESSON, O.F. MOSSBERG & SONS and INTERARMS IN SERIAL NUMBER RANGES: PN 00010 – PN87159, LS00001 – LS05150, LS 10001 – LS10309, B000001 – B028450, M000001 – M020422.

Howa Machinery Ltd. of Japan is performing a **safety upgrade** on all model 1500, 1550, and 1700LS series rifles manufactured between 1970 and 1993. The affected rifles bear serial numbers from one of the following series on the receiver. PN00010 – PN 87159, LS00001 – LS 05150, LS10001 – LS10309, B000001 – B028450, M000001 – M020422. The safety upgrade will replace the bolt sleeve to prevent misassembly of the bolt, which might occur on the affected rifles. Such misassembly might result in a hazardous condition in which the rifle can be accidentally discharged without the bolt being fully engaged, causing severe injury. These rifles were distributed by Smith & Wesson, O.F. Mossberg and Sons and Interarms. These rifles may bear the distributor trademark. Presently Howa firearms are imported by Interarms of Alexandria, Virginia.

RETIRED

If you own a Howa model 1500, 1550 or LS1700 series rifle with the serial number from one of the serial number series referred to above: **USE OF YOUR RIFLE MAY RESULT IN AN ACCIDENT AND SEVERE INJURY, STOP USING THE RIFLE IMMEDIATELY** and visit our website at www.regcen.com/howa or call Howa at 1 (800) 456-5131 to receive a Bolt Upgrade Kit. Only the bolt sleeve is being upgraded. Your bolt will be returned to you after the upgrade.

NO OTHER HOWA RIFLES ARE AFFECTED. CHECK YOUR SERIAL NUMBER.

Howa Machinery Ltd. Apologizes for any inconvenience to its customers resulting from this safety upgrade program, but it is important that owners of affected rifles take advantage of this free upgrade program and **DO NOT USE AN AFFECTED RIFLE** before it has been upgraded. All work will be done at no charge. Return the bolt only, not the entire rifle. Please do NOT return your bolt before contacting Howa Machinery Ltd. At either www.regcen.com/howa or 1 (800) 456-5131 for your Bolt Return Kit.

WARNING

Make: Glock
Model: all
Caliber: all
Source: www.glocktalk .com February 17, 2002;
www.GSSFonline.com

GLOCK, SEMIAUTOMATIC PISTOLS MANUFACTURED BETWEEN SEPTEMBER 2001 AND MAY 2002 WITH SERIAL NUMBERS STARTING WITH "E", "GSSF" OR "USA" REGARDLESS OF CALIBER HAVE BEEN EXPERIENCING FRAME RAIL FAILURES.

Glock pistols manufactured between September 2001 and May 2002 with serial numbers starting with "E", "GSSF" or "USA" regardless of caliber have experienced FRAME RAIL FAILURES.

The specific issue is the potential of breaking a rear frame rail. Glock notes that under most conditions the pistol will continue to function with three rails.

Glock, Inc. does not officially call this a recall, but an upgrade at no charge. The complete firearm should be returned to Glock for a replacement frames that will have the original serial number with a "1" prefix added to it.

If you believe you have a pistol affected by this notice contact Glock at (866) 225-4098**.**

RETIRED

WARNING

Make: Intratec
Model: Tec-9
Caliber: 9mm Luger
Source: California DOJ Criminalistics Institute Firearms Safety Bulletin, April 2002

INTRATEC, MODEL TEC-9, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

This pistol is subject to **FIRING WITHOUT PULLING THE TRIGGER AND/OR IN FULL AUTOMATIC MODE.**

In recent casework, it was noted a submitted firearm had the back portion of the polymer frame broken off. This damage allows the rear disk cap and pin to come out of the recess in the frame. The upper receiver pivots on the assembly pin and raises the back end of the upper receiver approximately 1/8". The striker disengages from the sear allowing the firing pin to fall and strike the chambered cartridge. The firearm may continue to fire in a FULL AUTOMATIC MODE until the striker is correctly repositioned by the upper receiver returning to the lower position in the frame. The receiver may be lifted manually or by gravity when the pistol is turned on its side.

It was not determined if the damage was intentional or accidental because there were no toolmarks present and no internal parts appeared to be modified.

Questions and comments should be directed to Mike Giusto At CAL DOJ Criminalistics Institute in Sacramento (916) 227-3575 or Michele Merrit CAL DOJ Lab in Riverside (90) 361-5000.

RETIRED

RECALL

Make: Remington
Model: 700, 600, 660, 721, 722, 40-X, XP-100 Target
Caliber:
Source: North American Hunter, Aug 2002, pg 103

REMINGTON -
MODEL 700, CENTER FIRE RIFLES MFG BEFORE 1982
MODEL 600, 660, 721, 722 40-X RIFLES MFG BEFORE MARCH 1982
MODEL XP-100 TARGET PISTOL MFG BEFORE FEBRUARY 1975

Remington Arms Company, Inc. is offering a safety modification program for certain bolt-action centerfire firearms manufactured prior to 1982, including the Model 700, Model 600, 660, 721, 722, 40-X bolt-action rifles (made before March 1982) and Model XP-100 target pistols made before February 1975.

These firearms have a feature known as a bolt-lock that requires the safety to be placed in the "off" position in order to unload the gun. If you participated in this program, your firearm will be modified to eliminate the bolt-lock feature. The operation of your gun will not be otherwise affected.

If you want assistance, call Remington's toll-free consumer hotline at (877) 387-6691. To learn more about the 1979 safety recall, check our website at www.remington.com

Offer valid through December 31, 2002

Remington
870 Remington Drive
Madison, North Carolina 27025-0700

RETIRED

RECALL

Make: Remington
Model: 710
Caliber:
Source: www.remington.com/safety/model_710_notice/ 2002

REMINGTON, MODEL 710, BOLT ACTION RIFLES

These rifles may be subject **to unintentional discharge** as they have been assembled with an improperly made "Safety Detent Spring."

Remington Arms Company, Inc. is voluntarily recalling a limited number of the MODEL 710 BOLT ACTION RIFLES as a result of its discovery, that some Model 710 rifles manufactured between July and October 2002 may have been assembled with an improperly made "Safety Detent Spring." Although unlikely, there is a possibility that the manual safety arm mechanism in such a rifle could fail to fully return to the "safe" or "on" position.

WARNING: Use of a rifle with an improperly functioning safety could lead to an accidental discharge, resulting in personal injury, death or property damage.

Contact Remington by calling 1-800-243-9700, or 1-336-548-8700, Monday through Friday 9 AM – 5 PM EST.
If you own a Model 710 rifle please enter your serial number on the Remington Website widget to determine if your firearm needs to be inspected or repaired as part of the recall.
Remington apologizes for this inconvenience, but wants its customers to enjoy the shooting sports safely. It is important, therefore, that owners of Model 70 rifles subject to this recall participate in this program and do not use their rifles before they have been inspected and/or repaired.

RETIRED

RECALL

Make: Norinco
Model: Dragunov
Caliber: 308 Winchester
Source: http://www.cdnninvestments.com/dragunovrecall.html

NORINCO, MODEL DRAGUNOV, 308 WINCHESTER CALIBER, RIFLE

It has been determined that certain commercial ammunition may produce a slam fire condition causing the firearm to fire out of battery. This can result in injury or death to a bystander or the shooter. A simple modification to the bolt assembly can easily remedy the situation.

Please have your serial number ready when you call.

CDNN Investments, Inc.
PO Box 6514
Abilene, TX 79608
Telephone: 800 588-9500
Fax: 325 695-4898

RETIRED

RECALL

Make: Blaser
Model: R93
Caliber: all
Source: SIGARMS, Inc. Website, Aug 5, 2002;
SIGARMS, Inc. Consumer Subscription E-Mail Newsletter, Vol C2, Num 3:9;
American Hunter, Oct 2002, pg 20;
American Rifleman, Nov 2002; pg 89;
Shooting Sports Retailer, Sept/Oct 2002, page 59

BLASER, MODEL R93 RIFLES

RETIRED

Warning: Use of your R93 rifle may result in an accident severe injury or death to the user or bystanders. **Stop using your R93 immediately.** Do not use your rifle until it has been inspected.

Blaser has been committed to designing and manufacturing the safest most reliable firearms in the world since 1963. In keeping with this commitment to our customers, Blaser is conducting a recall of its R93 rifles sold in the US.

What is the Problem?

The R93 trigger assembly uses stainless steel pins for corrosion resistance. We have learned that a small number of non-stainless steel pins were inadvertently used in some R93's. **Note: the UIT, CISM, and LRS/LRS2 are not affected by this recall.**

What could happen?

An accidental discharge from the off safe position may occur if an R93 with non-stainless steel pins is used and the pins have corroded due to moisture. In this situation, once the firing pin has been cocked (into the off-safe position), the rifle may discharge.

What should you do to ensure your safety?

Removal of the trigger housing and trigger subassembly for inspection is required. This inspection should only be made by SIGARMS Inc., Blaser's US representative, located in Exeter, New Hampshire. Call the recall hotline number below to coordinate the inspection of your R93 rifle: **Please contact us toll-free at1-877-442-7671 (8 a.m. - 5 p.m. EST).**

What will we do for you?

Upon calling, we will inform you how to return your R93 and give you an estimated time frame for return. Once we have your rifle in our facility, we will conduct a complete inspection of your R93 and replace your pins if they are not made from stainless steel. We will also clean and ensure your R93 is ready for more adventures. Please do not contact the dealer where you purchased the R93, as we will serve you directly. We will return your serviced R93 to you along with an expression of our appreciation.

We recognize the inconvenience this situation causes our Blaser R93 customers and offer you our sincerest apologies. We are passionate about your safety and we are confident that you would expect nothing less from Blaser.

WARNING

Make: Bersa
Model: 86
Caliber: 380 Auto
Source: Connecticut State Police Laboratory, Oct 22, 2002

BERSA, MODEL 86, .380 AUTO CALIBER, SEMIAUTOMATIC PISTOL

During laboratory examination and test firing this pistol slide separated from the frame. Examination of the slide disclosed the lower forward muzzle portion had sheared off during test firing.

This pistol serial number was 246184 and was imported by C.M.I. of Miami, Florida.

Caution should be exercised and safety rules should always be followed whenever firing any firearm.

RETIRED

RECALL

Make: Smith & Wesson
Model: SW 1911
Caliber: 45 Auto
Source: Gun Week, Oct 20, 2003, pg 3;
Smith & Wesson Website;
American Rifleman, July 2004:37;
Gun List, July 28, 2006

SMITH & WESSON, MODEL SW 1911, 45 AUTO CALIBER SEMI-AUTOMATIC PISTOL

These make and model pistols may have a defective firing pin safety plunger, which could cause the firearm to fail to fire reliably.

Smith & Wesson indicates the recall is prompted by reports that the firing pin safety plunger of the SW 1911 pistol can become disabled, creating a situation where a jam may occur, rendering the firearm inoperable. This only pertains to certain SW 1911 pistols bearing serial numbers JRD0000 to JRD4750.

In no case has this issue resulted in injury, and the company has not received any reports of accidental discharge associated with the functioning of the firing pin safety plunger. However, failure of the firing pin safety plunger may affect the reliability and safety of the firearm.

Call (800) 331-0852 to receive additional details on the free repair program for this recall.

RETIRED

WARNING

Make: North American Arms
Model: Revolvers
Caliber: 22 caliber
Source: NAA Company Notice, May 17, 2004

NORTH AMERICAN ARMS, INC., 22 CALIBER, REVOLVERS

This Product Performance Advisory Bulletin is issued due to the potential of an **INADEVERTANT, DOUBLE DISCHARGE** (two rounds simultaneously firing. One aligned with the barrel and the other out of battery). This notice is for the benefit of NAA's customers, owners and users of its 22 caliber revolvers. NAA has recently learned that the use of PMC 22 caliber ammunition (Magnum and LR) may affect the performance of its revolvers.

At this time, NAA recommends that its customers, owners and users of NAA 22 revolvers **DO NOT USE ANY PMC 22 CALIBER** ammunition (Magnum and/or LR) in any model NAA revolvers.

If you have any questions concerning this Product Performance Advisory Bulletin, please contact NAA at (800) 821-5783 or write to North American Arms, 2150 South 950 East, Provo, Utah 84606-6285, Attention General Manager.

RETIRED

RECALL

Make: Tikka
Model: Sako
Caliber:
Source: FirearmsID.com Website

TIKKA BRAND, MODEL SAKO, STAINLESS STEEL RIFLE

A limited number of recently delivered Sako and Tikka stainless steel rifles have been recalled due to a problem discovered in the steel used in the barrels.

The **lowest serial number** affected by this recall is **419140** and the **highest serial numbered** affected is **461951**; however, only a small percentage of rifles within this range of numbers is being recalled. In addition, no rifle shipped before February 2004 is involved in the recall.

Because only a limited number of recently shipped rifles are involved in the recall, customers being asked to return their rifles are being contacted directly.

If you have a stainless steel Sako or Tikka rifle within the serial number range listed above, received by you after February 28, 2004, and you want to check and determine whether it is being recalled, please call 1-800-503-8869.

RETIRED

WARNING

Make: Bryco Arms
Model: 58
Caliber: 9mm Luger
Source: AFTE Journal, Spring 2004, Vol 36, Num 2:149

BRYCO, MODEL 58, JENNINGS ARMS, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

A Jennings Arms, Bryco, model 58, 9mm Luger caliber, semiautomatic pistol was submitted for examination. During initial function and safety examination of the pistol, nothing was noted that would indicate the pistol was unsafe to test fire. During test firing the front portion of the slide broke completely off the pistol and landed inside the water tank with the recoil spring. This situation is extremely dangerous and could result in serious injury.

RETIRED

RECALL

Make: Kahr Arms
Model: PM9
Caliber: 9mm Luger
Source: Company Notice, Feb 2005

KAHR ARMS, MODEL PM9, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL BARRELS

Kahr Arms, model PM9, 9mm Luger caliber, semiautomatic pistol barrels within the serial number range below are being recalled in order to repair or replace barrels that were found to not properly feed some brands of hollow point ammunition, which under certain circumstances may be unsafe to the user. If you possess a Kahr PM(3" barrel within the serial number ranges listed below, please return the barrel only with a tag indicating the serial number of the gun. Barrels received will be repaired or replaced and shipped back within 7 business days.

Please return the barrel and your name and address to:
Kahr Arms
130 Goddard Memorial Drive

Worcester, MA 01603
Attn: Dorothy Herold

If you have any questions or comments regarding this notice, please contact David Konn, Director of Dealer Development at 561-656-1288.

RETIRED

MODELS	PM9093A	PM9093NA	PM9094A	PM9094NA
SERIAL #'S	VA0003 TO	VA9999	VB004 TO	VB0999

WARNING

Make: Glock
Model: 35
Caliber: 40 S&W
Source: FirearmsID.com Website, April 26, 2005

GLOCK, MODEL 35, 40 S&W CALIBER, PISTOL MAGAZINES

It was recently discovered that some magazines issued for the Glock, model 35, 40 S&W caliber semi-automatic pistol might cause a jam upon feeding the last cartridge from the magazine into the chamber of the pistol. This leaves the pistol with a slide partially open and the nose of the cartridge firmly jammed against the feed ramp.

The effected magazines have a number"5" molded into the follower. Glock has produced new magazines for these pistols that have a new follower that now has a number "6" molded into the follower. The new design of the follower now holds the last cartridge at a higher angle and should eliminate the feeding issue.

It is unknown at this time if a recall has been issued by the company or if they will replace the magazines at no cost to the customer.

RETIRED

WARNING

Make: Mossberg
Model: 500, 600
Caliber: 12 GA
Source: AFTE Website 2005

MOSSBERG, MODEL 500/600, 12 GAUGE, PUMP SHOTGUN

These Mossberg may be subject to JAR-OFF accidental discharge. Using a primed shotshell, the shotgun cocked, loaded and struck about the receiver assembly with a hard rubber mallet, the shotgun did not fire.

When the flat area of the plastic trigger assembly was struck just behind the trigger guard the gun fired the primed shotshell. This was tried on several different Mossberg 500/600 shotguns, all discharged.

RETIRED

RECALL

Make: FN
Model: FS2000
Caliber: 5.56mm
Source: Company Notice 7/21/2006

FN, MODEL FS2000, 5.56MM CALIBER, SEMI AUTOMATIC CARBINE RIFLES

These rifles may be subject **to unintentional SLAM-FIRE discharge** with civilian/commercial ammunition as they have been assembled with heavier designed military firing pins.

Should you have any questions regarding this recall, please contact Terri Rife at FNH USA by calling (800) 635-1321, extension 252. Shipping kits will be sent to customers with instructions on how to return their FN FS2000 firearms. The firearm will be upgraded at no charge and returned to the customer as soon as possible.

FNH USA LLC, PO Box 697 McLean, VA 22101, www.fnhusa.com

RETIRED

RECALL

Make: Smith & Wesson
Model: 329
Caliber: 44 Magnum
Source: Gun List, July 28, 2006;
Smith & Wesson Website, Dec 19, 2009

SMITH & WESSON, MODEL 329, 44 MAGNUM CALIBER, REVOLVER

It has been determined that damage to the frame may have occurred during assembly of the single piece barrel. As a result, there exists the possibility that the revolver may rupture during firing, causing failure of the firearm and creating a risk of physical injury.

You should immediately stop using the revolver and return it to Smith & Wesson. Upon return Smith & Wesson will issue a refund or a credit towards the purchase of a replacement firearm. Please contact Smith & Wesson directly at 1-800-331-0852 to arrange for return of your firearm.

This safety recall notice applies to the limited edition model 329 revolver manufactured at the Smith & Wesson performance center.

This safety recall notice applies to 270 revolvers bearing **SERIAL NUMBERS** – ranging from **BVA0004 to BVA0210** and **DEW0001 to DEW0099**.

RETIRED

RECALL

Make: Smith & Wesson
Model: 460 PC
Caliber: 460 S&W Magnum
Source: Shot Business, Dec 2006, pg 11;
Guns, Jan 2007, pg 66

SMITH & WESSON, MODEL 460 PC, 460 S&W MAGNUM CALIBER, REVOLVER

Barrel failures are limited to the Performance Center Model 460 Revolvers shipped prior top September 18, 2006. The affected are within the following serial number ranges:

EBD001 – EBD0524
EBF002 – EBF0251
LHZ0000- LHZ0511
LLL0000 – LLL0702
LRR001 – lRR0552
MMM0001 – MMM1809
XMC001 – XMC0109

Owner currently having these revolvers should immediately contact the company at (800) 331-0852 EX. 3800 or through the website at www.smith-wesson.com

RETIRED

WARNING

Make: Glock
Model: 22
Caliber: 40 S&W
Source: AFTE Website 2006

GLOCK, MODEL 22, .40 CALIBER, FULL AUTOMATIC PISTOL

On January 9, 2006 Charlotte-Mecklenburg Police Department seized a Glock, Model 22, .40 Caliber pistol. Laboratory examination revealed a small fin had been welded to the slide converting the pistol to FULL AUTOMATIC FIRING.

EXTREME COUTION SHOULD BE EXERCISED AS THIS PISTOL ONCE FIRED WILL CONTINUE TO FIRE UNTIL THE AMMUNTION SUPPLY IS EXHAUSTED OR THE GUN MALFUNCTIONS.

RETIRED

WARNING

Make: Smith & Wesson
Model: 4006TSW
Caliber: 40 S&W
Source: Gun Digest Magazine May 18, 2007:12

SMITH & WESSON, MODEL 4006TSW, 40 S&W CALIBER SEMIAUTOMATIC PISTOL

The California Highway Patrol will replace the sear catches on 3,000 new sidearms it purchased in 2006 from Smith & Wesson after two sears broke during training.

The sear catches on the Smith & Wesson 4006TSW pistol is being replaced as a precautionary measure in all of the guns delivered to the CHP since last summer under a 9,736 pistol, multiyear contract. The pistols will be returned to CHP gunsmiths, who will replace the parts with the assistance from Smith & Wesson.

RETIRED

RECALL

Make: Smith & Wesson
Model: i-Bolt
Caliber:
Source: Smith & Wesson Website, Jan 14, 2008

SMITH & WESSON, i-BOLT RIFLE

Smith & Wesson has discovered that the sear engagement pin in certain i-Bolt action rifles does not meet specifications. The sear engagement pin is part of the fire control unit for the rifle which is manufactured for Smith & Wesson by an outside vendor. In certain instances this may lead to a failure of this component and result in an unintended discharge of the rifle.

This recall applies to only 597 i-Bolt rifles manufactured between October 18, 2007 and December 12, 2007 with selected serial numbers between IBR0223 and IBR6992 To determine if your rifle is part of this recall, please check your serial number and contact Smith & Wesson directly at 1 (800) 713 0356. If your rifle is part of this recall, Smith & Wesson will inspect and replace the pin free of charge.

RETIRED

RECALL

Make: Ruger
Model: SR9
Caliber: 9mm Luger
Source: Ruger Website April 11, 2008;
Gun Digest, June 9, 2008

RUGER, MODEL SR9, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOLS

DO NOT USE YOUR RUGER SR9 PISTOL

We have determined that some Ruger SR9 pistols manufactured between October 2007 and April 2008 can, under certain conditions, fire if dropped with their manual safeties in the "off" or "fire" position. The pistols will not fire if the manual safety is in the "on" or "safe" position. We will retrofit all Ruger SR9 pistols starting with serial number prefix "330" (330-xxxxx) with these new parts **at no charge** to our customers.

In order to ensure correct fitting, the new parts must be installed at our Ruger factory in Prescott, Arizona. We will remove the old parts and install the new trigger group promptly, at no charge, and will return the pistol to you. The old parts will not be returned.

Step 1 - Contact us and provide your name, address, telephone number and SR9 serial number.

Provide your information by any of the following:	
Website:	SR9 Recall On-line Form
E-mail:	SR9Recall@ruger.com
Fax:	(928) 541-8873
Phone:	SR9 Recall Hotline **1-800-784-3701** (available Monday - Friday, 8:00 a.m. to 8:00 p.m. EDT)

Step 2 - When we are ready to retrofit *your* SR9, we will send you a shipping label and shipping box with instructions so you can return your pistol to us **FREE** of charge.

Step 3 - We will install the new trigger group in your SR9 and return it to you **FREE** of charge. When we do, we also will send you a FREE extra magazine as a "thank you" for your patience and cooperation. We will make every effort to return your pistol within one week, so we will not ask you to send it to us until we are ready to receive it. We expect to begin sending shipping labels and boxes in mid-May.

Do not load or fire your pistol until it has been factory retrofitted with these new parts! If you must fire your pistol, be sure to keep the manual safety in the "on" or "safe" position except when you are actually firing.
Thank you,
Sturm, Ruger & Co., Inc.

RETIRED

RECALL

Make: EAA Corp
Model: Witness
Caliber:
Source: Guns & Week, Aug 16, 2008, pg 6;
Guns & Week, Sept 1, 2008, pg 2

EAA CORP., WITNESS SEMI AUTOMATIC PISTOL

RETIRED

European American Armory Corporation has requested owners of Witness semi-automatic pistols bearing a **serial number between AE00000 to AE70000** immediately field strip the pistol and send the slide assembly to:
European American Armory Corp.
411 Hawk Street
Rockledge, FL 32955

Upon receipt they will replace the original firing pin with an upgraded firing pin for your pistol. If the original firing pin is installed incorrectly in an upside down position, it may adversely affect the operation of your pistol and its safeties. Please call European American Armory Corp. at (800) 536 4442 if you have any questions regarding this issue.

RECALL

Make: Smith & Wesson
Model: i-Bolt
Caliber:
Source: Smith & Wesson Website, November 26, 2008

SMITH & WESSON, i-BOLT RIFLE

Smith & Wesson has identified a condition that may exist with the bolt supplied with some rifles that would allow the striker to become disengaged from its locking tabs during the cycling of the bolt, allowing the rifle to fire without the trigger being pulled.

This recall applies to the bolts of all i-Bolt rifles manufactured prior to December 1, 2002. To facilitate the inspection and repair of your rifle bolt please contact Smith & Wesson customer service department to receive instructions and a call tag for the return of your bolt to Smith & Wesson.

When your rifle bolt is returned, you should note a punch dot on the handle of the bolt to confirm it is safe for use.

Please contact Smith & Wesson directly at 1 (800) 713 0356 if you have any questions about this recall.

RETIRED

RECALL

Make: Ruger
Model: LCP
Caliber: 380 Auto
Source: Ruger Website Dec, 2008;
Gun Digest, Dec 22, 2008

RUGER, MODEL LCP, 380 AUOT CALIBER, SEMIAUTOMATIC PISTOLS

Ruger has received a small number of reports from the field indicating that LCP pistols can **accidently discharge** when dropped onto a hard surface with a round in the chamber.

Ruger will retrofit upgrade hammer mechanisms at no charge to customers with LCP pistols bearing serial number prefix 370. Pistols marked with a diamond stamped on the flat portion of the slot just behind and below the hammer and those with the prefix 371 are not affected.

Contact Ruger's LCP Recall Hotline; (800) 784 3701, from 9 am to 8 pm EST, Monday through Friday. Or, send faxes to (928) 541 8873, e mail LCPRecall@ruger.com or log onto www.ruger-firearms.com:80/LCPRecall/index.html.

RETIRED

RECALL

Make: Smith & Wesson, Walther
Model: PPK, PPK/S
Caliber: 32 ACP, 380 Auto
Source: Smith and Wesson Website, Feb 20, 2009

SMITH & WESSON MANUFACTURED, WALTHER, 32 ACP/380 AUTO CALIBER, MODEL PPK SEMI AUTOMATIC PISTOLS & 380 AUTO CALIBER, MODEL PPK/S SEMI AUTOMATIC PISTOLS

THESE PISTOLS MAY PERMIT A ROUND TO BE DISCHARGED WITHOUT THE TRIGGER BEING PULLED.

Smith & Wesson has issued a recall of all Walther PPK and PPK/S pistol manufactured from March 21,2002 until February 3, 2009 because then the manual safety is disengaged the possibility exists in certain firearms that the lowering of the hammer may cause a chambered round to fire.

The serial number s of the pistols subject to this recall are as follows:

0010BAB – 9999BAB
0000BAC – 9999BAC
0000BAD – 9999BAD
0000BAE – 9999BAE
0000BAF – 9999BAF
0000BAH – 9999BAH
0000BAJ – 9999BAJ
0000BAK – 9999BAK
0000BAL – 5313BAL
0000BAM – 1320BAM
0000LTD – 0499LTD
0001PPK – 1500PPK
0026REP – 0219REP
0001WLE – 0459WLE

Contact Smith & Wesson customer service department directly at 1 800 331 0852 for replacement of the existing hammer block feature with a new part at no cost to the owner. A punch dot at the back of the frame will verify that the new hammer block has been installed and the pistol is safe to handle.

RETIRED

RECALL

Make: Smith & Wesson
Model: 22-A
Caliber:
Source: Smith and Wesson Website, May 29, 2009

SMITH & WESSON, MODEL 22-A SEMI AUTOMATIC PISTOL

Smith & Wesson has identified a condition that may exist in certain Model 22-A pistols that the slides manufactured from August 1, 2008 to February 19, 2009, may not meet the design specifications. This can create a situation where insufficient headspace exists creating a risk of unintended discharge.

This recall applies only to 22-A pistols within the following serial number range:

UBW0000 – UBW9999 7 UBY0000 – UBY4104

Please contact Smith & Wesson customer service department for instructions on the return of your pistol. When your pistol is returned, you should note a punch dot which appears on the center rear underside of the slide. This punch dot can be seen by locking the slide to the rear and looking at the underside.

If you have any questions about this recall, please contact Smith & Wesson at 1 800 331 0852 or e mail us at 22a@smith-wessom.com.

RETIRED

RECALL

Make: Knight Rifles
Model: Revolution and Revolution II
Caliber:
Source: Company Website Notice, July 2009

KNIGHT RIFLES, REVOLUTION & REVOLUTION II MUZZLELOADING RIFLES

Knight Rifles has received a small number of reports from the field of Revolution muzzleloading rifles **accidently discharging** as the user closed the action. Customers are asked to return their rifles for inspection. All Revolution and Revolution II rifles are being recalled for inspect and repair of the trigger mechanisms.

To register click on the web link “Add another rifle”, complete form and click the submit button. This will initiate a process whereby a trigger assembly kit with prepaid return labels will be sent to your address.

For more information check: www.knightrifles.com/revolutionrecall/

RETIRED

WARNING

Make: Magnum Research
Model: Magnumlite
Caliber: 17 HMR
Source: Company Notice, July 15, 2009; Company Website http://www.magnumresearch.com/MagnumLite_HMRNotice.asp, 4/28/10

MAGNUM RESEARCH, MODEL MAGNUMLITE, 17 HMR CALIBER, SEMI AUTOMATIC RIFLE

It has come to Magnum Research's attention that certain lots of 17 HMR ammunition may exhibit performance problems that could result in damage to your rifle and may be capable of causing injuries to shooters and/or bystanders.

For any customer who has purchased a Magnum Research 17HMR rifle, and has a concern about this issue, please contact our Customer Service Department at 1-800-772-6168, Monday-Friday 8:30- 4:30 CST. By your request, if you decide to return your rifle, Magnum Research will either refund the purchase price of the rifle, or replace the rifle with an equivalent one in a different caliber.

RETIRED

RECALL

Make: Colt
Model: 1911 WWI Replica, 1918 WWI Replica, Combat Elite, New Agent, Defender, Talo Night Defender
Caliber:
Source: Gun Digest Magazine; Aug 17, 2009, pg 5;
Gun Week: Aug 1, 2009

RETIRED

COLT,

MODEL 1911 WWI REPLICA (01911) SEMI AUTOMATIC, PISTOL

MODEL 1918 WWI REPLICA (01918) SEMI AUTOMATIC, PISTOL

MODEL COMBAT ELITE (08011XSE) SEMI AUTOMATIC PISTOL

MODEL NEW AGENT (07810D) SEMI AUTOMATIC PISTOL

MODEL DEFENDER (07000D) SEMI AUTOMATIC PISTOL

MODEL TALO NIGHT DEFENDER (07000NDF) SEMI AUTOMATIC PISTOL

Colt's Manufacturing Company is recalling certain Colt pistols sold since March 2007. Colt has determined that the Slide Lock Safety and/or Recoil Spring Guide Pad in these pistols were not manufactured to Colt specifications and must be replaced.

The Safety and/or Guide Pad in these models were recently found to be of improper hardness. As a result, the Safety may prematurely wear or be prone to break, especially if the pistol is dropped, and might not prevent an accidental firing. The Guide Pad might crack and possibly prevent the pistol from firing.

The range of serial numbers of these models covered by the recall is as follows:

Model	Serial # Range	Safety	Guide Pad
1911 WWI REP. (01911)	4597WMK to 5414WMK	X	
1918 WWI REP. (01918)	1001WWI to 3431WWI	X	
COMBAT ELITE (08011XSE)	CG10000E to CG11293E	X	
NEW AGENT (07810D)	G01001 to GT04505	X	X
DEFENDER (07000D)	DR33036 to DR35948		X
TALO NIGHT DEFENDER (07000NDF)	NDF0001 to NDF0400		X

Colt has offered to replace the affected parts at no cost to its customers. Instructions for obtaining the recall service or replacement parts are posted on Colt's website at : cltsmfg.com, and can also be obtained by e mailing Colt at: Recall@colt.com, or by calling Monday- Friday, 8:30 am - 12 pm or 1 pm – 5 pm EST, at: 800 962 2658, and pressing Option "2".

RECALL

Make: Bersa Lasergrips
Model: LG-442 for Thunder and Firestorm
Caliber:
Source: Gun Week Aug 1, 2009

BERSA, LASERGRIPS, FOR MODEL LG-442 THUNDER AND FIRESTORM, SEMIAUTOMATIC PISTOLS

The Lasergrips panel may allow the trigger transfer bar spring to completely detach from the trigger transfer bar, thus disarming the trigger and rendering the gun inoperable.
Customers have the option of choosing a new ALG-442 unit in exchange for the defective one, of they may choose another Crimson Trace laser sight model of equivalent value, once the defective unit has been returned to Crimson Trace. Customers should contact Crimson Trace at receive a return authorization and instructions on how to execute the no-cost recall: telephone: 800 442 2406 or email: LG442recall@crimsontrace.com.

Caution Lasergrip may make pistol inoperable.

RETIRED

WARNING

Make: Remington
Model: 597
Caliber: 17 HMR
Source: Company Notice, Aug 18, 2009;
Gun Week, Sept 15, 2009, pg 6

REMINGTON, MODEL 597, 17 HMR CALIBER, SEMI AUTOMATIC RIFLE

RETIRED

Remington has been notified by its supplier of 17 HMR ammunition that 17 HMR ammunition is not suitable for use in semi-automatic firearms. The use of this ammunition in a semi-automatic firearm could result in property damage or serious personal injury.

If you have a semi-automatic firearm chambered for 17 HMR ammunition, immediately discontinue use of Remington 17 HMR ammunition. If you have any Remington 17 HMR ammunition that you wish to return to Remington contact the Remington Consumer Service number below. Do not return the ammunition to the dealer. Remington will provide you with a $10.00 coupon for each complete box of 50 rounds of Remington branded 17 HMR ammunition you return to Remington. This coupon will be good for the purchase of any Remington ammunition at your local dealer.

In light of the ammunition manufacturer's notice, it is very important that you immediately stop using your Remington Model 597 17 HMR semi-automatic rifle. If you own a Remington Model 597 17 HMR semi-automatic rifle and wish to return it to Remington please contact the below Remington Consumer Service Number. In return for your Remington Model 597 17 HMR synthetic stock semi-automatic rifle, Remington will provide you a coupon valued at $200.00 good for the purchase of a replacement Remington firearm. If you have a laminate stock Remington Model 597 17 HMR semi-automatic rifle, Remington will provide you a coupon valued at $250.00 good for the purchase of a replacement Remington firearm. Remington will also reimburse you for the actual postage to return your Model 597 17 HMR semi-automatic rifle to Remington.

Please allow up to 6 weeks after Remington receives your Model 597 17 HMR semi-automatic rifle or your Remington branded 17 HMR ammunition for the appropriate coupons to arrive. Instructions for redemption of the coupons will be contained on the coupon.

For any consumer questions or instructions on how to return of your Model 597 17 HMR semi-automatic rifle or your Remington branded 17 HMR ammunition, please contact the Remington Consumer Service Department at 1-800-243-9700, Prompt #3.
We apologize for any inconvenience this may cause.

RECALL

Make: Romanian Tokarev
Model: TT33
Caliber:
Source: Company Letter: Jan 2010

ROMANIAN, TOKAREV, TT33 SEMI AUTOMATIC PISTOL

I.O. Inc. has become aware that a small number of Romanian Tokarev TT33 semi automatic pistols imported in October 2009 have defective safety devices.

I.O. Inc**.** has set up the following procedure outlined below for owners of these pistols:

1. Call the Customer Service team at (866) 882 1479 and request an RMA number.
2. You will be issued a Call Tag. When it arrives,
3. Package the pistol for shipping
4. FedEx will pick it up and return it
5. Your TT33 pistol will be returned to you once any necessary repairs have been made

I.O. Inc**.**
3305 Westwood Industrial Drive
Monroe, NC 28110
(704) 225 8843
(866) 882 1479

David J. Moses
Vice President

RETIRED

RECALL

Make: Tactilite
Model: T-1 Conversion Kit for AR-15 Style Rifles
Caliber: 50 BMG
Source: Tactilite Website, Feb 2010

TACTILITE, T-1 CONVERSION KIT FOR AR-15 STYLE, 50 BMG CALIBER RIFLES

Recall Notice – Potential for Very Serious Injury. If you have a Tactilite T1 50 BMG with a bell-shaped handle with 3/16" threading and a roller, there is the potential for out of battery detonation due to over tightened of the bolt handle which may bind the firing pin.

We ask that you contact us immediately to arrange for free replacement components.

www.tactilite.com

RETIRED

RECALL

Make: Sig Sauer - Lasergrips
Model: P230, P232 – LG-432
Caliber:
Source: Crimson Trace Corporation Website, April 2010

SIG SAUER. MODEL P230/P232 PISTOL REPLACEMENT LG-432 LASERGRIPS

Crimson Trace has received a small number of reports that the LG-432 Lasergrips for Sig Sauer P230 and 232 semi automatic pistols may allow the mainspring to detach when the magazine release is depressed without the magazine in place, **rendering the firearm inoperable**.

Crimson Trace is initiating a 100% recall of all units produced and an upgraded LG-432 grip design has been modified to prevent this from occurring. Customer should contact Crimson Trace to receive a return authorization and instructions on how to execute the no-cost recall:
Telephone : 1 800 442 2406

E Mail: LG432recall@crimsontrace.com

Fax: (503) 783 5334

RETIRED

RECALL

Make: Sig Sauer
Model: P238
Caliber:
Source: Sig Sauer Website, April 2010

SIG SAUER. MODEL P238, SEMI AUTOMATIC PISTOL

Sig Sauer has initiated a Mandatory Safety Upgrade for Model P238 semi automatic pistols between serial number DA000501 and DA003216 that may have safety levers that are not manufactures to factory specifications. Under certain conditions, it may be possible for the lever to not be completely engaged in the safe position. In this condition, the gun will not fire when the trigger is pulled. However, when the safety lever is moved to the off position, the hammer may fall, with the remote possibility that the gun could fire unintentionally, thus creating a risk of injury or death.

Call the UPGRADE HOT LINE at 1 (877) 446 1914 so an operator can take your information and confirm if your firearm is required to be returned at no cost to the owner.

If you have questions or would like further information, you may contact Sig Sauer Customer Service at 603 772 2302 (Choose Option # 3).

RETIRED

RECALL

Make: Beretta
Model: NEOS
Caliber: 22 caliber
Source: Beretta Website, Sept 2010

BERETTA, MODEL NEOS, 22 CALIBER SEMI AUTOMATIC PISTOL

Beretta USA Corp has discovered a potential condition with Beretta 22 caliber NEOS semiautomatic pistols in which the **pistol will fire even if the safety is activated** and, in some cases, the **pistol may fire if the safety is moved from the OFF to the ON position**.

If you have a Beretta NEOS pistol, please send it to our Accokeek Maryland Service Center for repair. Instructions on how to send your NEOS to Beretta is available at www.berettasupport.com/neos or by calling 1-800-BERETTA.

Your pistol will be retrofitted, at no cost to you, with a replacement firing pin assembly. The new firing pin assembly can be identified either by a circle on the back of the firing pin that includes a red dot in the center, or by a vertical line.

RETIRED

RECALL

Make: Bushmaster
Model: ACR
Caliber:
Source: Bushmaster Website, Oct 2010

BUSHMASTER, MODEL ADAPTIVE COMBAT RIFLE (ACR), RIFLE

FULLY AUTOMATIC FIRING WITH THE SINGLE PULL OF THE TRIGGER

Bushmaster has become aware of a possible firearms performance issue that may develop with a small number of the ACR rifles and request immediate discontinued use of this rifle. During routine test firing, Bushmaster discovered a design flaw which could result in multiple rounds continuously when the trigger is pulled.

Contact the Customer Service Department at 1 800 883 6229, (Monday – Friday 8:30 am – 6 pm EST). Return shipping instructions will be provided with a RMA# (Return Authorization Number). The Corrective Actions Plan will process your rifle at no cost to you.

Bushmaster Firearms International, LLC
999 Roosevelt Trail
Windham, ME 04062
customerservice@bushmaster.com

RETIRED

RECALL

Make: Remington
Model: 700, 40X
Caliber:
Source: Remington Website, Oct 2010

REMINGTON, MODEL 700, RIFLE AND MODEL 40-X, RIFLE

Remington Model 770 and Model 40-X made before March 1982 were manufactured with a bolt-lock mechanism. Model 770 and Model 40-X made after March 1982 were manufactured without a bolt-lock mechanism so they may be loaded or unloaded with the safety in the "S" or "on Safe" position.

If your Model 700 or Model 40-X has a bolt-lock mechanism, it is eligible for this bolt-lock Safety Modification Program

Call Remington toll-free at 1877 387 6691 and a service representative will help you determine if your firearm has a bolt-lock mechanism. Your firearm will be cleaned and inspected by a qualified gunsmith. If your firearm is in good condition the gunsmith will remove the bolt-lock feature so that your rifle can be loaded and unloaded while the safety remains in the "S" or "on Safe" position.

The total cost to you is $20 plus shipping.

RETIRED

RECALL

Make: Remington
Model: Versa Max
Caliber:
Source: Remington Website, Nov 12, 2010

REMINGTON, MODEL VERSA MAX, SHOTGUN

Remington has found a limited number of its Versa Max shotguns may have hammers out of specification. This condition may result in inconsistent firearm performance that may result in property damage, serious personal injury or death.

If you have a Remington Versa Max shotgun immediately discontinue use of the shotgun and contact Remington at 1800 243 9700, Prompt #4. Remington will arrange for the return shipment of your Versa Max shotgun, inspect, retrofit and return it at no cost to you. All retrofitted shotguns with the enhanced hammer will contain a "V" stamped that can be seen by looking in the ejection port or through the carrier.

RETIRED

RECALL

Make: Glock
Model: G-17, G-17T, G-19, G-21, G-22, G-23, G-31, G-32, G-34, G-35, G-37
Caliber:
Source: Glock Website, Sept 6, 2011

GLOCK, GEN4, MODEL G-17, G-17T, G-19, G-21, G-22, G-23, G-31, G-32, G-34, G-35, & G-37 SEMI AUTOMATIC PISTOLS

Glock Inc. announces that it is voluntarily exchanging the recoil spring assembly (RSA) on its new GEN4 pistols shipped between August, 2009 to July 21, 2011, to ensure each pistol's performance meets the company demanding standards.

The replacement of the GEN4 pistol RSA can be performed as part of the regular field-strip process; consumers do not need to take the GEN4 pistol to an armorer. Consumer's are urged to call 1 877 745 8523 to order their new recoil spring in exchange for a new RSA at no cost.

RETIRED

RECALL

Make: Smith and Wesson, Walther
Model: PK380
Caliber: 380 Auto
Source: Company Website Notice, Oct 4, 2012

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380 AUTO CALIBER, SMITH & WESSON, WALTHER PK380, SEMIAUTOMATIC PISTOLS

SERIAL NUMBERS: PK 101201 TO PK 112155

STOP USING THE PISTOL, UNLOAD IT IMMEDIATELY, EXERCISING EXTREME CARE AND RETUN IT TO WALTHERAMERICA AT ONCE.

RETIRED

Walther has identified a condition that may exist in certain PK380 pistols which may permit a round to be discharged if the trigger is pulled, despite engagement of the manual safety. Walther has found that manual safety may not prevent firing of a chambered round when the trigger is pulled.

This recall applies to Walther PK380, 380 ACP pistols manufactured by Carl Walther GmbH from May, 2012 to September, 2012 and which have a serial Number ranging from PK 101201 to PK 112155.

Any **unintended discharge** of a firearm has the potential for causing **injury or even death,** and we ask that you stop using the pistol immediately. When unloading the pistol, please take extreme care to ensure that the firearm is pointed in a safe direction.

To facilitate the inspection and repair, if necessary, of the pistol, please contact waltheramerica's customer service department at 1-800-713-0356 to receive instructions and a call tag for return of the pistol to Waltheramerica. Additional information will be posted on Waltheramerica.com. If you have any questions about this recall, please contact Waltheramerica at
1-800-713-0356 .

RECALL

Make: Smith and Wesson
Model: M&P Shield
Caliber:
Source: Company Website Notice, Aug 22, 2013;
AFTE Website, Aug 22, 2013

SMITH & WESSON, MODEL M&P SHIELD, SEMIAUTOMATIC PISTOLS MANUFACTURED BEFORE AUGUST 19, 2013

RETIRED

Smith & Wesson has identified a condition where the trigger bar pin could damage the lower trigger in certain M&P Shield s in a way that may affect the functionality of the drop safety feature of the firearm, potentially allowing the pistol to discharge if it is dropped. Any unintended discharge of a firearm has the potential to cause injury, and we ask that you to

STOP USING YOUR PISTOL IMMEDIATELY UNTIL IT HAS BEEN INSPECTED AND, IF THE CONDITION IS FOUND, REPAIRED.

This condition is largely limited to recently manufactured M&P Shield pistols. However, out of an abundance of caution, we are asking all consumers of all M&P Shields manufactured before August 19, 2013 immediately inspect their pistols for this condition.

With the **firearm unloaded, ensure the muzzle is pointed in a safe direction, the slide locked back and the manual safety engaged**:

1.) Hold pistol sideways with the muzzle pointed to the left. Look at the trigger. Note that the trigger is actually 2 pieces, with a pin in the middle that connects the 2 halves.
2.) Without touching the trigger, identify the plastic tab under & to the rear of the trigger.
3.) Slowly pull the trigger. The plastic tab should disappear into the upper trigger section.
4.) Slowly release the trigger. The tab should reappear completely on its own.
5.) Repeat steps 3 & 4 several times. If the small tab reappears each time, the pistol does **NOT** require repair.
6.) If the small tab does not consistently reappear the pistol **MUST BE RETURNED FOR SERVICE.**

If after inspection it is determined that the condition outlined in this safety alert exists, the firearm MUST be sent to Smith & Wesson. The firearm will be repaired at no cost to you and all shipping costs will be covered by Smith & Wesson.

Please contact Smith & Wesson directly at 877-899-6259 or at MPShieldSafetyAlert.com to arrange for repair, if necessary for your pistol.

RECALL

Make: Caracal
Model: C
Caliber:
Source: Internet Notice Issued March 28, 2013 Updated Sept 9, 2013; AFTE Website Sept 9, 2013

CARACAL, MODEL C, SEMIAUTOMATIC PISTOLS

PISTOL MAY EXPERIENCE CATASTROPHIC FAILURE DURING FIRING AND HAVE A DROP SAFETY ISSUE

Caracal reports that all model C pistols are being recalled after several slides separated from the frame during firing. Additionally it was determined that the model C pistol can fire when dropped on its muzzle.

The catastrophic failure and unintentional discharge of a firearm can cause serious injury or death.

If you have or own a Caracal Model C pistol, **PLEASE DO NOT LOAD OR FIRE THE PISTOL.** Please contact Caracal customer service immediately to arrange to have your Model C pistol returned. Caracal will provide you with a full refund of the purchase price of your pistol or vouchers for other Caracal products. Unfortunately, the potential safety issue cannot be addressed through a repair of the pistol and ALL MODEL C PISTOL'S MUST BE RETURNED FOR REFUND.

USA CUSTIMERS: Contact Caracal at 205-655-7050 or info@caracal-usa.com.
INTERNATIONAL CUSTOMERS: Contact Caracal at 971-4 21 22 055 or recall@caracal.ae

RETIRED

RECALL

Make: Springfield Armory
Model: 3.3 XD-S
Caliber: 9mm Luger, 45 Auto
Source: Company Website Notice, Issued-Aug 28, 2013 & Updated-Oct 18, 2013

9MM AND 45 ACP CALIBER SPRINGFIELD ARMOR, MODEL 3.3 XD-S, SEMIAUTOMATIC PISTOLS

RETIRED

Springfield Armory is initiating this voluntary safety recall to upgrade 3.3 XD-S 9mm and 3.3 XD-S 45 ACP pistols with new components, which eliminate the possibility of a potentially dangerous condition. We want to emphasize that no injuries have been reported to date.

Springfield has determined that under exceptionally rare circumstances, some 3.3 XD-S 9mm and 45 ACP caliber pistols could experience an **unintended discharge** during the loading process when the slide is released, or could experience a **double-fire** when the trigger is pulled once. The chance of these conditions existing is exceptionally rare, but if they happen, **serious injury or death could occur.**

STOP USING YOUR 3.3 XD-S 9MM OR 45 ACP PISTOL AND CAREFULLY UNLOAD IT !

To begin the upgrade process and for faster response click on the GET STARED link at the top of the company website safety recall webpage. On that page enter your serial number and click Next. If your pistol is subject to this recall, you will need to provide your name, address, phone number, and email address. The serial number is located on the frame beneath the dust cover (accessory rail). If you prefer, you may initiate the upgrade process by calling 800-680-6866. If you no longer own the 3.3 XD-S 9mm or 45ACP pistol, please advise Springfield of the current owner.

This Safety Recall applies only to:

SPRINGFIELD 3.3 XD-S 9MM PISTOLS – SERIAL # XS900000 TO XS938700 &
SPRINGFIELD 3.3 XD-S 45ACP PISTOLS – SERIAL # XS500000 TO XS686300

Springfield will send you a return authorization and mailing label, allowing you to return you 3.3 XD-S 9mm or 45 ACP pistol to Springfield at no cost to you. Springfield will upgrade your 3.3 XD-S 9mm 0r 45 ACP pistol with the new components and return it to you. Springfield will provide tracking numbers so you can monitor the receipt and return of your upgrades.

Visit www.springfield-armory.com for further information about this safety recall. You can reach the Springfield Armory Call Center at 800-680-6866. Springfield apologizes for this inconvenience, but your safety is our paramount concern.

We greatly appreciate your loyalty to Springfield products.

RECALL

Make: Taurus
Model: 24/7 DS
Caliber: 40 S&W
Source: Internet Notice Sept 26, 2013

TAURUS, 40 S&W CALIBER, MODEL 24/7 DS SEMIAUTOMATIC PISTOLS

Sao Paulo State Military Police (PMSP) have recalled all of the issued Taurus, 40 S&W caliber, Model 24/7 DS pistols after discovering that some could be discharged without the trigger being pulled.

RETIRED

WARNING

Make: Taurus
Model: PT145 Pro Millennium
Caliber: 45 Auto
Source: AFTE Website Oct 30, 2013

TAURUS, 45 AUTO CALIBER, MODEL MILLENNIUM PT145 PRO SEMIAUTOMATIC PISTOLS

EXTREME CAUTION - PISTOL MAY UNINTENTIONALLY FIRE FULL AUTO

The Tennessee Bureau of Investigation Nashville Crime Laboratory reports an unsafe condition involving a case with a Taurus, 45 Auto caliber, model Millennium PT145 Pro pistol. During test firing the pistol jammed, had a light primer strike and fired full auto.

Additionally a Taurus 40 S&W pistol was found to have the same issue.

RETIRED

RECALL

Make: Remington
Model: 700 and Seven
Caliber:
Source: Company Notice April 2014

PRODUCT SAFETY WARNING AND RECALL NOTICE REMINGTON MODEL 700™ AND MODEL SEVEN™ RIFLES

PRODUCTS: Remington Arms Company, LLC ("Remington") is voluntarily recalling Remington Model 700™ and Model Seven™ rifles with X-Mark Pro® ("XMP®") triggers, manufactured from May 1, 2006 to April 9, 2014.

DESCRIPTION OF THE HAZARD: Remington has determined that some Model 700 and Model Seven rifles with XMP triggers could, under certain circumstances, unintentionally discharge. A Remington investigation has determined that some XMP triggers might have excess bonding agent used in the assembly process. While Remington has the utmost confidence in the design of the XMP trigger, it is undertaking this recall in the interest of consumer safety to remove any potential excess bonding agent applied in the assembly process.

HOW TO DETERMINE IF YOUR RIFLE IS SUBJECT TO THE RECALL: Only Model 700 and Model Seven rifles with XMP triggers are being recalled. To determine if your rifle is subject to this recall, you should take the following steps:

I. Find the rifle's serial number where the barrel meets the receiver.
   a. For a right-handed rifle, the serial number is located on a user's left.
   b. For a left-handed rifle, the serial number is located on a user's right.

II. Identify the serial number and provide it to Remington's recall support team, either by entering it at xmprecall.remington.com or calling 1-800-243-9700 (Prompt #3 then Prompt #1) Monday through Friday, 9 a.m. to 5 p.m. EDT. You will be informed if your rifle is affected by this recall and supported with free resources to return the rifle for inspection and specialized cleaning.

III. You may also determine if your rifle is subject to the recall by a visual inspection.
   1) If the face of the trigger is ribbed, your rifle does not have an XMP trigger and is NOT subject to this recall.
   2) If the face of the trigger is smooth, your rifle has an XMP trigger and IS subject to this recall – in which case you should immediately seek further assistance at xmprecall.remington.com or by calling 1-800-243-9700 (Prompt #3 then Prompt #1) Monday through Friday, 9 a.m. to 5 p.m. EDT.

REMEDY/ACTION TO BE TAKEN: STOP USING YOUR RIFLE. Any unintended discharge has the potential to cause injury or death. Immediately cease use of recalled rifles and return them to Remington free of charge. Rifles will be inspected, specialty cleaned, tested, and returned as soon as possible, at no cost to you. DO NOT attempt to diagnose or repair recalled rifles.

TO PARTICIPATE IN THIS RECALL PROGRAM: For your safety, STOP USING YOUR RIFLE and immediately contact Remington. To participate in the recall, please follow the instructions below:

STEP 1 Visit xmprecall.remington.com or call 1-800-243-9700 (Prompt #3 then Prompt #1) Monday through Friday, 9 a.m. to 5 p.m. EDT. You will be asked to provide your name, address, telephone number, and rifle(s) serial number.

STEP 2 Upon receipt of the information requested in Step 1, Remington will send you pre-paid shipping tags, boxes and written instructions. Remington will cover all related shipping, inspection, and cleaning charges. Please ONLY return your rifle with the designated shipping tags and boxes, as they are marked to expedite the rifle to a dedicated Remington facility.

VERIFICATION OF CORRECTIVE ACTION: Upon return of your rifle, you will note a punch mark on the bolt release. This mark confirms your rifle has been inspected and specialty cleaned under this recall program. Remington has also corrected the XMP trigger assembly process to eliminate this problem in rifles made after April 9, 2014. Rifles made after April 9, 2014 will also have a punch mark on the bolt release.

Remington is deeply sorry for this inconvenience, but we believe in safety first. It is imperative that Model 700 and Model Seven rifles subject to this recall are not used until they have been inspected and specialty cleaned by Remington. The Remington team is committed to the quality and safety of its products.

RETIRED

RECALL

Make: Blackwater
Model: GSR 1911
Caliber: BB
Source: www.ammorecall.com October 1, 2014

Blackwater Metal Slide Series GSR 1911 Airsoft Pistol

GRAPEVINE, TX, Oct. 1, 2014 /CNW/ - Soft Air USA is recalling its Blackwater metal slide series GSR 1911 airsoft pistol – (product #75-2271).

The product, a recreational airsoft pellet gun with a 328 feet per second (fps) velocity, was shipped in error to some Canadian Tire stores and may have been available at other retailers across the country.
Affected units were sold between August 2014 and September 2014. The recalled product features all-black components and an orange tip.

The Blackwater metal slide series GSR 1911 airsoft pistol with transparent components on the barrel and handle of the product are NOT affected by this recall.

Customers who have purchased the recalled product should discontinue use and call 1-844-300-8936 to verify if their product is affected by this recall or have the item picked up and refunded.
SOURCE Soft Air USA

For further information: Joe Huston, Soft Air USA, 479-282-5013, jhuston@softairusa.com; Leah Gaucher, Canadian Tire, 416-544-7785, leah.gaucher@canadiantire.com

RETIRED

WARNING

Make: Winchester
Model: 1200, 1300, 120, 2200, 1400, 1500, 140, 2400; Sears 200, 300; Ted Williams 200, 300
Caliber:
Source: Company Notice October 1, 2014

PRODUCT SAFETY NOTICE – WARNING

RETIRED

WINCHESTER® Slide-Action Shotgun Models 1200, 1300, 120, and 2200; SEARS® Slide-Action Shotgun Models 200 and Ted Williams® 200; WINCHESTER Semi-Automatic Shotgun Models 1400, 1500, 140, and 2400; SEARS Semi-Automatic Shotgun Models 300 and Ted Williams 300

Olin Corporation, through its Winchester Division, is warning users of the above shotguns to follow owner's manual instructions to fully open the action before moving the safety to the "ON" position and to only use shotgun shells of 2-3/4 inch or 3 inch length.

Under certain conditions it may be possible to move the safety to the "ON" position while the shotgun's action is partially open, placing the hammer under control of the safety and not the trigger. Operating the safety in this manner is improper and contrary to the instruction manual. It has come to our attention that short length shotgun shells, including those known as "minishells", can be manually placed in the firearm's chamber while the action is partially open. With the hammer controlled by the safety and with a shotgun shell in the chamber, closing the action and moving the safety to the "OFF" position may accidentally fire the shotgun without a trigger pull. Accidental firing may result in property damage, serious personal injury or death.

IN THE WINCHESTER AND SEARS SHOTGUNS NAMED ABOVE, PLEASE –

- FULLY OPEN THE ACTION BEFORE MOVING THE SAFETY TO THE "ON" POSITION,
- ONLY USE SHOTGUN SHELLS OF 2-3/4 INCH OR 3 INCH LENGTH, and
- DO NOT USE "MINISHELLS".

This is not an ammunition or firearm recall. No other firearms are subject to this warning.
For Winchester firearm owner's manuals please go to
www.winchesterguns.com/customerservice/ownersmanuals
© 2014 WINCHESTER 600 Powder Mill Road East Alton, IL 62024-1273

WARNING

Make: Winchester
Model: 94, 1894, 9422, 9422M, 55 Sears, 54, Ted Williams 100, 64, 64A, Centennial '66, Canadian Centennial '67
Caliber:
Source: Company Notice October 2, 2014

PRODUCT SAFETY NOTICE – WARNING

WINCHESTER® Model 94 Family of Lever-Action
Rifles, Carbines, and Muskets with Half-Cock Safety

Winchester Model 94 (including commemoratives); Winchester Model 1894;Winchester Model 9422 and Model 9422M; Winchester Model 55 Sears® Model 54 and Ted Williams® Model 100; Winchester Model 64 and 64A; Winchester Centennial '66; Winchester Canadian Centennial '67

Olin Corporation, through its Winchester Division, is warning users of the above firearms that when there is a live cartridge in the chamber, **dropping, jarring or bumping the firearm may cause an accidental discharge**, which may result in property damage, serious personal injury or death to the user or others:

- With the hammer in the full-down position, a slight impact to, or bumping the hammer can cause a discharge.
- With the hammer in the half-cock safety position, a hard impact to the hammer, such as from dropping the firearm, can break the safety mechanism and cause a discharge.
- With the hammer in any position, a hard impact, such as from dropping or jarring the firearm, can cause a discharge, even if the hammer is not impacted.

To reduce the risk of accidental discharge, follow safe firearm handling practices, including:

- Always maintain control of the firearm and keep your firearm pointed in a safe direction at all times.
- Never place the hammer in the full-down position when there is a live cartridge in the chamber.
- Never rely on the half-cock safety to prevent accidental discharge due to dropping or jarring the firearm, or hard impacts to the hammer.
- When there is a live cartridge in the chamber, avoid activities that increase the risk of dropping or jarring the rifle, such as running, climbing, crossing a fence, and taking the firearm up and down from a tree stand.

RETIRED

This is not an ammunition or firearm recall. For Winchester firearm owner's manuals please go to www.winchesterguns.com/customerservice/ownersmanuals

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http://www.winchester.com/library/news/Pages/model-94-warning.aspx

RECALL

Make: Remington
Model: 887
Caliber: 12 GA
Source: Company Notice December 2014

PRODUCT SAFETY WARNING AND RECALL NOTICE REMINGTON MODEL 887™ Recall

RETIRED

PRODUCTS: Remington is voluntarily recalling Remington Model 887™ shotguns manufactured from December 1, 2013 to November 24, 2014.

DESCRIPTION OF THE HAZARD: Remington has determined that some Remington Model 887™ shotguns manufactured between December 1, 2013 and November 24, 2014 may exhibit a defect causing the firing pin to bind in the forward position within the bolt, which can result in an unintentional discharge when chambering a live round. While Remington has the utmost confidence in the design of the 887™ shotgun, it is undertaking this recall in the interest of consumer safety.

REMEDY/ACTION TO BE TAKEN: STOP USING YOUR SHOTGUN. Any unintended discharge has the potential to cause injury or death. Immediately cease use of recalled shotguns and return them to Remington free of charge. Shotguns will be inspected, repaired, tested, and returned as soon as possible, at no cost to you. DO NOT attempt to diagnose or repair recalled shotguns yourself. For your safety, STOP USING YOUR SHOTGUN and immediately contact Remington.

TO PARTICIPATE IN THIS RECALL PROGRAM: To participate in the recall, please follow the instructions below:

STEP 1 Visit http://887recall.remington.com or call 1-800-243-9700 (Prompt #3 then Prompt #2) Monday through Friday, 9 a.m. to 5 p.m. EDT. You will be asked to provide your name, address, telephone number, and shotgun(s) serial number.

STEP 2 Upon receipt of the information requested in Step 1, Remington will send you boxes and written instructions, and arrange for UPS to pick-up your shotgun(s). Remington will cover all related shipping, inspection, and repair charges. Please do not return your shotgun on your own. Remington will arrange for pick-up of your Model 887™.

VERIFICATION OF CORRECTIVE ACTION: Upon repair of your shotgun, you will note a punch mark on the bolt (see photo below). This mark confirms your shotgun has been inspected and repaired by Remington under the recall program. Remington has also corrected the manufacturing process to eliminate this potential firing pin problem in shotguns manufactured after November 24, 2014. Shotguns manufactured after November 24, 2014 will also have a punch mark on the bolt.

WARNING

Make: Jimenez Arms
Model: J.A. Nine
Caliber: 9mm Luger
Source: AFTE Contributor – 01/2015

Jimenez Arms Model J.A. Nine, 9mm Luger caliber semiautomatic pistol is subject to the same accidental discharge as the Bryco Arms, Model Jennings Nine listed below.

BRYCO ARMS, MODEL JENNINGS NINE, 9MM LUGER CALIBER, SEMIAUTOMATIC PISTOL

The Bryco Arms, model Jennings Nine, 9mm Luger caliber, semiautomatic pistol is subject to accidental discharge. The pistol **MAGAZINE SAFETY** is subject to **FAILURE** when the trigger is pulled and the magazine release button is depressed simultaneously. When this occurs the pistol will fire even though the magazine has been removed from the pistol.

RETIRED

RECALL

Make: Winchester
Model: SXP
Caliber: 12 GA
Source: Company Notice April 2015

IMPORTANT SAFETY NOTICE: WEATHERBY® 28 GA. SA-08 SEMI-AUTOMATIC SHOTGUN

Weatherby, Inc. of Paso Robles, California, is performing a **product safety recall** on a limited number of Weatherby® SA-08 model semi-automatic shotguns sold by Weatherby, Inc., beginning March 11, 2013. **The affected shotguns bear serial numbers on the receiver within the following series AN00001-AN02802 (inclusive).** The **safety recall** is based on reported incidents of **accidental discharge** upon bolt closure and chambering of a live shot shell. A corrective **safety upgrade** has been identified, which will be fully implemented upon completion of testing to achieve assurance that the **safety upgrade** prevents occurrences of **accidental discharge** as reported. ***Accidental discharge*** *of any firearm can result in* ***severe injury or property damage*** *if the muzzle of the firearm is pointed in an unsafe direction at the time of* ***accidental discharge****.*

RETIRED

WARNING: If you own a Weatherby® 28 Gauge SA-08 model semi-automatic shotgun with a serial number within the range of serial numbers referred to above, USE OF YOUR SHOTGUN MAY RESULT IN AN ACCIDENTAL DISCHARGE AND SEVERE INJURY. STOP USING YOUR SHOTGUN IMMEDIATELY and call Weatherby at 1-800-227-2018, extension 6, or 1-805-227-2600 or www.weatherby.com/28 to receive instructions on how to return the shotgun for the safety recall.

NO OTHER WEATHERBY® SHOTGUNS ARE AFFECTED BY THIS SAFETY NOTICE. CHECK YOUR SERIAL NUMBER.
The affected shotguns were originally sold through licensed wholesale distributors and gun dealers nationwide, and some may have been resold by the original owners through retail gun dealers, trade shows or privately. If you have sold, traded or given away an affected 28 Gauge Weatherby® SA-08 model semi-automatic shotgun, it is important for you to forward this Safety Notice to the current owner.

Weatherby apologizes for any inconvenience to its customers resulting from this safety recall program, but it is **important** that owners of affected shotguns take advantage of this free repair program and **do not use an affected shotgun** before it has been repaired. All work will be done at the factory at no charge. Please do not return your Weatherby® SA-08 28 Gauge shotgun to the factory before contacting us for return instructions.

1605 Commerce Way, Paso Robles, CA 93446

RECALL

Make: Winchester
Model: SXP
Caliber: 12 GA
Source: Company Notice April 2015

WINCHESTER SXP (3½" CHAMBER) SHOTGUN SAFETY WARNING AND RECALL NOTICE

Winchester Repeating Arms has discovered that a ***limited*** number of SXP (3 ½" chamber) shotguns (also called the Super X Pump) may, under certain circumstances, unintentionally discharge while closing the action. Failure to return any affected shotguns for inspection and/or repair may create a risk of harm, including serious personal injury or death.

If you own one of the following firearms, please immediately contact our Winchester Consumer Administrative Center to find out if your firearm is affected and should be returned. Please be prepared to provide the serial number of your firearm.

	CHAMBER	GAUGE	BARREL LENGTH
SXP WATERFOWL HUNTER	3 1/2"	12	26" or 28"
SXP BLACK SHADOW	3 1/2"	12	26" or 28"
SXP TURKEY HUNTER	3 1/2"	12	24"
SXP LONG BEARD	3 1/2"	12	24"

DO NOT LOAD OR SHOOT ANY OF THE SHOTGUNS LISTED ABOVE CURRENTLY IN YOUR POSSESSION OR CONTROL UNTIL YOU HAVE CONTACTED THE WINCHESTER REPEATING ARMS CONSUMER ADMINISTRATIVE CENTER.

RETIRED

If you have purchased one or more of the shotguns listed above, and have confirmed with the Winchester Consumer Administrative Center that it is an affected shotgun, please note that such shotguns should not, under any circumstance, be loaded or fired until they have been inspected and/or repaired by the Winchester Repeating Arms Service Center.

For further information and instructions, please contact the Winchester Consumer Administrative Center: CALL: 1-800-945-5372 OR Email: sxp@winchesterguns.com

HOW TO DETERMINE IF YOUR SXP HAS ALREADY BEEN INSPECTED AND/OR REPAIRED.

Upon inspection and/or repair by the Winchester Repeating Arms Service Center, all SXPs affected by this recall will be inconspicuously marked on the front of the receiver as shown below in the illustration. If an SXP already has this marking, the firearm has been inspected and/or repaired.
To view the mark use a flashlight or other light source to peer past the action bars to the front of the receiver. To view the mark more easily, you can remove the barrel. Remember to . . . ALWAYS KEEP THE MUZZLE POINTED IN A SAFE DIRECTION. BEFORE REMOVING THE BARREL OPEN THE ACTION AND VISUALLY INSPECT THE CHAMBER TO BE ABSOLUTELY CERTAIN THE FIREARM IS COMPLETELY UNLOADED. PLACE THE "SAFETY" IN THE ON SAFE POSITION. FAILURE TO FOLLOW THIS WARNING COULD RESULT IN SERIOUS INJURY OR DEATH.

- After assuring your shotgun is unloaded, unscrew the magazine cap.
- Pull the barrel forward and off the magazine tube, away from the receiver.
- Look for the indented dot on the front to the receiver, on the left side.

When the inspection is complete, return the barrel to its original position and tighten the magazine cap.

Searching headstamp records dialog

With the searching headstamp records dialog you can enter the criteria that must apply to headstamp records you are searching for.

You can pick from the list of available fields the desired criterion, press the --> button, and fill it out and press the **Search** button to start a search.

If there is already a criterion in the search on list you can remove it by using the <-- button.

When you check a criterion without filling it out, the **Search** command will be not be executed.

After you have filled out the criteria and pressed the **Search** button, CartWinPro will search the database for headstamp records that match the entered criteria. When CartWinPro has finished searching, all found records are shown in a search result window, from where you can open individual records in their own record window.

You can pick more than one criterion. The searched records must match all entered criteria in that case.
If none criterion is selected, the **Search** command will show ALL records of the headstamp database in a search result window.

You can search for every field in the headstamp record, and every field of all the records that are related to the headstamp-records.

The criteria marked with this sign are fast search fields.

The most significant criteria are:

Cat. #

Here you can enter the catalog number of the headstamp records you are searching for.
When you leave the second field empty and only fill out the first field, records with that catalog number will be searched.
You can search for a range of catalog numbers, by also filling out the second field.

Manufacturer relation

Here you can specify which manufacturer relation record must be linked to the headstamp records you are searching for.
You choose the manufacturer relation record, by pressing the **Select** button, which gives you a manufacturer record search dialog. After you have searched for manufacturer records, you can select the desired manufacturer relation in the manufacturer search result dialog, by double clicking it.
The chosen manufacturer relation will be identified by its catalog number, in the field next to the **Select** button.
When a manufacturer relation record was selected before, it will be replaced by the new selection.

Displayed headstamp

Country of origin

Headstamp type

RETIRED

Explanation code 1

Explanation code 2

Explanation code 3

Explanation code 4

Explanation code 5

Explanation code 6

Explanation code 7

Explanation code 8

Here you can select the search item of the headstamp records you are searching for.
The field where you enter the search item, is a list selection field.

From - Until

Notes

Here you can enter text that should be part of the chosen field of the headstamp records you are searching for.
The search is not case sensitive.

You can characterize the search as follows:

- **Starts with**
  The model name field of the headstamp records you are searching for, must start with the text you enter here.
- **Contains**
  The model name field of the headstamp records you are searching for, must contain the text you enter here.
- **Exactly**
  The model name field of the headstamp records you are searching for, must be equal to the text you enter here, including spaces and punctuation marks.
- **Roughly**
  The model name field of the headstamp records you are searching for, must contain text that is roughly the same as the text you enter here.
  This allows you to search for text when you are not sure about the exact spelling.

RETIRED

Code 1

Code 2

Code 3

Code 4

Code 5

Code 6

Code 7

Code 8

Latent search code

Here you can enter the code (marking) or the beginning of a code, of the headstamp records you are

searching for.
The field where you enter the code of headstamps, is a headstamp code field.

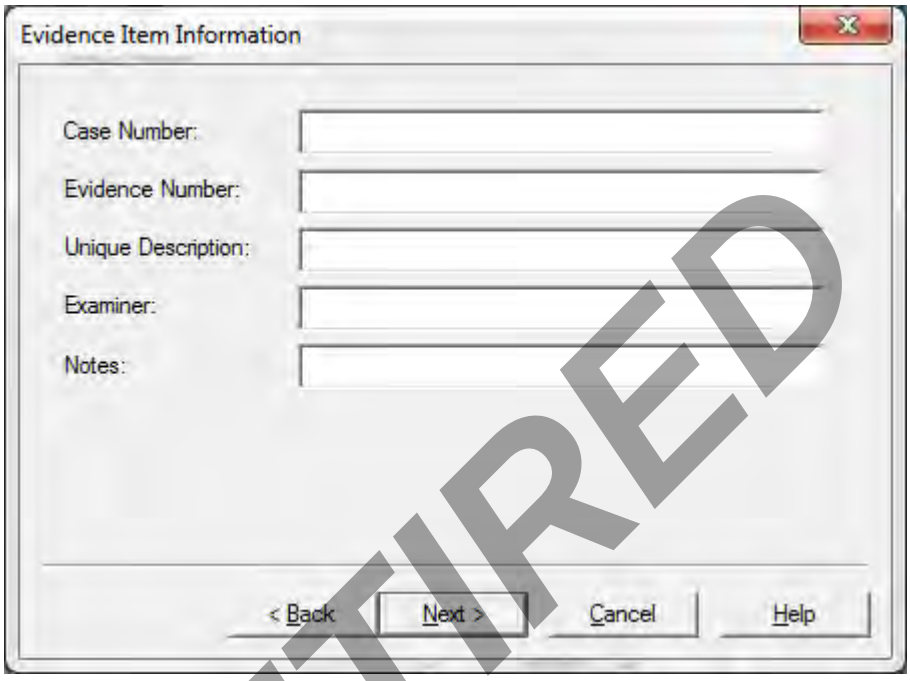
Besides the usual code fonts, this headstamp code field contains the option of selecting the 'international' font. Use this font to search for codes when you are not sure about the font of a certain code or marking.

When you enter **R** in the international font for example, CartWinPro will treat codes that start with **R** (CC LatinSans), **R** (CC GreekSans) or **R** (CC CyrillicSans) as matching your criterion.

- When you enter only one of the nine code criteria, CartWinPro will search for headstamp records, where one of the nine possible codes matches your entry.
- When you enter more than one code, CartWinPro will search for headstamp records, where two or more of the nine possible codes match your entry in any order.

RETIRED

1) During the process of imaging using FTK Imager 3.x, the following dialogue box will appear:



RETIRED

2) Use the following conventions to fill in each field:

   a) Case Number - This will be the unique identifier for the case. It consists only of the Laboratory Case Number of the evidence being acquired.

      For example: DSS-16-001234

   b) Evidence Number - This will be the unique identifier for that particular piece of media. It can be the Laboratory Submission Number (for example: “001”) or a sub-item number of the Laboratory Submission Number (for example: “001-1A”).

   c) Unique Description - This field should contain information about the particular piece of media. For example: Make, Model and Serial Number.

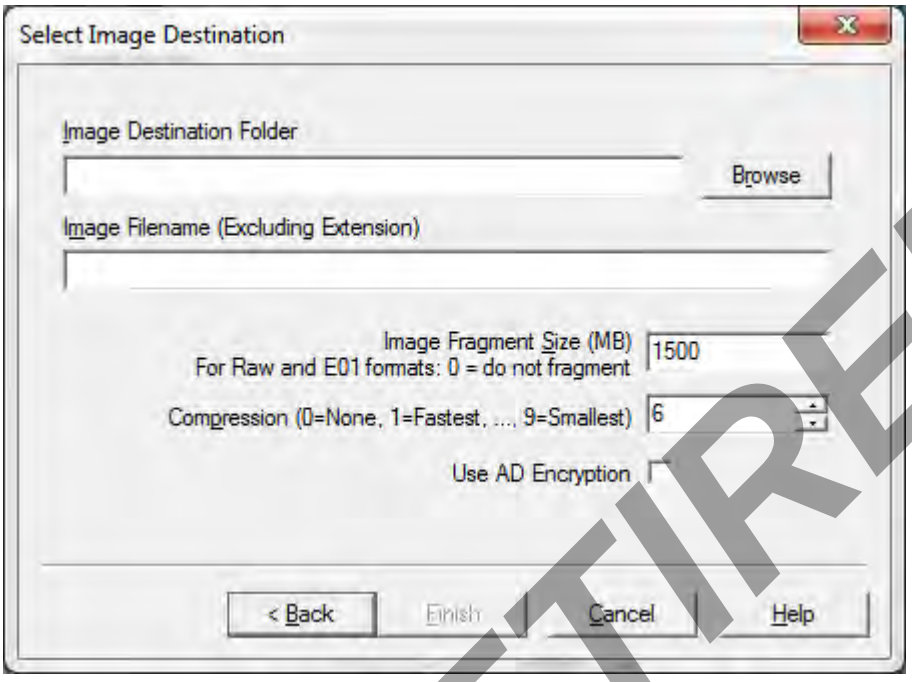
   d) Examiner - This will be the name of the analyst acquiring the specific piece of media.

      For example: “Jim Jackson”,”Jane Smith” or “Johnson”

      If two or more examiners share the same name they must be uniquely indentified.

      For example: “Jim Jackson” and “James Jackson”

   e) Notes - This filed is optional and may contain any information the examiner feels is relevant to the case.



Referring to the screenshot to the left, the following naming conventions are used when acquiring digital media with FTK Imager 3.x:

1. In the Image Destination Folder field change the destination path to the established staging drive.
2. In the Image Filename (Excluding Extension) field, the naming convention will reflect the Submission # along with the media number based on how many pieces of the particular media type* there are. For example, if a computer is submitted as Submission #003 and it contains two (2) hard drives, the naming convention would be: S3_HD1 and S3_HD2. Meaning;
   - S3 equals Submission #003
   - HD1 equals Hard Drive 1
   - HD2 equals Hard Drive 2
3. All other fields may be defined based upon the examiner's requirements.

RETIRED

* A complete list of abbreviations for the various types of media can be found in SOP-CC-31 – Sub-item Labeling Standards.

A. When filling in the fields contained in QR-CC-10 - Laboratory Report Template select from the following list the appropriate request for analysis.

1. Recovery of suspected child pornographic images either in still or video format
2. Recovery of data signifying the distribution of suspected child pornographic images or videos
3. Recovery of data signifying the importation of suspected child pornographic images or videos
4. Recovery of data signifying the production of suspected child pornographic images or videos
5. Recovery of data pertaining to an enticement investigation
6. Recovery of data pertaining to a homicide investigation
7. Recovery of data pertaining to a suicide investigation
8. Recovery of data pertaining to a fraud investigation
9. Recovery of data pertaining to a larceny investigation
10. Recovery of data pertaining to a burglary investigation
11. Recovery of data pertaining to a threatening/harassment investigation
12. Recovery of data pertaining to a financial fraud/counterfeiting investigation
13. Recovery of data pertaining to an e-mail threats/harassment/stalking investigation
14. Recovery of data pertaining to a narcotics investigation
15. Recovery of data pertaining to an identity theft investigation
16. Recovery of data pertaining to a network intrusion investigation
17. Recovery of data pertaining to a domestic violence investigation
18. Recovery of data pertaining to a voyeurism investigation
19. Recovery of cell phone data
20. Recovery of media device data
21. Hard drive restoration
22. Digital media restoration
23. Hard drive sterilization
24. Removable media sterilization
25. Recovery of DVR data
26. Imaging/acquisition of media
27. Administrative inquiries

RETIRED

28. Other - please specify

RETIRED

1) Upon initiation of acquisition using the Tableau Forensic Duplicator, a dialogue box will appear following the menu selection 1. Duplicate Disk, 2. Disk-to-File and Start which will prompt for a case ID, followed by Case Note, Subdir Name and File Base Name. The entries should be made as follows:

   a) Case ID - This will be the unique identifier for the case. It consists only of the Laboratory Case Number of the evidence being acquired.

      For example: DSS-16-001234

   b) Case Note - This field should contain information about the particular piece of media. For example: Make, Model and Serial Number and the examiners name.

   c) Subdir Name- leave as default.

   d) File Base name - will reflect the Submission # along with the media number based on how many pieces of the particular media type* there are. For example, if a computer is submitted as Submission #003 and it contains two (2) hard drives, the naming convention would be: S3_HD1 and S3_HD2. Meaning;

      S3 equals Submission #003

      HD1 equals Hard Drive 1

      HD2 equals Hard Drive 2

* A complete list of abbreviations for the various types of media can be found in SOP-CC-31 – Sub-item Labeling Standards.

RETIRED

A. Purpose:

To purpose of this procedure is to outline the steps taken to securely procure evidence being submitted to the Computer Crime and Electronic Evidence Unit through the Evidence Receiving Section of the DSS.

B. Responsibility:

Section supervisor or designee; assigned case examiner.

C. Definitions/Abbreviations:

Refer to SOP-CC-26 - Definitions and Abbreviations.

D. Procedure:

1. Upon notification of evidence being submitted to the Computer Crime and Electronic Evidence Unit through the Evidence Receiving Section of the DSS, the section supervisor or designee are, at their earliest convenience, to take custody of the evidence submission(s).
2. Update the chain of custody in the JusticeTrax system to reflect the individual receiving the evidence submission(s) from Evidence Receiving. In the event that the JusticeTrax system is down, maintain the chain of custody following the protocol established by the Evidence Receiving section.
3. Transport the evidence submission(s) along with the supporting documentation* to a designated secure location and create a new case file as described below in step 5.

   *Supporting documentation may include, but is not limited to:

   1. Request for Examination of Physical Evidence form (SOP-ER-02:1)
   2. Laboratory Evidence Receipt
   3. Joyce Warrant or Consent
   4. Incoming Evidence Checklist
4. Ensure that the Laboratory case number designator has been transcribed on all documentation.
5. Create a file folder and a hanging folder for the supporting documentation.
   a. Place all the supporting documentation into the file folder and affix the case generated barcode label (Laboratory case number) to the folder tab and place the file folder in the hanging folder.
6. Maintain the completed case file for case assignment.
7. Transfer the evidence submission(s) to a dedicated evidence storage area and update the chain of custody in the JusticeTrax system.
8. Complete case assignment through the JusticeTrax system.
9. Complete the appropriate milestone on the milestone sticker located on the outside cover of the case folder.
10. Following case assignment, the assigned examiner will review the submitted evidence and the supporting documentation to account for all of the submitted evidence.

RETIRED

a. The assigned examiner will ensure that the Laboratory barcode labels on all of the submitted evidence match with the Request for Examination of Physical Evidence form (SOP-ER-02:1) and the Laboratory Evidence Receipt. Any discrepancies will be resolved with the appropriate individual(s) prior to beginning the case examination. When it has been determined that no discrepancies exist, the examiner will check the box at the bottom of the Incoming Evidence Checklist and initial.

11. The assigned examiner will review the Incoming Evidence Checklist to ensure that it has been filled out properly and all questions have been answered by the submitting agency representative. Any discrepancies will be resolved with the appropriate individual(s) prior to beginning the case examination. When it has been determined that no discrepancies exist, the examiner will check the box at the bottom of that form and initial.
12. If additional documents (e.g. Search Warrants or Incoming Evidence Checklist) are provided at a later date, a copy of this documentation will be provided to Evidence Receiving for the main case file.

E. References:

1. JusticeTrax help file

RETIRED

A. Purpose:

To provide the steps taken to wipe all information from target drives used in forensic casework.

B. Responsibility:

CCEEU forensic examiners

C. Definitions/Abbreviations:

Refer to SOP-CC-26 - Definitions and Abbreviations.

D. Procedure:

1. Select a target drive that has sufficient storage capacity to hold the work product that will be produced through the course of the analysis.
2. Connect the target drive using authorized hardware to an authorized computer or by using the internal storage drive(s) in the analysis machine.
3. If using an external target drive, use an authorized wiping utility and following the manufacturer's guidelines, overwrite all information contained on the drive.
4. Using authorized software and following manufacturer’s guidelines, partition and format the wiped target drive.
5. Create folders on the target drive to organize the work product.

Note: A target drive may only contain material from a single case.

RETIRED

A. Purpose:

To outline the steps taken to generate a new case file and initiate processing in the specified forensic software application.

B. Responsibility:

CCEEU forensic examiners.

C. Definitions/Abbreviations:

Refer to SOP-CC-26 - Definitions and Abbreviations.

D. Procedure:

Note: The use of a particular forensic software application will be dependent on the specifics of the case request, for this reason case initiation may include one or more of the applications listed below. Additional approved forensic tools or software applications (Approved Software QR-CC-49) may also be utilized following case initiation:

<u>EnCase (Guidance Software)-new case file initiation and processing:</u>

1. Prepare a target drive as outlined in CC SOP-2 - Target Drive Preparation Protocol and attach this drive to a forensic computer or access the internal drive partition on the forensic computer.
2. Connect the staging drive(s) to the forensic computer or access the designated server staging partition to access the case evidence acquisition files.
3. Open the EnCase case file and follow the steps below that represent the method of acquisition used by the examiner.
    a. If the examiner created a new EnCase case file during the imaging process and added the acquired image file(s) directly to the new case proceed to Step 5.
    b. If the examiner created a new EnCase case file during the imaging process and did not add the acquired image file(s) directly to the new case proceed to Step 4.
    c. If the examiner has not created an EnCase case file follow the protocol outlined in the Reference Document, pertaining to the appropriate EnCase version, which outlines how to create a new case. When complete go to Step 5.
4. In order to add existing evidence files to the case follow the protocol outlined in the Reference Document pertaining to the appropriate EnCase version. When complete, proceed to Step 5.
5. The file verification process which is automatically initiated upon the addition of an evidence image file can be canceled. If during the examination process (Steps 6 and 7) an “Invalid Block Checksum” error message occurs, then data corruption in the evidence file has been indicated and the integrity of the sector group(s) cannot be verified as valid. If this error condition should occur then the verification process needs to be initiated and run to completion for the associated evidence file image so that a record of the errors can be generated and documented. After data corruption errors in the evidence file image have been confirmed then acquisition procedures for the source media should be repeated (Refer to the appropriate protocol -CC SOP-05, CC SOP-06, CC SOP-12, or CC SOP-13). Use Analysis Notes (QR-CC-6) to document the errors and actions taken during the process. Proceed to Step 6.

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6. Complete the below listed steps prior to any analysis of the data. Use Analysis Notes (QR-CC-6) or other appropriate QR as a documented narrative to describe actions and observations made during the process.
   a. Check to see that all of the media to be examined is present in the case file.
   b. Account for all sectors of the physical disk(s) to ensure there are no deleted or missing partitions. Make note of any read errors, missing sectors or CRC errors.
   c. Run a virus scan.
   d. Set-up processor options or run each of the processes below separately.
   e. Recover lost folders. (Note: EnCase version(6.x or prior) if the Operating System installed on the hard drive is Microsoft Windows Vista, Windows 7, Windows 8 or Windows 10 do not run recover lost folders. EnCase version(6.x or prior) will find multiple instances of the same files and folders, occupying the same physical locations on the media)
   f. Mount appropriate compressed files.
   g. Verify file signatures and compute hash values.
   h. Account for the time zone configuration.
   i. Check for any irregularities with file created, last written and last accessed dates.
7. Upon completion of Step 6 proceed with case specific tasks. Use Analysis Notes (QR-CC-6) or other appropriate QR as a documented narrative to describe actions and observations made while performing the case specific tasks. In addition, document in the Analysis Notes or other appropriate QR any pertinent discussions with the submitting agency relating to the examination.

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<u>FTK (AccessData)- new case file initiation and processing:</u>

1. Prepare a target drive on the forensic computer as outlined in CC SOP-2 - Target Drive Preparation Protocol and attach this drive to a forensic computer or access the internal drive partition on the forensic computer.

2. Connect the staging drive(s) to the forensic computer or access the designated server staging partition to access the case evidence acquisition files.

3. Begin processing a case by launching the FTK application version and providing the authentication parameters (User Name and Password) that were established during the FTK database preparation.

4. Follow the protocol and guidelines established in the Reference Document, pertaining to the appropriate FTK application version to create a new case.

   a. Enter the case information; select the built-in evidence processing parameters (file signature and hash analysis, expand compound files, recover deleted files and folders, data carving, registry reports) and indexing options. Account for the time zone configuration. Create the case.

b. Add the evidence acquisition files created by imaging protocols CC SOP-05, CC SOP-06, CC SOP-12, or CC SOP-13. Note: If an error condition occurs during case initiation when using evidence acquisition files generated in Encase then verify the hash values or run the verification process in Encase. If verification fails, the acquisition procedures for the source media should be repeated (Refer to the appropriate protocol -CC SOP-05, CC SOP-06, CC SOP-12, or CC SOP-13). Use Analysis Notes (QR-CC-6) to document the errors and actions taken during the process.
c. Initiate processing and run to completion.

5. Complete the below listed steps prior to any analysis of the data. Use Analysis Notes (QR-CC-6) or other appropriate QR as a documented narrative to describe actions and observations made during the process.

   a. Check to see that all of the media to be examined is present in the case file.
   b. Account for all sectors of the physical disk(s) to ensure there are no deleted or missing partitions. Make note of any read errors, missing sectors or CRC errors. Note: if the image was created in a different forensic application this information should be verified within that acquisition software.
   c. Mount the evidence file image to a drive in FTK and run a virus scan.
   d. Check for any irregularities with file created, last written and last accessed dates.

6. Upon completion of Steps 4 and 5 proceed with case specific tasks. Use Analysis Notes (QR-CC-6) or other appropriate QR as a documented narrative to describe actions and observations made while performing the case specific tasks. In addition, document in the Analysis Notes (QR-CC-6) or other appropriate QR any pertinent discussions with the submitting agency relating to the examination.

RETIRED

<u>IEF/Axiom (Magnet Forensics)- new case file initiation and processing:</u>

1. Prepare a target drive on the forensic computer as outlined in CC SOP-2 - Target Drive Preparation Protocol and attach this drive to a forensic computer or access the internal drive partition on the forensic computer.

2. Connect the staging drive(s) to the forensic computer or access the designated server staging partition to access the case evidence acquisition files.
3. If using AXIOM for the acquisition of the submitted evidence media follow the protocol and guidelines established in the Reference Document, pertaining to the appropriate Axiom version to create a new case and begin the acquisition process. Refer to the applicable unit imaging protocols - CC SOP-05, CC SOP-06, CC SOP-12, or CC SOP-13 for specific media types. Use Laboratory

Notes (QR-CC-5) as a documented narrative to describe actions and observations made while performing the acquisition tasks.

4. Begin processing a case by launching the IEF/Axiom application version and selecting the source media type - drive(write blocked), image, files and folders, volume shadow copies or mobile. Check to see that all of the media to be examined is present in the case file.

5. Follow the protocol and guidelines established in the Reference Document, pertaining to the appropriate IEF version or Axiom version to create a new case. Use Analysis Notes (QR-CC-6) or other appropriate QR as a documented narrative to describe actions and observations made during the process.

   a. Select the processing parameters for each source media type loaded in the case.
   b. Select the artifacts to be processed and parsed.
   c. Setup the Case Folder on the designated Target Drive and enter the case information.
   d. Initiate processing and run to completion.

6. Upon completion of Steps 4 and 5 proceed with case specific tasks. Use Analysis Notes (QR-CC-6) or other appropriate QR as a documented narrative to describe actions and observations made while performing the case specific tasks. In addition, document on the Analysis Notes (QR-CC-6) or other appropriate QR any pertinent discussions with the submitting agency relating to the examination.

RETIRED

<u>NetClean Analyze (Griffeye)- new case file initiation and processing:</u>

1. Prepare a target drive on the forensic computer as outlined in CC SOP-2 - Target Drive Preparation Protocol and attach this drive to a forensic computer or access the internal drive partition on the forensic computer.

2. Connect the staging drive(s) to the forensic computer or access the designated server staging partition to access the case evidence acquisition files.

3. Begin processing a case by launching the NetClean Analyze version.

4. Follow the protocol and guidelines established in the Reference Document, pertaining to the appropriate NetClean Analyze version to create a new case. Use Analysis Notes (QR-CC-6) or other appropriate QR as a documented narrative to describe actions and observations made during the process.

   a. Verify that the hash databases have been connected and enabled in the settings menu.
   b. Create a new case.

c. Select the source media to import- forensic image, C4All XML or folder contents. Check to see that all of the media to be examined is present in the case file.
d. Select the import options and filters.
e. Initiate processing and run to completion.

5. Upon completion of Steps 3 and 4 proceed with image/video categorization. Update the child abuse hash library upon completion of categorization. Use Analysis Notes (QR-CC-6) or other appropriate QR as a documented narrative to describe actions and observations made while performing the case specific tasks. In addition, document on the Analysis Notes (QR-CC-6) or other appropriate QR any pertinent discussions with the submitting agency relating to the examination.

RETIRED

E. References:

1. Guidance Software -EnCase user manual
2. Guidance Software -EnCase training documentation.
3. Access Data - Forensic Toolkit user manual
4. Access Data - Forensic Toolkit training documentation.
5. Magnet Forensics - Internet Evidence Finder - user manual
6. Magnet Forensics Internet Evidence Finder - training documentation.
7. Magnet Forensics Axiom – Axiom Examinations (AX200) – training documentation
8. Griffeye - NetClean Analyze- user manual.
9. Griffeye - NetClean Analyze- training documentation.

A. Purpose:

To outline the steps taken to report findings and conclusions.

B. Responsibility:

Forensic Examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Use Computer Crimes Report Template (QR-CC-10) when entering finding and conclusions based on the analysis of digital evidence. A "DRAFT" watermark will be included to indicate that the report is at the draft level. The draft level indicates the end of analysis and needs to be initialed and dated by the examiner upon completion.
    a. The suggested font style set for this report is Times New Roman 12. Exceptions to this would be when information in the report needs to be displayed in its native format for presentation, for example, an internet chat.
2. Fill in the Computer Crimes Report (QR-CC-10) Template fields as outlined below:
    a. Laboratory Case # - Use the ***Laboratory Case #*** as represented on the Evidence Receipt. Do not truncate.
    b. Agency - Use the ***Name of Submitting Agency*** as represented on the Request For Analysis (SOP-ER-02:1) form. In addition, include the address of the submitting agency in the report header.
    c. Agency Case # - Use the ***Agency Case #*** as represented on the Request For Analysis (SOP-ER-02:1) form.
    d. Request for Analysis - Select from Appendix A - Requests for Analysis.
    e. Evidence Submitted - List any evidence that was examined in the case. Document the ***Submission #*** and ***Description*** of the evidence as it appears on the Evidence Receipt.
    f. Findings and Conclusions of Examination - Record the findings and conclusions in a numbered order to reflect the following:
        i. Address each specific request for analysis accurately, clearly, unambiguously and objectively. In the case of a proficiency test, the examiner should answer each question posed by the proficiency provider on the report.
        ii. Include submission number(s) where evidentiary findings were recovered.
        iii. Address each item of evidence listed and analysis conducted along with results. Indicate if no analysis was conducted on an item (may include reason for no analysis).
    g. Examiner - The individual assigned to conduct the analysis as indicated in the Request for Analysis.

RETIRED

h. Reviewer - The individual assigned to the Technical Review

3. Create any attachments that will accompany the report. Cut and paste functions will not be utilized for case specific information during the creation of an electronic reporting format.
4. Upon draft report completion, update the JusticeTrax system (LIMS) to reflect that the request now is at the milestone of “Draft Complete”.
5. Submit the report along with the case file to the section supervisor or designee for technical review assignment.
6. Upon completion of the technical review and administrative review, the final report with the signatures and final date of report will be uploaded into Justice Trax.

RETIRED

A. Purpose:

To outline the use of case file records to record pertinent information pertaining to the examination of digital evidence.

B. Responsibility:

Forensic examiners

C. Definitions/Abbreviations:

Refer to SOP-CC-26 - Definitions and Abbreviations.

D. Procedure:

1. Case file records include, but are not limited to the following:
    a. Quality Records.
        i. Current versions of Quality Record templates can be accessed on the shared network drive. Ensure that current version of records have been utilized.
    b. Handwritten notes.
        i. Ensure that a unique case identifier and initials are present on all handwritten notes.
    c. Electronic records (e.g. CD/DVD/USB)
        i. Ensure that a unique case identifier and initials are present on all electronic records. If electronic records containing data/information that can be analyzed at a later date for more testing, these records shall be sub-itemed in Justice Trax and treated as evidence. These records shall be stored in a secure storage location.
2. Case file records shall contain the following information when applicable:
    a. Any communications with the submitting agency and/or other authorized individuals about information relevant to the investigation and/or request(s).
    b. Documentation of non-standard methods used in the examination process(Refer to GL 20).
    c. Documentation of any discrepancies within the evidence submission(s). This documentation should include communication with the appropriate parties.

E. References:

1. Case file

RETIRED

A. Purpose:

To outline the steps taken to report additional findings and conclusions.

B. Responsibility:

Forensic Examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Use Supplemental Computer Crimes Report Template (QR-CC-20) when entering additional findings and conclusions based on the analysis of digital evidence.
   a. The suggested font style set for this report is Times New Roman 12. Exceptions to this would be when information in the report needs to be displayed in its native format for presentation, for example, an internet chat.
   b. If more than one Supplemental Report has been issued on the case, the Supplemental Report title will be followed by the number indicating the chronological order of the reports issued.
2. Fill in the Supplemental Computer Crimes Report Template (QR-CC-20) fields as outlined below:
   a. Laboratory Case # - Use the ***Laboratory Case #*** as represented on the Evidence Receipt. Do not truncate.
   b. Agency - Use the ***Name of Submitting Agency*** as represented on the Request For Analysi (SOP-ER-02:1) form. In addition, include the address of the submitting agency in the report header.
   c. Agency Case # - Use the ***Agency Case #*** as represented on the Request For Analysis (SOP-ER-02:1) form.
   d. Request for Analysis - Select from Appendix A - Requests for Analysis.
   e. Evidence Submitted - List any evidence that was examined in the case. Document the ***Submission #*** and ***Description*** of the evidence as it appears on the Evidence Receipt.
   f. Findings and Conclusions of Examination - Record the findings and conclusions in a bulleted order to reflect the following:
      i. Address each specific request for analysis directly and concisely;
      ii. Include submission number(s) where evidentiary findings were recovered.
      iii. Address any evidence not examined.
   g. Examiner - The individual assigned to the Request for Analysis.
   h. Reviewer - The individual assigned to the Technical Review.

RETIRED

3. Create any attachments that will accompany the report. Cut and paste functions will not be utilized for case specific information during the creation of an electronic reporting format.
4. Upon report completion, affix a supplemental report milestone label to the corner of the case file folder and date and initial the Draft Complete section of the label.
5. Submit the report along with the case file to the section supervisor or designee for technical review assignment.
6. At the completion of the Administrative Review, the final signed report should be scanned and uploaded into Justice Trax.

RETIRED

A. Purpose:

To outline the steps taken when processing evidence in cases involving child pornography.

B. Responsibility:

CCEEU forensic examiners

Unless otherwise stated by an officer of the court (States Attorney, Prosecutor or Judge), the Supervisor, Deputy Director or Director of the Computer Crimes Unit, a maximum of 250 total image and/or videos suspected of being child pornography will be selected for further review and submitted as evidence.

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Open an existing case or create a new case following “CC SOP-8 - Case Initiation Protocol ”.
2. Create a new case record using “ Child Pornography Guidelines (QR-CC-7)”.
   a. Fill in the appropriate Start Date, Laboratory Case #, Submission #(s) and Examiner fields.
   b. Use this as a guide while performing the analysis.
3. Open or create a new case record using “Analysis Notes (QR-CC-6)”.
   a. Fill in, if necessary, the appropriate Start Date, Laboratory Case # and Examiner fields.
   b. Record information relevant to the analysis as encountered. In addition, document any pertinent discussions with the customer relating to the examination.
4. In addition to using techniques for analysis based upon the examiner’s training, knowledge and experience, other protocols can be found outlined and explained in the forensic software reference documents and training documentation pertaining to the forensic software utilized.
5. Initial the appropriate area alongside the task performed in the section(s) on the Child Pornography Guidelines worksheet pertaining to the forensic software utilized, after it has been completed or if it had been previously completed.
6. If the C4All categorizer application was used to review and categorize images and/or videos, then upon completion export the hash values so that they can imported into the laboratory database. This is carried out within the application by selecting *New Filter*, *Custom Filter*, Choose Categories 1,2,3 and 9 and select *Set Filter*. Then select *Data Migration, Export Work, From Current Filter, to Hash Report,* select file path to save to and select *Build.* The filename needs to be amended to include the case number at the start of the name. Finally, the file needs to be uploaded to your named folder within the C4ALLHASHES folder on the ISO shared drive.
7. Initial this form in the upper right corner to indicate completion.

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8. If additional requests for analysis were made for the case, or if based upon the examiner's training, knowledge, experience and familiarity with details of the case additional analysis is warranted, follow the appropriate SOPs.
9. If images/videos need to be reviewed by NCMEC or if possible newly identified victim images/videos need to be sent to NCMEC, follow "CC SOP-32 - NCMEC Requirements'.
10. Upon completion of the examination, proceed with "CC SOP-9 - Laboratory Report Protocol" to produce a Laboratory report.

E. References:

1. Guidance Software -EnCase user manual
2. Guidance Software -EnCase training documentation.
3. Access Data - Forensic Toolkit user manual
4. Access Data - Forensic Toolkit training documentation.
5. Magnet Forensics - Internet Evidence Finder - user manual
6. Magnet Forensics Internet Evidence Finder - training documentation.
7. Griffeye - NetClean Analyze- user manual.
8. Griffeye - NetClean Analyze- training documentation.

RETIRED

A. Purpose:

To outline the steps taken when processing evidence in cases involving check fraud and/or larceny.

B. Responsibility:

CCEEU forensic examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Open an existing case or create a new case following “CC SOP-8 - Case Initiation Protocol ”.
2. Create a new case record using “Check Fraud and Larceny Guidelines (QR-CC-8)”.
   a. Fill in the appropriate Start Date, Laboratory Case #, Submission #(s) and Examiner fields.
   b. Use this as a guide while performing the analysis.
3. Open or create a new case record using “- Analysis Notes (QR-CC-6)”.
   a. Fill in, if necessary, the appropriate Start Date, Laboratory Case # and Examiner fields.
   b. Record information relevant to the analysis as encountered. In addition, document any pertinent discussions with the customer relating to the examination.
4. In addition to using techniques for analysis based upon the examiner’s training, knowledge and experience, other protocols can be found outlined and explained in the forensic software reference documents and training documentation pertaining to the forensic software utilized.
5. Initial the appropriate area alongside the task performed on the Check Fraud and Larceny Guidelines worksheet pertaining to the forensic software utilized after it has been completed or if it had been previously completed.
6. Initial this form in the upper right corner to indicate completion.
7. If additional requests for analysis were made for the case, or if based upon the examiner’s training, knowledge, experience and familiarity with details of the case additional analysis is warranted, follow the appropriate SOPs.
8. Upon completion of the examination, proceed with “CC SOP-9 - Laboratory Report Protocol” to produce a Laboratory report.

E. References:

1. Guidance Software -EnCase user manual
2. Guidance Software -EnCase training documentation.
3. Access Data - Forensic Toolkit user manual
4. Access Data - Forensic Toolkit training documentation.
5. Magnet Forensics - Internet Evidence Finder - user manual

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6. Magnet Forensics Internet Evidence Finder - training documentation

RETIRED

A. Purpose:

To outline the steps taken when processing evidence in cases involving counterfeiting.

B. Responsibility:

CCEEU forensic examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Open an existing case or create a new case following “CC SOP-8 - Case Initiation Protocol ”.
2. Create a new case record using “ Counterfeiting Guidelines (QR-CC-9)”.
   a. Fill in the appropriate Start Date, Laboratory Case #, Submission #(s) and Examiner fields.
   b. Use this as a guide while performing the analysis.
3. Open or create a new case record using “ Analysis Notes (QR-CC-6)”.
   a. Fill in, if necessary, the appropriate Start Date, Laboratory Case # and Examiner fields.
   b. Record information relevant to the analysis as encountered. In addition, document any pertinent discussions with the customer relating to the examination.
4. In addition to using techniques for analysis based upon the examiner’s training, knowledge and experience, other protocols can be found outlined and explained in the forensic software reference documents and training documentation pertaining to the forensic software utilized.
5. Initial the appropriate area alongside the task performed on the Counterfeiting Guidelines worksheet after it has been completed or if it had been previously completed.
6. Initial this form in the upper right corner to indicate completion.
7. If additional requests for analysis were made for the case, or if based upon the examiner’s training, knowledge, experience and familiarity with details of the case additional analysis is warranted, follow the appropriate SOPs.
8. Upon completion of the examination, proceed with “CC SOP-9 - Laboratory Report Protocol” to produce a Laboratory report.

E. References:

1. Guidance Software -EnCase user manual
2. Guidance Software -EnCase training documentation.
3. Access Data - Forensic Toolkit user manual
4. Access Data - Forensic Toolkit training documentation.
5. Magnet Forensics - Internet Evidence Finder - user manual
6. Magnet Forensics Internet Evidence Finder - training documentation.

RETIRED

A. Purpose:

When contraband evidence (i.e. child pornography) is encountered while examining evidence submitted to the laboratory, a sub-item needs to be created in LIMS in order to track the chain of custody of such evidence and to ensure its integrity. The following outlines the steps taken to securely maintain contraband evidence that is either submitted to the laboratory or located on evidence during the examination process.

B. Responsibility:

Section supervisor and forensic examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Transcribe the following information onto the disc(s) or removable media either before or after the data is copied:
   a. Laboratory case #
   b. Investigating agency
   c. Agency case #
   d. Date
   e. Indicate how many disc(s) comprise the final product (e.g. Disk 1 of 2).
   f. Identify the disc(s) by describing the contents (e.g. Attachment One).
2. Burn a disc(s) containing the contraband data or copy the contraband data to the removable media.
3. After the disc(s) has been created, verify that the disc(s) or removable media contains the data and demonstrate that it is accessible by opening a couple of files.
4. Upon successful completion, initial the disc(s) or removable media.
5. Create a sub-item barcode label following the steps outlined in JusticeTrax's help file under "Itemizing Evidence" following these criteria:
   a. Create the sub-item (child) within the first CCEEU submission (parent) in the case. Do not inherit the properties of the parent submission.
   b. Use the sub-item numbering convention as outlined in SOP GL4 to generate the sub-item number.
   c. Describe the contents in the "Description" field.
   d. Create a new sub-item for each additional disc or removable media drive.
   e. Affix the barcode label to the disc sleeve or to the removable media packaging.
   f. Affix an evidence/contraband label (CCEEL-50 - Contains Contraband Labels) to the disc sleeve or removable media packaging.
   g. Seal the disc sleeve or the manila envelope containing the removable media with evidence tape. Initial and date the evidence tape.

RETIRED

6. Continue tracking the chain of custody in LIMS and maintaining secure storage locations while the sub-item is in the laboratory.

E. References:

1. JusticeTrax help file

RETIRED

A. Purpose:

To outline the steps taken to analyze cell phones. A Cell phone analysis request means that the internal memory of the device will be extracted, as well as onboard memory card(s)s /SIM card(s). The extraction(s) will be parsed using a software program(s). Searches and other in depth analyses will be conducted on the parsed data. A laboratory report of the analysis results is produced and provided to the requesting agency.

B. Responsibility:

Forensic Examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. The technique used for the examination of cell phones should be determined by, but not limited to, the examiners training knowledge and experience.
2. Current acceptable techniques for data extraction and analysis include:
   a. Using the CelleBrite UFED Touch Unit or UFED 4PC software to extract data contained on the cell phone and using the UFED Physical Analyzer software to parse and analyze data contained on the cell phone by following the manufacturer's protocol, as well as, the examiner's training and experience;
   b. Using Oxygen Forensic Suite software to extract data contained on the cell phone by following the manufacturer's protocol, as well as, the examiner's training and experience;
   c. Manually scrolling through the cell phone and recording relevant information pertaining to the request for analysis.
   d. Graykey can used for extraction of data from iPhone devices.
3. Every effort should be taken to ensure that the cell phone is protected from receiving an external signal. For example, using the Ramsey Shielded Test Enclosure.
4. If a different method is used to analyze the cell phone, thoroughly document it in the Cell Phone Worksheet\Notes (QR-CC-16) and all other appropriate records. In addition, bring this method to the Technical Lead and/or the Unit Supervisor's attention for further review.
5. If a passcode locked Apple device has been submitted for analysis refer to CC SOP-50 and CC SOP-53.
6. If a custom passcode or pattern locked device has been submitted for analysis and current methods cannot bypass or determine the code, submit a Cell Phone Passcode Needed Notification Letter (QR-CC-51) to the submitting agency. If the passcode cannot be provided within 5 business days, the evidence should be returned to the agency with a report stating the analysis could not be conducted.

References:

1. CelleBrite UFED Touch user manual or training documentation.

RETIRED

2. Oxygen Forensic Suite user manual or training documentation.

RETIRED

A. Purpose:

To outline the steps taken to ensure that a forensic computer being used to examine and store digital evidence is secure and working properly.

B. Responsibility:

Forensic examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations

D. Procedure:

1. Turn on the forensic computer and verify that the POST / boot sequence executes properly.
2. Ensure that the forensic computer requires a logon password for access.
3. Ensure that there is no case related material opened or accessible on the system and temporarily enable the internet network connection:

   Review operating system updates available and install if necessary.

   Ensure that the anti-virus and malware definitions have been updated.
4. Restart the system and verify that the POST / boot sequence executes properly; also verify that the operating system updates installed properly by reviewing the update history.
5. Disable the internet network connection on the forensic system.
6. In the event that verification of the system fails, resolve the issue before proceeding with any examinations using this computer.
7. Troubleshooting procedures include, but are not limited to the following:
   a. Training, knowledge and experience.
   b. Technical references.
   c. Consulting with co-workers and technical support.
8. If the issue cannot be resolved, the computer cannot be used for digital evidence analysis and a “Out of Service” sticker should be placed on the computer.
9. Document the verification process by documenting the verification date in the forensic system’s maintenance log book along with a note that this check was performed..
10. A notation that software and/or hardware maintenance was performed on the system is to be recorded in the individual examiner’s maintenance log book.
11. The system POST / boot sequence check, any necessary operating system updates and antivirus updates must be performed prior to starting a new case and/or following system repairs.

E. References:

1. Technical references

RETIRED

A. Purpose:

To outline the steps taken to ensure that primary forensic software used for the analysis of digital media is functioning properly. The initial determination of suitability needs to be done by conducting a validation. After the initial validation, any software updates may be checked using a performance check. GL-22 should be used as guidance to determine what is needed for a validation or performance check.

B. Responsibility:

Forensic examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Install the forensic software according to the manufacturer's guidelines to a verified performance checked forensic computer (Refer to CC-SOP-19).
2. Obtain the controlled media image files and associated guidelines from the shared directory- i.e. DCFL Control Standard or other. Using the approved validation plan or performance check, this control media will be used to conduct the necessary check.
3. Prepare a " QC Worksheet - Software (QR-CC-23)" record by filling in the date and examiner fields.
4. Initiate a QC case using the software and load the media image files.
5. If applicable, perform a hash of the controlled media images using the software being performance checked. Record the information in the appropriate fields on the worksheet.
6. Follow the control standard guidelines and verify the artifacts listed are present and located at the given offsets. Conduct the necessary processing of the images using the software to verify the elements listed in the guidelines. Record the results in the Notes section of "QC Worksheet - Software (QR-CC-23)", including errors or deviations from expected results.
7. Complete the record by filling in the appropriate information and hash values.
8. If completed successfully (i.e. YES=Pass), the software has been successfully performance checked.
9. In the event that the software fails the performance check or validation, redo the process in an attempt to rule out any processing errors.
10. If a second attempt fails, drawing upon your training, knowledge and experience, as well as, consulting with co-workers and technical support, attempt to isolate the issue and/or define the functional limitations of the software. Record any limitations in the Notes section of "QC Worksheet - Software (QR-CC-23)".

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11. If the issue cannot be resolved and there are no defined functional limitations, the software cannot be used for case examination.
12. Retain a copy of the filled out " QC Worksheet - Software (QR-CC-23)" for your records. The records may also be archived electronically in the designated examiner folder located in the "iso directory" of the unit server.
13. Whenever new software is purchased, a validation plan should be conducted to determine the suitability for casework. If a previously validated software has an update, a quality control performance check needs to be conducted prior to the updated software being used. If the previously validated software has a major software update, a validation should be considered depending on the effect of the major update relates to casework.

E. References:

1. Manuals and guides for applicable forensic software.

RETIRED

A. Purpose:

To outline the steps taken to ensure that the write block hardware and/or software used when working with digital media is functioning properly.

B. Responsibility:

Forensic examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations

D. Procedure:

1. Prepare a "QC Worksheet - Write Blocks" (QR-CC-24) record by filling in the date and examiner fields.
2. Prepare an appropriate QC media device from files on the "iso folder" shared network directory or other suitable source. An example would be as follows:
   a. If testing a USB write block device or software, copy the appropriate files onto a USB storage device.
   b. Place the USB storage device into the write blocked port.
3. Perform a pre-QC hash of the QC media using an approved hashing tool. Record the information in the appropriate fields on the worksheet.
4. Perform the following tasks:
   a. Attempt to delete files on the QC media (at least two (2)).
   b. Attempt to copy files to the QC media (at least two (2)).
   c. Attempt to alter files on the QC media (at least two (2)).
   d. Attempt to create a file onto the QC media (at least two (2)).
   e. Attempt to wipe the QC media.
5. Perform a hash of the QC media. Record the information in the appropriate fields on the worksheet.
6. Complete the record by filling in the appropriate information.
7. If completed successfully (i.e. YES=Pass), the write block has been successfully validated.
8. In the event that the write block fails to validate, redo the process in an attempt to rule out any processing errors.
9. If a second attempt fails, drawing upon your training, knowledge and experience, as well as, consulting with co-workers and technical support, attempt to isolate the issue.
10. If the issue cannot be resolved, the write blocker cannot be used on that computer.

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11. A hardcopy of the completed record "QC Worksheet - Write Blocks" (QR-CC-24) needs to be retained by each examiner in a notebook. The records may also be archived electronically in PDF format in the designated examiner folder located in the " iso directory" of the unit server.
12. This quality control check should be performed prior to initially being used and prior to re-use of a repaired device.

RETIRED

A. Purpose:

To outline the steps taken to technically review an analysis case request or a cell phone extraction only request.

B. Responsibility:

Forensic examiners

C. Definitions/Abbreviations:

Refer to SOP-CC-26 - Definitions and Abbreviations.

D. Procedure:

1. Prepare a “Technical Review Guidelines (QR-CC-15) ” record for an analysis case request or a “Cell Phone Extraction Technical Review Guidelines (QR-CC-53)” record for a cell phone extraction only case request. Begin by filling in the appropriate Laboratory Case #, Reviewer and Start Date fields.
2. Follow the steps outlined in this quality record to complete the technical review process.
3. Notes generated by the technical reviewer must be recorded in the Notes section of the “Technical Review Guidelines (QR-CC-15) ”. The notes must be dated and initialed by the technical reviewer and maintained in the case folder (Refer to GL-18 for guidance).
4. The examiner will initial each deficiency listed in the notes to acknowledge that it has been reviewed and addressed.

E. References:

1. GL-18

RETIRED

A. Purpose:

To provide definitions and abbreviations to clarify terminology referenced in the CCEEU protocols.

B. Responsibility:

All CCEEU Staff

C. Procedure:

1. Definitions:

a. **Attachment** - An attachment is work product that is referenced in the associated Laboratory Report. It contains information pertaining to the request and is presented in an electronic format on optical disk media /other media or hard copy format.

b. **CFE –** Computer Forensic Examiner

c. **Child Pornography** - State Statute 53a-193

Prior law restricted child pornography to material involving a live performance or photographic or other visual reproduction of a live performance which depicts a minor in a prohibited sexual act. The act, instead, defines it as any visual depiction, including any photograph, film, videotape, picture, or computer-generated image or picture, whether made or produced by electronic, mechanical, or other means, of sexually explicit conduct. The production of the depiction must involve use of a person engaging in such conduct who was under age 16 at the time of its creation. It specifies that the trier of fact must decide disputes about the actor's age.

Sexually explicit conduct. The act defines "sexually explicit conduct" as actual or simulated:

(a) sexual intercourse, including genital-genital, oral-genital, anal-genital, or oral-anal physical contact between persons of either sex or with an artificial genital;

(b) bestiality;

(c) masturbation;

(d) sadistic or masochistic abuse; or

(e) lascivious exhibition of the genitals or pubic area of any person.

d. **Data Card** - Removable digital storage found in cell phones.

e. **DCFL Control Standard** – a Computer Reference Data Set for digital evidence from NIST and developed by the Defense Computer Forensics Laboratory. Currently known as the Defense Cyber Crime Center(DC3), Department of Defense (DoD).

f. **Digital Media** - Items of evidence that store or transmit in binary form.

g. **Evidence Drive** - Hard drives submitted to the Laboratory as evidence.

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h. **Forensic Computer** - A computer containing approved forensic hardware and software used for the analysis of digital evidence.

i. **Forensic Image** - A bit for bit copy of the original digital media that can include proprietary data within the image file(bit plus copy).

j. **JusticeTrax** - The laboratory information management system (LIMS) currently used to track cases throughout the laboratory sections/units within the DSS.

k. **Optical Disk** - Includes all types of standard and mini CD, DVD and Blu-ray disk media.

l. **Removable Media** - Includes optical disks, flash cards, floppy disks, thumb drives and zip disks.

m. **Staging Drive** - Storage media that contains acquisition(image) files.

n. **Target Drive** - Storage media that contains work product data.

o. **Work Product** - Files containing information(data) pertinent to the specific case request being examined.

2. **Abbreviations:**

a. **CCEEU** - Computer Crime and Electronic Evidence Unit

b. **COC** - Chain of Custody.

c. **CRIS** – Child Recognition Identification System

d. **DC** - Digital Camera - Includes all dedicated camera devices capable of taking digital images and/or videos but does not include any other device capable of these actions (i.e. cell phones, iDevices, tablets)

e. DESPP - Department of Emergency Services and Public Protection

f. **DSS** - Division of Scientific Services

g. **FC** - Flash Card - Includes all non-USB removable modules. Examples of flash card memory include: SD Cards, Memory Sticks and CompactFlash.

h. **FD** - Floppy Disk - Includes all types of 3.5” and 5.25” floppy disks.

i. **HD** - Hard Drive - Includes all internal and external Parallel, Serial, SCSI and Solid State hard drives.

j. **LBA** - Logical Block Address- a hard drive parameter which may or may not be designated on the label and can be used to calculate the drive capacity.

k. **LIMS** - Laboratory Information Management System (Currently JusticeTrax).

l. **MD** - Media Device - Includes portable media player devices such as an iPod.

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m. **MP** - Cellular Phone - Includes; Basic, Camera, Music/MP3, Video, Smart and PDA (Personal Digital Assistant) phones

n. **ND** - Not Defined - Includes any digital media not previously defined.

o. **NCMEC** - National Center For Missing and Exploited Children

p. **OD** - Optical Disk - Includes all types of standard and mini CD, DVD and Blu-ray disks

q. **SC** - SIM Card - Found primarily in GSM cell phones. However, SIM cards may be found in certain CDMA (Code Division Multiple Access) cell phones.

r. **TAB** - Tablet devices - Includes Apple iPads and Android based tablets

s. **TD** - Thumb Drive - Any direct USB digital media device.

t. **ZD** - Zip Disk - Includes any size 3.5” removable disk drive from Iomega.

RETIRED

A. Purpose:

To unify the maintenance and indexing process for hardware and software used for the examination of digital evidence.

B. Responsibility:

Technical lead and forensic examiners

C. Definitions/Abbreviations:

Refer to CC-SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. All hardware and software purchased by the CCEEU is commercially available and industry standard. A quality control check and performance check is conducted on the hardware and software as described below.
2. Quality control check existing hardware and software based on the intervals specified in the appropriate protocol:
   a. CC-SOP -19 - QC Protocol - Forensic Computer
   b. CC-SOP -20 - QC Protocol - Software used to Analyze Digital Media
   c. CC-SOP -21 - QC Protocol - Write Blocks
   d. CC-SOP -36 - QC Protocol - Non-Standard Methods - Software
3. Approved software is catalogued in "Approved Software" (QR-CC-49) . A hardware list will be maintained on the unit server (currently "iso folder" shared directory).
4. When possible, approved software setup files are maintained in a central location (currently "iso folder" shared directory) that is accessible by the forensic examiners, the technical lead and supervisors. Software setup files can also be downloaded by the examiner from the vendor's internet sites and hash checked upon completion of the download.
5. Electronic copies of the manufacturer's reference material (e.g. user guides, training manuals) will be made available in a central location (currently "iso folder" shared directory) when possible. When electronic copies of the manufacturer's reference material are unavailable, hard copies will be available in the CCEEU's library and/or with the individual forensic examiners and supervisors.
6. Approved virus protection software shall be maintained on all forensic computers based on the manufacturers specifications. Virus software shall be updated according to protocol established in CC-SOP-19.
7. When updating any software on a forensic computer, ensure that no case work files are opened prior to the update by closing all open forensic applications.
8. When repairs/updates are necessary to a forensic computer (e.g. hard drive crash, software updates, addition and/or removal of hardware), the computer should be taken out of service and

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not be used for digital evidence analysis until a quality control check has been performed to verify that the forensic computer is working properly. Refer to CC SOP-19.

9. A maintenance log book will be provided to each forensic examiner to record the following types of maintenance:
   a. Hardware installations and removals
   b. Software installations and updates
   c. Repairs (e.g. replacing a crashed hard drive)

   The dates of the maintenance shall be recorded within this log.

E. References:

1. Manufacturer's reference material (e.g. user guides, training manuals)

RETIRED

A. Purpose:

To outline the steps taken to ensure that the integrity and chain of custody of evidence is maintained.

B. Responsibility:

CCEEU forensic examiners and section supervisor

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Upon notification of evidence being submitted to the CCEEU through the Evidence Receiving section the section supervisor or designee transports the evidence from Evidence Receiving to a secure location in the CCEEU and a chain of custody is maintained.
2. During the evidence examination process, remove the evidence from the secure evidence storage location and update the chain of custody in LIMS.
3. If the evidence is unattended, secure it in a secure area until the examination process is complete. When the examination is complete, transport the evidence to a secure evidence storage location and update the chain of custody in LIMS.
4. Prior to the evidence being return, ensure that it has been properly packaged.
5. When the evidence is returned, transport it to the Evidence Receiving section and maintain the chain of custody through LIMS.

E. References:

1. SOP GL13

RETIRED

A. Purpose:

To outline the steps taken to ensure that secondary viewer software used to analyze digital media is functioning properly.

B. Responsibility:

Forensic examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations

D. Procedure:

1. When a file needs to be examined, the examiner may use available secondary viewer software that supports viewing the contents of that file type.
2. As a verification check, another software application, should also be used to examine the file type for its' contents.
3. If both software packages display the same information to the examiner and it is reproducible, the viewer software is considered performance checked. This should be documented in "QC Protocol-File Viewers" (QR-CC-28) and a copy should be retained by the examiner in a record format. The records may also be archived electronically in PDF format in the designated examiner folder located in the " iso directory" of the unit server.
4. When a secondary viewing software is used in an examination, the name and version number of the software should be noted in the examiners Analysis Notes (QR-CC-6).

RETIRED

A. Purpose:

To uniquely identify sub-items of digital media submitted to the CCEEU.

B. Responsibility:

CCEEU Examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. When processing evidence submitted to the CCEEU and an item(s) of digital media is located with that submission that is not uniquely referenced on the submission barcode label, may be identified with a unique reference abbreviations. If an item has multiple components, these may be referenced in the description together. (001-001 Cell phone S1_MP1, SIM card S1_SC1 and flash card S1_FC1)
2. The following table lists the abbreviations to be assigned to the corresponding digital media:

DC	Digital Camera	ND	Not Defined
FC	Flash Card	OD	Optical Disk
FD	Floppy Disk	SC	SIM Card
HD	Hard Drive	TAB	Tablet Computer
MD	Media Device	TD	Thumb Drive
MP	Mobile Phone	ZD	Zip Disk

3. If the item(s) of digital media will be separated from the parent item and will take a separate path during the analysis (i.e. tower and hard drive separated and tower will stay in evidence room), the item will be sub-itemized in Justice Trax using guidance from GL4 “LIMS”.
4. The following are examples explaining the process of creating create sub-items.
   a. A sealed paper bag with a Laboratory barcode label indicating “Submission #003” and containing “two (2) cell phones” is taken in to be examined by the CCEEU.

      Since the parent item barcode label does not uniquely identify the two (2) cell phones, the phones will be described as S3_MP1 and S3_MP2 in the case notes. If the cell phones will be analyzed at different times and locations, they will be itemized in Justice Trax as 003-001 and 003-002 using the description language of S3_MP1 and S3_MP2. The naming

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convention for the item description can be S3_MP1 and S3_MP2. The "S" represents the Laboratory Submission Number and the MP represents each unique cell phone. The underscore separates the two.

Each phone will have transcribed on it:

Phone #1 - The Laboratory Case Number - e.g. ID-00-123456
The sub-item number - 003-001 if itemized in Justice Trax and/or S3_MP1
The analyst's initials

Phone #2 - The Laboratory Case Number - e.g. ID-00-123456
The sub-item number - 003-002 if itemized in Justice Trax and/or S3_MP2
The analyst's initials

b. A sealed paper bag with a Laboratory barcode label indicating "Submission #006" and containing "one (1) hard drive" is taken in to be examined by the CCEEU.

Even though the barcode label does uniquely identify the hard drive a sub-item needs to be created to comply with the acquisition syntax.

The hard drive will have transcribed on it:

The Laboratory Case Number - e.g. ID-00-123456
The sub-item number - "S6_HD1" and if the hard drive travels a different path from the exterior case, it will also be itemized in Justice Trax as 006-001 (description S6_HD1)
The analyst's initials

RETIRED

A. Purpose:

1. To outline the steps taken to submit images/videos to be reviewed for identified children by NCMEC.
2. To outline the steps taken to submit newly identified victim(s) to NCMEC.

B. Responsibility:

CCEEU forensic examiners.

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

One of the two methods below can be used to submit images and videos to NCMEC for review:

1. Electronic Submission to NCMEC:

a. Follow the instructions outlined in the current NCMEC policy document for submitting images/videos electronically for review by the Child Victim Identification Program (CVIP). The current NCMEC policy document will be saved on the shared server located in the ISO folder share directory.
b. Create a sub-item (child) within the first CCEEU submission (parent) in the case for the electronic submission.
c. Use the sub-item numbering convention as outlined in SOP GL4 to generate the sub-item number.
d. Give a general description of the sub-item in the "Description" field (i.e. - electronic submission file(s) containing images/videos for NCMEC). Do not reference child pornography in the description.
e. Transfer the sub-item to the virtual storage location – Electronic Submission to NCMEC upon uploading to NCMEC. Note this storage location is virtual and does not contain physical items, its purpose is to track the date at which the file(s) were uploaded to NCMEC.
f. Generate a request in JusticeTrax (LIMS) for NCMEC CRIS –Electronic Submission. Leave the request unassigned. Associate the NCMEC sub-item to the request.

2. Mail Transport to NCMEC:

a. Burn an optical disk (CD/DVD) containing the images/videos to be sent to NCMEC or copy files to an alternate type of media if a greater capacity is required- i.e. Thumb drive, hard drive.
b. Label the optical disk or alternate storage media with the appropriate NCMEC CD label template that has been filled in with the proper identifying information or manually record the proper identifying information on the alternate storage media to include Investigating Agency, Agency Case#, Laboratory Case#, Date and Content description.

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c. Verify the information on the optical disk or alternate storage media and initial the "Verified:" area of the label to indicate the appropriate information is on the media.
d. Depending on the request(s), fill out the appropriate NCMEC paperwork that will accompany the optical disk or alternate storage media. This will be either one or both: "USPIS Template Letter-CRIS Reviews" and/or "USPIS Template - Letter Identified Victim Submission". These template letters are located in the ISO folder share directory.
e. Place the optical disk or alternate media, as well as, the above paperwork in a CD mailer or other suitable container.
f. Create a sub-item barcode label following the steps outlined in JusticeTrax's help file under "Itemizing Evidence". Affix the label on the optical disk container or media container. Follow these criteria to generate the sub-item:
    1. Create the sub-item (child) within the first CCEEU submission (parent) in the case.
    2. Use the sub-item numbering convention as outlined in SOP GL4 to generate the sub-item number.
    3. Give a general description of the sub-item in the "Description" field (i.e. – optical disk/alternate media - external hard drive containing images/videos for NCMEC). Do not reference child pornography in the description.
    4. Seal the CD mailer or container securely with evidence tape.
g. Obtain a shipping envelope / container, a USPS Certified Mail Receipt and a Return Receipt and follow the steps outlined below:
    1. Fill out the required information on the postal shipping receipts and apply them to the shipping envelope/container.
    2. Record the Laboratory Case Number at the bottom of the postal Return Receipt.
    3. Transfer the barcoded optical disk container or media container to Evidence Receiving for mail transport to complete the chain of custody update.
    4. Place the optical disk/paperwork package in a shipping envelope and seal the envelope.
h. Generate a request in JusticeTrax (LIMS) for NCMEC CRIS. Leave the request unassigned. Associate the NCMEC media sub-item to the request. Submit the optical disk or media to the Unit Supervisor for mail transport

3. Upon receiving a NCMEC CRIS report via email, send a copy to the Unit Supervisor. The number of images and videos that are indicated as known victims and those that were not identified will be tracked by the associated case number in a spreadsheet located on the shared drive. The hash values for the images and videos of known victims as well as the unidentified images will also be saved in text files to be uploaded and included in the appropriate laboratory hash database categories.

E. References:

1. NCMEC-Submitting Images Electronically for Review by NCMEC'S Child Victim Identification Program (CVIP)

RETIRED

2. NCMEC-CVIP General Submissions Forms pdf
3. JusticeTrax help file

RETIRED

A. Purpose:

To outline the steps taken to produce a Report Attachment on media.

B. Responsibility:

Forensic Examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. If the attachment media is an optical disk, select one of the approved Attachment disc label templates and fill in the appropriate information in the corresponding fields.
2. If no labels are available or another type of attachment media is used, transcribe the following information onto the media either before or after the Attachment media is created:
   a. Laboratory case #
   b. Investigating agency
   c. Agency case #
   d. Date
   e. Indicate how many discs or media items comprise the Attachment (e.g. Disc 1 of 2).
   f. Identify the disc(s) or media content. For example, write "Attachment Disc" on them.
3. Create the attachment media containing the information relevant to the case report. Cut and paste functions will not be utilized for case specific information during the creation of an electronic reporting format.
4. After the media has been created, verify that the media contains the attachment information and demonstrate that it is accessible by opening files on the media.
5. Upon successful completion, initial the media.
6. Create a sub-item barcode label following the steps outlined in JusticeTrax's help file under "Itemizing Evidence" following these criteria:
   a. Create the sub-item (child) within the first submission (parent) in the case. Do not inherit the properties of the parent submission.
   b. Use the sub-item numbering convention as outlined in SOP GL4 to generate the sub-item number.
   c. Describe the Attachment Media in the "Description" field.
   d. If the Attachment media contains contraband, adhere an evidence/contraband label (CCEEU-50 - Contains Contraband Labels) to the media container and seal the container with evidence tape. Initial the evidence tape.
   e. Affix the barcode label to the media container and transfer the sub-item(s) to the appropriate storage location in JusticeTrax.

E. References:

RETIRED

1. JusticeTrax help file
2. SOP GL4

RETIRED

A. Purpose:

To outline the steps taken to address evidence discrepancies and suitability issues.

B. Responsibility:

Forensic examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Examples of evidence discrepancies and suitability issues include, but are not limited to the following:
   a. When comparing the contents of a submission to the SOP-ER-02:1 (Request for Examination of Physical Evidence) record any inaccuracies found. Examples of this would be that the evidence description is wrong or the number of items in the submission is inaccurate. These types of evidence discrepancies or errors will be documented in the associated Laboratory Notes (QR-CC-5). Case Management also needs to be notified by email of the discrepancies or errors so that the main case file in Evidence Receiving can be updated or corrected.
   b. When processing a submission and it is determined that the submission is unsuitable for testing. An example of this would be a hard drive that you determine does not work. This type of processing problem will be recorded in the associated Laboratory Notes (QR-CC-5) (Refer to CC-SOP-5).
   c. When processing a submission and it is determined that the proper equipment (hardware and/or software) necessary for the analysis is not available. This type of analysis problem will be recorded in the Laboratory Notes (QR-CC-5) and/or Analysis Notes (QR-CC-6). The Laboratory Report (QR-CC-10) will also include a statement that the submission item could not be processed and/or analyzed due to the unavailability of compatible hardware or software.
2. When these types of criteria exist, the submitting agency should be notified and alternative options should be discussed and documented. If an alternative method is agreed upon and it deviates from a standard method refer to CC-SOP-10, "Case File Records" and GL-20.

RETIRED

A. Purpose:

To outline the steps taken to produce a Work Product Archive.

B. Responsibility:

Forensic Examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. If the archival media is an optical disk, select one of the approved work product disc label templates and fill in the appropriate information in the corresponding fields. Produce a label for the disk.
2. If no labels are available or another type of archival media is used, transcribe the following information onto the media either before or after the work product media is created:
   a. Laboratory case #
   b. Investigating agency
   c. Agency case #
   d. Date
   e. Indicate how many discs or media items comprise the work product (e.g. Disk 1 of 2).
   f. Identify the disc(s) or media content by writing "Work Product" on them.
3. Create the archival media including the following case related information when it exists:
   a. Quality Records (e.g. Laboratory Notes, Analysis Notes, etc.)
      - Any completed Quality Record (e.g. Lab Notes, Analysis Notes, etc.) which has been edited, updated or includes supplementary information will have all pages electronically scanned and included on the Work Product archival media in a PDF or similar format.
   b. Submission photos
   c. Attachment material
   d. NCMEC material (images, videos and correspondences)
   e. Laboratory report
   f. Encase case file
   g. Extracted cell phone data
   h. Cell phone screenshot photos
4. After the archival media has been created, verify that the media contains the information and demonstrate that it is accessible by opening files on the media.
5. Upon successful completion, initial the media.
6. Create a sub-item barcode label following the steps outlined in JusticeTrax's help file under "Itemizing Evidence" following these criteria:

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a. Create the sub-item (child) within the first submission (parent) in the case. Do not inherit the properties of the parent submission.
b. Use the sub-item numbering convention as outlined in SOP GL4 to generate the sub-item number. Type “Work Product” in the “Description” field.
c. If the work product media contains contraband, adhere an evidence/contraband label (CCEEU-50 - Contains Contraband Labels) to the media container.
d. Affix the barcode label to the media container and transfer the sub-item(s) to the appropriate storage location in JusticeTrax.

7. Secure the media to the inside of the case folder with tape. The work product media will remain in the case file folder when not being reviewed.

E. References:

1. JusticeTrax help file
2. SOP GL4

RETIRED

A. Purpose:

To outline the steps taken to quality control check that non-standard / newly obtained software is capable of performing the task that it was designed to do with respect to the analysis of digital evidence.

B. Responsibility:

Forensic examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Assemble a QC media device with the appropriate controlled media to test the software. Controlled media image files and associated guidelines can be obtained from the shared directory- i.e. DCFL Control Standard or other.
2. Prepare a "QC Protocol - Non-Standard - Software" (QR-CC-27) record by filling in the date and examiner fields.
3. Perform a pre-QC hash of the QC media using an approved hashing tool. Record the information in the appropriate fields on the worksheet.
4. Connect the QC media to the forensic computer that will be testing the software.
5. Run the software on the QC media device. Follow the control standard guidelines and verify the artifacts listed are present and located at the given offsets. Conduct the necessary processing of the images using the software to verify the elements listed in the guidelines. Record all pertinent information listed on the QC worksheet (QR-CC-27) and additional information in the Notes section including errors or deviations from expected results.
6. Perform a post-QC media hash. Record the information in the appropriate fields on the worksheet.
7. Repeat the process.
8. The software successfully passes the QC validation if the two (2) following criteria are met:
   a. The pre-QC hash values and the post-QC hash values match.
   b. The expected results were obtained with consideration of defined functional limitations and were reproducible.
9. In the event that the QC test fails, repeat the validation process in an attempt to rule out any processing errors.
10. If a second attempt fails, drawing upon your training, knowledge and experience, as well as, consulting with co-workers and technical support, attempt to isolate the issue and/or define the functional limitations of the software. Record any limitations in the Notes section of "QC Worksheet (QR-CC-27).

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11. If the issue cannot be resolved and there are no defined functional limitations, the software cannot be used for case examination.
12. Retain a copy of the filled out "QC Protocol - Non-Standard Methods - Software" (QR-CC-27) for your records. The records may also be archived electronically in PDF format in the designated examiner folder located in the " iso directory" of the unit server.
13. This quality control check should be performed on non-standard software prior to being incorporated as approved software and re-evaluated when major software versions are released.

E. References:

1. Specific software's user guide and manuals

RETIRED

A. Purpose:

To outline the steps taken to analyze optical disk media.

B. Responsibility:

Forensic Examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. It is not necessary to acquire or hash optical disk media.
2. The technique used for the examination of optical disks should be determined by, but not limited to, the examiners training knowledge and experience.
3. Current acceptable techniques for analysis include:
   a. Acquiring the media into a case using one of the established methods in place at the Laboratory;
   b. Use the Rimage Evidence Disk System to export any information contained on the optical media by following the manufacturer's protocol, as well as, the examiner's training and experience;
   c. Previewing the media using an approved forensic tool, for example, EnCase;
   d. Previewing the media using an appropriate approved application such as Windows Explorer.
4. Every effort should be taken to ensure that the optical disk media is write protected. For example, a read only CD drive.
5. If a different method is used to analyze the optical disk media, thoroughly document it in the Laboratory Notes (QR-CC-5) and all other appropriate records.

E. References:

1. CC SOP-29 - Evidence Retrieval Storage and Return
2. Rimage Evidence Disk System user manual

RETIRED

A. Purpose:

To outline the steps taken to reissue a report to replace a previously released report.

B. Responsibility:

Forensic Examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Use the Revised Laboratory Report Template (QR-CC-21) when there have been identified revisions required to a released Computer Crimes Report (Refer to GL-9 Quality Action Requests).
   a. The suggested font style set for this report is Times New Roman 12. Exceptions to this would be when information in the report needs to be displayed in its native format for presentation, for example, an internet chat.
2. Fill in the Revised Laboratory Report Template (QR-CC-21) fields as outlined below:
   a. Laboratory Case # - Use the ***Laboratory Case #*** as represented on the Evidence Receipt. Do not truncate.
   b. Date of Report - This date reflects the date the report is submitted for Technical Review. The date format is: mm-dd-yyyy.
   c. Agency - Use the ***Name of Submitting Agency*** as represented on the Request for Analysis form. In addition, include the address of the submitting agency in the report header.
   d. Agency Case # - Use the ***Agency Case #*** as represented on the Request For Analysis form.
   e. Request for Analysis - Select from Appendix A - Requests for Analysis.
   f. Evidence Submitted - List any evidence that was examined in the case. Document the ***Submission #*** and ***Description*** of the evidence as it appears on the Evidence Receipt.
   g. Findings and Conclusions of Examination - Record the findings and conclusions in a bulleted order to reflect the following:
      i. Address each specific request for analysis accurately, clearly, unambiguously and objectively;
      ii. Include submission number(s) where evidentiary findings were recovered;
      iii. Reference the report that this report replaces.
   h. Examiner - The individual assigned to the Request for Analysis.
   i. Reviewer - The individual assigned to the Technical Review.
3. Create any attachments that will accompany the report. Cut and paste functions will not be utilized for case specific information during the creation of an electronic reporting format.

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4. Upon report completion, affix a revised report milestone label to the upper left corner of the case file folder and date and initial the Draft Complete section of the label.
5. Submit the report along with the case file to the section supervisor or designee for technical review assignment.

RETIRED

A. Purpose:

To outline the steps taken to return a completed case to Evidence Receiving.

B. Responsibility:

Forensic examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. After a completed case has been administratively reviewed by the section supervisor or designee, it is considered ready to be returned to the submitting agency through Evidence Receiving.
2. Ensure that the original laboratory report is signed by both the person performing the examination and the technical reviewer.
3. The original signed laboratory report is submitted to and retained by Evidence Receiving. A copy of the original report is provided to the submitting agency and a copy is also retained in the case file folder to be securely stored in a designated storage location within the Unit.
4. A copy of the report is scanned and then appended to the “case images” area of Justice Trax. Refer to GL-11 for more guidance.
5. Any hard copy attachments or digital media that is to be given to the submitting agency needs to be made in duplicate with a copy placed in the case file to be securely stored in a designated storage location. Duplicate digital media attachment copies can be saved to the Work Product optical disk.
6. Update the chain of custody in LIMS of any evidence or sub-items that will be turn over to Evidence Receiving.
7. Transport the evidence and associated paperwork to Evidence Receiving and update the chain of custody in LIMS
8. The case file can now be stored in a designated storage area.

E. References:

1. JusticeTrax help file

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A. Purpose:

In the event that a particular piece of digital media cannot be accessed directly for data acquisition, the following protocol outlines the steps to be taken to acquire that media while maintaining the integrity of the evidence.

B. Responsibility:

Forensic examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations

D. Procedure:

1. Methods developed to acquire the media should rely on, but not be limited to the following:
   a. Training, knowledge and experience
   b. Technical references (electronic and print)
   c. Consulting with co-workers and technical support personnel.
   d. Contacts at other laboratories
2. If a method or methods are established to acquire the digital media, thoroughly document in the Laboratory Notes (QR-CC-5) and all other appropriate records the steps taken in the process, as well as any technical data and results.
3. Depending upon the result, success or failure, proceed with one of the following:
   a. If a successful acquisition was the result, proceed with processing the case.
   b. If the acquisition process fails, the submitting agency should be notified and alternative options should be discussed and documented.
4. Proceed with processing the case.

E. References:

1. Technical references (electronic and print)
2. CC SOP-29

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A. Purpose:

To outline the steps taken when previewing electronic evidence that has been submitted to the Computer Crimes Unit prior to or in place of full analysis while maintaining the integrity of the evidence.

B. Responsibility:

CCEEU forensic examiners.

C. Definitions:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. Retrieve the evidence and update the chain of custody.
2. Prepare Laboratory Notes (QR-CC-5) for the submitted evidence. Record a description and /or relevant information about the evidence:

   Computer Information - Make, Model and Serial Number;

   Hard Drive Information - Subitem#, Make, Model, Serial Number, Interface Type, Manufacturer's Total Sectors and capacity;

   Removable media/Other - Subitem#, Make, Model, Serial Number, Media type, Manufacturer's capacity.
3. Initial the submission barcode label.
4. Use the Laboratory Notes (QR-CC-5) as a narrative to describe actions and observations made during the entire preview process.
5. If the submission is packaged, photograph, with a unique identifier present, the unopened package focusing on:
   a. Evidence labels and any other identifying markers
   b. Notable marks or damage
6. Remove the packaging.
7. Photograph, with a unique identifier present, the exterior of the computer focusing on:
   a. Top and front face
   b. Rear and all connectors
   c. Serial numbers, evidence labels and any other identifying markers
   d. Notable marks or damage
8. If applicable, open the computer case and note your access into the computer by putting the Laboratory Case Number, Submission Number and your initials on the frame inside the computer. In the event of a laptop or similar device, note your access inside the battery compartment. If not, locate a suitable place on the laptop exterior and record this information in your notes.
9. Photograph, with a unique identifier present, the interior of the computer focusing on:

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a. Overall view of the inside of the computer
b. Location of the hard drive(s)
c. Close-up of the hard drive(s)
d. Anything notable inside the case

10. Remove power and data cables, if necessary, from the hard drive(s) and write the following on the cables to indicate the hard drive(s) that the cables were connected to:
    a. Your initials
    b. Hard drive designator (e.g. “HD1” for hard drive 1, “HD2” for hard drive 2)
11. Remove hard drive(s) and photograph, with unique identifier present, the hard drive(s) focusing on:
    a. Top of hard drive(s)
    b. Jumper settings
    c. Make, model, serial number, LBA and any other identifying markers
12. Note your access to the hard drive(s) with the following information transcribed on the hard drive(s):
    a. Laboratory Case Number
    b. Sub-item number following the naming convention below:
        Evidence Submission Number Hard Drive Number
        For example: S1_HD1.....S1_HD2.....
    c. Your initials
13. If applicable, sub-itemize evidence in LIMS; updating chain of custody.
14. If applicable, process the BIOS as follows:
    a. With hard drives removed from the computer connect the computer to a monitor and keyboard
    b. Turn the computer on
    c. Break into the setup screens by entering the appropriate keystrokes
    d. Record the appropriate BIOS information on the in the Laboratory Notes (QR-CC-5).
    e. In certain instances it may not be possible to gain access to the BIOS settings. In these cases, document any attempts made and make note in the Laboratory Notes (QR-CC-5) that attempts to access the BIOS were unsuccessful.
15. Connect the evidence drive or media to a forensic computer using an approved read-only hardware device or software. Document the make, model and serial#/Tag# of the write block / read-only hardware device using Laboratory Notes (QR-CC-5).
16. Conduct the preview examination using only approved software and established examination protocols.
17. Complete Laboratory Notes(QR-CC-5)/Analysis Notes (QR-CC-6).
18. Any hard drive, media or submitted evidence where a preview examination is conducted and found to contain items of evidentiary value must undergo a full forensic examination in order to

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officially report out the positive findings. If a preview examination results in negative findings, (1) a full forensic examination may be conducted or (2) a statement that "only a preview examination was conducted on the evidence item with negative results" must be included in the final report.

19. Reassemble the computer leaving the data and power cables disconnected from the hard drive(s).
20. Repackage the computer if necessary.
21. If additional digital media was found, appropriately package it and adhere it to the computer or place it within the computer's packaging.
22. Update the chain of custody in JusticeTrax and return the computer to the evidence area.

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A. Purpose:

To outline the procedures to be utilized to add expedited cell phones and other electronic devices as a case submission.

B. Responsibility:

Computer Crimes Unit Personnel

C. Procedure:

In cases in which an expedited examination (cell phone data extraction or GrayKey Software) is requested on cell phones or other electronic devices, these items might be submitted outside of the DSS core work hours or during core hours where there is a need to immediately process the cell phone. In such cases, Computer Crimes Unit personnel may create a new case or add additional evidence to a previously opened case following guidance below.

In cases in which an expedited examination is requested on cell phones or other electronic devices, the device will be physically submitted to the laboratory to Computer Crimes Unit personnel. The submitting agency will also include the Request for Analysis form (SOP-ER-02:1) along with the Unit's Incoming Evidence Checklist (QR-CC-1).

The following steps will be followed to create a case following guidance for ER SOP-02 "General Evidence Submission" (please refer for more details):

1. Review the "Request for Analysis" (RFA) form (SOP-ER-02:1) to ensure that essential information is provided. Review the submission to ensure that it is properly labeled and shown to be tamper proof as received.

2. In Justice Trax, using the Search – Agency Information tabs, enter in the local case number provided (make sure contain box is checked off) and search to see if this case was already opened at the Laboratory. If not opened previously, continue to step 2. If the case has been previously submitted, do step 4 and then proceed to step 8-9.

3. Select create a new case and choose the submitting agency name and enter in the case number with no spaces or dashes. Please include any "0" that are place holders. The results should indicate not case was submitted and allows a choice of "new case".

4.3. After selecting "New Case" the main case window will open and use the tabs located on the top of the window for the next steps.

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5. In the Case Info tab's Synopsis section – enter in "Exigent analysis – electronic evidence".

6. In the Agency tab – verify that the submitting agency and the case number are correct.

7. In the Offense tab – right click and choose "add offense". Fill in the Offense and Date fields.

8. In the Individual's tab – right click and add related individuals in this case. The fields of Last Name, First Name, type of individual, race of individual, gender and DOB.

9. In the "Evidence" tab - right click mouse and select "add evidence". Fill in the agency representative submitting the evidence to analyze. Add the description of the evidence.

   Under the initial transfer location, select "Agency & Representative". In the "From" tab, using the drop down, select the submitting agent. If the person is not listed, click the (+) to add this information. The "To" line should be populated with your name.

10. In the "Requests" tab, right click and select "Add Request". If the Badge Rep is not populated, add the name of the representative sending you the email. Select Computer Crimes & Electronic Evidence" in the Section box and then proceed to add the appropriate requests.

    When performing exigent analysis, select "Cell phone expedite".

    Once the requests are completed, click OK. A second window will open to pick the evidence to be added to the request. Click on the evidence to add and then select the down arrow. Click OK and this tab is completed.

11. When all the fields have been completed, click Apply and select "System Generated". This function will select the next available case number in Justice Trax to apply to this case. An evidence intake receipt will be printed and signed by the submitting agency's representative. The submitting agency will receive a copy of this receipt.

    A barcode label shall be generated for each piece of evidence submitted to the Laboratory. Whenever practical, the barcode shall be affixed to the submission as

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it is created in JT. *Prior to affixing the barcode, one last check is done that the information on the evidence package, the RFA form, and the barcode are consistent.
The generated paperwork (Request for Analysis, copies of warrant/consent, incoming evidence checklist, signed intake receipt and signed return receipt) in this case will be provided to the Evidence Receiving Unit.

12. Verify that the evidence has been appropriately transferred into the examiner's possession and begin expedited analysis. The examiner should follow applicable protocols (SOPs) and use the Cell Phone Worksheet/Notes (QR-CC-16) to document the examination process. Any data that was extracted should be placed on storage media and a sub-item created in Justice Trax.

13. After analysis is completed and the data is compiled to provide to the submitting agency, a technical review should be conducted prior to transferring of the evidence and extracted data.

14. When the technical review has been completed in Justice Trax and reflected on the Technical Review Guidelines (QR-CC-15), the evidence may be transferred back to the awaiting officer. An evidence return receipt will be printed and signed by the submitting agency's representative.

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References:

CC SOP-44 Cell Phone Data Extraction

CC SOP-50 GrayKey Software Technique

ER SOP-02 General Evidence Submission

A. Purpose:

This SOP outlines the steps to be taken when a request for extraction of data from cell phones is made by an agency. A cell phone data extraction request means that the extraction files are provided to the submitting agency and there is no search or analysis conducted on the extracted data. No laboratory report will be generated in the event of a data extraction since no analysis is being conducted.

B. Responsibility:

Forensic Examiners

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

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D. Procedure:

1. The technique used for the extraction of data from cell phones should be determined by, but not limited to, the examiners training knowledge and experience.

2. Current acceptable techniques for data extraction and analysis are delineated in CC SOP-18.

3. If the submitting agency has only requested a data extraction, the service request type should be confirmed as “cell phone – data extraction”.

4. Once the data has been extracted from the device, the data should be transferred onto storage media such as DVD or thumb drive. Included on this storage media will be a reader application for the data and an instruction document or a complete extraction report. A copy of the recovered data will be maintained on the Work Product CD.

5. If a different method is used to extract data from the cell phone, thoroughly document it in the Cell Phone Worksheet\Notes (QR-CC-16) and all other appropriate records. In addition, bring this method to the Technical Lead and/or the Unit Supervisor’s attention for further review.

6. If a passcode locked (simple passcode) Apple device has been submitted for analysis refer to CC SOP-50 and/or SOP-53.

7. If a custom passcode or pattern locked device has been submitted for analysis and current methods cannot bypass or determine the code, submit a Cell Phone Passcode Needed Notification Letter (QR-CC-51) to the submitting agency. If the passcode cannot be provided within 5 business days, the evidence should be returned to the agency with a report stating the analysis could not be conducted.

8. No laboratory report will be generated in the event of a data extraction since no analysis is being conducted. The technical reviewer will review the case file for technical content and verify the data disk being provided contains the extraction image file(s) and reader, or a complete data extraction report and instructions (a guidance letter). Administrative Review of the included letter will also be conducted by the Technical Reviewer.

9.In the event that a cell phone data extraction is requested as an exigent request and evidence receiving personnel are not available, refer to CC SOP-43.

References:

1. CelleBrite UFED 4PC manual or training documentation.

2. Oxygen Forensic Suite user manual or training documentation.

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A. Purpose:

Analysis of skimmer-type devices using Exeba-Comm software

B. Responsibility:

Examiners assigned to the Computer Crimes Electronic Evidence or Multimedia and Image Enhancement Units.

C. Background:

As skimmers are often unique in design and implementation, examination processes vary depending upon the category and/or type of device.
In general, skimmers can be broken down into the following three categories:
1. Hand-held
2. Altered hand-held
3. Custom

Analysis of these types of skimmers depends on the device submitted and the manner in which the stored information is encoded. In the case of hand-held skimmers, data extraction from these devices is accomplished by connecting the skimmer to a computer via a data cable. Once connected, a program such as Exeba-Comm is used to extract the stored track data located on the device.

In some cases, an altered hand-held skimmer and custom-made skimmer devices may be submitted for examination. These devices should be handled much like the hand-held skimmer and if possible, connected to the computer via a data cable.

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D. Procedure:

1. Upon receiving this device, photographs should be taken documenting the condition of the device and its various components including the port.

2. The examiner's computer system will be checked for virus updates.

3. The target drive will be prepared by formatting the USB drive.

4. A folder will be created on the target drive with the case number information as its title.

5. After determining the appropriate data cable to use, the device should be attached to a USB port in the computer.

6. Once attached, the Exeba software will be opened and the appropriate device will be selected. If the device name is unknown, the examiner should try the various device types until a COM link is detected.

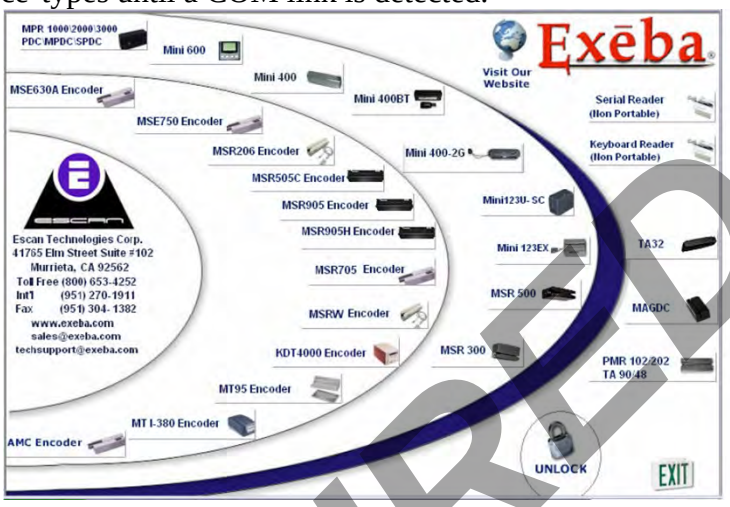

Figure 1: Menu of Exeba to select device type

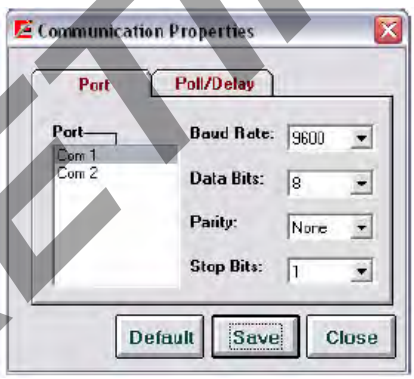

Figure 2: Communication link detection

4. After making sure that the device is recognized in the correct communication port, the examiner will go to the "Configure" drop-down menu and will select "Get Password". The below window will open:

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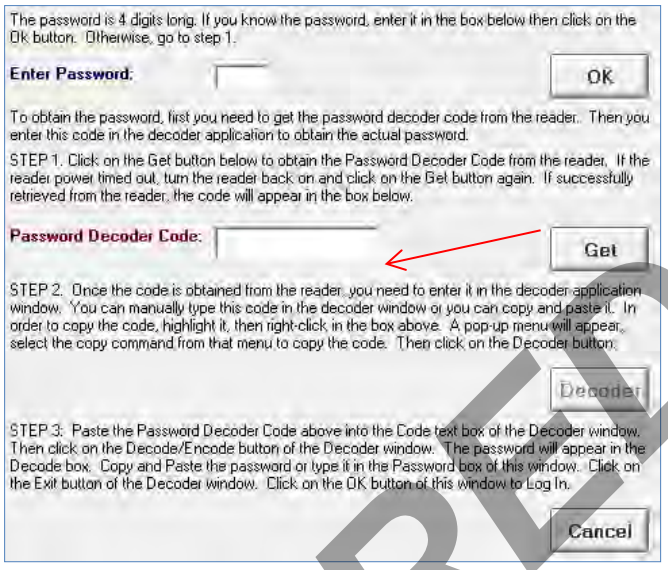

Figure 3: Passcode decoder window in Exeba

The "Get" bottom will be selected and the decoder value will appear in the box. Copy the code from this box and then click on the "Decoder" button. Paste the code into the decoder box and then the password will appear in the box.

5. The examiner will select the "Portable Reader Data" drop down menu and will select the "Poll to Database" selection.

Figure 4: Poll to database screen

The examiner will choose to poll the data in two formats: Raw and Formatted. The "Raw" polled data will be in the same format as the reader saved it. The "Formatted" data will be in placed in Comma Delimited Fields that will allow the data to be imported into a database.

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6. Once the data is extracted, the examiner will then save the data to the folder located on the target drive. This data will be saved as database text file with the case number and submission number included in the file name.

7. The analyst will then open a blank Microsoft excel spreadsheet and will import the data into an organized format following steps.

8. Select "Data" and "From Text" indicating the link to the text file in the folder located on the desktop for this case.

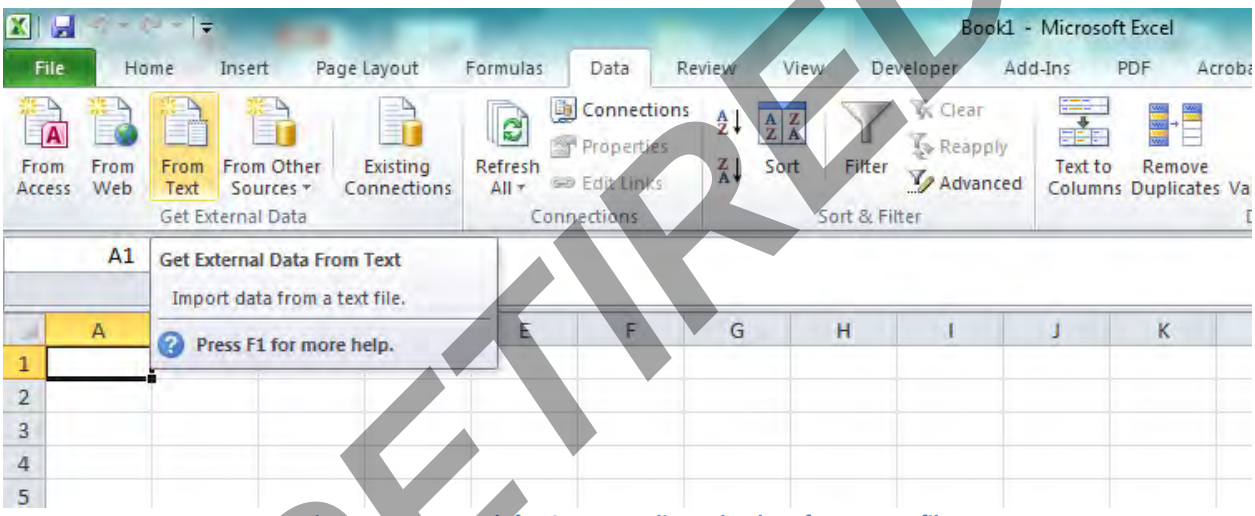

Figure 5: Import tab for Comma Delinated Values from a text file

9. Once the link is made for the source of the file, the Text Import Wizard should open. The following screenshots indicate the settings that should be used to import the information.

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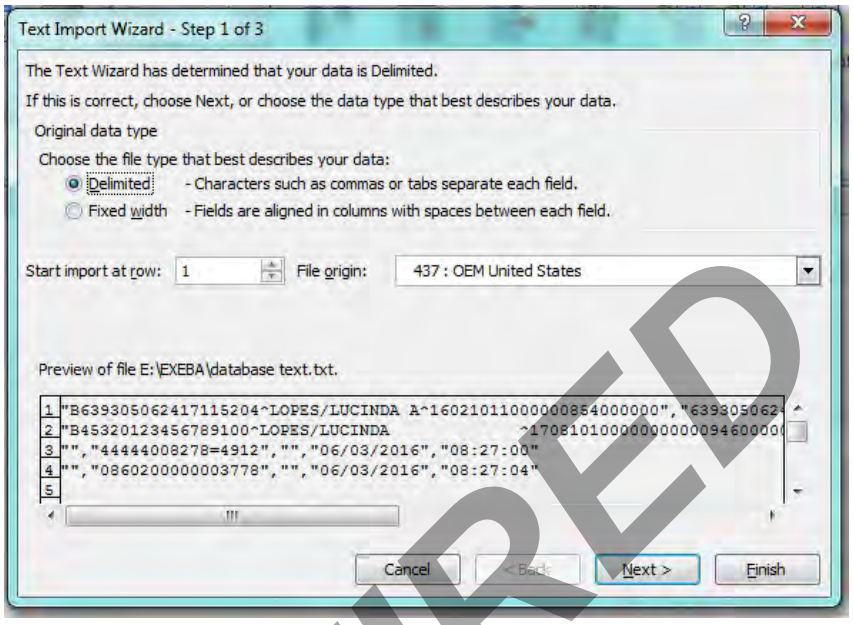
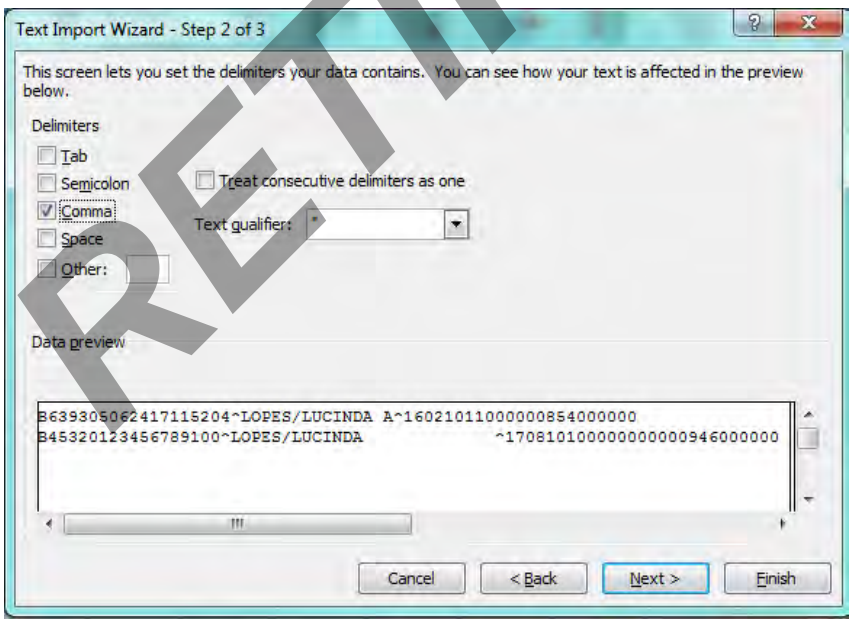

Text Import Wizard - Step 1 of 3
The Text Wizard has determined that your data is Delimited.
If this is correct, choose Next, or choose the data type that best describes your data.
Original data type
Choose the file type that best describes your data:
Delimited - Characters such as commas or tabs separate each field.
Fixed width - Fields are aligned in columns with spaces between each field.
Start import at row: 1
File origin: 437 : OEM United States
Preview of file E:\EXEBA\database text.txt.
1 "B6393050624171152O4^LOPES/LUCINDA A^160210110000008540000000","639305062
2 "B4532012345678910O^LOPES/LUCINDA ^170810100000000000946000000
3 "","4444008278=4912","","06/03/2016","08:27:00"
4 "","0860200000003778","","06/03/2016","08:27:04"
5
Cancel
< Back
Next >
Finish


Text Import Wizard - Step 2 of 3
This screen lets you set the delimiters your data contains. You can see how your text is affected in the preview below.
Delimiters
Tab
Semicolon
Treat consecutive delimiters as one
Comma
Text qualifier:
Space
Other:
Data preview
B6393050624171152O4^LOPES/LUCINDA A^160210110000008540000000
B4532012345678910O^LOPES/LUCINDA ^170810100000000000946000000
Cancel
< Back
Next >
Finish

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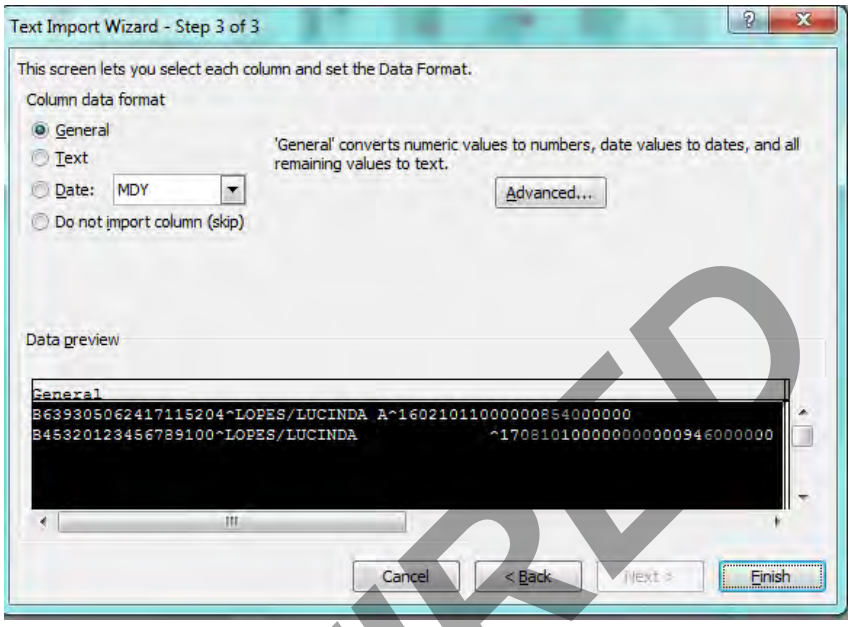

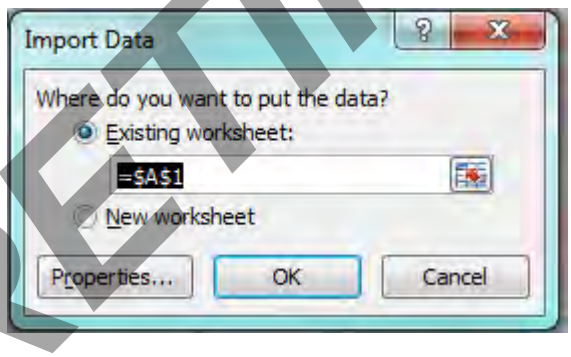

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The resulting information will be assembled into columns. Headings may be inserted above the first row.

	A	B	C	D	E	F
1	B6393050624171152O4^LOPES/LUCINDA A^160210110000008540000000	639305062417115204=160210110000854		6/3/2016	8:26:46	
2	B4532012345678910O^LOPES/LUCINDA ^17081010000000000946000000	4532012345678910=17081010000100000946		6/3/2016	8:26:55	
3		44444008278=4912		6/3/2016	8:27:00	
4		860200000003778		6/3/2016	8:27:04	
5						
6						
7						
8						
9						
10						

Figure 6: Screen capture of data from Exeba-Comm software organized by columns

9. The resulting spreadsheet should be saved to the folder on the desktop with the case number and submission number present in the file name.

10. The Laboratory Notes (QR-CC-5) should be used to document the evidence-device specifics; Exeba software version, the extraction procedure specifics and any errors encountered.

11. A compact disk will be burned to the submitting agency containing the excel spreadsheet file.

12. A second disk will be generated for the case file containing all working files including images taken of the device.

13. These cases will normally be processed as a data extraction with the service request titled “Skimmer data extraction”. This request will be released at the technical review milestone with no report issued. In the event that additional analysis is needed, a “data analysis” request will be added and a report will be issued.

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A. Purpose:

To outline the steps required when a Discovery or FOIA (Freedom of Information Act) request is made to provide a redacted (i.e. removal of child pornographic images and/or videos) copy of work product or attachment media.

B. Responsibility:

Forensic Examiners (however titled)

C. Procedure:

1) Upon receiving a discovery request from an outside agency, the examiner will notify the Unit Lead and the request will be referred to Case Management per (CM WI-06) and GL-11 (Control of Records).

2) Prior to release of the copy requested, a careful review of the attachment media data contained on the Work Product media will be made using an approved forensic software tool. The examiner will complete Quality Record CC-52 "Verification Record – Discovery/FOIA Request for Redacted Attachment Data".

3) All image and video type files contained within the attachment data will be reviewed for suspected child pornographic content. In circumstances, where triage type reports are included as attachment materials, all associated exported data will be reviewed. There may also be reports containing embedded image data (i.e. C4All reports) which may not be directly viewable upon loading into a forensic tool; in these cases, a new report excluding the image data will be generated.

4) All files which contain suspected child pornography will be identified and removed from the attachment data and a new copy of the attachment will be generated on the appropriate media. Following completion of the new copy, the examiner will review the final product to confirm removal of all child pornographic containing files.

5) Upon completion and review of the redacted copy of the attachment by the examiner, the case will be reviewed by the technical reviewer of the case or if not available by another examiner. The media copy will be initialed as verified by the examiner and reviewer.

6) Procedures for control and returning the discovery media to the requesting agency will be followed as detailed in CC SOP-29. A copy of the media produced will be retained in the case jacket and itemized in Justice Trax.

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The itemized media that is to be released will be transferred to Evidence Receiving for the completion of the Discovery Request.

7) In Case Management WI-06, the examiner notification form is normally signed by Legal Representative receiving the Discovery materials. In the case of electronic media being requested, the evidence receipt will act as the acknowledgement of receipt of materials.

8) In the case of a FOIA, the media produced will be stored in the case jacket rather than on the S: drive. The Unit Lead will review the media and generate and sign the "True and Accurate" letter. The electronic media that was produced for the requestor, will be handled as indicated in Section 6 of this SOP.

   A copy of the "True and Accurate" letter will be scanned into the S: drive and the original will be placed into the main case file in Evidence Receiving. A copy of the letter may be retained in the Unit case jacket. The electronic media produced for a FOIA will be transferred in Justice Trax to Legal Affairs personnel or into "mail transport" for delivery.

RETIRED

A. Purpose:

To outline the use of the general checklist, Parole and Probation Guidelines (QR-CC-55) for the examination of submitted evidence.

B. Responsibility:

CCEEU Forensic Examiners

C. Procedure:

1. A general checklist of items that are searched during an examination for Probation and Parole cases is listed in QR-CC-55.

2. The examiner will use the list as a guide for search criteria which are based on conditions of the Computer Access Agreement (JD-CR-143) and non-compliance of conditions set forth by the Sex Offender conditions of Probation (JD-CR-131).

3. The examiner will search and examine the evidence for all of the items on the list and check off those items that yield case search results.

4. The assigned technical reviewer will ensure that the items checked have been included in the findings section of the report.

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E. References:

1. Computer Access Agreement (JD-CR-143)

2. Sex Offender conditions of Probation (JD-CR-131)

3. CC SOP-23-Technical Review Protocol

Purpose:
To outline the steps taken to conduct the chip-off technique and direct physical connection techniques for forensic examination of memory chips.

Chip-off forensics is an advanced digital data acquisition procedure which involves physically removing the non-volatile integrated circuit (*i.e. flash memory chips from a device capable of storing* data). The chip-off process is performed to remove the internal memory chip from damaged or destroyed devices not accessible by extraction tools, devices unsupported by commercial tools and/or unsupported by advanced data extraction methods. The data can be read directly on an external specialized reader.These chips are removed, cleaned and prepared for reading by the compatible reader adapters that support specific BGA, eMMC, eMCP and UFS memory chip types. Data extraction software and programmers are used to extract the data from the memory chip which can be parsed and examined by mobile forensic software tools.

Other processes can also be employed to access and extract data from damaged NAND based devices (i.e.USB Flash drives, SD cards, micro SD cards, compact flash cards and others) that do not require removal of the memory chip. These methods utilize specialized software and hardware tools to allow for a direct physical connection to the memory chip / PCB (printed circuit board) bypassing damaged controllers and achieving potential data recovery.

A laboratory report of the data extraction and analysis results is produced and provided to the requesting agency.

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Responsibility:
Forensic Examiners or other analysts working in the Computer Crimes Forensic Unit.

Definitions/Abbreviations:
Refer to CC SOP-26 - Definitions and Abbreviations.

Safety: The Chip-off procedure should be conducted in a hood with the Sentry Air System Series 200 Exhaust in operation. The “use log” located in the binder will be updated for the days of use of the Sentry Air System Series 200 to monitor replacement of filters. The filters will be changed in the Sentry Air System Series 200 after 30 uses or after one (1) year, whichever is sooner.

Procedure:

Chip-off Technique:

Identify the evidence device make and model and confirm that no other full physical memory extraction is possible by other means. When performing the Chip-Off Technique, the information on the device, chemicals and readers used should be recorded on QR-CC-56 (Chip-off Record).

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1. To aid in determining the type and location of the flash memory chip, the FCC ID number located on a sticker underneath the battery or on the back of the device should be identified and recorded. The FCC site should be checked to obtain the information specific to the FCCID# at the following address: http://transition.fcc.gov/oet/ea/fccid/.
    a. The information for the device is located within the detail summary.
    b. Select, "Internal Photos" within the attachment list to view image photos of the internal components and their locations for the device. Details about the main board and the lay out are shown in these photos and can be used as a guide to take apart the phone and access the main board.
2. For devices without FCCID#'s attempt to find information for the device or the flash memory chip once the device is dismantled from internet searches.

   If possible, determine whether the device model employs encryption by default, if so the data stored on the chip will not be readable and the chip-off technique would not be a useful procedure.

3. If the chip-off technique is advisable, dismantle the device to gain access to the main board.
    a. Using the information from above, locate the position of the flash memory chip and begin removing the tin shield covering.
    b. To remove the tin covering, apply liquid solder flux around the entire perimeter of the shield. The solder flux will serve to spread and transmit the applied heat evenly around the edges of the plate.
    c. Heat is applied to the area using a hot air gun. Gently lift the shield up using a metal prying stick until it is completely removed and the chip(s) underneath are visible.
    d. Identify the chip type and determine if a compatible reader adapter/programmer is available.

4. Different methods for removing the chip are used depending on the device and how the chip is set on the board. Factors include the type of chip; the amount of epoxy present around and underneath the chip; and what other parts of the mainboard are in close proximity to the chip.

   Three primary methods can be used to carry out the chip detachment from the board:
   a. Use a heat gun 3-4 inches above the chip being careful not to apply too much heat.
   b. Use heat applied beneath the chip (Gordak 853 High Power ESD BGA Rework Station).
   c. Use Infrared heat (320° C) above the chip with heat applied beneath the chip (200° C)(T-862 BGA Rework Station).

   IMPORTANT NOTES AND CONCERNS:
   - Carefully score around the chip edges using an exactor knife before heating. When you are heating the chip/board, you need to work away at the epoxy on the side of the chip and open some air pockets. Low heat for longer period of time is better than high heat for short time.
   - Do not use force to pry the chip, let the chip come off with light pressure, once the solder and epoxy are broken down, the chip will come off easily. Use a wide flat exactor knife on the sides of the chip to pry the chip loose. If the chip is forced off, some of the BGA pins will be damaged and a read will not be possible.
   - Use protective eye glasses or only look at board/chip through the protective screen when using Infrared heating.
   - Perform these procedures in a hood with a filtration system and /or venting.

5. Clean the flash memory chip after a successfully removing from the board. Using a solder iron with a chisel tip, apply pressure against the epoxy residue and push forward slightly as the epoxy breaks down. Repeat this process turning chip 90 degrees. A magnifying scope or glasses can be used to view the chip for remaining residues left behind.

   IMPORTANT NOTES AND CONCERNS:
   - Do not tilt the chisel tip as pressure is applied-this will scratch the chip surface.
   - Use short strokes and clean the solder tip between scrapings; during the process, brush with alcohol to check how much epoxy remains on the chip.

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6. After the cleanup step has been completed, the chip needs to be re-tinned or re-balled.
   a. Solder paste is applied to the surface of the chip focusing on the BGA pins that the adapter will read from.
   b. Use the hot air from the re-work station and apply hot air to the solder paste for just seconds, until all the solder is attracted to the BGA pins.
   c. Use the solder iron tip to clean away excess solder and large balls that can build up in the previous step.
   d. Use a tooth brush and alcohol 99.9% to clean the chip for reading.

7. Following re-tinning or re-balling, the chip should be readable by a compatible reader adapter programmer. If a read is not achieved repeat the re-tinning/re-balling procedure above and attempt to read the data again.

The selection of a reader type will depend on the type of memory chip.
eMMC153 and eMMC169 chips can be read by a Sireda using a SD adapter to a USB interface on the forensic system. A physical acquisition can be conducted using a forensic application such as Encase or FTK Imager.
Other BGA types or UFS types can be read and imaged using a programmer – the UP828P using the appropriate adapter or the DediProg NuProg-E UFS programmer.

8. The image file produced from the acquisition will be examined using a forensic application (EnCase, FTK, IEF - Refer to CC SOP-08 Case Initiation Protocol) or the image will be examined by a cell phone data analyzer application (Cellebrite's Physical Analyzer-Refer to CC SOP-18 Cell Phone Data Extraction).

9. The Cell Phone Worksheet/Notes (QR-CC-16) should be used to document the evidence device specifics; the chip type identified; the chip-off technique specifics employed; the reader type adapter and /or programmer used; the forensic application or data analyzer application version; and any errors, technical problems, deviations encountered.

10. Produce a report and attachment data of the results following the laboratory reporting protocol (Refer to CC SOP-09 Laboratory Report Protocol).

Direct Physical Connection Techniques:

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1. PC-3000 with Spider Board Adapter to recover data from a damaged or non-functional monolithic device - SD or microSD card.
   a. Fix the card down on the table with double sided adhesive tape.
   b. Carefully remove the outer coating on the bottom side of the card opposite to the contacts using very fine sand paper (1000-2500)or grind with a fiber glass pen; if sanding usethe coarser sand paper to start and change to the finer size as the copper layer of contacts become visible on the PCB board. Be very careful not to over sand (or grind) and damage the contacts and circuitry on the PCB board.
   c. Determine the pinout scheme using the Global Solution Center source (PC3000FLASH.COM) as a resource.
   d. After the copper layer and contacts have been fully exposed attach the card to the Spider Board adapter. Solder the power and ground wires to the standard contact locations on the card and attach to the spider board adapter connectors.
   e. Using a microscope connect to the appropriate pinouts using the specialized needles.
   f. Connect the spider board adapter to the PC-3000 Flash reader through the extension port; use the PC-3000 software to set the needle to pin configuration and test the outputs for duplication and short circuiting.
   g. Following verification of pin settings read and acquire the data.
2. The Cell Phone Worksheet/Notes (QR-CC-16) or Analysis Notes (QR-CC-6) should be used to document the evidence device specifics; technique specifics employed; the reader and /or programmer used; the forensic application or data analyzer application version; and any errors, technical problems, deviations encountered.
3. The image file produced from the acquisition will be examined using a forensic application (EnCase, FTK, IEF - Refer to CC SOP-08 Case Initiation Protocol) or the image will be examined by a cell phone data analyzer application (Cellebrite's Physical Analyzer-Refer to CC SOP-18 Cell Phone Data Extraction).
4. Produce a report and attachment data of the results following the laboratory reporting protocol (Refer to CC SOP-09 Laboratory Report Protocol).

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References:

1. Teel Technologies Advanced Chipoff Training documentation.
2. T-862 BGA Rework Station, Gordak 853 High Power ESD BGA Rework Station, JBC CD-S Soldering Station Kit, Circuit Specialist Digital Hot Air Rework Station w/

soldering iron, UP828P BGA Chip Programmer and adapters, SD Chip Reader Kit instruction manuals and documentation.

3. Teel Technologies PC-3000 Flash and Spider Board Adapter Training documentation.
4. Cellebrite UFED Touch, UFED4PC software user manual or training documentation.
5. SAS Sentry Air System Series 200 Operations and Maintenance Manual

RETIRED

A. Purpose:

To outline the steps taken to retrieve and analyze data stored in cloud storage. Evidence may be submitted with a cell phone analysis request or other request that applies to devices that use cloud storage for backup. The internal memory of these devices will be extracted, as well as onboard memory card(s) /SIM card(s). The extraction(s) will be parsed using approved software program(s). Searches and other in-depth analyses will be conducted on the parsed data including the data related to information stored in cloud storage. A laboratory report of the analysis results will be produced to provide to the requesting agency.

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B. Responsibility:

Forensic Examiners or analysts assigned to the Computer Crimes Forensic Unit

C. Definitions/Abbreviations:

Refer to CC SOP-26 - Definitions and Abbreviations.

D. Procedure:

1. The technique used for the examination of cell phones or other mobile devices should be determined by, but not limited to, the examiner's training knowledge and experience.

2. Current acceptable techniques for data extraction and analysis include:
   a. Using the Cellebrite UFED Touch Unit, UFED 4PC or comparable approved software to extract data contained on the cell phone or mobile device and using the UFED Physical Analyzer software to parse and analyze data recovered from the device by following the manufacturer's protocol, as well as, the examiner's training and experience.

   b. Using the Cellebrite UFED Cloud Analyzer software to extract the data from cloud storage accounts that were identified and parsed out by the UFED Physical Analyzer software. An Account Package folder containing the storage account credentials is produced.

3. Once the cloud storage account information is recovered through the use of the UFED Physical Analyzer software, the Account Package folder can be copied to an external

hard drive which will then be connected to a computer with Internet access and the UFED Cloud Analyzer software installed on it. If the examiner is using their forensic system, access to the Unit's intranet should be disabled before enabling Internet access. For guidance, refer to CC SOP-19 (QC Protocol – Forensic Computer).

If the credentials are provided in a different manner, this information can be entered and used for the recovery of cloud storage account information.

4. Following the software import procedures, the Account Package data will be imported into the UFED Cloud Analyzer software and relevant case information will be input into the software.

5. A recovery of the data contained in the cloud storage accounts will be conducted and the extracted data will be saved to the external hard drive. Using the software a UFDR report will be generated for this data to be later analyzed by the examiner using the UFED Physical Analyzer software.

6. A report and attachment data of the results will be produced following the laboratory reporting protocol (Refer to CC SOP-09-Laboratory Report Protocol).

RETIRED

References:

Cellebrite UFED Touch, UFED4PC, and UFED Cloud Analyzer software user manual or training documentation.

CC-SOP-18: Cell phone analysis protocol

Note:

UFED Cloud Analyzer usage for probation and parole cases:

The UFED Cloud Analyzer software will be used on an as needed basis for probation and parole cases based on the examiner's training and experience along with the examination of information that was recovered by the UFED Physical Analyzer software. If another agency becomes

involved in the case, refer to the next paragraph for guidance.

UFED Cloud Analyzer usage for non-probation/parole cases:

If a search and seizure warrant is not provided that refers to cloud data, the examiner should refer this case to Case Management to seek guidance from the submitting agency or the State's Attorney/U.S. Attorney's Office on whether analysis may proceed.

RETIRED

A. Purpose:

To acquire data from infotainment/telematics systems previously removed from vehicles. Refer to CC SOP-51 for the on-scene vehicle retrieval guidance.

Many of the newer vehicles encountered today are equipped with an Infotainment and or a Telematics system. The Infotainment system is a combination of information and entertainment for use within the vehicle. Pairing to this system occurs through Bluetooth® or USB connection to the vehicle system and data is transferred or streamed. Other information contained may also include vehicle performance, maintenance and status. The telematics system is a combination of the telecommunication and information for interaction of the vehicle to exterior sources. This interaction can occur via wireless connectivity by utilizing a paired mobile device or onboard cellular connectivity to the internet or a dedicated service. Depending on the vehicle, these may be one hardware system or separate modules.

The vehicle's manufacturer and OEM (original equipment manufacturer) will determine how much data and the type of data that is contained in these systems. The following data may be present:

- Global Positioning System events and possible locations (not available is navigation is not included in the vehicle model). List of entered addresses, routes or track points captured at various intervals including possible velocity.
- Vehicle information such as serial/part number of infotainment/telematics system, software version of the operating system and original vehicle identification number (VIN).
- Loaded entertainment files such as audio, video or images.
- Connected devices such as media players, USB devices, SD cards with some containing unique serial numbers or identification numbers.
- Bluetooth or Wifi connections with names of connected devices that may be personalized along with MAC address.
- Call logs, contacts and SMS messages with possible time stamps included.
- Vehicle events such as gear shifting, lights on/off, doors opening/closing, odometer readings, pairings and attachments and system reboots.

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In cases in which the data cannot be downloaded via USB or through the diagnostic system onboard the vehicle, but must be downloaded directly from the Infotainment system. In these cases, the system is removed and the submitting agency will be provided the removed system to submit directly to the Laboratory.

B. Responsibility:

Forensic Science Examiners assigned to the Multimedia and Image Enhancement and/or Computer Crimes Unit or performing casework in the Unit.

C. Procedure:

1. The request type for this analysis will be "Vehicle Data Acquisition/Analysis". The entire

infotainment/telematic system will be transferred to the examiner to begin working on this request.

2. The examiner will attempt the data extraction from the infotainment/telematic system by accessing the internal components as instructed in the iVE Berla Software. If successful, the data will be analyzed in the iVE software and a report will be generated providing the data information requested by the requesting agency.

3. In some cases, the unit may need to be disassembled to connect to the internal components using the DIB (device interface board). In these cases, if the power is supplied to the various components, the analysis is to take measures to prevent electrocution or compromising the system with excess voltage.

4. Once the connection is successfully made, the analyst can proceed to perform a logical or physical acquisition to extract the data into the software. The physical extraction is the preferred method. Depending on the vehicle, sometimes only a logical acquisition can be performed.

5. In some cases, the infotainment/telematic system may need a "chip off". Prior to conducting this process, the submitting agency will be notified that this is a destructive process and that they are giving us consent to move forward with this procedure. In this case, the analyst will add the "Chip-Off Analysis" request to this submission.

D. References:

iVE Vehicle Forensics Certification Coursework Book, Version 2.0, May 2018

RETIRED

A. Purpose:

This SOP outlines the steps to be taken when there is a request for unlocking of an Apple iPhone or Android cell phone using the Cellebrite Premium UFDR technology. Cellebrite Premium software and hardware by Cellebrite is a product that conducts a brute force attack on iPhones and supported Android devices.

The ability of Cellebrite Premium to access a phone depends on the encryption technology resident on the device.

The encryption technology found within iOS on Apple devices is built on the security of two separate processors: the Secure Enclave (SEP) and also, on some devices, the Secure Element (SE). Additionally, a principal factor in the nature of the brute force is whether the device is in the Before First Unlock (BFU) or After First Unlock (AFU) state. Devices that are in the After First Unlock state have been unlocked at some point in the past since being turned on. Regardless of how long the phone has been running, if it has maintained power continuously since the first unlock, it is in the AFU state. This is different from a phone that may have been powered down for storage after being seized for evidence. The large majorities of evidentiary phones are in the BFU state, as they have been powered down and stored or have lost their battery charge and need to be recharged prior to analysis.

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Before iOS 10.3.2, the speed of the Cellebrite Premium brute force was always the same whether the phone was in the AFU or BFU state. The anticipated maximum brute force time for a 4 digit passcode was about 25 minutes, and the maximum brute force time for a 6 digit passcode was about 2 days with approximately 1 million attempts. When iPhones 6 was introduced to the market, these came with the “Secure Element” that is embedded in the NFC chip. This was used for secure communication with the device and Apply Pay terminals. The use of this Secure Element started with iOS 10.3.2, on devices that are equipped with the NFC chip. The use of the SE is not automatic for new users of iPhone 6 or later models. After the device is first set up for use, the SE is tested for at least 2 weeks prior to activating live. During this two week trial period, the behavior of the Cellebrite Premium is the same as before. However, if the evidence phone has been in use for longer than the two week period, which is the majority of the devices, the brute force speed is affected. The brute force time is increased due to the SE now being included in the iOS authentication process. Once the two week grace period has passed, this new authentication mechanism can be referred to as an “SE-Bound Passcode”.

B. Definitions/Abbreviations:

- SEP – Secure Enclave

- SE – Secure Element
- BFU – Before First Unlock
- AFU – After First Unlock
- DFU – Device Firmware Update

C. Procedure

Note: The request for this device in Justice Trax is designated as "Cellebrite Locked Device".

All information regarding the device and the device lock status will be recorded on QR-CC-57 (Passcode Agent Install/Extraction Worksheet).

<u>Apple Devices:</u>

RETIRED

1. Upon receipt of the device, determine if the device is powered "on" and/or needs to be charged.
   If the device was submitted powered on, determine if the device is supported for AFU extraction. If it is supported connect the device to the AFU adapter to perform a file system extraction within the Cellebrite Premium software running on the laptop. Verify the extraction completed successfully using Cellebrite Physical Analyzer. After verifying, proceed to remove the AFU agent by initiating the reboot process. This will bring the device into the BFU state. Proceed with the steps below if the brute force agent will be installed.

2. If the device is not supported for AFU extraction or if the device was submitted powered off, then perform the following steps to install the brute force agent on the device. Ensure that the device is fully charged and is powered "off" in BFU.

3. The device should be placed in Recovery mode and DFU mode.

4. Connect the device to the appropriate adapter.

5. Begin installation of the agent and start the Autonomous Brute Force detection process.

6. Remove the device from the adapter and connect to a power source.

7. Once the process is completed, the device is connected to the laptop and the Cellebrite Premium software and extraction process is initiated.

8. Save the full file system extraction to an external drive and then proceed as indicated in CC SOP-18 (Cell Phone Analysis Protocol) or CC-SOP-44 (Cell Phone Data Extraction).

Android Devices:

1. Upon receipt of the device, determine the lock status of the device.

2. All information regarding the device and the device lock status will be recorded on QR-CC-57 (Passcode Agent Install/Extraction Worksheet).

3. Determine the chipset of the android model using one of the online mobile phone assessment websites: i.e phonedb.net;gsmarena.com; phonescoop.com and/or imei.info.

4. Begin exploit and initialization for the identified model device and chipset following the on screen instructions. Ensure that the device is an approved vendor before proceeding with the method steps.

5. Connect the cable to the adapter (BE CERTAIN THAT IT IS THE ANDROID ADAPTER) and then connect the cable to the device. Follow on screen instructions.

6. Complete the extraction following the on screen instructions and save to a designated folder.

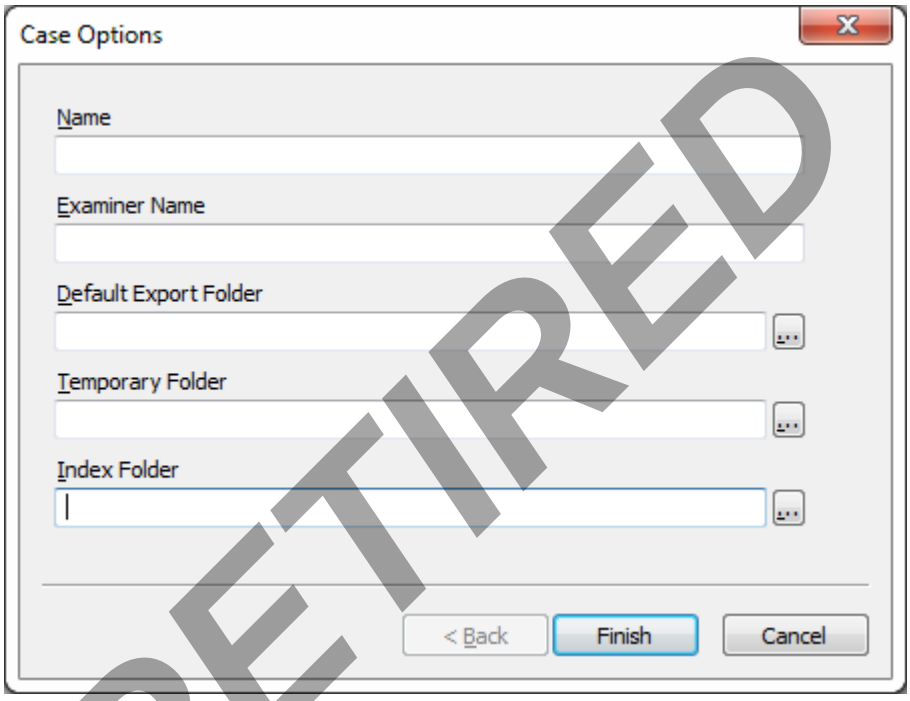
7. Save the physical extraction to an external drive and then proceed as indicated in CC SOP-18 (Cell Phone Analysis Protocol) or CC-SOP-44 (Cell Phone Data Extraction).

RETIRED

Encase Naming Conventions

EnCase 6.x

1) When versions EnCase 6.x are initially opened, the following dialogue box will appear:



RETIRED

2) Use the following conventions to fill in each field:

   a) Name - This will be the unique name of the EnCase case file for this particular case. It consists only of the Laboratory Case Number of the evidence acquired or to be acquired into the case.

      For example: DSS-16-001234

   b) Examiner Name - This will be the name of the analyst creating the new case file.

      For example: “Jim Jackson”, ”Jane Smith” or “Johnson”

      If two or more examiners share the same name they must be uniquely indentified.

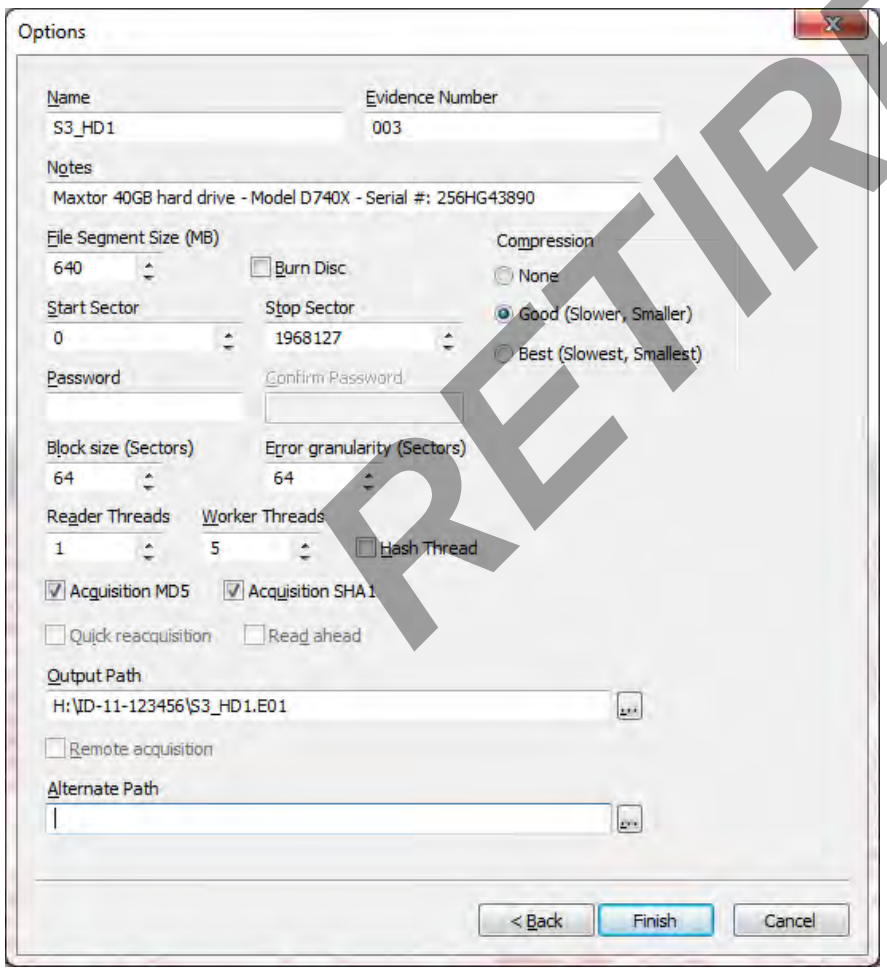
      For example: “Jim Jackson” and “James Jackson”

   c) Default Export Folder - This folder will be named “EnCase Export” and the path to it will be the target drive.

d) Temporary Folder - This folder will be named “EnCase Temp” or “EnCase Temporary” and the path to it will be the target drive.

e) Index Folder - This folder will be named “EnCase Index” and the path to it will be the target drive.

RETIRED



Referring to the screenshot to the left, the following naming conventions are used when acquiring digital media with EnCase 6.x:

1. In the Name field, the naming convention will reflect the Submission # along with the media number based on how many pieces of the particular media type* there are. For example, if a computer is submitted as Submission #003 and it contains two (2) hard drives, the naming convention would be: S3_HD1 and S3_HD2. Meaning;
   - S3 equals Submission #003
   - HD1 equals Hard Drive 1
   - HD2 equals Hard Drive 2
2. The Evidence Number field is the actual 3 digit Laboratory Submission Number.
3. The Notes field contains information about the particular piece of media. For example: Make, Model and Serial Number.
4. The Output Path field is the path to the Staging Drive.
5. Both MD5 and SHA1 acquisition hash boxes should be check.
6. All other fields may be defined based

upon the examiner's requirements.

Notes: The screenshot above depicts a version of EnCase 6.x. The same Name, Evidence Number, Notes and Output Path fields exist in previous version of EnCase and should be treated the same.

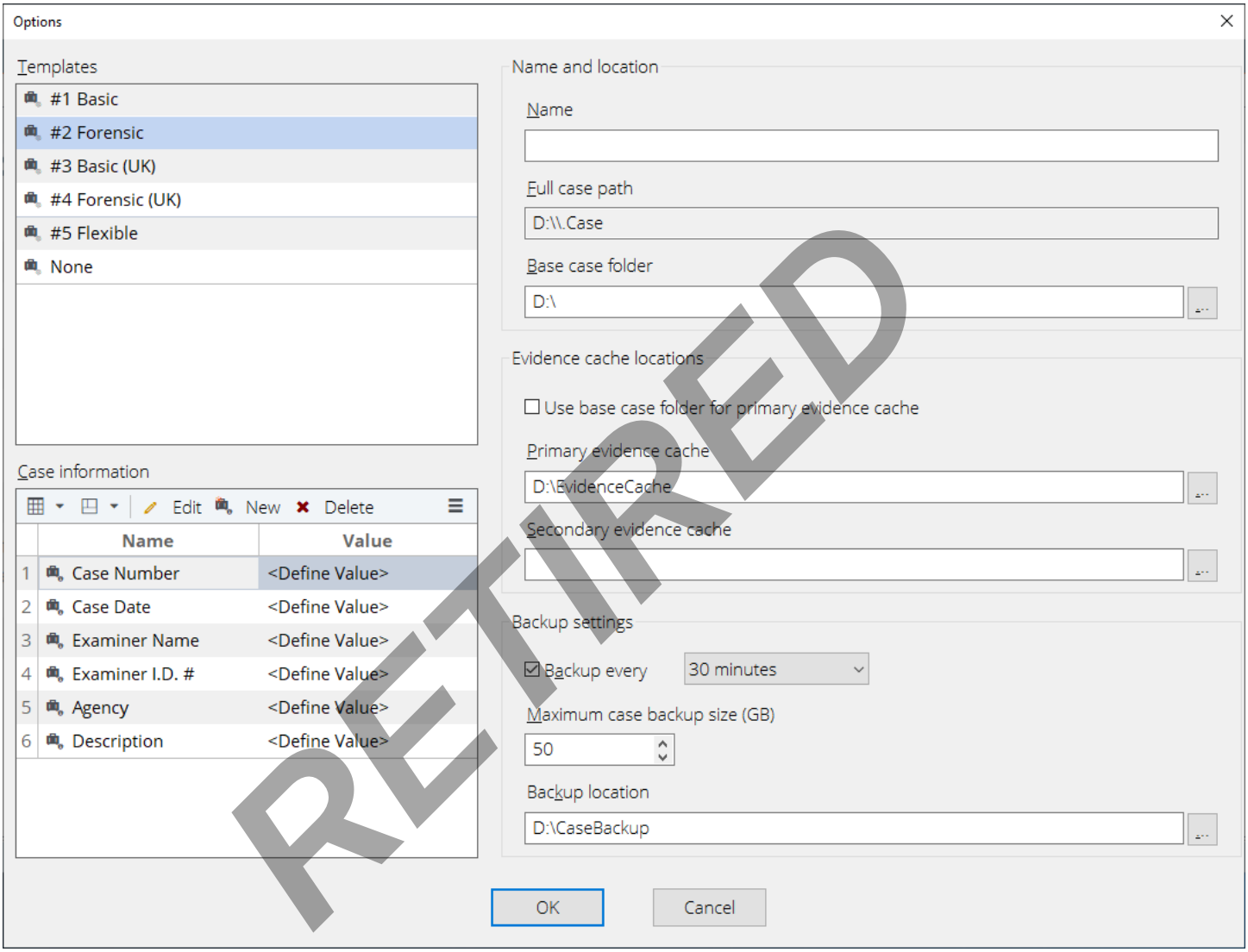
Both the MD5 and SH1 acquisition boxes are absent in earlier versions of Encase. However, a MD5 hash is automatically generated in the earlier versions.

* A complete list of abbreviations for the various types of media can be found in SOP-CC-31 – Sub-item Labeling Standards.

RETIRED

EnCase 8.x

1) When versions EnCase 8.x are initially opened, the following dialogue box will appear:



RETIRED

2) Use the following conventions to fill in each field:

Within Case Information complete the minimum:

a) Case Number – DSS-YY-###### Example DSS-19-001234.

b) Examiner Name - This will be the name of the analyst creating the new case file.

For example: “Jim Jackson”, “Jane Smith” or “Johnson”

If two or more examiners share the same name they must be uniquely indentified.

For example: “Jim Jackson” and “James Jackson”

Name and location:

c) Name - This will be the unique name of the EnCase case file for this particular case. It consists only of the Laboratory Case Number of the evidence acquired or to be acquired into the case.

For example: DSS-19-001234

d) Base Case Folder – path where the case file is saved.

e) Primary evidence cache - path where the data processing elements are saved.

f) Backup location - path where the backup file for the case is saved.

The following naming conventions are used when acquiring digital media with EnCase 8.x:

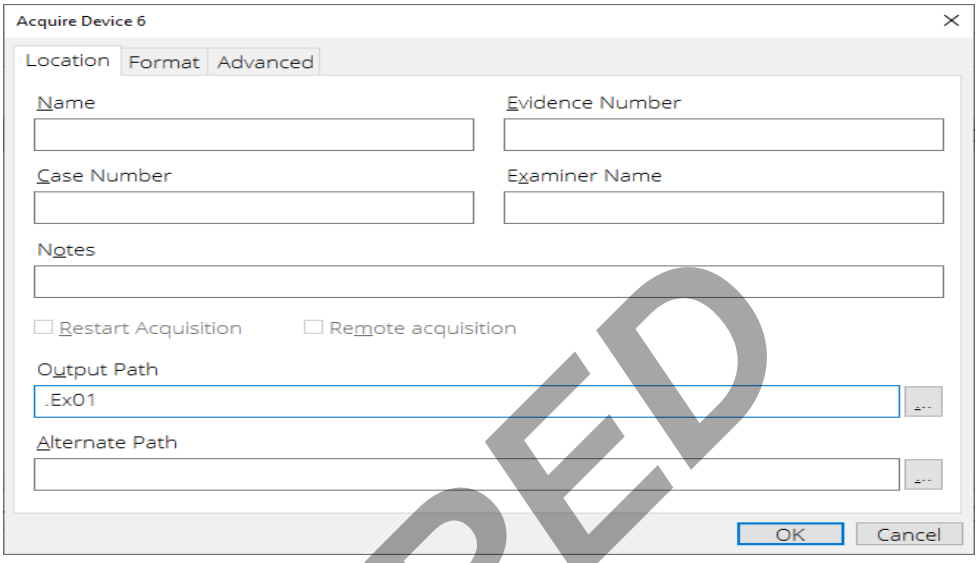
1. In the Name field, the naming convention will reflect the Submission # along with the media number based on how many pieces of the particular media type* there are. For example, if a computer is submitted as Submission #003 and it contains two (2) hard drives, the naming convention would be: S3_HD1 and S3_HD2. Meaning;
   -S3 equals Submission #003
   -HD1 equals Hard Drive 1
   -HD2 equals Hard Drive 2

2. The Evidence Number field is the actual 3 digit Laboratory Submission Number.

3. The Notes field contains information about the particular piece of media. For example: Make, Model and Serial Number.

4. The Output Path field is the path to the Staging Drive or Partition.

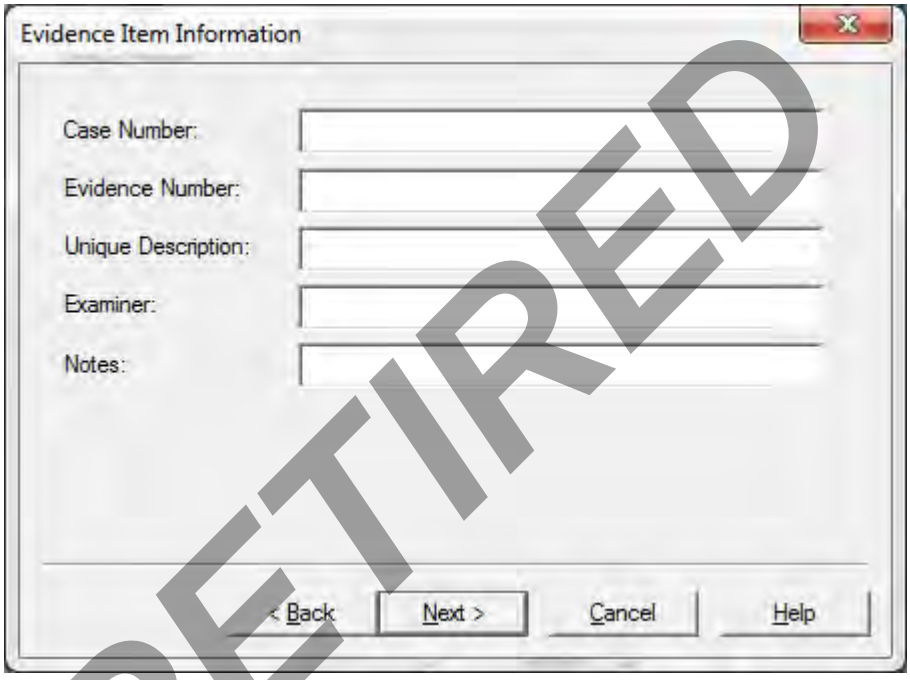
RETIRED


Acquire Device 6
Location
Format
Advanced
Name
Evidence Number
Case Number
Examiner Name
Notes
Restart Acquisition
Remote acquisition
Output Path
.Ex01
Alternate Path
OK
Cancel

RETIRED

FTK IMAGER 4.x NAMING CONVENTIONS

1) During the process of imaging using FTK Imager 4.x, the following dialogue box will appear:



RETIRED

2) Use the following conventions to fill in each field:
   a) Case Number - This will be the unique identifier for the case. It consists only of the Laboratory Case Number of the evidence being acquired.

      For example: DSS-16-001234

   b) Evidence Number - This will be the unique identifier for that particular piece of media. It can be the Laboratory Submission Number (for example: “001”) or a sub-item number of the Laboratory Submission Number (for example: “001-1A”).

   c) Unique Description - This field should contain information about the particular piece of media. For example: Make, Model and Serial Number.

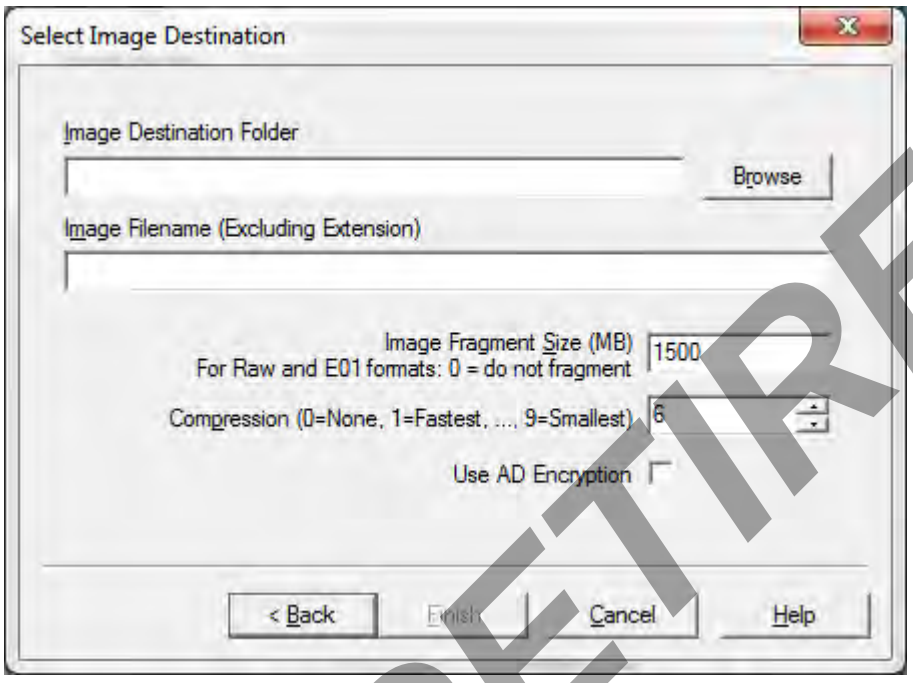
   d) Examiner - This will be the name of the analyst acquiring the specific piece of media.

      For example: “Jim Jackson”,”Jane Smith” or “Johnson”

      If two or more examiners share the same name they must be uniquely indentified.

For example: “Jim Jackson” and “James Jackson”

e) Notes - This filed is optional and may contain any information the examiner feels is relevant to the case.



Referring to the screenshot to the left, the following naming conventions are used when acquiring digital media with FTK Imager 4.x:

7. In the Image Destination Folder field change the destination path to the established staging drive.
8. In the Image Filename (Excluding Extension) field, the naming convention will reflect the Submission # along with the media number based on how many pieces of the particular media type* there are. For example, if a computer is submitted as Submission #003 and it contains two (2) hard drives, the naming convention would be: S3_HD1 and S3_HD2. Meaning;
   - S3 equals Submission #003
   - HD1 equals Hard Drive 1
   - HD2 equals Hard Drive 2
9. All other fields may be defined based upon the examiner’s requirements.

RETIRED

* A complete list of abbreviations for the various types of media can be found in SOP-CC-31 – Sub-item Labeling Standards.

Tableau Forensic Duplicator (TD1) NAMING CONVENTIONS

3) Upon initiation of acquisition using the Tableau Forensic Duplicator, a dialogue box will appear following the menu selection 1. Duplicate Disk, 2. Disk-to-File and Start which will prompt for a case ID, followed by Case Note, Subdir Name and File Base Name. The entries should be made as follows:

   a) Case ID - This will be the unique identifier for the case. It consists only of the Laboratory Case Number of the evidence being acquired.

      For example: DSS-16-001234

   b) Case Note - This field should contain information about the particular piece of media. For example: Make, Model and Serial Number and the examiners name.

   c) Subdir Name- leave as default.

   d) File Base name - will reflect the Submission # along with the media number based on how many pieces of the particular media type* there are. For example, if a computer is submitted as Submission #003 and it contains two (2) hard drives, the naming convention would be: S3_HD1 and S3_HD2. Meaning;

      S3 equals Submission #003

      HD1 equals Hard Drive 1

      HD2 equals Hard Drive 2

* A complete list of abbreviations for the various types of media can be found in SOP-CC-31 – Sub-item Labeling Standards.

RETIRED

REQUESTS FOR ANALYSIS

When filling in the fields contained in QR-CC-10 - Laboratory Report Template select from the following list the appropriate request for analysis.

1. Recovery of suspected child pornographic images either in still or video format
2. Recovery of data signifying the distribution of suspected child pornographic images or videos
3. Recovery of data signifying the importation of suspected child pornographic images or videos
4. Recovery of data signifying the production of suspected child pornographic images or videos
5. Recovery of data pertaining to an enticement investigation
6. Recovery of data pertaining to a homicide investigation
7. Recovery of data pertaining to a suicide investigation
8. Recovery of data pertaining to a fraud investigation
9. Recovery of data pertaining to a larceny investigation
10. Recovery of data pertaining to a burglary investigation
11. Recovery of data pertaining to a threatening/harassment investigation
12. Recovery of data pertaining to a financial fraud/counterfeiting investigation
13. Recovery of data pertaining to an e-mail threats/harassment/stalking investigation
14. Recovery of data pertaining to a narcotics investigation
15. Recovery of data pertaining to an identity theft investigation
16. Recovery of data pertaining to a network intrusion investigation
17. Recovery of data pertaining to a domestic violence investigation
18. Recovery of data pertaining to a voyeurism investigation
19. Recovery of cell phone data
20. Recovery of media device data
21. Hard drive restoration
22. Digital media restoration
23. Hard drive sterilization
24. Removable media sterilization

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25. Recovery of DVR data
26. Imaging/acquisition of media
27. Administrative inquiries
28. Other - please specify

RETIRED

A. Purpose

a. To provide a new employee with the training guidelines to conduct competent examinations of firearm and toolmark evidence, to render opinions relating to the examinations, and to provide expert witness testimony in a professional and unbiased manner.

b. To provide guidance for a new examiner with prior firearms examination experience.

c. To provide National Integrated Ballistic Information Network (NIBIN) technicians with the appropriate training to conduct acquisitions and correlation review of test fired and evidence cartridge cases.

d. For current examiners, to stay abreast of new technology, current legal trends, and the latest research developments relevant to the discipline of firearm and toolmark examination.

e. To provide guidance on procedure to complete the competency testing and request authorization for an employee to begin casework in the Firearms discipline.

RETIRED

B. Responsibility

Assigned Unit staff

C. Procedure

a. New examiners shall follow the Connecticut Division of Scientific Services Firearm and Toolmark Unit Training Manual (QR FA-15) and complete the New Examiner Achievement Record (QR FA-15-1).

i. This training manual is composed of twelve modules. New examiners will complete each module as assigned by the Unit lead. The modules do not need to be completed in numerical order, and some modules may skipped based on the operational needs of the Division. They may be addressed at a later time. As an example, the following may be areas that examiners may be trained:

1. NIBIN – acquisition and correlation
2. Operability
3. Serial Number Restoration
4. Microscopic Comparison

ii. This training may be supplemented by the training modules on the National Forensic Science Training Center (NFSTC) website.

iii. Acceptance into the ATF's National Firearms Examiner Academy (NFEA) may replace parts of the DSS Firearm and Toolmark Unit Training Manual.

1. Any new examiner accepted into this program is still responsible for completing DSS-specific modules within the Firearm and Toolmark Unit Training Manual (e.g. Orientation, Safety, Evidence Handling).
2. Any new examiner accepted into this program is also responsible for reading all DSS standard operating procedures relating to the Firearms Unit and to the laboratory as a whole.

iv. Training for new examiners may also be provided via a contractual service with a qualified trainer. In this circumstance, the qualifications of the trainer will be reviewed by a DSS employee with technical knowledge, Deputy Director and Director. The trainer will provide a training program as to the topic areas that will be covered. At a minimum, these topic areas should cover the similar areas as listed in the Unit Training Manual.

v. Upon completion of training, the training files are provided to the Quality Manager for review prior to providing a competency test. Once reviewed and found acceptable, new examiners shall be competency tested in the appropriate categories of testing prior to beginning independent casework. This is to evaluate their analytical skills and their ability to clearly communicate their findings in a report. The competency testing shall include an oral or written evaluation of the areas tested with the competency test. Refer to GL-14 on grading parameters for tests issued during the training process.

The competency testing shall include a revolver and a handgun for the appropriate categories of testing.

vi. A memo shall be written to the Deputy Director stating that the analyst has successfully completed their training and passed the competency test.

1. The Deputy Director will review the training files and generate a workflow request in Qualtrax to request that the new examiner be authorized to conduct work in the specified categories of testing. Supporting documents may be appended to this workflow.
2. A Qualtrax workflow will be initiated for authorization of the examiner for casework. The workflow may include the following in which the analyst is being authorized to participate in conducting:

    1. Handle evidence
    2. Perform examination and/or analytical methods
    3. Utilize all applicable equipment in methods described in SOPs
    4. Report, review and authorization of results

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5. Perform administrative reviews
6. Perform validations in the discipline
7. Assist in the development of new methods
8. Modification of methods
9. Verification of methods

vii. The new examiner's trainer or the Unit's technical responsibility/designee will be his/her verifier/technical reviewer of casework for a period of up to six (6) months. The verifier/technical reviewer will evaluate 100% of the evidence items and all conclusions reached. At the end of this period, the new examiner's work will be assessed. If at this assessment, the new examiner's work still needs 100% review, then the review will continue up to two (2) months and then reassessed. Once the new examiner has successfully been assessed, other examiners in the Unit may perform any verifications/technical reviews for the new examiner.

viii. The new examiner may conduct verifications/technical reviews of others' casework in a category of testing after a period of up to six (6) months and a sufficient number of cases of their own independent casework in that category of testing.

1. Once the examiner has completed the evaluation period, a memo shall be written to the Deputy Director stating the examiner has successfully completed the evaluation period and has been deemed competent to conduct technical reviews and verifications in that category of testing.
2. The Deputy Director, after review, may endorse the memo and generate a workflow request in Qualtrax requesting that the examiner be authorized to conduct technical reviews and verifications based upon the examiner's casework experience in the specified categories of testing. Supporting documents may be appended to this workflow. The analyst may also be authorized to participate in conducting administrative reviews (if not previously authorized).

b. New examiners with prior experience shall review all General Laboratory SOPs and Unit-specific SOPs.

i. New examiners with prior experience shall be competency tested along with an oral/written evaluation in the appropriate categories of testing prior to beginning independent casework.

c. NIBIN technicians/users shall be trained in the operation of the Integrated Ballistics Information System (IBIS) equipment by an approved instructor and shall follow the appropriate training modules within the Firearm and Toolmark Unit Training Manual.

RETIRED

d. New and current examiners shall attend training when possible to stay abreast of new topics within the forensic discipline. This can include conferences, presentations, armorer's schools, webinars, etc.

   i. Any certificates of attendance attained for training courses shall be kept with the examiner or analyst and a copy uploaded to Qualtrax under the Personnel-Electronic Filing Cabinet.

   ii. Where possible, training agendas should be included with certificates and uploaded to the Qualtrax Electronic Filing Cabinet.

e. Remediation and Retraining

   i. Refer to GL-14 for guidance when a deficiency has been identified.

D. References

a. QR FA-15: Connecticut Division of Scientific Services Firearm and Toolmark Unit Training Manual

b. QR FA-15-1: New Examiner Achievement Record

c. National Forensic Science Training Center website (http://projects.nfstc.org/firearms/)

d. General Laboratory SOP 14, General Training

RETIRED

A. Purpose:

To describe the safety rules for handling firearms in the Unit.

B. Responsibility:

Forensic Science Examiners and other laboratory personnel assigned to the Firearms Unit.

C. Safety:

1. Eye protection shall be worn when test firing a firearm.
2. Ear Protection – dual ear protection will be worn when firing weapons.

Refer to FA SOP 2A 'Firearms Safety – Lead Conformance' for further guidance.

D. Procedure:

1. General Safety Rules
   a. All firearms handled in the laboratory will be treated as if they are loaded.
   b. The muzzle of the firearm will always be pointed in a safe direction.
   c. The analyst will keep their finger off of the trigger of a firearm until they are ready to fire.
   d. Firearms will only be loaded in the designated safe areas: the water recovery tank room and the indoor range.
2. Loaded firearms submitted to the laboratory will first be transferred to the Firearms Unit to be rendered safe. The indoor range is the closest and most safe area to work. Once the firearm is unloaded, it may then be released to the appropriate unit for examination.
3. Evidence and/or destruction firearms
   a. The safe condition of a firearm must be established upon completion of the examination.
   b. The source of ammunition must be removed and the chamber(s) must be checked to ensure it is clear of ammunition.
   c. The firearm will be then placed in its original submitted container. If desired, a zip tie may be placed through the action of the firearm.
4. For Work Practices, Engineering Controls, Housekeeping and PPE related to minimize lead exposure for activities in the Firearms Unit refer to FA SOP 2A 'Firearms Safety – Lead Conformance'.

E. References:

RETIRED

1. GL 2, Safety Manual
2. OSHA fact Sheet 'Protecting Workers from Lead Hazards at Indoor Firing Ranges'
3. CFR29 1910.1025
   https://www.osha.gov/pls/oshaweb/owadisp.show_unique?p_table_name=STANDARDS&p_unique_file=1910_1025

RETIRED

A. Purpose:

Proper evidence storage and maintenance of chain of custody is essential to protect the integrity of all evidence in the Division. The following describes the documentation of and procedures for retrieving, storing, and returning evidence in the Firearm and Toolmark Unit and the documentation/maintenance of the chain of custody in JusticeTrax (LIMS).

B. Responsibility:

Assigned Unit personnel

C. Procedure:

1. The chain of custody will be maintained at all times to document the location of the evidence.
    a. Person-to-person transfers require the use of Personal Identification Numbers (PINs) from both parties and will be documented in JusticeTrax.
    b. Person-to-storage location transfers require the analyst's PIN and will be documented in JusticeTrax.
2. Evidence transfers shall be documented at the time they occur.
3. Analysts assigned to the Firearm and Toolmark Unit shall retrieve items from the Evidence Receiving Unit as soon as possible upon notification.
    a. When an analyst in the Firearms Unit is called to the Evidence Receiving Unit to render a firearm safe, the evidence shall be transferred to the analyst conducting the safety check.
4. Evidence retrieved from the Evidence Receiving Unit, or from a member of another laboratory unit, shall be transferred to the appropriate storage location (typically Firearms Storage 1) in the Firearms Unit.
5. In-progress case work shall be secured in an evidence locker assigned to the analyst or in Firearms Evidence Storage 1. If evidence is stored in Firearms Storage 1, the chain of custody will be appropriately updated to reflect a transfer.
6. When evidence is transferred to another analyst for the purpose of verification, the chain of custody will be updated in JusticeTrax.
    a. If a verification is conducted in the presence of the analyst without an evidence transfer, it must be documented in the notes, preferably on the Verification Sheet (QR FA-5).
7. After all examinations of evidence are complete, the items shall be stored in a secure location.

RETIRED

8. After the case file has been technically and administratively reviewed, the evidence can be returned to the Evidence Receiving Unit. The chain of custody is updated and the evidence is stored in Evidence Receiving Unit until it is picked up by the submitting agency.

D. References:

1. GL-13 General Evidence Handling

RETIRED

A. Purpose:

1. To describe the process for recording the results of an examination electronically in the JusticeTrax (LIMS) system so that a report may be generated.

B. Responsibility:

Assigned unit personnel

C. Procedure:

1. Open an existing case in JusticeTrax.
2. Click on the Requests tab and select the Firearms request.
   a. This may be listed as "Firearms Analysis," "Firearms Analysis – Other," "NIBIN Operability," "Serial Number Restoration," "Toolmarks," etc.
3. Right click on the request → Edit Findings. This opens the Firearms Analytical Module.

a. Submission Results

i. Right click the submission number → Add Result → Select Submission Result from the drop-down menu under Result Type.

ii. Enter the text for submission results in the white box at the bottom of the dialog box.

iii. Click Apply to save results.

b. Firearms Analysis/Firearms Analysis – Other/Serial Number Restoration/Etc.

i. Right click the firearms request → Add Result → Select Results of Examination from the drop-down menu under Result Type.

ii. Enter the text for the results of the examination in the white box at the bottom of the dialog box.

iii. Click Apply to save results.

c. Operability

i. Operability for firearm(s) submitted in the case may be added to either the Firearms Request or to the submission number containing an evidence firearm.

ii. Right click the Firearms Request or the submission which contains the evidence firearm → Add Result → Operability. The white box at the bottom of the dialog box will be left blank.

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iii. Click Apply to save.

d. Firearms Data

i. Open an existing case in JusticeTrax.

ii. Click on the Evidence tab, and select the submission(s) which contain a firearm.

iii. Right click → Firearms Data → Firearm

iv. Enter the firearms data in the applicable fields. Click Apply to save.

v. The firearms data may be printed if desired (QR FA-3).

vi. This information will auto-populate in any Operability modules that are selected for the case.

e. Completing the case

i. Once all findings have been entered, right click the request → Set Milestone → Draft Complete.

ii. A draft copy of the report is then printed to put into the case file.

(a) Right click the firearms request → Print Final Report → Select printer

iii. The completed case file may then be turned in for Technical Review.

RETIRED

D. Equipment:

1. Any networked DSS computer with JusticeTrax access

E. References:

1. GL 4 – LIMS

Purpose:

To generate contemporaneous notes on the evidence examined, and to generate a report stating the findings and opinions of the analyst.

Responsibility:

Forensic Science Examiners and other laboratory personnel assigned to the Firearms Unit.

Procedure:

A. General

An examination report will be completed for each case analyzed by the Firearms Unit. The report will be saved in JusticeTrax (LIMS) and will be prepared as soon as possible after completion of the examination process. The report should be scientific, easy to read, and grammatically correct. The conclusions of the examination must be conveyed in a manner that is reported accurately, clearly, unambiguously and objectively. The report will include all the information of the analysis conducted and the methodology used.

There are various types of analysis conducted in the Firearms Unit and there are different levels of reporting for these different testing methodologies. The analyst should refer to the following SOPs for guidance on the reporting for the following:

a. Microscopic comparison – refer to FA SOP-25 (Range of Conclusions)
b. Operability testing – refer to FA SOP-08 (Testing for Operability)
c. Serial number restoration – refer to FA SOP-26 (Serial Number Restoration)
d. NIBIN acquisition and correlation – refer to FA SOP-22 (NIBIN)

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B. Report Contents

The report may include the following information (Note: Starred items are required per GL 18):

a. Name and address of the Division*
b. Title*
c. Division case number*
d. Name and address of the submitting agency*
e. Agency case number*
f. Town of incident
g. Date of request*
h. Date of report*
i. Individual requesting report
j. Description of the item(s) tested*
k. Identification of the test method used*

l. Results obtained*
m. Disposition of the evidence
n. Opinion/conclusion statement*
o. Name, title, and signature[1] of analyst and technical reviewer*

C. The case file will contain all pertinent information to support the findings. This may include:
   a. A copy of the completed report
   b. Technical and Administrative Review Checklist (QR FA-9)
   c. Firearms Unit worksheet(s)
   d. Notes
   e. Photographs
   f. NIBIN data, if applicable
   g. Verification worksheet (QR FA-5), if applicable
   h. Documentation of the barcode label on the evidence packaging
   i. Evidence receipt(s)
   j. Request for Analysis sheets (SOP-ER-02)
   k. Other supporting documentation as needed

D. All digital files and pictures will be stored on the analyst's H-drive and backed up by the IT Unit.

E. Communications with the customer, such as a detective, attorney, etc., should be documented either in the Case Info section of JusticeTrax or in a note in the case jacket.

F. Upon completion of the case and report, the case jacket will be forwarded to the Technical Reviewer and Administrative Reviewer for their evaluation. This may sometimes be the same individual. Refer to GL-18 for guidance on technical and administrative reviews. The reviewers will use the QR FA-9 worksheet to document their technical review and administrative review, and will release the milestone in JusticeTrax upon completion of each review.

G. At the completion of the Administrative Review and the final report has been added to the case folder, the entire case jacket will be scanned and saved in the analyst's H drive to be available for court discovery requests.

RETIRED

References:

1. GL 13 General Evidence Handling
2. GL 18 Case Reviews

Footnotes:

1. Signatures of examiner and technical reviewers may be electronic if applicable

RETIRED

Purpose:

To establish a list of abbreviations commonly used within the Firearms Unit. Universally accepted abbreviations such as chemical symbols and measurement designations may be utilized but need not be included on this list.

Responsibility: Forensic Science Examiners or other employees assigned to the Firearms Unit.

ACP	Automatic Colt Pistol
add'l	additional
AFTE	Association of Firearm and Tool Mark Examiners
appx.	approximately
au	Auto
BBL, bbl	barrel
BEB, beb	brass enclosed base
BFM, bfm	breechface marks
bio	biological, biology
BLS	blood-like substance
BPB, bpb	brown paper bag
Br.	brass
BT, bt	boattail
carts	cartridges
CC, cc	cartridge case -or- class characteristics
char	characteristic(s)
CGIC	Crime Gun Intelligence Center
COLLECT	CT On-Line Law Enforcement Communications Teleprocessing
cont.	containing
con't.	continued
c/n	case number
CPB, cpb	clear plastic bag
ctgs	cartridges
ctrl	control
ccw	counter clockwise
cw	casework -or- clockwise
DA	double action
DAO	double action only
DBM, dbm	detachable box magazine
DD	distance determination

DL	download, downloaded
ECC, ecc	evidence cartridge case
EB, eb	evidence bullet
ellip	elliptical
EM	evidence marker
EJ, ej	ejector
EJM, ejm	ejector mark
ER	Evidence Receiving Unit
evid	evidence
EXT, ext, ex	extractor, extractor mark
FB	Forensic Biology Unit
Fed	Federal
FDE	flat dark earth
FMC, fmc	full metal case
FMJ	full metal jacket
FNB, fnb	flat nose bullet
FP, fp	finger print -or- firing pin
FPA, fpa	firing pin aperture
FPAS, fpas	firing pin aperture shearing
FPI, fpi	firing pin impression
FTE	failure to eject
FTF	failure to feed
FRT	Firearms Reference Table
ga, ga.	gauge
GAP	Glock Automatic Pistol
GEA	Groove Engraved Area
GIMP, GI	groove impression
GR	granular
GRC	General Rifling Characteristics
GSD	gunshot detection
GSR	gunshot residue(s)
hemi	hemispherical
HSPB, hspb	heat-sealed plastic bag

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IBIS	Integrated Ballistics Information System
IC, ic	individual characteristics
IFL	Integrated Forensic Laboratories
I/S, i/s	inside
JFN, jfn	jacketed flat nose
JRN, jrn	jacketed round nose
JHP, jhp	jacketed hollow point
JSP, jsp	jacketed soft point
JT	JusticeTrax
(L)	left
L/LR	Long/Long Rifle
L&G	lands and grooves
LEA	Land Engraved Area
LEB	large evidence bag
LIMP, LI	land impression
LIMS	Laboratory Information Management System
LP	latent print; Latent Prints Unit
LRN, lrn	lead round nose
M, m	magazine
mag	magazine
man. env.	manila envelope
MEB	medium evidence bag
mfr.	manufacturer
mic	microscope
MPI	Magnetic Particle Inspection
MROS	Minimum Required Operating Standards
NaRho	sodium rhodizonate
NCV	no comparison value
NCIC	National Crime Information Center
NEATT	Not Examined At This Time
NFSTC	National Forensic Science Training Center
NIBIN	National Integrated Ballistic Information Network
NQH	NIBIN Quick Hit
OAL	Overall length
oblit	obliterated

OCME	Office of the Chief Medical Examiner
OD, ODG	olive drab, olive drab green
O/U	over/under
// or ||	parallel
par	parallel
PbHP	lead hollow point
PbRN	lead round nose
PFT	Precision Forensic Testing
PI, Pi	pistol
PLB	plastic bag
poly	polygonal
poss.	possible
PR, Pr	revolver
proj.	projectile
(R)	right
RCMP	Royal Canadian Mounted Police
rec'd.	received
Rem	Remington
RF	rimfire
RFC	rimfire circle
RFR	rimfire rectangle
RI, Ri	rifle
RC, Rc	carbine
rxn	reaction
S/	sealed
SA	single action
SEB	small evidence bag
SP, Sp	pump shotgun
SJHP, sjhp	semi-jacketed hollow point
SJSP, sjsp	semi-jacketed soft point
S&B	Sellier & Bellot
S&W	Smith & Wesson
SN, S/N	serial number
SNR	serial number restoration
SoRho	sodium rhodizonate
SS, ss	stainless steel
St.	steel
Sub or Sub.	submission
SWC, swc	semi wad cutter

RETIRED

SxS	Side by side
T#	test fire #
TF	test fire
TFCC, tfcc	test fired cartridge case
TMJ, tmj	total metal jacket
unk.	unknown
Wbox	white box
Win, WW, WCC	Winchester
WPB	white pill box
VIN, vin	vehicle identification number

1°	primary
2°	secondary
(+)	positive
(+) swab	fouling present in firearm barrel
(-)	negative
(-) swab	no fouling present in firearm barrel
=	fired in, made by, cycled through (indicates identification)
≠	not fired in, not made by, not cycled through (indicates elimination)

RETIRED

A. Purpose:

To determine the functioning condition of a firearm and to obtain fired ammunition components for microscopic comparison examinations and/or NIBIN entry.

B. Responsibility:

Forensic Science Examiners or other laboratory personnel assigned to the Firearms Unit.

C. Safety:

1. Eye and ear protection shall be worn. Additional PPE may be worn at the analyst's discretion.
2. Test firing must be conducted in a designated safe laboratory area.
3. Firearms may only be loaded in the designated safe laboratory areas.
4. Examiners should refer to FA SOP-02 and SOP-02A for safety guidelines.

D. Procedure:

1. An overall photograph of the firearm with a scale should be taken and included in the documentation.
2. Complete a physical examination of the firearm to determine its operability.
    a. For a brief examination, use QR FA-17 Test Fire Operability Label. This may include cases such as Illegal Possession, Found Property, NIBIN Entry, etc.
    b. For an in-depth examination, use a QR FA-17 and a QR FA-2 Firearms Worksheet. This may include cases such as Homicide, Officer-Involved Shooting, or at the analyst's discretion.
    c. Two (2) copies of the QR FA-17 label should be generated.
        i. One copy is adhered to the test fire container (a #4 coin envelope or similar)
        ii. The second copy is adhered to a worksheet in the case jacket.
3. Select the appropriate caliber and type of ammunition. The ammunition may be from the laboratory supply. Consideration should be taken in the choice of ammunition if evidence exists for comparison examinations.
    a. Evidence ammunition may be used at the discretion of the analyst.
    b. The use of evidence ammunition must be documented in the analyst's notes and indicated in the final report.

RETIRED

4. A minimum of two (2) tests should be fired, unless extenuating circumstances apply. Additional tests may be fired at the discretion of the analyst.
5. Render the firearm safe after test firing.
6. Recover the test fired ammunition components.
    a. Test fires from evidence firearms are placed in the #4 coin envelope with the QR FA-17 Label.
    b. Test fires from destruction firearms are placed in a stamped coin envelope that includes caliber, make, model, serial number, SLFU identifying number, and analyst's name.
7. For evidence firearms, sub-itemize the test fires in JusticeTrax.
    a. Log onto JusticeTrax
    b. Open the appropriate case.
    c. Click on the Evidence tab, then select the appropriate firearm submission.
    d. Right click → Itemize Evidence
        i. JusticeTrax will automatically assign the sub-item the correct, sequential number, such as 001-001.
        ii. Select "Test Fires" under the "Evidence Type" dropdown menu.
        iii. Indicate in the description box that the items are test fires, then click "Apply."
        iv. A barcode label will print out. Adhere the barcode to the test fire container.
8. Test fired specimens are maintained in the appropriate storage location in the Firearms Unit.
9. Upon completion of the examination, return the firearm to its original container. Containers for evidence firearms shall be sealed and initialed appropriately.

RETIRED

Note: For cases in which two (2) or more of the same make/model firearms are submitted (e.g. officer involved shootings), or for cases in which items cannot be differentiated by class characteristics alone, the following procedure should be followed:

1. Only one firearm will be opened and processed at a time.
2. The serial number of the firearm and the lab submission number will be scribed onto the ammunition being test fired (if space permits).
3. The examiner will enter the test fire area with the following:
    a. Firearm to be test fired.
    b. Firearm packaging (evidence box).

c. The Firearm Worksheet (QR FA-2/FA-17).

d. The test fire container.

4. At the time of test firing, a second examiner will verify the above information. This will be documented by initialing the appropriate area on the Firearm Worksheet.

Note 2:

For this SOP, air guns are treated as firearms and functionality will be determined. Documentation will follow the standard documentation process, including the condition of the CO2 canister if present. The examiner will report the condition of the air gun as received and if a charged CO2 canister was used to make the weapon operable.

E. **Reporting:**

The examiner will indicate in the report if the weapon was operable as received. In the event the weapon is not operable, the report should indicate if the weapon was made operable. The report should indicate any issues that may have been present on the weapon as submitted. If the weapon was determined to be inoperable, the examiner will indicate this in the report.

F. References:

1. GL 2 - Safety Manual
2. GL 4 – LIMS
3. FA SOP-02 General Firearms Safety
4. FA SOP-32 Weapons Destruction

RETIRED

A. Purpose:

To provide an alternate method to test the operability of a firearm, if during the examination it is determined to be unsafe for test firing. If it is not necessary to obtain test standards for comparison purposes, the working condition of the firearm can be established by using a primed empty cartridge case or shotshell to primer fire the firearm.

RETIRED

B. Responsibility:

Assigned Unit staff

C. Safety:

1. Follow all safety rules for handling firearms.
2. Appropriate hearing and eye protection must be used.
3. Perform the testing in a safe designated laboratory area.

D. Procedure:

1. Obtain a primed empty cartridge case in the desired caliber or gauge.
   a. If a primed cartridge case is not available, create one by removing the bullet and gun powder from the cartridge case, retaining only the primed cartridge case.
   b. For shotguns, a primed shotshell can be created by cutting open a live shotshell, removing all components, and retaining only the primed shotshell.
2. Ensure that the ventilation exhaust system is turned on.
3. Turn on the hallway warning device.
4. Load the primed cartridge case or primed empty shotshell into the chamber of the firearm.
5. Primer fire the firearm inside the water recovery bullet trap, the snail bullet trap, or the indoor range.
6. Repeat the primer firing process if the firearm has more than one action.
7. Recover all primer fired components.

E. References:

1. FA SOP-02 General Firearms Safety
2. GL 2 Safety Manual

RETIRED

A. Purpose:

Due to limitations of the Firearm and Toolmark Unit's bullet recovery devices and to collect test fired bullets suitable for comparison, it may be necessary to reduce the powder load of a cartridge.

B. Responsibility:

Analysts assigned to the Firearm and Toolmark Unit.

C. Safety:

1. Proper caution must be exercised and the use of personal protective equipment must be considered.
2. Appropriate hearing and eye protection must be used.

D. Procedure:

1. Pull the bullet out of the cartridge using an inertia bullet puller or a reloading press.
2. Remove the gun powder and weigh it.
3. Remove 1/3 to ½ of the gun powder, and replace the remaining ½ to 2/3 of the gun powder back into the empty cartridge case. Dispose of the removed gun powder.
4. It may be necessary to loosely pack a small piece of tissue or similar material into the cartridge to fill the gap between the bullet and the powder.
5. Seat the bullet back into the cartridge case using a rubber or leather mallet, or a reloading press.
6. When using downloaded ammunition, it is imperative that the analyst checks the barrel for obstructions between each firing.
7. Indicate in the notes that downloaded cartridges were fired.

E. Equipment:

1. Inertia bullet puller
2. Reloading press
3. Balance

RETIRED

4. Weigh boat
5. Rubber or leather mallet

RETIRED

A. Purpose:

To measure the barrel and overall length of a firearm.

The State of Connecticut defines a pistol or a revolver as any firearm having a barrel less than 12 inches. Federal statutes define short-barreled shotguns to have an overall length of less than 26 inches and a barrel length of less than 18 inches. Federal statutes define short-barreled rifles to have an overall length of less than 26 inches and a barrel length of less than 16 inches. Measurements for altered barrels should include the longest measured length.

RETIRED

B. Responsibility:

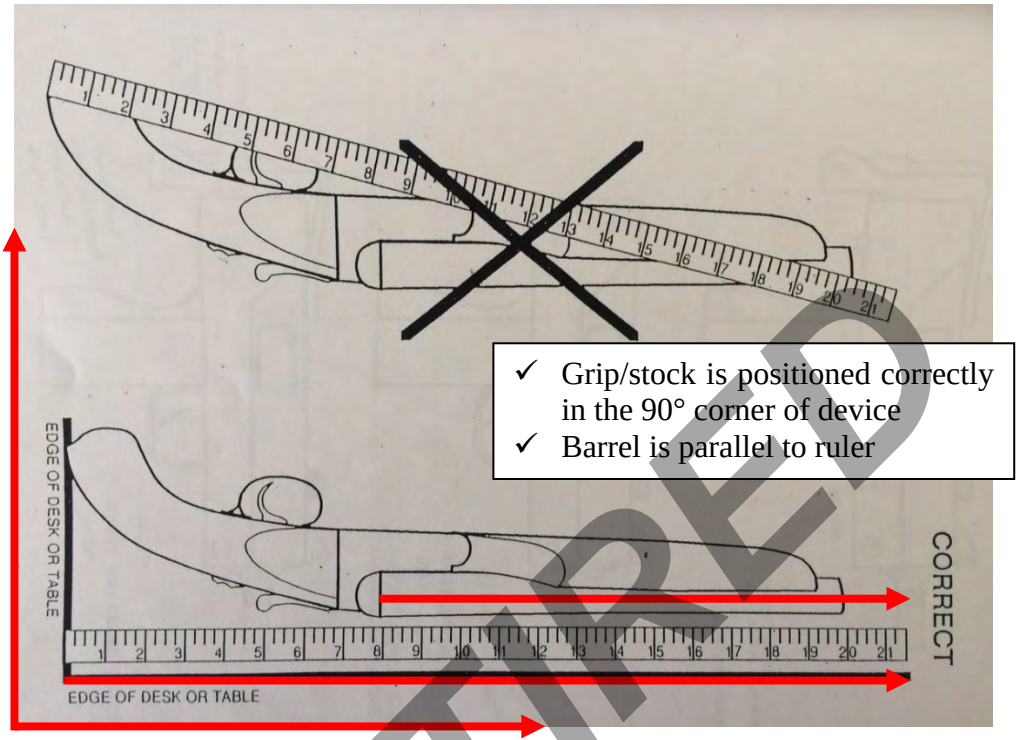
Assigned Unit staff

C. Safety:

1. Follow all safety rules for handling firearms.
2. Personal Protective Equipment (PPE) can be used at the discretion of the analyst.

D. Procedure:

1. Overall Length
    a. When measuring the overall length of a firearm, the measurement is taken along a line which is parallel to the axis of the bore from a perpendicular tangential line which touches the rearmost point of the firearm to the muzzle.
    b. Using an Overall/Barrel Measuring Device, place the rear of the firearm so that the rearmost portion of the firearm is contacting the rear fixed plate.
    c. Align the firearm in the measuring device so that the barrel is parallel to the side plate.
    d. Gently slide the movable plate until it contacts the barrel.
    e. Looking perpendicularly to the ruler, observe the reading on the ruler, and record it on the Weapon Worksheet (QR FA-2) or the Operability Test Fire Label (QR FA-17).



RETIRED

2. Barrel Length
    a. Integral Chamber Barrels
        i. The distance parallel to the bore axis from the muzzle to the breechface with the action closed represents the length of a barrel.
        ii. With the action of the firearm in the closed position, gently insert the metal measuring rod into the barrel until it encounters the breechface.
        iii. Slide the adjustable collar until it touches the tip of the barrel and lock in the collar with the thumb screw.
        iv. Remove the barrel measuring rod and place it on the measuring device parallel to the ruler and flush against the rear plate.
        v. Record on the Weapon Worksheet (QR FA-2) or the Operability Test Fire Label (QR FA-17) the measurement on the ruler where the collar is positioned.
    b. Revolvers
        i. The distance parallel to the bore axis from the muzzle end to the end of the forcing cone represents the length of the barrel. The cylinder is not included in the measurement.

ii. A block or magnet at the muzzle or forcing cone, perpendicular to the axis of the bore, can represent the starting point for the measuring rod to determine barrel length.

iii. With the block or magnet in place, follow steps iii through v above.

3. For any modified firearm whose barrel and/or overall length is found to be less than the federal requirements, a second examiner will verify the measurement(s) and initial the appropriate area on the Weapon Worksheet.
4. Uncertainty of Measurement (UoM) has been calculated for this analysis. This information may be found in the Unit's uncertainty budget, located within the Firearms Unit.

E. Equipment:

1. Precision Forensic Testing Overall/Barrel Measuring Device

F. References:

1. *Instructions for Use of the MD-36/MD-48 Overall/Barrel Measuring Device from Precision Forensic Testing* instruction manual
2. "SWG-GUN Guidelines for Barrel and Overall Length Measurements for Firearms." https://www.nist.gov/sites/default/files/documents/2016/11/28/guidelines_for_barrel_and_overall_length_measurements_for_firearms.pdf
3. Bureau of Alcohol, Tobacco, Firearms & Explosives (ATF) *Firearms and Ammunition Identification Guide*
4. Connecticut General Statutes § 53a-3 (18); 53a-211
5. National Firearms Act of 1934: 26 U.S.C. Chapter 53
6. GL 1.5 ASCLD/LAB Uncertainty Policy
7. Firearms Unit Uncertainty Budget

RETIRED

A. Purpose:

To provide guidance when handling and examining a rusty firearm. Rusty firearms or those recovered from a body of water may be submitted for examination. Immediate attention must be given to prevent further damage to the firearm. If a firearm is recovered from water, the submitting agency should be advised to submit the firearm in a container with the same water it was recovered from (salt water, fresh water, etc.).

RETIRED

B. Responsibility:

Forensic Science Examiners assigned to the Firearms Unit.

C. Safety:

1. Protective eyewear must be worn during this examination process. It is at the discretion of the examiner to use any other personal protective devices.
2. Any firearm that cannot be unloaded must be examined in a designated safe area in the laboratory such as the water trap recovery room or the indoor range.

D. Procedure:

1. Ensure that the firearm is unloaded.
   a. If it cannot be readily verified as unloaded, it must be examined in a designated safe area of the laboratory.
   b. Determining whether a firearm is unloaded may necessitate a complete disassembly, or in some cases destruction, by means of cutting the firearm into pieces.
2. Ensure that the firearm is not subjected to extreme rough handling and that the muzzle is always pointed in a safe direction.
3. Determine to what level the firearm must be restored, (i.e., for test firing, recovering manufacturer information, determining serial number, etc.).
4. Saturate the rusted firearm with penetrating oil, de-rusting solvents, or a similar material. Disassembly of the firearm may be necessary.
5. Periodically check the firearm until the firearm functions, or until the desired information can be recovered.

6. Swab the bore, breech face, or slide, by alternating solvent soaked patches and dry patches until those areas are as clean as possible. Only clean parts of the firearm necessary to allow it to function.
7. Only use non-marring items to clean the barrel and breech area of the firearm.
8. Once the firearm is cleaned, functioning properly, checked for safety, and all pertinent information is recorded, proceed to test fire the firearm if necessary.

E. Equipment:

1. Safe designated laboratory work area, water trap recovery room, or indoor range
2. Sink with running water
3. Solvents, patches, brushes, and non-marring tools

F. References:

1. GL 2 Safety Manual
2. FA SOP-02 General Firearms Safety
3. Gunsmith books and videos

RETIRED

A. Purpose:

Submitted firearm evidence may contain trace material such as blood, tissue, plaster, sand, etc., which may need to be removed for further examination.

B. Responsibility:

Analysts assigned to the Firearm and Toolmark Unit.

C. Safety:

Personal protective equipment (PPE) may be utilized if necessary.

D. Procedure:

1. If the debris is found on a firearm, follow all firearm safety rules when handling the firearm.
2. Document the debris that may need to be removed by written notes, drawings, or photographs.
3. If necessary, contact the appropriate section such as Latent Prints, or Forensic Biology for consultation or removal of the trace material.
4. Once removed, secure the debris in an appropriate container. This container should be packaged in the original evidence packaging along with the evidence item.
5. Once any debris has been documented and removed, the evidence item may then be cleaned if needed.
6. Collected and retained debris should be indicated in the final report. If the trace material is being retained at the Laboratory, this shall be created as a sub-item in JusticeTrax for evidence tracking.
7. It may not be necessary to maintain the debris. In this case, documentation of the debris is the only step required.

E. Equipment:

1. Containers for debris (pill box, paper for druggist fold, etc.)

RETIRED

A. Purpose:

To replicate the interior surface of the chamber or bore of a firearm. Occasionally, firearms are received for which the caliber may not be known, or may be different than is designated on the firearm and in the literature. It may also be necessary to evaluate the bore for subclass characteristics. Creating casts of the chamber and/or the bore may facilitate this process.

B. Responsibility:

Forensic Science Examiners assigned to the Firearms Unit.

C. Safety:

Proper caution must be exercised and the use of personal protective devices should be considered by the examiner, such as gloves, clothing and eyewear when performing this procedure.

D. Procedure:

1. Ensure that the firearm is unloaded.
2. Open the action and remove the bolt or bolt assembly.
3. Check the bore to make sure it is clear.
4. Push a cleaning patch in the barrel from the muzzle end until it is ½ inch to ¼ inch from the beginning of the chamber.
5. Lubricate the chamber with WD 40®, gun oil, silicone spray, or other similar substance.
6. Prepare the casting material as per the manufacturer's instructions.
7. Fill the chamber with the casting material. Do not allow it to flow into the breech.
8. When the casting material is set, gently tap it with the end of a cleaning rod to loosen the cast from the chamber and remove it from the breech.
9. The same steps may be used in the casting of the bore. The entire length of the bore does not need to be filled with casting material; a few inches from the end are sufficient.

E. Equipment:

1. Casting material

RETIRED

F. References:

1. Casting material manufacturer's instructions

G. Quality Control:

When a chemical/reagent is utilized for the first time, the examiner opening/preparing the chemical or reagent will ensure that the container is properly labeled as to its contents, its safety information (NFPA or GHS) and the ability of the performance of the chemical/reagent). The examiner will also place the date opened/prepared and his/her initials on the container. Most chemicals/reagents are purchased as packaged kits. Per GL-21 General Laboratory Equipment, chemicals/reagents are considered "equipment" as they can influence the correct performance of laboratory activities (i.e. creating test impressions or enhancement).

The very first control test impression developed with a newly opened or prepared casting material will demonstrate the ability of the performance of that lot of chemical/reagent. A log will be kept of this control test impression. The log will include the chemical/reagent name, the date opened/prepared, the lot number, the initials of the analyst who opened/tested it, if the control test impression was acceptable and the manufacturer's expiration date, if there is one. This information will be logged on the Reagent Log form (QR-FA-19) . Once the chemical/reagent has been found to be acceptable, a colored dot will be initialed and dated and "OK" or "OK for use" will be written on it. This dot will be taped to the chemical/reagent bottle and will signify the chemical/reagent is okay to use. Before any chemical/reagent is used, every analyst should check for this sticker. If it is not present, the above stated procedure must be followed.

The quality control procedure for the casting material will be to apply a small amount of this casting material to a cartridge casing. It is preferable if the cartridge case has a Glock-type or other similar distinct firing pin impression. If the cast once removed replicates the markings on the head stamp of the cartridge case, the result is considered acceptable. The quality control check will conducted once on the initial opening of the casting material. If at any point, the casting material appears to have hardened, the quality control should be repeated or a new tube should be opened and tested.

RETIRED

A. Purpose:

To describe the procedures for documenting and classifying bullet/projectile evidence.

B. Responsibility:

Forensic Science Examiners assigned to the Firearms Unit.

C. Safety:

To avoid exposure to any potential bio-hazardous material, bullet/projectile evidence should be decontaminated.

D. Procedure:

The characteristics and condition of bullet evidence will be documented in the notes, using QR FA-11 Projectile Worksheet, QR FA-13 Notes Page, or QR FA-14 Fillable Blank Notes Page.

Some of the details that will be recorded on the QR FA-11 Projectile Worksheet will include whether the bullet evidence is suitable for microscopic comparison.

1. Caliber determination
   a. Measure the base diameter of the evidence bullet using a measuring device such as calipers. Damage may preclude this measurement.
   b. Caliber may also be determined by the use of the following equation (formula for determining the circumference of a circle). This calculation will be verified during technical review.

$$\mathbf{(L + G)n \approx \pi d}$$

(L + G) is the measurement of one land impression plus the measurement of one groove impression in inches

n is the number of lands/grooves

π is approximately 3.14

d is the diameter of the bullet in inches.

   c. Physical characteristics of the evidence bullet, such as weight, bullet shape, composition, nose design, and number and placement of cannelures may aid in caliber determination.
   d. Caliber is written as a numerical term and may be written with or without the decimal point.
2. Other Characteristics

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a. Any trace material (refer to FA SOP-15 Removing Debris and FB SOP-19 Trace.HLF Examination for guidance).

b. Caliber

c. Weight in grains

d. Number of lands and grooves

e. Dimensions of lands and grooves

f. Direction of twist

g. Composition of bullet

h. Type of bullet

i. Base description

j. Possible manufacturer of bullet

k. Cannelures

l. Any other physical description of the bullet's condition

3. Measuring Land and Groove Dimensions

a. The land and groove dimensions may be measured in two ways on a comparison microscope: live image or by photograph. This data may be used to determine possible suspect weapons with the assistance of a general rifling characteristics database. The NIST-traceable stage micrometers may be used as needed to ensure the measuring tools are measuring accurately.

b. Measuring should be done on any suitable land and/or groove impressions.

i. If a bullet is identified as having been fired in specific firearm, L&G measurements are not necessary.

ii. Some unusual or common rifling characteristics, such as 4R, 5R, 7L, 7R, 9L, etc., do not require measurements to determine possible suspect weapons.

c. Mount the bullet on a microscope stage.

d. Adjust the lighting as needed.

e. Using the imaging software, follow the steps below for the chosen method.

To measure by live image:

1. Click the Measure tab.

2. Choose the Distance Line Tool.

To measure by photograph:

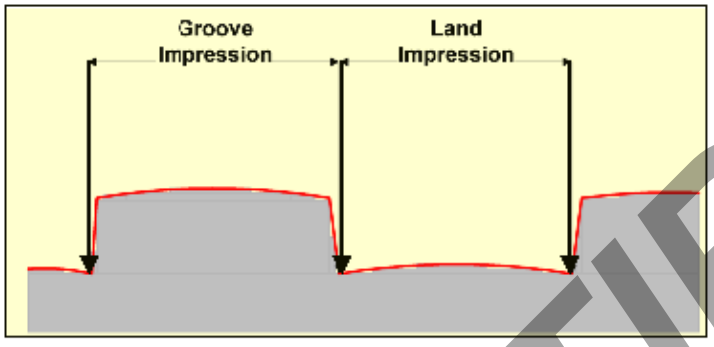
1. Capture an image of the bullet by clicking on the "Acquire" button. Save the image in the appropriate folder.

2. Click Process tab → Annotate tab →

RETIRED

Extended Annotation toolbox.

3. Measure the dimensions of the land or groove impression from shoulder to shoulder (see diagram below).
3. Using the Distance Line Tool, measure the dimensions of the land or groove impression from shoulder to shoulder (see diagram below).
4. Record the data in case notes.
4. Click Merge to add the measurement annotations to the image of the bullet. Save the image in the appropriate folder.



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4. Using the Land/Groove Measurements to Determine Suspect Weapons
    a. The acquired measurements may be input into the FBI GRC Database.
    b. Examiners will use a +/- 0.005" input tolerance for entry into the GRC database. More information on the entry into the FBI's GRC File document is located in Qualtrax under Firearms Unit – Controlled Electronic Reference Documents.
    c. The search results list will be printed and kept in the case jacket.
    d. When reporting possible suspect weapons, it should be indicated that the list is being provided. A copy of the GRC list will be attached to the report provided to the requesting agency.

E. References:

1. AFTE Glossary
2. GL 2 Safety Manual
3. FA SOP-15 Removing Debris
4. Forensic Biology SOP-19 Trace.HLF Examination
5. General rifling characteristics databases (FBI and AFTE)
6. General Rifling Characteristics File document from the Firearms/Toolmarks Unit FBI Laboratory

RETIRED

A. Purpose:

To examine recovered shot pellets and wadding in order to determine the shot size and gauge of the firearm from which these items were fired. This determination can be made by comparing the fired ammunition components with ammunition components found in live shotshells, or by comparing the fired ammunition components to submitted fired shotshells.

B. Responsibility:

Forensic Science Examiners assigned to the Firearms Unit.

C. Safety:

The use of personal protective devices is at the discretion of the analyst.

D. Procedure:

1. Shot Pellet Examination
   a. Determine the total number of pellets received.
   b. Determine the composition of the pellets (lead, steel, bismuth, etc).
   c. Determine the weight of the pellets.
   d. Note the pellet condition.
   e. Determine the size of the shot. The following methods may be used:
      i. Compare side by side the evidence pellets with known shot sizes.
      ii. Compare the weight of the evidence pellets to the weight of known standards.
      iii. Using the least damaged pellet specimen, measure the diameter using calipers.
   f. Record the results of the findings in the notes.
   g. The shot size/weight tables in the AFTE Glossary may be useful in determining shot size.
2. Wad Examination
   a. Gauge determination
      i. Directly compare the evidence wadding to known examples of similar manufacture and composition. Do this by comparing the base of evidence to the bases of the standards until a similar size is found.

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ii. Measure the base diameter of the wad and compare it to the base diameter of known gauge measurements.

iii. Measurements may be obtained by utilizing calipers or imaging software associated with the comparison microscopes.

b. If evidence shotshells are submitted, it may be necessary to disassemble one of them to determine the gauge or the manufacturer of the unknown component.

c. Manufacturer's data may also be determined by locating information stamped into the wad or by comparing the wad to known examples.

d. Microscopic examination may reveal striations suitable for identification of the wad back to the shotgun that fired it.

e. Record the results of the findings in the notes.

RETIRED

E. References:

1. AFTE Glossary, current edition

A. Purpose:

To describe the process of documenting fired cartridge case and fired shotgun shell evidence.

B. Responsibility:

Forensic Science Examiners or laboratory-authorized personnel assigned to the Firearms Unit.

C. Safety:

Personal protective equipment (PPE) shall be used at the analyst's discretion.

D. Procedure:

Using a QR FA-10 NIBIN, QR FA-12 Cartridge Case, QR FA-13 Notes Page, or QR FA-14 Fillable Notes Page, the following information should be documented:

1. The presence of any trace material. Refer to FA SOP-15 Removing Debris for guidance.
2. The caliber or gauge/bore
3. Headstamp information
4. Any information printed on the hull (shotgun shells)
5. Type of ignition system
    a. Centerfire
    b. Rimfire
    c. Other
6. Composition of primer and case body
7. Any artifacts of firing, including:
    a. Shape of firing pin impression
    b. Type of breechface marks
    c. Extractor/ejector marks
    d. Chamber marks
    e. Anvil marks
8. Resizing marks

RETIRED

9. Magazine lip marks
10. Ejection port marks
11. Any other pertinent characteristic(s) found on the item of evidence

E. References:

1. GL 2 Safety Manual
2. FA SOP-15 Removing Debris
3. AFTE Glossary

RETIRED

A. Purpose:

To describe the steps for screening cartridge case evidence and entering it into the National Integrated Ballistic Information Network (NIBIN) computer database. This allows the analyst to provide a submitting agency with possible linkages to other cases.

B. Responsibility:

Forensic Science Examiners and other laboratory-authorized individuals who have been trained by ATF, Forensic Technology, Inc., and/or a NIBIN Authorized Trainer to use the equipment.

C. Safety:

PPE shall be used at the analyst's discretion.

D. Procedure:

1. Write the laboratory case number and the analyst's initials on the outer packaging of the evidence submission(s).
2. Scribe the case number, the submission number designation, and the analyst's initials onto all of the items in the package(s).
3. Using a stereo microscope, evaluate the fired cartridge cases to determine which item(s) should be entered into NIBIN. The following class characteristics should be considered:
   a. Caliber
   b. Breech face marks
   c. Firing pin impressions
4. If the class characteristics are the same, select the best exemplar of the cartridge cases.
5. If class characteristics vary, an exemplar from each group should be selected.
6. The QR FA-10 NIBIN, QR FA-12 Cartridge Case, QR FA-13 Blank Note Page, or QR FA-14 Fillable Blank Note Page worksheets may be used for documentation of the evidence.
7. Enter the cartridge case evidence into NIBIN following the procedures outlined in FA SOP-22 NIBIN.
8. When the acquisition is completed, return the evidence to its original packaging.

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9. Refer to the procedures outlined in FA SOP-22 NIBIN for correlation review, generating hits, and case reports.

E. Equipment:

1. Stereo microscope
2. BrassTrax HD3D acquisition station

F. References:

1. FA SOP-22 NIBIN
2. BrassTrax HD3D training guide

RETIRED

Purpose:

The focus of the National Integrated Ballistic Information Network (NIBIN) is to provide federal, state and local law enforcement agencies with investigative leads by comparing digital images of fired cartridge cases from different crime scenes. The NIBIN database utilizes BrassTrax HD3D, an automated acquisition station for capturing images of fired cartridge cases, and MatchPoint+, a viewing station for reviewing correlation results. High-resolution images captured by the BrassTrax HD3D acquisition station are stored on regional servers maintained by the Bureau of Alcohol, Tobacco and Firearms and Explosives (ATF). Utilizing MatchPoint+, an analyst can review the correlation results and compare the images side by side on a computer monitor. If a potential association (a lead) is located during the review process, the associated agency(cies) are notified via a NIBIN Lead memo. If the agency desires, confirmation of the lead can be conducted by an examiner using a comparison microscope.

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ATF Minimum Required Operating Standards (MROS)

1. Quality Assurance Program

The Division of Scientific Services (DSS) has a NIBIN terminal in its facility and DSS has a documented quality system that is appropriate to the NIBIN acquisition and correlation processes that comply with ASCLD-LAB and ISO/IEC 17025 and the ATF Standards. The quality system will include the following:

- Goals and Objectives as indicated in the General Laboratory SOPs along with the Firearms Unit SOPs (Standard 1.1.1)
- Organization and Management as represented in GL-1 (Standard 1.1.2)
- Personnel as represented in the General Laboratory SOPs (GL-1, GL-2, GL-3, GL-5, GL-14 and GL-15) and FA SOP-1 which include training, ethics, safety and security (Standard 1.1.3)
- Facilities including the practices or procedures for security to maintain the integrity of firearms evidence and its analysis as indicated in GL-3 and GL-13 (Standard 1.1.4)
- Acquisition, Correlation, NIBIN Lead Dissemination Procedures are articulated in Section 5 of this SOP (Standard 1.1.5)
- Evidence control including the DSS procedures on handling and preserving evidence as indicated in GL-4, GL-12, GL-13 (Standard 1.1.6)
- Validation of new methods or equipment as represented in GL-22 and FA SOP-37 (Standard 1.1.7)
- Equipment calibration and maintenance and the procedures for conducting performance checks as indicated in GL-21, FA SOP-33 and FA SOP-35 (Standard 1.1.8)

- Procedures on how reports are generated and confidentiality as indicated in GL-1, GL-5, GL-18, FA SOP-05 (Standard 1.1.9)
- Review of casework prior to release of results as designated in GL-18 and FA-SOP-06 (Standard 1.1.10)
- Corrective Actions and the Quality System of DSS as described in GL-1 and GL-9 (Standard 1.1.11)
- Participation in internal and external audits as described in GL-7 (Standard 1.1.12)
- Procedure on training and maintenance of reports of training, competency and authorization of NIBIN Users as described in GL-11, GL-14, FA SOP-1 and FA SOP-22 (Standard 1.1.13)
- Procedures in regards to the safe handling of firearms and general safety procedures as described in GL-2, FA SOP-2 and FA SOP-4 (Standard 1.1.14)
- DSS does participate in outsourcing of samples for DNA analysis as described in GL-18, but no outsourcing of firearms testing is being conducted at this time (Standard 1.1.15)

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2. Organization and Personnel

NIBIN Program Administrator: full-time examiner employed by the Division of Scientific Services the NIBIN site. This individual is designated to communicate with all parties (i.e submitting law enforcement agencies, ATF Crime Gun Intelligence Centers {CGICs}, ATF NIBIN Unit) involved in the NIBIN process. The NIBIN Program Administrator will work with the Case Management Unit and Deputy Director in communicating to the submitting agencies. The NIBIN Program Administrator must be a qualified NIBIN user in good standing and trained by an ATF-approved acquisition and correlation course provided by the ATF, Forensic Technology, Inc. and/or by a NIBIN Authorized Trainer.

The NIBIN Program Administrator will work in conjunction with Management and the Quality Section in implementing and directing policies, procedures and operations of the NIBIN site along with any subsequent audits. The NIBIN Administrator will recommend to Management to initiate, suspend and resume NIBIN operations for the site or an individual. In general, the NIBIN Program Administrator is usually the individual designated as having technical responsibility for the Firearms Unit. (Standards 2.1, 2.2 and 2.2.1 and 2.2.3)

In the event that the NIBIN Program Administrator position is vacated and there is no employee present who meets the requirements of the above standard, DSS will immediately contact the ATF and submit a contingency plan to the ATF within 14 days for approval. No new casework involving the use of NIBIN can be started until such plan is approved. (Standard 2.2.4.1)

If the NIBIN Program Administrator position is vacated, the Deputy Director will notify the Director and Quality Manager. The Director and Deputy Director will, within 7 days of the

NIBIN Program Administrator's position being vacated, designate an individual from the Firearm Unit as the Interim NIBIN Program Administrator. A memo from DSS Laboratory Management will be generated informing the appointee of the appointment. The Interim NIBIN Program Administrator must meet the requirements as listed in Standard 2.2.4.1.

The ATF will be contacted with the name and qualifications of the named Interim NIBIN Program Administrator. Once the contingency plan is approved, this individual will named the NIBIN Program Administrator.

If the NIBIN Program Administrator does not meet the training requirements of a Qualified NIBIN User for both acquisition and correlation, the site may also have a Technical NIBIN Administrator, who is a qualified NIBIN User trained in both acquisition and correlation and is designated to assist the NIBIN Program Administrator with technical operations. (Standard 2.2.1.2)

NIBIN Users: Assigned Unit personnel or other laboratory-authorized individuals who have successfully completed an ATF-approved acquisition and correlation course provided by the ATF, Forensic Technology, Inc., and/or a NIBIN Authorized Trainer to use the equipment to perform Acquisition and/or Correlation. (Standard 2.3)

Records of training, qualifications, skills and experience of the NIBIN Administrator and NIBIN Users are maintained by the DSS Quality Section (Standard 2.3, 2.4 and GL 11).

RETIRED

3. Facility and Security of NIBIN Equipment:

DSS will comply with all ATF, DOJ and/or Federal security requirements related to the NIBIN program including its network and systems to ensure the integrity of the NIBIN analysis as well as the evidence. (Standard 3.1)

DSS will contain the NIBIN equipment in a room that has a locked door and access limited to personnel assigned to the Firearms Unit or other employees as designated by the Director. DSS has proximity cards and keys assigned to all employees. The proximity cards have permission levels that are set that limit access to the NIBIN area as designated by the NIBIN Administrator and Management. (Standards 3.2, 3.3, 3.4, 3.6, 3.7/ GL-3)

Employees no longer authorized to use the NIBIN equipment will be limited to room containing the NIBIN equipment via a change in the proximity access level and/or removal of the keys. (Standards 3.5, 3.8 / GL-3)

DSS is a secure facility that has security monitoring and limited access via proximity cards. This facility is alarmed when employees are not in the building. Main doors to the operational areas are locked and require an appropriate proximately card to acquire access. This security system is tested quarterly and records of testing are maintained. (Standards 3.3 / 3.4 / GL-3)

Visitors to DSS are are escorted and required to follow the guidance as provided in GL 3 "Security" Section 2 "DSS Facility Access to Visitors". (Standard 3.9)

DSS accepts NIBIN related evidence through its Evidence Receiving Unit. Submitting agencies are granted access by staff in Evidence Receiving area to enter the location. This procedure is outlined in GL-3 Section 1. B. ii. (Standard 3.5)

4. Evidence Control:

The Firearms Unit uses GL-13 "General Evidence Handling" and FA SOP-4 as guidance on the proper handling of evidence to main the forensic integrity of case materials. Evidence in this unit will be handled in a manner that appropriately documents custody and storage locations. (Standard 4.1 / 4.3)

Evidence submitted to DSS is given a unique identifier as to the case number submitted to the case along with a unique identifier for the evidence submitted. Guidance for the case numbering, submission numbering and evidence tracking is clarified in GL-4 "LIMS". The evidence when submitted to DSS is given a barcode that is used for tracking of this evidence to personnel or evidence storage locations. (Standard 4.1)

The Chain of Custody for the items of evidence submitted for NIBIN entry are tracked electronically via the barcode of the evidence and a unique PIN for each examiner. A record of the chain of custody is maintained in the Justice Trax database and can be printed when needed. (Standard 4.2)

Access to evidence locations are limited to personnel authorized by the Director or their designee. This access is designated in the Justice Trax permissions and only authorized personnel can transfer the evidence into or out of specific storage locations. (Standard 4.4)

RETIRED

5. Procedure:

1) NIBIN Entry Protocols (Standard 5)

If the case has multiple exhibits, the examiner should refer to FA SOP-21 "Screening Cases for NIBIN". (Standard 5.2.1.)

a. Exhibits that may be entered:

i. Cartridge cases fired in semiautomatic and fully automatic pistols.

ii. Cartridge cases fired in semiautomatic, fully automatic, bolt action, pump/slide action and lever action rifles.

iii. Shotgun shells fired in semiautomatic, slide/pump action, lever action and bolt action shotguns.

iv. Any firearm that is magazine fed and/or designed to eject cartridge cases after firing.

b. Exhibits that should not be entered:

i. Cartridge cases or shotgun shells that lack sufficient or repeatable characteristics within the breech face and firing pin impressions to assist in the correlation review. The analyst's discretion will be used to make this determination.

ii. Images of cartridge cases or shotgun shells fired in revolvers, break open shotguns, derringers and single shot firearms.

iii. Evidence cartridge cases that are consistent with having been fired in the above type weapons.

iv. Any law enforcement officer's duty weapon, unless extenuating circumstances apply.

2) Entering Case Information into BrassTrax HD3D

a. Creating case records (Standard 5.2.1.4)

i. The procedure outlined in the BrassTrax HD3D users guide will be utilized to create a new case record and to enter the case information.

ii. Case ID – The "DSS" or "ID" number section or the eleven digit Weapon Destruction Unit number. A dash (-) separating DSS or ID and the case number digits is optional.

Examples: DSS13-1234, ID-13-9876, or 40001652345

iii. Event Type – Will be selected based on the information provided by the submitting agency. The event type should be the offense associated with the case.

iv. Occurrence Date – The date of offense provided by the submitting agency.

v. Case Supervisor – The analyst responsible for the case.

vi. Originating Agency – The police agency submitting the evidence. If the agency is not listed, a note of the submitting agency should be made in the comment field.

vii. Originating Agency Contact – The name of the lead investigator listed in the case.

viii. Originating Agency Reference – The submitting police agency's case number.

ix. High Profile – Shall be used at the discretion of the analyst.

x. Comment – Any additional comments about the case can be entered at the discretion of the analyst.

b. Creating cartridge case exhibit records

i. The procedure outlined in the BrassTrax HD3D users guide will be utilized to create a new cartridge case exhibit and to enter the exhibit information.

ii. Exhibit number:

a) Cartridge cases from an unknown firearm will use the case submission number or the number designated by the analyst.

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b) Test fired cartridge cases will include the submission number of the firearm, the test fire number, and "TFCC".

iii. The following fields will be added in the cartridge case exhibit screen:

Caliber

Make of ammunition

Firing Pin shape

Firearm exhibit number (if applicable)

Composition (composition of cartridge case: nickel-nickel, brass-nickel, etc.)

Breech face class characteristics

Reception date

Category:

- Crime Evidence (items collected from a crime scene)
- Test Fire – Returned (use for firearms which will be returned to their rightful owner, or otherwise released from law enforcement custody)
- Test Fire – Terminated (use for all other firearms). If test fires are obtained from a firearm submitted in the case, the event type for the cartridge case exhibit will be changed to "test fire".

c. Creating firearm exhibit records

i. The procedure outlined in the BrassTrax HD3D users guide will be utilized to create a new firearm exhibit and to enter the exhibit information.

ii. Firearm exhibit number - Use the submission number of the weapon or the number designated by the analyst.

iii. The following fields will be added in the firearm exhibit screen:

Caliber

Make

Model (if known)

Type

Serial Number

- If obliterated and later restored, the serial number field shall be updated.
- If the serial number could not be restored, enter "Obliterated" or "Oblit."
- If the firearm does not have a serial number, enter "N/A" or "none."

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d. Acquisition Parameters

i. **Centerfire Cartridge Cases/Shotshells**

a) The analyst will acquire the following regions of interest:

Breech Face

Full Headstamp

Firing Pin

Ejector marks (at the discretion of the analyst)

ii. **Rimfire Cartridge Cases**

a) The analyst will acquire the following regions of interest:

Full Headstamp

Firing Pin

iii. **Other Acquisition Parameters**

a) The analyst will follow the BrassTrax HD3D acquisition protocols outlined in the BrassTrax HD3D users guide to orient the cartridge case/shotgun shell and capture the digital images.

b) If a cartridge case/shotgun shell is oriented outside of the above parameters, a remark shall be made in the comment field stating the reason for the deviation.

c) In the event that reference collection parts (e.g. firing pin, slide, etc.) are used to obtain test fired cartridge cases/shotgun shells, the images of the respective areas need not be captured. A remark shall be made in the comment section describing which parts were replaced.

e. Correlation Results (Standard 5.3)

i. Acquired exhibits will automatically correlate against the CT site and other surrounding area servers. In the event an agency requests a search of other geographical locations, the analyst will perform a manual correlation for the region requested. A manual correlation may also be conducted to search the CT only site.

ii. Using the Rank Sort feature, the analyst will review at minimum the first thirty (30) images of the correlation.

iii. Once the correlation results have been reviewed and there are no leads, the Rank Sort results will be printed and kept in the case file. The date of review will be indicated on the worksheet and/or printout to the correlation results.

iv. The correlation request may be deleted from the correlation results list.

f. Generating Leads

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i. If the analyst reviewing the correlation results locates a potential lead, they will mark the cases as an unconfirmed lead according to the MatchPoint+ users guide protocols after a second analyst has reviewed the potential lead.

ii. If a lead has been microscopically confirmed by a firearms examiner, the analyst will mark the lead as "confirmed," using the steps outlined in the MatchPoint+ users guide. The analyst will note in the comments field who confirmed the lead.

g. Case Records and Memos (Standard 5.4)

i. A preliminary NIBIN Lead memo will be issued for unconfirmed hits.

a) NIBIN Lead memos are generated via the MatchPoint+ Unit. The case information for each part of the lead is auto-populated by the equipment.

b) The memo will be reviewed and signed by the analyst and the reviewer of the NIBIN lead.

c) The original memo will be furnished to the main case file located in the Evidence Receiving Unit.

d) A hardcopy of the memo will be sent to the associated agencies.

e) A scanned copy will be sent via email to the associated agencies.

f) A copy of the memo along with the email will be maintained in the firearms case file.

g) The "Related Laboratory Cases" field in JusticeTrax will be used to link together cases in a NIBIN lead.

h) If the unconfirmed lead involves a destruction gun from the Special Licensing and Firearms Unit, the analyst will fill out the Weapon Destruction Unit form letter and attach it to the memo.

i) If a candidate for comparison is not found, the case can remain in the custody of the laboratory until either the case is completed, or a report is issued stating that NIBIN entry was made, no hits were developed, the evidence is being returned to the submitting agency unworked, and if further examination is required, to contact the laboratory.

h. NIBIN Maps

i. If the submitting agency requests a NIBIN map, a NIBIN map may be created using a word processing program and will be saved in the folder "NIBIN Hit Maps" located on the network drive. The map will be part of the NIBIN Lead memo and is subject to review.

ii. Each box on the map shall contain the laboratory case number, the agency and agency case number, the type of crime, and the caliber(s) of submitted cartridge cases.

a) Firearms submitted in the case will have the caliber/make/model/serial number listed.

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b) Different colored fonts and arrows will represent cartridge cases that share/may share a common origin.

c) Dotted arrows will indicate unconfirmed hits. Solid arrows will indicate confirmed hits.

d) Maps will be updated as additional hits are made and/or hits are confirmed on the microscope.

e) Updated maps will be added to the associated case jackets as additional documentation.

i. Maintenance and Quality Assurance

i. The NIBIN monthly check will be completed, by the fifth of each month, by the assigned analyst. The Rank Sort correlation results and case information pages will be printed and stored in the NIBIN Monthly Control Log binder located in the NIBIN room. Refer to FA SOP-33 Equipment Maintenance Schedule for instructions.

ii. Every fifty (50) correlations, the BrassTrax unit will undergo an automatic self-check. In the event that BrassTrax fails the self-check, the unit will be marked as out of service and Forensic Technology, Inc. will be notified via customer support (1-866-384-4247).

iii. Tests and Calibrations activities are maintained in the BrassTrax HD3D software. This report can be generated at will.

6. Corrective Action

The NIBIN Site is incorporated in the Firearms Unit of DSS and will follow guidance provided in GL-9 in regards to corrective actions, root cause analysis, remediation, implementation and evidence of effectiveness.

7. Audits (Standard 7)

The NIBIN Site is incorporated in the Firearms Unit of DSS and will follow guidance provided in GL-7 in regards to participation in internal and external audits.

Equipment:

1. BrassTrax HD3D acquisition station
2. MatchPoint+ viewing station

References:

1. BrassTrax HD3D users guide (located on the BrassTrax computer)

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2. MatchPoint+ users guide (located on the MatchPoint+ computer)
3. FA SOP-33 Equipment Maintenance Schedule
4. Minimum Required Operating Standards (MROS)

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A. Purpose:

To describe the steps to perform an examination of evidence using a comparison microscope. These procedures apply to both the examination of fired ammunition components and to tool marks.

B. Responsibility:

Forensic Science Examiners assigned to the Firearms Unit.

C. Safety:

Evidence items for comparison should be decontaminated prior to using a comparison microscope.

D. Procedure:

The procedure steps below do not have to be performed in the order listed; however, all steps should be considered and/or addressed:

1. Ensure that the objectives are set for equal magnification for both stages of the comparison microscope.
2. Adjust the illumination as needed. Oblique lighting is usually preferred.
3. Adopt a consistent procedure for comparing knowns to unknowns, e.g. tests on the left stage and evidence on the right stage, or vice versa.
4. If a firearm or a tool are included as evidence, consider comparing test-to-test to ensure reproducibility of microscopic characteristics.
5. Consider the possibility of subclass characteristics on tests or on the evidence.
6. Compare the unknown evidence to either another piece of unknown evidence or to a known standard.
7. The entire unknown should be considered.
8. If a conclusion cannot initially be reached, the analyst should consider the following factors:
    i. The angle of lights
    ii. The type of lights
    iii. The need for additional tests
    iv. The position of the test and/or evidence on the microscope

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v. The possibility of needing to clean the firearm

vi. The possibility that the firearm or tool itself has changed

9. Comparisons of evidence will be adequately documented to support the conclusions reached. This can be in the form of photographs, written notes, or a combination of both.
10. If an examination results in either an identification or an elimination based on individual characteristics, a second examiner must verify the findings. Refer to the steps outlined in FA SOP-24 Verifications.

E. References:

1. GL 2 Safety Manual
2. FA SOP-25 Range of Conclusions
3. FA SOP-24 Verifications

RETIRED

A. Purpose:

To describe the process of verifying an analyst's findings. If a comparison of firearm and/or toolmark evidence results in an identification or in an elimination based on individual characteristics, the findings must be verified by a second firearms examiner. He or she will conduct a separate, independent microscopic comparison of the evidence and document their findings. All serial number restorations and distance determination/gunshot residue analyses will also be verified by a second examiner. Legal length measurements that fall below the state and/or federal requirements will be verified by a second examiner.

Guidance is provided for the instances where examiner and verifier have conflicting opinions.

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B. Responsibility:

Forensic Science Examiners assigned to the Firearms Unit.

C. Procedure:

1. Firearm and toolmark evidence
    a. The analyst will transfer the items to be verified to the second analyst via JusticeTrax. Alternatively, the evidence may stay in the custody of the first analyst if the verification is being conducted in their presence. This will be so noted on the QR FA-5 Verification Worksheet.
    b. The verifier will conduct an independent microscopic comparison of the evidence.
        i. Their findings will be documented on the QR FA-5 Verification Worksheet.
        ii. Documentation may be in the form of written notes, photographs, or both.
    c. If the evidence was transferred via JusticeTrax, the second analyst will transfer the evidence back to the first analyst.
2. Serial number restoration and distance determination/gunshot residue
    a. The verifier will initial the appropriate area on the Serial Number Restoration Worksheet (QR FA-8) as results are observed.
    b. The verifier will initial and date the Notes Pages (DD SOP 1.1, QR FA-13, or QR FA-14) when verifying distance determination.
    c. Serial number and distance determination verifications may be conducted in the presence of the initial examiner. The evidence does not need to be transferred between examiners.
3. Legal lengths

a. In the event the barrel and/or overall length of a rifle or shotgun is below the state and/or federal requirements, a second examiner will verify the measurements and initial the appropriate area on the Weapons Worksheet (QR FA-2).

4. The verifier's documentation will be kept in the case jacket.

D. Conflict Resolution

1. If, during the verification process, the verifier disagrees with the examiner's findings, the two shall meet to discuss the differences. Additional examination of the evidence may be done if it assists in bringing a resolution to the differences.
2. If additional work is being performed, the appropriate documentation will be kept in the case jacket.
3. If the issue cannot be resolved, it will be brought to the attention of the unit supervisor/lead. The supervisor/lead will determine the appropriate resolution.
4. The supervisor/lead will inform the Deputy Director of Identification of the conflict and the proposed resolution. The Deputy Director will review the proposed resolution and approve or make additional suggestions if needed.
5. In the event the unit supervisor/lead is part of the conflict, the Deputy Director will determine the appropriate resolution and may discuss with other members of Administration.
6. The supervisor/lead who handled the issue will place a memo in the case jacket, indicating that a conflict occurred and was resolved. In the event the unit supervisor/lead is part of the conflict, the Deputy Director will author the memo. The memo shall contain the following:
   a. Names of examiners involved and each of their findings
   b. A description of the issue
   c. Final action
   d. Name of the supervisor/lead who resolved the conflict

E. References:

1. QR FA-5 Verification Worksheet
2. QR FA-13, QR FA-14 Notes Pages
3. DD SOP 1.1 Distance Determination Notes Page
4. QR FA-2 Weapons Worksheet

RETIRED

A. Purpose:

The following range of conclusions and definitions were developed by the Association of Firearm and Tool Mark Examiners (AFTE) to use as a guideline for reporting the results when conducting a microscopic comparison. The descriptions of such results in an examination report may be worded as the examiner sees fit.

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B. Responsibility:

Forensic Science Examiners assigned to the Firearms Unit.

C. Range of Conclusions:

1. Identification:

   Agreement of all discernible class characteristics and sufficient agreement of a combination of individual characteristics where the extent of agreement exceeds that which can occur in the comparison of toolmarks made by different tools and is consistent with the agreement demonstrated by toolmarks known to have been produced by the same tool.

   When an association is made the report shall indicate that the identification was made based on similar class characteristics and sufficient agreement of individual characteristics.

2. Inconclusive:

   i. Agreement of all discernible class characteristics and some agreement of individual characteristics, but insufficient for an identification.

   ii. Agreement of all discernible class characteristics without agreement or disagreement of individual characteristics due to an absence, insufficiency, or lack of reproducibility

   When an inclusive finding is made, the report shall include the reason for such result.

3. Elimination:

   Disagreement of discernible class characteristics and/or individual characteristics.

   When an elimination is made, the report should indicate the reason for this elimination.

4. Unsuitable:

   Unsuitable for examination.

D. References:

1. AFTE Glossary

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A. Purpose:

To describe the techniques that can be utilized to recover an obliterated serial number.

B. Responsibility:

Forensic Science Examiners or other employees assigned to the Unit.

C. Safety:

1. The analyst shall use appropriate PPE (eye protection, gloves, lab coat).
2. Serial number restorations shall be conducted in a fume hood.

D. Procedure:

- Operability testing shall be conducted prior to any serial number restoration attempts.
- The condition of the obliterated area(s) and any visible characters shall be documented in the notes by photographs and/or sketches prior to restoration attempts.
- The notes shall demonstrate the progression of the restoration attempt(s).
- During any stage of the restoration, a second analyst must verify the restored characters by initialing the appropriate space on the Serial Number Worksheet (QR FA-8, 8a).

1. Preparation
   a. Polishing
      i. Can be done with fine grit sand paper, emery cloth, or an electric polishing tool such as a Dremel.
      ii. Depending on the extent of the obliteration, continue polishing until the surface is mirror-like, removing the scratches. If the obliteration is severe, it may not be possible or desirable to remove all of the scratches.
      iii. Any characters that become visible shall be documented.
   b. If the serial number is not visible, or only a portion of the serial number is visible after polishing, chemical and/or magnetic processing may be conducted.
2. Magnetic Particle Inspection

Magnetic particle inspection is generally used on magnetic or ferrous metals, but can also be used on other types of metals. It is considered a non-destructive method of serial number restoration.

a. Spray the Magnaflux oil suspension (red or black) into a small cup or beaker.

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b. Place the poles of the magnet on either side of the obliteration. Placement may be adjusted as needed. Press the power button.

c. Using a disposable pipette, drip the oil suspension onto the area of obliteration.

d. Document any characters that become visible.

e. This method may be used alone or in conjunction with chemical reagents.

3. Chemical Processing

a. Determine the most appropriate reagent for the type of metal.

b. Using a cotton swab, apply the reagent to the area of obliteration.

c. Document any characters that become visible.

d. Polishing may be used in between applications of the reagent.

e. When complete, flush the area with water to neutralize the chemicals. Then dry the area and apply oil to inhibit oxidation.

4. Electrolytic Method

Applying voltage to the chemical reagent process speeds up the reaction between the acid and the metal.

a. Using an alligator clamp, attach the positive terminal of the low voltage power supply to the frame or receiver of the firearm.

b. Hold the negative terminal of the low voltage power supply to a cotton swab moistened with the desired reagent.

c. Turn on the power supply to low.

d. Swab the obliterated area, taking care to not touch the frame/receiver with the negative terminal on the cotton swab.

e. Document any characters that become visible.

f. Polishing may be used in between applications of the reagent.

g. When complete, flush the area with water to neutralize the chemicals. Then dry the area and apply oil to inhibit oxidation.

5. Reporting Results

a. Unrestorable characters shall be represented by a "?"

b. Characters which could be multiple possibilities shall be represented by a "*" with the possibilities listed. e.g. *The "*" represents a character that could be a 3 or an 8.*

6. QC Check

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a. Prior to using any chemical reagent, a quality control check shall be conducted and documented on the Serial Number Restoration Worksheet.

b. Two (2) pistol slides are located in the fume hood. One is a ferrous material, the other is non-ferrous. These will be used to perform the QC check.

c. Using a cotton swab moistened with the desired reagent, dab a small amount onto the appropriate slide.

d. The appearance of bubbles is a positive control which indicates that the reagents are working properly.

e. If the reagent does not bubble as expected, the reagent has failed the QC check. This should be documented in the Firearms Chemical Reagent Log.

f. The failing reagent should be disposed of properly and remade.

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E. Available Reagents

Reagents that are prepared in the laboratory shall be logged in the reagent log book. Refer to GL-2 Safety Manual for proper labeling.

In general, Fry's Reagent, Turner's Reagent, and Davis Reagent are used on ferrous materials. 25% Nitric Acid, Acidic Ferric Chloride, Ferric Chloride, 10% Sodium Hydroxide, Hubball Reagent, and Phosphoric/Nitric Acid are used on non-ferrous materials.

a. Fry's Reagent (for ferrous material)
   i. 90g cupric chloride ($CuCl_2$)
   ii. 100mL distilled water (dH_2O)
   iii. 120mL hydrochloric acid (HCl)

b. Turner's Reagent (for ferrous material)
   i. 2.5g cupric chloride ($CuCl_2$)
   ii. 25mL ethanol (C_2H_6O)
   iii. 30mL distilled water (dH_2O)
   iv. 40mL HCl (Hydrochloric acid)

c. Davis Reagent (for ferrous material)
   i. 5g cupric chloride ($CuCl_2$)
   ii. 50mL distilled water (dH_2O)

iii. 50mL HCL (Hydrochloric acid)

d. 25% Nitric Acid (for non-ferrous material)
   i. 75mL distilled water (dH_2O)
   ii. 25mL nitric acid (HNO_3)

e. Acidic Ferric Chloride (for non-ferrous material)
   i. 25g ferric chloride ($FeCl_3$)
   ii. 100mL distilled water (dH_2O)
   iii. 25mL Hydrochloric acid (HCl)

f. Ferric Chloride (for non-ferrous material)
   i. 25g ferric chloride ($FeCl_3$)
   ii. 100mL distilled water (dH_2O)

g. 10% Sodium Hydroxide (for non-ferrous material)
   i. 10g sodium hydroxide (NaOH)
   ii. 90mL distilled water (dH_2O)

h. Hubball Reagent (for non-ferrous material)
   i. 10g potassium dichromate ($K_2Cr_2O_7$)
   ii. 60mL distilled water (dH_2O)
   iii. 50mL C_2H_6O (Ethanol)
   iv. 80mL concentrated HCL (Hydrochloric Acid)

   Directions: Measure the powder on a balance contained in a hood. Add the powder to a glass bottle and add the dH_2O, mixing well to dissolve the powder. In small amounts, add in the Ethanol stirring slowly. Once this step is completed, begin to slowly add in the Hydrochloric Acid to this mixture. This mixture will have an exothermic reaction and will need to cool overnight.

i. Phosphoric/Nitric Acid (for non-ferrous material)
   i. 98mL 85% phosphoric acid (H_3PO_4)

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ii. 2mL concentrated nitric acid (HNO_3)

-or-

iii. 50mL concentrated phosphoric acid (H_3PO_4)

iv. 3mL concentrated nitric acid (HNO_3)

F. Equipment:

1. PPE
2. Fume hood
3. Polishing tools
4. Chemical reagents
5. Cotton swabs
6. MagnaFlux magnet and supplies
7. Low voltage direct current power supply

G. References:

1. GL 2 Safety Manual
2. FA SOP-02 General Firearms Safety
3. ATF Serial Number Structure Guide

RETIRED

A. Purpose:

Before they can be destroyed, all handguns submitted to the Connecticut State Police (CSP) Special Licensing and Firearms Unit (SLFU) must be test fired by the Firearm and Toolmark Unit.

B. Responsibility:

It is the responsibility of the examiner assigned to this task to retrieve the handguns from the SLFU. These firearms must be test fired, and if the caliber is suitable, entered into the National Integrated Ballistic Information Network (NIBIN) database.

C. Safety:

1. Personal protective devices, such as hearing and eye protection, will be used during test firing.
2. Additional personal protective devices will be worn when examining and test firing firearms that have potentially come into contact with bio-hazardous materials.

D. Procedure:

1. The firearms received from the SLFU will be checked by the examiner against the document presented by the SLFU to ensure that all the firearms are present in the container.
2. The firearms will then be checked to determine if any have been previously test fired in the laboratory during normal casework. Those firearms will not be further tested. A red band placed around the trigger guard of the handgun will indicate that it has previously been test fired in the laboratory.
3. The remaining firearms that have not previously test fired during normal casework will be screened to determine if they are safe to handle and test fire.
4. Firearms which are not determined to be safe will not be test fired.
5. Handguns determined to be in a safe and operable condition will be test fired twice using laboratory ammunition.
6. The two tested fired bullets and cartridge cases will be placed in a coin envelope with the following pertinent information on the face of the envelope: caliber, type, make, model, serial number, DES # (SLFU identifying number) and examiner.
7. If the test fires are from a suitable caliber for NIBIN entry, a cartridge case will be entered into the database for correlation.

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8. No further report writing is necessary for the handling of SLFU test fires, unless a preliminary hit is made in the NIBIN database. In that instance, a NIBIN preliminary hit memo will be sent to the associated agency(ies).
    a. In the rare event in which the associated agency requests confirmation of the preliminary NIBIN hit, the test fires will become evidence and will be handled like a normal case.
9. The test fires will be maintained in the sealed envelope and stored by caliber and year of testing with the Firearm and Toolmark Unit of the Laboratory.
10. The firearms will be returned to the SLFU in the container in which they were received by the examiner assigned this duty.
11. A copy of the attached handguns submitted to the lab will be kept on file with the Firearm and Toolmark Unit of the laboratory.

E. Equipment:

1. PPE
2. Test firing equipment
3. NIBIN BrassTrax HD3D acquisition unit

F. References:

1. GL-2 Safety Manual
2. FA SOP-02 General Firearms Safety
3. FA SOP-08 Test Firing for Operability
4. FA SOP-09 Test Firing Equipment
5. FA SOP-22 NIBIN

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A. Purpose:

To establish an internal quality control check of the NIBIN (National Integrated Ballistic Information Network) database, the BrassTrax 3DHD acquisition station, and the analysts in the Firearm and Toolmark Unit. On a monthly basis two issues must be addressed: 1) is the cartridge case acquisition and correlation software performing adequately, and 2) are the analysts properly acquiring fired cartridge cases in accordance with Forensic Technology, Inc. best practices. This quality check shall also be completed when the hardware or software is upgraded by Forensic Technology, Inc. (FTI).

B. Responsibility:

The assigned analyst is responsible for entering the standard cartridge case specimen into the NIBIN database by the 5th day of each month. The analyst is also responsible for reviewing, printing, and filing the correlation results and case information page in the NIBIN Monthly Control Log. In the event of a hardware/software upgrade, this check is to be done as soon as possible following the completion of the upgrade.

C. Procedure:

1. Following the procedure outlined in FA SOP-22 NIBIN, create a Case ID.
   a. Case Number: Bench Test 1/1/2015 (insert date of monthly check)
   b. Originating Agency: CT-SP Forensic Science Lab Meriden
   c. Agency Contact: Analyst conducting monthly check
   d. Event Type: Other
   e. Supervisor: Analyst conducting monthly check
   f. Occurrence Date: date of monthly check
2. Following the procedure outlined in FA SOP-22 NIBIN, create an Exhibit.
   a. Exhibit Number: 001 or 1
   b. Caliber: 9mm
   c. Make: Remington
   d. Firing Pin Shape: Circle
   e. Composition: Nickel-Nickel
   f. Category: Crime Evidence

3. Acquire images of the standard cartridge case; then Submit and Synchronize.
4. Using the Rank Sort feature, the analyst will review the Top 20 results and ensure that previous entries entitled "Bench Test xx/xx/xx" are within the Top 20.
    a. If no previous Bench Test entries are within the Top 20, this is considered out of acceptable range, and the Unit supervisor should be notified.
5. Print the images of the entry and the Correlation Results, initial and date the pages, and store them in the NIBIN Monthly Control Log.
    a. At the end of a calendar year, the printed images and correlation results will be scanned and saved on a network drive that is regularly backed up.
6. Delete the correlation once it has been reviewed.
7. In order to minimize the number of Bench Tests circulating in the database, the following steps shall be taken.
    a. After the start of a new calendar year, the monthly entries of two years prior shall be deleted from the database, with the exception of January of that year.
    b. For example: In 2019, the entries of February through December 2017 will be deleted. New monthly entries for 2019 will correlate against all entries for 2018 and the January entries for January 2013 through 2017.

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D. Equipment:

1. Standard cartridge case specimen (located in NIBIN room)
2. BrassTrax 3DHD acquisition station
3. MatchPoint+ viewing station

E. References:

1. FA SOP-22 NIBIN
2. BrassTrax users guide (current edition)
3. MatchPoint+ users guide (current edition)

RETIRED

A. Purpose:

The Reference Collection serves the Firearms Unit in several ways. For training new examiners, it is a tool to demonstrate how various types of firearms operate. Those in training have the opportunity to disassemble and reassemble many types of firearms to gain a detailed understanding of how the internal parts work together to make the firearm operate.

For casework, the Reference Collection serves as a parts library to assist in rendering an inoperable evidence firearm operational for test firing. It also functions as a serial number structure reference for the restoration of obliterated characters on evidence firearms.

B. Responsibility:

Forensic Science Examiners and other laboratory personnel assigned to the Firearms Unit.

C. Safety:

1. Firearms in the Reference Collection will be stored in an unloaded condition.
2. No live ammunition is to be brought into the Reference Collection.
3. Only authorized laboratory personnel may enter the Reference Collection.

D. Procedure:

1. Adding firearms to the Reference Collection
    a. In conjunction with the Weapons Destruction Unit (WDU) of the Special Licensing and Firearms Unit (SLFU) of the Connecticut State Police, the laboratory has been granted the right to request that any firearm slated for destruction be maintained in the reference collection.
    b. It is the responsibility of the Unit supervisor or designee to ensure that firearms added to the collection are properly logged into the Reference Collection database, securely stored on the laboratory T-drive.
    c. Firearms will be tagged with a plastic band, marked with the Reference Collection database record number and storage location.
2. Removing firearms from the Reference Collection
    a. A firearm may be temporarily checked out of the Reference Collection for use in casework by tagging its location with a white tube. It should be checked back in as soon as possible.

b. If a firearms is permanently removed from the Reference Collection, it is the responsibility of the Unit supervisor or designee to update the Reference Collection database with the date of removal, and to arrange its transportation to the WDU for destruction.

3. The Reference Collection will be audited yearly by a Firearms Unit designee and a member of another laboratory unit.

E. References:

1. FA SOP-02 General Firearms Safety
2. GL 2 Safety Manual
3. Firearms Reference Collection database

RETIRED

A. Purpose:

To ensure that all standard methods and equipment employed in the Firearms Unit are suitable for the customer's request(s).

B. Responsibility:

Forensic Science Examiners or other laboratory personnel assigned to the Firearms Unit.

C. Procedure:

1. All methods and equipment used to fulfill customer requests shall be deemed suitable by the Unit supervisor/lead and approved by the Director or his/her designee. This includes the range and accuracy of the results obtained being relevant to the customer's needs. All methods are based on standard methods described and developed by professional organizations – eg. Association of Firearm and Tool Mark Examiners (AFTE), Scientific Working Group for Firearms and Tool Marks (SWG-GUN), Organization of Scientific Area Committees (OSAC) Subcommittee on Firearms and Toolmarks.

2. Before a new method or new piece of equipment is introduced, it must be validated according to the appropriate parameters. A validation plan shall be developed and approved by the Unit supervisor/lead and the Deputy Director of Identification. All documentation of the new method and the validation plan shall be stored in the Unit's Validation Plans binder, which is kept in the Firearms Unit main work area. Also refer to GL 22. Where applicable, the reliability of the new method shall be compared to published results and become part of the validation file.

3. Any changes to an existing method must be validated as above.

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D. References:

1. Firearms Unit Validation Plan binder
2. GL 22 Policy on Validation and Performance Checks

IM SOP-16 Electronic Submission of Digital Images

Purpose: To allow for the electronic submission of digital images

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate.

Procedure:

Digital images of imprints/impressions, shoes and tires are often utilized in casework. Sometimes these images are emailed to an examiner for review for use in casework. In some circumstances, an investigator may submit an image to inquire if it is suitable for analysis. If the examiner determines that the electronic image is suitable, the examiner may suggest to the investigator that the case can be submitted electronically. In these instances the following procedure will be followed:

1. The agency requesting to submit digital images will first contact the Imprint Unit and Case Management Unit (ct.forensiclab@ct.gov). The email should contain the agency name, requestor, agency case number and contact information.

2. If the request is accepted, the submitting agency will be notified to email an electronically completed DSS Request for Analysis Form (SOP-ER-02:1) along with the attached digital images.

3. Upon receipt of the completed request form and digital images, the digital images will be scanned for viruses and then a disc (CD or DVD) will be made.

4. The case will be entered into JusticeTrax by the Evidence Receiving Unit.

5. Once submitted as a case, the images may now be reviewed and analyzed as a typical case. (See IM SOP-3 Evidence Procedures)

Sources of Error: N/A
Quality Assurance: N/A
References: See bibliography.

RETIRED

Purpose: Introduction

The procedures presented in this technical manual are designed to serve as a basic foundation for conducting imprint examinations. The methodologies and procedures contained in this manual are not all encompassing. The wide variety of imprint problems, which can be encountered by the imprint examiner, precludes using this manual as a "cookbook" for examination purposes. Certain cases may require divergence from, and/or adaptations to these written procedures (See GL-20).

The competent Imprint Examiner must also rely on his or her experience and training, reference materials available in various scientific and technical papers, publications and texts, online resources, consultations with experts in a specific field and/or new developments within the imprint examination field.

The exact procedures(s) employed by an imprints examiner is dependent upon the evidence submitted and the type(s) of examinations requested. All non-standard methods will be validated prior to being implemented. Deviations from standard methods must be approved by the Deputy Director or designee prior to proceeding and documentation maintained in the case record.

RETIRED

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: N/A

Procedure: N/A

Sources of Error: N/A

Quality Assurance: N/A

References: See bibliography.

Purpose: The following is a list of specialized instrumentation, tools, equipment and materials maintained at the Division of Scientific Services which are commonly used by the examiner(s) conducting routine imprint examinations.

A. Optical Enhancement Devices

1. Hand-held magnifiers (various magnification powers)

2. Stand [fingerprint] magnifiers (various magnification powers)

3. Microscopes (variable magnification powers)

B. Alternate Light and Filtering Equipment/Devices

1. Video Spectral Comparator (VSC-4 and VSC-8000)

a. Ultraviolet light source with various filters

b. Infra-red light source with various filters

c. Transmitted light source

d. Side light source

SPECIAL NOTE: Calibration(s) and/or repairs to the VSC-4 and VSC-8000 will be performed by the manufacturer/factory representative(s). Proper documentation referencing the calibration(s) and/or repairs will be maintained by the Imprints/Questioned Documents Unit.

C. Photographic/Duplication Equipment

1. Digital camera(s) and accessories

2. Tripod(s)

3. Photographic Copy Stand

4. Scanner(s)

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5. Color/black and white printers

6. Color/black and white photocopiers

D. Electrostatic Dust Print Lifter

E. Electrostatic Detection Apparatus (ESDA)

F. Miscellaneous Tools

1. Protractors

2. Calipers

3. Scales/rulers

4. Angle finder

G. Miscellaneous Materials

1. Casting material

2. BIO-FOAM™

3. Fingerprint powders

4. Fingerprint ink

5. Inkless imprint kit

6. Display board/butcher paper

7. Adhesive lifters

8. Electrostatic lift film

9. Gelatin lifters (white, black, and transparent)

10. Transparencies

RETIRED

11. Spray paint/snow print wax

12. Fixative spray/hair spray

NOTE: Operation manuals for instrumentation, equipment and tools maintained by the Imprint Unit will be kept on file within the Unit.

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate.

Procedure:

A. Purchasing of Supplies

1. Supplies will be purchased from approved vendors through section supervisory personnel and administrative personnel.

2. Upon receipt of the item, a visual inspection of the packaging will be performed to ensure that the integrity of the product is intact and the correct item has been received.

3. Prior to the use of a supply item or consumable, a visual inspection will be performed.

4. The examiner will use their discretion based on their training and experience to determine if a product is suitable to use. A control test will be performed on all reagents prior to use in casework. A record of the control test will be maintained in the case notes or in other documentation within the case file.

5. If a product is unsuitable for use, the product will be discarded and another product unit will be used.

6. Supplies and consumables will be stored in a clean, dry environment or an environment suggested by the manufacturer.

7. All records of purchases are maintained in the purchasing files located in the Administration area. Packing lists are initialed and dated upon receiving items and returned to Administration.

Sources of Error: N/A

RETIRED

Quality Assurance: N/A

References: See bibliography.

RETIRED

Purpose: Evidence Procedures

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate.

Procedure:

A. Taking Custody of Evidence

1. Review the Submitting Agency's request for analysis and the Laboratory's evidence receipt paperwork and the evidence containers (packages) at the Evidence Receiving Office. Verify that the number and description of items listed on both the forms, for examination by the Imprints Unit, are present and accounted for.

2. Review the Submitting Agency's request for analysis and/or any other form of request such as a letter of request or accompanying documents (eg. search warrants) and photographs.

    a. Determine the type(s) of examination(s) requested and if they might require special handling of evidence.

    b. Determine if evidence should be reviewed or examined by another Unit of the Laboratory prior to being assigned to the Imprints Unit (i.e. trace, forensic biology, etc.).

3. If problem exists with evidence (i.e. all evidence listed as being submitted to Laboratory is not accounted for) or if evidence should be assigned to another Division Unit prior to the Imprints Unit, advise Case Management personnel of the situation.

4. Take custody of the evidence and obtain copies of all paperwork.

B. Secondary Receipt Procedures

1. Verify that the DSS barcode information, including case number, is consistent with the information on the evidence packaging and the request for analysis. This is documented on the worksheet QR-IM1.

2. Open each container and/or package containing evidence and take physical inventory of evidence contained therein.

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a. Write initials on laboratory barcode.

b. If evidence is to be subsequently processed by another Unit of the Division (i.e. Latent Prints Unit), ensure proper procedures are followed so as not to destroy or contaminate evidence.

c. After opening a container or package, appropriately identify and initial each piece of evidence received.

d. If any evidence listed on "Evidence Receipt" form is not received (i.e. receipt lists "One taped envelope containing four (4) impression lifts"; however, when the envelope is opened it is found to contain only three (3) lifts):

- Notify Case Management Unit of the discrepancy.
- Contact submitting agency and advise them of the discrepancy. Request clarification and/or correction concerning the discrepancy. (This may be done by the Case Management Unit.)
- Make note for file, and/or in LIMS, including date, time, person spoken to, etc. relative to explanation of missing evidence by submitting agency. (This may be done by the Case Management Unit.)
- If discrepancy cannot be corrected, reseal evidence in its original container(s) and/or package(s) and return it to the Evidence Receiving Unit for return to the submitting agency.

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3. Initiate Imprints Unit case file on new case.

a. Write Laboratory's case number, town of origin and submitting agency's case number on tab of a file folder or place JusticeTrax label on tab of file folder.

b. Initial and date copies of all paperwork and place it into the file folder.

c. Initiate filling out an "Imprints/Impressions Examination Worksheet" (QR-IM1). This worksheet and any additional notes will contain the case number, examiner's initials and date. If the analysis takes more than one day, the worksheet will reflect all dates analysis was conducted.

d. Any clerical errors that are made on any items of written documentation will be stricken through with a single line and initialed.

C. Documentation of Evidence

1. Generate an image of the "Evidence" labels, stickers or markings on evidence package(s) and/or container(s) which will serve as a documentation of the contents. Initial the image and place it in the case file.

2. Photographs of evidence will be taken as deemed necessary and appropriate by the examiner.

D. Securing and Control of Evidence

All imprint evidence will be properly secured in the approved evidence storage cabinets or areas. Evidence may be left unattended for a short period of time during the examination process only when it is secured in a locked approved examination area. At no time will any evidence submitted for examination be left unsecured and/or unattended outside of the Imprints Laboratory or evidence storage areas.

Sources of Error: N/A

Quality Assurance: N/A

References: See bibliography.

RETIRED

Purpose: To document evidence using photography.

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate.

Procedure:

RETIRED

Materials:

1. Camera
2. Memory card
3. Tripod or copy stand
4. Light source/flash
5. Scale/ruler
6. Angle finder

Procedures:

1. If the item of evidence contains an impression first examine the item of evidence to determine the location(s) of interest.
2. Determine the best type of lighting to be used (direct, reflective, oblique, transmitted, etc.).
3. Determine if tripod or copy stand should be used. Set up appropriate apparatus. Ensure that camera is parallel with the item of evidence or the imprint/impression.
4. Place appropriate scale adjacent to and at same depth as the item of evidence or imprint/impression.
5. Take photographs filling the frame of the camera view finder with the item of evidence or imprint/impression.
6. Enhance the evidence or imprint/impression with a light source at the appropriate angle for maximum detail.

7. Take several photographs with the light source at different angles.

8. Digital photographs are downloaded to the examiner's computer. A printed copy of the photographs is included in the case file. The date that the photographs were generated will be on all pages of the printed copies. Photographs of impressions that cannot be lifted or otherwise collected will be saved to a CD or DVD. The CD or DVD will be packaged in an appropriate evidence container, tape sealed, sub-itemized in JusticeTrax, barcoded, and returned to the submitting agency along with the original evidence.

   If a disk is generated containing the working files including digital images, this disk will be treated as evidence. The disk will be sub-itemized in JusticeTrax and retained in a secure storage location.

9. After downloading, have selected photographs enlarged to natural size (1:1) for comparison purposes, as applicable.

Results:

A permanent record of the item of evidence is obtained for comparison purposes.

Sources of Error: N/A

Quality Assurance: N/A

References: See Bibliography.

RETIRED

Purpose: To recover imprints in dust or residue from suspect items.

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate. Precautions should be taken when using the Electrostatic Lifting Device and the Electrostatic Detection Apparatus due to the potential for electrical shock. The examiner should avoid touching the metal electrical contacts on the Electrostatic Lifting Device and the corona wire on the Electrostatic Detection Apparatus when the devices are in use.

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Procedure:

Materials:

1. Electrostatic lifting device
2. Grounding material
3. Lifting film
4. Oblique lighting source
5. Large transparent adhesive lifters
6. Large white backed adhesive lifters
7. Large black backed adhesive lifters
8. Large transparent gelatin lifters
9. Large white backed gelatin lifters
10. Large black backed gelatin lifters
11. Electrostatic Detection Apparatus (ESDA)

Procedures:

1. Examine area using an oblique light source to identify location(s) of any visible dust or residue imprint(s).

2. Determine which area(s) are to be processed with an electrostatic lifting device or adhesive/gel lifters.

3. Photograph area(s) where lifts are to be conducted.

4. When using the electrostatic lifting device, place lifting film – black side down – on top of surface where imprint(s) are to be lifted, set up appropriate ground device, and continue with electrostatic lift procedure(s) following the instructions in the manual.

5. When using adhesive or gel lifters, remove the cover from the lifter, place the lifter over the imprint, use your hands or a roller to ensure that the lifter contacts the entire imprint, and remove lifter. After photographing the lift place the cover back over the imprint.

6. When using the Electrostatic Detection Apparatus (ESDA) follow the SOP for the Questioned Documents Unit regarding the proper use of the ESDA.

7. Photograph lifted images.

8. Secure lifts in proper container.

9. Mark container with appropriate identification markings, including case number, item number and description.

10. Sub-itemize the lift in JusticeTrax and place a barcode on the evidence container. Lifts will be returned to the submitting agency.

RETIRED

Results:

Permanent record of dust or residue imprint(s).

Sources of Error: N/A

Quality Assurance: N/A

References: See bibliography.

Purpose: To obtain a three dimensional record of an impression.

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate. SDS on file.

Procedure:

RETIRED

Materials:

1. Dental stone casting material
2. Water
3. Plastic zip lock bag/mixing container
4. Scale/ruler
5. Frame material (optional)
6. Fixatives (optional)
    - Hairspray
    - Spray (clear) shellac or lacquer
7. Highlighting materials (optional)
    - Primer spray paint

Procedures:

1. Photograph all impressions prior to casting (see IM SOP-4 Photography).
2. Prepare impression for casting.
    - If necessary, use appropriate fixatives.

- If necessary, use appropriate highlighting materials.
- If appropriate place framing material around impression.

3. Mix dental stone material and water to desired consistency.
4. Pour prepared dental stone mix into and over impression.
5. Before cast hardens, scratch appropriate identifying information on the backside of cast (i.e. initials, date, case number, direction, etc.) or write identifying information on the backside of cast with permanent marker after hardening.
6. After cast hardens, remove from casting location. The cast may be photographed (see IM SOP-4 Photography).
7. After 48 hours the cast may be cleaned and re-photographed (see IM SOP-4 Photography).
8. Secure cast in proper container.
9. Mark container with appropriate identification markings, including case number, item number and description.
10. Sub-itemize the cast in Justice Trax using guidance from GL-4 and place a barcode on the evidence container. Casts will be returned to the submitting agency.

RETIRED

Results:

Three-dimensional reproduction of impression.

Quality Control:
When a chemical/reagent is utilized for the first time, the examiner opening/preparing the chemical or reagent will ensure that the container is properly labeled as to its contents, its safety information (NFPA or GHS) and the ability of the performance of the chemical/reagent (see section a. below). The examiner will also place the date opened/prepared and his/her initials on the container. Most chemicals/reagents are purchased as packaged kits.

Per GL-21 General Laboratory Equipment, chemicals/reagents are considered “equipment” as they can influence the correct performance of laboratory activities (i.e. creating test impressions or enhancement). The very first control test impression developed with a newly opened or prepared chemical/reagent will demonstrate the ability of the performance of that lot of chemical/reagent. A log will be kept of this control test impression. The log will include the chemical/reagent name, the date opened/prepared, the lot number, the initials of the analyst who opened/prepared it, if the control test

impression was acceptable and the manufacturer's expiration date, if there is one. This information will be logged on the Reagent Log form (QR-IM Reagents/Chemicals). Once the chemical/reagent has been found to be acceptable, a colored dot will be initialed and dated and "OK" or "OK for use" will be written on it. This dot will be taped to the chemical/reagent bottle and will signify the chemical/reagent is okay to use.

Before any chemical/reagent is used, every analyst should check for this sticker. If it is not present, the above stated procedure must be followed.

The dental stone used in this procedure is considered a one-time use product and historically will work by hardening with the addition of water. The original evidence is not affected by this product since the dental stone does not come into contact with the submitted tire or footwear. It is suggested that this product be purchased in a quantity that has the same lot if possible. One of these bags from the lot will be tested and then the lot will be considered acceptable for casework if it passes the quality control test. The quality control test should be conducted by using an item to create an impression in BioFoam or other similar substrate, and following the same procedure as creating a cast. The quality control for this product is considered acceptable if the dental stone product hardens creating a cast of the object used to create the impression.

Sources of Error: N/A

Quality Assurance: N/A

References: See Bibliography.

RETIRED

Purpose: To detect and enhance latent or patent imprints using fingerprint powders.

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate.

Procedure:

Materials:

1. Contrasting color fingerprint powders
2. Fingerprint powder brush
3. Large transparent adhesive lifters
4. Large white backed adhesive lifters
5. Large black backed adhesive lifters
6. Large transparent gelatin lifters
7. Large white backed gelatin lifters
8. Large black backed gelatin lifters
9. Oblique lighting source

RETIRED

Procedure:

1. Physical examination of item to identify area(s) where suspect imprint(s) are located.
2. Use of oblique lighting of item to identify area(s) where suspect imprint(s) are located.
3. Photograph the imprint(s) using the procedures outlined in IM SOP-4 Photography.
4. Apply powder to suspect imprint(s) to develop detail.
5. Remove excess powder surrounding imprint(s).

6. Re-photograph imprint(s) using the procedures outlined in IM SOP-4 Photography.

7. Using appropriate lift product, lift imprint(s).

8. The lift(s) should be photographed (see IM SOP-4 Photography).

9. Secure lift(s) in proper container.

10. Mark container with appropriate identification markings, including case number, item number and description.

11. Sub-itemize the lift(s) in JusticeTrax and place a barcode on the evidence container. Lifts will be returned to the submitting agency.

Quality Assurance:
When a chemical/reagent is utilized for the first time, the examiner opening/preparing the chemical or reagent will ensure that the container is properly labeled as to its contents, its safety information (NFPA or GHS) and the ability of the performance of the chemical/reagent (see section a. below). The examiner will also place the date opened/prepared and his/her initials on the container. Most chemicals/reagents are purchased as packaged kits.

RETIRED

Per GL-21 General Laboratory Equipment, chemicals/reagents are considered “equipment” as they can influence the correct performance of laboratory activities (i.e. creating test impressions or enhancement). The very first control test impression developed with a newly opened or prepared chemical/reagent will demonstrate the ability of the performance of that lot of chemical/reagent. A log will be kept of this control test impression. The log will include the chemical/reagent name, the date opened/prepared, the lot number, the initials of the analyst who opened/prepared it, if the control test

impression was acceptable and the manufacturer's expiration date, if there is one. This information will be logged on the Reagent Log form (QR-IM Reagents/Chemicals). Once the chemical/reagent has been found to be acceptable, a colored dot will be initialed and dated and "OK" or "OK for use" will be written on it. This dot will be taped to the chemical/reagent bottle and will signify the chemical/reagent is okay to use.

Before any chemical/reagent is used, every analyst should check for this sticker. If it is not present, the above stated procedure must be followed.

RETIRED

Magnetic Powder Quality Control:

Quality Control for powders consists of minimizing contamination and exposure to moisture. The only quality control check on powders will be conducted on the initial opening of the stock container. To perform the quality control test, the new lot of magnetic powder will be applied to a rubber stamp. The stamp will then be pressed against a gel lift. If the impression of the stamp made in powder is visible, this result is considered acceptable and this lot of powder may be used for casework.

For casework, a small amount of powder should be removed from the stock or working container for each use. Powder removed for use should NEVER be added back to a container. Excessive moisture may cause clumping of the powder. Do not expose powders to high humidity or moisture. Keep all containers closed as much as possible.

The date the original stock container is opened shall become the lot number of the powder. The lot number will be in the following six digit month, day, year format: mmddyy (i.e. 010115). Smaller working containers of powder may be made from the stock container. Any working container will be labelled with the name and lot number of the stock container.. The shelf life of development powders is indeterminable; however, if clumping of the powder is observed, it shall be discarded.

RETIRED

Sources of Error: N/A

References: See bibliography.

Purpose: To detect and enhance latent or patent imprint/impressions using various chemical processes.

Responsibility: Forensic Science Examiners assigned to the Imprints Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate. Fume hoods should be utilized whenever possible.

Procedure:

RETIRED

Special Note:

All chemical enhancement processing will be coordinated and conducted with the assistance of the Latent Print and/or other Unit(s) of the Laboratory.

Procedure:

1. Review information provided by submitting agency and conduct preliminary examination of items to determine if chemical enhancement processing is necessary.

2. Photograph all items prior to processing. (See IM SOP-4 Photography.)

3. All chemicals and reagents will be stored and kept in appropriate containers. Containers will be properly labeled as to the contents, date of preparation, and expiration date, as applicable. Chemicals and reagents that have expired or have passed their shelf-life will be discarded.

4. The selection of the chemical or reagent is at the discretion of the examiner and may be based on several factors, including the material from which the imprint is made, the color of the substrate, the background reactivity of the substrate and the amount of blood, dirt or other material in the imprint. It is ultimately the responsibility of the examiner to determine the best possible application or sequential applications of various techniques based on previous experience, training and type of evidence being examined.

5. Before a chemical/reagent can be used on any item of evidence, it shall be tested to assure that its shelf-life has not passed and that expected results will be achieved. The substrate shall also be tested for possible background interference. The results of the test(s) shall be recorded in the examiner's notes and in any log book as applicable. Any chemical/reagent that does not produce a positive result of an acceptable strength shall be discarded. A new supply source shall be tested and utilized only if positive results are obtained.

6. Chemicals/reagents and powders utilized by the Latent Prints Unit are approved for use by the Imprints Unit. Whenever possible premixed solutions or premixed kits will be purchased. When an examiner needs guidance as to the application of development techniques or the preparation of chemicals not purchased as premixed, the "Guidelines for the Application of Powders, Chemicals and Reagents" in LP SOP-04 Appendix 2 should be referenced. If a chemical/reagent will be made at the Laboratory, the examiner should refer to the appropriate "formula" information in LP SOP-04 Appendix 1.

7. Any new reagents or chemicals not listed in this SOP or LP SOP-04 will first be validated after conferring with the Deputy Director of Identification and/or the Quality Manager.

8. In addition to the chemicals and reagents in LP SOP-04, the following reagent is also approved for use by the Imprints Unit.

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Potassium Thiocyanate

Iron will react with thiocyanate ions in an acid solution. If iron is present in the residue of an imprint, a positive reaction will produce a reddish-brown color. Iron is found in some soils. The procedure works well for wet residue and muddy impressions. A premixed solution can be used or the potassium thiocyanate can be prepared according to the following formula:

Potassium Thiocyanate:

15 g potassium thiocyanate
120 mL acetone
15 mL distilled water
8.5 mL sulfuric acid (dilute)

To 120 mL of acetone and 15 mL of water add 15 g of potassium thiocyanate. Then add 8.5 mL of dilute sulfuric acid. Make sure that you add the sulfuric acid to the acetone/water mixture. A milky mixture will result, which, on standing, will separate into two layers. When the layers have separated, remove the top layer, which is clear. This is the solution to be sprayed on the impressions. The reagent can be stored for three months in a cool dry place. This reagent is light sensitive and should be stored in a dark bottle.

Procedure of Application

1. Spray the impression with the potassium thiocyanate reagent using a fine spray in light successive coatings until satisfactory development is obtained. Be careful to not over

spray, as running of the reagent can occur.

Before application of the reagent on the evidence, a control test will be run to determine if the reagent is performing properly and that the expected results will be achieved. An imprint on paper, or on another surface as applicable, will be made with a soil sample known to contain iron. The reagent will be applied to this test imprint and the results of the test shall be recorded in the examiner's notes and in any log book as applicable. Any chemical/reagent that does not produce a positive result of an acceptable strength shall be discarded. A new supply source shall be tested and utilized only if positive results are obtained.

9. The evidence and any imprint(s) developed should be photographed after enhancement. (See IM SOP-4 Photography.)

Results:

Dependent on results of chemical enhancement processing.

Sources of Error: N/A

Quality Assurance: N/A

References: See bibliography.

RETIRED

Purpose: To enhance digital images for comparison purposes.

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate.

Procedure:

A. Enhancement of Images

1. All images received by the Imprints Unit as evidence will be copied onto the hard drive of the examiner's computer. All CD's, DVD's and other media submitted to the laboratory will be scanned for viruses using proper security software before files are downloaded to the examiner's computer.

2. Image enhancement will only be performed on a copy file, which has been made from the original file. The original image is never touched.

3. The history of any enhancement is maintained for each image that is enhanced, either within the software program used, the case notes, or other appropriate documentation. Available software programs include Adobe Photoshop, Corel PhotoPaint and Corel Draw.

4. Adjustments and enhancements using the following filters/applications may be applied: levels, curves, deinterlace, channel mixer, reduce noise, sharpen/unsharpen, sharpen more and invert, desaturate, remove moiré, crop/stitch and resampling, difference/averaging filters.

   The following descriptions are filters and other applications that may be used in the examination and enhancement of digital image files. However, this is not an all-inclusive list of tools that may be used.

   Blur: This effect softens the edges or reduces detail in an image.
   Brightness & Contrast: This effect adjusts the brightness and contrast of the color tones located in the image.
   Channel Mixer: Adjustment of color in an image by choosing a RGB (red, green, blue) channel to adjust the intensity and saturation of that hue in an image.
   Deblur (lens and motion): This filter attempts to correct the pixel alignment to adjust a blurred image due to the lens focus being incorrect or the motion of an object in the image.
   Desaturate: Desaturation is the adjustment of the red, green and blue channels towards the gray spectrum. Creates a black and white in the RGB mode.
   Difference/Averaging Filter: Traditional photographic technique used to enhance the detail of

RETIRED

an image by reducing the amount of noise or static. Combines two or more exposures of the same
scene to bring out detail and obtain a richer and more vivid photograph.
Dodge/Burn: Traditional photographic technique to light or darken specific areas of a print by regulating the exposure.
Invert: This effect inverts the color information of an image. This technique may be used to bring out patterns or details from an image.
Levels/Curves: The levels effect adjusts the brightness/contrast of a clip. It combines the functions of the color balance, gamma correction, brightness, contrast and invert effects.
Lucis Pro®: Photographic filter that enhances images by adjusting high, medium and low contrast
areas and corrects exposure levels.
Resampling: the addition or removal of pixels to prevent the loss of image details during the enlargement of an image.
Reduce Noise: Image noise appears as random extraneous pixels that are not part of the image detail. Image noise can appear in two forms: luminance (grayscale) noise that makes an image appear grainy and color noise that appears as colored artifacts. The filter reduces luminance noise
through adjustment of the blue channel. Color noise is reduced by the filter by adjusting the color channels.
Reduce Moiré: Moiré is patterns caused by interference between two sets of grids. These are commonly encountered in scanned images and in images with fine linear patterns. The filter adjusts the horizontal and vertical lineup of the pixels and softens the edges of the pixels.
Scale or Magnify: The ability to zoom in or zoom out of an image.
Sharpen/Sharpen more filters: Improves image clarity and emphasizes small details and sharp contrasts.
Shadow/Highlight: Photographic technique that adjust the exposure levels in highlighted or shadowed area of an image.
Stiching: Allows the creation of photos with higher resolution or wider view. This stitching provided a seamless joining of multiple digital images.
Unsharpen: Filter applied to soften the pixel edges.

5. The examiner will never use tools that can clone, copy or add pixels to the same image.

6. All steps of the digital processing/enhancement will be recorded in the case file.

Sources of Error: N/A
Quality Assurance: N/A

References: See bibliography.

RETIRED

Purpose: To obtain test impressions of patterns present on suspect item(s) for inter-comparison purposes with questioned imprint/impression.

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate.

Procedure:

RETIRED

Materials:

1. Fingerprint or printer's ink
2. Fingerprint powders/brushes/magnetic powder applicator
3. Backing board
4. Large transparent adhesive acetate lifters
5. Large white backed adhesive acetate lifters
6. Large black backed adhesive acetate lifters
7. Large transparent cover sheets
8. Large transparent gelatin lifters
9. Large white backed gelatin lifters
10. Large black backed gelatin lifters
11. BIO-FOAM®
12. MicroSil
13. Bubber
14. Inkless pad

15. Chart board/foam board

16. Petroleum jelly/clear shoe polish

17. Fixative Spray

18. Other, as needed

Procedure:

1. Conduct preliminary physical and, if necessary, microscopic review of evidence to determine appropriate test impression process to employ.

2. Photograph all items prior to processing.

3. Two-dimensional test impressions may be made by applying a thin layer of ink or fingerprint powder medium on the item (shoe outsole, tire, etc.) and transferring an imprint of that item onto an appropriate receiving medium (adhesive acetate lifter, gel lifter, foam board, etc.). Alternatively, a layer of petroleum jelly, clear shoe polish, etc. can be applied to the item and transferred to an appropriate receiving medium (foam board, paper, etc.). This clear imprint can be enhanced using fingerprint powders to make a test impression. Test impressions of tires should be made with the tire on the vehicle if possible.

   Test impressions of evidence that may be processed for DNA or may contain blood or other body fluids can be made using a magnetic powder applicator and magnetic fingerprint powder. The magnetic powder applicator can be cleaned before and after use utilizing a bleach solution in order to prevent contamination.

4. Three-dimensional test impressions can be made by impressing the shoe/tire into BIO-FOAM® or Bubber. This may be photographed using acceptable methods (See SOP-IM-4 Photography). Additionally, a cast may be made of the three-dimensional impression (See SOP-IM-6 Casting Impressions).

5. Other methods for making test impressions may be utilized as necessary.

6. Test impressions may photographed, scanned, photocopied, or otherwise documented.

Retention:

RETIRED

Test impressions will be packaged in an appropriate evidence container, sealed, sub-itemized in JusticeTrax, barcoded, and returned to the submitting agency along with the original evidence.

Quality Assurance:
When a chemical/reagent is utilized for the first time, the examiner opening/preparing the chemical or reagent will ensure that the container is properly labeled as to its contents, its safety information (NFPA or GHS) and the ability of the performance of the chemical/reagent (see section a. below). The examiner will also place the date opened/prepared and his/her initials on the container. Most chemicals/reagents are purchased as packaged kits.

Per GL-21 General Laboratory Equipment, chemicals/reagents are considered "equipment"as they can influence the correct performance of laboratory activities (i.e. creating test impressions or enhancment). The very first control test impression developed with a newly opened or prepared chemical/reagent will demonstrate the ability of the performance of that lot of chemical/reagent. A log will be kept of this control test impression. The log will include the chemical/reagent name, the date opened/prepared, the lot number, the initials of the analyst who opened/prepared it, if the control test impression was acceptable and the manufacturer's expiration date, if there is one. This information will be logged on the Reagent Log form (QR-IM Reagents/Chemicals). Once the chemical/reagent has been found to be acceptable, a colored dot will be initialed and dated and "OK" or "OK for use" will be written on it. This dot will be taped to the chemical/reagent bottle and will signify the chemical/reagent is okay to use.

Before any chemical/reagent is used, every analyst should check for this sticker. If it is not present, the above stated procedure must be followed.

RETIRED

Fingerprint or Printer's Ink Quality Control:
The quality control procedure for fingerprint/printer's ink will be to apply a small amount of this ink to a stamp and then transfer the impression to the surface such as paper. If the impression of the stamp is visible and legible, then the result is considered acceptable and this fingerprint/printer's ink is considered acceptable and this lot of fingerprint/printer's ink may be used in casework. The quality control will conducted once on the initial opening of the tube of ink. Once tested, that tube is considered acceptable for use in casework. If at any point, the ink appears to have hardened, the quality control should be repeated or a new tube should be opened and tested.

Powder and Cast Material Quality Control:
Please refer to IM-SOP-07 for the quality control procedure for magnetic powder and IM-SOP-06 for the quality control procedure for dental stone.

Sources of Error: N/A

Quality Assurance: N/A
References: See Bibliography.

RETIRED

Purpose: To attempt to find the manufacturer and model of tread design patterns on footwear outsoles and tires found in questioned impressions.

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protective equipment will be used as appropriate.

Procedure:

RETIRED

A. Solemate® FPX

1. The Solemate® FPX database can be searched for footwear outsole designs.

2. The computer containing the Solemate® FPX will be password protected.

3. The Solemate® FPX database is no longer receiving updates, however, the database will be updated if they are received in the future. The imprint examiner will update the database by following the instructions supplied by the vendor. The database will then be checked for proper functioning by entering the coding shapes for a test image into the search options. The test image should be one of the results obtained after the coding steps are entered. The database results are printed (include examiner initials and date on the printout) and added to a binder which is kept in the laboratory near the Solemate® FPX database computer. The results are also recorded in a log book which is kept in the laboratory near the Solemate® FPX database computer.

4. Before a search of the database is conducted, the database is checked for proper functioning by entering the coding shapes for a test image into the search options. The test image should be one of the results obtained after the coding steps are entered. The database results are printed (include examiner initials and date on the printout) and added to a binder which is kept in the laboratory near the Solemate® FPX database computer. The results are also recorded in a log book which is kept in the laboratory near the Solemate® FPX database computer. If the database performed as expected, the results are also recorded on the QR-IM1 worksheet by checking the "Controls OK" box under "SoleMate".

5. If the test image is returned using the coding shape search the database check is successful and the database can be searched for footwear outsole designs. If this does not occur, then the database check has failed, then the test will be re-run. If this second test also fails, and the analyst cannot determine the reason and resolve the issue, the database search program will be taken out of use and clearly marked as such. Foster & Freeman will be contacted to resolve the problem. Once the problem is resolved and the database check is successful, the database search program will be put back in service and will return to normal use.

6. If a potential matching footwear outsole design is located, the agency requesting the examination will be supplied with photographs of the outsole design and uppers as well as any other information on the shoe present in the database.

B. Other References/Online Searches

1. A search may be conducted for the tread design on tires or outsole design on footwear utilizing other databases as available. The Imprints Unit currently has the Tread Assistant Database, from Tire Guides, Inc. This database no longer receives updates.

2. Footwear outsole designs and tire tread designs may be searched utilizing websites containing images of the tread/outsole designs (e.g. Zappos.com, Michelinman.com, etc.).

3. Tire tread designs may also be searched using the Tread Design Guide books available from Tire Guides, Inc.

2. If a potential matching footwear outsole design or tire tread design is located, the agency requesting the examination will be supplied with photographs of the outsole design and uppers or the tire as well as any other information on the shoe/tire and the reference site information. A note should be placed in the report that the information provided was retrieved from a non-forensic database and that the information provided should be verified with the source.

Sources of Error: N/A

Quality Assurance: N/A

References: See bibliography.

RETIRED

Purpose: To attempt to find the manufacturer and model of tread design patterns on footwear outsoles and tires found in questioned impressions.

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protective equipment will be used as appropriate.

Procedure:

RETIRED

A. Solemate® FPX

1. The Solemate® FPX database can be searched for footwear outsole designs.

2. The computer containing the Solemate® FPX will be password protected.

3. The Solemate® FPX database is no longer receiving updates, however, the database will be updated if they are received in the future. The imprint examiner will update the database by following the instructions supplied by the vendor. The database will then be checked for proper functioning by entering the coding shapes for a test image into the search options. The test image should be one of the results obtained after the coding steps are entered. The database results are printed (include examiner initials and date on the printout) and added to a binder which is kept in the laboratory near the Solemate® FPX database computer. The results are also recorded in a log book which is kept in the laboratory near the Solemate® FPX database computer.

4. Before a search of the database is conducted, the database is checked for proper functioning by entering the coding shapes for a test image into the search options. The test image should be one of the results obtained after the coding steps are entered. The database results are printed (include examiner initials and date on the printout) and added to a binder which is kept in the laboratory near the Solemate® FPX database computer. The results are also recorded in a log book which is kept in the laboratory near the Solemate® FPX database computer. If the database performed as expected, the results are also recorded on the QR-IM1 worksheet by checking the "Controls OK" box under "SoleMate".

5. If the test image is returned using the coding shape search the database check is successful and the database can be searched for footwear outsole designs. If this does not occur, then the database check has failed, then the test will be re-run. If this second test also fails, and the analyst cannot determine the reason and resolve the issue, the database search program will be taken out of use and clearly marked as such. Foster & Freeman will be contacted to resolve the problem. Once the problem is resolved and the database check is successful, the database search program will be put back in service and will return to normal use.

6. If a potential matching footwear outsole design is located, the agency requesting the examination will be supplied with photographs of the outsole design and uppers as well as any other information on the shoe present in the database.

B. Other References/Online Searches

1. A search may be conducted for the tread design on tires or outsole design on footwear utilizing other databases as available. The Imprints Unit currently has the Tread Assistant Database, from Tire Guides, Inc. This database no longer receives updates.

2. Footwear outsole designs and tire tread designs may be searched utilizing websites containing images of the tread/outsole designs (e.g. Zappos.com, Michelinman.com, etc.).

3. Tire tread designs may also be searched using the Tread Design Guide books available from Tire Guides, Inc.

2. If a potential matching footwear outsole design or tire tread design is located, the agency requesting the examination will be supplied with photographs of the outsole design and uppers or the tire as well as any other information on the shoe/tire and the reference site information. A note should be placed in the report that the information provided was retrieved from a non-forensic database and that the information provided should be verified with the source.

Sources of Error: N/A

Quality Assurance: N/A

References: See bibliography.

RETIRED

Title: Examination Process

Purpose: To perform a comparison of a questioned impression to a known item of evidence.

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate.

Procedure:

A. Comparison of Questioned Impression with Known Item of Evidence

NOTE: The following is the recommended procedure for comparing questioned impressions with known evidence.

1. Comparisons can be performed by several methods:

    - Side-by-side – Examining the Questioned imprint/impression and the test impression while they are side-by-side
    - Overlays – Placing the test impression over the Questioned imprint/impression
    - Like-to-like – Examining a cast to a cast, a lift to a lift, etc.
    - Via the Internet/computer – Examining images of the Questioned imprint/impression with images of the test impression on a computer monitor

2. The method in which the comparison is performed remains at the discretion of the imprint examiner.

3. The examiner will take proper and detailed notes at every stage of the examination process. Any discussions with the requesting agency will be documented in the notes.

4. The examiner shall follow ACE-V methodology in all comparative examinations. The ACE-V methodology is the application of the scientific method to identification sciences. The terms ACE-V stands for:

    - Analysis – The initial examination of the Questioned imprint/impression and the Known item and test impression. The Questioned imprint(s)/impression(s) should be analyzed first, prior to the analysis of the Known item(s). The date (and time, if necessary) of analysis, should be recorded on the worksheet and/or in the case notes. The examiner may look at the quality of the imprint/impressions, the class, wear, manufacturing and individual characteristics present and any other information that can assist in the examination.

RETIRED

- Comparison – The process whereby the examiner will compare the characteristics found in the Questioned imprint/impression with the Known item and test impression to determine if there are similarities or differences.
- Evaluation – The process whereby the examiner will take the information obtained during the analysis and comparison phases of the examination and determine the appropriate conclusion to report.
- Verification – The technical review of the case file by another imprint examiner. The technical reviewer in the case of an identification or elimination will look at the original or generated evidence (such as lifts) and will verify the findings. The technical reviewer will indicate that they are in agreement with these findings by initialing and dating and writing "OK" in the Evaluation/Conclusion section of the case notes.

5. All imprints cases shall be reviewed prior to a formal report being issued by the laboratory. Two types of reviews will be conducted for each case, the technical review and the administrative review.

    a. Technical Review

        1. The technical review shall be performed by second imprint examiner. It will consist of reviewing all case documentation and evidence to determine whether or not the examiner of record has drawn the proper scientific conclusion(s). Both the initials of the technical reviewer and the date of the review will be documented in the case file on QD-IM QR1.

        2. An examiner shall not perform the technical review on any case where he/she is the examiner of record.

    b. Administrative Review

        1. The administrative review may be performed by another Forensic Science Examiner or employee in the laboratory. It will consist of reviewing the report for correct grammar, spelling, etc. Both the initials of the administrative reviewer and the date of the review will be documented in the case file on QD-IM QR1.

Sources of Error: N/A

Quality Assurance: N/A

References: See bibliography.

RETIRED

Purpose: Examination Reports

Responsibility: Forensic Science Examiners assigned to the Imprints Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate.

Procedure:

A. General

An examination report will be completed for each case examined by the Imprints Section. The report will be saved in JusticeTrax (LIMS) and will be prepared as soon as possible after completion of the examination process. The report should be scientific, easy to read and grammatically correct.

The conclusions of the examination must be conveyed in clear, concise and accurate manner. Opinions rendered in the report will be qualified so as to express the proper weight that can be assigned to the evidence submitted for examination. Only those opinions outlined in the "Opinion Terminology" section below will be rendered.

B. Basis for Opinion

There are three (3) types of characteristics which the imprint examiner must concern himself or herself with during the examination process to render an opinion.

1. Class Characteristics - Those features which are general in nature and which all similar items have in common. (i.e. size, shape, general pattern or tread design, manufacturing feature, etc.). These features can also be described as intentional or unavoidable characteristics which are repeated during the manufacturing process and are shared by more than one of the same item.

2. Individual Characteristics - Characteristics which result when something is randomly added to or taken away from the item that either causes or contributes to making the item unique (i.e. cuts, scratches, gouges, holes, tears, rocks, etc.). They are often called accidental characteristics or randomly acquired characteristics.

3. Wear Characteristics - These characteristics are normally associated with examinations involving articles of footwear and tires. Wear can be described as a continuous changing of class characteristics and certain accidental characteristics that result in individualistic features.

RETIRED

The more extensive the wear, the more original the appearance when compared to another footwear outsole or tire tread of the same design.

C. Opinion Terminology

1. Identification – This is the highest degree of association expressed by a footwear and tire impression examiner. The questioned impression and the known footwear or tire share agreement of class and randomly acquired/individual characteristics of sufficient quality and quantity.

2. High Degree of Association – The questioned impression and known footwear or tire must correspond in the class characteristics of design, physical size, and general wear. For this degree of association there must also exist: (1) wear that, by virtue of its specific location, degree and orientation make it unusual and/or (2) one or more randomly acquired/individual characteristics.

3. Association of Class Characteristics – The class characteristics of both design and physical size must correspond between the questioned impression and the known footwear or tire. Correspondence of general wear may also be present.

4. Limited Association of Class Characteristics – Some similar class characteristics are present; however, there were significant limiting factors in the questioned impression that did not permit a stronger association between the questioned impression and the known footwear or tire. These factors may include but are not limited to: insufficient detail, lack of scale, improper position of scale, improper photographic techniques, distortion or significant lengths of time between the date of the occurrence and when the footwear or tires were recovered that could account for a different degree of general wear. No confirmable differences were observed that could exclude the footwear or tire.

5. Indications of Non-Association – The questioned impression exhibits dissimilarities when compared to the known footwear or tire; however, the details or features were not sufficient to permit an exclusion.

6. Exclusion – This is the highest degree of non-association expressed in footwear and tire impression examinations. Sufficient differences were noted in the comparison of class and/or randomly acquired/individual characteristics between the questioned impression and the known footwear or tire.

7. Lacks Sufficient Detail

RETIRED

a. No Comparison Was Conducted – The examiner determined there were no discernible questioned footwear/tire impressions or features present. This opinion applies when there is insufficient detail to conduct any comparison.

b. A Comparison Was Conducted – The examiner determined that there was insufficient detail in the questioned impression for a meaningful conclusion. This opinion only applies to the known footwear or tire that was examined and does not necessarily preclude future examinations with other known footwear or tires.

D. Report Contents

The report may include the following information (Note: Starred items are required per GL 18):

1. Name and address of the Division*
2. Title*
3. Division case number*
4. Name and address of the submitting agency*
5. Agency case number*
6. Town of incident
7. Date of request*
8. Date of report
9. Individual requesting report
10. Description of the item(s) tested*
11. Description of request for analysis
12. Identification of the test method used*
13. Results obtained*

RETIRED

14. Disposition of the evidence

15. Opinion/conclusion statement:*

    This report reflects the test results, conclusions, interpretations and/or the findings of the analyst and technical reviewer as indicated by their signature below.

16. Name, title and signature of analyst and technical reviewer*

D. Distribution

1. Original report to be maintained in case file at Evidence Receiving Unit.

2. First photocopy, with original initials, to be provided to requesting agency.

3. Second photocopy, with original initials, to be maintained in case file.

4. With the exception of the following, additional copies of the examination report <u>will not be provided</u> without the expressed approval of the Agency who originally requested the examination.

    a. A lawful subpoena.

    b. Request from the appropriate prosecutor or States Attorney's office. (Except those cases involving examinations which were conducted at the request of the Public Defenders' Office).

    c. FOIA requests which will be handled by Division of Scientific Services Administration

5. In the case of an amended report, the report shall be uniquely identified and shall contain a reference to the original that it replaces. The distribution of the report will follow the same procedure outlined previously.

6. In the case of a supplemental report, the report shall be uniquely identified. The distribution of the report will follow the same procedure outlined previously.

E. Retention/Storage

1. Examination case files will be stored in appropriate storage containers. These containers will be maintained and secured at the Division of Scientific Services facilities.

RETIRED

2. No examination case file(s) and/or related material(s) will be removed from the facility without the approval and knowledge of the appropriate supervisor.

3. The case file will contain all pertinent information to support the findings, these may include:

   a. Evidence Receipt(s)

   b. Request form(s)

   c. Worksheet(s) (QR-IM1)

   d. Documentation of the label on the packaging of the item(s) of evidence

   e. Photograph(s)

   f. Copy of test impressions

   g. Notes

   h. Copy of the report (including amended or supplemental report if applicable)

   i. Draft report(s)

   j. Case Review Form (QD-IM QR1)

   k. Other documents as applicable

4. A backup of all digital files and pictures will be stored on one of the Laboratory's backed up drives or on another drive/device as needed. Additionally, a CD or DVD will be created containing the digital case file. This CD or DVD will be packaged in an appropriate evidence container, tape sealed, sub-itemized in JusticeTrax, barcoded, and transferred to "QD/IM Archive Storage".

5. Photographs of impressions that cannot be lifted or otherwise collected will be saved to a CD or DVD. The CD or DVD will be packaged in an appropriate evidence container, tape sealed, sub-itemized in JusticeTrax, barcoded, and returned to the submitting agency along with the original evidence.

Sources of Error: N/A

RETIRED

Quality Assurance: N/A

References: GL 1 Quality Manual

GL 13 General Evidence Handling

GL 18 Case Reviews

RETIRED

Title: Training Guidelines

Purpose: To provide an overview summary of specific requirements and knowledge needed to perform the functions of an imprint examiner.

Responsibility: Forensic Science Examiners assigned to the Imprints Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate.

Procedure:

Introduction: The following outline will be followed for all persons newly assigned to the Imprint Section. The amount of time necessary to achieve proficiency in any area will depend on the previous experience and training of the individual examiner. The new examiner and his/her supervisor will sign and date each section as completed. Not all sections will be necessary to complete a new examiner's training. The sections that are not applicable will be determined by the supervisor and will be marked as such.

Note: Any examiner's being cross-trained, previously trained or with experience in conducting casework in the Imprint disciple will need to focus on completing the training guidelines that are specific to DSS or relating to technology that is new for that analyst.

As part of the training and competency testing guidelines, feedback is needed as each task is completed and any testing will need to be graded with a passing grade of 80% or better.

RETIRED

A. Introduction to the Laboratory: To familiarize the examiner with the forensic science laboratory operation, LIMS system, and individual responsibilities.

1. Tasks:

Date	Initials	Trainer's Initials	
			Orientation to the laboratory facility and personnel
			Instruction of the organization structure, code of ethics, and chain of command
			Security and confidentiality requirements
			Introduction to quality control and quality assurance including required documentation
			Review of Ethics

			Safety procedures – chemical and biohazard, incident reports, fire/emergency procedures

Completion of this portion of the training is accompanied by a completed General Laboratory Training form (GL-14:1) and the Safety Checklist form (GL-2.2).

2. Reading:

Date	Initials	Trainer's Initials	
			All General Laboratory SOPs
			DESPP A&O Manual
			Unit SOPs

B. Evidence Handling: To handle evidence in a manner appropriate for preservation and analysis. To properly preserve evidence which may be analyzed by other units of the laboratory.

1. Tasks:

Date	Initials	Trainer's Initials	
			Observe and understand the procedures in place for the intake and release of evidence submitted to the laboratory for analysis
			Handling evidence in an appropriate manner to ensure preservation of evidence and chain of custody

2. Team approach to evidence processing:

Date	Initials	Supervisor's Initials	
			Understands the team concept of processing evidence in concert with other units of the laboratory and the importance of communication
			Use of JusticeTrax to update information in Case Synopsis
			Understands how to itemize evidence, transfer evidence

Assessment for this portion of the training is done by completion of a competency test in the use of JusticeTrax to include transferring of evidence, creation of sub-items, completion of a case including up

RETIRED

to milestone of draft complete. Upload of reports to JusticeTrax. Addition of comments to Case Synopsis area.

C. Footwear/Tire Imprint-Impression Training Program

NOTE: A federal training program or other acceptable training program may be substituted for the following internal training program (e.g. training program through the Federal Bureau of Investigation (FBI), National Institute of Justice (NIJ)/National Forensic Science Technology Center (NFSTC), etc.). Additionally, certification as a footwear examiner by the IAI may also substitute for the following training program.

1. Active Casework Reviewed:

Date	Initials	Trainer's Initials	
			Reviewed cases:
			Validation Studies Reviewed:
			Discussion and understanding of cases reviewed.

2. Practical Casework Exercises

Date	Initials	Trainer's Initials	
			Mock case work – the number of cases required is based on the examiner's prior experience and how the examiner is performing
			Mock casework (case number and feedback):
			Competency test(s):
			Oral/Written Exam:

The completion of mock cases will include the complete working of the cases from case assignment in JusticeTrax to notes/documentation and completing with writing the report and completing milestones in JusticeTrax.

RETIRED

3. Final Competency Test

Date	Initials	Trainer's Initials	
			Competency test(s):
			Oral/Written Exam:

4. Required Text Reading: To develop a contextual understanding, each of the following texts will be read from cover to cover in the following order. (Record progress on "Daily Training Log")

Date	Initials	Trainer's Initials	
			Forensic Footwear Evidence – William Bodziak
			Footwear Impression Evidence, 2nd Edition – William Bodziak
			Footwear Identification – Michael J. Cassidy
			Footwear Evidence – John Reginald Abbott
			Footwear: The Missed Evidence – Dwane Hilderbrand
			Tire Tread and Tire Track Evidence - William Bodziak
			Tire Imprint Evidence – Peter McDonald
			Forensic Tire Impression Identification – Lawren Nause
			Shoe Image Capture and Retrieval (SICAR)/ Solemate – Foster & Freeman

RETIRED

5. Monthly Written Research Papers

Date	Initials	Trainer's Initials	
			Forensic Science General
			The History of Footwear

			The Footwear Examiner
			The Theory of Impression Identification
			Manufacturing Processes of Footwear
			Wear Characteristics
			Class Characteristics of Footwear
			Randomly Acquired Characteristics of Footwear
			Three Dimensional Footwear Impressions
			Two Dimensional Footwear Imprints
			Barefoot Impressions
			The History of Tires
			Anatomy and Mechanics of Tires
			Sidewalls
			Wear Patterns of Tires
			Documenting Tire Imprints and Impressions
			Testing Tire Imprints and Impressions
			Comparison and Identification of Tire Impressions
			Shoe Image and Capture Retrieval (SICAR) / Solemate
			The Scientific Working Group on Shoe Print and Tire Tread Evidence (SWGTREAD) and National Institute of Standards and Technology (NIST) Organization of Scientific Area Committees (OSACs) Footwear and Tire subcommittee
			The Footwear Examiner in Court
			Tire Impression Evidence and the Courts

RETIRED

6. Monthly Oral Examinations/Moot Court

Date	Initials	Trainer's Initials	
			Forensic Science General

Date	Initials	Trainer's Initials	
			The History of Footwear
			The Footwear Examiner
			The Theory of Impression Identification
			Manufacturing Processes of Footwear
			Wear Characteristics
			Class Characteristics of Footwear
			Randomly Acquired Characteristics of Footwear
			Three Dimensional Footwear Impressions
			Two Dimensional Footwear Impressions
			Barefoot Impressions
			The History of Tires
			Anatomy and Mechanics of Tires
			Sidewalls
			Wear Patterns of Tires
			Documenting Tire Imprints and Impressions
			Testing Tire Imprints and Impressions
			Comparison and Identification of Tire Impressions
			Shoe Image and Capture Retrieval (SICAR) / Solemate
			The Scientific Working Group on Shoe Print and Tire Tread Evidence (SWGTREAD) and National Institute of Standards and Technology (NIST) Organization of Scientific Area Committees (OSACs) Footwear and Tire subcommittee
			The Footwear/Tire Track Examiner in Court
			Moot Court

RETIRED

7. Daily Training Log

It is recommended that the trainee maintain a daily log book to record the details of how the trainee's day was spent. This should include contextual textbook reading progress, cases reviewed, practical exercises completed, and any information that the apprentice deems to be a learning experience or "moment".

D. Completion of Training

At the end of the training program, the training binder and the training guidelines will be provided to the Quality Manager for review for completeness. Upon completion if GL-14.3 (Quality Section Review of Training Records), the analyst may be administered a competency test. This competency test will

mimic casework and will include a written report. The analyst will also be administered an oral or written test of the topic areas surrounding the training which will be graded. The passing grade for this assignment is 80% or better.

Upon passing the competency case and oral/written test, a memo/email shall be written to the Deputy Director stating that the analyst has successfully completed their training and has been administered a competency case that evaluated their analytical skills and their ability to clearly communicate their findings in a report.

The Deputy Director may review the training files or simply endorse this memo/email and generate a workflow request in Qualtrax to the Quality Section requesting that this analyst be authorized to perform casework in that discipline.

A Qualtrax workflow will be initiated for authorization of the examiner for casework. The workflow may include the following in which the analyst is being authorized to participate in conducting:

1. Handle evidence
2. Perform examination and/or analytical methods
3. Utilize all applicable equipment in methods described in SOPs
4. Report, review and authorization of results
5. Perform administrative reviews
6. Perform validations in the discipline
7. Assist in the development of new methods
8. Modification of methods
9. Verification of methods

RETIRED

The analyst will perform casework and after gaining experience in conducting analysis and reporting findings in a variety of evidence type/conclusions in the discipline, their experience will be evaluated for the ability to conduct technical reviews. This evaluation period may include technical review ghosting with an experienced examiner. Once the analyst has completed the evaluation period, a memo/email shall be written to the Deputy Director stating that the analyst has successfully completed their evaluation period and has been deemed competent to conduct technical reviews in that discipline. The analyst at this time should be given a moot court if not previously conducted. After successful completion of evaluation period and moot court.

The Deputy Director, after review, may endorse this memo/email and generate a request to the Quality Section requesting that this analyst be authorized to perform technical reviews based upon the analyst's casework experience in that discipline.

A Qualtrax workflow will be initiated for the authorization of the analyst to be able to participate in the following: perform technical review of cases and testimony and/or perform administrative reviews (if not previously authorized).

NOTE: In the event of a retraining situation (extended leave of absence, technical reviews issues, etc.), SOP-IM-14 Training Guidelines will be utilized as directed by GL-14.

Sources of Error: N/A

Quality Assurance: N/A

References: See bibliography.

RETIRED

Purpose: Procedure for the Validation of a New Method or Technology

Responsibility: Forensic Science Examiners assigned to the Imprint Unit are responsible to follow the guidance of this procedure.

Safety: All proper personal protection equipment will be used as appropriate.

Procedure:

1. Before a new method, software or technology is introduced a validation of the method will be performed. A plan of validation will be proposed to and approved by the Deputy Director and the Director prior to conducting the validation. If during the validation there is a change in the plan, the changes will also be proposed to and approved by Deputy Director and/or Director prior to continuing with the validation procedures. (Refer to GL22)

2. All methods used to fulfill customer requests are deemed suitable by the Director and their designee and technical responsibility. This includes the range and accuracy of the results obtained being relevant to the customer's needs. All methods are based on standard methods described and developed by national organizations.

   In the case of a slight variation for a current method, the Unit will treat it as a deviation of testing and have the procedure approved by the Director. The Deputy Director or their designee will create a workflow in Qualtrax explaining the need for the deviation. Until the deviation is approved, no further work should be conducted on that item/case.

3. Any new method, software or technology that is to be added to routine casework must be validated according to the appropriate parameters. All documentation of the new method validation shall be saved in Administration. If possible, the reliability of the new method shall be compared to published results and become part of the validation file.

4. Any changes to an existing method must be validated as above.

Sources of Error: N/A
Quality Assurance: N/A
References: GL-1

RETIRED

A. Purpose:

Guidelines regarding the documentation of physical evidence received for analysis by the latent print section.

B. Responsibility:

Latent Print Section Examiners

C. Procedure:

1. Upon receiving evidence for analysis each assigned examiner will verify the listed items on the request for analysis form, submission evidence label, laboratory bar code and the laboratory receipt form for consistency as compared to the actual item(s) received. Any discrepancy indicating that the represented contents is in error will be noted on the examiner's worksheet or bench notes. Case Management will also be notified of any found errors.

2. A record of every submission examined will be made on the latent print examiner's worksheet or bench notes.

3. Received evidence shall be either photographed or photocopied. This will be at the option of the assigned examiner.

4. All latent print evidence of value will be photographed or digitally scanned and made part of the examiner's case file. Latent impressions of value being digitally scanned do not require that a scale be present but will be uniquely indexed. All latent prints of value that are being photographed will include a scale and will be uniquely indexed. Indexing can be reflected through a variety of methods some of which include: the direct marking of evidence prior to photography; placards or labels used with photography; and the marking of printed photographs. The method to be utilized is at the option of the examiner. Regardless of the method chosen, there should be no doubt as to the latent print in question.

5. All submitted lifts and photographs utilized for comparison purposes should be labeled with the Laboratory Case #, Submission #, Item # and the examiner's initials. Some submissions may not contain sufficient space for this information. If this is the case, the submission should be photographed, digitally scanned or photocopied and the pertinent information can then be added to the printed photograph, or photocopy.

6. Known exemplars are considered materials for comparison unless a submission number is assigned. In either event, a photograph, photocopy or digital image of the exemplar will be

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retained as part of the case file. This includes elimination prints and prints obtained from AFIS, SPBI, FBI or any other source.

7. Copies of the final report, bench notes, worksheets, and any other pertinent paperwork will be kept in the examiner's case file. Any electronic media contained in the case file with case images of latent prints will be sub-itemized in Justice Trax and tracked as evidence including transfer to a secure storage location.

8. All pages of the complete case file excluding the final report will be consecutively numbered by the examiner prior to technical review. Memorandum received after the file has been assigned for technical review need not be numbered but should contain documentation as to when it was received. The purpose of numbering case file pages is for the logical reassembly of the case file and materials associated with specific technical and administrative reviews when such reviews have occurred on more than one occasion.

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A. Purpose:

To establish guidelines for the examination of latent print evidence.

B. Responsibility:

Latent Print Examiners

C. Procedure:

1. When processing latent print evidence, lab coats, masks and gloves will be worn. Other items such as sleeves or splash protectors will be worn as needed based on case and/or personal protection requirements.
2. Eye protection will be worn whenever there is a possibility of injury to the eyes.
3. Fume Hoods and Down Flow Workstations will be utilized whenever possible during the application of various chemical and powdering techniques.
4. For evidence that has both Latent Print and DNA requests, all equipment which will be handled during the examination process will be wiped down with an appropriate decontamination solution (refer to GL-2). This equipment includes writing instruments, cameras, tweezers and magnifying glasses. The examiner will be mindful of contamination.
5. The examination process will be documented on the appropriate worksheet (i.e. QR-LP1a, QR-LP1c, QR-LP1d, QR-LP-10, QR-LP11, QR-LP12, QR-LP-13, QR-LP-14). Worksheets will be completed on every case which involves an examination. In instances where a submission is received and not examined, the acronym "NEATT" may be written on a photocopy or photograph containing the evidence labeling of such item to indicate "No Examination At This Time". The NEATT document will be kept in the case file.
6. A virtual case folder will be created to store digital files used for analysis. The virtual case folder will incorporate the laboratory case number for identification. This folder is used as the working file and will also be used by the technical reviewer.
7. All examinations will start with a thorough visual examination of the submitted evidence. After the initial visual examination, the examiner will determine the appropriate sequential method(s) of processing to be utilized. Each processing step will be documented on the appropriate worksheet (i.e. QR-LP1c or QR-LP-13), including the use of alternate light sources and lasers. Friction ridge skin impressions of value will also be documented on the worksheet.
8. Any friction ridge skin impressions of value must be captured in a manner that makes them suitable for future analysis, meaning they must be captured in a manner that protects them from being compromised. Developed impressions may fade or be otherwise compromised through other testing techniques (i.e. DNA). Impressions of value may be lifted, digitally photographed, or scanned.

8.1 The determination to obtain lifts after processing is at the option of the examiner. The lifting of a latent impression will only be executed after adequate photographs have been taken. All generated

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lifts shall contain the laboratory case number, item number and the examiner's initials. Laboratory generated lifts will be itemized in LIMS and will be treated as evidence, including tracking all transfers in LIMS. Laboratory generated lifts should be transferred to the submitting agency after they are no longer needed for examiner review. If the lifts are being retained in-house, they will be treated as evidence and transferred into a secure storage location.

8.2. Latent impressions of value may be captured using digital imaging, which may include scanning. It is recommended that the images be both in JPEG and RAW format when using a digital camera. If using a scanner, RAW format might not be available, refer to LP SOP-12 for other available formats.

9. After the completion of technical review, the contents of the virtual folder will be write-protected. The virtual folder will be copied to digital media [i.e. disc(s)] which will be finalized and write-protected. All disc(s) containing digital files will be labeled with the case number, item number and the initials of the examiner creating the disc(s). The LIMS description of the disc(s) should indicate the that this is the archive file of the latent print case. An index sheet of the digital files located on the disc(s) will be printed and placed into the case jacket attached to the Case Review Form (QR-LP4). The administrative reviewer will check the printed index sheet to verify that files were transferred onto digital media to be retained.

10. The disc(s) should be transferred to Latent Print File Storage. At a future time, the disc(s) may be transferred to an archive storage area of the laboratory.

11. In cases in which the submitting agency submits lifts and the examiner documents the lifts with a digital image, these images are considered as working/examination files/records. Refer to LP SOP-12 for further guidance on digital image management.

12. When the examination is completed, all evidence should be marked by the examiner for identification at a later time. There will be times when marking evidence is neither realistic or appropriate. This may be more common with some types of evidence than others. When evidence is not marked the examiner will make every effort to assure that it is properly documented for identification at a later time. The examiner will also mark the outer packaging that the evidence is contained in with his/her initials.

13. If further analysis is needed at a later date to images previously saved from the initial examination, the examiner may work on the images contained in the secure virtual folder located on the server or on the archived Latent Print Case File disk.

    a. If the examiner is using images located on the server, it shall be clearly indicated on their worksheet (QR-LP11) the source location of the image being used for their additional analysis (i.e. source is the virtual folder).

    b. If the examiner is planning to use the images located on the Latent Print Case File disk, the examiner will need to transfer this disk into their possession prior to conducting the additional analysis. The worksheet (QR-LP11) will indicate the source of the image being used (i.e. source is the archive disk).

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A. Purpose:

To outline the procedures to be utilized by the Latent Print Section to ensure that chemicals and reagents are of sufficient quality to produce expected results.

B. Responsibility:

Latent Print Examiners

C. Procedure:

1. Fingerprint development and enhancement chemicals/reagents are commercially purchased through an approved vendor. All chemicals and reagents will be stored and kept in appropriate containers. This will include items that need to be refrigerated or kept under other environmental safe guards.
2. When restocking supply chemicals/reagents, the new chemical/reagent packages will be marked with the initials of the receiving examiner and the date they are received. New stock should be placed behind the older stock. Older chemicals/reagents should always be utilized first unless a manufacturer expiration date has passed. Any expired chemicals/reagents should be discarded.
3. When a chemical/reagent is utilized for the first time, the examiner opening/preparing the chemical or reagent will ensure that the container is properly labeled as to its contents, its safety information (NFPA or GHS) and the ability of the performance of the chemical/reagent (see section a. below). The examiner will also place the date opened/prepared and his/her initials on the container. Most chemicals/reagents are purchased premixed or in kits.

   a. Per GL-21 General Laboratory Equipment, chemicals/reagents are considered "equipment" as they can influence the correct performance of laboratory activities (i.e. processing). The Latent Print Unit will satisfy all applicable requirements for this "equipment". Except for cyanoacrylate, a control test impression will be developed before use on any evidence. The very first control test impression developed with a newly opened or prepared chemical/reagent will demonstrate the ability of the performance of that lot of chemical/reagent. A log will be kept of this control test impression. The log will include the chemical/reagent name, the date opened/prepared, the lot number, the initials of the analyst who opened/prepared it, if the control test impression was acceptable and the manufacturer's expiration date, if there is one. This information will be logged on the Reagent Log form (QR-LP3). Once the chemical/reagent has been found to be acceptable, a colored dot will be initialed and dated and "OK" or "OK for use" will be written on it. This dot will be taped to the chemical/reagent bottle and will signify the chemical/reagent is okay to use. Before any chemical/reagent is used, every analyst should check for this sticker. If it is not present, the above stated procedure must be followed. In the case of cyanoacrylate, when a new bottle is opened, a control test impression must be developed BEFORE being used for any evidence. The first control test impression developed with cyanoacrylate cannot be run simultaneous with evidence. The Reagent Log will be used to record the development of the control test impression.

   b. Chemicals/reagents that are purchased premixed can be used without additional preparation. The date opened will be the lot number (absent a manufacturer's lot number). The date opened

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shall be in the following six digit month, day, year sequence: mmddyy i.e. 010115 If there is an expiration date, it will also be placed on the container. The acceptability of the first control test impression developed must be documented on QR-LP3 (Reagent Log form).

c. Chemicals/reagents (such as Indanedione) purchased as a kit need to be prepared for use. The date prepared will be the lot number and will follow the same month , day, year format as above. If there is an expiration date, it will also be placed on the container. The preparation and the acceptability of the first control test impression developed must be documented on QR-LP3 (Reagent Log Form).

d. Although not a chemical/reagent, the lot number of DNA free fingerprint brushes will be recorded on QR-LP17 (Brush Log). These brushes are bought in large amounts so only one lot number will be in use at a time. The start of use date and end of use dates will be logged under the appropriate columns. When a new lot of brushes is placed into use, any remaining brushes from a previous lot will be marked "Not for Casework". Generally, only these brushes will be used for the application of dry fingerprint powders. If any other kind of brush is used for the application of dry fingerprint powders, it must be noted on the Latent Print Processing form for the case.

4. A control test impression will be developed on each day of use to ensure that the chemical/reagent is still working in an acceptable manner; the procedure in Section C.3.a. above does not take the place of the day of use control test impression development unless it is the first day of use.

   a. A control test impression will be developed on the day of use by the examiner that is using the chemical/reagent. Separate cases shall require separate test impressions. The examiner will create a test impression using a similar substrate (i.e. porous, non-porous, adhesive, etc. as the evidence being tested) capable of retaining a test impression and reacting with the chemical/reagent that is going to be used in the case at hand. The examiner will place an impression on the substrate using a Latent Print Reference or Standard pad to ensure that a proper matrix has been deposited. The chemical is then applied as required.

   b. An acceptable result is the development of a latent print with definitive contrast against the background. The examiner will photograph the test impression, and will document the results and the chemical/reagent's lot number on the processing worksheet. The digital image file of the control test impression will be made part of the case file.

      This process of conducting a test impression must be done for each case, when the examiner uses chemicals/reagents.

   c. An unacceptable result is when there is no development of an impression. The chemical/reagent that didn't produce an acceptable result shall be discarded. The examiner will document the unacceptable result in Reagent Log book and indicate that the chemical was discarded. The examiner will then repeat the process with a new lot of the chemical/reagent. If the new lot does not produce an acceptable result, the Unit Lead or Deputy Director will be notified.

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d. The only exception to the preceding shall be cyanoacrylate (superglue). The control test impression will be run simultaneously with the processing of evidence.

If the control test impression is acceptable (development of an impression), a photograph of the control test impression will be taken and made part of the case file. If the control test impression is not acceptable (no development of an impression) and there is no development on the evidence, a new batch of cyanoacrylate (superglue) will be used and processing of the control test impression will be repeated. If the control test impression is not acceptable, but there is development on the evidence the Unit Lead or Deputy Director will be contacted to determine the next step.

5. Controls should be re-run by an examiner if one of the following occurs:
   a. Examiner is processing an item of evidence with cyanoacrylate and begins using a different chamber from the original control test impression.
   b. If it has been noted that a chemical was improperly stored or possibly compromised between use on the same day in between testing of items in that examiner's case.
   c. The original chemical has run out and a new lot/bottle needs to be used.
   d. The cyanoacrylate processing bottle was refilled from the stock bottle during the same day.

6. Latent Print Development powder lots are tested upon opening the first bottle of the lot (refer to section C.3.a). When testing the powder upon opening, a test impression should be placed on a non-porous surface using a Latent Print Reference or Standard pad to ensure that a proper matrix has been deposited. A small amount of powder is then used to develop the latent print. If the impression is visible after the application of the powder, this lot is considered acceptable for casework. For casework, a small amount of powder should be removed from the stock or working container for each use. Powder removed for use should NEVER be added back to a container. Excessive moisture may cause clumping of the powder. Do not expose powders to high humidity or moisture. Keep all containers closed as much as possible.

   The date the original stock container is opened shall become the lot number of the powder. The lot number will be in the following six digit month, day, year format: mmddyy (i.e. 010115). Smaller working containers of powder may be made from the stock container. Any working container will be labelled with the name and lot number of the stock container.. The shelf life of development powders is indeterminable; however, if clumping of the powder is observed, it shall be discarded.

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A. Purpose:

To list approved chemicals, reagents, powders and guidelines for their use.

B. Responsibility:

Latent Print Examiners

C. Safety:

1. When using chemicals, reagents and powders; lab coats, masks and gloves will be worn. Other items such as sleeves or splash protectors should be worn as needed based on case and/or personal protection requirements.
2. Eye protection will be worn whenever there is a possibility of injury to the eyes.
3. Fume Hoods and Down Flow Workstations will be utilized whenever possible.

D. Procedure:

Although general guidelines are available in this SOP for the application of various powders, chemicals and reagents, it is ultimately the responsibility of the examiner to determine the best possible application or sequential applications of various techniques based on previous experience, training and type of evidence being examined.

The following reagents and powders are approved for use by the latent print section (see Appendix 1). Whenever possible premixed solutions or premixed kits will be purchased. When an examiner needs guidance as to the application of development techniques or the preparation of chemicals not purchased as premixed, the "Guidelines for the Application of Powders, Chemicals and Reagents" in Appendix 2 should be referenced.

If a chemical/reagent will be made at the Laboratory, the examiner should refer to the appropriate "Formula" information in Appendix 1 of this SOP.

Any new reagents or chemicals not listed in this document will first be validated after conferring with the Deputy Director of Identification Services and/or the Quality Manager.

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E. References:

1. **FBI Latent Print Operations Manual**
2. **Home Office Handbook of Fingerprint Development Techniques**
3. **http://www.cbdiai.org/Reagents/main.html**
4. **Ron Smith & Associates Appendix B - Approved Processing Methods 6/11/13 Rev.7**

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APPENDIX 1

Authorized Powders, Chemicals, Reagents & Formulas

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Fingerprint Powders:

Many types of powders are available for the development of latent prints. Powders will combine or adhere to friction ridge residue which can be of natural or foreign substances. Powders are available for both conventional and alternate light source documentation. Magnetic powders offer an advantage for processing porous materials such as paper. The following powders may be utilized by the latent print section; however this list is not all inclusive or limiting:

Standard Powders such as but not limited to: White, Gray or Black

Magnetic Powders

Bi Chromatic Powders

Fluorescent Powders

Chemicals & Reagents:

Amido Black (Aqueous & Methanolic) [blood]

Aqueous Amido Black solution can be used to enhance or develop friction ridge detail matrices containing blood. (Premixed rinse/destains may also be used.) This chemical is stored at room temperature.

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Guidelines for Application

1. Be certain that the blood is 'dried' prior to application.
2. Squirt, Spray bottle or Tray application - Apply Amido Black solution for 30 to 90 seconds
3. For Methanolic based formula only: Apply rinse solution for approximately 1 minute.
4. Apply a final distilled water rinse
5. Allow the item to air dry.
6. Forced air or blot dry.
7. Photograph any latent prints of value.

Developer & Rinse Solution Formula for Methanolic Solution

DEVELOPER SOLUTION:

1 2g Amido black dye
2 100 ml Glacial acetic acid
3 900 ml Methanol
4 (Combine and mix with a stirring device for 30 minutes.)

RINSE SOLUTION:

1 100 ml Glacial acetic acid
2 900 ml Methanol

FINAL RINSE:

1 use distilled water

Ardrox [CA dye stain]
Ardrox is a fluorescent liquid dye used to enhance the visualization of friction ridge detail which was processed with superglue fuming. ALS settings: 365nm, 450-480nm utilizing the best contrasting barrier filter. This chemical is stored at room temperature.

Guidelines for Application

1. Tray immersion or squirt or spray bottle for 5 seconds.
2. Water rinse – using Squirt bottle or gently running stream of tap water for 10 seconds.
3. View under an Ultra-violet lamp 365nm range or Forensic Light Source in the 435 nm to 480 nm range.
4. Photograph latent prints of value using an appropriate filter.

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Basic Yellow [CA dye stain]

Basic Yellow dye is designed to enhance latent fingerprints developed with cyanoacrylate on non-fluorescent, multi-colored surfaces. Prints fluoresce bright yellow/green between 365nm and 485nm with an alternate light source or a UV light. This chemical is stored at room temperature.

RETIRED

Guidelines for Application

1. Tray immersion or spray to cover item for at least 5 seconds.
2. Water Rinse - Squirt bottle application or gently running stream for 10 seconds
3. View under an Ultra-Violet lamp 365 nm a Forensic Light Source in the 450 nm to 485 nm range.
4. Photograph with appropriate filter.

CyanoBlue [CA dye stain]

CyanoBlue is a non-flammable, water-based cyanoacrylate dye which bonds rapidly to cyanoacrylate fumed latent prints, providing a highly bright fluorescence. CyanoBlue works immediately upon contact with the print and does not require long-term exposure. This chemical is stored at room temperature.

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Guidelines for Application

1. Tray immersion or spray to cover item for at least 5 seconds.
2. Water Rinse - Squirt bottle application or gently running stream for 10 seconds
3. View under an Ultra-Violet lamp 365 nm a Forensic Light Source in the 450 nm to 485 nm range.
4. Photograph latent prints of value using an appropriate filter.

Crystal Violet [adhesives / oil]

Crystal Violet is a staining process using a water-based working solution. Used for non-porous evidence and the adhesive side of tape. The evidence is repeatedly stained and rinsed until optimum development occurs. The working solution may also be applied by brushing. Enhanced detail may be observed under a forensic light source at 505nm to 570nm range using red viewing goggles. This chemical is stored at room temperature.

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Crystal Violet (Gentian Violet) Formula (A):

Stock Solution

1.5gm Crystal Violet
100ml Ethyl Alcohol

Working Solution

2ml Stock Solution
100ml Distilled Water

Crystal Violet (Gentian Violet) Formula (B):

Stock Solution

5gm Crystal Violet
10gm Phenol (caution hazardous)
50ml Ethyl Alcohol

Working Solution

1ml Stock Solution

Add distilled water constantly swirling the mixture until the gold film on the surface disappears.

Guidelines for Application

1. Tray immersion

for 1 to 2 minutes.

2. Cold tap water rinse - 30 seconds.

3. Photograph latent prints of value.

Cyanoacrylate (see Superglue)

DFO [amino acid]

Reacts to amino acids present in latent print residue on porous surfaces. A fluorescence of the prints is obtained after baking the item in an oven at 50 to 100 degrees C (120 to 212F), then viewing under a forensic light source. A hair dryer or a DRY steam iron may be substituted for a baking oven, if necessary. Do not allow steam to contact the item. Strict control of the humidity is not required. View the item at 500 nm to 590 nm range using red colored goggles to reduce background fluorescence, if necessary. This chemical is stored at room temperature.

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Guidelines for Application

1. Submerge or spray the item - 5 seconds.

2. Air-dry the item in a fume hood.

3. Process the item a second time &
Air-dry the item in a fume hood.

4. Oven bake or hair dryer @ 120 to 212 degrees F
for 10 to 20 minutes.

5. View under a forensic light source
at 495 nm to 550 nm range. Absorption Max
is usually around 514 nm.

6. Photograph latent prints of value
using an appropriate filter.

Fluorescein [blood]
Fluorescein is a chemical mixture that causes a catalytic reaction to occur between the hemoglobin in blood and oxygen. The reaction results in a fluorescence that is visible under UV light. This chemical is stored at room temperature.

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Guidelines for Application

1. Spray suspect area with mixed reagent (A+B). Allow to Dry
2. Spray suspect area with part (C). Allow to Dry
3. View under UV Light (365nm) or ALS at around 450nm range with orange or yellow filter.
4. Photograph latent prints of value using an appropriate filter.

Gentian Violet [adhesives / oil]

See crystal violet.

Iodine [grease / fats]

The iodine fuming method is used on surfaces that are generally impractical for dusting with fingerprint powder. On greasy surfaces the iodine fumes may be absorbed at different rates by different fatty or oily residues, and the latent impressions may be visible. Most paper surfaces, and especially hard smooth surfaces, may show high quality latent prints when fumed with iodine. This chemical is stored at room temperature.

Guidelines for Application

1. Prepare the photographic set-up by pre-setting camera lighting, aperture

and shutter speed.

2. Pass the fumes over the surface to be examined. Utilize container or pipette method.

   Note: Low temperature heat is required to sublimate the iodine crystals into fumes.

3. Photograph any latent prints of value ***immediately***.

LCV (Leuco-Crystal Violet) [blood]

LCV is used to enhance blood through the catalytic oxidation of the dye, while simultaneously fixing and enhancing the blood impression. Other blood enhancement techniques such as Amido Black may be applied after this technique. This chemical is stored at room temperature.

Guidelines for Application

1. Spray the blood impression or suspected using a fine-mist sprayer.

   Development should occur in 30 seconds.

2. Photograph latent prints of value.

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Liqui-Drox [CA dye stain]
Liqui Drox is effective for dark-colored adhesive tapes, the Liqui-Drox method is a post-cyanoacrylate process involving brushing the reagent unto tape, rinsing, then viewing the result under long-wave ultra-violet light. This reagent is composed of a mixture of a fluorescent agent, a detergent and water. This chemical is stored at room temperature.

Liqui-Drox is prepared in the laboratory. See formula below:

Liqui-Drox Formula**:**

200 ml Ardrox P-133D
400 ml Liqui-Nox
400 ml distilled water

Combine and stir the chemicals thoroughly. A thick milky-yellow solution should result.

Guidelines for Application

1. Using a camel hair brush, apply the reagent onto both sides of the tape until a lather is produced.

2. Wait approximately 10 seconds.

3. Rinse under a gentle stream of cold tap water.

4. Allow the tape to air dry or low temp hair dryer.

5. View under a Forensic Light Source or Ultra-Violet lamp able to produce a long-wave ultra-violet light output.

6. Photograph latent prints of value using an appropriate filter.

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MBD 7-(PMethoxybenzlamino-4Nitrobenz-2-Oxa-1,3-Diazile) [CA dye stain] MBD is a yellow fluorescent dye stain used to enhance the visualization of friction ridge detail which was processed with superglue fuming. ALS settings: Various wavelengths utilizing the best contrasting barrier filter. This chemical is stored at room temperature.

Guidelines for Application

1. Spray, immerse or use a squirt bottle to apply the M.B.D. solution to the item.

2. View under a Forensic Light Source: Absorption Max is should be around 450 nm range.

3. Photograph latent prints of value using an appropriate filter.

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Ninhydrin [amino acid]
Ninhydrin is a reagent for developing prints on porous materials like paper and cloth by reacting with amino acids contained in latent print residue. This chemical is stored at room temperature.

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Ninhydrin Formula:

FORMULA #1:

12.5g Ninhydrin crystals
dissolved in 1L alcohol solvent.

FORMULA #2:

Use a magnetic stirring device.

5 g ninhydrin crystals
dissolve in 30 ml Methanol
add - 40 ml 2-Propanol
add - 930 ml Petroleum ether.

Guidelines for Application

1. a) Tray immersion of item
   - 5 seconds
   b) Brush solution onto item
   - until coated.
   c) Spray solution onto item
   - until coated.
2. Warning: Do not use heat or steam for Thermal Ninhydrin applications.

   Heat up to 80 degrees C
   & humidity exposure
   60% - 70% relative humidity.
   Monitor for development, or use
   a steam iron or use no heat or humidity
   and wait up to 72hrs for development.

3. Photograph the developed detail using a green colored filter.

4. View under Forensic Light Source Generally at 450-590nm range.

5. Photograph latent prints using an appropriate filter.

Ninhydrin Analogs [amino acid]

Indanedione does not produce strong-colored prints. They are typically pale pink in color, but prints do fluoresce strongly when examined by an alternate light source. Prints developed with 1,2-indanedione will fluoresce under green light (optimum about 530 nm range), similar to DFO. Optimum viewing and photographing is done with dark orange glasses/filters (cut on point 570-590 nm range). This technique is may prove more successful on multicolored porous materials. This chemical is stored at room temperature.

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Guidelines for Application

1. Dip, spray or wash the item in the reagent.

2. Air-dry the item (3 minutes).

3. Oven bake at 100 degrees C for 10 - 20 minutes at 60% relative humidity or with no additional humidity added.

4. View under a forensic light source:

   For most papers .....
   View @ 515 nm range (green light) with orange barrier filter.

   For manila, brown paper bags, cardboard items & craft paper ....
   View @ 515 - 570 nm range with orange or red barrier filters.

5. Photograph latent prints of value using an appropriate filter.

Oil Red O [lipids]

A lipid-specific dye stain technique for use on porous items; and porous items which have become wet. Oil Red O may be used after ninhydrin processed items or in lieu of, or as a precursor to, Physical Developer. This chemical is stored at room temperature.

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Guidelines for Application

1. Tray immersion in stain solution and soak completely. It is optional to agitate the solution on a shaker platform.

   - Ridge detail should begin to develop in 5 min.
   - Weak fingerprints (poor lipidic content) may require 60 to 90 minutes of development time.

2. Remove & drain item - immerse in the buffer solution.

3. Remove item from buffer solution - rinse in distilled water.

4. Dry item at room temperature - or heat in an oven at 50 degrees C or use a hair dryer.

5. Photograph latent prints of value.

Physical Developer [lipids]

Physical Developer is a silver-based liquid reagent which reacts with lipids, fats, oils and waxes present in fingerprint residue. Paper items should be treated with Ninhydrin, and then Physical Developer. This chemical is stored at room temperature.

Guidelines for Application

1. Maleic Acid Pre-wash for 10 min
2. Working solution
   (approximately 20 minutes)
3. Rinse (5 minutes) w/Appropriate Solution
4. Rinse (5 minutes) w/Appropriate Solution
5. Photograph latent prints of value.

RETIRED

RAM (Rhodamine 6G, Ardrox, MBD) [CA dye stain]
RAM is a combination of Rhodamine 6G, Ardrox and MBD. It is a fluorescent liquid dye used to enhance the visualization of friction ridge detail which was processed with superglue fuming. ALS settings: Various wavelengths utilizing the best contrasting barrier filter. This chemical is stored at room temperature.

Guidelines for Application

1. Spray, dip, or use a squirt bottle to apply R.A.M. to the item then rinse w/water.
2. Examination under a UV light 365nm or Forensic Light Source at 415-530 nm range.
3. Photograph latent prints of value using an appropriate filter.

RETIRED

RAY (Rhodamine 6G, Ardrox, Basic Yellow) [CA dye stain]
RAY is a combination of Rhodamine 6G, Ardrox and Basic Yellow. It is a fluorescent liquid dye used to enhance the visualization of friction ridge detail which was processed with superglue fuming. ALS settings: 450-550nm range utilizing the best contrasting barrier filter. This chemical is stored at room temperature.

Guidelines for Application

1. Spray, dip, or use a squirt bottle to apply R.A.Y. then rinse w/water.
2. Examination under a laser or Forensic Light Source at 450 nm to 550 nm range.
3. Photograph latent prints of value using an appropriate filter.

Rhodamine 6G [CA dye stain]
Rhodamine 6G is a fluorescent liquid dye used to enhance the visualization of friction ridge detail which was processed with superglue fuming. ALS settings: 495-540nm range utilizing the best contrasting barrier filter (generally orange or red). This chemical is stored at room temperature.

Guidelines for Application

1. Spray, dip, or use a squirt bottle to apply the Rhodamine solution to the item then rinse w/water.

2. Allow the item to dry.

3. Examination under a laser or Forensic Light Source at 495 nm to 540 nm range. Absorption Max is at 525 nm range. Use orange or red colored goggles.

4. Photograph latent prints of value using an appropriate filter.

RETIRED

Silver Nitrate [Salts]

Silver Nitrate is typically used on porous materials after Ninhydrin processing to produce a dark brown color. Silver Nitrate reacts to the chlorides present in latent prints, and requires exposure to sunlight or ultraviolet light in order to develop. This chemical is stored at room temperature. Precaution should be used when using this chemical since it highly flammable and a skin irritant.

Guidelines for Application

1a. Tray immersion of item for 5 seconds.

1b. Reagent solution brushed onto item until coated.

2. Air dry for 20 minutes.

3. Expose item to Sunlight or U.V. Light 365 nm for ten to sixty minutes.

4. Continuously monitor for development.

5. Photograph latent prints of value.

RETIRED

Small Particle Reagent [wet surfaces / oil / fats]

Small Particle Reagent (SPR) is also known as Molybdenum Disulfide (MoS2). Small Particle Reagent is composed of finely ground particles suspended in a detergent solution. These particles adhere to the fatty constituents of latent fingerprints to form a visible deposit. Small Particle Reagent is typically used to develop latent prints on wet surfaces, such as vehicles wet with rain or recovered from bodies of water. Latent prints developed using this method may be lifted using standard lifting mediums. This chemical is stored at room temperature.

RETIRED

Guidelines for Application

1a. Tray Immersion Method:
keep Stationary for 1 minute.

- or -

1b. Spray Bottle Method:
Shake well and apply.
Repeat as necessary to keep
wet for about 1 minute.

2a. Tray Immersion Method:
Rinse excess reagent in
tap water for 15 seconds.

- or -

2b. Spray Bottle Method:
Rinse under running tap
water for 15 seconds.

3. Allow the item to dry
at room temperature.

4. Photograph latent prints of value

5. Lifts may be taken after latent prints

of value are photographed.

Sudan Black [grease / wax]

Sudan Black is best used for processing smooth or rough non-porous surfaces contaminated with greasy or sticky substances. Works best on greasy or waxy non-porous surfaces such as glass, metal, milk cartons, interiors of gloves, and candles. This chemical is stored at room temperature.

RETIRED

Guidelines for Application

1. Tray immersion in Working solution for 2 minutes.
2. Cold Tap water rinse - remove excess dye.
3. Dry item at room temperature.
4. Photograph latent prints of value.

Superglue (cyanoacrylate) [hard non-porous surfaces]

Superglue vapor polymerizes with friction ridge residue to form a white deposit which will visualize a latent print. The superglue technique is most effective on non-porous materials. Superglue can be stored at room temperature, refrigeration may be used to extend the usable viscosity of the liquid cyanoacrylate.

RETIRED

Guidelines for Application

1. Place item(s) into enclosed chamber.

2. Add glue to cover bottom of aluminum dish or other dispersion source. Glue may be heated to accelerate development.

3. Humidify w/cup of warm water (except under vacuum)

4a. For Small Chamber
Fume at least 10 minutes or
until ridge development occurs,
monitor for development.

4b. For Automated Chambers follow
manufacturer's instructions.

4c. For processing under vacuum
run vacuum pump for approx. 1min 30sec
and vapor is visible from pump exhaust portal.
Fume under vacuum for at least 30 minutes.

5. Photograph latent prints of value.

6. Continue processing with powder or
Dye-stain if necessary.

Wet Powders [adhesives surfaces]

Wet powders are applied to adhesive surfaces to develop latent impressions. This chemical is stored at room temperature.

Guidelines for Application

1. With a camel hair style brush apply wet powder to the adhesive surface side of item being processed.
2. Rinse under mild stream of tepid to cool tap water.
3. Allow item to dry (adhesive side up).
4. Photograph latent prints of value.

RETIRED

RETIRED

Zinc Chloride [ninhydrin treatment]

Zinc chloride is applied as post-ninhydrin , post 1,2-Indandione & post 5-MTN treatments in order to improve the strength of the fluorescence of the ridge detail for viewing and photography. View @ 515 - 570 nm range with orange or red 600(BP 35) barrier filters. This chemical is stored at room temperature.

Zinc Chloride Formula #1:

30 g Zinc chloride dissolved in
500 ml methyl-tert-butylether (MTBE)
20 ml of anhydrous Ethanol.

Use magnetic stirrer until completely dissolved. Dissolution may be slow.
Add 10 ml Glacial acetic acid.
Dilute with 500 ml Petroleum ether.

Zinc Chloride Formula #2:

*** ZINC CHLORIDE **STOCK SOLUTION** ***

8 grams Zinc chloride crystals
180 ml Ethanol
20 ml Glacial Acetic acid

Combine and stir with a magnetic stirrer until ALL the ingredients are dissolved.

*** ZINC CHLORIDE **WORKING SOLUTION** ***

6 ml Zinc chloride stock solution
100 ml Petroleum ether or Pentane or Heptane
(cont. next page)

Guidelines for Application

1. Spray the item lightly.
2. Air-dry the item.
3. Process & dry a second time.
4. Oven bake at about 80-100 degrees C at about 65% humidity for 40 minutes.
5. View light source at around 490nm to 515nm range.
6. Photograph latent prints of value using an appropriate filter.

RETIRED

RETIRED

APPENDIX 2

Guidelines For The Application Of Powders, Chemicals, And Reagents

Note: The case examiner has the final discretion to determine the best possible application or sequential applications of various processing techniques based on previous experience and training and type of evidence being examined.

Porous

- Visual Examination
- ALS (to be utilized sequentially as determined appropriate)
- Iodine
- DFO or Indandione
- Ninhydrin
- Oil Red O
- Physical Developer

Non-porous

- Visual Examination
- ALS (to be utilized sequentially as determined appropriate)
- Cyanoacrylate Fuming or Powder
- Dye Staining
- Powder

RETIRED

Blood Stained-Porous

- Visual Examination
- ALS (to be utilized sequentially as determined appropriate)
- Ninhydrin / LCV / Amido Black
- Physical Developer

Blood Stained Non-Porous

- Visual Examination
- ALS (to be utilized sequentially as determined appropriate)
- LCV / Amido Black / Acid Fuchsin / Fluorescein
- Cyanoacrylate Fuming
- Dye Staining
- Powder

Cardboard (non-glossy)

- Visual Examination
- ALS (to be utilized sequentially as determined appropriate)
- Ninhydrin
- Silver Nitrate

RETIRED

Rubber Gloves (special circumstance processing only)

- Visual Examination
- ALS (to be utilized sequentially as determined appropriate)
- Wetwop -or- Cyanoacrylate
- Magnetic Powder
- Dye Stain
- Ninhydrin
- Physical Developer

Tape Non-Adhesive Side (protect adhesive side prior to processing ie clean acetate)

- Visual Examination
- ALS (to be utilized sequentially as determined appropriate)
- Cyanoacrylate Fuming
- Dye Staining
- Powder

Adhesive Surfaces

- Wet Powder Processing –or– Gentian Violet

– OR –

- Cyanoacrylate
- Dye Stain

RETIRED

Glossy Paper

- Visual Examination
- ALS (to be utilized sequentially as determined appropriate)
- Cyanoacrylate Fuming
- Powder
- Ninhydrin
- Dye Stain

A. Purpose:

To define symbols, abbreviations and acronyms that are commonly used in the latent print section.

B. Responsibility:

Latent Print Examiners

RETIRED

Abbreviation	Key
Δ	Change to
Ø	Identified
#	Number
?	Undetermined at this time
+	Positive
-	Negative
\	Right Sloped Loop
/	Left Sloped Loop
A/R	Administrative Review
ABP	Amino Acid based processing
AEL (laser)	Accessible Emission Level*
ALS	Alternate Light Source
AR	Administrative Review
ARCH ? P	Undetermined if Plain Arch
ARCH ? T	Undetermined if Tented Arch
AW	Accidental whorl
BBC	Black Backer Card
BC	Backer Card (BBC or WBC)
BI	Bi-chromatic
B/P or BP	Black Powder
BER	Blood Enhancement Reagent
BHL	Black Hinge Lifter
BLK	Black
BLK PDR	Black Powder
BLS	Blood Like Stain
BMAG	Black Magnetic Powder
BSA	By Submitting Agency
BP	Black Powder
CA	Superglue / Cyanoacrylate
CAE	Superglue / Cyanoacrylate Ester
CHL	Clear Hinge Lifter

CM	Case Management
COMP	Comparison
CT-AFIS	Connecticut AFIS Database
CV	Comparison Value
CW (laser)	Continuous Wave*
CW	Central Pocket Loop Whorl
DEV	Developed
dH_2O	Distilled Water
DOB	Date of Birth
DPP	Dry powder processing
DSP	Dye stain processing
DW	Double Loop Whorl
ENF	Eliminations needed first
FA	Firearms
FB	Forensic Biology
F/B	Front & Back
FCR	Full Case Review
FLR	Fluorescent
FP	Fingerprint
FRAGS	Fragments
FRD	Friction Ridge Detail
FRDD	Friction Ridge Detail - Documented
FT	Fingertip
GMAG	Grey Magnetic Powder
GP or G/P	Grey Powder
GRY	Grey
GYR	Green, Yellow, Red mark-up per SOP-LP-18
ID	Identification
IMP	Impression(s)
INC	Inconclusive
INSUFF	Insufficient
IQQ	Insufficient Quantity and Quality
IR	Infrared
IV	Identification Value
J (laser)	Joule*
JNT	Finger joint/phalanges
L	Left
L/L	Latent Lift
L/P or LP	Latent Print
LAT REG	Latent Registered into Unsolved Latent Database
LFFS	Latent File Feature Search
LFIS	Latent File Image Search
LI	Left Index

RETIRED

LJ	Lower Joint
LL	Left Little
LM	Left Middle
LP	Latent Print
LPP	Left Palm Print
LR	Left Ring
LS/	Left Sloped
LSO (laser)	Laser Safety Officer*
LSP (laser)	Laser Safety Plan*
LT	Left Thumb
MCP	Major case prints
MPE (laser)	Maximum Permissible Exposure*
N.G.	Negative
N/A	Not Applicable
NAQ	Not AFIS Quality
NDVF	No Friction Ridge Detail of Value Found
NEATT	Not Examined At This Time
Neg	Negative
NG	Negative
NGI	Next Generation Identification
NHLR	No Hit Latent Registered
NHZ (laser)	Nominal Hazard Zone*
NI	Not Identifiable
NIN	Ninhydrin
NL	No Latents Found
NM	Nanometers
NV	No Value
OML	Oblique Magnified Lighting
ORO	Oil Red O
OV	Of Value
PC	Photocopy
PDR	Powder
POS	Positive
POSS	Possible
PP	Palm Print
PPC	Palm Print card
PW	Plain Whorl
QUAL	Quality
R or Rt	Right
R/B	Reddish/Brown
RC	Ridge Characteristics
RF or REF	Reference
Rev.	Reversed

RETIRED

RI	Right Index
RI	Rhode Island
RL	Right Little
RM	Right Middle
RPP	Right Palm Print
RPT	Report
RR	Right Ring
RS\	Right Sloped
RT	Right Thumb
RT	Right
SAF	Sample Accounted For (when using FB worksheets)
SBBHL	Sirchie Black Backer Hinge Lifter
SBP	Sebaceous based processing
sdH_2O	Sterile Distilled Water
SG	Superglue / Cyanoacrylate
SI	Superimposed
SID	State Identification Number
SPBI	State Police Bureau of Identification
SPR	Small Particle Reagent
SQQ	Sufficient Quantity and Quality
STHL	Sirchie Transparent Hinge Lifter
SUB	Submission
SWBHL	Sirchie White Backer Hinge Lifter
TIP	Finger tips
TL	Tape Lift
TL (laser)	Threshold Limit*
T/P	Test Print
T/R, T.R. or TR	Technical Review
THL	Transparent Hinge Lifter
TOT	Turned over To
TP	Test Print
TPC	Ten Print Card
TR	Technical Review
TRANS	Transparent
UAL	Unknown Anatomical Location
UCN	Universal Control Number
ULD	Unsolved Latent Database
ULDB	Unsolved Latent Database
ULF	Unsolved Latent File
UNK	Unknown
UV	Ultraviolet
VAL	Value
VE	Visual Examination

RETIRED

VER or VR	Verification or Verification Review
VIS	Visible
w/	With
W	Whorl
WBC	White Backer Card
WHL	White Hinge Lifter
WHT	White
WP	White Powder
WPP	Wet powder processing

*** denotes any abbreviations associated with the Laser and its use. Refer to LP SOP-28 and 29 for definition of abbreviations.**

RETIRED

A. Purpose:

Guidelines for preparing reports of findings and case reviews by the latent print examiners.

B. Responsibility:

Latent Print Examiners

C. Procedure:

1. All latent print comparisons, regardless of the findings, will be verified by another competent latent print examiner, who will also technically review and countersign the report.

2. All items examined will be listed.

3. Latent prints and known prints will be indexed or listed in the examiner's report, in such a way, that there is no confusion as to what is being represented in the examiner's findings.

4. The result of all comparisons and or conclusions, regardless of the findings, should be clearly explained in the examiner's report. These conclusions will be based on the information contained on the case jacket worksheets, which may include examination activity conducted, sequence of the activities, development techniques, photography/digital imaging used, AFIS/NGI searches conducted, known exemplars used, comparisons conducted and the conclusion of such searches and/or comparisons.

   The results of all comparisons and processing shall be provided in a report in a manner that is accurate, clear, unambiguous and objective. The report shall include all the information as requested by the Request for Analysis. The report will include all information necessary for the interpretation of the results and the methodology used to obtain the results. Refer to LP SOP-18 (Friction Ridge Skin Comparison Methodology) for conclusions that may be provided in a report.

5. The current latent print examination report should adhere to the following outline. However, if the form or outline does not clearly or adequately reflect the examiner's findings, the report may be modified to a more appropriate format or style, which must include all pertinent information. The typical outline for a latent print report is as follows:

   Laboratory Case Number
   Submitting Agency and Address
   Agency Case Number
   Date of Request
   Date of Report
   Report To
   Evidence Submitted
   Submission Itemization
   Examination/Processing Method(s)

RETIRED

Known Impressions Obtained For Comparison (if applicable)
Results Of Item/Impression(s) Of Value Documented, Searched & Compared
Analyst Comments (if applicable)
Disposition of the evidence
Analyst's Signature
Technical Reviewer's Signature
Abbreviation/Definition Key

6. Supplemental Reports

6.1 Reports which are amended to add additional information due to additional work being performed on the case, will be clearly marked as "Supplemental Reports".

7. Revised Reports

7.1 A report which has been re-issued due to a clarification or correction will be clearly marked as "Revised Report". A cover letter may be added to indicate the reason for the revision.

8.0 Completed Reports – Upload
Once the final report has been signed, the final report will be scanned and uploaded into Justice Trax as a PDF document.

9.0 When an identification is made, the report may be emailed to the requesting investigator. A copy of the email sent will be kept in the case jacket. This is considered an administrative document and will need the case examiner's initials and case number on the printed document in the case jacket.

RETIRED

A. Purpose:

To illustrate the general progressive methodology of processing evidence in the latent print section. The term AFIS also includes NGI.

B. Responsibility:

Latent Print Examiners

C. Procedure:

RETIRED

Note: All questioned evidence should be independently evaluated prior to comparing to known exemplars provided. During the evaluation of the questioned evidence, the analyst should refer to LP SOP-18.

AFIS SEARCH FLOW CHART

Enter Demographic Information

↓

Enter Latent Print Of Value Into AFIS

↓

Execute Search Of Print

↓

Generate List of Candidates for Review

↓

Compare Candidates Against Searched Impression

Examiner's shall be responsible for reviewing the top 10 generated candidates. The examiner is allowed to stop the reviewing process when a hit occurs. A copy of the AFIS candidate list and transaction number will be placed in the case file along with a photo of the searched impression. When a hit occurs, a photo of the candidate's known impression will also be made part of the case file. If no hit proceed to the next step.

↓

Enter Unidentified Latents Of Sufficient Quality Into Unsolved Latent Database

↓

Report Results Of AFIS Search To Requesting Agency

RETIRED

A. Purpose:

To define certain words and acronyms utilized by the Latent Print Section.

B. Responsibility:

Latent Print Examiners

C. Glossary:

RETIRED

ACE-V	The acronym for the methodology utilized during friction ridge examinations: Analysis, Comparison, Evaluation and Verification.
AFIS	The acronym for **A**utomated **F**ingerprint **I**dentification **S**ystem - A computer-based system for reading, cataloging, searching, matching and storing fingerprints and related data.
Analysis	The first step of the ACE-V methodology. The assessment of an impression to determine suitability for comparison.
Anchor Point	An anchor point is a concrete group of friction ridge features that allow the examiner the ability to focus in on a section of an impression for the purposes of making an identification or to eliminate a known impression from a questioned impression. Examples of anchor points are clearly defined major creases, distinctive ridge flow, deltas, cores, triradius, loops, whorls and pattern vestigia. Anchor points in many instances can help the examiner determine the anatomical location or orientation of a partial latent impression.
Arch – Plain	A fingerprint pattern in which the friction ridges enter on one side of the impression and flow, or tend to flow, out the other side with a rise or wave in the center.
Arch – Tented	A type of fingerprint pattern that possesses either an angle, an upthrust, or two of the three basic characteristics of the loop.
Bench Notes	Handwritten notations made by the examiner during the examination of the submitted items of evidence.
Bifurcation	The point at which one friction ridge divides into two friction ridges.
Bridge	A connecting friction ridge between, and generally at right angles to, parallel running friction ridges.

Characteristics	Features of the friction ridges. Commonly referred to as Galton details, minutiae, points, ridge endings, dots and bifurcations.
Classification	The alpha numeric formula of finger and palm print patterns used ~~as a~~ for filing and searching.
Comparison	The second step of the ACE-V methodology. The observation of two or more impressions to determine the existence of discrepancies, dissimilarities, or similarities.
Comparison Value	References a friction ridge impression which does not meet the criteria to be referenced as "identification value". Some impressions referenced as comparison value may ultimately not be able to be identified and may be downgraded as to their value. Comparison value impressions are entered into AFIS at the discretion of the examiner.
Complete Friction Ridge Exemplars	Also known as major case prints. A systematic recording of all friction ridge detail appearing on the palmar sides of the hands. This includes the extreme sides of the palms; and joints (phalanges), tips, and sides of the fingers.
Core	The approximate center of a pattern.
Court Charts	Photographic or digital enlargements in a side-by-side relationship of latent print/inked print. Identifying characteristics are plotted in such a manner as to assist the examiner in demonstrating the method of identification to members of a court during legal proceedings.
Discrepancy	The presence of friction ridge detail in one impression that does not exist in the corresponding area of another impression.
Dissimilarity	A difference in appearance between two friction ridge impressions.
Dissociated Ridges	1. Disrupted, rather than continuous, friction ridges. 2. An area of friction ridges units that did not form into friction ridges.
Distal	1. Farthest away from the center point of attachment. 2. The direction away from the body.

RETIRED

Distortion Variances in the reproduction of friction ridge skin detail caused by pressure, movement, force, contact surface, and so forth.

Dot An isolated friction ridge unit whose length approximates its width in size.

Edgeoscopy The study of the morphological characteristics of the contour or shape of the edges of friction ridge skin and their resulting impressions.

Electronic Imaging Capturing an image digitally. Once digitized, the image can be processed, saved for future use and/or printed.

Elimination Prints Exemplars of friction ridge skin detail of persons known to have had legitimate access to an item.

Enclosure A single friction ridge that bifurcates and rejoins after a short course and continues as a single friction ridge.

Ending Ridge A single friction ridge that terminates within the friction ridge structure.

Evaluation The third step of the ACE-V methodology. At this step an examiner assesses the value of the details observed during the analysis and comparison steps and thereby results in a conclusion.

Exclusion The determination by an examiner that there is sufficient quality and quantity of detail in disagreement to conclude that two areas of friction ridge impressions did not originate from the same source.

Exemplar The known prints of an individual, recorded electronically, photographically, by ink, or by another medium.

Fingerprint An impression of the friction ridges of all or any part of the finger.

Focal Points

1. In classification, those areas that are enclosed within the pattern area of loops and whorls. They are also known as the core and the delta.

2. In ACE-V, the areas selected for comparison purposes (see Anchor Point and Target Area).

Friction Ridge A raised portion of the epidermis on the palmar or plantar skin, consisting of one or more connected ridge units.

RETIRED

Friction Ridge Detail An area comprised of the combination of ridge flow, ridge characteristics, and ridge structure.

Friction Ridge Unit A single section of ridge containing one pore.

Furrows Valleys or depressions between friction ridges.

Galton Details see "characteristics"

Henry Classification A system of fingerprint classification named for Sir Edward Richard Henry (1850 - 1931).

Highly Probable Evidence is very persuasive, yet some critical feature or quality is missing so that an identification is not in order.

Hyperhidrosis Excessive sweating the hands and feet.

Hypohidrosis A disorder in which a person exhibits diminished sweating in response to appropriate stimuli.

Hypothenar Meaty portion of the palm below the little finger extending to the wrist.

IAFIS The acronym for Integrated Automated Fingerprint Identification System, the FBI's national fingerprint database. IAFIS has been replaced by NGI.

Impression Number The unique case specific number or number/letter combination which indicates a particular friction ridge impression for bench note referencing and reporting purposes. (see index below).

Index Index, Indexed or Indexing refers to the unique case specific number or number/letter combination which indicates a particular friction ridge impression for bench note referencing and reporting purposes. Index is also synonymous with impression number.

Identification
1. See Individualization.
2. In some forensic disciplines, this term denotes the similarity of class characteristics.

Identification Value References the quality of a friction ridge impression that meets the criteria as outlined in LP SOP 18.

RETIRED

Incipient Ridge A friction ridge not fully developed that may appear shorter and thinner than fully developed friction ridges.

Inconclusive During Evaluation, the conclusion reached that neither sufficient agreement exists to individualize nor sufficient disagreement exists to exclude.

Individualization The determination of an examiner that there is sufficient quality and quantity of detail in agreement to conclude that two friction ridge impressions originated from the same source.

Inked print A deliberate and intentional recording of the friction ridge details present on the fingers, hands, and feet for the purpose of comparison to latent prints or for personal identification records. This recording is normally accomplished by applying a substance such as black printers ink to the friction ridge area and then bringing it in contact with a suitable receiving surface such as a fingerprint card or piece of white paper.

Known Print (Finger, Palm, Foot) A recording of an individual's friction ridges with black ink, electronic imaging, photography, or other medium on a contrasting background. See inked print.

Latent print
1. Transferred impression of friction ridge detail not readily visible.
2. Generic term used for questioned friction ridge detail.

Lateral Reversal A mirror image.

Level 1 Detail Friction ridge flow and general morphological information.

Level 2 Detail Individual friction ridge paths and friction ridge events (e.g., bifurcations, ending ridges, and dots).

Level 3 Detail Friction ridge dimensional attributes (e.g., width, edge shapes, and pores).

Lift or Lifter An adhesive or other medium on which recovered friction ridge detail is preserved.

Matrix The substance that is deposited or removed from friction ridge skin when leaving an impression.

NGI Next Generation Identification; the FBI biometric database which replaced IAFIS.

RETIRED

No Value	References a friction ridge impression that is not suitable for identification or comparison.
Target Group	Is a group of level two friction ridge detail which can be associated with an anchor point in an effort to effect a conclusion Level three detail may also be utilized in conjunction with level two detail (but only when in both impressions it is clear and distinct).
Thenar	Meaty portion of the palm below the thumb.
Tonal Reversal	Pertaining to friction ridge analysis it is the inversion of shading and color tone.
Polydactlyism	The presence of supernumerary digits (one or more extra fingers or toes).
Preservation	A method by which a latent print is recovered for comparison with known inked prints. Preservation can be accomplished by proper handling, photography, lifting or any other acceptable method.
Verification	The fourth step of the ACE-V methodology. The independent opinion of an examiner as to the conclusion effected by another qualified examiner.

RETIRED

A. Purpose:

To establish maintenance protocols for the latent print section's superglue processing chambers.

B. Responsibility:

Latent Print Examiners

C. Procedure:

1. For superglue chamber operation and maintenance refer to the operator's manual kept in the latent print processing laboratory.

2. A log will be kept for each processing chamber to record the date that filters were changed.

3. Chambers not operating properly will be removed from service until repaired.

RETIRED

A. Purpose:

To establish a maintenance schedule for the replacement of particulate filters for the latent print section's downflow workstations.

B. Responsibility:

Latent Print Examiners

C. Procedure:

1. At least once every six months, the filters for downflow workstations will be replaced with new filters.

2. A log will be kept for each downflow workstation to record the date that filters were changed.

RETIRED

1. Purpose:

To assure the accuracy of latent print entry and consistent candidate target hits of the Connecticut Automated Fingerprint Identification System (CT-AFIS).

2. Responsibility:

CT-AFIS Administrator

3. Procedure:

1. The Latent Print Unit Supervisor/Lead shall appoint an administrator to function test the CT-AFIS latent workstations.

2. On a quarterly basis a sample print is searched through a selected AFIS latent workstation(s). Workstations shall be selected on a rotational bases. It is expected that at each workstation will be function tested no less than once a year.

3. The target candidate of the sample print must be included within the top 3 generated candidates. If the target candidate is not found within the top 3 candidates, the sample print searched will be checked to make sure that it was entered into the system properly. If it is determined that the latent workstation or CT-AFIS itself is not functioning properly, then the CT-AFIS Administrator will notify the Latent Print Unit Supervisor/Lead or who in turn will notify the following:

    a. DSS Deputy Director of Identification Services
    b. Director of DSS
    c. DESPP/DSS Chief Information Officer (CIO)

4. Workstations found not to be functioning properly will be taken out of service until repaired or replaced.

5. The results of each search test will be kept in a log book in the CT-AFIS administrator's office along with the known target sample.

RETIRED

A. Purpose:

To set guidelines and procedures for Digital Image Management

B. Responsibility:

Latent Print Examiners

C. Procedure:

1. General Evidence Procedure for Submitted Digital Media

1.1 All submitted digital images will be scanned for viruses in a laboratory workstation containing up-to-date antivirus software regardless of whether or not enhancements will be performed. This step is necessary to protect against computer viruses and potential corruption of the AFIS database and the DSS Local Area Network (LAN).

1.2 Upon receiving media containing digital images of latent print evidence, the assigned examiner will inspect the physical media for any visible or obvious signs of damage. Any damage will be noted on an appropriate worksheet. If necessary, the submitting agency will be contacted prior to attempting any recovery or repair of the submitted digital media found to be damaged.

1.3 Once the inspection and virus check of the submitted media has been performed, the digital images will be reviewed. If the submission is found to contain all images of no value or insufficient quality/quantity, a contact sheet should be printed out for the case jacket and no archive copy of the images will be retained. This contact sheet will have the date the sheet was printed present. If the images have some value, they will be copied to an appropriate "read only" storage device such as CD-R or DVD-R media or transferred as “read only” image files into a virtual case folder. These digital images that have been transferred to virtual case folders are considered working copies and not considered evidence. These images may now be enhanced utilizing approved software (see Appendix 2). The enhancement process will be documented either through metadata, history files or notes. Image files that have been enhanced or modified in any other fashion will be renamed and saved in a virtual case folder.

1.4 A virtual case folder will be created for storage of computer files created by the latent print examiner or submitted to the laboratory for analysis. The virtual case folder shall incorporate the laboratory case number. Upon completion of the case being examined, the virtual case folder shall be transferred to a secure server and also written to write once only digital media which will be filed in the latent print file storage area until such time that they are transferred to the laboratory’s archive file storage area. The write once digital media will be created as a sub-item

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in Justice Trax and tracked through chain of custody. This media should be transferred into the Latent Print File Storage upon completion of the case.

1.5 Please refer to LP SOP-26 for electronically submitted latent prints.

2. General Evidence Procedure for Submitted Lifts

2.1 Upon receiving latent transfer lift(s) for analysis or comparison, the examiner shall determine if the lift(s) will be digitized and entered into a virtual case folder on the computer being utilized (the main or parent folder shall contain the laboratory case number). Lifts containing friction ridge impressions of value will be imaged using a flatbed scanner or digital camera. The examiner shall determine the appropriate digital imaging capture method. The following are recommended scanner & camera settings:

Scanner Reflected Images
1000 ppi or the minimum acceptable AFIS input (whichever is greater)

Scanner Transparent Images
1000 ppi or the minimum acceptable AFIS input (whichever is greater)

Digital Camera & Scanner Settings (file type utilized is at the option of the examiner)

Image/Capture size set to:	Largest size possible
JPG File Type:	(set to highest quality setting available)
Tiff File Type:	(set to highest quality setting available)
RAW File Type:	(set to highest quality setting available)
JPG+RAW Combination:	(highest quality setting available)

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2.2 Upon completion of the documentation process, all digital images shall be made part of the case file. Digital images shall be managed as specified in sections 1.3 and 1.4 above.

2.3 Photocopying or digital imaging can be utilized for the initial documentation of latent transfer lifts. The method of choice is at the option of the examiner.

2.4 Any printed images in the case jacket will have the date they were printed located on the page.

3. General Evidence Procedure For Submitted Film Negatives

3.1 Film negatives may be digitized by using a Latent Print Unit flatbed scanner. The examiner shall determine the appropriate scanning method. The following recommended scanner settings are:

Scanning Film Negatives
1000 ppi or the minimum acceptable AFIS input (whichever is greater)

Capture File Types (type utilized is at the option of the examiner)

JPG File Type	(set to highest quality setting available)
Tiff File Type	(set to highest quality setting available)
RAW File Type	(set to highest quality setting available)

3.2 Scanned images may be saved as a TIFF, high quality JPG or any other format providing equal or better quality than a JPG image.

3.3 Upon case completion, scanned images of value will be managed as specified in sections 1.3 and 1.4 above.

4. General Evidence Procedure For Submitted Physical Evidence For Processing

4.1 Visible or developed friction ridge impressions determined to be of value will be documented utilizing digital imaging.

4.2 Upon completion of the documentation process all photographs and digital images shall be made part of the case file.

4.3 Latent print examiners wishing to document evidence during the examination process will use a laboratory issued digital camera or flatbed scanner. Once digital images have been captured, images on the captured card will be transferred as into a virtual folder. When the transfer is complete the images should be marked as read only. Upon completion of the case being examined, the virtual case folder shall be managed as specified in section 1.4 above.

4.4 Media cards may be reformatted after digital images contained therein have been transferred into a virtual folder.

5. Case File Documentation

5.1 Either history logs, notes or metadata will be used to record changes made to digital images for enhancement purposes. This will be the responsibility of the examiner performing such enhancements.

6. File Integrity And Retention

6.1 Upon case completion digital case files will be stored on a secure laboratory server in addition to the creation of a write once only archive disc.

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6.2 Archive disks and/or packaging will contain at minimum the following information:

a. Division of Scientific Services Lab ID Number
b. Creators Initials
c. Other descriptive information as determined by the examiner.
d. Barcode label affixed to the exterior of the CD envelope

7. Flatbed Scanner Function Test

7.1 An optical target containing known measurements will be used on a bi-annual basis to test and assure flatbed scanner accuracy. An offset of more than 1mm in one square inch shall be considered unacceptable.

7.2 A log book containing the results of such test and the optical target shall be kept in the latent print section.

7.3 Any equipment found not to perform correctly either through external testing or system diagnostics shall be immediately removed from service until repaired or replaced.

8. Best Practices for the Digital Processing and Enhancement of Latent Print Evidence

8.1 The latent print examiner will determine if image enhancement of latent print evidence is required. Enhancements shall be performed on "read only" or "duplicate" images unless the enhancement software (such as CSIpix) automatically renames the file on a save command. Duplicate copies of original images shall be known as "working copies" or "working images". Original source images shall not be altered.

8.2 Only approved software shall be utilized for enhancing digital images (see Appendix 2). Tools that are part of the State of Connecticut AFIS or Federal NGI ULW are also authorized for use.

8.3 When performing image enhancements the examiner will refer to the Image Processing Guidelines when applicable (see Appendix 1)

9. AFIS Digital Image Entry

9.1 When needed only laboratory provided thumb drives will be utilized when transferring digital images into the DESPP CT-AFIS/NGI database.

9.2 Such thumb drives shall be dedicated to laboratory use only.

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9.3 Digital images imported into the CT-AFIS/NGI database will be appropriately calibrated as required by the system software. This is to ensure that accurate searches are conducted.

10. Function Testing of New Enhancement Software

All new latent print Unit software utilized for enhancing images will be checked for consistency and repeatability of results prior to being used in casework. Validations will be conducted when the Unit receives new software for use in casework. This SOP is to act as guidance for the validation plan; if modifications are required for the plan, these will be approved by the Deputy Director and Director. The validation plan may also be used for software upgrades that reflect a major change. Proprietary AFIS systems not under the control of DSS shall not apply to this section.

In the case of minor updates to software, a performance check is sufficient to determine if the new upgrade is acceptable for use in casework. A performance check will also be conducted if a piece of equipment is sent out for repairs and is now ready to be placed back into service. Performance checks may also be conducted when casework software is loaded for the first time onto a computer. Refer to GL-22 for guidance.

Validation Procedure:

1. A plan will be written detailing the steps of the validation procedure.
2. The plan will be reviewed by the Supervisor or their designee, Assistant Director, Deputy Director and Director prior to commencement of the validation. The Deputy Director or their designee will complete the Laboratory Method Validation Form. After the validation plan is reviewed, the Deputy Director or their designee and the Director will approve the plan by completing the plan approval portion of the form. Approval may also have the initials of the plan approver on the printed pages of the validation plan.
3. This plan may be updated during the process as new information or results of the validation require changes to the original plan. Any changes to the plan will need to be re-approved Deputy Director and or their designee and the Director.
4. After completion of the validation, the results will be clearly communicated in the validation and the results will be submitted for review by the Supervisor or their designee, Assistant Director and Deputy Director.
5. These validation results will be evaluated to determine if SOPs changes are needed prior to casework use. The approved software list will be updated with the validated software.
6. A final memo will be issued by the Deputy Director or their designee of the review of the validation and its results. The memo will have a statement indicating if the software has successfully passed its validation and is acceptable to be placed into service or use for casework. This memo will be approved by the Quality Manager and the Director.

Performance Check Procedure:

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a. Samples images will be imported into the software.

b. Filters utilized for enhancement will be applied to the images and the settings will be recorded.

c. The enhanced images from the series of testing will be saved.

d. This above process will be repeated for each filter utilizing the same settings two additional times for a total of three images. The computer being utilized for the testing will be restarted prior to each of the two additional test cycles.

e. The processed images will be analyzed to determine any unacceptable differences. A digital imaging difference filter or its equivalent or a hashing program may be utilized to make this determination.

f. Tested software and filters which produce unacceptable results will not be used for case work.

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APPENDIX 1

Image Processing Guidelines

The following is a guideline of filters and tools (or their equivalents) which can be used to enhance an image. As such it is ultimately up to the examiner to determine which types of tools and filters are to be utilized for a particular enhancement based on his/her experience.

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1. Do not do anything that can change an original image (the original image should be write protected or duplicated).
2. Consider using the a color mode to remove one color value:
    a. RGB
    b. Chromatic FFT
    c. CMYK
    d. Lab Color (Lightness channel, Channel a (green + red) or Channel b (green + blue) when using LAB mode it is necessary to adjust the levels in Channel A and Channel B to properly evaluate the information contained within those two channels.
3. The following tools or their equivalent can be used to remove two or more color values as well as adjust the color values so that a color channel may be used to eliminate the background.
    a. Hue & Saturation (eliminates two or more color values: used in conjunction with Calculations to eliminate background noise)
    b. Color Balance
    c. Black & White
    d. Variations
    e. Photo Filter
4. The following tools can be used to adjust tonal range and contrast:
    a. Levels
    b. Curves
    c. Layer and Object Filters
    d. Shadow/Highlight (balance tonal range and contrast)

e. Exposure (balance tonal range)

f. Burn and Dodge (see Guidelines for Brush Tools below)

g. Brightness/Contrast

5. The following filters can be used to sharpen an image:

   a. Unsharp Mask

   b. Smart Sharpen

   c. Sharpen Edges

6. Guidelines for Brush Tools (Burn and Dodge)

   a. Always choose a soft (feathered) round brush.

   b. Always choose a brush size that is at least four to five ridges in diameter.

   c. Always apply the brush at a right angle to the ridges. Do not follow ridge flow.

7. Feathering Guidelines for Area of Interest Tools (Marquee and Lasso Tools)

   a. Determine image resolution

   b. From the tool bar choose the appropriate area of interest tool.

   c. Use the mouse to select the area of interest to be processed.

   d. Go to Modify > Feather

      i. If resolution is less than 1500ppi, multiply the number of hundreds by 3. The sum is the "Feather Radius" value you must use (i.e. 1000ppi = 3000).

      ii. If resolution is more than 1500ppi, multiply the number of hundreds by 4. The sum is the "Feather Radius" value you must use (i.e. 2400ppi = 9600).

   Note: If you select the area first then try to enter the feather radius in the Feather field on the tool options bar, it will have no effect on the current selection; it will only effect future selections.

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APPENDIX 2

Approved Software

AccessData FTK Imager
For the forensic imaging of digitally captured latent impressions determined to be evidence.

Adobe Photoshop
For viewing, analyzing and enhancing digital images and preparing exhibits.

CorelDraw Graphics Suite
For viewing, analyzing and enhancing digital images and preparing exhibits.

CSIpix
For viewing, analyzing and enhancing digital images and preparing exhibits.

Epson Scan Software
For digitizing reflective and transparent materials including film negatives.

Jdirprinter
For printing a list of files contained within a directory and/or subdirectory.

PieStudio
For extracting & write protecting digital images from storage media and also used for preparing and printing proof sheets.

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Appendix 3

Focus Stacking Capture Guidelines

Focus stacking is an acceptable technique to reveal detail in an image that exhibits distortion due to depth of field issues. The following are image capture guidelines to use when an examiner determines that focus stacking is appropriate for documenting impression evidence of potential value:

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Capturing Images for Focus Stacking

1. The camera must be mounted on a copy stand, tripod or other device with the ability to make minor lens to target distance adjustments. The Latent Print Unit has a "focusing rail" available for such use.

2. Mount the camera on the appropriate device, the camera should be completely in manual mode (for both exposure and focusing).

3. Focus the camera to the middle distance of the object of interest and manually adjust the exposure to the desired result.

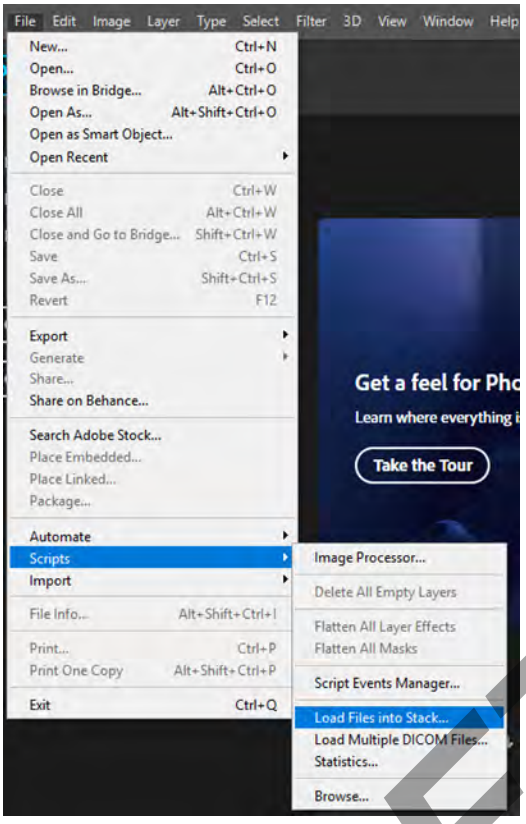
4. Once the proper exposure has been set, focus to either the closest, or the furthest part of the object of interest. Take the first photograph as part of a stack of images. From this point on do not adjust the camera lens manual focusing ring. Focusing will be accomplished by either decreasing or increasing the distance between the camera lens and object of interest.

5. After the first photograph has been taken, either slightly increase or decrease the camera lens distance, as appropriate, to partially bring into focus another part of the object of interest that was previously not in focus. If using the Latent Print Unit Focusing Rail, a ¼ turn of the focusing knob will displace the camera's lens to target distance approximately 1mm. One complete rotation of the focusing knob will displace the camera's lens to target distance approximately 4mm.

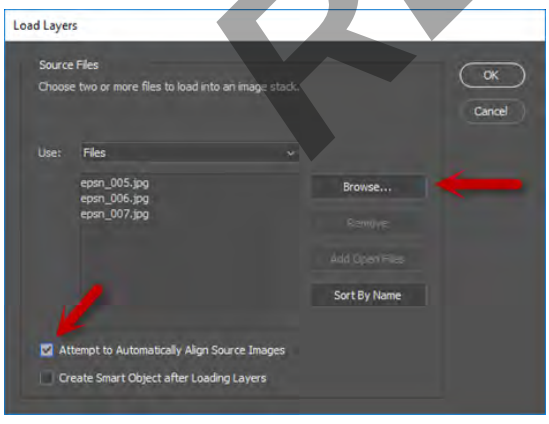
6. Continue to adjust the distance of the camera's lens in the same direction, at approximately the same increments, until a series of photographs has captured (in focus) the complete object of interest. The captured photographs (images) may now be imported into appropriate image stacking software.

Focusing Stacking with Adobe Photoshop

1. **File > Scripts > Load Files Into Stack**



1.1. Load your images and make sure "***Attempt to Automatically Align Sources***" is selected, click "OK".



2. **Select > All Layers**

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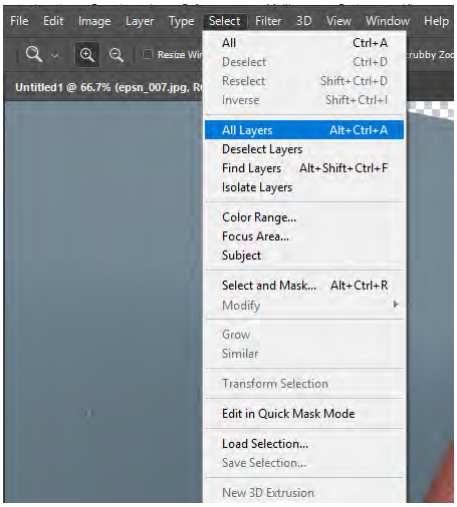
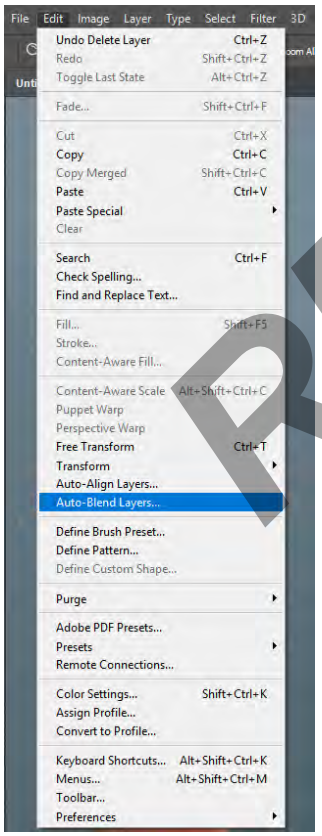


3. Edit > Auto-Blend Layers



3.1. Select “Stack Images”, select “***Seamless Tones and Colors***”, click OK

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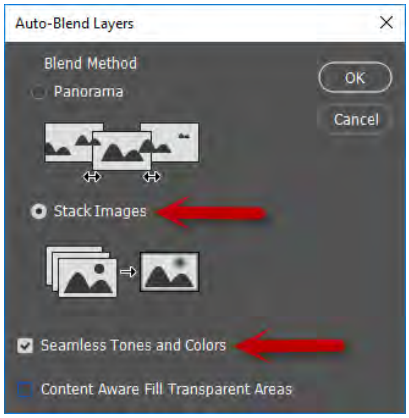
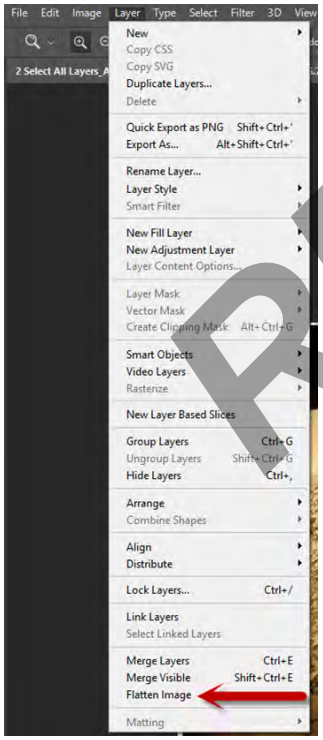


4. **Save the stacked image file as a "psd" and retain in the case file.**

5. **Layer > Flatten Image**

   5.1. Once the image has been flattened save as an uncompressed png, bmp or tiff.



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Purpose:

To index statutory requirements and guidelines which may apply to the latent print section.

Responsibility:

Latent Print Examiners

Procedure:

A. The following Connecticut General Statutes specifically reference fingerprints and fingerprint records.

29-7b	Division of Scientific Services
29-11	State Police Bureau of Identification
29-12	Arrested Persons
29-12a	Fingerprints in Electronic Format
29-14	Duties of SPBI
29-15	Return of Fingerprints
54-76l	Records of Youthful Offenders

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Purpose:
To provide an overview summary and specific training modules for the complete training of a latent print examiner.

Responsibility:
New Latent Print Examiners

Procedure:
The following outline of training modules will be used for assessing the qualifications and/or training of all new persons assigned to the Latent Fingerprint Unit. The amount of time necessary to achieve competency in any one area will depend on the new examiner's previous training and experience. Previous training and experience will be consider when assessing the completion of each training module.

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1. **Training Modules**
   - Module 1: Introduction to the Laboratory
   - Module 2: History of Personal Identification
   - Module 3: The Biology of Friction Ridge Skin
   - Module 4: Current Methods of Fingerprint Identification
   - Module 5: Recording Known Standards
   - Module 6: Introduction to Latent Prints
   - Module 7: Latent Print Processing
   - Module 8: Latent Print Capture Methods
   - Module 9: Comparison of Friction Ridge Impressions
   - Module 10: Automated Fingerprint and Palm Print Identification System
   - Module 11: Preparation of Demonstrative Fingerprint Evidence
   - Module 12: Written and Oral Communication
   - Module 13: Supervised Case Work and LIMS
   - Module 14: Expert Witness Testimony and Moot Court
   - Module 15: Latent Print Comparison Competency
   - Module 16: Latent Print Processing Competency

2. **Training Program**

2.1. The above listed training modules will be incorporated into a document, which is made part of this SOP and titled: Training Program for Latent Print Examiner

2.1.1. The latent print unit supervisor shall be responsible for reviewing, and as necessary updating, the training manual prior to the training of a new employee.

2.1.2. The training may be conducted in a step fashion with Latent Print Comparison being completed and the analyst being authorized to conduct casework in that area while still completing the training for Latent Print Processing.

3. Assignment of Case Work

3.1. Since training may be conducted in a step fashion and can be separated as comparison casework versus processing casework, if the supervisor of the latent print unit determines that a new examiner is ready to start case work prior the completion of all the training modules, then the supervisor may submit the training binder to the Quality Manager to review the areas completed. Once the binder is reviewed and the training considered sufficient, form GL-14.3 will be completed by the Quality Section and the new examiner may be given a competency test. If the examiner passes the competency test and oral/written examination, then a memo will be written to the Director of Identification Services indicating the examiner successfully completed the competency. Once reviewed by the laboratory administration, a Qualtrax workflow will be initiated for the authorization of the analyst in the areas they are deemed competent to conduct casework. The workflow may include the following in which the analyst is being authorized to participate in:

3.1.1. Handle evidence,
3.1.2. Perform examination and/or analytical methods,
3.1.3. Utilize all applicable equipment in methods described in SOPs,
3.1.4. Report, review and authorization of results
3.1.5. Perform administrative reviews
3.1.6. Perform validations in the discipline
3.1.7. Development of new methods
3.1.8. Modification of methods
3.1.9. Verification of methods

3.2. New examiner's conducting case work will receive two independent technical reviews in addition to an independent administrative review on all cases for a minimum of six months. This will be done to determine if further training is warranted. If there are no training or other issues that need to be addressed after six months have elapsed, then the second technical review may be terminated. If on the other hand, issue(s) are evident, then these issues will be identified and corrective training actions taken and documented until the issue(s) is resolved.

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4. Notification of Completed Training

4.1. At the end of the entire training program, the supervisor will submit the training binder to the Quality Manager for review. Once the binder is reviewed and the training considered sufficient, form GL-14.3 will be completed by the Quality Manager and the examiner will be given a competency test. Refer to Section 3.1.

4.2. If the examiner does not pass the competency case and oral and/or written examination. The area of deficiency will be determined and the examiner will be retrained.

4.3. Once authorized, the analyst will perform casework and after gaining experience in conducting analysis and reporting findings in a variety of evidence type/conclusions in the discipline, their experience will be evaluated for the ability to conduct technical reviews. This evaluation period may include technical review ghosting with an experienced examiner. Once the analyst has completed the evaluation period, a memo/email shall be written to the Deputy Director stating that the analyst has successfully completed their evaluation period and has been deemed competent to conduct technical reviews in that discipline.

4.4. The Deputy Director, after review, may endorse this memo/email and generate a request to the Quality Section requesting that this analyst be authorized to perform technical reviews based upon the analyst's casework experience in that discipline. A Qualtrax workflow will be initiated for the authorization of the analyst to participate in the following: perform technical reviews of cases and testimony and/or administrative reviews (if not previously authorized).

5. Retraining

In the event that an examiner requires retraining, the area of retraining will be identified. The retraining will be provided and a competency / evaluation period will be initiated as determined by the Deputy Director of Identification along with the Unit Supervisor and the Quality Section. Refer to SOP-GL-14 and SOP-GL-1 for further guidance.

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Training Program for Latent Print Examiner

Introduction

The training program for a latent print examiner position shall be comprised of formal internal instruction coupled with practical experience based apprenticeship and mentoring. Training may also include external instruction as determined by the unit supervisor/lead or Deputy Director. Previous practical experience and training will be considered by the latent print unit supervisor and approved by the Deputy Director of Identification Services and the Director of the Division of Scientific Services if found to be acceptable. Upon administrative approval, module areas covered by previous experience and training can be marked as complete by the Unit supervisor/lead.

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External instruction may be provided through online courses, such as those provided by the National Institute of Justice, and specific courses, which are offered through a number of educational conferences and vendors, such as but not limited to: the International Association for Identification, the Sirchie Fingerprint Laboratories and Ron Smith & Associates.

Internal instruction will be accomplished by assigning the new examiner to senior latent print examiners who will apprentice and mentor the trainee through all outlined modules which have not been administratively accepted as complete upon the initial review of the new latent print examiner's credentials.

The new latent print examiner's training will be reviewed monthly by the latent print unit supervisor/lead. Details as to the trainee's current status and progress will be documented through practical exercises and observations.

Depending on the trainee's previous level of experience and education (in addition to his/her ability to master the required performance objectives), the completion of the required module related tasks and

external training can range anywhere from thirty days for the experienced latent print examiner; and up to eighteen months or more for the inexperienced examiner.

MODULE TRAINING

Module 1: Introduction to the Laboratory
Module 2: History of Personal Identification
Module 3: The Biology of Friction Ridge Skin
Module 4: Current Methods of Fingerprint Identification
Module 5: Recording Known Standards
Module 6: Introduction to Latent Prints
Module 7: Latent Print Processing
Module 8: Latent Print Capture Methods
Module 9: Comparison of Friction Ridge Impressions
Module 10: Automated Fingerprint and Palm Print Identification System
Module 11: Preparation of Demonstrative Fingerprint Evidence
Module 12: Written and Oral Communication
Module 13: Supervised Case Work and LIMS
Module 14: Expert Witness Testimony and Moot Court
Module 15: Latent Print Comparison Competency
Module 16: Latent Print Processing Competency

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Module 1: Introduction to the Laboratory	
Trainee:	
Trainer	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Orientation to Laboratory Facility and Personnel	Give complete tour of the laboratory and introduce trainee to all management and staff as appropriate.		
Instruction of Organization Structure, Chain of Command, and Code of Ethics	Provide trainee a copy of the organizational chart of DSS. Make sure the trainee is aware of his/her immediate supervisor. Provide pertinent contact phone numbers and email addresses. Provide trainee a copy of GL-5 and Guiding Principles to read and understand.		
Laboratory Security	Assure that background check has been completed. Issue trainee security ID Card and Proximity Reader Card to access the laboratory. Provide keys to office & personal storage locker.		
Read Appropriate Laboratory Manuals and Receive Instruction with Appropriate Safety Equipment	Read Laboratory Quality Manual on Qualtrax Server and meet with Quality Manger. Explain the quality assurance & control program and stress its importance to the overall operation of the laboratory. Read DPS A&O Manual on the laboratory intranet. Read Emergency & Safety Procedures GL2 on Qualtrax Server and meet with the Safety Officer. Review the fire alarm system and building exits. Explain specific procedures for handling hazardous situations such as chemical spills, bio hazards and proper disposal of sharps. Trainee is informed of the Hepatitis B vaccine and the need to be protected. Trainee is made aware of, and instructed in, the proper use of personal protective equipment. Trainee is made aware of chemical and eyewash		

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	stations. Trainee is made aware of fire extinguisher locations. Review the LP SDS manual and make sure that trainee knows where it is located. Review LP Unit SOPs Meet with Firearms Unit for instruction on firearm safety and review FA SOP 3.		
Discuss the following laboratory policies.	Case confidentiality State of CT Ethics Policy Inquiries from the press Inquiries from defense attorneys Receipt of a subpoena Providing copies of case files Reexamination of evidence		
Evidence Storage	Show and explain evidence storage areas in the LP Unit including evidence and case file storage.		
Discuss office supply purchases	Explain the supply system and set up new trainee with proper supplies and equipment.		
Suggested Resources	SDS Sheets for applicable chemicals. Safety for the Identification Specialist by Nancy Masters. OSHA Website: www.osha.com Hepatitis Website: www.hepnet.com Work Place and Safety Health: www.cdc.gov		
COLLECT Security	Complete COLLECT Security Awareness Level 1		
20 Question Test	Trainee will be given a 20 question test pertaining to the above materials (80% shall be passing).		
Completed forms GL-2.2 and GL-14.1	The trainee will initially meet with the Quality Manager to begin these forms and will later complete the remainder of the forms after reading and videos are completed.		

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Module 2: History of Personal Identification	
Trainee:	
Trainer:	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Research Early History of Personal Identification	Prepare a timeline illustrating the various types of identification methods.		
History and Current methods of Friction Ridge Identification	Prepare a 15 to 30 minute PowerPoint presentation of the history and current methods of friction ridge identification.		
Suggested References	Quantitative-Qualitative Friction Ridge Analysis, by David Ashbaugh The Fingerprint Source Book, by NIJ Advances in Fingerprint Technology, by Lee & Gaensslen		

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Module 3: The Biology of Friction Ridge Skin	
Trainee:	
Trainer:	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Friction Ridge Skin Anatomy	Read : Ridgeology by David Ashbaugh http://onin.com/fp/ridgeology.pdf Friction Ridge Skin, by James Cowger Fingerprint Source Book, chapters 2 & 3 www.ncjrs.gov/pdffiles1/nij/225320.pdf		
Oral Exercise	Be able to explain the growth properties of friction ridge skin and its relation to the fundamental principle of uniqueness.		
Presentation	Demonstrates the ability to explain friction ridge development and the principal functions of the Eccrine, Apocrine and Sebaceous glands.		
Illustration Exercise	Researches and then prepares a series of hand drawn illustrations depicting the various areas and zones of the palmar surface to include ridge flow and flexion crease characteristics.		

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Module 4: Current Methods of Fingerprint Identification	
Trainee:	
Trainer:	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Research and Understands Fingerprint Pattern Definitions	Displays a fundamental knowledge of the Henry system and pattern classification: Arch, Tented Arch, Ulnar Loop, Radial Loop, Plain Whorl, Double Loop Whorl, Central Pocket Loop and Accidental Whorl.		
Short Paper	Prepares a short paper on the Henry System.		
NCIC	Understands what NCIC stands for and why it was established.		
III	Understands what III stands for and how it is utilized.		
Suggested Reference	FBI: The Science of Fingerprints Chapters 2,5,8		

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Module 5: Recording Known Standards	
Trainee:	
Trainer:	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Fingerprinting Methods	Understands various methods utilized to record friction ridge skin (inked, inkless, powder, live-scan).		
Major Case Prints	Understands the proper recording methods which are necessary to obtain major case prints.		
Practical Exercise	Practices taking good quality impressions, including major case prints, utilizing materials available at the laboratory.		
Post-Mortem Exemplars	Understands the methods utilized in the recording of post-mortem prints including the re-hydration of mummified friction ridge skin.		
Suggested References	The Use of Embalming Fluids in the Restoration of Mummified Fingers, S. Cook, JFI 46(5) RE: The Use of Embalming Fluids in the Restoration of Mummified Fingers, S. Cook, JFI 46(5) by G. Laskowski JFI 47(1)		

RETIRED

Module 6: Introduction to Latent Prints	
Trainee:	
Trainer:	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Latent Print Residue	Understands the composition of latent print residue.		
Latent Print Persistency	Researches factors which affect the persistency of latent print residue.		
Forgery & Fabrication	Researches and understands methods of friction ridge forgery and fabrication including techniques for their detection.		
Presentation	Prepares a 20 to 30 minute PowerPoint presentation relating to the above completed tasks.		

RETIRED

Module 7: Latent Print Processing	
Trainee:	
Trainer:	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	**Requirement**	**Date Completed**	**Trainee/Trainer Initials**
SOP LP 04	Read and Understand		
Research Processing Methods	Process the following items: Glass Notebook paper Waxed paper Plastic bags Handguns and/or Long-arms Thermal paper Unfinished wood Finished wood Fabric Rough surfaced items Adhesive side of different types of tape Non-adhesive side of different types of tape Blood Impression (synthetic blood may be used) Grease / Oil Impression		
Sequential Processing	Understands the benefits of properly using sequential processing.		
Team Approach to Evidence Processing	Understands the team approach of processing evidence through multiple disciplines and what types of LP analysis can be detrimental to other forensic disciplines.		

RETIRED

Module 8: Latent Print Capture Methods

Trainee:

Trainer:

Date Module Completed: **Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Photographic Principles/Concepts	The trainee will be familiar with the following photographic principles and concepts: a. The relationship between shutter speed and f-stop b. F-stop and image sharpness c. The inverse square law d. Diffused lighting e. Direct lighting f. Side lighting g. Fill light h. Reflected lighting i. Tent lighting j. Transmitted lighting k. Transmitted lighting –dark field l. Transmitted lighting – reflected top fill		
Laser, UV & Alternate Light Sources	Safety Training & Practical experience utilizing a Laser, UV and ALS with appropriate safety goggles and barrier filters.		
Digital Images	Proper capture and preservation of digital images. Understands the effects of image compression and the difference between lossy and lossless image file types. Proper techniques for digitally enhancing friction ridge evidence. Review and understand SOP LP 12, Digital Image Management. Display a fundamental understanding of all applicable digital imaging software programs utilized in the latent print unit. Read and understand State v. Swinton.		
	Review of validations of software used in the latent		

RETIRED

	print unit.		
Suggested References	Polaroid Photomacrography with the MP-4 Nikon SLR Cameras, by Shipman Digital Imaging, by Adrian Davies Police Photography, by Larry Miller		

RETIRED

Module 9: Comparison of Friction Ridge Impressions
Trainee:
Trainer:
Date Module Completed: **Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Fingerprint Orientation	Trainee will indicate correct orientation for viewing partial fingerprint impressions.		
Analysis	Trainee will understand the qualitative – quantitative analysis of friction ridge evidence.		
Friction Ridge Detail	Trainee will research and give a 5 minute oral presentation on the various details utilized by latent print examiners in assessing friction ridge impressions.		
Anchor Point and Target Group	Trainee will demonstrate an understanding the meaning of the terms Anchor Point and Target Group.		
ACE-V	Trainee will prepare a short report as to why ACE-V qualifies as a scientific method. Read Meeting the Fingerprint Admissibility Challenge in a Post-NAS Environment - JFI 430/61(5)2011		
Friction Ridge Distortion	Trainee will have a practical understanding of friction ridge distortion and acceptable tolerances of distortion. A PowerPoint presentation will be presented as to what was learned through experimentation.		
Color Reversal and Positional/Lateral Reversal	The trainee will be able to explain the following conditions: Color Reversal and Positional/Lateral Reversal.		
Conclusions	The Trainee will understand and know when to apply the various conclusions applicable to a latent print examination/analysis.		

RETIRED

Module 10: Automated Fingerprint and Palm Print Identification System	
Trainee:	
Trainer:	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Research	Research and prepare a short paper on AFIS and NGI.		
Capture Requirements	Understand the required capture requirement for AFIS entry of latent impressions and live scan capture.		
Feature Marking	Understands and demonstrated the ability to correctly mark friction ridge features for AFIS search entry.		
Matching Process v. Comparison	Can explain the difference between the AFIS matching process and a comparison conducted by an examiner.		

RETIRED

Module 11: Preparation of Demonstrative Fingerprint Evidence	
Trainee:	
Trainer:	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Fingerprint Chart/Exhibit	The trainee will produce a fingerprint chart with software utilized by the Latent Print Section.		
Oral Exercise	The trainee will present the chart and explain the process utilized.		

RETIRED

Module 12: Written and Oral Communication	
Trainee:	
Trainer:	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Mock Case Reports	Five mock cases will be analyzed by the trainee of which the following scenarios will be reported: a. A suspect identification b. An exclusion c. An inconclusive result in which additional inked standards are needed. d. An inconclusive result due to the quality of the latent impression e. No latent prints of value were developed/found.		
30 Minute Presentation	Trainee's choice: Prepare a 30 minute presentation on any forensic topic.		
Public Speaking Techniques	Research public speaking techniques and give a PowerPoint presentation of at least 20 min.		

RETIRED

Module 13: Mock Casework	
Trainee:	
Trainer:	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Justice Trax LIMS-Plus System	Trainee will be assigned mock cases and become proficient in using Justice Trax LIMS-Plus System		
Evidence Processing	As part of the mock casework, the trainee will be asked to process evidence under the supervision of a latent print examiner. As part of this casework, a report will be generated.		
Latent Print Comparisons	As part of the mock casework, the trainee will be asked to review latent impressions to known impressions. As part of this casework, a report will be generated.		

RETIRED

Module 14: Expert Witness Testimony and Moot Court	
Trainee:	
Trainer:	
Date Module Completed:	**Unit Supervisor:**

Task/Objective	Requirement	Date Completed	Trainee/Trainer Initials
Verbal Skills/Direct Examination	The trainee will be judged on his/her ability to explain laboratory protocols for processing and analyzing evidence including the ACE-V methodology.		
Cross Examination	The trainee will be judged on his/her ability to respond to difficult questioning while maintaining his/her composure. The trainee will be judged on his/her ability to explain their judgment on casework.		

RETIRED

Module 15: Latent Print Comparison Competency
Trainee:
Trainer:
Completed: **Pass** **Fail** **Unit Supervisor:**

1. The competency test shall consist of at least 5 latent impressions and 3 known sets of exemplars.
    a. Passing shall be that 100% of all identifiable friction ridge impressions were attributed to their source with no erroneous identifications being made.

2. Oral or written examination consisting of twenty questions.
    a. Passing shall be that 80% of all questions were answered satisfactorily.

Note: upon the successful completion of the competency test and oral/written examination, the examiner will be authorized to conduct casework. For a period up to three months, the examiner will have their cases technically reviewed by two other competent examiners.

RETIRED

Module 16: Latent Print Processing Competency
Trainee:
Trainer:
Completed: **Pass** **Fail** **Unit Supervisor:**

1. The processing competency test shall consist of at least one porous and one non-porous object.
    a. Passing shall be the successful development of all target impressions (target impression need not be of value for identification).

2. Oral examination consist of twenty questions.
    a. Passing shall be that 80% of all questions were answered satisfactorily.

Note: upon the successful completion of the competency test and oral/written examination, the examiner will be authorized to conduct casework. For a period up to three months, the examiner will have their cases technically reviewed by two other competent examiners.

RETIRED

Purpose:

To establish a validation procedure for chemicals/techniques being introduced to the latent print unit as a new method of enhancing/developing friction ridge detail. Additional guidance is located in GL-22 (Policy on Validation and Performance Checks).

Responsibility:

Latent Print Examiners

Procedure:

1. Chemicals/Reagents

Any new chemical/reagent being introduced to the latent print unit as a new method of developing or enhancing friction ridge detail will first be validated prior to actual use on case work.

Validation may include the following substrates: porous materials, non-porous materials, adhesive surfaces and/or bloody surfaces. If the new method is substrate specific, then only that particular substrate will be tested.

a. Examples of Porous Materials
    1. Copy Paper
    2. News Paper
    3. Brown Paper Bag
    4. Craft or Wrapping Paper
    5. Plain Envelope
    6. Manila Envelope
    7. Cardboard
    8. Wood

b. Examples of Non-Porous Materials
    1. Glass
    2. Plastic
    3. Glossy Materials (photo etc)
    4. Back Side of Tape
    5. Metal
    6. Painted Material

RETIRED

c. Examples of Adhesives Surfaces

1. Scotch Tape
2. Duct Tape
3. Masking Tape
4. Electrical Tape

2. DNA Sensitivity

The TL of the DNA Unit will be contacted regarding the proposed chemical/technique and its effect in regards to DNA testing. It should be determined if there is literature/research available regarding the use of a proposed chemical/technique and its effect on DNA testing. If there is literature/research available, this will be included in the validation plan. Depending on the literature/research, this will determine whether internal DNA testing is needed.

RETIRED

Purpose:

To outline the methods utilized by the latent print examiner for the comparison of friction ridge skin impressions.

Responsibility:

Latent Print Examiners

Definitions:

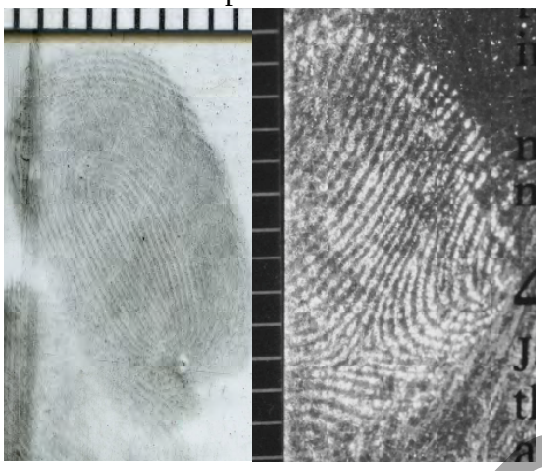
High Quality Friction Ridge Impression:
Level 1 detail is distinct
Level 2 details are distinct
Level 3 details are abundant and distinct

RETIRED

Medium-High Quality Friction Ridge Impression:
Level 1 detail is distinct
Level 2 details most are distinct
Level 3 details if present are minimal but distinct

Medium-Low Quality Friction Ridge Impression:
Level 1 detail is distinct

Level 2 details few are distinct
Level 3 details if present are minimal



Low Quality Friction Ridge Impression:
Level 1 detail may not be distinct
Level 2 details most are indistinct
Level 3 details are not distinct

RETIRED

Verifier: A competently trained analyst that will conduct an independent verification when requested by the primary examiner.

Procedure:

Indexing Friction Ridge Impressions of Value

1.1.
Friction ridge impressions selected for their potential to effect an identification will be documented and indexed on an appropriate worksheet (e.g. QR-LP1b, QR-LP1d, QR-LP-14 or other). These impressions will be clearly marked by the examiner. There should be no confusion as to the selected friction ridge impression and its associated index number, letter, or number letter combination. Examiners are not required to document or make comparisons to friction ridge impressions that are assessed as IQQ.

2.
ACE-V Methodology Overview

2.1.
The comparison of friction ridge skin impressions are conducted utilizing the Analysis, Comparison, Evaluation, and Verification (ACE-V) methodology. This includes both qualitative and quantitative analysis of the following three levels of friction ridge detail:

Level 1 Detail
Overall ridge flow / pattern shape
Cannot be used alone to individualize.

Level 2 Detail
Individual ridge path and events (bifurcations, ending ridges, and dots) used in conjunction with level one detail to individualize as well as to exclude.

Level 3 Detail
Ridge dimensional attributes (width, edge shape, pores) used in conjunction with level one and level two detail to form a conclusion.

RETIRED

2.1.1
Other Features Associated with Friction Ridge Impressions

a. Creases
b. Injury related features both temporary and permanent (scars/cuts/blisters)
c. Skin ailments such as warts

Note: Other features may be used in conjunction with friction ridge detail to individualize or exclude.

3.
EXECUTING ACE-V

3.1.
Analysis

3.1.1.
Friction ridge impressions selected as being of value will be indexed. Ridge events will include ridge endings, bifurcations, dots and other articulable ridge attributes that can be used to support a conclusion. Generated sufficiency worksheets (QR-LP-14) which were annotated as part of determining ridge event sufficiency for further review and comparison potential will be made part of the examiner's case file. The annotations shall be made so there is no question as to the marked details (see 3.1.3). Open fields may also be given weight and noted as a characteristic to document and illustrate areas lacking minutiae. The following classifications will be used to demonstrate the quality assessment of a latent impression of value.

a. **Identification Value (IV):** When an impression is assessed by the primary examiner to be Identification Value it shall:

1. Have a quality rating of High or Medium-high and;
2. Contain a minimum of (8) clear and distinct (green) minutiae/ridge events for finger impressions or a minimum of (10) clear and distinct (green) minutiae/ridge events for palm impressions and;
3. Contain a known anchor point such as a core, delta, or other distinct ridge formation or ridge flow which can be associated to its anatomical region. For palm impressions this shall include areas indicative of the Interdigital, Thenar, Hypothenar, Central Area, Carpal Delta or outer edge areas of the palm such as the writer's palm.
4. With the above criteria in mind, the analyst has the discretion to classify an impression as CV if in their opinion documented distortion, interference or other issues in the reviewed impression makes a comparison more complex (i.e. substrate interference).

b. **Comparison Value (CV):** When an impression is assessed by the primary examiner to be Comparison Value it shall:

1. Have a quality rating of Medium Low or Low or;
2. The impression contains less than eight (8) clear and distinct (green) minutiae/ridge events for finger impressions or less than ten (10) clear and distinct (green) minutiae/ridge events for palm impressions or;
3. The impression lacks a known anchor point or other distinct ridge formation or ridge flow which can be associated to its anatomical region or;
4. The impression contains distortion, interference or other issues in which the examiner determines a CV rating is more appropriate.

3.1.2.
When the primary analyst wishes to move forward for comparison an impression that has less than (8) eight level two detail in total for finger impressions and less than ten (10) level two detail in total for palm impressions, this impression shall be reviewed by a second analyst for sufficiency using QR-LP-14

RETIRED

(Sufficiency Worksheet). In reviewing the friction ridge impression for sufficiency, the second analyst will document all viable ridge events on the impression being reviewed and whether the impression is sufficient or insufficient to move forward to the comparison phase. The annotated analysis including the sufficiency decision will be printed out and provided to the primary examiner for the case file.

3.1.3.
Ridge events marked for sufficiency will be traced or plotted and color coded based on the examiner's confidence level of the feature being marked using laboratory software. Images should be calibrated to 1000ppi prior to analysis. Tools similar to the FreeHand or Dot tool will be the primary tools used to annotate the impression. In general the marking tool opacity should be set to an acceptable contrast level of 70% or higher. The following color codes will be used:

Green: High Confidence Level

Yellow: Medium Confidence Level

Red: Low Confidence Level (areas circled in red will indicate unsuitable for analysis)

Note: If another color code other than the above is used, the examiner will indicate the designations with a color code key on the printed copy in the case file.

RETIRED

3.2.
Comparison

3.2.1.
After a thorough analysis has been made of the unknown or questioned friction ridge impression(s) of value, it may be compared to the known impression(s) or exemplars. Exemplars that have not been opened, examined or compared will be reported as such.

3.2.2.
The comparison will be accomplished by comparing the friction ridge features documented during the analysis phase of the first friction ridge impression to the features of the 2nd friction ridge impression (which under most conditions will be obtained under controlled circumstances i.e. major case prints).

3.2.3.
Documented ridge events in the final comparison chart for identification should be marked in red, however the examiner may use an additional color to mark a place holder or to illustrate ridge counts or ridge flow.

3.2.4.

Types of Comparison

3.2.4.1.
The following types of comparison may be conducted:

a. Unknown vs Known

b. Known vs Known

c. Unknown vs Unknown

3.2.4.2.
Known Prints / Exemplar Impressions

3.2.4.2.1.
Known prints or exemplar impressions are those from a known source generally recorded under a controlled environment. Examples of known prints are:

a. Ten Print, Palm Print and Major Case Print Cards recorded by a law enforcement agency.

b. Elimination Print Submissions

c. John/Jane Doe Prints (serve as both known and unknown)

3.2.4.3.
Unknown Prints

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3.2.4.3.1.
Unknown prints or impressions are of unknown or questionable origin. Examples of unknown prints are:

Prints obtained from crime scenes (unknown)

John/Jane Doe Prints (serve as both known and unknown)

3.3
Evaluation

3.3.1.

The primary focus is to first determine if an identification can be effected. If an identification cannot be effected, or a situation as listed in 3.3.4 below has not become apparent, then the next phase of the evaluation is to determine if any of the exemplars can be excluded as containing the source impression. If an exclusion is not possible, then the reason for an inconclusive result will be reported.

3.3.2
When conducting AFIS database searches only candidate identifications will be reported out; except in the event that there is an inconclusive candidate in which it is highly probable that better exemplars could effect an identification. In this instance, the result to that candidate may be reported out as inconclusive with a request for better exemplars to be provided.

3.3.3.
When a cursory review of multiple exemplars to a specific friction ridge impression results in an identification to a specific source, it is acceptable for the examiner to terminate any pending comparisons and evaluations. In this instance, in addition to reporting the identification, it shall also be reported which other exemplars were compared and the status of those comparisons. It shall also be reported as to which exemplars were not compared.

3.3.4.
During a full comparison or cursory review of multiple exemplars that results in the focus of a particular region of friction ridge detail to a specific exemplar with a high level of agreement, but falls short of an identification (inconclusive finding) due to a lack of sufficient comparable areas in either the latent or exemplar; the examiner may terminate the comparison and evaluation of all other exemplars submitted for review. In this instance, in addition to reporting the inconclusive region of friction ridge detail, the results of all other comparisons shall be reported including the disclosure of exemplars not compared.

RETIRED

3.3.5
The following conclusions can be reported out as a result.

a. **That an identification was made**. When an identification is made to a friction ridge impression, the impression index number and source candidate are reported. If no identification is made, then the submitting agency's requested candidate exemplars which were examined will be reported out as excluded or inconclusive.

b. **That the questioned source was excluded.** An exclusion will only be reported when one of the following conditions have been met and sufficient exemplars have been provided.

    1. A known anchor point such as a loop, whorl or delta is available in both impressions which encompasses sufficient ridge events for a conclusion to be made. If an anchor point cannot be documented then the result will be reported as inconclusive.

2. Any ridge flow or formation which can be associated to its anatomical region (e.g. finger, palm, thenar) and sufficient ridge events for a conclusion to be made are present. If the anatomical region of the latent impression cannot be determined then the result will be reported as inconclusive.

c. **That the results of the examination were inconclusive.** The reason(s) for an inconclusive result will be documented and reported. Reasons for inconclusive results are as follows:

1. Inconclusive due to a lack of sufficient detail and or clarity in the latent impression.

2. Inconclusive due to insufficient exemplars with a notation indicating that exemplars of a specific anatomical region may provide a more definitive conclusion (e.g. major case prints or tips). This may be reported as the exemplar was unsuitable for analysis or that the exemplar was incomplete and a full examination could not be conducted.

3.3.6.
Documentation of an identification or inconclusive result to specific anatomical region shall be available in the case file on quality record QR-LP-11.

3.3.7.
Documentation of an exclusion or inconclusive result other than an inconclusive result to a specific anatomical region shall be available in the case file on quality record QR-LP-18.

RETIRED

3.4.
Verification

3.4.1
All identifications shall be verified; the primary examiner will create a digital case folder titled “VR” for the verification review of all identifications made by the primary examiner. Digital images of the friction ridge impressions which were identified and the accompanying known impressions will be placed in this digital folder.

3.4.2
The primary analyst’s assessed impression value rating will determine the minimum number of verifiers needed to release an identification or inconclusive result to a specific anatomical region. Exclusions will only require the verification of the technical reviewer.

a. When a friction ridge impression is assessed by the primary examiner to be "Identification Value" there will be one verification step before an identification or inconclusive result to a specific anatomical region can be released.

b. When a friction ridge impression is assessed by the primary examiner to be of "Comparison Value" there will be two verification steps before an identification or inconclusive result to a specific anatomical region can be released. One of the verifiers will be an independent examiner and the second will be the technical reviewer.

3.4.3
Examiners verifying identifications or inconclusive results to specific anatomical regions will independently analyze the indexed friction ridge impression in question as to its quality and observed ridge events prior to the comparison phase. The verifier will generate their own independent annotated comparison chart(s). The annotated comparison chart(s) will be placed in the case file and will be initialed and dated by the verifying examiner.

3.4.4
Aside from an identification or inconclusive result to a specific anatomical region which requires two verifiers, all other conclusions shall be verified by the assigned technical reviewer, unless the primary examiner requests additional verifications.

3.4.5
The results of all evaluations, verifications and conclusions made by the primary examiner and the verifier shall be reviewed by the technical reviewer. If the technical reviewer agrees with the primary examiner, he/she shall sign (by hand or electronically) the report prepared by the primary examiner. The technical review is the last step after the verification process.

3.4.6
Reports shall not be officially signed by the primary examiner until the administrative review process has been completed.

RETIRED

4.
SIMULTANEOUS IMPRESSIONS

4.1
Friction ridge impressions are simultaneous if they are deposited with one touch to an item or surface. The most obvious example of this would be impressions from adjoining fingers from one touch of the hand.

4.2
Individual segments of simultaneously placed impressions may or may not have sufficient value to arrive at a conclusion of identity.

4.3
It shall be the policy of the latent print unit, that at least one segment or area of detail, of what may appear to be simultaneously placed impressions, be able to stand alone in arriving at a conclusion of identity. An aggregate of detail from multiple impressions will not be used in concert to effect an identification.

4.4
Absent some unusual circumstance from which no other conclusion can be drawn, the latent print unit will not report a conclusion of multiple friction ridge impressions being deposited on an object simultaneously in one touch. In the rare instance such a conclusion is reported, proper documentation supporting the conclusion shall be made part of the case file.

5.
CONFLICT RESOLUTION

5.1
GL 18 will outline the technical and administrative review process.

5.2
When conducting a technical review, deference should always be given to the primary analyst's decision making discretion. It should be kept in mind that no two analysts will always technically approach the same problem in exactly the same manner.

If during the technical review, there appears to be deviation from standard operating procedures or substandard work, the Unit Lead/Supervisor shall be consulted.

5.3
If any reviewer has a difference of conclusion with a case that they are reviewing, the primary analyst will first be consulted on the matter. Additional work on evidence will not be performed without the approval of the primary analyst. If additional work is performed, the appropriate documentation will be placed in the case file. The reviewer and analyst will make every professional effort to resolve the issue.

If the issue cannot be resolved, the matter at hand will be brought to the attention of the unit supervisor/lead. Once the issue has been referred to the unit supervisor/lead, the technical reviewer must also provide documentation to support their differing conclusion. The documentation may be a chart or a document with clear explanations supporting the differing opinion. These documents will remain in the case jacket.

The unit supervisor/lead will determine the appropriate resolution. In the event the supervisor/lead is part of the conflict, then the Deputy Director of Identification Services will determine the appropriate resolution.

RETIRED

a. The lead or supervisor who handled the conflict will place a memo in the case file indicating that a conflict occurred and was resolved. The memo shall contain the following information:
   1. Names of the examiners in disagreement.
   2. A description of the issue at hand.
   3. Final action taken.
   4. The name of the lead or supervisor who resolved the conflict.

b. If a conflict resolution has been applied in the case, the report should indicate that the finding that was the result of a conflict resolution. This can be accomplished with a notation in the Comment Section of the report.

5.4
Erroneous Identifications and Exclusions

5.5.1
An erroneous identification or exclusion shall be defined as an incorrect conclusion of identity or exclusion which escapes the technical review process and is released in an official report. The laboratory has set strict standards for its examiners to follow in an effort to minimize erroneous findings. All examiners are expected to follow these standards. If it is found that an erroneous identification or exclusion has occurred, then this is a serious matter which will be referred to the Deputy Director of Identification Services and the Quality Section of the laboratory. A Quality Action Record will be opened and the following may be a remediation:

a. Retraining
b. Re-evaluation of prior casework.
c. Retraining and re-evaluation of prior case work
d. Retraining and removal from case work for a predetermined amount of time.
e. Self-initiated medical vision examination.
f. Any other appropriate action based on the results of a root cause analysis conducted Quality Section in conjunction with the Latent Print Unit Supervisor and the Deputy Director.

The customer will be notified by the release of an amended report and depending on the root cause, further communication may be needed.

RETIRED

Purpose:

To establish a policy regarding access to the Latent Print Unit examination areas to limit the possibility of contamination of evidence.

Responsibility:

Latent Print Examiners

Procedure:

1. Evidence sent for forensic laboratories often require multi-section analysis. Contamination must be kept to an absolute minimum. In the event that non-laboratory personnel must have access to this area, GL-3 will be followed for guidance.
2. The laboratory area must be cleaned after any work is performed by a vendor.
3. Aseptic techniques will be undertaken when conducting casework that needs DNA analysis.

RETIRED

Purpose:

To provide guidelines for the non-examination and return of submitted evidence.

Responsibility:

Latent Print Examiners

Procedure:

1. If a request is cancelled by the submitting agency and no work has been done on the case, it will be documented in the Case Synopsis Section in Justice Trax. The documentation must include the date the request was cancelled and the agency representative who communicated to cancel the request. Case Management will be notified. Generally, Case Management will be responsible for cancelling the request in Justice Trax and informing other Units that may be affected. The evidence will be returned to the submitting agency and no report needs to be written.

2. If a request is cancelled by the submitting agency and work has been started on the case, but the case has not been completed, it will be documented in the Case Synopsis section in Justice Trax. The documentation must include the date the request was cancelled and the agency representative who cancelled the request. Analysis of the case will stop and no further analysis will be conducted. A report will be written as to what had been done before the cancellation. This report will go through the normal technical and administrative reviews.

   Case Management will be notified. Generally Case Management will be responsible for informing other units and determining if there are other requests that need to be cancelled. The evidence and final report will be returned to the submitting agency in the same manner as fully examined cases.

3. The Latent Print Unit may determine that evidence is not suitable for examination. If evidence is deemed not suitable for examination, communication should be made with the submitting agency by Case Management, the Latent Print Unit Supervisor/Lead or a laboratory designee detailing the reason for the non-examination. This communication will be documented in the Case Synopsis area in Justice Trax (See LP SOP-24 Case Communication). If all the evidence in the case is not examined, no final report is required. If other evidence in the case was examined, the item that was not examined and the reason for the non-examination will be documented on the final report for the case. The evidence will be returned to the submitting agency. Some examples of evidence that may not be suitable for examination are (but is not limited to):

   a. Improperly marked evidence that needs to be returned for clarification prior to analysis
   b. Physical condition of item that renders it unsuitable for examination
   c. Any other reason which could compromise evidence integrity or analysis

RETIRED

4. Certain types of evidence submitted for latent print processing either have a non-existent or extremely low chance of developing friction ridge impressions of value. The Latent Print Unit will not process or examine the following types of evidence without prior approval by the Latent Print Unit Supervisor/Lead or the Deputy Director of Identification Services.

    a. Rocks, Stones, Bricks, Concrete Block or Similar Masonry Type Products
    b. Bullets/Cartridges (live and spent)
    c. Cigarettes/E-Cigarettes or small hand-held smoking devices (*i.e.* crack pipes)
    d. Cords, Ropes and Wires
    e. Cloth and Woven Fabric and Leather-type items
    f. Coins/Currency (*does not include counterfeit paper currency*)
    g. Dye Packs
    h. Footwear
    i. Glassine Bags
    j. Heavy Gauge Rubber Gloves (*latex/nitrile or vinyl gloves are accepted*)
    k. Pens, Pencils or other Writing Instruments
    l. Rusted Weapons/Rusted Metal Instruments/Tools
    m. Straws
    n. Syringes

5. Narcotics packaging other than types of evidence listed above may be submitted to the latent print unit for processing.

RETIRED

Purpose:
To outline the latent print unit's policy pertaining to evidence storage.

Responsibility:
Latent Print Examiners

Procedure:

1. Evidence under the control of a latent print examiner shall be kept in a secure location. Secure locations shall include any of the following:

   a. Latent evidence storage room (any evidence).

   b. A securable latent print examiner's office or securable cabinet/desk (for the temporary storage of case files, latent lifts, photographs and image disks only).

   c. Assigned cabinet in the latent print processing lab (any evidence).

   d. An examiner may leave evidence that is actively being examined on the examination bench during the work day in an appropriate manner that prevents compromise or loss of that evidence. This evidence must be returned to a secure storage location at the end of the workday.

2. Evidence which has been transferred to the Latent Print Unit, but has not yet been assigned to an examiner shall be kept in the latent print evidence storage room.

3. Upon case completion, the evidence shall be properly re-packaged and returned to evidence receiving or transferred to the next appropriate Unit or examiner for analysis.

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A. Purpose:

To give guidance regarding the documentation required when contact is made regarding a submitted case, whether that contact was initiated by the laboratory or an outside individual.

B. Responsibility:

Latent Print Section Examiners

C. Procedure:

1. The preferred method of documenting communications regarding cases is to put a note in the Case Synopsis area in Justice Trax. All documentation should include the date of the communication, the subject of the communication and the names of the people who were involved in the communication (laboratory and outside individuals). Examples of when the communication must be documented in Justice Trax are (but are not limited to):
   a. Non-examination of evidence (decision by laboratory) – include how this was communicated to the submitting agency (i.e. e-mail, phone call, etc.)
   b. Cancellation of case by submitting agency
   c. Any deadlines involved in the case
   d. A request is made for further examination after the evidence has already started being examined
   e. A new suspect in the case
   f. If other units could be affected by the subject of the communication

2. If communication is made that is relevant only to the Latent Print Unit, it is acceptable to document the communication in the latent print case jacket only. Communication will be documented on 8-1/2" X 11 paper with the case number and initials of the examiners legibly present. Examples of this are (but are not limited to):
   a. Agency contacted to ask about previous processing of evidence
   b. Agency representative calls to ask for a clarification about the final report. If this clarification is generic in nature and does not involve another unit, it is acceptable to document in Justice Trax only
   c. Contact with a State's Attorney pertaining to Latent Print testimony only
   d. Case communication between examiners.

3. If the Latent Print Unit receives a possible candidate for an Unsolved Latent Database impression (CT-AFIS or NGI systems), there are instances when a memo is written to the submitting agency (see LP SOP-32 ULD Entry and Candidate Review).

4. If any communication is done via e-mail and it may affect another unit, copies of the e-mail(s) will be placed in the Latent Print case folder and copies will be given to Case Management. It

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will be Case Management's responsibility to inform the other units and to add the e-mail communication to the main case folder in Evidence Receiving.

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A. Purpose: To establish a protocol for function testing the oven used in the Latent Print Unit.

B. Responsibility:

Latent Print Examiners

C. Procedure:

1. Evidence processed with chemicals or reagents where higher temperatures are recommended for the development of friction ridge evidence may be placed in the oven located in the latent print processing area.

2. Biannually every year, the latent print unit oven will be checked using a validated thermometer.

   A validated thermometer will be placed in the oven. The oven will be set to a temperature between 50 and 100 degrees centigrade. The oven will be allowed to run at the set temperature for at least 60 minutes. If the thermometer reading is within ±2 degrees of the set temperature, the results will be recorded on the oven function test log and no further action is required. If the difference is not within the acceptable limits, appropriate adjustments will be made and the results will be documented.

3. Biannually, the humidity of the latent print oven will be checked using a certified hygrometer.

   Set the oven at 100 degrees centigrade and make sure that the recirculation system is off. Leaving the door slightly ajar, allow the oven to stay at these settings for one hour. This will allow the oven to evaporate most of any residual water that may be in the tray.

   Once an hour has passed, set the temperature to a range between 30 to 50 degrees centigrade. Set the humidity to a setting of 40-80%. The oven will be allowed to run at the set temperature and humidity for at least 60 minutes. If the hygrometer's humidity reading is within ±10 of reading reflected on the oven, the results are acceptable and will be recorded on the oven function test log and no further action is required. If the difference is not within the acceptable limits, appropriate adjustments will be made and the results will be documented.

4. If the oven has to be adjusted to reflect an accurate temperature/humidity setting, the oven shall be turned off, allowed to cool down and rechecked utilizing the appropriate procedures as outlined in steps 2 or 3. If after the adjustment the oven still does not fall within acceptable limits, it shall be taken out of service until repaired or replaced.

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5. The results of these checks will be recorded on QR-LP-6. This form will be attached to the oven or maintained in a log book.

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A. Purpose:

To establish a procedure for the electronic submission of friction ridge impressions.

B. Responsibility:

Latent Print Examiners

C. Procedure:

In cases where an expedited examination is requested on a friction ridge impression(s) collected as part of an official investigation, the digital image(s) may be electronically submitted to the laboratory. The following procedure will be followed:

1. The agency requesting to submit digital images of friction ridge impressions will first contact the latent print unit by phone to initiate the request.

2. If the request is accepted, the submitting agency will be notified to email an electronically completed DSS Request for Analysis Form (SOP-ER-02:1) along with attached digital images of the friction ridge impressions that they wish to submit. The DPSLATENT email account will be utilized for receiving electronic submissions.

3. Upon receipt of the completed request form and the emailed images, a virtual folder will be created and the images will be transferred to that folder. Prior to opening the images, a virus scan will be conducted. If there are no threats identified, the images will be burned to digital media (CD/DVD). The submitting agency's name and case number will be written on the media along with the initials of the person who created the media and date the media was created. The case will be entered into Justice Trax and a DSS laboratory number will be generated (see LP-SOP-26.1 - Appendix1 for Justice Trax Entry). The DSS case number and item number will be written on the disc.

   If a threat is detected, a Supervisor is to be notified so that the threat can be assessed and if the system is compromised.

4. The images may now be reviewed and analyzed as a typical case.

5. If an identification occurs, a Tentative ID Preliminary Report may be released via email providing that a technical and administrative review has been completed. Charts by the examiner and the verifier(s) must be prepared prior to the Preliminary Report being sent. The requirements as to when a second verifier is delineated in LP SOP-18. The first verifying

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examiner shall be both the technical and administrative reviewer for emailed Preliminary Reports. The email shall include the following:

a. Laboratory Number
b. Name of Requesting Agency and Requesting Agency Case Number
c. Type of Case
d. That a tentative identification has been made to a friction ridge impression(s) depicted in submitted digital image file with the name of the file included.
e. The name of the individual identified (do not release the anatomical source)
f. The SID or UCN of the individual identified.
g. The following notice will be added to the email:

> "NOTICE: This email notification is not an official report, but considered a preliminary report. The verifying examiner acts as both the technical reviewer and administrative reviewer. The official report will be generated upon case completion and will follow the Latent Print Unit's standard technical and administrative review processes."

h. The name of the examiner and technical/administrative reviewer.
i. The examiner and technical/administrative reviewer will both review the email prior to it being sent. A printed copy of the email along with the initials of the examiner and technical/administrative reviewer will be placed in the case file.

6. Upon completion of the case, the generated disc will be turned over to the submitting agency along with a printed copy of the examiner's report.

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APPENDIX 1

The following steps will be followed to create a case in Justice Trax:

1. In Justice Trax, click on Search and then Agency Information. Fill out the agency name and the agency case number (make sure to check the "contain" box). This will search Justice Trax to see if the case has been already opened. If the results indicate the case has not been opened previously, continue to step 2. If the results indicate the case has been previously opened, go to step 3, skip steps 4-6, and proceed to step 7.

2. Select File, then New. Fill out the agency name and case number. Justice Trax will search the system again. As long as the case is not found during this search, an option to create a new case will come up. Select New Case. A main case window will open. Use the tabs located on the top of the window for steps 3-8.

3. Click on the Case Info tab. In the Synopsis section, enter "Electronic submission".

4. Click on the Agency tab. Verify the submitting agency and their case number auto-populated correctly.

5. Click on the Offense tab. Right click and choose "add offense". Fill in the Offense and Date fields. All other fields are optional.

6. Click on the Individual tab. Right click and choose "add individual". If the information is known, fill out the last name, first name, type of individual (i.e. victim/suspect, etc.), race of individual, gender and DOB. Do this for each individual associated with the case.

7. Click on the Evidence tab and right click and choose "add evidence". Fill in the agency representative (the representative who requested the examination). The submission number should auto-populate. Fill out the description of the evidence (i.e. disc with electronic image file). At the bottom of the page is the Chain of Custody information. Under the initial transfer location, select Agency Representative. The agency should auto-populate, then select Email or Electronic Submission. Under the first "To" location, select your name (Lab Staff Member or enter your employee number). Under the "Then To" location, select Storage Location and then select an appropriate storage area. (i.e. Latent Evidence Storage). Justice Trax will not let a case be created without a second transfer so the "Then To" field must be filled out. Click apply.

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8. Click on the Request tab. Right click and choose “add request”. Fill in the Rep field with the name of the representative who is requesting the examination. Under Section, select the appropriate unit (i.e. Fingerprint) then select the appropriate Service. Do this for each request associated with the case. Once all requests have been entered, click OK. Windows will open to select evidence and offenses related to the request(s). Click on the appropriate evidence or offense and then select the down arrow to relate the evidence or offense to the request. Once done click OK. Print bar code labels if able to now. Apply appropriate bar code labels to all evidence and paperwork. If the labels do not print, this can be done at the end.

9. Once all tabs have been completed, click Apply and select System Generated. This will give the case the next available case number in Justice Trax.

10. Click on Transfer then Print Inbound Evidence Receipts. Click “uncancel” selected. Find the case that was just created and check the box. Click Print and then to Screen. Click File then Print. Verify the receipt has printed correctly. Do not close the receipt screen window until you have verified the receipt has printed. Sign the receipt. Verify the submitting agency case number was entered into Justice Trax correctly. Check the box on the receipt.

11. If bar code labels have not been previously printed, print bar code labels and apply appropriate bar code labels to all evidence and paperwork.

12. The original paperwork (i.e. Request for Analysis, copies of warrants/consent, intake receipt, etc.) will be given to the Evidence Receiving Unit. Copies of all paperwork will be made for Unit case files.

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1.0 PURPOSE

The Laser Safety Program has been developed to provide guidance and oversight for the safe use of lasers and laser systems being utilized by the Latent Print Unit at the Department of Emergency Services and Public Protection's Division of Scientific Services (DSS).

2.0 SCOPE

The Laser Safety Program applies to all latent print examiners and those DSS employees who may require access to the Latent Print Unit's laser.

3.0 RESPONSIBILITY

3.1 Laser Safety Officer (LSO)

a) The Latent Print Unit supervisor/lead shall be responsible for obtaining and providing classification information concerning the Unit's laser system;
b) Implement or provide laser safety training;
c) Determine necessary safety information (evaluation and control measures) critical for the implementation of the DSS Latent Print Unit's laser system as specified by OSHA Section III: Chapter 6 Laser Hazards;
d) Provide information concerning the purchase of necessary laser safety materials (signs, labels, safety eyewear, etc);
e) Monitor and enforce program requirement;
f) Conduct annual review and survey of the laser safety program and make appropriate changes if necessary (see 6.7).

3.2 Laser Users:

a) Know all laboratory hazards and laboratory procedures for the safe use of laser(s) and laser system(s) in the work area;
b) Attend required training(s) as specified by a supervisor or the LSO;
c) Complete all aspects of laser usage in accordance with the laser safety program and good safety practices;
d) Use all personal protective equipment as specified in prescribed training or required by a supervisor or the LSO;
e) Immediately notify a supervisor, the LSO or section safety officer of any hazards encountered.

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4.0 REFERENCES

OSHA Section III: Chapter 6 Laser Hazards
National Institute of Health Laser Safety Program
NIST Laser Safety Program

5.0 DEFINITIONS

Accessible Emission Limit (AEL): The maximum accessible emission level permitted within a particular laser hazard class.

Authorized Personnel: Any individual approved to work with a laser or laser system.

Aversion Response: An involuntary movement of either the head and /or eye to avoid exposure to a visible laser beam.

Blink Reflex: The involuntary closing and opening of the eye(s) as a result of external stimulation.

Continuous Wave (CW): A laser beam with an output greater than 0.25 seconds.

Controlled Area is an area or location, such as a safe room, which encompasses or contains the hazardous conditions of an operating laser.

Diffuse Reflection: The reflecting of a laser beam in many directions by a surface.

Divergence: The splitting of a laser beam so that the beam diameter increases with distance traveled.

Embedded Laser: A laser of a specific class that is reduced to a lower class due to the mechanisms and devices (engineering controls) that remove potential for contact.

Fail-Safe Interlock: An engineering control where the failure of a single mechanical or electrical component of the interlock will cause the system to go into, or remain in, a safe mode.

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Infrared: In this standard, the region of the electromagnetic spectrum between the long- wavelength extreme of the visible spectrum (about 0.7 m) and the shortest microwaves (about 1 mm).

Interlock: See "Fail-Safe Interlock"

Intrabeam viewing: The viewing condition whereby the eye is exposed to all or part of a laser beam.

LSO: Laser Safety Officer

LSP: Laser Safety Program

Maximum permissible exposure (MPE): The level of laser radiation to which an unprotected person may be exposed without adverse biological changes in the eye or skin.

Nominal Hazard Zone (NHZ): The space within which the level of the direct, reflected, or scattered radiation may exceed the applicable MPE. Exposure levels beyond the boundary of the NHZ are below the appropriate MPE.

Non-Beam Hazard: A class of hazards that result from factors other than direct human exposure to a laser beam.

Protective Housing: An enclosure that surrounds the laser or laser system and prevents access to laser radiation above the applicable MPE. The aperture through which the useful beam is emitted is not part of the protective housing. The protective housing limits access to other associated radiant energy emissions and to electrical hazards associated with components and terminals, and may enclose associated optics and a workstation.

Specular Reflection: A mirror-like reflection.

Temporary Controlled Area is a temporary area or safe room which encompasses or contains the hazardous conditions of an operating laser. It is not a permanent or semi-permanent area or location.

Thermal Effect: Temperature elevation caused by exposure to a laser beam.

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Threshold Limit (TL): The term is applied to laser protective eyewear filters, protective

windows, and barriers. The TL penetration of a laser protective device. This is generally related by the Threshold Limit (TL) of the protective device, expressed in W cm -2 or J cm -2 . It is the maximum average irradiance or radiant exposure at a given beam diameter for which a laser protective device provides adequate beam resistance. Thus, laser exposures delivered on the protective device at or below the TL will limit beam penetration to levels at or below the applicable MPE.

Ultraviolet radiation: In this standard, electromagnetic radiation with wavelengths between 0.18 and 0.40 m (shorter than those of visible radiation).

Wavelength: The distance in the line of advance of a sinusoidal wave from any one point to the next point of corresponding phase (e.g., the distance from one peak to the next).

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6.0 PROGRAM

6.1 Laser Safety Program

The Laser Safety Program (LSP) is established to promote health and safety in the use of Class 4 lasers by the Latent Print Unit of the Department of Emergency Services and Public Protection's Division of Scientific Services (DSS). The LSP is administered by the laser safety officer (LSO). The LSP at DSS is a program with a mandate to ensure the proper training for all users and ensure the proper response to accidents and incidents involving the DSS Latent Print Unit laser.

6.2 Laser Classification

A. Class 1 cannot emit laser radiation at known hazard levels (typically continuous wave: cw 0.4 μW at visible wavelengths). Users of Class I laser products are generally exempt from radiation hazard controls during operation and maintenance (but not necessarily during service). Since lasers are not classified on beam access during service, most Class I industrial lasers will consist of a higher class (high power) laser enclosed in a properly interlocked and labeled protective enclosure. In some cases, the enclosure may be a room (walk-in protective housing) which requires a means to prevent operation when operators are inside the room.

B. Class 1A a special designation that is based upon a 1000-second exposure and applies only to lasers that are "not intended for viewing" such as a

supermarket laser scanner. The upper power limit of Class I.A. is 4.0 mW. The emission from a Class I.A. laser is defined such that the emission does not exceed the Class I limit for emission duration of 1000 seconds.

C. Class 2 low-power visible lasers that emit above Class I levels but at a radiant power not above 1 mW. The concept is that the human aversion reaction to bright light will protect a person. Only limited controls are specified.

D. Class 3A intermediate power lasers (cw: 1-5 mW). Only hazardous for intrabeam viewing. Some limited controls are usually recommended.

E. Class 3B moderate power lasers (cw: 5-500 mW, pulsed: 10 J/cm^2 or the diffuse reflection limit, whichever is lower). In general Class IIIB lasers will not be a fire hazard, nor are they generally capable of producing a hazardous diffuse reflection. Specific controls are recommended.

F. Class 4 High power lasers (cw: 500 mW, pulsed: 10 J/cm^2 or the diffuse reflection limit) are hazardous to view under any condition (directly or diffusely scattered) and are a potential fire hazard and a skin hazard. Significant controls are required of Class IV laser facilities.

6.3 Laser Hazard Control Measures

A. Controls for Class 3b and Class 4 Lasers

1. Posting – At minimum, the entryway of a controlled laser safety area hereinafter referred to as "Controlled Area" must be posted for the class of laser, laser power, and laser wavelength(s) of the laser being utilized in that area. Additionally, it may be necessary to post inside the laser safety area to further define the hazard.

2. Authorization – Only individuals who have been trained by a designated official may enter and utilize a laser of class 3b or 4. No other individuals shall have the means to utilize the laser or laser system. The DSS Latent Print Unit laser is a class 4 laser.

3. Beam trajectory and control – The laser may only be utilized after the beam manner is well characterized and understood. The beam should be controlled to not be at either standing or sitting height of the operator. A laser beam will not be directed at any individual. Additionally, an appropriate beam stop must be utilized to terminate the beam. The DSS

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Latent Print Unit dual beam laser can have its beam terminated by the use of the supplied "Enable" Key. The DSS Latent Print Unit dual beam laser utilizes a diffused beam.

4. Non-beam hazards – All non-laser materials that have the potential to come into contact with the laser beam must be analyzed to determine if any hazards are created upon contact over a period of time. When no written information is available to describe conditions created by contact, the responsible party should assume a hazardous product and ensure a proper means to eliminate the hazard. The most common means of controlling non-beam hazards is the use of local exhaust ventilation, essentially a mechanical means of purging an atmosphere through the use of controlled, directional air flow. This is necessary for beams that will be utilized for industrial purposes such as cutting or etching metal or other materials.

5. Personal Protective Equipment (PPE) – All class 3b and 4 laser usage areas must have a written document providing the required PPE to be used while working with the laser. The most common PPE for use with lasers is protective eye wear. It is critical that eye wear be appropriate to the laser used. All PPE must be inspected before use for conditions that would negate the effectiveness of the protective device.

6. Containment – All class 3b and 4 laser(s) and laser system(s) should utilize a means of containment that fully encloses the beam path and any potential reflections of the beam off of surfaces. Building materials utilized in a laser area(s) should be analyzed for reflective potential, and when possible altered to decrease reflectance to as low a level as achievable. Windows to spaces outside of the laser area should be removed or covered to block transmission of the laser. Engineering controls like laser activation entry lights and key switches should be used at all times to ensure effective containment and control. The DSS Latent Print Unit utilizes an enable key in addition to an interlock installed to the doorway of the controlled area.

7. Rapid Egress and Emergency Access – All class 4 laser area(s) must have a controlled means of rapid egress and admittance for emergency conditions. The controlled area will not be locked when the dual beam laser is in use.

6.5 Laser Related Non-Beam Hazards & Control Measures

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A. Electrical Hazards – Many incidents and accidents related to laser(s) and laser system(s) stems not from the laser beam, but from user interaction with electrical components required for the laser to function. Most class 3b and 4 lasers utilize high voltages and large capacitors which have a high potential for electrical accidents. DSS employees will not disassemble the Laser Unit. Only authorized service technicians may disassemble the laser unit.

B. Laser Dyes – Dyes used as lasing mediums are often classified as toxic, carcinogenic and/or flammable. These chemicals must be handled appropriately and material safety data sheets for each must be on file within the laser use area. The DSS Latent Print Unit dual beam laser does not use toxic, carcinogenic and/or flammable lasing mediums.

C. Laser Generated Air Contaminants (LGAC) – Contact between a laser beam and a material can cause specific; yet, sometimes unknown contaminants to be released into the atmosphere of the laser use area. Periodically this release of air contaminate may occur without noticeable signs, such as smoking of the material contacted. Proper room ventilation or local exhaust ventilation is critical in any laser use area, and must be evaluated before the installation of a class 3b or 4 laser. The use of respiratory protection is not an accepted means of controlling the generated airborne hazard. The DSS Latent Print Unit dual beam laser utilizes a diffused beam and is not manufactured for industrial cutting or etching purposes, therefore this hazard is not present.

D. UV, Visible-Radiation and Plasma Emissions – Evaluations for the discharge of radiation, both visible and UV; and, plasma formation must be completed before the use of a class 3b or 4 laser. Identification of any of these conditions requires a review of the PPE used for skin protection to ensure adequate safety coverage. Additionally, laser and laser system components must be periodically surveyed for damage from these conditions. The DSS Latent Print Unit dual beam laser emits in the visible color spectrum at 445nm and 532nm respectively and therefore this hazard is not present. However the DSS Latent Print Unit dual beam laser will be visually inspected on an annual basis for signs of damage or wear.

6.6 Laser Safety Training

All users must successfully complete Laser Safety Training before using a class 3b or 4 laser or laser system. Refresher training shall be mandatory on a yearly basis.

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6.7 Laser Safety Review &Survey

A laser safety review and survey shall be completed annually for all class 3b and 4 laser and laser systems by the LSO. The results of the safety review and survey shall be maintained by the LSO.

The following posting and security checks will be completed:

Safety Program Review:

a. Review Safety Program to determine if is meets OSHA laser safety guidelines.

Label and Security:

a. Are entrances properly posted?
b. Is room security adequate?
c. Is there a laser status indicator outside of room?
d. Is laser class posted?
e. Is Danger Class 4 Laser In Use Placard Posted?

Laser Unit Safety Controls:

f. Is protective housing secure?
g. Is beam shutoff (enable switch) working properly?
h. Is beam indicator working properly?

Engineering Safety Controls

i. It there an entry way interlock switch installed to terminate the laser beam?
j. Is there a visual or audio warning when laser is active?
k. Are reflective materials removed from beam path?
l. Are appropriate goggles available both in and outside of the controlled area?
m. Does the laser unit show any signs of wear or damage that would require servicing?

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6.8. Medical Surveillance and Laser Accidents

a. In the event of any accidents or incidents involving a class 3b or 4 laser, the user shall immediately notify their supervisor/lead. The supervisor lead shall notify the Deputy Director of Identification Services and the Safety Officer.
b. The injured employee shall receive immediate medical attention.

LP SOP-29 Latent Print Unit Laser System

Purpose:

The Division of Scientific Services utilizes a laser system as a method of developing and documenting latent friction ridge skin impressions. The device is a Laser Light Solutions BrightBeam DUAL High Power Blue/Green Forensic Laser System. The guidelines below will be followed to safely and properly operate this system.

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1. Safety

1.1. Lasers pose unique hazards in the laboratory, and those hazards must be identified, understood, and controlled before using these systems. Lasers can operate across a broad spectrum, from the ultraviolet (UV) to the infrared (IR). Not all wavelengths present the same hazards. Additionally, hazards may vary depending on the specific nature of the lasers.

1.2. The most notable hazard is ocular. The lens and cornea of the human eye focus optical wavelengths between 400 nm and 1400 nm directly onto the retina. However, the human eye is only sensitive to wavelengths between 400 nm and 700 nm. The near-IR range (700 nm to 1400 nm) can present an increased hazard—in this range, light is still focused on the retina but is not visible. As a result, the blink reflex (or aversion response) is not present at these wavelengths. Retinal burns are the most common significant injuries that occur with lasers in the ocular wavelength range (400 nm to 1400 nm), higher power lasers in this range can cause thermal burns to the skin as well. The Latent Print Unit's dual beam laser emits radiation in the following wavelengths:

445nm	(blue)	InGaN diode based semiconductor
532nm	(green)	Nd: YAG solid-state based

1.3. No persons shall be allowed to operate the Latent Print Unit's dual beam laser until they have received proper safety training pertaining to the use of class 4 lasers. The training shall be in compliance with OSHA guidelines and LP SOP-28 Latent Print Unit Laser Safety Program.

1.4. No persons shall be allowed to operate the Latent Print Unit's dual beam laser until they have been trained in its proper use. The Latent Print Unit Supervisor/Lead or his/her designee shall be responsible for training Latent Print Unit Examiners.

1.5. The area where the laser is being utilized shall be designated a "Controlled Area". A controlled area shall display proper warning and danger signs as designated below:

1.5.1. **Danger Laser In Use** sign shall be displayed on the exterior and interior doorway to the specific laboratory giving access to a Controlled Area where the Latent Print Unit's dual beam laser is in use. It shall also be displayed at Temporary Controlled Areas. This sign shall also indicate the laser wavelength and power output.

1.5.2. **Controlled Area** sign shall be posted on the exterior Controlled Area door of the Latent Print Unit. The sign shall display "Controlled Area" and instruct reader to stop, knock and announce before entering. It shall also indicate that goggles and a lab coat are mandatory if the laser is being operated. An illuminating warning sign adjacent to the entrance door to the laser room (Controlled Area) is currently present and shall be turned on (illuminated) when the laser is in use in order to alert employees who may wish to enter the Controlled Area. For a **Temporary Controlled Area** a printed sign shall be posted and display "Controlled Area" and instruct the reader not to enter and that goggles and lab coat are mandatory if laser is

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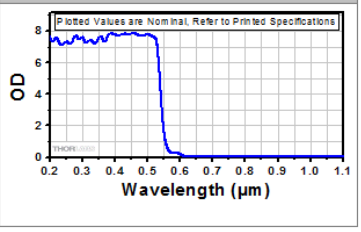
being operated.

1.5.3. **Danger Class 4 Laser Safety Check** sign shall be displayed over the laser warning sign which will be illuminated when the laser is in use. The warning sign shall be on the exterior wall of the Controlled Area entrance way.

1.6. **Laser Glasses/Goggles** shall be available in both the "Controlled Area" and "Temporary Controlled Area" in a clearly visible location adjacent to such area's entrance way. Glasses/goggles for the BrightBeam Blue/Green Laser shall meet or exceed the following specifications as recommended by the manufacturer:

OD Specs (ANSI Z136)	L-Rating Specs (EN 207)[a]	Optical Density vs. Wavelength
180 to 532 nm, OD = 7+	180 to 315 nm (D LB7 + IR LB4)	
	>315 to 532 nm (DIRM LB6)	

Manufacturer recommended suppliers are:

Thorlabs (www.thorlabs.com)

NOIR (www.noirlaser.com)

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1.7. **Direct Skin or Eye Exposure:** Direct skin or eye exposures are to be avoided and are not authorized. Under no circumstances should the laser beam be intentionally pointed or aimed at any individual. Appropriate eye protection and a lab coat (or other outer skin covering) will be worn while operating a class 4 laser. The laser will be immediately turned off if anyone without proper protection enters the Controlled Area.

1.8. **Medication, Drugs & Alcohol:** Under no circumstances is the laser to be operated by any individual who is under the influence of medications that may affect balance, perception, and decision making processes, illicit drugs or alcoholic substances.

1.9. **Incidents or Injury:** Any incidents or injury involving the Latent Print Unit's dual beam laser are to be immediately reported to the Latent Print Unit Supervisor/Lead, the Deputy Directory of Identification Services and the Identification Section's Safety Officer. Immediate medical attention will be provided to the injured person.

1.10. **Entrance Way Interlock Device:** The Latent Print Unit Controlled Area entrance has an interlock device installed to terminate the laser beam in the event an accidental entrance occurs. Temporary controlled areas will either incorporate an entrance way interlock device or other physical security measure to assure that unauthorized and unprotected persons do not enter the Temporary Controlled Area.

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2. BrightBeam Operation

2.1. Prior to Start Up: Make sure laser safety precautions are in place.

2.1.1. Make Sure Evidence Viewing Area is Clean and Prepared
2.1.2. Check that Laser Unit Power is set to OFF
2.1.3. Check that Laser Unit Enable key switch is set to OFF
2.1.4. Set Up Camera Equipment (if needed)
2.1.5. Place Barrier Filter on Camera Lens (if needed)

2.1.6. Make Sure Evidence is Dry of any Flammable Liquids or Solutions
2.1.7. Place Evidence in Controlled Area
2.1.8. Make Sure Personal Protective Equipment is in Place (Goggles & Lab Coat)

2.2. Laser Start Up

2.2.1. Make sure at least one pair of laser goggles are hanging on the exterior wall adjacent to the control area door
2.2.2. Power on the illuminating warning sign to alert lab personnel that the laser is in use
2.2.3. Make sure entrance door to controlled area is closed
2.2.4. Press Power rocker switch to on position to enable power to laser
2.2.5. The Power LED will illuminate
2.2.6. The fan will momentarily go to maximum speed
2.2.7. The Enable LED will flash during power up and then go off
2.2.8. The Interlock and Temp LED will be green
2.2.9. The LCD will display: Disabled Key On to Enable
2.2.10. Turn enable key switch to on position (this allows the lasers to be turned on and enables the hand controls)
2.2.11. The Enable LED will illuminate (Both Lasers will still of OFF)
2.2.12. The LCD will display: Blue Off 000 GRN Off
2.2.13. Select appropriate laser (blue/green) on hand piece and gradually increase power setting to achieve proper illumination.
2.2.14. Once the proper illumination has been achieved, use this light source to locate any latent prints and document findings on the appropriate quality record.

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2.3. Laser Shut Down

2.3.1. Turn enable key to off position
2.3.2. Press Power rocker switch to Off position
2.3.3. Turn off illuminating warning sign to alert lab personnel that laser is no longer in use

3. References

3.1. Laser Light Solutions BrightBeam DUAL High Power Blue/Green Forensic Laser System Users Manual
3.2. OSHA Section III: Chapter 6 Laser Hazards https://www.osha.gov/dts/osta/otm/otm_iii/otm_iii_6.html

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Purpose:

This procedure is used to establish guidance for the role of the Division of Scientific Services' CT-AFIS Latent Print Search Terminal Administrator.

Responsibility:

Latent Print Unit Supervisor/Lead and/or Designee

Procedure:

1. The Latent Print Unit Supervisor/Lead shall have the responsibility of overseeing the operator function and functionality testing of the DSS's CT-AFIS latent print terminals. The Latent Print Unit Supervisor/Lead may appoint a designee to perform some or all of the associated tasks listed in Section 2. In such cases the designee shall report his/her activities and findings to the Latent Print Unit Supervisor/Lead (or Deputy Director if the Supervisor/Lead is not available).

2. Latent Print Search Terminal Administrator operator and functionality oversight shall consist of the following:
   a. Perform function tests on each DSS latent print search terminal at least once annually with a target impression (see LP SOP-11).
      1) A log shall be kept of all function tests and the result of each test.
      2) If a functionality tests fails, the Deputy Director of the Identification Section of DSS, the Administrator of SPBI and the DESPP Chief Information Officer (CIO) will be notified.
   b. Prepare or assist with the preparation of qualifications which are needed to be latent print search terminal operator.
   c. Assist with the preparation of municipal Memorandum of Understanding (MOU) documents.
      1) Upon request of DESPP, may administer screening tests in compliance with DESPP and municipal MOU. The MOU shall dictate the parameters of the test.
      2) The results of such testing shall be provided to the test taker in an official letter or e-mail. If the test taker is part of a municipal agency, a copy of the letter or e-mail will be provided to the highest ranking officer of the agency, and the DESPP CJIS Systems Officer.
   d. Assist with the technical requirements for the procurement or upgrades of the State of Connecticut AFIS System (CT-AFIS).

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3. Any issues associated with DSS laboratory operators of the CT-AFIS will be handled based on the DSS's GL's and the Latent Print Unit's SOP's.

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PURPOSE:
To describe the procedure for confirming a candidate match generated by a CT AFIS or NGI unsolved latent match and issuing a report.

RESPONSIBILITY:
Latent Print Unit Personnel

OVERVIEW:
During the process of casework analysis, latent prints may be developed or submitted that are of value. These latent prints are searched through the CT AFIS and NGI. In the event that an identification is not reported at the time of the search, the examiner will generally enter the latent print for continued searching in the CT AFIS's Unsolved Latent Database (ULD) or the NGI's Unsolved Latent File (ULF). A transaction number is associated with the initial search of the latent print and that same transaction number is carried forward upon entry into the ULD or ULF.

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ULD/ULF CANDIDATE EVALUATION/REPORTING PROCEDURE

When an examiner determines that a ULD/ULF transaction has resulted in a viable candidate, the examiner should adhere to the following guidelines in the evaluation of the candidate. These are general guidelines and depending on case circumstances, a deviation from these guidelines may occur with approval from the Unit Lead or Deputy Director.

CT AFIS ULD Candidate Procedure:

1. The ULD transactions are reviewed in the CT AFIS database. This can be done by any qualified personnel. At least once per month, the Latent Print Unit Lead will assure ULD transactions are being reviewed.
2. When the candidate list is accessed, the examiner will review the images for potential associations. If the examiner determines that there is not an association, the examiner will select "Confirm No" and will go on to the next latent, if present. At the completion of all latent reviews for the candidate, the examiner will click "End Transaction" in the CT AFIS software.
3. If the examiner determines that there is a tentative identification, the examiner will forward the transaction information to the original case examiner. If the original examiner is not available, the technical reviewer of the case will be forwarded the information. If

neither the original examiner nor the technical reviewer is available, the information will be forwarded to the Latent Print Unit Lead/Supervisor for assignment.

4. The case examiner will evaluate the print. If it is agreed there is a tentative identification, a "ULD Hit – Report" request will be created in Justice Trax and assigned to that examiner. If it is found that the identification has previously been reported out, a new request does not need to be created and the unsolved latent will be removed from the ULD.
5. The case examiner will request a copy of the candidate's exemplar card (finger/palm) from SPBI (State Police Bureau of Identification). If SPBI confirms that there is a releasable card, the examiner will continue with the examination as follows:
    a. If the date of the incident in the case jacket is greater than 5 years from the date of the ULD hit, a memo will be written to the submitting agency – refer to Quality Record QR-LP-15. The memo will state that there is a candidate to an unsolved latent print from the case and that is has not been confirmed at this time. If confirmation is required, they must contact the laboratory. This memo will be itemized in Justice Trax and transferred to the submitting agency via Justice Trax for tracking purposes. A copy of this memo will be forwarded to the State's Attorney's office via email. A copy of this memo (and the email) will also be placed in the case jacket with a screen shot of the CT AFIS transaction information stapled to it. This transaction information must contain the transaction number of the latent. Exceptions to this are cases identified as homicides, sexual assaults, kidnappings, home invasions or arsons. If any of these crimes are listed on the Request for Analysis then an examination will proceed as listed in 5b below.
    b. If the date of the incident in the case jacket is less than 5 years from the ULD hit, the examiner will examine all unidentified prints of value in the case to the ULD candidate. A report will be generated indicating the results of such examinations.
6. If SPBI reports that the card is not releasable, a report will be generated indicating the possibility of an identification, but the unavailability of a record for confirmation.
7. Once an identification is reported out, the transaction will be removed from the CT AFIS ULD.

NGI ULF Candidate Procedure (direct connectivity):

1. When a candidate is developed in the NGI ULF, an email is automatically generated through NGI. The subject line of the e-mail will have the acronym "ULM" (Unsolved Latent Match). This email is available to all examiners. The examiner that opens the ULM

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email will determine the original examiner of that print. At least once per month, the Latent Print Unit Lead will assure the ULM's are being reviewed.

2. Once the original examiner is identified, the transaction information will be forwarded to that examiner for follow up. If the original examiner is not available, the technical reviewer of the case will be forwarded the information. If neither the original examiner nor the technical reviewer is available, the information will be forwarded to the Latent Print Unit Lead for assignment.
3. The examiner will access the ULM transaction information and review the images for potential associations. If the examiner determines that there is not an association, the examiner will not go any further.
4. If the examiner determines there is a tentative identification, a "ULF(NGI) Hit – Report" request will be created in Justice Trax and assigned to that examiner. If it is found that the identification has previously been reported out, a new request does not need to be created and the unsolved latent will be removed from the ULF.
5. The examiner will request the candidate's fingerprint card through NGI.
6. Once the card is obtained, the keeper of the record must be determined. If the keeper of the record is any CT agency, CT SPBI must be contacted to obtain an exemplar card (finger/palm).
7. If the candidate is identified, the examiner will proceed to generate a report indicating the identification. *(Refer to 5 a. and 5 b. in the CT AFIS ULD Candidate Procedure to determine which unidentified latent prints in the case will be examined).*
8. If a candidate's card is not available through NGI, the FBI's Latent Support Unit will be contacted for assistance.
9. Once an identification is reported out, the transaction will be removed from the NGI ULF.

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REFERENCES:

Purpose:

To establish a validation procedure or performance check procedure for the use of new equipment or software being introduced to the Latent Print Unit.

Validations will be conducted when the Unit receives a new piece of equipment or new software for use in casework. This SOP is to act as guidance for the validation plan; if modifications are required for the plan, these will be approved by the Deputy Director. The validation plan may also be used for software upgrades that reflect a major change.

In the case of minor updates to software, a performance check is sufficient to determine if the new upgrade is acceptable for use in casework. A performance check will also be conducted if a piece of equipment is sent out for repairs and is now ready to be placed back into service. Performance checks may also be conducted when casework software is loaded for the first time onto a computer.

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Responsibility:

Forensic Science Examiner assigned to the Latent Print Unit or conducting casework in the Unit.

Validation Procedure:

1. A plan will be written detailing the steps of the validation procedure. This plan will be reviewed by the Deputy Director, Quality Manager and Director.
2. This plan may be updated during the process as new information or results of the validation require changes to the original plan. Any changes in the validation plan will need to be approved.
3. After completion of the validation, the results will be clearly communicated in the validation and submitted for review by the Deputy Director.
4. The Deputy Director will review the validation and will issue a statement if the equipment or software has successfully passed its validation and is acceptable to be

placed into service or use for casework. Software or equipment is considered acceptable for casework if it can produce reliable and repeatable results.

5. This validation will be reviewed to determine if SOPs or Quality record changes are needed prior to casework use.
6. Record of all steps in the validation process will be recorded on the "Laboratory Method Validation Summary Form".

Reference:

GL1 – Quality Manual

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Purpose:

To provide guidance to Latent Print Examiners when collecting DNA samples via swabbing from evidence that is being processed for latent prints.

Responsibility:

Forensic Science Examiners, however titled

Note:

This information is a general guideline for the collection of evidence for DNA testing. A trained analyst should always use their judgement and experience when examining evidence. The analyst should always take into account the type, condition, and quantity of the evidence being examined.

The following should be determined as the latent print analyst proceeds forward with the examination:

1. If there is a reasonable expectation that more sample remains on the item and could be collected in the future, then there is no consumption issue.
2. If blood is also present, the evidence should be sent first to the Forensic Biology Unit for processing.
3. Forward appropriate samples to DNA according to the case scenario and refer to LP SOP-34.1 (Evidence Sampling guidance) for suggested number of samples to collect and collection location.
4. LP-Sample Prep will be the request associated to evidence that is being processed for latent prints and samples collected for DNA testing by Latent Print personnel.

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Procedure:

Physical evidence will be examined and swabbing(s) will be performed based on the examiner's knowledge, training, experience and according to the submitting agency requests, case information and the condition of the evidence.

A. Cleaning of Equipment and Laboratory Area

1. Clean utensils and bench top supplies during use as needed and between each case and case submission. Use appropriate disinfecting solution such as stabilized bleach. Utensils will be rinsed with ethanol following the disinfecting solution to ensure aseptic conditions; dH_2O may be used between the disinfecting solution and ethanol.

2. Containers used to clean/soak utensils in disinfecting solution, dH_2O and ethanol, are replaced weekly. The disinfecting solution, dH_2O and ethanol are replaced daily or more often, as necessary.

3. Clean with disinfecting solution the camera and other electronic equipment during use as needed and between each case and in between the processing of each case submission to ensure aseptic conditions.

4. Clean bench top using the appropriate disinfecting solution and replace examination paper between each submission or more often as necessary, to ensure aseptic conditions.

B. Personal Protective Equipment

Examiners must wear lab coats, masks, gloves, disposable sleeves and hair nets while processing and handling evidence in the laboratory examination area.

C. Evidence Examination

1. When possible, leave the submitting agency seal intact when opening the package.

2. Record information on the appropriate Quality Record Worksheet.

   a. If processing the evidence for latent prints and samples are collected for DNA analysis, use QR-LP-16 to record the samples collected. The item description, disposition and processing information including if samples were collected will be recorded on the appropriate worksheets (QR-LP1a, QR-LP1c, QR-LP-12 or QR-LP-13).

   b. If assisting the Forensic Biology Unit with FB Sample Prep cases only, use FB QR-17 (Swab only worksheet) and FB-QR-18 (page 2 of swab only) or FB QR-23 (Swab only – multi submission worksheet) or FB-QR-19 (BLST Worksheet) and/or FB-QR-20.

3. All documentation will be dated. If completed on the same day, the date on each page of the worksheet is sufficient. If additional examinations and/or testing were conducted on a subsequent day, then it will be dated and initialed on the worksheet accordingly.

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4. If processing the evidence for latent prints first, proceed as indicated in LP SOP-02 (Processing guidelines).

D. Sample Collection for DNA

When the examiner is ready to proceed with the DNA sample collection, sample selection is conducted considering the substrate and the type/amount of sample present. Samples for touch/wearer DNA analysis may also be collected based on the submitting agency requests, case information and type of evidence.

1. Pour off a small amount of dH_2O into a sterile Eppendorf tube. The dH_2O that is used for collection of samples will be dH_2O previously quality control checked by the DNA Unit. The lot number present on the approved dH_2O will be entered on the worksheet used for sample collection.

2. When an area is swabbed with the intention of separating the swabs (i.e. retaining half and sending half for DNA analysis), the swabs must be collected simultaneously.

3. Label the unopened swab package with the case number, item number and description of area swabbed prior to sampling. Remove the appropriate number of swabs from the swab package(s) and retain the package(s).

4. Moisten the swab(s) with dH_2O.

5. Using pressure, swab the area turning the swab(s) to ensure that all sides come in contact with the area. It is not necessary to mark the evidence for touch or wearer samples collected. This is indicated in the worksheet as location of collection.

a. If swabbing the area for touch or wearer, swab the entire location.

b. If only collecting DNA from a specific location to determine handler, refer to LP SOP-34.1 Appendix 1 for guidance.

c. If collecting a sample from an area with a latent print of value, collect a sample from the designated area, using one swab. If there is more than one designated area, it is the discretion of the examiner to collect each area separately using one swab or to collect multiple areas simultaneously using 1 or 2 swabs.

6. Place the swabs back into an appropriately labeled swab package with the moistened swab tip facing outward for drying. Itemize the samples collected using the itemization guidance provided in GL-4 for guidance on sub-item creation in Justice Trax and generation of transfer sheets.

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7. If possible, air dry the swab(s) over-night in a designated area. During this time the sample(s) will remain in the custody of the examiner. Moist samples may be transferred directly for DNA analysis when necessary.

8. If trace material was noted on the evidence, the QR should indicate what type of trace material was observed. In the case of evidence from a homicide, if the trace material is hair-like fibers, these should be collected and placed into a druggist fold and returned with the evidence in the original packaging.

9. Once the swabs have air-dried, samples can be retained in a properly labeled sterile plastic tube which is then sealed into a properly labeled plastic bag.

a. Remove the swab cotton from the stick and place in a properly labeled plastic tube.

b. No more than three swabs are to be placed into one tube for DNA analysis. Place any additional swabs into a separate tube and notify DNA.

c. Heat seal the plastic bag, initial the heat seal and place in freezer storage (Freezer Storage - DNA sample).

10. Any samples not going forward to DNA should be packaged as noted in line 9 above and placed in a separate plastic bag. Heat seal the plastic bag, initial the heat seal and place in freezer storage (further analysis).

11. Each case will be evaluated to determine which of these samples, if necessary, should be forwarded for DNA analysis.

a. No Suspect/Suspect (no arrest): Send what was collected to DNA referring to the Submission Guidelines for the appropriate number of samples to forward.

b. Suspect (arrested): Send what was collected to DNA (consumption issue).

12. The number of swabs being forwarded for DNA and any swabs remaining should be indicated on the appropriate Quality Record Worksheets and in LIMS.

Indicate on the Quality Record Worksheet, the disposition of the samples collected and the disposition of the original evidence.

13. Once the examination(s) is/are complete, place the evidence back in the original packaging, seal and initial the seal. If the original packaging is not suitable for re-packaging, do not discard. Place with evidence in new packaging, seal and initial the seal. Store the evidence in the designated storage area.

14. Create the samples collected from the evidence in the LIMS computer system according to GL-4 (LIMS/Justice Trax) using the designated itemization.

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15. Store samples in designated, secure and temperature appropriate areas or transfer to other Units of the Laboratory using the LIMS computer system according to GL-4 (LIMS/Justice Trax). Print the LIMS transfer sheets as needed.

16. If forwarding sample(s) to DNA, create the appropriate DNA request(s) using the LIMS computer system according to GL-4 (LIMS/Justice Trax).

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Appendix 1: Evidence sampling guidance

The following is guidance on sample collection depending on the type of sample that may be encountered. Samples for touch/wearer DNA analysis may be collected based on submitting agency request, case information, results from latent print processing and the type of evidence.

The appropriate number of samples will be forwarded to DNA as follows. Additional samples may be included as determined by case information.

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A. Objects with handles (i.e knives)

1. If the knife has been previously fumed, swab vigorously on the areas when collecting the sample to penetrate beneath the cyanoacrylate layer.

2. If there is a latent print developed, collect a sample from the area with the latent print, as necessary, using one swab. If there is more than one designated area, it is the discretion of the examiner to collect each area separately using one swab or to collect multiple areas simultaneously using 1 or 2 swabs.

3. Collect a sample from the handle area using one or two swabs.
   a. No Suspect/Suspect (no arrest): send what was collected to DNA
   b. Suspect (arrested): send what was collected to DNA as "consumption issue"

   A consumption issue is defined by the Superior Court – Procedure in Criminal Matters Practice Book Section 40-9 "Presence during Tests and Experiments" as the following: *If a scientific test or experiment to be performed upon any object which…may preclude or impair any further tests or experiments, the opposing party and any other person known to have or believed to have an interest in the matter shall be given reasonable notice and opportunity to be present and to have an expert observe or participate in the test or experiment, unless the judicial authority for good cause shall order otherwise." (P.B 1978-1997, Sec. 738)*

4. If requested or necessary, collect a sample from the blade or area that may have come in contact with question body fluid or skin cells using one or two swabs.
   a. No Suspect/Suspect (no arrest): send what was collected to DNA
   b. Suspect (arrested): send what was collected to DNA as "consumption issue"

B. Gloves (latex/nitrile or vinyl gloves)

1. Collect a sample from the interior of each using two swabs.
   a. No Suspect/Suspect (no arrest): send what was collected to DNA
   b. Suspect (arrested): send what was collected to DNA as "consumption issue"

2. If the interior of a glove cannot be determined, collect a sample from the interior and a sample from the exterior of the glove as it was received, each with two swabs.

a. No Suspect/Suspect (no arrest): send what was collected to DNA
b. Suspect (arrested): send what was collected to DNA as "consumption issue"

C. Other items such as cash drawers, tools, or items handled by individuals may be sampled for touch and the samples are forwarded to the DNA Unit at the discretion of the examiner with input from Case Management.

D. Paper Items (i.e. notes, cards, sheets of paper, etc)

1. If there is a latent print developed and it is necessary to sample separately, collect a sample from the area with the latent print, using one swab. If there is more than one designated area, it is the discretion of the examiner to collect each area separately using one swab or to collect multiple areas simultaneously using 1 or 2 swabs.

   If there is no need to collect the latent print area separately, follow the guidance in step 2.

2. Collect a sample from the remaining paper surfaces as necessary using two swabs.
   a. No Suspect/Suspect (no arrest): send what was collected to DNA
   b. Suspect (arrested): send what was collected to DNA as "consumption issue"

E. Envelopes with sealed flap

1. If there is a latent print developed and it is necessary to sample separately, collect a sample from the area with the latent print, using one swab. If there is more than one designated area, it is the discretion of the examiner to collect each area separately using one swab or to collect multiple areas simultaneously using 1 or 2 swabs.

   If there is no need to collect the latent print area separately, follow the guidance in step 2.

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2. Collect a sample from the remaining paper surfaces as necessary using two swabs.
   a. No Suspect/Suspect (no arrest): send what was collected to DNA
   b. Suspect (arrested): send what was collected to DNA as "consumption issue"

3. Using forceps, carefully, begin at one corner of the envelope and gently attempt to pry apart the flap from the envelope. If the flap does not separate easily, proceed to step 4.

4. Steam may be applied to the flap area for up to 5 minutes. After apply the steam, use forceps to carefully pry apart the flap from the envelope beginning at one end of the envelope. The examiner may need to work in segments to completely loosen the entire flap.

5. Collect a sample from the adhesive area of both surfaces using one or two swabs.
   a. No Suspect/Suspect (no arrest): send what was collected to DNA
   b. Suspect (arrested): send what was collected to DNA as "consumption issue"

6. If unable to separate the flap from the envelope, collect a cutting along the adhesive area of the flap. This cutting should measure approximately 4cm X 1cm in size. This cutting should include the both the flap and envelope layers.

   No Suspect/Suspect (no arrest)/Suspect (arrested): send half of the cutting sample to DNA. (Refer to Appendix 2 regarding designation of a "*" sample).

7. If the envelope has a stamp that has been removed during the latent print processing steps, collect a sample with one swab the adhesive area of the stamp and the location on the envelope where the stamp was affixed. Do not send this sample for analysis initially and retain at the lab.

   If unable to remove the stamp from the envelope, a sample is collected by cutting through the stamp and envelope layers. This cutting should measure approximately 4cm X 1cm in size. Do not send this sample for analysis initially and retain at the lab.

F. Bottles/Cans

1. If there is a latent print developed and it is necessary to sample separately, collect a sample from the area with the latent print, using one swab. If there is more than one designated area, it is the discretion of the examiner to collect each area

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separately using one swab or to collect multiple areas simultaneously using 1 or 2 swabs.

If there is no need to collect the latent print area separately, follow the guidance in step 2.

2. Collect a sample from the exterior mouth opening and interior cap (if present) using two swabs.
   a. No Suspect/Suspect (no arrest): send what was collected to DNA
   b. Suspect (arrested): send what was collected to DNA as "consumption issue"

3. If requested or necessary, collect a sample from the exterior body (sides) of the bottle using two swabs.
   a. No Suspect/Suspect (no arrest): send what was collected to DNA
   b. Suspect (arrested): send what was collected to DNA as "consumption issue"

4. If multiple bottles/cans are submitted for one case, collect samples from each mouth/cap area individually. There is no need to collect from the sides of each bottle/can.

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Modified Training Plan for Collection of DNA Samples from Evidence for Latent Print Examiners:

Trainee: _____

Trainer: _____

S**tart Date**: _____

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Training Goal:
To provide practical instruction on routine DNA collection from Evidence following the Standard Operating Procedures of the Forensic Biology Unit and the use of worksheets and procedures currently in place in the Latent Print Unit.

Tasks:
1. To complete training in and pass the competency test for Standard Aseptic Technique administered by DNA personnel.

2. Training will be conducted through a series of demonstration, observed practice and side-by-side examination of evidence with a Lead examiner or designee. The Trainee will observe a minimum of three (3) cases and perform the examination of at least 5 mock cases or at least 5 mock items of evidence during training. More cases can be observed or completed by the trainee as necessary. It is the trainee's responsibility to document the DSS case number, technique(s) utilized and trainer throughout this process. It is recommended that when the examiner is performing their examination under the guidance of their trainer, the sampling should take place in the Latent Print work area.

3. The Trainee is expected to review a minimum of ten (10) cases, including worksheets, photographic documentation and LIMS transfer sheets generated by a competent analyst.

4. Training for this procedure is fulfilled upon successful completion of a competency examination. The competency examination will include a minimum of three (3) items that have been previously handled. The analyst will independently collect samples from these items, prep and forward them to the DNA Unit. DNA analysis will be conducted to determine if they are free from analyst contamination and if the handled DNA profile is present in the samples collected.

5. Since this is an "FB Sample Prep" technique, FB report writing is not required for this training. The Latent Print Examiners will need to indicate in their report that samples were collected for DNA analysis (list of item numbers collected) and which samples were forwarded to the DNA Unit and which were retained.

6. Upon completion of this Modified Training Plan, Administrative approval will be sought and confirmed prior to the assigning of casework.

7. Since no FB report is generated, a Forensic Biology Lead or designee will review a minimum of the first five (5) cases independently completed by the analyst for sample collection and itemization.

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Related SOP's:

SOP Number	Title	Date Complete / Trainee Initials	Trainer Initials
FBSOP-1	Physical evidence exam		
FBSOP-3	Guidelines for collection and forwarding		
FBSOP-4	Definitions/Abbreviations		
LP SOP-34	LP DNA sample collection		
LP SOP-34.1	Appendix 1: Evidence sampling guidance		

Aseptic Technique Training and Competency: (required for new employees)[1]

Date Administered	By Whom:	Comments*
Training:		
Training:		
Competency:		* see related documentation

[1] Employees currently assigned to the Latent Print Unit as of the release of this document have already been trained in Aseptic Techniques and have been conducting their analysis using these techniques.

Examinations Performed by Trainer and Observed by Trainee:

Lab Case Number	Type of Case	Submission # / Description	Techniques observed	Date Complete / Trainee Initials	Trainer Initials

Examinations Performed by Trainee and Observed by Trainer:

Lab Case Number	Type of Case	Submission # / Description	Techniques observed	Date Complete / Trainee Initials	Trainer Initials

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Case Review by trainee of worksheets and Justice Trax itemization:

Lab Case Number	Type of Case	Submission # / Description	Date Complete / Trainee Initials	Trainer Initials

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Competency to include mock report indicating samples collected and forwarded:

Date Administered	By Whom:	Comments*
		* see related documentation

Review of First (5) Cases Independently Completed by Analyst:

Lab Case Number	Type of Case	Submission # / Description	Lead Initials	Comments

Court Testimony Review:

Date Administered	By Whom:	Comments*
		* see related documentation

RETIRED

PURPOSE:

To describe the procedure for confirming an offender's fingerprint record when that offender is requesting their own DNA profile.

RESPONSIBILITY:

Latent Print Unit Personnel

OVERVIEW:

The CODIS Administrator or their designee may request confirmation of an offender via fingerprint comparison to the offender's known fingerprint record. The fingerprint examiner will use this SOP and DNA SOP-10.8.5.2 for guidance.

Using CT-AFIS or NGI to Obtain Fingerprint Records for Comparison

When conducting a fingerprint comparison as required by DNA SOP-10.8.5.2, Latent Print Unit Examiners shall follow section 10.8.5.2 of the DNA SOP. The examiners may use CT-AFIS and/or NGI to conduct a database search or request a ten-print record by using the provided demographic information to obtain records for comparison. All paperwork pertaining to the comparison will be turned over to the DNA CODIS Administrator or designee for retention and filing purposes.

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A. Purpose:

To establish guidelines for the examination of post mortem friction ridge impressions received from the Office of the Chief Medical Examiner (OCME).

B. Responsibility:

Latent Print Examiners

C. Procedure:

The OCME during the course of a death investigation may obtain post mortem friction ridge impressions via a live scan device. When the OCME requires the identification of captured friction ridge impressions the outlined procedure will be followed.

1. OCME captured friction ridge impressions will be emailed to the DPSOCME email account which is shared by State Police Bureau of Identification (SPBI) Fingerprint Section, the Division of Scientific Services (DSS) Latent Print Unit and the OCME.
2. The SPBI Fingerprint Section will first attempt a ten print search of the submitted impressions. The result of this search will be emailed back to the OCME and the DSS Latent Print Unit via the DPSOCME email account.
3. If the SPBI Fingerprint Section is unable to identify the impressions submitted by the OCME; then the DSS Latent Print Unit Supervisor/Lead, the DSS Deputy Director or their designee will contact the OCME to determine if follow up is needed by the Latent Print Unit.
4. If no follow up is needed by the Latent Print Unit, it will be documented via the DPSOCME email account and nothing further needs be done.
5. If follow up for identification is requested, then the case will be taken in as outlined in LP SOP 26 starting at step 3. The Request for Analysis Form will be completed by the person taking in the OCME case.
6. If follow up is requested and the email discloses that the live scan impressions are of no value for the required task; then the OCME will be notified that the post mortem friction ridge detail will need to be photographed. The digital images will be submitted through the regular evidence intake process if they cannot be emailed. OCME post mortem friction ridge detail evidence received through the regular intake process will follow guidelines 7 and 8 of this SOP if a CT-AFIS and/or NGI search is conducted.
7. If a CT-AFIS or NGI hit occurs, a tentative email will be sent to OCME as outlined in LP SOP 26. The associated SID or UCN will be run through COLLECT or III to determine if alias names and dates of birth are associated with the provided SID or UCN. If alias information is revealed, this information will be printed out and retained in the case file; and will also be reported out to the OCME to assist with their investigation.

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8. If CT-AFIS and NGI searches result in no hit, then an email may be sent to the OCME indicating that no hit occurred and that an official report will follow.

D. References

1. CGS 29-7b (b): Authorizes that the Division of Scientific Services be made available to the OCME.

2. CGS 19a-406: Authorizes Chief Medical Examiner to investigate human deaths.

3. CGS 54-142g(b): Defines the Office of the Chief Medical Examiner as a Criminal Justice Agency.

RETIRED

A. Purpose:

This SOP is to provide guidance in the scanning of latent print case jacket files for discovery purposes.

B. Abbreviations:

1. CJI – Criminal Justice Information
2. COLLECT – Connecticut On-Line Law Enforcement Communications Teleprocessing
3. III – Interstate Identification Index
4. NICS– National Instant Criminal background check System
5. ORI – Originating Agency Identifier
6. PII – Personally Identifiable Information
7. SID – State Identification Number
8. SPBI – State Police Bureau of Identification
9. UCN – Universal Control Number

C. Procedure:

At the completion of the case, the examiner will scan the entire case jacket as a PDF and upload it to the designated folder on the S: drive "Latent Print Case Folder". The examiner will name the PDF in the following format: "DSS-YY-NNNNNN LP Case Jacket" or "ID-YY-NNNNNN LP Case Jacket" (depending on the Justice Trax designation).

Guidance for following circumstances:

1. Two sided pages
   a. If the page being scanned is a two page document, a copy of the back side of the page will be made and then used to scan in the copier.
   b. Once the scan is completed and the PDF document is reviewed to make sure all pages were scanned, the copy of the back page can be discarded.
2. Non-Releasable Record
   a. A non-releasable record is a fingerprint record in which SPBI has returned a response indicating that the fingerprint card being requested is categorized as "non-releasable". In the event that the case jacket contains non-releasable record information, the following will be redacted:
      1) The non-releasable person's name, DOB and SID (FBI number can remain).
      2) Any fingerprint card associated with the non-releasable record.

RETIRED

a. A blank page will be inserted in place of the redacted fingerprint card page. The blank page will have the page number which it replaces along with the language "Redacted Info – Non-Releasable Record".

The UCN can remain on the worksheets, provided that the non-releasable person's name and DOB is redacted and is not present with this information.

"The following type of data are exempt from the protection levels required for CJI: transaction control type numbers (e.g., ORI, NIC, UCN, etc.) when not accompanied by information that reveals CJI or PII." Reference: Criminal Justice Information Services (CJIS) Security Policy (CJISD-ITS-DOC-08140-5.8) Version 5.8, Release date: 06/01/2019, page 10.

3. Collect Records and III Records
Since these records are being provided to the State's Attorney, there is no need to redact this Collect or III information unless the record pertains to a non-releasable.

RETIRED

Method of Redaction:

The redaction can be done by making a copy of the page that needs redacting and using a black marker to obscure the information. Once the page with the obscured data is scanned, the page may be discarded.

Another method of redacting is to put a portion of a sticky note over the information that cannot be released and scan the page.

Note: This SOP pertains to Discovery Requests. Additional redactions are needed in Freedom of Information Requests, GL-11 (Control of Records) should be consulted for this redaction information.

A. Purpose: Multimedia and Image Enhancement Section provides technical support and services for the State of Connecticut, Connecticut State Police and the Connecticut law enforcement community in the areas of digital imaging, video and audio analysis and forensic imaging.

B. Responsibility: Forensic Science Examiners assigned to Unit or conducting analysis in Unit

C. Procedure:

1 Goals, Objectives and Performance Measures

The goals of the Multimedia and Image Enhancement Unit follows the requirements as set forth in Section 29-7b of the Connecticut General Statues by providing forensic services that meet our specifications and comply with the ISO 17025:2017 & ANAB requirements while meeting or exceeding the expectations of the criminal justice community.

2 Personnel Responsibilities

All personnel assigned to the Multimedia Unit will follow all applicable Federal, State and local laws and department policies. The personnel assigned to this section will perform their duties free from any internal or external inappropriate influence/pressure as detailed in SOP GL-05 “Ethics”.

Responsibilities will include the following:

1. Digital Image support
2. Video duplication for State and Local Law Enforcement Agencies and the Federal/State Attorney’s Office
3. Forensic Video Imaging and Audio reproductions and enhancement
4. Audio Duplication for the State and Local Law Enforcement Agencies and the Federal/State Attorney’s Office
5. Storing and maintaining analog, digital and other media files
6. Providing analog, digital media and video analysis assistance to department personnel
7. Participate in the Division’s quality assurance program
8. Assist law enforcement agencies in the acquisition of video and audio evidence

3 Training, Development and Proficiency Testing

The Multimedia and Image Enhancement Section will follow training and proficiency testing guidelines as set forth in the Department of Emergency Services and Public Protection, Division of Scientific Services Forensic Laboratory’s Quality Manual SOP GL-14 “General Training”, SOP GL-16 “Proficiency Testing” and SOP-MMIE-26.

RETIRED

4 External/Internal Audits

The Multimedia and Image Enhancement Section will cooperate with inspections to ISO 17025:2017 & ANAB standards, when conducted for accreditation.

The Multimedia and Image Enhancement Section will cooperate with the internal audit inspections as set forth in SOP GL-1 Section 4.14. The Section will comply with changes or correction of findings as addressed in the QAR.

5 Quality Assurance

The Multimedia and Image Enhancement Unit will comply with the Department of Emergency Services and Public Protection's Division of Scientific Services' Forensic Laboratory's Quality Assurance Program as specified in the Quality Manual. It is the goal of the quality assurance program to provide a system that ensures reliable and accurate results and objective interpretations of data; it also encourages regular evaluation and reviewing of the policies and procedures of the section.

6 Records, Management and Special Reporting

The Multimedia and Image Enhancement Unit will follow the guidelines as set forth in the Department of Emergency Services and Public Protection's Division of Scientific Services Forensic Laboratory's Quality Manual SOP GL-18 (Case Review System), SOP GL-11 (Control of Records) and SOP GL-9 (Quality Action Reports) and Department of Emergency Services and Public Protection's A&O Manual.

RETIRED

D. Sources of Error: Not applicable

E. References:

Quality Manual – General Laboratory Standard Operating Procedures

Connecticut Department of Emergency Services and Public Protection Administrative and Operations Manual.

A. Purpose:

The Multimedia and Image Enhancement Unit will take precautions to maintain the security and integrity of evidence submitted for examination.

B. Responsibility:

Forensic Science Examiners assigned to the Multimedia and Image Enhancement Unit or conducting casework in this Unit will follow and maintain security measures as detailed in the Department of Public Safety, Division of Scientific Service Forensic Laboratory's Quality Manual's SOP GL-3 "Security".

RETIRED

C. Procedure:

.1 All work product in the Multimedia and Image Enhancement Unit will be securely maintained by each employee to prevent the loss, damage or deterioration of the evidence.

Work product is defined as the media generated or a digital copy of the original evidence submitted.

.2 Digital media will be stored on the casework computer and external hard drives, a copy will be transferred to CD/DVD and maintained in a designated locked cabinet storage area of the section. This location is termed as "Video Storage" in Justice Trax.

.3 The case storage area consists of cabinets with a lock. Only examiners assigned to this Unit will have a key and access to this file storage area.

.4 All media will be stored in a designated storage cabinet/locker in the Unit in an area safe from magnetic field interference.

.5 All video media submitted to this Unit will be inspected prior to analysis and if possible to ensure that the media's "write protect" feature is utilized to prevent the loss, deterioration of damage of the evidence.

.6 All media will be inspected; any physical damage will be documented on QR: MMIE General worksheet prior to any further analysis. If necessary, the submitting agency will be notified of the documented damage according to SOP-GL-4.4.2. The submitting agency will be notified of physical damage is this prevents the analysis of the evidence.

.7 All work product and evidence in the Multimedia and Image Enhancement Unit will handled according to the standards set forth in the Laboratory's Quality Manual SOP GL-13 "General Evidence Handling" and SOP GL-4 "Lims/Justice Trax".

.8 Integrity of casework will be maintained through the use of password protected computer systems and evidence transfers.

D. References:

Division of Scientific Services, General Laboratory Standard Operating Procedures

Justice Trax User Manual

RETIRED

A. Purpose:

All personnel will adhere to the quality assurance standards as set forth in Division of Scientific Service's (DSS) Quality Manual. The following are the guidelines for Multimedia and Image Enhancement Section quality assurance program.

RETIRED

B. Procedure

1. Technical and Administrative Reviews

Technical reviews will be conducted on all reports generated in which case analysis was performed. The technical reviewer will be denoted by the signature on the right side of the final report. The technical reviewer will date and initial the milestone sticker and technical/administrative review checklist (QR: MMIE Checklist). After completion of the technical review, the case jacket will be forwarded to a third party for the Administrative review. The Administrative Reviewer will check for spelling, grammatical or numerical errors or any other discrepancies as necessary. The administrative reviewer will date and initial the milestone sticker and complete their portion of the review checklist (QR: MMIE Checklist).

Reports are generally not issued for duplication requests when the entire media is being copied. Reports are also not generally issued for video retrievals service requests unless additional enhancement/editing type analysis was performed to duplicate the evidence submitted. A review is conducted of the paperwork and the request is released by the reviewer completing the Technical Review milestone in Justice Trax/LIMS. Since there are no technical findings, a report is not issued and an Administrative Review is not conducted.

2. Lab Case Folders

Lab Case folders shall be maintained when an item of evidence is submitted for analysis. Case folders shall include case notes, request for examination forms and evidence receipts, reports or any other documentation at the discretion of the person completing the report.

3. Report Writing

Reports generated will be written according to the guidelines set forth in the DSS Quality Manual SOP GL-11. The results of analysis will be communicated on the report in an accurate, clear, unambiguous and objective manner. All reports will be issued on letterhead and they will contain the following:

- Laboratory Case Number
- Submitting Agency

- Submitting Agency Address
- Submitting Agency Case Number
- Date of Request of Examination
- Date of Completed Report
- Name of person or commanding officer requesting testing
- Evidence examined by examiner
- Methodology of testing
- Results of Examinations
- Conclusion of testing if applicable
- Samples or items created and the disposition of the evidence

The report should indicate the testing conducted as requested on the Request for Analysis (RFA).

4. Supplemental Reports

Additional evidence and forensic analysis requested for the same Laboratory Case will be reported on a report titled "Supplemental Report". A numerical designation will be added to the report title to indicate the chronological sequence of the additional supplemental reports.

5.Amendments to Reports

If a report must be amended to provide revised/corrected information, the amended report shall be titled with the wording "REVISED REPORT". Included with this report shall be a cover letter detailing the area(s) of revision. This "REVISED REPORT" shall be addressed to the customer and all other interested parties (State's Attorney, Public Defenders, etc).

RETIRED

A. Purpose:

All personnel will adhere to the quality assurance standards as set forth in Division of Scientific Service's (DSS) Quality Manual. The following are the guidelines for Multimedia and Image Enhancement Section quality assurance program.

RETIRED

B. Procedure

1. Technical and Administrative Reviews

Technical reviews will be conducted on all reports generated in which case analysis was performed. The technical reviewer will be denoted by the signature on the right side of the final report. The technical reviewer will date and initial the milestone sticker and technical/administrative review checklist (QR: MMIE Checklist). After completion of the technical review, the case jacket will be forwarded to a third party for the Administrative review. The Administrative Reviewer will check for spelling, grammatical or numerical errors or any other discrepancies as necessary. The administrative reviewer will date and initial the milestone sticker and complete their portion of the review checklist (QR: MMIE Checklist).

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Lab Case folders shall be maintained when an item of evidence is submitted for analysis. Case folders shall include case notes, request for examination forms and evidence receipts, reports or any other documentation at the discretion of the person completing the report.

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- Methodology of testing
- Results of Examinations
- Conclusion of testing if applicable
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Additional evidence and forensic analysis requested for the same Laboratory Case will be reported on a report titled "Supplemental Report". A numerical designation will be added to the report title to indicate the chronological sequence of the additional supplemental reports.

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If a report must be amended to provide revised/corrected information, the amended report shall be titled with the wording "REVISED REPORT". Included with this report shall be a cover letter detailing the area(s) of revision. This "REVISED REPORT" shall be addressed to the customer and all other interested parties (State's Attorney, Public Defenders, etc).

RETIRED

A. Purpose:

General standard operating procedure for the forensic science examiner assigned a multi-media image enhancement case.

B. Responsibility:

Forensic Science Examiners assigned to Unit or conducting casework in the Unit.

C. Procedure:

1. A new case evidence will be picked up from Evidence Receiving and transferred to the Video Storage- Incoming location, unless the examiner will begin to work on the evidence.

2. When beginning a case, the examiner shall transfer the evidence from its storage location into the examiner's possession.

3. The transfer shall occur in the Justice Trax/Lims program and a secure PIN will be used as part of this transfer. The start date of the case shall be designated as the first date that analysis began in the case as reflected on the appropriate worksheet and on the milestone sticker located on the exterior of the case jacket.

   Evidence not immediately being examined will be transferred into the examiner's locker in the Unit.

2. Photocopies or photographs of evidence labeling and sealing information will be made for the case file and documented on the appropriate worksheet.

3. The examiner will verify that the barcodes and labeling information correspond.

4. Prior to beginning examination, the examiner should note if there may be the presence of biohazardous materials present and proper precautions should be employed.

   In the event that such substances are present, the examiner will follow safety precautions as detailed in SOP GL-02 "Safety".

5. Prior to beginning examination, verify that the media's erasure prevention mechanism is implemented.

RETIRED

6. Inspect physical media for any damage. Any damage should be noted and recorded on the appropriate worksheet. If necessary, contact the submitting agency prior to repairing the evidence.

7. Any media that will be inserted into a computer should be checked for viruses and malware using proper security software.

8. Record the serial/lot number or any other identifiers on the worksheet.

9. Begin examination of evidence as requested on the Request for Physical Examination Form SOP-ER-02:1provided by the submitting agency according to the procedures listed in the appropriate section.

10. After completion of examination, all worksheets, media and reports (if applicable) generated must be reviewed by the technical reviewer and administrative reviewer. Refer to the GL-1 Section 5.10 "Reporting the Results." The end date of the case is the date that the report is Administratively Reviewed in Justice Trax/LIMS, which is documented on the report and the outside of the case jacket. Note: Administrative reviews are not conducted in duplications or video retrievals, unless a report is generated.

11. All media generated should have the examiner's initials, the Laboratory's Case Number identifier present and the evidence's Submission/Item number with a barcode present on the external packaging.

RETIRED

D. Sources of Error:

Not applicable

E. References:

Division of Scientific Services, General Laboratory Standard Operating Procedures

A. Purpose:

To duplicate submitted video evidence for distribution to other agencies in a format that is recognizable and accessible.

B. Responsibility:

Forensic Science Examiner

C. Procedure:

1. Evidence will be submitted to the Laboratory according to the Evidence Receiving Standard Operating Procedures.

2. Inspect the physical media for any damage. Any damage shall be documented in the case file's quality record (MMIE QR: General Worksheet). If necessary, the submitting agency/customer may be notified of any noted damage and possible repairs. Communication with the customer will be indicated in Justice Trax Case Synopsis area.

3. Any repairs made shall be noted on the appropriate worksheet.

4. Load the evidence into the appropriate player and locate the segment of video to be duplicated. Record the devices used on the appropriate location on the worksheet.

5. Load the appropriate media into the recording device and begin to record. If media is to be inserted into a computer, this media should be checked for viruses and malware using proper security software.

6. After the duplication process has been completed, verify that the duplication copy is working properly and that the final product is the best quality attainable.

7. If possible, an archive copy of the duplicated material will be generated and retained at the Laboratory.

8. Label the original submission and its duplications with the Laboratory's identifiers (case number, submission number and examiner initials). A barcode should be generated and affixed to the external packaging.

RETIRED

9. Document on the appropriate worksheet: the type of media for the original submission, the equipment used to perform the duplication and the resulting media.

10. A sub-item will be created in the *LIMS JusticeTrax System*. For any duplications generated to be disseminated to the requesting agency, this sub-item will be created as the next corresponding sub-item number available under the parent item. Refer to GL-4 for guidance.

11. No report is issued if the duplication request is for the entire contents of the media submitted.
    A report will be issued for VHS tapes reflecting a portion of the recorded video (incident of interest) was duplicated.
    A report will be issued if there are issues with the duplication of the evidence and/or additional enhancement/editing-type analysis was performed to duplicate the evidence.

12. Return the original evidence and duplication copy(ies) to Evidence Receiving.

D. Sources of Error:

Error may occur due to equipment malfunction or condition of submitted evidence

E. References:

Division of Scientific Services General Laboratory Standard Operating Procedures

RETIRED

A. Purpose:

Video evidence when submitted to the Laboratory may be in a format that is unable to be played or viewed by the submitting agency. In cases where the evidence is time-lapsed or multiplexed, the submitting agency may request that procedures be taken to clarify the video evidence for the purpose of viewing and continuing their investigation. In some cases, the video may be difficult to view due to the lighting conditions or other factors. The purpose of enhancing video is primarily to capture, isolate, adjust the speed or camera sequencing and clarify (sharpen, highlight) the video to provide the best quality images for the submitting agency. In some cases, images may be captured from the video and printed for the submitting agency.

There may be cases in which the video with associated audio may need to be captured from a source and then converted to a file format that is able to be enhanced. In this type of case, it is recommended that equipment/software be used that can capture the video with associated audio in a format that has the least amount of compression.

Similarly, there may be occasions in which an audio file associated with a video or free-standing may be of quality that does not lend the listener to understand the content of the audio. Filters may be employed to lessen or remove the interference.

When analyzing a video/audio file, the following software applications (or similar working software) may be used for the purposes of capturing, processing and enhancing the video/audio or photographic file generated from the submitted evidence:

- Adobe Photoshop/Bridge/Clear ID
- Adobe Premiere Pro
- Camtasia
- Corel
- iNPUT Ace
- SnagIt
- StarTech USB 3.0
- Adobe Soundbooth/Audition
- Audacity
- Izotope

These software applications may be used to apply the following filters: capture of video/audio, adjusting audio levels, video de-interlacing, single and multiple frame exporting, playback speed adjustment, frame averaging, levels or brightness/contrast adjustment, highlighting, sharpening,

RETIRED

inverting, magnifying or inserting informational text into a video or photographic file and providing picture-in-picture display. Additional features include the ability to mask or track subject/objects in a video.

The following descriptions are filters and other applications that may be used in the examination of video evidence:

Audio Adjustment: Involves controlling the audio frequency, bandwidth and levels of multiple bands.
Blur/DeBlur: Filters that make pixels in an image or video become unclear/less distinct or improve the sharpness of the edges of objects in an image or video
Brightness & Contrast: This effect adjusts the brightness and contrast of the color tones located in the segment or frame of video.
Canvas Editor: Process of combining files on a canvas to create synchronized side-by-side and multi-file views.
Concatenate: Process of stitching multiple videos together
Crop: Filter that allows an area to be selected and either remove a portion or focus in on an area of the video/image
De-Interlacing: The ability to view either the odd or the even field of a video frame.
De-noise (advanced settings), for finer control over the noise reduction process, including Adaptive noise reduction mode, for accurately tracking a changing noise spectrum.
Dodge/Burn: Traditional photographic technique to light or darken specific areas of a print by regulating the exposure.
Extract Frames: A filter that extracts all frames or specific frame types from a segment of video and saves them individually as a separate file type.
EQ Match/Equalization: Filter to allow for clips recorded differently the same sonic feel as one another .
Fields to Frames: Taking captured video frames and separating them into the odd and even fields and making them a unique frame by themselves to be viewed consecutively and in proper order. This technique, in effect, doubles the size of the video file.
Frame Averaging: Generates an image by adding together selected multiple frames. This technique removes dynamic noise from stationary objects in an image.
Gain/Volume – change in the decibel level of an audio track or segment
Highlighting: To bring out a selected area or zone of a video frame so that it stands out or apart from the non-selected area or zone.
Invert: This effect inverts the color information of an image. This technique may be used to bring out patterns or details from an image.
Levels/Curves: The levels effect adjusts the brightness/contrast of a clip. It combines the functions of the color balance, gamma correction, brightness, contrast and invert effects.
Lucis Pro®: Photographic filter that enhances images by adjusting high, medium and low contrast areas and corrects exposure levels.

RETIRED

Masking – the ability to apply a full blur or a highlight to an subject/object
Multiple or Single Frame Exporting: The function of exporting a video clip as a sequence of still image files or a single still image file.
Noise Removal (audio) – reduction of a frequency or artifact in an audio track. (see De-noise)
Picture in Picture: The ability to display to separate video files or to have a video file in the background and include a still photo image in the foreground to illustrate an area of interest.
Pitch Contour/Pitch: for remapping pitch inconsistencies using variable resampling.
Position: The ability to reposition a video frame to bring an item of interest into view.
Resize: using software to add or subtract pixels, and is called resampling.
Scale or Magnify: The ability to zoom in or zoom out of a video frame.
Sharpen filters: Improves image clarity and emphasizes small details and sharp contrasts.
Sharpen/Focus: Filters that make pixels in an image or video improve the contrast of the edges of objects in an image or video.
Shadow/Highlight: Photographic technique that adjust the exposure levels in highlighted or shadowed area of an image/video.
Silence – removal of audio from a portion of the timeline.
Speed/Duration Adjustment/Timing: The speed of a clip is the playback rate compared to the rate at which it was recorded. Initially, a clip plays back at its normal, 100% speed. Changing a clip's speed causes its source frames to either be omitted or repeated during playback; thereby making the video or audio play faster or slower. A change in speed results in a corresponding change in duration.
Stabilize: process of making a section of video less shaky
Tracking – ability to follow a subject/object and use masking to either obscure or highlight.
Transcoding: process of converting an audio or video file from one encoding format to another in order to increase the number of compatible target devices a media file can be played on.

RETIRED

B. Responsibility:

Forensic Science Examiners assigned to Unit or conducting casework in the Unit

C. Procedure:

Refer to specific standard operating procedure for type of analog or digital evidence received.

D. Sources of Error:

Not applicable

E. References:

See MMIE SOP-24 Multimedia & Image Enhancement References

Appendix: 6.1 “Photoshop CS3 for Forensics Professional”

RETIRED

A. Purpose:

General operating procedure for handling the enhancement of analog-type video evidence

B. Responsibility:

Forensic Science Examiners assigned to the Multimedia & Image Enhancement Unit or performing casework in the Unit

RETIRED

C. Procedure:

1. Inspect the analog media to ensure that there is no damage and the media is in good working condition.

2. If the media is damaged, note the damage on the worksheet and with photographs if possible. If necessary, contact the submitting agency to discuss the damage. Repair the damage if possible and note the repairing measures on the applicable worksheet.

3. After implementing erasure prevention practices, insert the analog media into the appropriate player (TL/RT).

4. Verify that the input/output signals are operating correctly.

5. In the examiner's computer documents, a working folder should be created with the laboratory case number as the identifier for all files/media generated.

6. Open the appropriate capturing software (Camtasia, SnagIt, StarTech H264 or applicable software) and activate the capture mode according to the user manual instructions.

7. Capture the relevant section of video information as noted in the Request for Examination form.

8. Save the captured segment as a video (.avi, .mpg, .mov, etc.) file with a case number or other identifier. *e.g. ID-06-12345capture 1.avi or bankrobbery.avi*

9. Analyze the captured file using the editing software, refer to MMIE SOP-06 for a list of software and filters that may be used.

10. Any files generated from the digital media should be saved using the highest quality setting.

11. Exported files can be created and labeled in the following manner: camera/time/date stamp information (*c# mmddyy hhmmss.tif, c# hhmmss.tif, mmddyy hhmmss.tif or hhmmss.tif)* or submission number (*Example: S001 1.tif (graphic files)* or other descriptor.

12. After analysis of the video evidence is completed, save any video files or exported image files in the examiner's case folder on the computer and a copy of this file should be transferred onto a CD/DVD for archiving.

13. The submitting agency may receive a set of printed images or a CD/DVD containing the image files.

14. If photographs or a media containing electronic files are generated to be disseminated to the requesting agency or retained as archives, a sub-item will be created in the *LIMS JusticeTrax System*. Refer to GL-4 for sub-itemization guidance.

15. A report will be issued of enhancements/clarifications made on the submitted evidence.

D. Sources of Error:

Equipment malfunction or condition of evidence

E. References:

See MMIE SOP-24 Multimedia & Image Enhancement References

RETIRED

A. Purpose:

General operating procedure for handling CD/DVD or other digital evidence for enhancement.

B. Responsibility:

Forensic Science Examiners assigned to the Multimedia and Image Enhancement Unit or performing casework in the Unit

C. Procedure:

1. Inspect the digital media to ensure that there is no damage present and it is in good working condition.

2. If the media is damaged, note the damage on the worksheet and with photographs if possible. If necessary, contact the submitting agency. Repair the damage if possible and note the repairing measures on the applicable worksheet. If damage is observed, the submitting agency should be contacted and a notation added to the Justice Trax Case Synopsis of this communication

   Precautions should be taken to ensure that the digital media is virus-free and malware-free prior to opening files in a computer.

3. Make a duplicate/working copy of the submitted media and all work performed will be made off of this copy. The working copy of the media should be write protected and a hash value should be taken and compare to the original evidence.

4. In the examiner's computer documents, a working folder should be created with the laboratory case number as the identifier for all files/media generated.

5. Use the Laboratory software programs (Adobe Photoshop, Adobe Premiere Pro, Cognitech, iNPUT ACE) or if applicable the pre-installed viewer software present to access and view the video or photo files.

6. Document all files and sub-files located on the digital evidence submitted.

7. If possible, import and analyze the digital files using editing software, refer to MMIE SOP-06 for guidance. Record software settings used for the enhancements.
   In some circumstances, due to the nature of the video, enhancements may need to be conducted on screen captures.

RETIRED

8. Any files generated from the digital media should be saved using the highest quality setting.

9. Save any captured segment as a video (.avi/.wmv) file using the Laboratory identification number as the file identifier (*ID-06-12345 capture 1*.avi) or any other descriptor (*bank robbery.avi*).

10. Exported files can be created and labeled in the following manner: camera/time/date stamp information (*c# mmddyy hhmmss.tif, c# hhmmss.tif, mmddyy hhmmss.tif or hhmmss.tif)* or submission number (*Example: S001 1.tif (graphic files)* or other case descriptor.

11. After analysis of the video evidence is completed, save any image or video files in the examiner's case folder on the computer and a copy of this file should be transferred onto a CD/DVD for archiving.

12. The submitting agency may receive a set of printed images or a CD/DVD containing the image files.

13. Media containing electronic files that is generated to be disseminated to the requesting agency or retained as archive will have a sub-item created in the *LIMS Justice Trax System*. Please refer to GL-4 for the sub-itemization guidance.

14. A report will be issued reflecting any enhancements/clarifications made on the submitted evidence.

RETIRED

D. Sources of Error:

Equipment malfunction or condition of evidence submitted.

E. References:

See MMIE SOP-24 Multimedia & Image Enhancement References

A. Purpose:

Standard operating procedures for analysis and enhancement of video from digital video recorders (DVRs).

B. Responsibility:

Forensic Science Examiners assigned to the Multimedia and Image Enhancement Unit or performing casework in the Unit

C. Procedure:

1. Inspect the digital video recorder to ensure that there is no damage present and it is in good working condition.

2. If the digital video recorder is damaged, note the damage on the worksheet and photograph if possible. If necessary, contact the submitting agency. Repair the damage if possible and note the repairing measures on the applicable worksheet (DVR worksheet). Communication with the submitting agency/customer should be documented according the GL-1 Section 4.4.2.

3. If possible obtain an operations manual for the Digital Video Recorder prior to operating. Obtain any necessary passwords/codes for the operation of the equipment.

   Note: Some DVRs may begin recording once it is plugged in, if possible, research how to turn off the record mode prior to proceeding with the analysis.

4. Prior to beginning analysis on the DVR, the examiner should try to establish if the time on the working DVR is accurate to real time. This may be accomplished by comparing the time on the DVR to the NIST time by accessing the following website: http://www.time.gov/ or if internet is not accessible when the DVR is being examined (ie scene) then the examiner may use the satellite time displayed on a cellphone. The examiner should indicate which reference was used when assessing any time differences.

5. If possible, export the requested video using the DVR's export menu and appropriate recording media. If a USB drive or other type of rewritable media is being used for the export, this media should be formatted prior to use.

   If the DVR does not have the appropriate output slots or is not able to make an exported copy, the examiner may make a working copy of the video segment by transferring the video information onto a miniDV tape with a capture device using firewire (IEEE) or analog/BNC connections to

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obtain the signal. Refer to Section 7 of this SOP for the procedure to import the video from the MiniDV.

Other options that the examiner may use to capture the video signal would be using StarTech, Camtasia or SnagIt software or using the line out/monitor out feed and plugging it into a DVD video recorder.

In the event that the video from the DVR/NVR cannot be exported by menu option, the examiner may proceed with the removal of the hard drive to examine using recovery software such as DVR Examiner or other similar software. Once the hard drive has been removed, a write block device should be used protect the evidence from erasure of data.

The retrieval of video directly from the hard drive using recovery software may be used in the event that the agency is requesting a large time frame of video. It should be noted that when the hard drive has been removed, the submitting agency should be advised that placing the hard drive back into the DVR may lead to reformatting of the hard drive.

6. After collecting the required segment of video/video file, document the segments captured on the appropriate worksheet (DVR worksheet).

7. If the video was captured from the monitor feed and recorded to a miniDV tape and further editing or analysis needs to be conducted in Adobe Premiere Pro software, the following procedure may be used to import the video from miniDVr:

Using a miniDV tape:

a. Attach the firewire (IEEE 1394) cable from the capture device to the firewire import slot on the computer.
b. Open Adobe Premiere Pro and input the project name and destination files. Select AVI as file type.
c. Turn on the capture device, the computer will recognize the device and Adobe Premiere Pro will display a monitor with the video contents located on the capture device.
d. Using Adobe Premiere Pro, set the Capture: In/Out.
e. After capturing the video segment, name the .avi file with the Lab Identifier and any other descriptor.

Another option is to record directly from the miniDV player output to the input signal of a DVD recorder. The record option should be set to highest quality recording.

If not using the miniDV tape, open the exported file(s) using Adobe Premiere Pro import option or use the DVR's proprietary player if downloaded at time of export and select the appropriate

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media (CD, DVD, Memory Stick, External Hard Drive, etc). Prior to opening the file in the computer, every effort should be made to ensure that the media is virus-free.

8. After importing the .avi/.mov/.wmv or other compatible video files, analyze the digital files using an editing program. Refer to MMIE SOP-06 for guidance on editing software. Record software settings used for enhancements.

9. Any files generated from the digital media should be saved using the highest quality setting, unless the resulting video file is too large to fit on standard recording media (CD/DVD). In such case, the submitting agency can be contacted to request a larger storage media device or the file may be saved at a lower compression as required. If the recording is larger than 64 GB, the agency will be notified in the report that a copy is retained at the Laboratory for 6 months. The original evidence would need to be resubmitted if another recording is necessary.

10. Save any captured segment as a video file using a Laboratory identification number or other identifier as the video name. *e.g. ID-06-12345 capture 1.avi or bankrobbery.avi*

11. File folders will be created in a documents folder in a drive that is backed up to a server. These folders should be labeled in the following manner: Laboratory case number (eg. 11-000123) and then if there are multiple submissions, the submissions will be placed in a separate electronic folder designated by the submission number (S00# or Sub #).

12. After analysis of the video evidence is completed, save any image or video files in the examiner's case folder on the computer and a copy of this file should be transferred onto a CD/DVD.

13. Exported files should be created and labeled in the following manner: camera/time/date stamp information (*c# mmddyy hhmmss.tif, c# hhmmss.tif, mmddyy hhmmss.tif or hhmmss.tif)* or submission number (*Example: S001 1.tif (graphic files)* or any other designator description.

14. The submitting agency may receive a set of printed images or some type of electronic media (*e.g.* CD/DVD, USB drive or external hard drive) containing the image files and video files.

15. If media containing electronic files is generated to be disseminated to the requesting agency or retained as archive files, a sub-item will be created in the *LIMS JusticeTrax System*. This sub-item will be created as designated in GL-4..

16. A report will be generated providing information on any enhancements/clarifications or file information retrieved from the DVR.

D. Sources of Error:

RETIRED

Equipment malfunction or condition of evidence submitted

E. References:

See MMIE SOP-24 Multimedia & Image Enhancement References

RETIRED

A. Purpose:

To duplicate submitted audio evidence for distribution to other agencies in a format that is recognizable and accessible.

B. Responsibility:

Forensic Science Examiners

C. Procedure:

1. Evidence will be submitted to the Division of Scientific Services according to the Quality Manual protocols.

2. Inspect the physical media for any damage. Any damage should be documented in the case file. Any repairs made should be noted on the appropriate worksheet and the submitting agency/customer may be notified, if necessary, of such repairs.

3. Load the evidence into the appropriate player and locate the segment of audio to be duplicated.

   If the audio is located on digital media and will be loaded into a computer, every effort will be made to ensure that the media is virus-free.

4. Load the appropriate media into the recording device and begin to record.

5. After the duplication process has completed, verify that the duplication copy is properly working and that the recording is of good quality.

6. An archive copy will be maintained at the Forensic Laboratory.

7. Label the original submission and its duplications with the Laboratory's identifiers (case number, submission number and examiner initials).

8. Document on the appropriate worksheet (MMIE: General): the original submission's serial/lot number or other identifying information and the equipment used to perform the duplication.

9. Return the original evidence and duplication copies to Evidence Receiving after the case has been completed.

RETIRED

10. No report is issued for duplications of the entire media. A report is issued when: requested by the submitting agency, or only a portion of the evidence was duplicated by request or if enhancement/editing techniques were needed to clarify the audio during the duplication service.

11. A sub-item will be created in the *LIMS JusticeTrax System* for any media generated for submitting agencies and internal archives following guidance from GL-4.

D. Sources of Error:

Equipment malfunction or condition of evidence

E. References:

See MMIE SOP-24 Multimedia and Image Enhancement References

RETIRED

A. Purpose:

Audio evidence when submitted to the Division of Scientific Services may be in a format or quality that is unable to be played or audibly intelligible to the submitting agency. The purpose of enhancing audio is to make the sound of interest more intelligible to the listener. This generally includes digitally capturing an audio segment of interest. The enhancement can be accomplished by capturing, isolating, adjusting the playback speed, bandwidth or decibel levels of the signal to provide the best quality audio sound for the submitting agency.

When analyzing an audio file, the following software applications (or similar) may be used for the purposes of capturing, processing and enhancing the audio signal generated from the submitted evidence:

- Adobe Soundbooth /Photoshop / Audition / Audacity
- Adobe Premiere
- Izotope

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B. Responsibility:

Forensic Science Examiners assigned to the Unit or conducting casework in the Unit.

C. Procedure:

1. Inspect the analog or digital media to ensure that there is no damage and that it is in good working condition.

2. If the media is damaged, note the damage on the appropriate worksheet (MMIE: General) and contact the submitting agency. If possible, photograph the damage for the case notes. Repair the damage if possible and note the repairing measures on the applicable worksheet. If necessary, the submitting agency/customer may be notified of such repairs.

3. After implementing erasure prevention practices, insert the analog/digital media into the appropriate player (standard audio player, microcassette player, compact disk player).

   If the media is to be inserted into a computer, the media should be checked to ensure that it is virus-free and malware-free.

4. Verify that the input/output signals are operating correctly.

5. Import/capture the audio signal using at least 44,100Hz (44.1mHz) as the sampling rate.

6. Designate the imported with the laboratory case number and save this as a duplicate/working copy of the submitted media and all work performed will be made off of this copy.

7. In the examiner's computer documents, a working folder should be created with the laboratory case number as the identifier for all files/media generated.

8. Adjustments to the audio files may comprise but are not limited to the following:
   - Decibel (dB) level adjustments may be applied to make the audio intelligible.
   - Extraction of interfering signals
   - Application of filters to reduce hum, hiss or other interference
   - Application of filters to clarify specific wavelengths

9. Specific audio duplications or redactions can also be made off the working copy.

10. Record software settings used for the enhancements.

11. Any files generated from the digital media should be saved using the highest quality setting.

12. Save the captured segment as a Windows® Media Audio (wma) or waveform audio format (wav) file using the Laboratory case number, case specific identification or other case descriptor. *e.g. ID-06-12345 capture 1.avi*

13. File folders will be created in a folder that is secure and backed up. These files will be labeled in following manner: Lab case number (YY-NNNNNN) and if multiple submissions are present, each submission will have a separate folder labeled Submission number (S00# or Sub #)) and then followed by the numerical order or other descriptor of files created. *Examples: S001 .wav (audio files) or .wma*

14. After analysis of the audio evidence is completed, save any audio or waveform files in the examiner's case folder on the computer and a copy of this file should be transferred onto a CD/DVD for archiving.

15. If any media containing electronic files are generated to be disseminated to the requesting agency or archived internally, a sub-item will be created in the *LIMS JusticeTrax System*.

D. Sources of Error:

Equipment failure or condition of evidence

RETIRED

E. References:

See MMIE SOP-24 Multimedia and Image Enhancement References

RETIRED

A. Purpose:

Images are often limited/confined to the number of pixels per inch (ppi) to provide the best resolution. Other limitations are set by the camera settings and quality of the optical device. Due to these limitations, enhancements are usually applied by using specific filters to improve the visibility of subtle or latent details to provide the best visual image possible for viewing.

B. Responsibility:

Forensic Science Examiners assigned to the Multimedia and Image Enhancement Unit or performing casework in the Unit.

C. Procedure:

1. Digital image files will be imported with the appropriate media device. If images are provided on a rewritable digital media, precautions will be taken to prevent information from being written onto the submitted evidence during acquisition of files. The use of a write blocker may be used in these circumstances to protect the evidence.

   If media is to be inserted into a computer, every effort will be made to check for viruses using proper security software.

2. The original file shall be saved as a copy and adjustments/enhancements shall be performed on the working copy.

3. Adjustments and enhancements using the following filters/applications may be applied: levels, curves, deinterlace, channel mixer, reduce noise, sharpen/unsharpen, sharpen more and invert, desaturate, remove moiré, crop/stitch and resampling, difference/averaging filters. These filters may be named differently depending on the software used. Refer to MMIE SOP-6 for a list of software that may be used. Record software settings used for the enhancements.

   The following descriptions are filters and other applications that may be used in the examination and enhancement of digital image files:

   Blur: This effect softens the edges or reduces detail in an image.

   Brightness & Contrast: This effect adjusts the brightness and contrast of the color tones located in the segment or frame of video.

   Channel Mixer: Adjustment of color in an image by choosing a RGB (red, green, blue) channel to adjust the intensity and saturation of that hue in an image.

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Deblur (lens and motion): This filter attempts to correct the pixel alignment to adjust a blurred image due to the lens focus being incorrect or the motion of an object in the image.
DeInterlacing: The ability to view either the odd or the even field of a video image frame.
Desaturate: Desaturation is the adjustment of the red, green and blue channels towards the gray spectrum. Creates a black and white in the RGB mode.
Difference/Averaging Filter: Traditional photographic technique used to enhance the detail of an image by reducing the amount of noise or static. Combines two or more exposures of the same scene to bring out detail and obtain a richer and more vivid photograph.
Dodge/Burn: Traditional photographic technique to lighten or darken specific areas of a print by regulating the exposure.
Invert: This effect inverts the color information of an image. This technique may be used to bring out patterns or details from an image.
Levels/Curves: The levels effect adjusts the brightness/contrast of a clip. It combines the functions of the color balance, gamma correction, brightness, contrast and invert effects.
Lucis Pro®: Photographic filter that enhances images by adjusting high, medium and low contrast areas and corrects exposure levels.
Resampling: the addition or removal of pixels to prevent the loss of image details during the enlargement of an image.
Reduce Noise: Image noise appears as random extraneous pixels that are not part of the image detail. Image noise can appear in two forms: luminance (grayscale) noise that makes an image appear grainy and color noise that appears as colored artifacts. The filter reduces luminance noise through adjustment of the blue channel. Color noise is reduced by the filter by adjusting the color channels.
Reduce Moire: Moire is patterns caused by interference between two sets of grids. These are commonly encountered in scanned images and in images with fine linear patterns. The filter adjusts the horizontal and vertical lineup of the pixels and softens the edges of the pixels.
Scale or Magnify: The ability to zoom in or zoom out of a image.
Sharpen/Sharpen more filters: Improves image clarity and emphasizes small details and sharp contrasts.
Shadow/Highlight: Photographic technique that adjust the exposure levels in highlighted or shadowed area of an image/video.
Stiching: Allows the creation of photos with higher resolution or wider view. This stitching provided a seamless joining of multiple digital images.
Unsharpen: Filter applied to soften the pixel edges.

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4. After analysis of the image media is completed, save any image files in the examiner's case folder on the computer and a copy of this file should be transferred onto a CD/DVD for archiving in the video storage area which is a secure storage location.

5. The submitting agency will receive a compact disk containing the enhanced image files.

6. If media containing electronic files is generated to be disseminated to the requesting agency/customer, a sub-item will be created in the *LIMS JusticeTrax System* using GL-4 as guidance for sub-itemization. A sub-item will be created for the archive copy being retained at the Laboratory.

D. References:

See MMIE SOP-24 Multimedia and Image Enhancement References

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RETIRED

A. Purpose:

The Multimedia and Image Enhancement Unit may assist law enforcement agencies in enhancing and capturing video/audio evidence for investigative or evidentiary purposes from video/audio systems and also provide image comparison with known exemplars provided by the submitting agency or evidentiary items submitted to the Laboratory. Image comparison is an assessment of the correspondence between features in images and known objects or images retrieved from video and compared to known objects/reference images for the purpose of rendering an expert opinion regarding identification or elimination. The Unit may also produce a demonstrative exhibit of such comparisons. In some cases, an opinion/report may consist of a composite image showing demonstrative areas of consistent features.

B. Responsibility:

Forensic Science Examiners assigned to the Multimedia and Image Enhancement Unit or performing casework in the Unit.

C. Methodology:

There are a number of critical practices, processes, and factors used to ensure and demonstrate validity in forensic image comparison. The relative importance of any one of these may vary among cases. Regardless of the protocol used in conducting photographic comparisons, the methodology used should be documented:

Class vs. Individual Characteristics

The concept of class vs. individual characteristics is fundamental to forensic image comparison. Class characteristics are used to subdivide things into groups or classes. Individual characteristics allow one to differentiate objects within a class from one another. Individual characteristics may be used to uniquely characterize an object. They arise from such events as random natural or commercial processes, intentional alteration, and wear-and-tear. In the case of vehicle image comparison, the basic body type/style might be used to be included in a class characteristic or eliminated.

The ability to identify an individual person or object requires a correspondence of individual characteristics. The number of such characteristics necessary for such an identification is a function of the subject matter, the quality and quantity of details in the images, and the expertise of the analyst; no arbitrary number of characteristics is required. A correspondence of class characteristics may be useful for establishing candidate subjects. Likewise, a lack of corresponding class and/or individual characteristics can be used to eliminate potential subjects. In the case of a vehicle image comparison,

this might be the comparison of damage or missing parts to the vehicle. In the case of comparisons of a person, this might include the comparison of tattoos or scars or other biometric features.

ACE-V

A commonly accepted protocol applied to image comparisons is ACE-V (Analysis, Comparison, Evaluation – Verification). Not all image comparisons invoke this method. The Analysis stage involves a thorough assessment of the properties and attributes of the features contained in the images under examination.

In the Comparison stage, an assessment is made of the correspondence/discordance of the characteristics identified in the Analysis stage.

In the Evaluation stage, a tentative conclusion is reached and the correspondences/discordances are tested against it. All discordances are evaluated to determine if they reflect true differences between the subjects.

Three types of discordance may be observed:
(1) *Explainable differences*. Discordances may result from the imaging process (compression artifacts, watermarks, lens aberration) or conditions from the scene or object.
(2) *Unexplainable differences*. Discordances exist, but are of unknown source and significance.
(3) *Exclusionary differences.* Discordances that reflect a true difference between the objects under comparison. Such a difference establishes an elimination.

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D. Procedure:

1. Prior to beginning the image comparison, the image selected for use in the comparison should be analyzed for the presence of artifacts. In order to accurately interpret the content of an image under examination, it is imperative that the examiner not mistake artifacts of the imaging process as reflective of physical properties of the subject depicted. The image should be analyzed to determine if resolution, compression, optical defects, sensor defects, lighting conditions, atmospheric conditions, and motion, among others, may introduce artifacts or other objects that may prevent correct interpretation. The images for comparison should be the same scale for accurate interpretation.

   If it is necessary to return to the original scene/recording device, measures should be taken to ensure that there have been no upgrades in software, maintenance of hardware that may have affected the position of the equipment or replacement of equipment. If one of these situations is noted, it should be noted that conditions are not optimal for image comparison purposes.

2. Image comparisons commonly involve an examination and evaluation of features observed in a submitted image or exported from a video to be compared to features of a known subject/object. This process may require image/video processing to enhance features for comparison. These processes should be carried out as referenced in SOP-MMIE-06 and SOP-MMIE-17.
3. Often it is necessary to determine that issues of image creation, lighting, and composition do not create artifacts that may affect the comparison. Reconstruction of the circumstances of the questioned image acquisition may be necessary especially if the image/video was captured/recorded using a camera with an infrared filter or the camera that captured the image was positioned in an angle that may affect the size and quality of the objects being compared. This reconstruction may consist of photographing or recording the object under comparable conditions (same digital/analog recording system) as the original submission was presented. If this avenue for reconstruction is no longer possible, then the examiner will note this in the report that this factor may affect the final image comparison results.
4. The examiner will import the selected images into Adobe Photoshop to provide the background canvas (composite) for the images to be compared. It is the examiners discretion on how he/she will demonstrate the corresponding objects/items being compared. Some acceptable visual markers may include numbers/letters, arrows, overlays and side by side visual comparisons.

   In cases in which an image is being compared to an image of a vehicle's make/model which was researched from common source vehicle manufacturer website; if this is being used as a comparative image, the website will be clearly marked with the address of the image. Additionally, the report will clearly note that this image was produced from a website and that the investigator must follow-up on the validity of the image posted.
5. After the examiner has determined which features/objects will be compared, the examiner will use one of the previously listed methodologies to evaluate these items. After evaluation, the examiner can render an opinion based on the findings as depicted below:

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IMAGE COMPARISON LEVEL OF FINDINGS CONTINUUM	
Individualizing Characteristics Present	**Individualization**
Similar Characteristics *– cannot individualized to that object only*	**Identification**
Similarities Noted – No Conclusion *– cannot distinguish if artifacts are present*	
No Conclusion *– markings noted, but not enough detail to determine if similar*	
Dissimilarities Noted – No Conclusion *– cannot distinguish if artifacts are present*	
Dissimilar	
Elimination	**Elimination**
No Comparison Possible *– quality of image not suitable for image comparison*	**No Examination Conducted**

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6. If the examiner has identified enough features to render a conclusion of an elimination or an identification. The composite image and the features used to render this conclusion must be verified by a second competent examiner. The verifying examiner will note on the printed composite that they agree with the conclusion by initialing the page, writing "OK" and the date the verification was conducted.
7. In cases in which the examiner uses class characteristics present to produce a possible make and model of candidate vehicles, the examiner should include the information of what open-source sites may have been used to generate the candidate list. The agency requesting the examination will be supplied with photographs as well as any other information on the shoe/tire and the

reference site information. A note should be placed in the report that the information provided was retrieved from a non-forensic open-source site and that the information provided should be verified with the source.

8. If the examiner notes any findings that may affect the image comparison, these findings will be listed in the report for the submitting agency.

E. References:

See MMIE SOP-24 Multimedia and Image Enhancement References

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A. Purpose:

In order to provide and maintain the quality of the work expected in the Multimedia and Image Enhancement Unit, it is necessary to ensure that the equipment is in good working condition. Routine quality control measures and equipment maintenance are used to accomplish this. Any quality control measures listed below are generally considered to be the minimum appropriate in each case, providing that the equipment is of good quality and of proven stability and the laboratory has both the equipment capability and expertise to perform adequate internal checks. More frequent checks are not discouraged.

If there is any question concerning the reliability of an instrument or piece of equipment, a maintenance check should be performed immediately. If the instrument or piece of equipment is deemed unreliable, this instrument or piece of equipment will be taken off-line until repairs or other appropriate measures are taken.

If the malfunctioning equipment was used to produce a product for the submitting agency/customer, the customer will be notified. Additionally, the Quality Manager will be notified and an incident report or corrective action may be needed.

B. Responsibility:

Forensic Science Examiners assigned to the Multimedia and Image Enhancement Unit or conducting casework in the Unit.

C. Documentation

Documentation of quality control will be made on the designated worksheet (MMIE: General). A logbook (Equipment Logbook) was maintained for the quality control of all designated equipment prior to 2014. This logbook is now used to cite documentation of date of maintenance or repair, other relevant observations or date taken out of service.

D. Procedure

1. Computers

This Unit utilizes several computers to perform casework and these computers contain various editing and enhancement software programs and hardware to generate working copies of audio, video and data files for each case.

01. Quality Control Procedure

It is the responsibility of employees to report any malfunction to the Supervisor and the Information Technology Department.

It is the responsibility of employees to ensure that the computers are operating properly and appropriate updates and virus checks are performed in a timely manner.

02. Maintenance Procedure

If the computers are not functioning properly, a qualified service technician shall repair the equipment or the computer shall be replaced with a comparable model. This equipment will be taken out of service until the issue is corrected.

2. Video Cameras

The Unit utilizes the video cameras to playback and create a digital storage file of video information. This camera may be used to import digital video/audio files by fire-wire (IEEE) into video/audio editing and enhancement software.

01. Quality Control Procedure

On days of use, the camera shall be inspected to ensure that all controls are functioning. A record of this inspection shall be maintained on the MMIE General Worksheet.

It is the responsibility of employees to report any malfunctioning of these video cameras to the Supervisor. This equipment will be taken out of service until the issue is corrected.

It is the responsibility of employees to ensure that the video cameras are working properly and that any malfunctions will be repaired or replaced in a timely manner.

02. Maintenance Procedure

If the video cameras are not functioning properly, a qualified service technician shall repair the instrument or this camera shall be replaced with a similar working model. Information on any maintenance will be noted in the Equipment Logbook. Records must be kept regarding repairs and/or corrective actions.

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3. VCR/DVD Players and Recorders

The Unit uses the various VCR/DVD players/recorders for the playback or recording of DVD, SVCD, VCD, VHS and audio CDs. These pieces of equipment may also be used to playback compact disks containing JPEG and MP3 files. This equipment is used primarily as the player in duplication procedures.

01. Quality Control Procedure

On days of use, these units shall be inspected to ensure that all controls are functioning. A record of this inspection will be maintained on the MMIE General Worksheet. All copies made by this equipment will be verified.

It is the responsibility of employees to report any malfunction of this player to the Supervisor. This equipment will be taken out of service until the issue is corrected.

It is the responsibility of employees to ensure that the player is working properly prior to beginning casework and that any malfunction will be repaired or replaced in a timely manner.

02. Maintenance Procedure

If the player is not functioning properly, a qualified service technician shall repair the instrument. Maintenance logs must be kept regarding repairs and/or corrective actions.

03. Safety

Visible and invisible laser radiation is used in some of these instruments. There is a danger of eye injury. Only qualified service personnel shall remove the cover or attempt repairs.

4. Televisions and Monitors

This Unit utilizes monitors for viewing/monitoring video evidence
when being placed through various recorders. This unit allows input through Video/Audio, S-Video and Component CMY lines.

01. Quality Control Procedure

RETIRED

It is the responsibility of employees to report any malfunctions occurring with the television to the Supervisor. This equipment will be taken out of service until the issue is corrected.

It is the responsibility of employees to ensure that the television is working properly prior to starting casework and that any malfunctions are repaired or replaced in a timely manner.

02. Maintenance Procedure

If the monitor is not functioning properly, a qualified service technician shall repair the instrument. Maintenance logs must be kept regarding repairs and/or corrective actions.

5. MiniDV and Hi-8mm Players/Recorders

The Multimedia Unit utilizes the MiniDV and Hi-8mm players/recorders to capture, record and play-back digital video images and audio for video/audio enhancement and duplications. The digitized video is imported into the appropriate editing/enhancing software using a fire-wire (IEEE) port.

01. Quality Control Procedure

On days of use, this unit shall be inspected to ensure that all controls are functioning. A record of this inspection will be maintained on the MMIE General Worksheet
It is the responsibility of employees to report any malfunctioning of these units to the Supervisor. This equipment will be taken out of service until the issue is corrected.

It is the responsibility of employees to ensure that the MiniDV/Hi-8mm player/recorders are working properly and that any malfunctions are repaired or replaced in a timely manner.

02. Maintenance Procedure

If this equipment is not functioning properly, a qualified service technician shall repair this unit. Maintenance logs must be kept regarding repairs and/or corrective action.

6. Standard Audio and Microcassette-Transcriber Player/Recorders

The Multimedia Unit utilizes the microcassette player/recorder to record and play-back microcassette tapes for audio enhancement and duplications. The audio signal from this

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equipment is imported into the appropriate editing/enhancement software using the headphone (line-out) and microphone (line-in) pathway.

01. Quality Control Procedure

On days of use, these units shall be inspected to ensure that all controls are functioning. A record of this inspection will be maintained on the MMIE General Worksheet

It is the responsibility of employees to report any malfunctioning of this equipment to the Supervisor. This equipment will be taken out of service until the issue is corrected.

It is the responsibility of employees to ensure that the equipment is working properly and that any malfunctions are repaired or replaced in a timely manner.

02. Maintenance Procedure

If this equipment is not functioning properly, a qualified service technician shall repair this unit. Maintenance logs must be kept regarding repairs and/or corrective action.

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7. Tableau Forensic USB Bridge (Ultrablock) / Forensic SATA/IDE Bridge (Tableau Ultrablock 2)

The Multimedia Unit utilizes the Tableau Forensic Ultrablocks to protect (write-block) any evidentiary media which includes, but is not limited to: thumb drives, SD cards, miniSD cards, MS, SM (smart media), microSD, camera flash media, hard drives and any memory media/data storage devices connected with a USB port.

01. Quality Control Procedure

On days of use, this unit shall be inspected to ensure that all controls are functioning. A record of this inspection will be maintained on the MMIE General Worksheet
It is the responsibility of employees to report any malfunctioning Tableau Forensic Ultrablock to the Supervisor. This equipment will be taken out of service until the issue is corrected.

It is the responsibility of employees to ensure that the Tableau Forensic Ultrablock is working properly and that any malfunctions are repaired or replaced in a timely manner.

02. Maintenance Procedure

If the Tableau Forensic Ultrablock is not functioning properly, a qualified service technician shall repair this unit. Maintenance logs must be kept regarding repairs and/or corrective action.

8. Software

The Multimedia and Image Enhancement Unit utilizes various types of software to capture, edit and enhance video/images/audio. The Unit will verify that this software is working properly according to the manufacturers specifications and that the computer in which it was installed has the minimum system requirements.

Any upgrades in versions will at a minimum have a performance check conducted depending on the magnitude of the changes made with the upgrade if they affect filters and other features used for casework. Any major updates will require a validation of the software before using in casework.

RETIRED

E. Sources of Error:

F. References:

Refer to instruction/operation manuals for each piece of equipment/software located in the Multimedia and Image Enhancement Unit or in the Controlled SOPs Folder located on the S drive.

A. Purpose:

General quality records, worksheets and a maintenance log should to record any processing and documentation of evidence handled in the Multimedia and Image Enhancement Section. The Multimedia and Image Enhancement Section will issue reports of analysis using a general laboratory report template located in the Justice Trax system.

RETIRED

B. Responsibility:

Forensic Science Examiners assigned to the Multimedia and Image Enhancement Unit or conducting casework in the Unit.

C. Quality Records Used by Examiners:

Worksheet Name or Description:	Quality Record Number
General Worksheet	QR: MMIE General
Review Checklist:	QR: MMIE Checklist
Scene Retrieval Worksheet	QR: Scene Retrieval
DVR Worksheet	QR: DVR Worksheet
Vehicle Comparison Worksheet	QR: MMIE Vehicle Comparison Worksheet

The controlled Quality Records are located on the S: drive in the Controlled Documents folder.

D. Quality Control Log:

Quality Control checks are documented on the QR: MMIE General on days of use.

Any repairs or issues with the equipment will be documented in the Unit's Equipment Logbook.

E. Sources of Error:

None

F. References:

Equipment User Manuals

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A. Purpose:

To assist law enforcement agencies in capturing video/audio evidence for investigative or evidentiary purposes from video/audio systems.

B. Responsibility:

Forensic Science Examiners assigned to the Multimedia and Image Enhancement Unit or performing casework in the Unit.

C. Procedure:

1. Laboratory personnel shall assist the law enforcement agency in processing and retrieving video evidence in accordance to the law. The burden of consent or search rights will be the responsibility of the requesting agency.

   Upon request for video retrieval assistance, the Deputy Director shall be informed of the request and will issue approval if the Unit will provide assistance. All contact information will be recorded on the Crime Scene Assistance Form (current version is located in the Controlled SOPs folder – Other Forms).

2. The capture process of video and audio evidence shall be conducted as defined in SOP-MMIE-07, SOP-MMIE-08, SOP-MMIE-09 and other applicable SOPs depending on the case.

3. Examiners will make arrangements to meet law enforcement personnel at the scene of the retrieval. Law enforcement personnel must be present at all times on-scene when the Unit is conducting a retrieval.

4. The examiner will document capture techniques utilized in obtaining evidence. All documentation and information on the system and actions taken at the scene will be documented on the MMIE Scene Retrieval Worksheet.

   If video files are able to be extracted, the requesting agency will be provided a compact disk or provided a thumb drive containing the retrieved data. The requesting agency will be requested to fill out a Request for Analysis Form (SOP-ER-02:1).

5. The compact disk containing the retrieved files/video will be turned over to the law enforcement agency's scene commanding officer to maintain chain of custody. This officer will sign for the media that was provided to him on the MMIE Scene Retrieval Worksheet. The original download will be contained on the Unit laptop which is password protected.

6. Upon return to the Unit, the examiner will download the recovered files to some type of electronic media and can submit these files as a case to the Evidence Receiving Unit. If the analyst has been

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trained in the intake of evidence by the Evidence Receiving Unit, the analyst may create the case in Justice Trax and transfer the evidence to a storage location. A laboratory case will be initiated or continued in Justice Trax and the submitted evidence will be transferred to video storage for archival storage. The files on the Unit laptop may be deleted at this time.

7. If the video retrieval was unsuccessful, this information will be documented on the Crime Scene Assistance form and a report may be issued to the agency, if requested.

   In the event that the DVR system must be removed from the scene, this system will be disassembled and turned over to the law enforcement agency representative. The law enforcement agency will handle all chain of custody, consent/warrant and other paperwork relating to the seizing of the system. The law enforcement will be responsible for submitting the DVR system to the laboratory to be examined.

8. A case jacket will be maintained in the MMIE Unit containing at a minimum: the MMIE Scene Retrieval Worksheet, the evidence receiving paperwork, any notes and a copy of the Crime Scene Assistance form.

D. References:

State of Connecticut, Department of Public Safety, Administration and Operations Manual

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A. Purpose:

As the field of video and image enhancement expands, the user must be able to understand the terminology that is routinely applied to the field. The following contains technical terminology that may appear in the examiner's worksheets and reports.

B. Responsibility:

Forensic Science Examiners assigned to the Unit or performing casework in the Unit.

C. Terminology:

Administrative Review
A procedure used to check casework for consistency with agency/laboratory policy and for editorial practice.

Algorithm
A step-by-step procedure for solving a problem or accomplishing some end. [*Webster's Dictionary*]

Archive Copy
A copy of data placed on media suitable for long-term storage, from which subsequent working copies can be produced. The archive copy may be stored in a high capacity media device or on CD/DVD and filed in a secure, locking filing cabinet or password protected.

Archive Image
Any image placed on media that is suitable for long-term storage.

Archiving
The process of storing data in a manner suitable for long term availability and retrieval.

Artifact
(a, i, v) A visual/aural aberration in an image, video, or audio recording resulting from a technical or operational limitation. Examples include speckles in a scanned picture or "blocking" in images compressed using the JPEG standard.
(c) Information or data created as a result of the use of an electronic device that shows past activity.

Aspect Ratio
(i, v) The width to height ratio of an image.

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Audio Enhancement
Processing of recordings for the purpose of increased intelligibility, attenuation of noise, improvement of understanding the recorded material and/or improvement of quality or ease of hearing.

Authentication
The process of substantiating that the data is an accurate representation of what it purports to be.

Capture
The process of recording data, such as an image, video sequence, or audio stream.

Capture card/frame grabber
A piece of computer hardware that accepts an analog or digital signal and outputs the signal as digital data.

Capture Device
A device used in the recording of data.

CD/DVD (compact disc/digital versatile disc)
Optical disc formats designed to function as digital storage media.

Chain of Custody
The chronological documentation of the movement, location and possession of evidence.

Codec
A device or computer program capable of encoding or decoding a digital data stream or signal; commonly referred to as compressor/decompressor

Cognitive Image Analysis
(i, v) The process used to extract visual information from an image.

Colorimetry
The quantification of the color of an object.

Color Range
The range of colors that can be detected by a sensor.

Compression

RETIRED

The process of reducing the size of a data file. (See also, "Lossy Compression" and "Lossless Compression".)

Compression ratio
The size of a data file before compression divided by the file size after compression.

Copy
An accurate reproduction of information.

Data
Information in analog or digital form that can be transmitted or processed.

Data Analysis
The assessment of the information contained within the media.

Data Extraction
A process that identifies and recovers information that may not be immediately apparent.

Deblurring
(i, v) A type of image restoration used to reverse image degradation, such as motion blur or out-of-focus blur. It is accomplished by applying algorithms based on knowledge or an estimate of the cause of the original degradation.

Deinterlacing
(v) Separating an interlaced frame into two discrete fields.

Demonstrative Comparison
(v) A method of presenting the similarities and/or differences among images and/or objects without rendering an opinion regarding identification or exclusion.

Digital CCTV Retrieval
(v) The process of retrieving video/images from digital CCTV systems.

Digital Evidence
Information of probative value that is stored or transmitted in binary form.

Digital Image

RETIRED

(i) An image that is represented by discrete numerical values organized in a two-dimensional array. [Taken from the "*Encyclopedia of Photography*" 3rd Edition] When viewed on a monitor or paper, it appears like a photograph. (c) See definition for "Image".

Directory Listing
(c) A list of files contained within an object. It may also contain other information such as the size and dates of the files.

Duplicate
An accurate and complete reproduction of all data objects independent of the physical media.

Dynamic Range
(i) The difference between the brightest highlight and darkest value that a sensor (e.g. film or CCD) can detect and record in a single image.
(a, v) The ratio of the strongest (undistorted) signal to that of the weakest (discernible) signal in a unit or system as expressed in decibels (dB). A way of stating the maximum signal to noise ratio.

Erased File Recovery
(c) The process for recovering deleted files.

Field
(v) An element of a video signal containing alternate horizontal lines. For interlaced video, the scanning pattern is divided into two sets of spaced lines (odd and even) that are displayed sequentially. Each set of lines is called a field, and the interlaced set of the two sets of lines is a frame.

File Format
The structure by which data is organized in a file.

File Slack
(c) The data between the logical end of a file and the end of the last storage unit for that file.
Ex: For the FAT (file allocation table) file system, the data between the logical end of the file and the end of the cluster.

Forensic Audio
A subdiscipline of Digital & Multimedia Evidence, which involves the scientific examination, analysis, comparison, and/or evaluation of audio.

Format

RETIRED

(v) One or several combined elements that may be used to describe the video recording method. These include tape width (e.g. 8mm, ½ inch, ¾ inch, 1 inch), signal form (e.g. composite, Y/C, component), media (e.g. VHS tape, DVD, CD), data storage type (e.g. analog/digital, AVI/MPEG), and signal standard (e.g. NTSC, PAL, SECAM).

Format Conversion
(a, i, v) To transfer audio and/or video information from one media type to another and/or from one recording method to another.

Frame
(v) Lines of spatial information of a video signal. For interlaced video, a frame consists of two fields, one of odd lines and one of even lines, displayed in sequence. For progressive scan (non-interlaced) video, the frame is written through successive lines that start at the top left of the picture and finish at the bottom right.

There are three major frame types found in video evidence: I, P and B frames.

I – frames are the lease compressible and are usually the coding frame for subsequent frames following.

P – frames use data from the previous frame to decompress and are more compressible than I-frames.

B- frames uses both the previous and forward frames for data reference to get the highest amount of data compression.

The I frame is an "Intra-coded picture' frame, while the P and B frames only hold part of the original image information. The P frame (aka Delta frames) is a "Predicted picture" that holds only changes from the previous frames. Finally, the B frame is a "bi-directive picture" that save more memory space by using the differences between the current frame and the preceding and following frame.

When the need to establish a frame count or time count in a video sequence, it is best to start with the I-frame if this frame is able to be located to determine the coding sequence.

Free Space
Data storage areas available for use by the computer. The area may already contain previously stored information. Also referred to as Unallocated Space.

Gaussian Blur
(i, v) A function typically used to reduce image noise and detail using a specific mathematical function known as the "Gaussian Kernel" or "bell-curve". The visual effect of this technique is a smoothing of image features as if viewing the image through a translucent filter.

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Hash or Hash Value
Numerical values, generated by hashing functions, used to substantiate the integrity of digital evidence and/or for inclusion /exclusion comparisons against known value sets.

Hashing Function
An established mathematical calculation that generates a numerical value based on input data. This numerical value is referred to as the hash or hash value.

Image
(c) A bit stream duplicate of the original data.
(i, v) An imitation or representation of a person or thing, drawn, painted, photographed, etc.

Image Analysis
A sub-discipline of Digital & Multimedia Evidence, which involves the application of image science and domain expertise to examine and interpret the content of an image and/or the image itself in legal matters.

Image Averaging
(i, v) The process of averaging similar images, such as sequential video frames, to reduce noise in stationary scenes.

Image Comparison (Photographic Comparison)
(i) The process of comparing images of questioned objects or persons to known objects or persons or images thereof, and making an assessment of the correspondence between features in these images for rendering an opinion regarding identification or elimination.

Image Content Analysis
(i) The drawing of conclusions about an image. Targets for content analysis include, but are not limited to: the subjects/objects within an image; the conditions under which, or the process by which, the image was captured or created; the physical aspects of the scene (e.g., lighting or composition); and/or the provenance of the image.

Image Data Recovery
(i) The process of retrieving viewable image(s) from a data set.

Image Enhancement
(i, v) Any process intended to improve the visual appearance of an image or specific features within an image.

RETIRED

Image Output
(i) The means by which an image is presented for examination or observation.

Image Processing
(i) Any activity that transforms an input image into an output image.

Image Processing Log
(i) A record of the steps used in the processing of an image.

Image Restoration
(i, v) Image restoration is any process applied to an image that has been degraded by a known cause (e.g., defocus or motion blur) to partially or totally remove the effects of that degradation.

Imaging Technology
(i, v) Any system or method used to capture, store, process, analyze, transmit, or produce an image. Such systems include film, electronic sensors, cameras, video devices, scanners, printers, computers, etc.

Image Transmission
(i, v) The act of moving images from one location to another.

Integrity verification
The process of confirming that the data presented is complete and unaltered since time of acquisition.

Intermediate Storage
Any media or device on which data is temporarily stored for transfer to permanent or archival storage.

Interlaced Scan
(v) A technique of combining two television fields in order to produce a full frame. The two fields are composed of only odd and only even lines, which are displayed one after the other but with the physical position of all the lines interleaving each other, hence interlace. (*CCTV*, Vlado Damjanovski, Butterworth-Heinemann. 2000)

Internet Protocol (IP) Address
A unique string of numbers separated by periods that identifies each computer or electronic device to communicate over a network.

RETIRED

Interpolation
(i, v) A method of image processing whereby one pixel, block, or frame is displayed or stored based on the differences between the previous and subsequent pixel, block, or frame of information. [Taken from the *Encyclopedia of Photography* 3rd Edition] This is often done to increase the apparent clarity of an image.

Log File
A record of actions, events, and related data.

Lossy Compression
Compression in which data is lost and cannot be retrieved in its original form.

Lossless Compression
Compression in which no data is lost and all data can be retrieved in its original form.

Media
Objects on which data can be stored.

Media Characterization
The process of inspecting, identifying, and noting the properties of the media.

Metadata
Data, frequently embedded within a file, that describes a file or directory, which can include the locations where the content is stored, dates and times, application specific information, and permissions.

Multiplexer/Demultiplexer
(v) A device used to combine multiple video signals into a single signal or separate a combined signal. These devices are frequently used in security and law enforcement applications for recording and/or displaying multiple camera images simultaneously or in succession.

Multimedia Evidence
Analog or digital media, including, but not limited to, film, tape, magnetic and optical media, and/or the information contained therein.

Native File Format
The original form of a file. A file created with one application can often be read by others, but a file's native format remains the format it was given by the application that created it. In most cases

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the specific attributes of a file (for example, fonts in a document) can only be changed when it is opened with the program that created it. [*Newton's Telecom Dictionary*]

Noise
(i, v) Variations or disturbances in brightness or color information in an image that do not arise from the scene. Sources of noise include film grain, electronic variations in the input device sensor and circuitry, and stray electromagnetic fields in the signal pathway. It frequently refers to visible artifacts in an image.

NTSC
National Television Standards Committee.

Original Image
(i) An accurate and complete replica of the primary image, irrespective of media. For film and analog video, the primary image is the original image.

PAL
Phase Alternation Line. [European Broadcast Union]

Partition
User defined section of electronic media.

Password Recovery
The process of locating and identifying a series of characters used to restrict access to data.

Photogrammetry
The art, science, and technology of obtaining reliable information about physical objects and the environment through the processes of recording, measuring, and interpreting photographic images and patterns of electromagnetic radiant energy and other phenomena [*The Manual of Photogrammetry*, 4th Edition, 1980, ASPRS].
In forensic applications, Photogrammetry, sometimes called "*mensuration*," most commonly is used to extract dimensional information from images, such as the
height of subjects depicted in surveillance images and accident scene reconstruction. Other forensic photogrammetric applications include visibility and spectral analyses. When applied to video, this is sometimes referred to as "videogrammetry".

Photogrammetric Analysis
The process of obtaining dimensional information regarding objects and people depicted in an image.

RETIRED

Photometry
The measurement of light values of objects in an image.

Physical Copy
(c) An accurate reproduction of information contained on the physical device.

Playback Optimization
(a, v) The process of determining the most suitable equipment and settings for analyzing the output signal.

Playback
Recorded material viewed and heard as recorded, facilitated by camcorder, cassette recorder, or other device.

Primary Image
(i, v) Refers to the first instance in which an image is recorded onto any media that is a separate, identifiable object. Examples include a digital image recorded on a flash card or a digital image downloaded from the Internet.

Processed Image
(i, v) Any image that has undergone enhancement, restoration or other operation.

Production Switcher
(a, v) A device and/or software used to mix video and/or audio signals from two or more sources (e.g. cameras, videocassette recorder/players, character generators) for dissolves, wipes, and other transition effects.

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Proficiency Test
A test to evaluate analysts, technical support personnel, and the quality performance of an agency.

Progressive Scan
(v) Display scan pattern where each line of the frame is scanned out sequentially.

Proprietary File Format
Any file format that is unique to a specific manufacturer or product.

Quality Assurance

Planned and systematic actions necessary to provide sufficient confidence that an agency's/laboratory's product or service will satisfy given requirements for quality.

Quantitative Image Analysis
(i, v) The process used to extract measurable data from an image.

Reconstruction
The process of repairing damaged media in order to allow the retrieval of data.

Reference Materials
Refers to items such as published literature, hardware and software documentation, hash sets, header sets, etc.

Reliability
The extent to which information can be depended upon.

Reproducibility
The extent to which a process yields the same results on repeated trials.

Residue
(c) Data that is contained in unallocated space or file slack.
(a) The residue of a filtered signal is the algebraic difference between the filter output and its signal input. [*Diamond Cut Users Manual*]

Resolution
(i, v) The act, process, or capability of distinguishing between two separate but adjacent parts or stimuli, such as elements of detail in an image, or similar colors. [Taken from the *Encyclopedia of Photography*, 3rd Edition]

Restoration
(i, v) Restoration is any process applied to an image that has been degraded by a known cause (e.g., defocus or motion blur) to partially or totally remove the effects of that degradation.
(c) The process of restoring data from an image.

Routing Switcher
(a, v) A device and/or software used to direct the path of one or more signals into one or more devices.

Sharpening

RETIRED

(i, v) A process used to emphasize edge detail in an image by enhancing the high frequency components.

Standard Conversion
(v) The transformation of one television system signal to another. For example, NTSC to PAL.

Source Code
The list of instructions written in a programming language used to construct a computer program.

Storage Media
Any object on which data is preserved.

Technical/Peer Review
An evaluation conducted by a second qualified individual of reports, notes, data, conclusions, and other documents.

Time-base Corrector (TBC)
(v) An electronic device used to correct timing inconsistencies and stabilize the playback of the video signal for optimum quality. It also synchronizes video sources allowing image mixing.

Time Lapse Video Recording
(v) Process by which images are recorded at less than the standard rate of frames per second (NTSC – 29.97; PAL – 25.00) thus extending the period of time that can be covered by the storage medium.

Timeline Sequence Reconstruction
The process of relating images, audio, or other data to one another in a chronologically ordered succession.

Traditional Enhancement Techniques
(i) Techniques that have direct counterparts in traditional darkrooms. They include brightness & contrast adjustment, color balancing, cropping, and dodging & burning.

Unallocated Space
(c) Data storage areas available for use by the computer. The area may already contain previously stored information. Also referred to as *Free Space*.

Validation

RETIRED

The process of performing a set of experiments, which establishes the efficacy and reliability of a tool, technique or procedure or modification thereof.

Validation Testing
An evaluation to determine if a tool, technique or procedure functions correctly and as intended.

Vectorscope
(v) An electronic device that measures a video signal's chrominance (color) performance.

Verification
1. The process of confirming the accuracy of an item to its original.
2. Confirmation that a tool, technique or procedure performs as expected.

Video
The electronic representation of a sequence of images, depicting either stationary or moving scenes. It may include audio.

Video Analysis
A subdiscipline of Digital & Multimedia Evidence, which involves the scientific examination, comparison, and/or evaluation of video in legal matters.

Video Distribution Amplifier
(v) A device used to divide single video signals, while boosting their strength for delivery to multiple video devices.

Video Enhancement
Any process intended to improve the visual appearance of video sequences or specific features within video sequences.

Video Stabilization
(v) The process of positioning individual frames so that a selected object or person will remain in the same location as the video is played.

Waveform Monitor
(v) An electronic device that provides a graphic display of a video signal.

Work Copy
A copy or duplicate of a recording or data that can be used for subsequent processing and/or analysis.

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Write Block/Write Protect
Hardware and/or software methods of preventing modification of media content.

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A. Purpose:

The Multimedia and Image Enhancement Section maintains reference material for research, processing of evidence and preparation for court testimony.

B. Responsibility:

Forensic Science Examiners

C. References:

For Software User Manuals, see Controlled SOPs/Qualtrax

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A. Purpose:

When processing evidence and generating worksheets, abbreviations may be used to consolidate time and writing. This SOP provides a list of commonly used abbreviations in the Multimedia and Image Enhancement Section.

B. Responsibility:

Forensic Science Examiners

C. References/Abbreviation List:

AVI	Audio Video Interleave, is a multimedia container format introduced by Microsoft in November 1992 as part of its Video for Windows technology. AVI files can contain both audio and video data in a file container that allows synchronous audio-with-video playback.
BMP	BMP file format, sometimes called bitmap or DIB file format (for device-independent bitmap), is an image file format used to store bitmap digital images, especially on Microsoft Windows and OS/2 operating systems.
B&W	Black and white (no color present; grayscale)
CAM	May be a shortened version for “camera” often seen used in reference to Body Cameras.
CD	Compact Disc is an optical disc used to store digital data, originally developed for storing digital audio.
CD-R	Compact Disc-Recordable is a Write Once Read Many (WORM) optical Medium.
Chap.	Chapter
DVD	“Digital Versatile Disc" or "Digital Video Disc" is a popular optical disc storage media format. Its main uses are video and data storage. DVD-R has a five-year lead on DVD+R. As such, older or cheaper DVD players (up to 2004 vintage) are more likely to favor the DVD-R standard. When creating DVDs for distribution where the playing unit is unknown or older

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	the DVD-R format would normally be preferable.
DVD+R	See Above
DVD-R	See Above
DVR	Digital video recorder
Evid.	Evidence
EXE	Filename extension for Executable files, is a program file / software
FF	frames (when used above a number set)
HDMI	High-Definition Multimedia Interface, is audio/video interface for transmitting uncompressed video and audio data from a device to a compatible device
HH	hours
HZ	Hertz
IP	Internet Protocol
IPS(ec)	Internet Protocol Security
ISP	Internet Service Provider
IVR	Internet Video Recording a proprietary file extension of RealPlayer.
JPEG	A file extension of a commonly used method of compression for photographic images developed by the Joint Photographic Experts Group.
JPG	See Above
MD5	Message Digest Algorithm 5 (128-bit hash value)
MIN	minutes
MM	minutes

RETIRED

MP4	Filename extension for MPEG-4 (Motion Pictures Expert Group – 4), is multimedia container format for video and audio data
MV	Motor Vehicle
MVA	Motor Vehicle Accident
No.	number
NVR	Network Video Recorder
PIN	Personal Identification Number
REC'D	received
QUAD	quadrant display format
SEC	seconds
SHA	Secure Hash Algorithm
SN or S/N	Serial Number
SS	seconds
SUB	Submission
TIFF	Tagged Image File Format is a file format for storing images, including photographs and line art.
TL/RT	Time-lapsed / Real-time
TXT	Filename extension for a Text file, is a computer file with a human-readable sequence of characters and the words text which is maintained in computer-readable formats
USB	Universal Serial Bus, is an interface standard for connecting peripheral devices to a computer

RETIRED

WAV	(or WAVE), short for Waveform audio format, is a Microsoft and IBM audio file format standard for storing an audio bit-stream on PCs. It is the main format used on Windows systems for raw and typically uncompressed audio.
WMA	Window Media Audio – audio file that is able to be recognized and played back in Windows Media Player
WVM	Windows Video Media
W/	with
W/1	with (number designation) in this case one
@	at

There are multiple proprietary filename extensions. These filename extensions are proprietary to a specific company and can only be interpreted by specific software / hardware that the company developed, therefore the definition of the filename extension is unknown (proprietary).

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Purpose:

The methods and procedures presented in this outline address the areas which should be focused upon to assist the examiner in the proper examination and processing of video, digital and multimedia related evidence. The examination pathway followed by the examiner to achieve proper evidentiary results is further augmented and directed by the General Laboratory Standard Operating Procedures and the appropriate technical references maintained within the Multimedia and Image Enhancement Unit.

Responsibility:

Forensic Science Examiners assigned to the Unit

Procedure:

The following outline (documented on MMIE-SOP-26 Appendix 1) should be used for all persons newly assigned to the Multimedia and Image Enhancement Unit. In general, the trainee should observe between two to five cases for each section of the training. Mock cases will include a written report and use of Justice Trax unless not required by SOPs.

1. Overview of Laboratory

Overview of Laboratory	Date Completed	Initials of Trainee	Trainer's initials
GL 2.1 Laboratory Safety Checklist			
GL 14.1 General Laboratory Training Checklist			
Introduction to LIMS			
Introduction to Evidence Receiving			
Statement of Qualification completed			
CV and Transcripts provided to Quality Manager			

2. Analog Video Analysis

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In this section of the training, the trainee will learn about analog video and how these recordings are made and the analysis involved with this type of video. The trainee should complete three mock cases.

Analog Video Analysis	Date Completed	Initials of Trainee	Trainer's initials
Overview of analog video and assigned readings			
Types of analog video/audio			
Introduction to equipment used for analog video casework			
Repairing an analog tape (video/audio)			
Types of analysis for analog video			
Introduction to worksheets and wording used for analog video			
Introduction to sub-itemization and derivatives created for analog video/audio			
Reading of MMIE SOPs 02, 03, 04, 05 and 07			
Review of video duplications – analog evidence			
Review of audio duplications – analog evidence			
Cases Observed:	Dates of Observation:		

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Mock Cases duplications with analog evidence both video/audio:	Comments by Trainer on Cases:
Competency Test Administered:	Comments of Trainer on Competency Test:

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3. Digital Image Analysis (Refer to MMIE SOP-02, 03, 04, 05, 06, 08, 09, 12, 13, 14 and 28)
   In this section of the training, the analyst will learn about different image formats and how to acquire images from video. The analyst will practice on three (3) mock cases of acquiring an image and then doing adjustments such as brightening or layering/frame averaging.

Digital Image Analysis	Date Completed	Initials of Trainee	Trainer's initials
Overview of still images and image analysis			
Review of SOPS: 02, 03, 04, 05, 06, 08, 09, 12, 13, 14, 14 and 28			
Protecting digital media using write blockers			
Hash value review			
Metadata associated with digital media			

Digital File Formats: JPEG, TIFF, RAW, BMP, MPEG, etc			
Different ways of acquiring/extracting still images			
Methods and software used for the analysis and enhancement of still images			
Sub-itemization of generated still images			
Worksheets and reports for image analysis;			
Cases Observed:	Dates of Observation:		
Mock Cases still images captured/acquired with report:	Comments by Trainer on Cases:		
Competency Test Administered:	Comments by Trainer on Competency Test:		

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4. Digital Video Analysis (Refer to MMIE SOP-02, 03, 04, 06, 08, 09, 12, 14, 16, and 28)

   In this section of the training, the analyst will learn about different video formats and how to acquire video and audio from a file. The analyst will practice on three (3) mock cases of acquiring a video and doing adjustments such as levels adjustments.

Digital Video Analysis	Date Completed	Initials of Trainee	Trainer's initials

Overview of digital video, compression and codecs			
Software programs used for analysis			
Proper methods for video analysis			
Acquiring segments of video and audio from a video file			
Converting video to different formats			
Creating a picture – in – picture of two videos			
Creating sub-items of video analysis cases			
Worksheets used for video analysis			
Cases Observed:	Dates of Observation:		
Mock cases completed with report:	Comments on Cases by Trainer:		
Competency Test Administered:	Comments on Trainer on Competency Test:		

RETIRED

5. Redactions

The analyst will understand the use of redactions and when it is used. The analyst will practice redactions on three (3) cases with the following types of scenarios: video redaction only, audio redaction only and combination of both.

Digital Video Redaction	Date Completed	Initials of Trainee	Trainer's initials
Overview of digital video, compression and codecs			
Use of Media Info			
Software programs used for redaction			
Proper methods for conducting a redaction			
Acquiring still images and audio from video from Arbitrator			
Converting video to different formats			
Understanding of Body Cam Policy from A&O manual 13.15.11			
Creating sub-items of redacted video cases			
Worksheets used for redactions			
Mock cases completed with report:	Comments on Cases by Trainer:		

RETIRED

Competency Test Administered:	Comments on Trainer on Competency Test:

6. Audio Analysis (Refer to SOP-02, 03, 04, 07, 08, 10 and 11)

In this section of the training, the analyst will learn about different audio formats and how to acquire audio from a file or analog media. The analyst will practice on five (5) mock cases of acquiring audio and doing adjustments such as equalization of volume, removal of noise, and clarification of speech.

RETIRED

Audio Analysis	**Date Completed**	**Initials of Trainee**	**Trainer's initials**
Overview of audio files, bit rates and types of codecs			
Introduction to software programs used of audio analysis			
Proper methods of audio analysis			
Converting audio to different formats			
Redacting audio files			
Sub-itemization of audio files			
Cases Observed:	Dates of Observation:		
Mock cases completed with report:	Comments on Cases by Trainer:		

Competency Test Administered:	Comments on Trainer on Competency Test:

7. DVR Analysis (Refer to MMIE SOP-02, 03, 04, 09 and 16)

In this section of the training, the analyst will learn about digital video recorders (DVR) and how to extract surveillance video from these recorders. The analyst will practice on five mock cases in total. Two (2) mock cases will consist of acquiring an video using the Unit's DVR. As part of the this section's training, the trainee will be evaluating video information from previously worked cases in which an archive disk is on file. The analyst will practice on three (3) such cases in which there is a time difference in the recording to real time.

RETIRED

DVR Analysis	**Date Completed**	**Initials of Trainee**	**Trainer's initials**
Overview of DVRs			
Types of DVRs (including manufacturers)			
Important considerations with DVRs: overwriting, date/time			
Proper methods of DVR analysis			
Photographing a DVR			
Exporting video from a DVR			
Other types of video capture			
Use of DVR Examiner			

Worksheets used for DVR analysis			
Cases Observed:	Dates of Observation:		
Mock cases completed with report:	Comments on Cases by Trainer:		
Competency Test Administered:	Comments on Trainer on Competency Test:		

RETIRED

8. Video Retrievals (Refer to MMIE SOP-16)

Video Retrievals	**Date Completed**	**Initials of Trainee**	**Trainer's initials**
Initial call/email for a video retrieval and information needed from requestor			
Considerations of DVRs when scheduling with agency			
Equipment needed for retrieval			
Documentation needed on-scene			
Submission of case to lab and forms needed completed			
How to document video retrieved			

at scene on worksheet			
Crime Scene Assistance Form			
Observation of five (5) retrievals:	Dates of Observation along with DVR model:		
Three (3) video retrievals with senior analyst observation:	Comments on Cases by Trainer:		

RETIRED

9. Comparative Analysis (Refer to MMIE SOP-02, 03, 04, 06, 12 and 13)

Comparitive Analysis	**Date Completed**	**Initials of Trainee**	**Trainer's initials**
Overview of comparisons			
How to set up a comparison			
Software used in comparisons			
Creating a comparison chart - Vehicle - Human subject - Clothing			
Limitations in comparisons			
Three (3) mock cases using a vehicle, human subject and	Comments by Trainer on Mock Cases:		

clothing including report written.	
Competency Test Administered:	Comments by Trainer on Competency Test:

10. Court (Refer to SOP-GL-17)

Court Testimony	Date Completed	Initials of Trainee	Trainer's initials
Review of qualification and ethical responsibilities			
Dress and Demeanor			
Court Monitoring Procedures			
Technical Testimony (includes reading of Swinton ruling)			
Observations of Court Testimony:	Dates of Observations and brief synopsis:		
Mock Court:	Comments from Trainer:		

RETIRED

11. Competency and Approval for Casework and Technical Reviews

At the end of the training program, a memo/email shall be written to the Deputy Director stating that the analyst has successfully completed their training and has been administered a competency case that evaluated their analytical skills and their ability to clearly communicate their findings in a report.

The Deputy Director may review the training files or simply endorse this memo/email and generate a request to the Quality Unit requesting that this analyst be authorized to perform casework in that discipline.

The analyst will perform casework and after gaining experience in conducting analysis and reporting findings in a variety of evidence type/conclusions in the discipline, their experience will be evaluated for the ability to conduct technical reviews. This evaluation period may include technical review ghosting with an experienced examiner. Once the analyst has completed the evaluation period, a memo/email shall be written to the Deputy Director stating that the analyst has successfully completed their evaluation period and has been deemed competent to conduct technical reviews in that discipline.

The Deputy Director, after review, may endorse this memo/email and generate a request to the Quality Unit requesting that this analyst be authorized to perform technical reviews based upon the analyst's casework experience in that discipline.

RETIRED

11. Retraining

In the event it is identified that an examiner has failed a proficiency or technical and administrative reviews have identified an issue in the casework, the employee shall complete a retraining and competency period as detailed in SOP-GL-14 and SOP-GL-1.

References:
See MMIE SOP-24 Multimedia and Image Enhancement References

RETIRED

A. Purpose:

The Multimedia and Image Enhancement Section will take measures to document or prepare memos regarding case discussion.

B. Responsibility:

Forensic Science Examiners assigned to the Multimedia and Image Enhancement Section

C. Procedure:

1. Whenever an examiner is contacted or contacts a customer/submitting agency or their designee, the content of the conversation pertaining to the discussion should be documented and placed in the case file. (Refer to GL-1 Section 4.4.2)

2. Examples of when a memo should be filed are as follows, but not limited to:
   - Evidence was compromised or not suitable for examination
   - Examiner/Requestor feels that an unapproved technique should be explored
   - A new suspect or addition evidence is obtained that may be used for comparisons
   - Any other documentation that the examiner deems necessary

3. Any notations may be made directly on the worksheet either typed or handwritten or if using a separate paper, it shall be noted on a standard 8-1/2" X 11" paper and the Laboratory ID number and the examiner's initials shall be present on this document.

D. Sources of Error:

E. References:

RETIRED

Purpose:
To establish a validation procedure or performance check procedure for the use of new equipment or software being introduced to the Multimedia and Image Enhancement Unit.

Validations will be conducted when the Unit receives a new piece of equipment or new software for use in casework. This SOP is to act as guidance for the validation plan; if modifications are required for the plan, these will be approved by the Deputy Director and Director. The validation plan may also be used for software upgrades that reflect a major change.

In the case of minor updates to software, a performance check is sufficient to determine if the new upgrade is acceptable for use in casework. A performance check will also be conducted if a piece of equipment is sent out for repairs and is now ready to be placed back into service. Performance checks may also be conducted when casework software is loaded for the first time onto a computer. Refer to GL-22 for guidance.

RETIRED

Responsibility:
Forensic Science Examiner assigned to the Multimedia and Image Enhancement Unit or conducting casework in the Unit.

Validation Procedure:

1. A plan will be written detailing the steps of the validation procedure.

2. The plan will be reviewed by the Technical Lead, Assistant Director and Deputy Director and Director prior to commencement of the validation. The Deputy Director or their designee will complete the Laboratory Method Validation Form. After the validation plan is reviewed, the Deputy Director or their designee and the Director will approve the plan by completing the plan approval portion of the form. Approval may also have the initials of the plan approver on the printed pages of the validation plan..

3. This plan may be updated during the process as new information or results of the validation require changes to the original plan. Any changes to the plan will need to be re-approved Deputy Director and or their designee and the Director.

4. After completion of the validation, the results will be clearly communicated in the validation and the results will be submitted for review by the Technical Lead, Assistant Director and Deputy Director.

5. These validation results will be evaluated to determine if SOPs changes are needed prior to casework use. The approved software/equipment list will be updated with the validated software/equipment.

6. A final memo will be issued by the Deputy Director or their designee of the review of the validation and its results. The memo will have a statement indicating if the equipment or software has successfully passed its validation and is acceptable to be placed into service or use for casework. This memo will be approved by the Quality Manager and the Director.

Performance Check Procedure:

1. Using a known file or image, or a completed case file, the updates to software will be evaluated to determine if the same results are obtained after the upgrade or if the software will still work correctly.

2. A memo indicating that the performance check was completed and if it passed successfully will be issued and kept on file in the Deputy Director's office.

3. Software updates will be documented in the Computer's log book as to the version installed and date of install.

Reference:

GL1 – Quality Manual

Laboratory Method Validation Summary Form

RETIRED

Purpose:

This SOP will be used to provide guidance for requests for redaction of a video or audio file. The request for a redaction will be accompanied by a letter, email or other document detailing the areas of redactions and what type of redaction is needed.

Responsibility:

Forensic Science Examiner assigned to the Multimedia and Image Enhancement Unit or conducting casework in the Unit.

Redaction Procedure:

1. The analyst will use approved methods to open or obtain the media file needing redaction. This may consist of the media file being directly submitted to the Evidence Receiving Unit or of a digital download from the Arbitrator Backend Client System.

   In the event that the request is directly from the Arbitrator Backend Client System, a Request for Analysis (RFA) form must be completed and an email or other document indicating the redaction request will accompany the RFA. The video will be downloaded and then placed on media to be submitted to Evidence Receiving. The kit "Arbitrator Download" will be used to create the evidence in Justice Trax.
2. Redactions will be conducted as requested by the submitting agency. If there is a question on the redactions needed, the requesting agency will be contacted and documentation will be contained in the case jacket of the discussion.
3. Redactions are conducted using the approved software. Documentation of software used and type of filters used for the redactions will be noted on MMIE General Worksheet.
4. Redactions will be assigned a Video/Audio Redaction request and this request will be released at Administrative Review milestone. A report will be generated indicating the media being requested to be redacted and that the redactions were completed.
5. The redacted media will be saved to electronic media (e.g DVD or a USB/External drive) depending on the size of the video/audio generated. The generated media will be created in Justice Trax as a sub-item. An archive copy of the original and redacted file will be created and maintained in Video Storage unless the file size exceeds any available storage media. This archive copy will be created in Justice Trax as a sub-item. When the file size exceeds any available storage media, a copy will be maintained on a server that has back-up safeguards and a notation will be placed in the case jacket as to its location.

RETIRED

Reference:

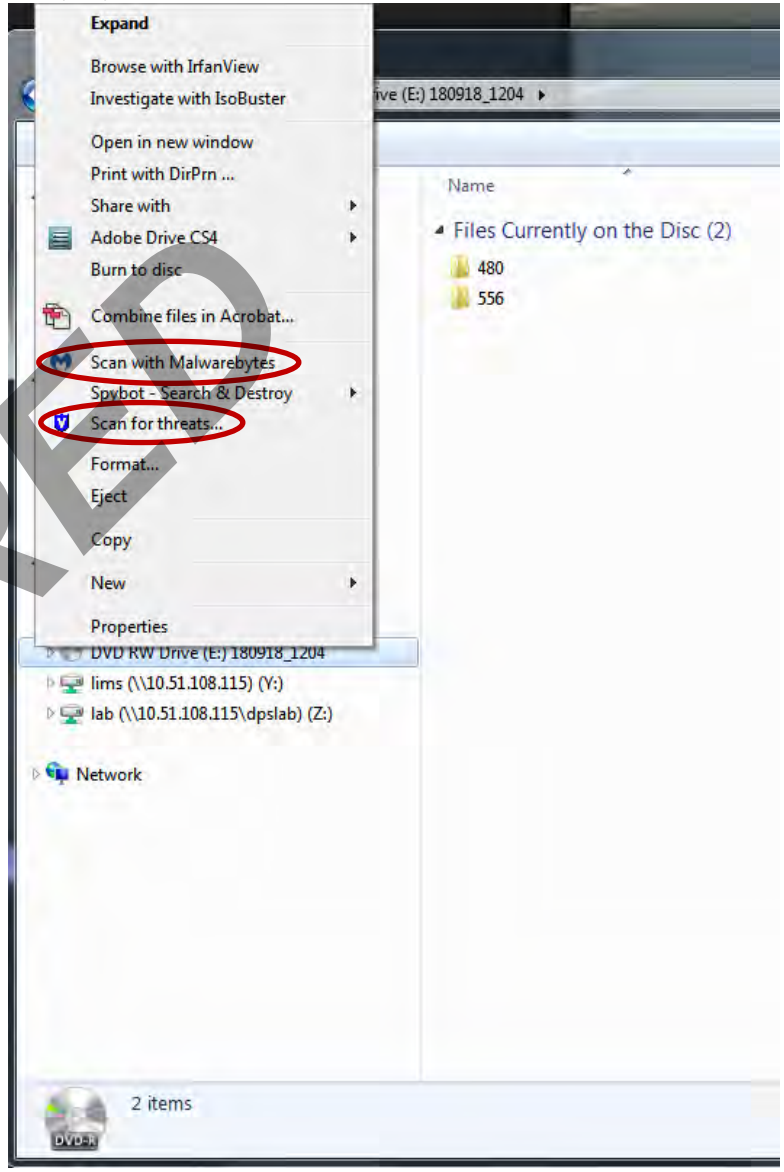
GL1 – Quality Manual

C.G.S. § 29-6d

Work Instructions for Redactions

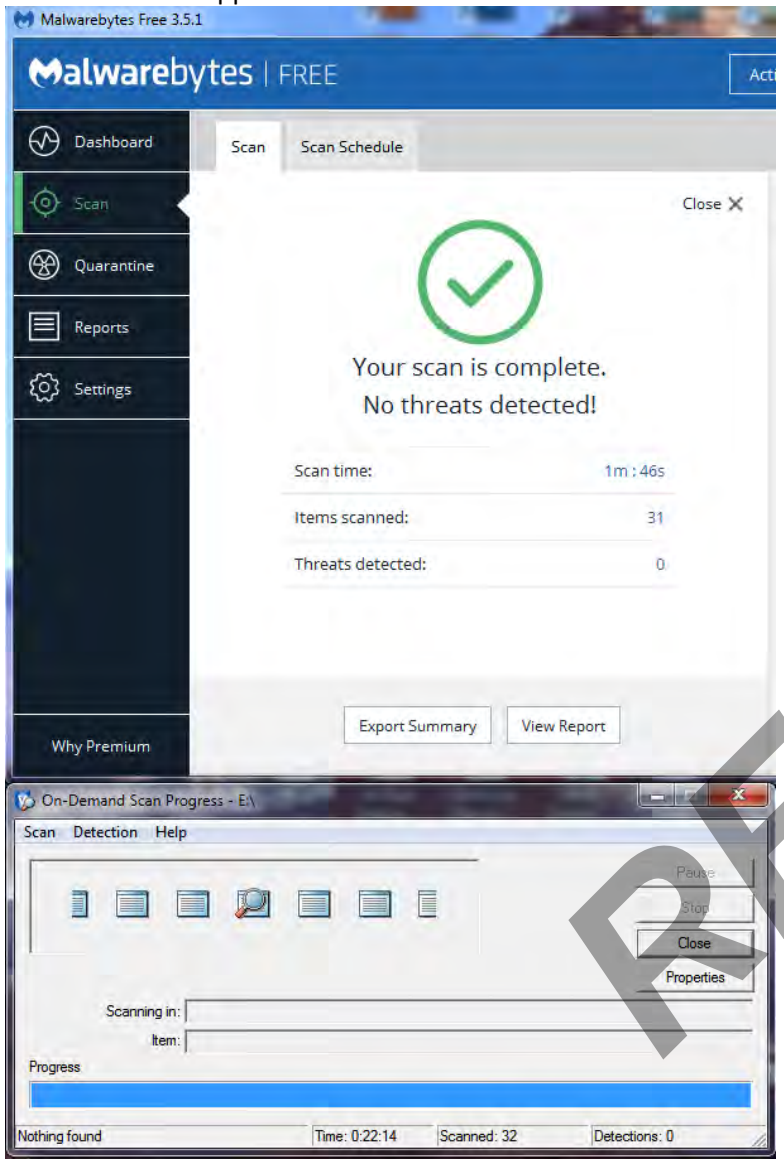
RETIRED

1. If a folder hasn't already been made, create a folder on the Documents folder with the DSS Case Number and the Town of Incident. When the media is inserted/downloaded into the computer, right click on the media and run both a Malwarebytes Scan and a McAfee Virus Scan.



2. Once the scan is complete, screenshot the results for the purpose of documentation and save that into the document folder.

RETIRED



screen captures including the sub-folders. A copy of the submitted media should be placed into the document folder.

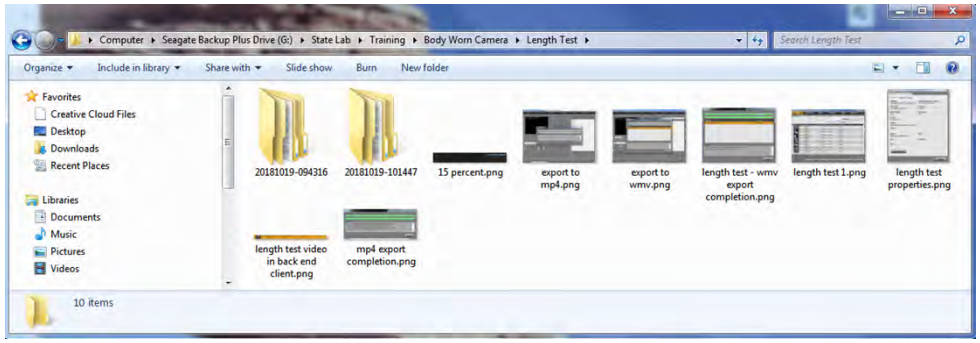
Downloaded files should first be write-protected. To accomplish this, right click on the file/folder and under "Properties" in the "General" tab, select the and check off "read only". Click "Apply" and then "Ok".

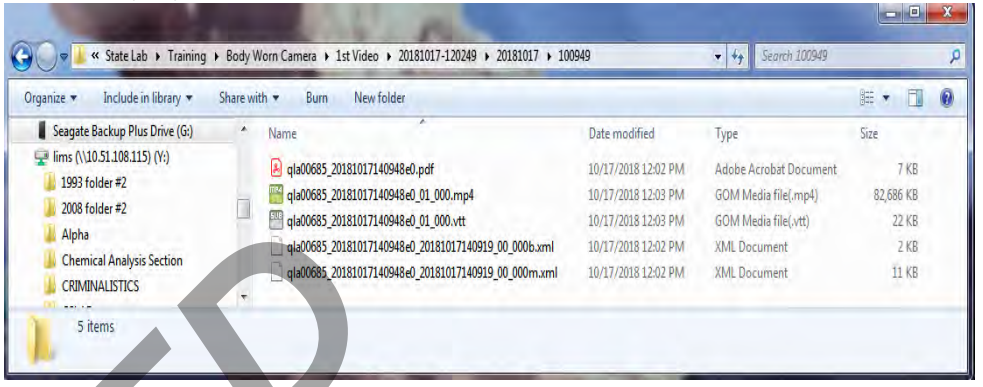
After the media has been write-protected, a hash value of the original and the copy should be obtained to verify that it is an exact copy. A screen shot of the hash values should be placed into the case documentation.

Note: if downloading from the "Arbitrator Back-End Client" this original hash value cannot be obtained.

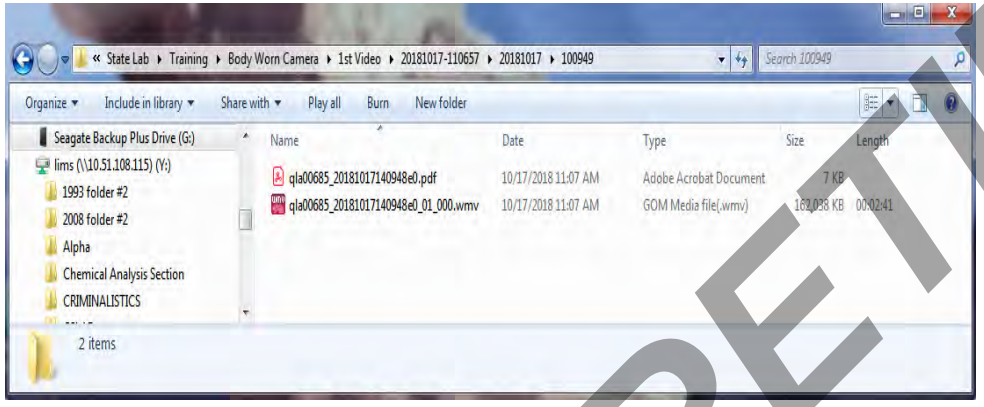
RETIRED

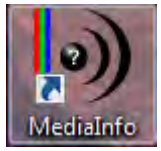
3. After making sure that there are no viruses and malware is present on the media, document what is present on the submitted media by performing





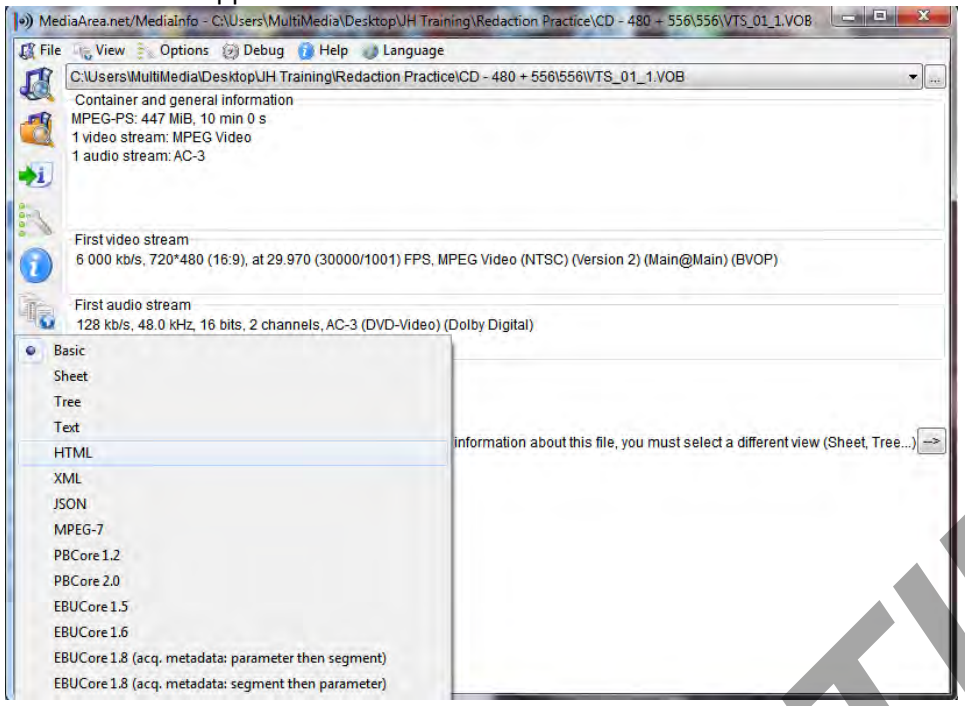
4. Once the media has been copied, drag the working files into “Media Info”





View the information in HTML (or whichever format is easier for you to read), and screenshot/print the information.

RETIRED



Print out this information and highlight the following:

Video

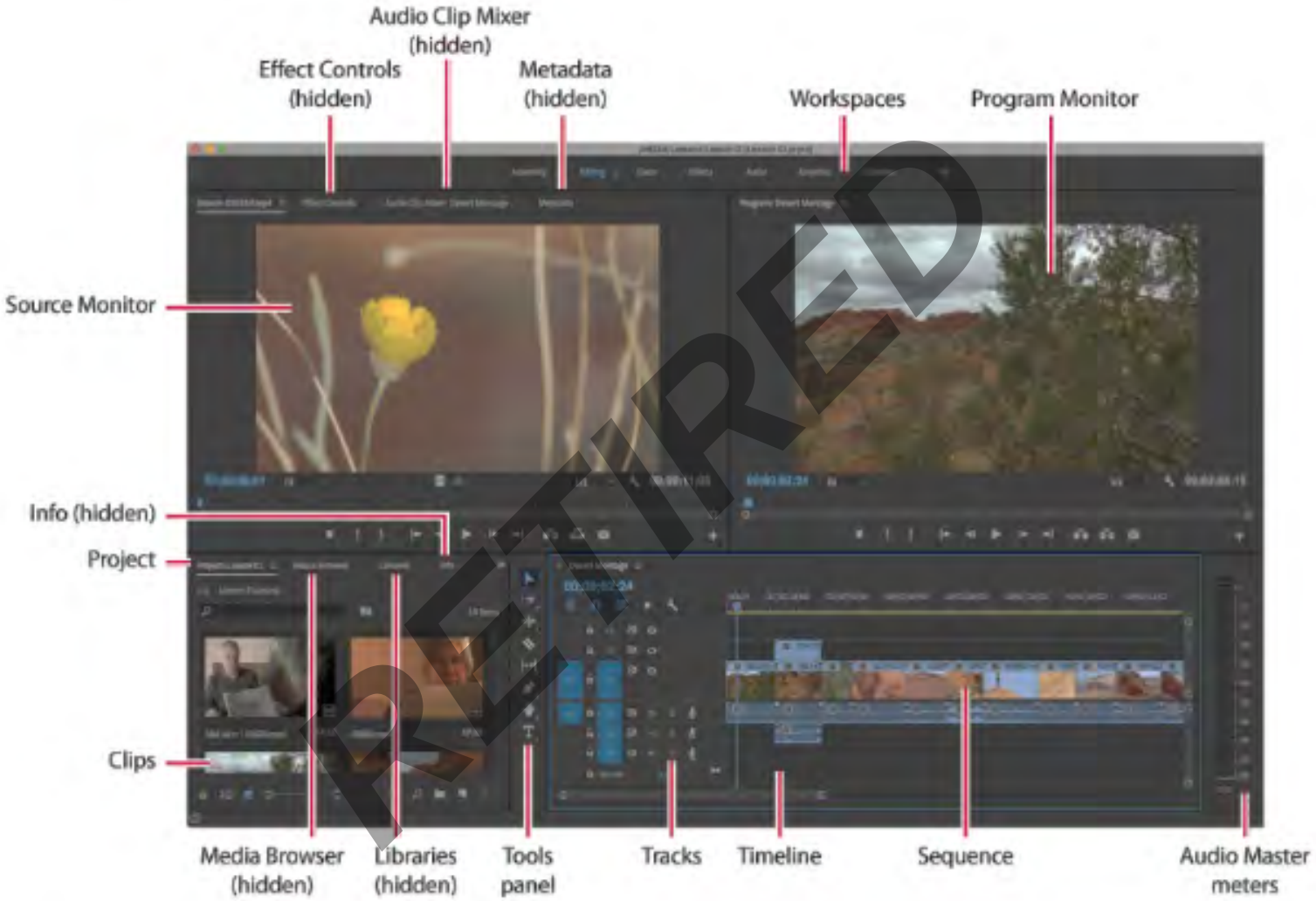
ID :	2
Format :	WMV2
Codec ID :	WMV2
Codec ID/Info :	Windows Media Video 8
Description of the codec :	Windows Media Video V8
Duration :	2 min 45 s
Bit rate mode :	Constant
Bit rate :	8 000 kb/s
Width :	1 280 pixels
Height :	960 pixels
Display aspect ratio :	4:3
Frame rate :	29.970 (29970/1000) FPS
Nominal frame rate :	30.000 FPS
Bit depth :	8 bits
*Bits/(Pixel*Frame) :*	0.217
Stream size :	157 MiB (97%)
Language :	English (United States)
NumberOfFrames :	4932

Audio

ID :	1
Format :	PCM
Codec ID :	1
Duration :	2 min 45 s
Bit rate mode :	Constant
Bit rate :	128 kb/s
Channel(s) :	1 channel
Sampling rate :	8 000 Hz
Bit depth :	16 bits
Stream size :	2.52 MiB (2%)
Language :	English (United States)

RETIRED

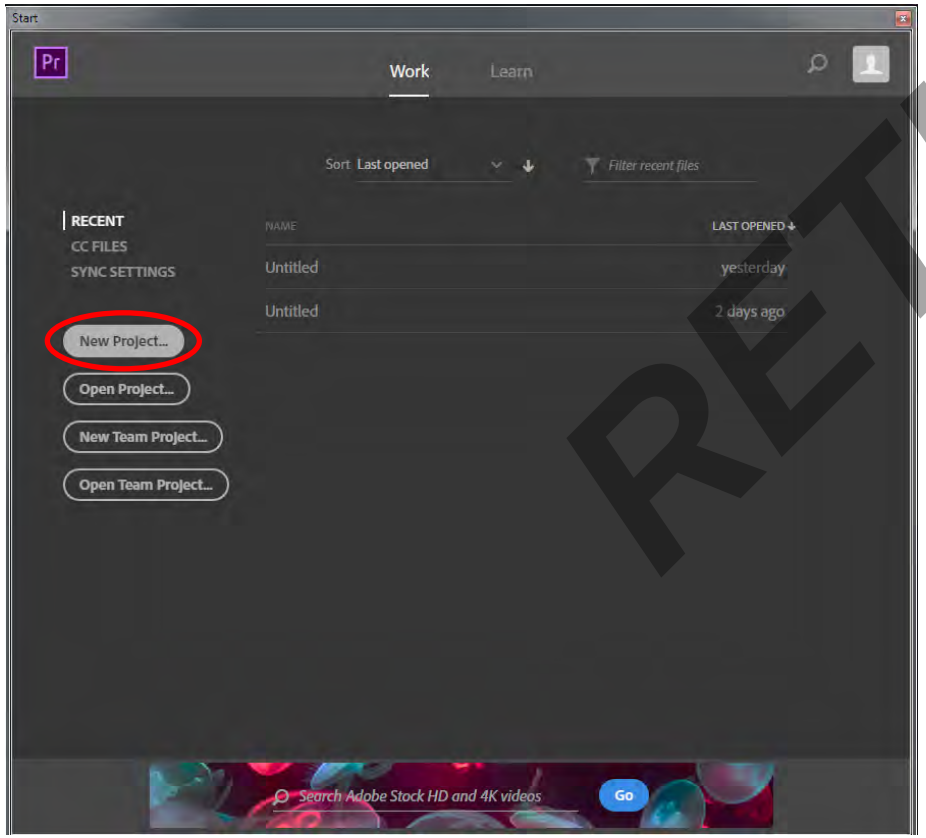
Premiere Pro CC - basic workspace layout for reference.



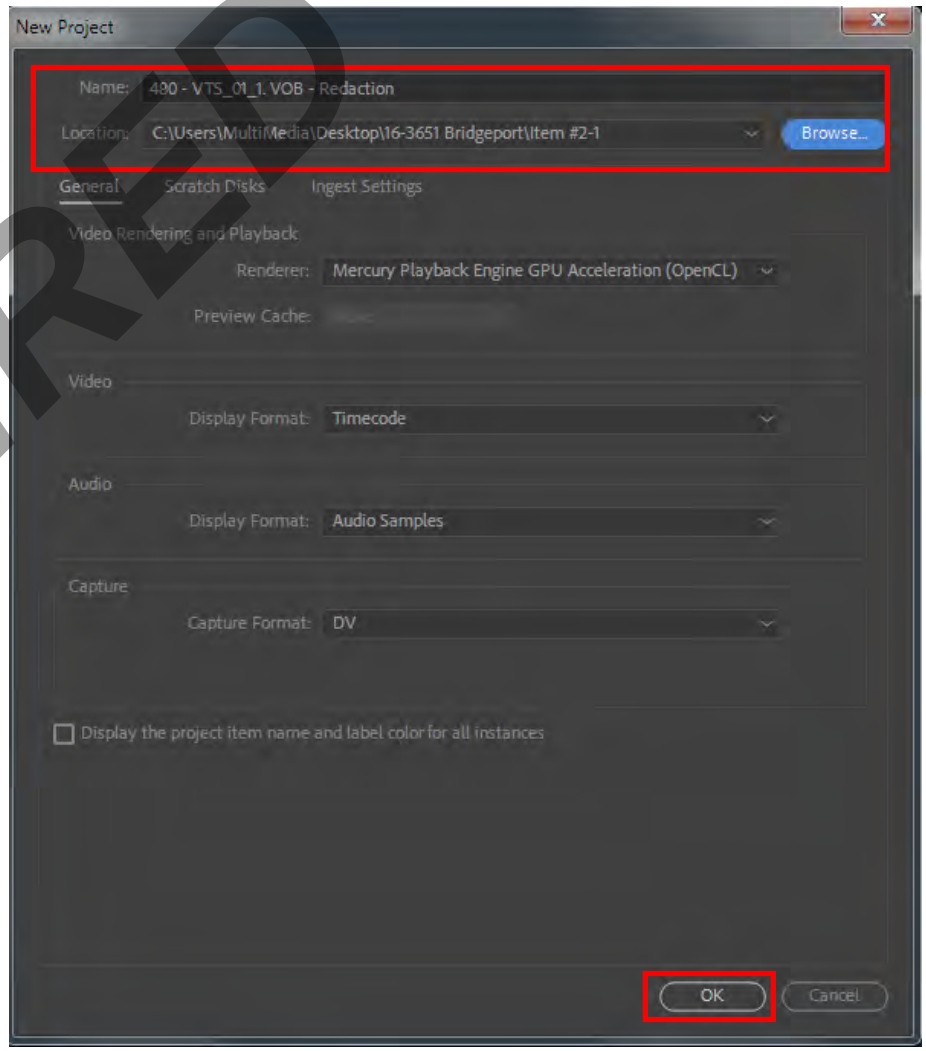
RETIRED

5. Open Adobe Premiere Pro CC:

Select: new project.

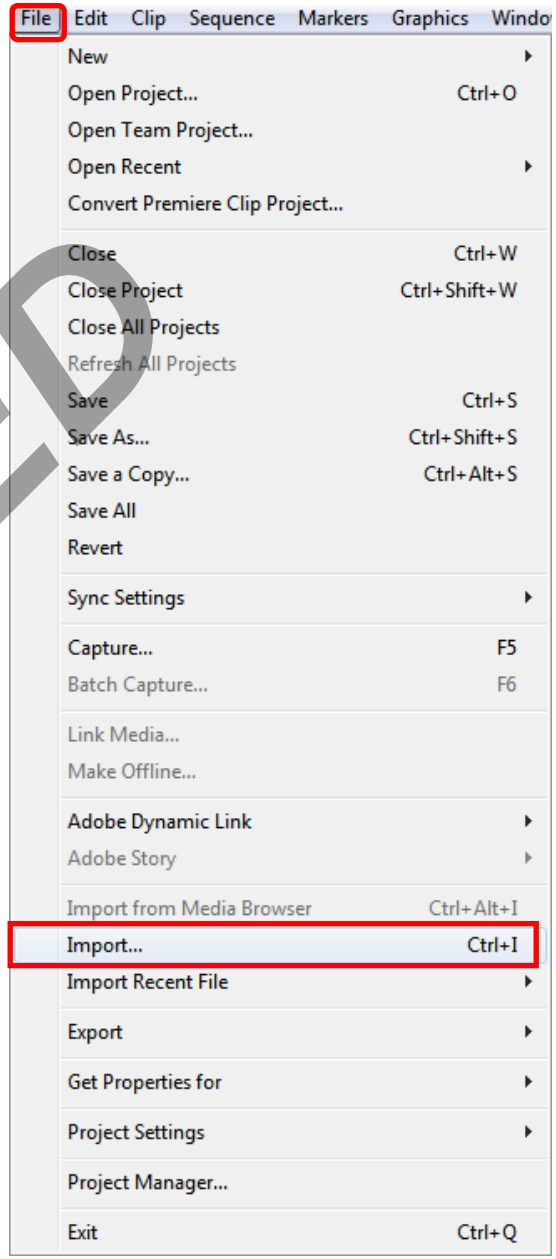


6. The following image depicts the setup of the menu when you select “New Project”. In the Name Section, add a name for your project. This is usually the case number and other identifiers. Select “browse” to be able to select the designated folder created in Step 1. Once that is completed select “OK”.



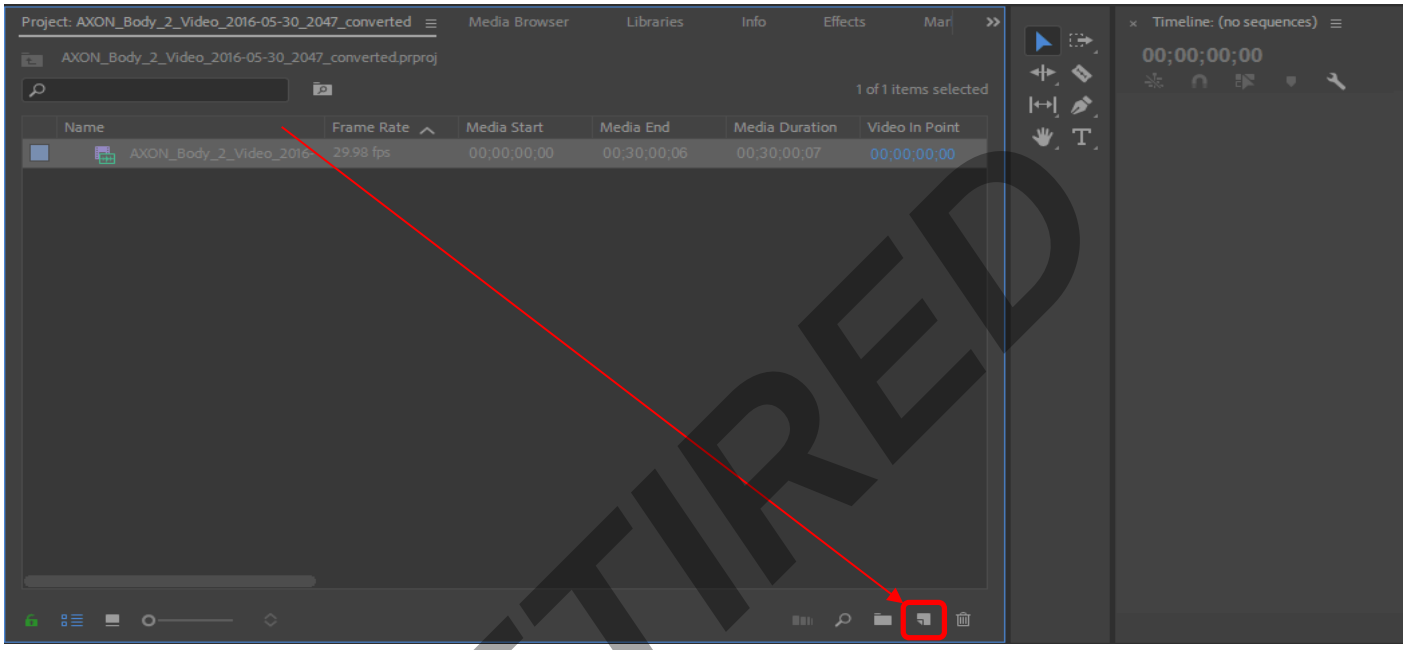
RETIRED

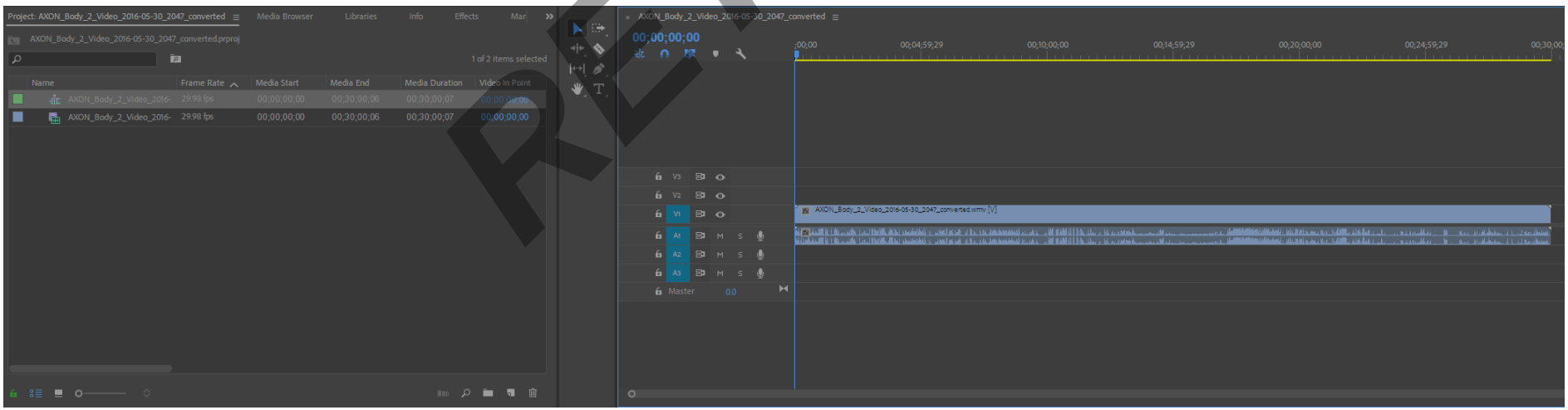
7. Once the workspace opens, import the media file to be redacted. Use the following to perform this import: File → Import and then browse for the folder on your desktop containing the copy of the submitted media. This step would also be used to access media that was downloaded directly from the Arbitrator program and placed in the document folder.



RETIRED

8. Once the media is imported, click and drag the media to the "**Add Item**" button (shape of a sticky note). This will create a sequence that fits the settings of your original video.

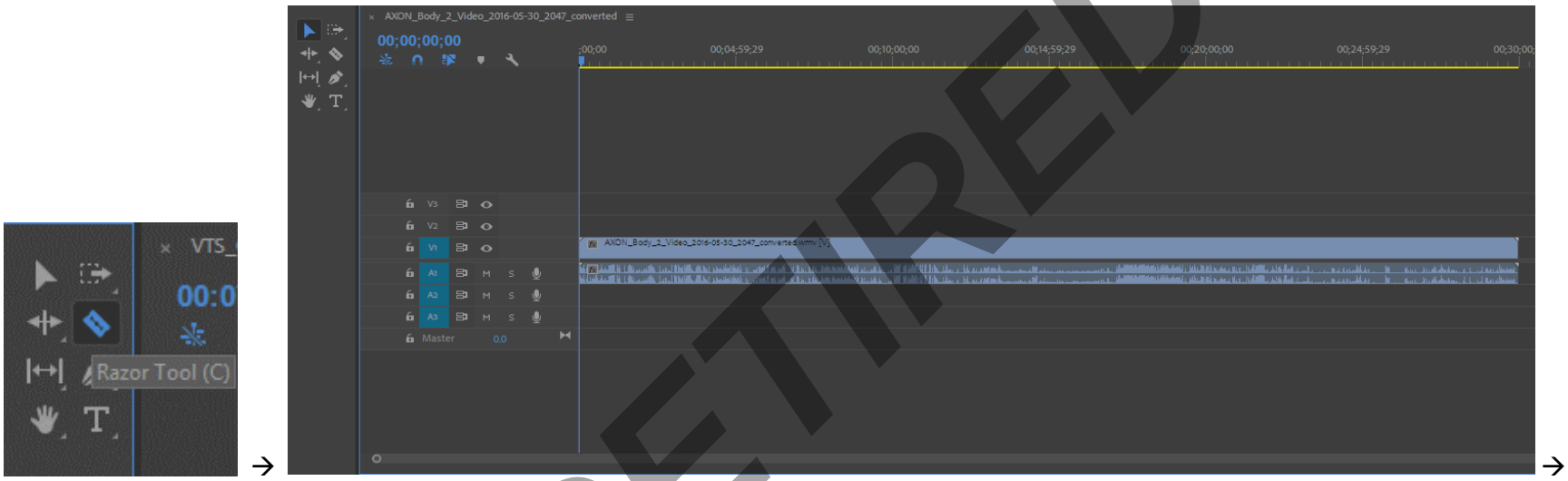




RETIRED

9. If redactions are needed to both audio and video, refer to page 16 of this work instruction. If no audio redaction is needed, proceed to Step 10. If redactions are needed only for audio, refer to page 16 of this work instruction.

10. Review the video to determine what needs to be redacted by the requesting agency. Once it is clear what areas of the video need to be redacted, pause the video at the beginning of where the redaction should take place. In the tools menu, select the "**Razor Blade**" tool to cut the video, scroll to the end of the portion of the video needing redaction and again cut the video using the "Razor Blade" tool. The selected segment of video enclosed within the "razor blade" cuts should be the area in which the redaction is going to be applied. The left and right cursor arrows move the video frame by frame for a more accurate location to cut using the "Razor Tool".



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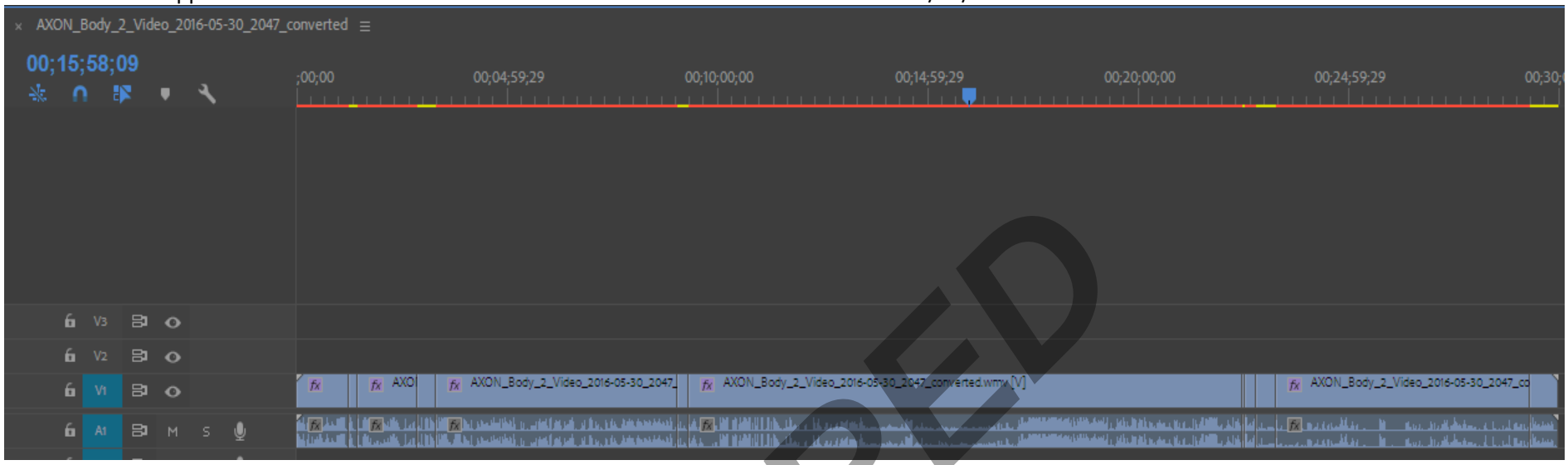
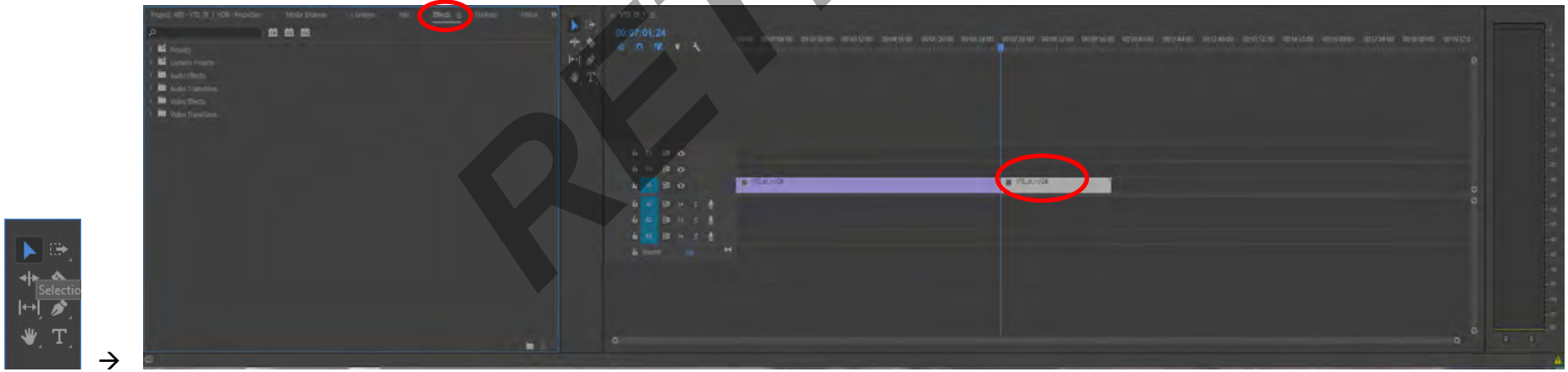
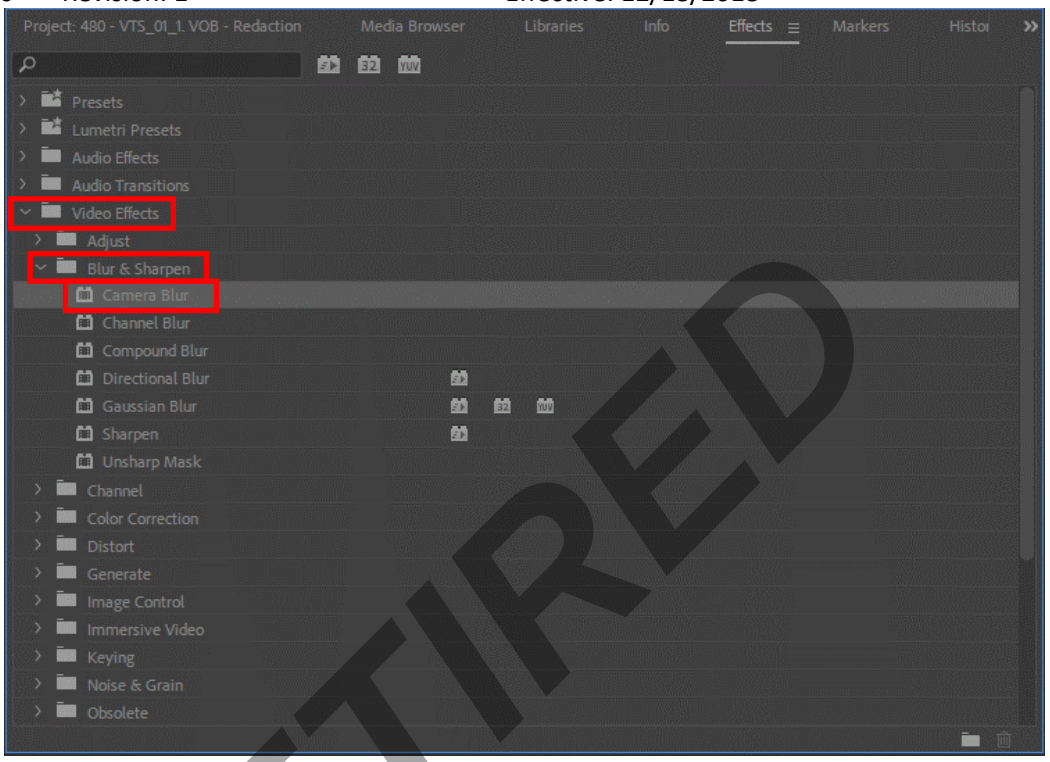


RETIRED

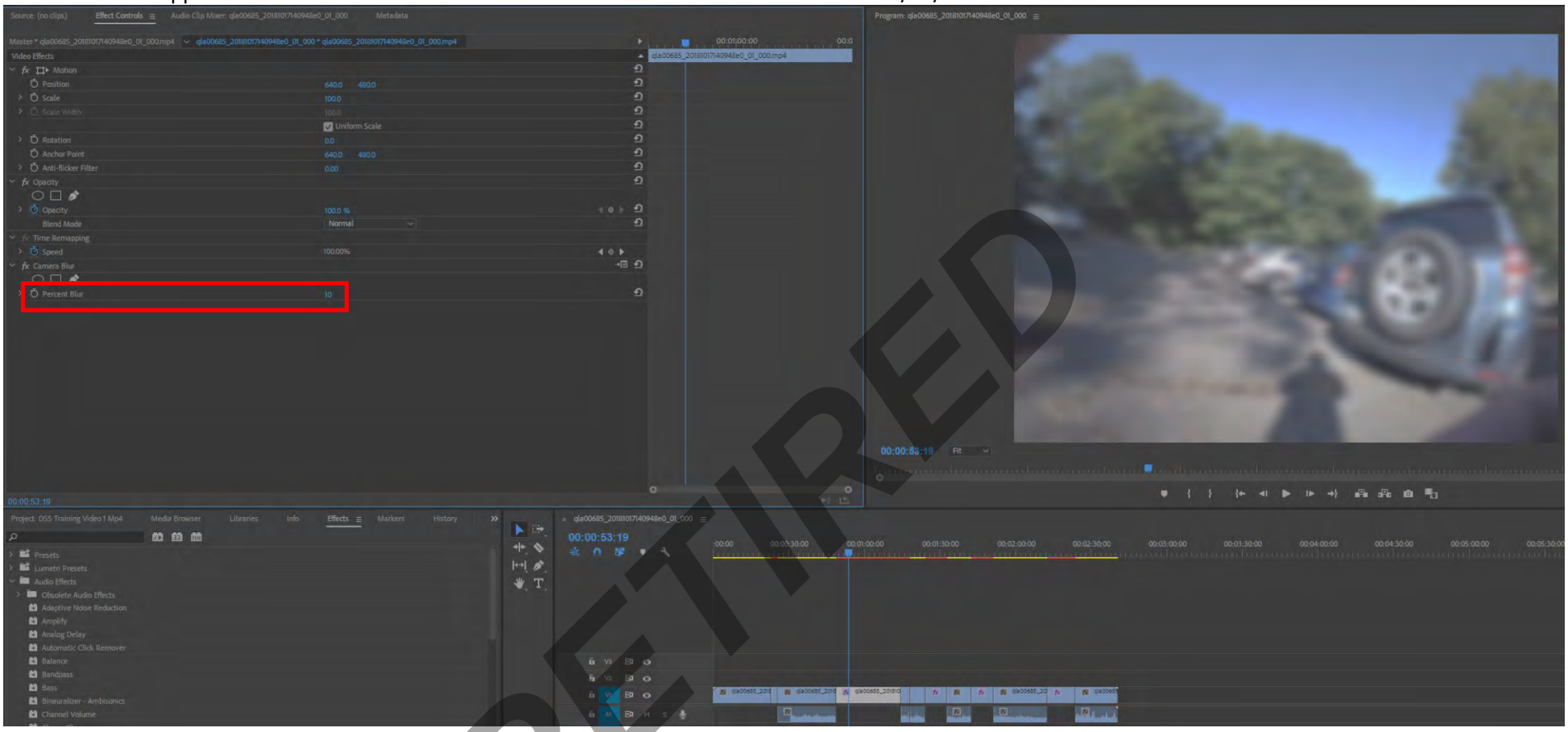
11. Once the video selection is isolated by the razor cuts, choose the arrow selection tool and go to the **Effects section**. In the "Effects" section select → **Blur & Sharpen → Camera Blur**. Once Camera Blur is chosen **Click and Drag** that effect and drop it onto the section of the video that you have isolated to redact.



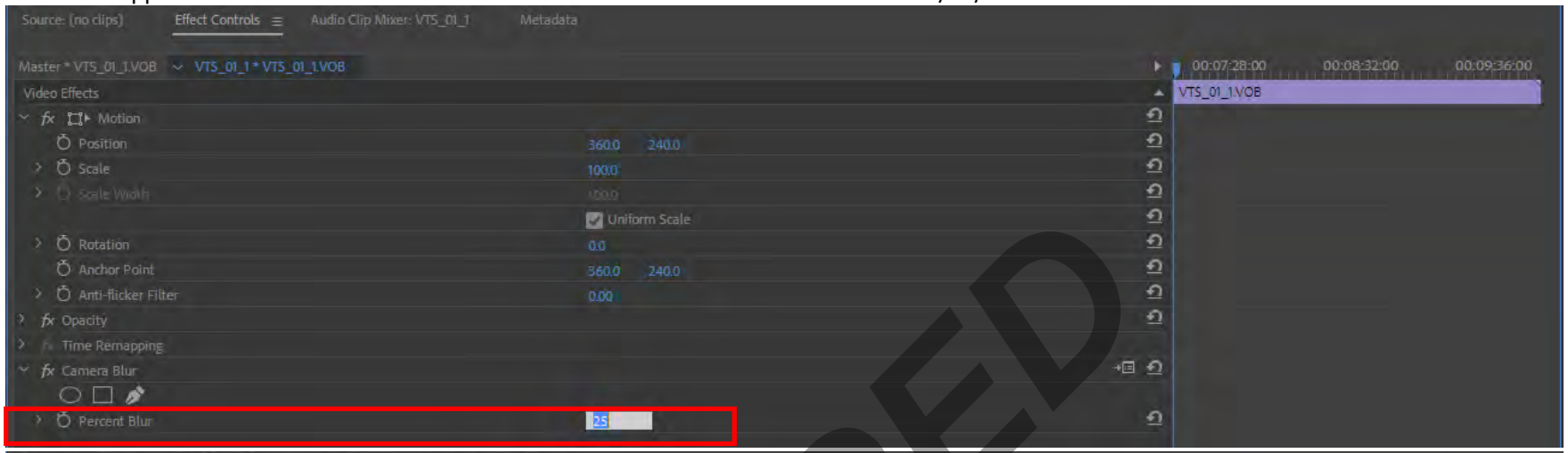


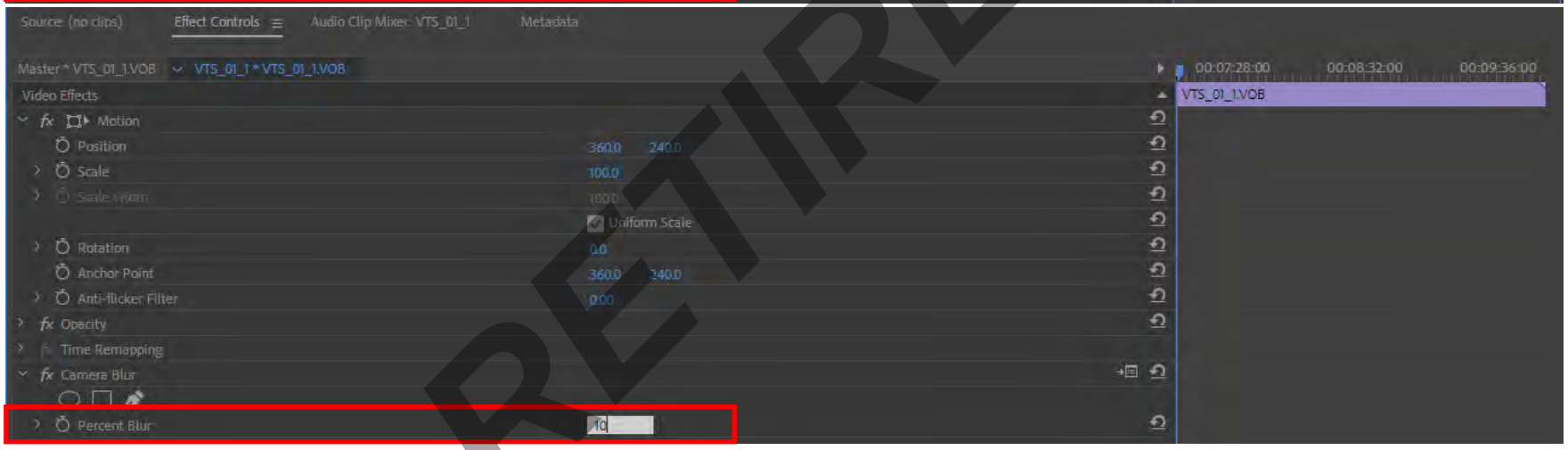
RETIRED

12. Once that effect is place on that section of the video, go to the effects control menu here you will need to adjust the “Percent Blur”. You can select a range from “25%” to “10%” as the blurring to apply to the entire video. It is preferable to use 15%, but if the details of the image are still legible, you may need to increase the blur up to 25%


Effect Controls
Audio Clip Mixer: qla00685_20181017140948e0_01_000
Metadata
Video Effects
Motion
Position
Scale
Uniform Scale
Rotation
Anchor Point
Anti-flicker Filter
Opacity
Blend Mode
Normal
Time Remapping
Speed
Camera Blur
Percent Blur
Program: qla00685_20181017140948e0_01_000
Project: OSS Training Video 1 Mp4
Media Browser
Libraries
Info
Effects
Markers
History
Presets
Lumetri Presets
Audio Effects
Obsolete Audio Effects
Adaptive Noise Reduction
Amplify
Analog Delay
Automatic Click Remover
Balance
Bandpass
Bass
Binauralizer - Ambisonics
Channel Volume
00:00:53:19

RETIRED


Source: (no clips)
Effect Controls
Audio Clip Mixer: VTS_01_1
Metadata
Master * VTS_01_1.VOB
VTS_01_1 * VTS_01_1.VOB
00:07:28:00
00:08:32:00
00:09:36:00
Video Effects
VTS_01_1.VOB
Motion
Position 360.0 240.0
Scale 100.0
Uniform Scale
Rotation 0.0
Anchor Point 360.0 240.0
Anti-flicker Filter 0.00
Opacity
Time Remapping
Camera Blur
Percent Blur 25


Source: (no clips)
Effect Controls
Audio Clip Mixer: VTS_01_1
Metadata
Master * VTS_01_1.VOB
VTS_01_1 * VTS_01_1.VOB
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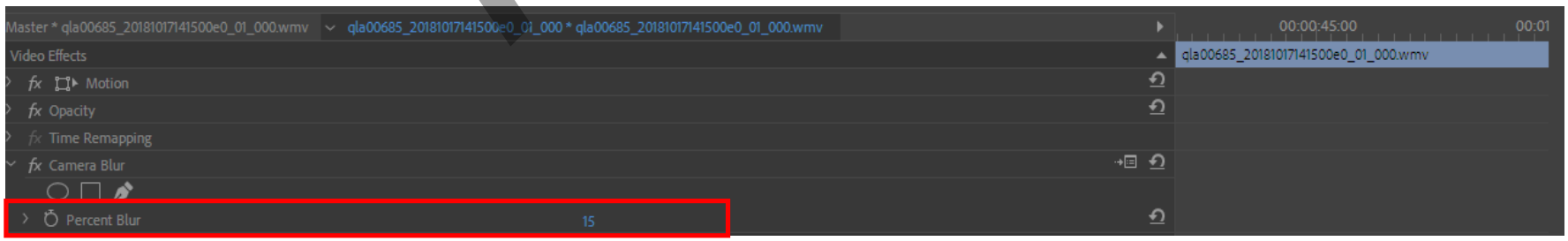
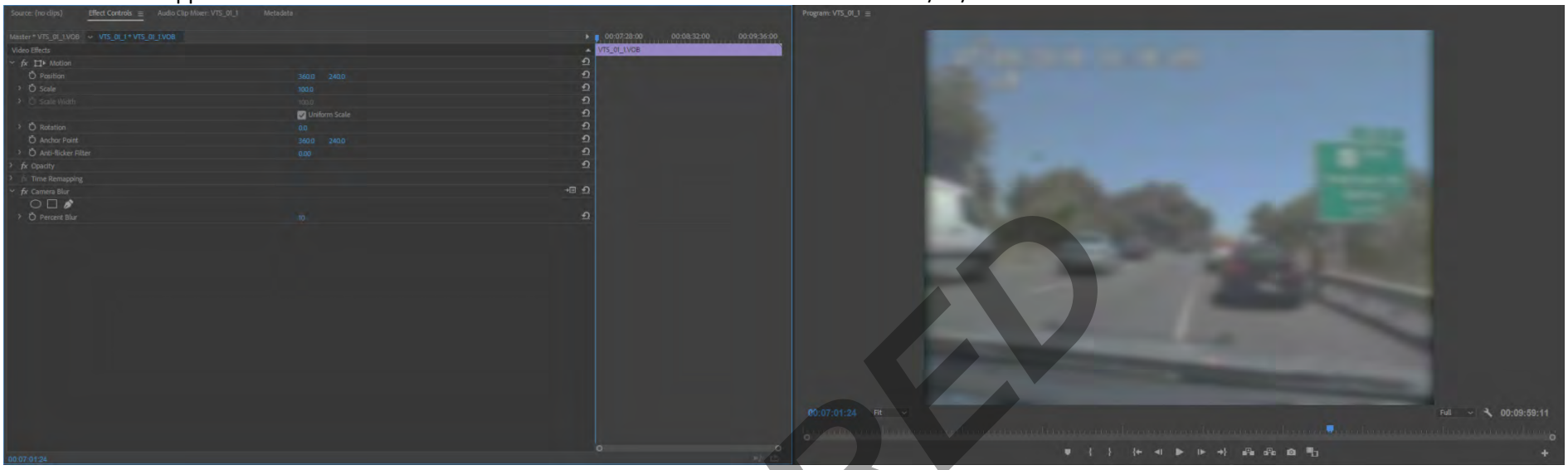
00:08:32:00
00:09:36:00
Video Effects
VTS_01_1.VOB
Motion
Position 360.0 240.0
Scale 100.0
Uniform Scale
Rotation 0.0
Anchor Point 360.0 240.0
Anti-flicker Filter 0.00
Opacity
Time Remapping
Camera Blur
Percent Blur 10


Master * qla00685_20181017141500e0_01_000.wmv
qla00685_20181017141500e0_01_000 * qla00685_20181017141500e0_01_000.wmv
00:00:45:00
00:01
Video Effects
qla00685_20181017141500e0_01_000.wmv
Motion
Opacity
Time Remapping
Camera Blur
Percent Blur 15

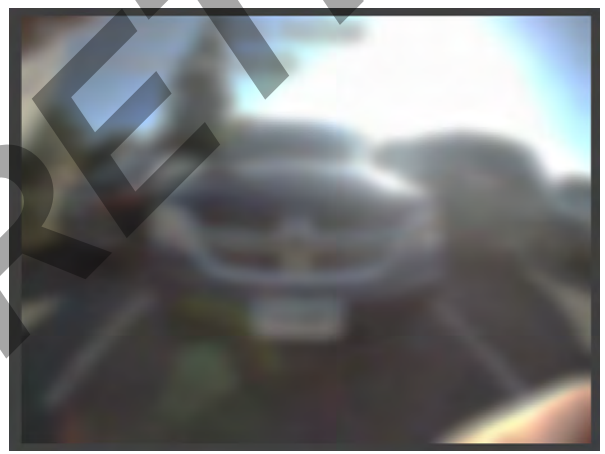
RETIRED



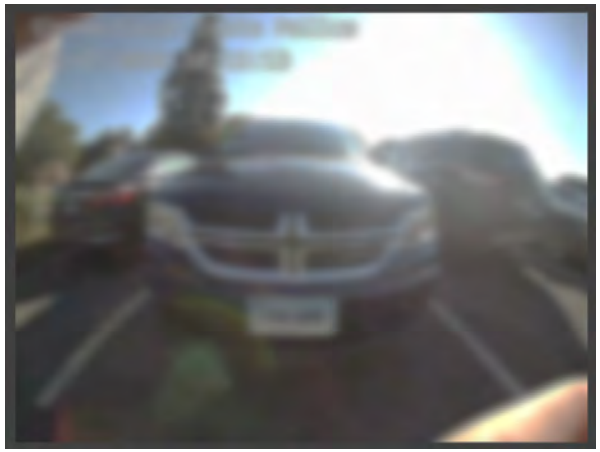
The images below illustrate the final results when using different levels of blurring percentage.

RETIRED

25% Blur

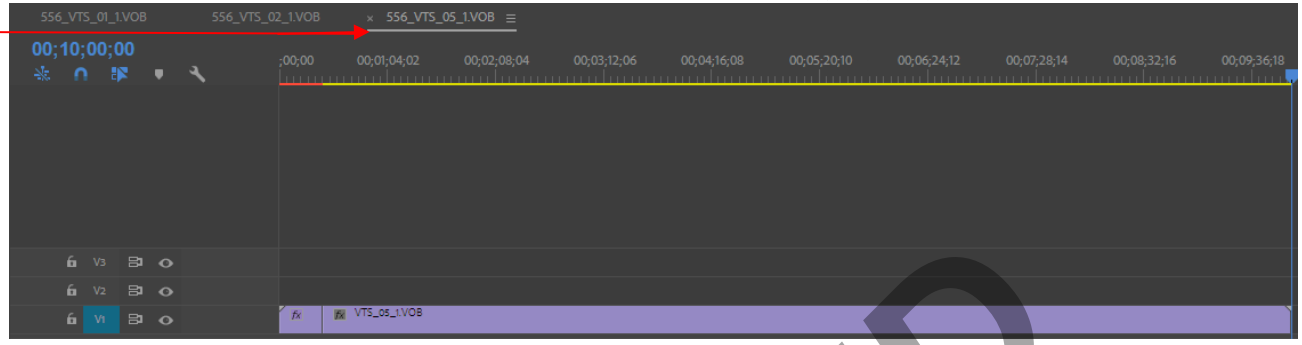


vs. 15% Blur

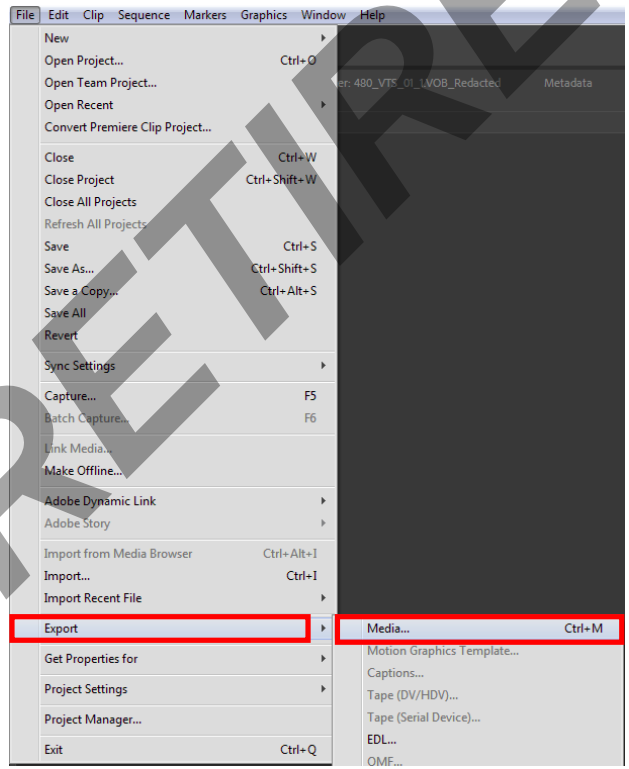


vs. 10% Blur

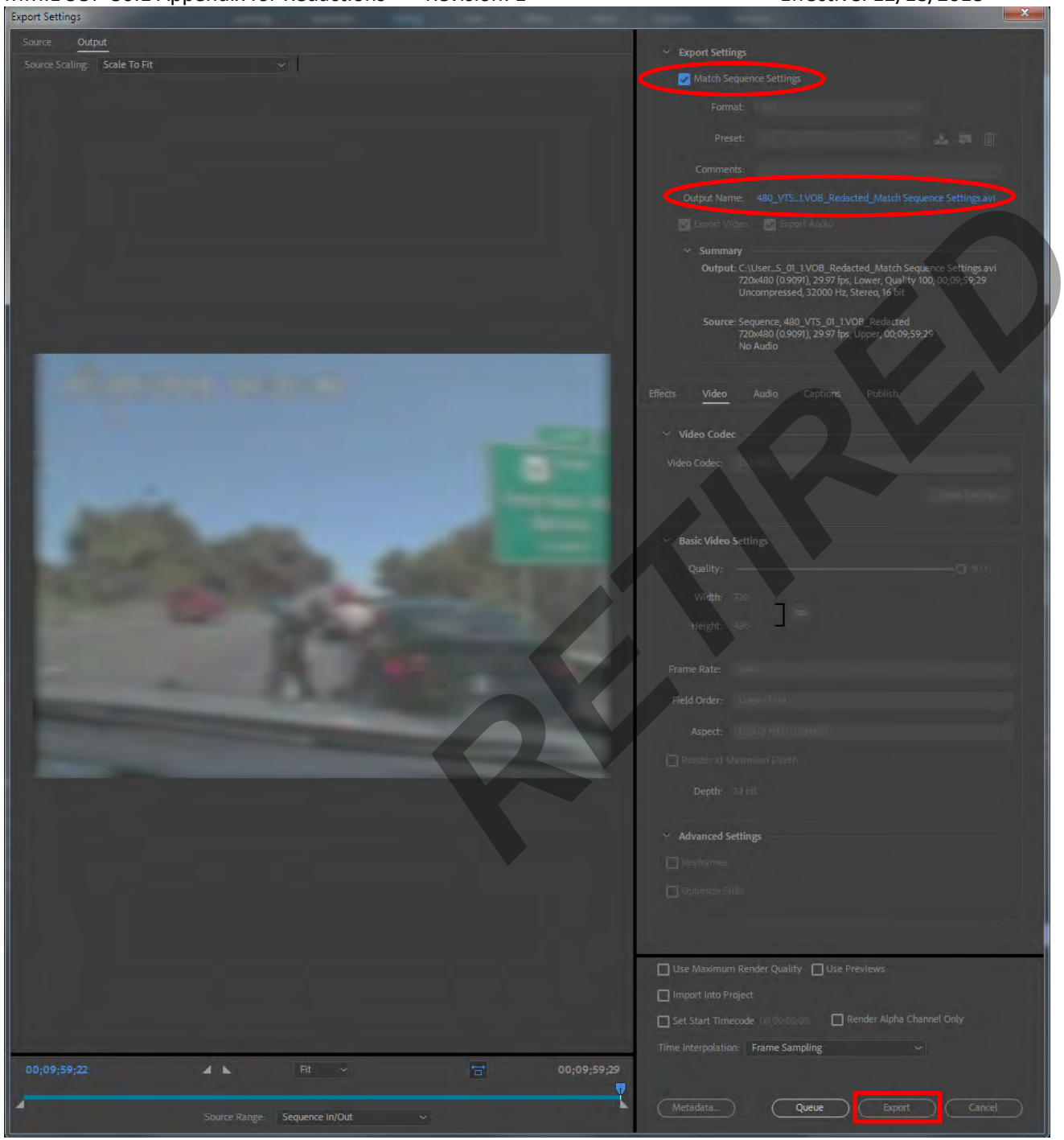
13. When there are multiple videos for the same case that require redactions, open up **"new sequence**". This will create a **new tab** in your workspace under the name of that original case file. Use the following steps to achieve this: File → Import, and follow steps 8-11.



14. Once the video is redacted, **export the video** in a format that the requesting agency will be able to playback on their computers or devices available to them, using the following steps: **File → Export → Media.**



RETIRED



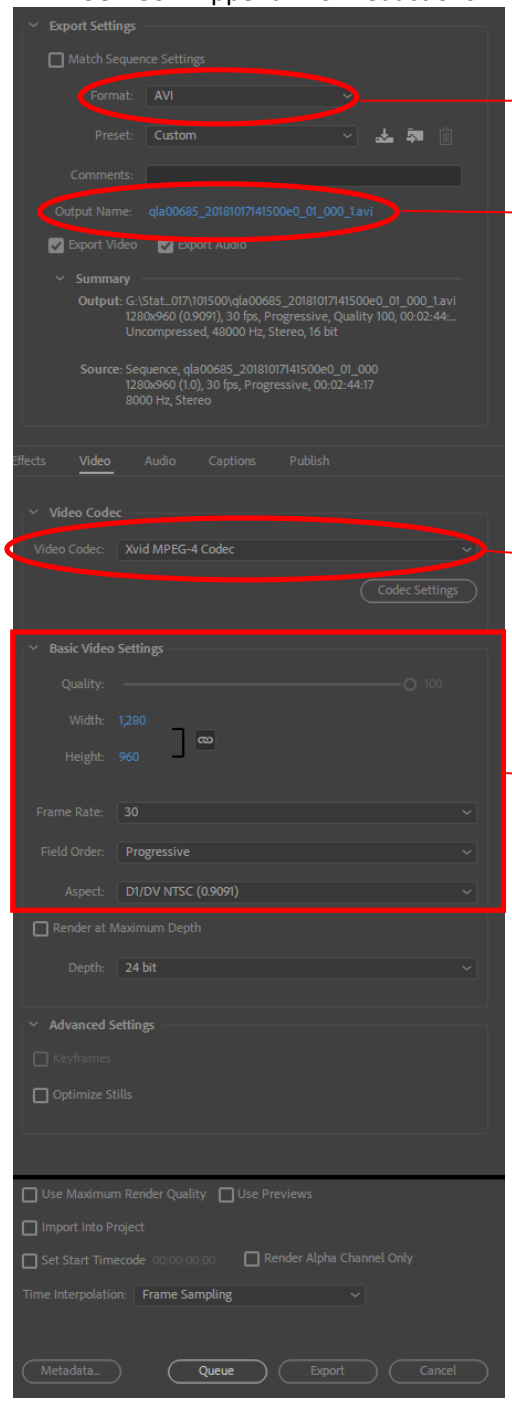
15. When exporting the MP4 file, check off the box that says "**Match Sequence Settings"** under export settings***.

Go to **"Output Name"** select the blue hyperlink in that section to reinsure that when the content is exported it is going to the correct folder and is named properly.

For example, if the media was originally labeled as "408_VTS_011.VOB" the media being exported out would be "408_VTS_01.1.VOB_Redacted". This way you will be able to tell which is the media you produced vs the file that came in for a redaction.

If you have more than one video in the project make sure to export each one. They all have to be individual exports.

******See below if exporting an imported WMV video***. **Note: MP4 is the prefable format to export.**



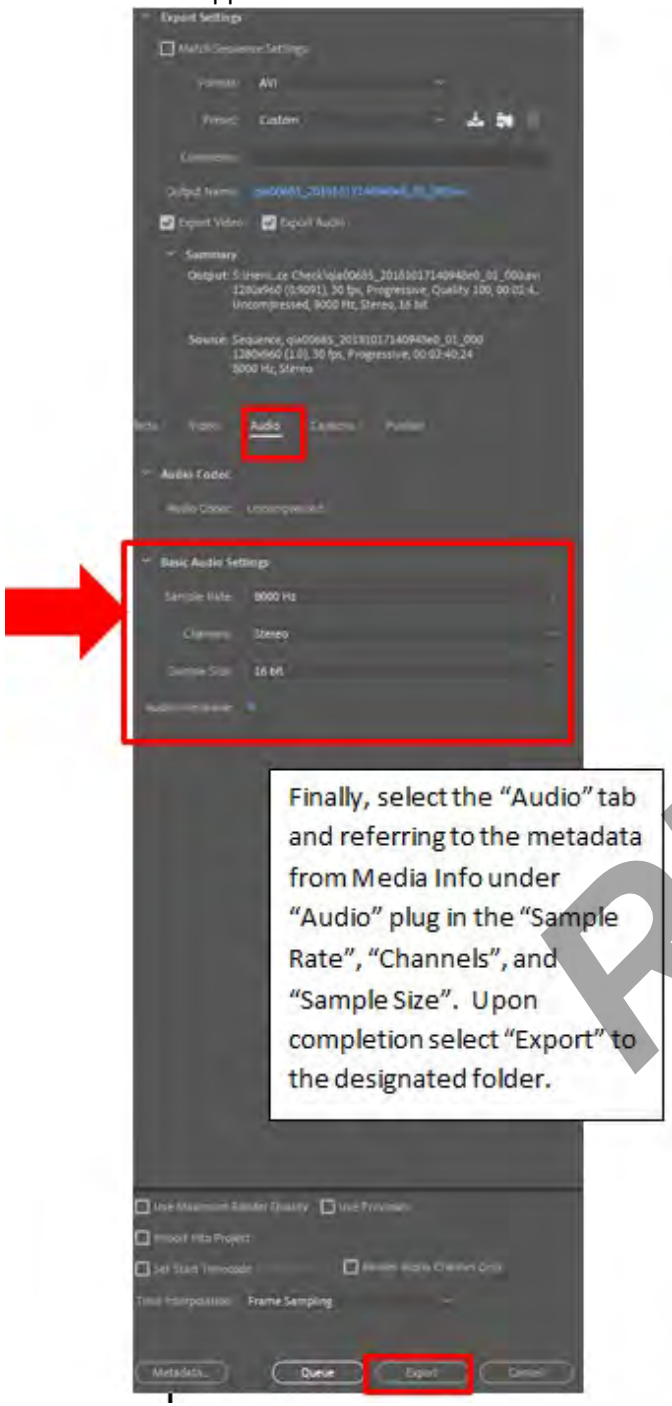
Select the format for the exported video to be an AVI

Change the output name to “the title of your video_redacted”, and select the designated folder for the case to save the video in.

Scroll to make the Video Codec is “Xvid MPEG-4”

Referring to the metadata from Media Info, plug in the Width, Height, Frame Rate, and Field Order of the video.

RETIRED

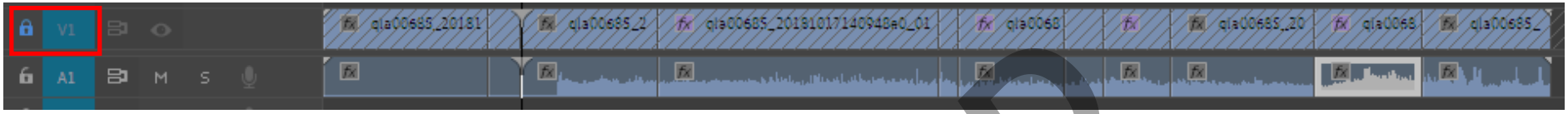


Finally, select the "Audio" tab and referring to the metadata from Media Info under "Audio" plug in the "Sample Rate", "Channels", and "Sample Size". Upon completion select "Export" to the designated folder.

RETIRED

Redacting the Audio in video using Premiere Pro CC

When redacting audio in a video. You want to "lock" the video by clicking the icon that looks like a pad lock. This prevents the video from being affected by your next cuts to the audio and next redactions.



Review the audio to determine what needs to be redacted as detailed by the requesting agency. Once it is clear what areas of the audio need to be redacted, pause the video at the beginning of where the audio redaction should take place. In the tools menu, select the "**Razor Blade**" tool to cut the audio at the place of the start of the redaction. Scroll to the end of the portion of the audio needing redaction and again cut the audio using the "Razor Blade" tool. The selected segment of audio enclosed within the "razor blade" cuts should be the area in which the redaction is going to be applied. Using the cursor, highlight the razor cut area and then hit "delete".

Once the audio is redacted you can export your now redacted video and audio by following the instructions under number 14.

Note: when needing to conduct both video and audio redactions on the same file, it is preferable to "unlink" the video from the audio and lock the segment that is not being redacted at that time. This will present extra "razor" cuts from being applied to both areas (video and audio) of the timeline simultaneously.

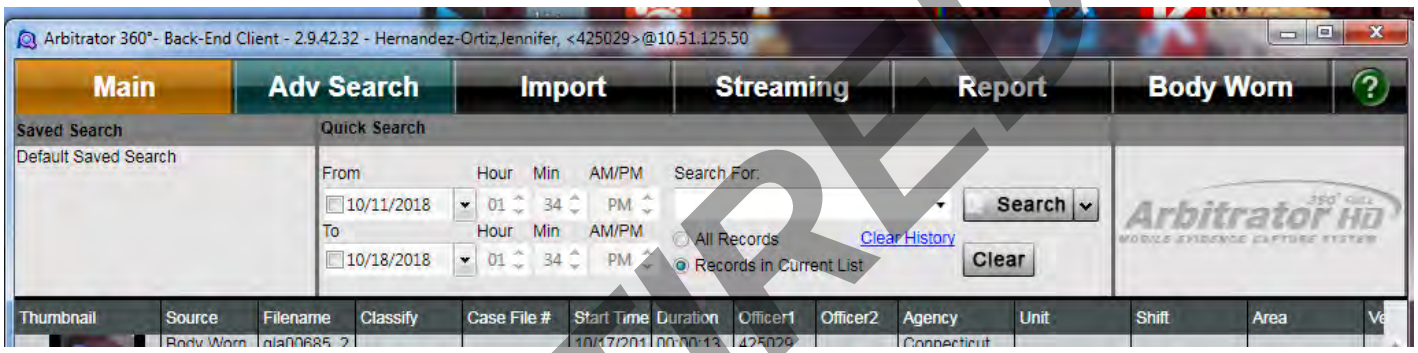
RETIRED

Acquiring Video from Arbitrator 360° Backend Client

1. If a folder hasn't already been made, create a folder in the Documents folder with the appropriate case number and any other designator.

2. If the request for a redaction is submitted via an RFA to acquire the video from the Arbitrator 360° Backend Client, log into Arbitrator 360° Backend Client using your credentials.

3. Search for video needed by using the "Quick Search Fields" and the provided information on the Request for Analysis.

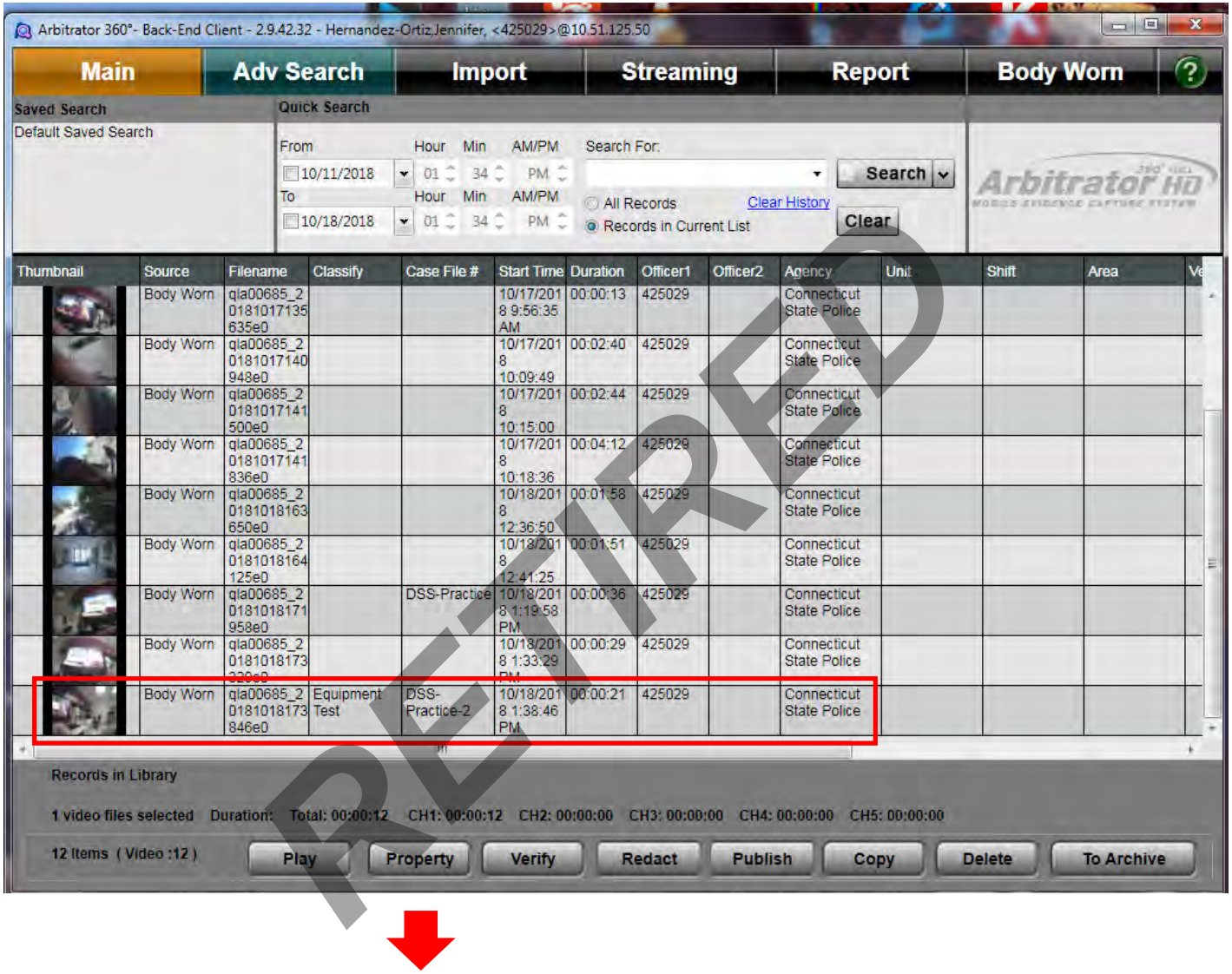


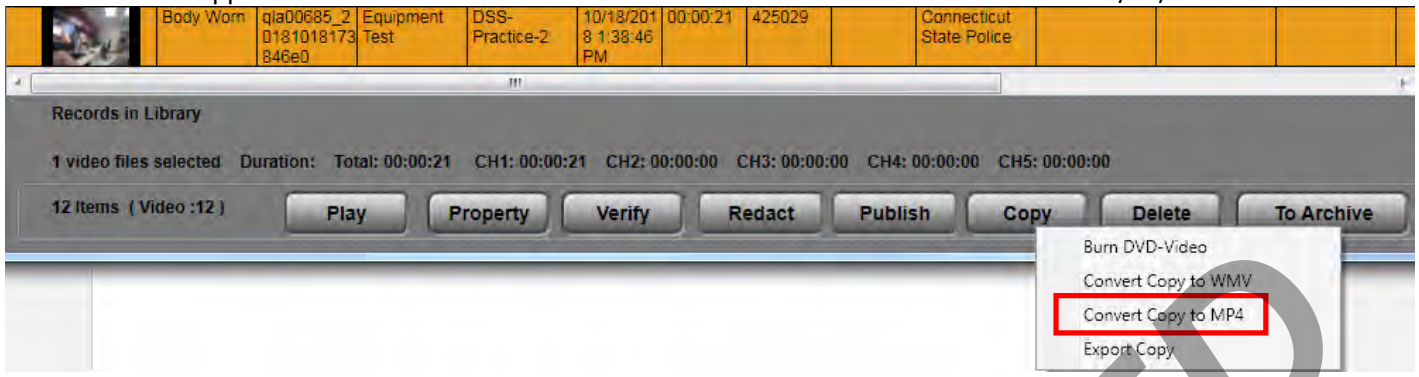
4. Once the requested video is located, select the file from the file list. You may have to refresh the list for the new video to show up. After the video is selected, use the "copy" tab from the options on the bottom of Back-End Client screen, and select "Convert Copy to MP4". The video will download into your selected folder, and you can then work with it in Premiere Pro.

Follow the highlighted inserts in the images.

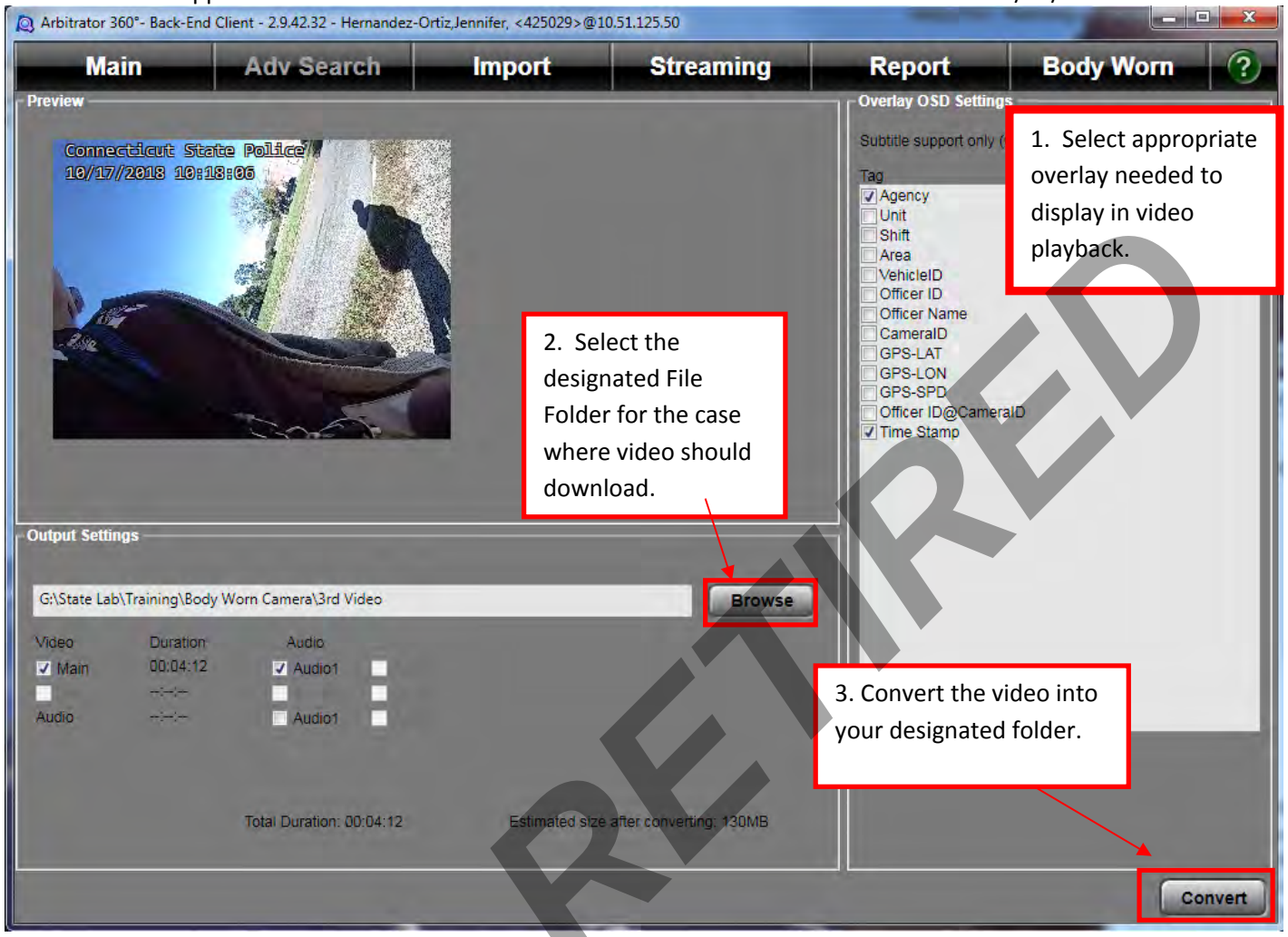
Note: MP4 is a higher quality video and preferable over the WMV format.

RETIRED


Arbitrator 360°- Back-End Client - 2.9.42.32 - Hernandez-Ortiz,Jennifer, <425029>@10.51.125.50
Main
Adv Search
Import
Streaming
Report
Body Worn
Saved Search
Default Saved Search
Quick Search
From
10/11/2018
Hour Min AM/PM
01 34 PM
To
10/18/2018
Hour Min AM/PM
01 34 PM
Search For:
Search
All Records
Clear History
Records in Current List
Clear
Arbitrator HD
Thumbnail
Source
Filename
Classify
Case File #
Start Time
Duration
Officer1
Officer2
Agency
Unit
Shift
Area
Body Worn qla00685_20181017135635e0 10/17/2018 9:56:35 AM 00:00:13 425029 Connecticut State Police
Body Worn qla00685_20181017140948e0 10/17/2018 10:09:49 00:02:40 425029 Connecticut State Police
Body Worn qla00685_20181017141500e0 10/17/2018 10:15:00 00:02:44 425029 Connecticut State Police
Body Worn qla00685_20181017141836e0 10/17/2018 10:18:36 00:04:12 425029 Connecticut State Police
Body Worn qla00685_20181018163650e0 10/18/2018 12:36:50 00:01:58 425029 Connecticut State Police
Body Worn qla00685_20181018164125e0 10/18/2018 12:41:25 00:01:51 425029 Connecticut State Police
Body Worn qla00685_20181018171958e0 DSS-Practice 10/18/2018 1:19:58 PM 00:00:36 425029 Connecticut State Police
Body Worn qla00685_20181018173 10/18/2018 1:33:29 PM 00:00:29 425029 Connecticut State Police
Body Worn qla00685_20181018173846e0 Equipment Test DSS-Practice-2 10/18/2018 1:38:46 PM 00:00:21 425029 Connecticut State Police
Records in Library
1 video files selected Duration: Total: 00:00:12 CH1: 00:00:12 CH2: 00:00:00 CH3: 00:00:00 CH4: 00:00:00 CH5: 00:00:00
12 Items (Video :12)
Play
Property
Verify
Redact
Publish
Copy
Delete
To Archive
RETIRED


Body Worn
qla00685_20181018173846e0
Equipment Test
DSS-Practice-2
10/18/2018 1:38:46 PM
00:00:21
425029
Connecticut State Police
Records in Library
1 video files selected Duration: Total: 00:00:21 CH1: 00:00:21 CH2: 00:00:00 CH3: 00:00:00 CH4: 00:00:00 CH5: 00:00:00
12 Items (Video :12)
Play
Property
Verify
Redact
Publish
Copy
Delete
To Archive
Burn DVD-Video
Convert Copy to WMV
Convert Copy to MP4
Export Copy

RETIRED



RETIRED

Once the video is downloaded into the appropriate folder on your computer, you may begin redactions and or enhancements as needed

Purpose: Introduction to Question Documents Unit

Responsibility: Forensic Science Examiners assigned to Unit or performing casework in Unit

Overview of Procedures:
The procedures presented in these Standard Operating Procedures (SOPs) manual are in general, the duties of a Forensic Science Examiner, performing casework in the discipline of Questioned Documents.

The methodologies and procedures contained in this manual are not all encompassing. The wide variety of questioned document cases which can be encountered by the Questioned Document Unit precludes using this manual as an all-encompassing handbook for examination processes. Certain cases may require divergence from, and/or adaptations to, these written procedures. All non-standard methods when possible shall be validated before examinations are conducted. The Deputy Director shall be notified of deviation of methods to address prior to proceeding.

The competent examiner must also rely on his or her experience and training, reference materials available in various scientific and technical papers, publications and texts, consultations with experts in a specific field and/or exploring new developments within the questioned documents examination field.

The exact procedure(s) employed by an examiner of questioned documents is dependent upon the evidence submitted and the type(s) of examination(s) requested.

RETIRED

Sources of Error: Not applicable

References: See Bibliography/Reference Materials and SWGDOC

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SOP-QD-2	Table of Contents
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SOP-QD-6	Terminology Relating to the Examination of Questioned Documents
SOP-QD-7	Terminology for Expressing Conclusions of Forensic Document Examiners
SOP-QD-8	Procedure for Examination of Handwritten Items
SOP-QD-9	Procedure for Examination of Altered Documents
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SOP-QD-15	Procedure for Preservation of Liquid Soaked Documents
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SOP-QD-17	Procedure for Examination of Fracture Patterns and Paper Fiber Impressions on Single-Strike Film Ribbons and Typed Text
SOP-QD-18	Procedure for Non-destructive Examination of Paper

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SOP-QD-19 Procedure for Examination of Documents Produced with Liquid Ink Jet Technology

SOP-QD-20 Procedure for Examination of Documents Produced with Toner Technology

SOP-QD-21 Procedure for the Validation of a New Method or Technology

SOP-QD-22 Reference Materials

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Purpose: Evidence Procedures

Responsibility: Forensic Science Examiners assigned to Unit or performing casework in Unit

Procedure:

Taking Custody of Evidence

1. When evidence is submitted to the Laboratory for Questioned Documents analysis, a request is placed in the Justice Trax Laboratory Information Management System (LIMS) and the examiners in the Unit are notified of the case needing analysis.
2. The evidence is transferred into the examiners possession and if the examiner is not beginning casework at that time, the evidence will be placed into a secure storage location.

   Take custody of evidence and obtain copies of "Evidence Receipt" and "Request For Analysis" forms.
3. When beginning casework, review "Request For Analysis" form and/or any other form of request such as a letter of request.
   1) Verify that the agency case number, item/submission number, and description of items listed on both the forms for examination by Questioned Document Section, are present and accounted for.
   2) Determine what type(s) of Document examination(s) are requested which may require special handling of evidence.
   3) Determine if evidence is to be reviewed or examined by other Units of the Laboratory.
   4) Determine if case or evidence should be reviewed and/or examined by another Unit prior to being examined by the Questioned Document (QD) Unit. If examination is needed by another Unit prior to examination in QD, the Case Management Unit will be notified.

Beginning of Analysis

1. When opening the evidence, an inventory of the evidence contained in the packages is compared to the Request for Analysis form and Evidence Receipt. If a problem exists with evidence (i.e. all evidence listed as being submitted to Laboratory is not accounted for), the Case Management Unit will be notified to contact the submitting agency.
2. Open each container and/or package containing evidence and take physical inventory of evidence contained therein.
   1) Write initials and date on evidence barcode sticker on package.
   2) Verify that evidence and barcode information correspond.

RETIRED

3) If evidence needs to be subsequently processed by another Unit of the Laboratory (i.e. Latent Prints), ensure proper procedures are followed so as not to destroy or contaminate evidence.
4) After opening a container or package, appropriately identify and initial each piece of evidence received. If evidence is to be processed by another Unit (i.e. Latent Prints or DNA) this step may be skipped in order to avoid contamination or obstruction of potential evidence on the item.

Documentation of Evidence

1. Information describing the actual contents of the evidence container and/or a description [of the evidence] appearing on the evidence labels, stickers or markings on the evidence container will be recorded on the Questioned Document Worksheet (QR-DOC-2).
2. Photocopies of all evidence will be made (when practical) and placed in case file.
3. Digital photographs and/or scans of evidence will be taken as deemed necessary and appropriate by the examiner. Copies of all photographs and/or scans will be maintained in appropriate case file. When scanning document, the scanner should be set at a minimum of 200 ppi. It should be noted that the scans are used for documentation in the laboratory notes and that the actual analysis takes place using the original evidence.
4. All instrumental analysis shall be recorded on Documents worksheets (QR-DOC-2).

RETIRED

Use of JusticeTrax (LIMS)

1. JusticeTrax will be utilized in the event of sub-itemization of evidence.
    1) If more than one item is present in a Submission, the items may be sub-itemized in JusticeTrax. For Submission 001, this would be represented as 001-1, 001-2, etc.
    2) For items that are created, such as Electrostatic Detection Apparatus (ESDA) lifts or items that are removed from the original evidence container, they may be sub-itemized as indicated in GL-4.
2. JusticeTrax will also be utilized to document the chain of custody of the evidence, to assign cases to an examiner and to document the technical and administrative reviews.

Securing and Control of Evidence

1. All evidence will be secured in approved Questioned Document storage area or container.
2. At no time will any evidence submitted for examination be left unsecured and/or unattended outside of Questioned Document Unit.

Completion of Case and Review

1. After completion of analysis, a report will be generated by the analyst, the report may include the following information (Note: Starred items are required per GL 18):

   a. Name and address of the Division*

   b. Title*

   c. Division case number*

   d. Name and address of the submitting agency*

   e. Agency case number*

   f. Town of incident

   g. Date of request*

   h. Date of report

   i. Individual requesting report

   j. Description of the item(s) tested*

   k. Description of request for analysis

   l. Identification of the test method used*

   m. Results obtained*

   n. Name, title and signature of analyst and technical reviewer*

2. This report and the case file will be provided to the technical reviewer for their evaluation. The case file will contain all pertinent information to support the findings, these may include:

   a. Evidence Receipt(s)

   b. Request form(s)

   c. Worksheet(s) (QR-DOC-2)

   d. Documentation of the label on the packaging of the item(s) of evidence

   e. Photograph(s), scan(s), photocopies, etc.

   f. Notes

   g. Copy of the report (including amended or supplemental report if applicable)

   h. Case Review Form (QD-IM QR1)

   i. Other documents as applicable

RETIRED

3. A backup of all digital files and pictures will be stored on the Laboratory's X Drive or on another drive/device as needed.

Sources of Error: N/A

Quality Assurance: N/A

References: GL 1 Quality Manual
GL 13
GL 18

RETIRED

Purpose: Scope of Work of Forensic Document Examiners

This overview provides guidance regarding the duties of forensic document examiners, also referred to as Questioned document examiners and provides guidance to anyone analyzing forensic document examination.

Responsibility: Forensic Science Examiners assigned to the Questioned Document Units or performing casework in the Unit.

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The Forensic Science Examiners assigned to this Unit make scientific examinations, comparisons, and analyses of documents in order to:

1. establish genuineness or non-genuineness, or to expose forgery, or to reveal alterations, additions, or deletions,
2. identify or eliminate persons as the source of handwriting,
3. identify or eliminate the source of typewriting or other impression, marks, or relative evidence,
4. write reports or give testimony, when needed, to aid the users of the examiner's services in understanding the examiner's findings.

Examiners in this field are sometimes known referred to by the term "handwriting experts." Forensic document examination includes expertise in handwriting identification. Handwriting includes cursive or script style writing, hand printing, signatures, numerals, and other written marks or signs.

Forensic document examination does not involve the employment of calligraphic or engrossing skills, nor does it involve a study of handwriting in an attempt to create a personality profile or otherwise analyze or judge the writer's personality or character.

General Examination Overview:

Forensic analysis about documents arise in business, finance, civil and criminal trials, or in any matter affected by the integrity of written communications and records. The DSS examines evidence related to criminal matters or investigations that may lead to criminal charges.

Forensic questioned document analysis generally encompasses the following:

1. The identification of handwriting and typewriting.
2. The identification or elimination of the source of and the output of other mechanical or electronic imaging devices such as printers, copying machines, facsimile equipment, and the like.
3. The identification or elimination of ink, paper, and writing instruments.

4. The establishment of the date, source, history, sequence of preparation, alterations or additions to documents, and relationships of documents.
5. Other analysis may require the decipherment and sometimes the restoration, or both, of obscured, deleted, or damaged parts of documents.
6. The analysis may include a study of the information carried by a document for discovery of evidence of spuriousness, identification of persons, or to show significant relationships.
7. Document examination also includes the recognition and preservation of other relevant physical evidence that may be present on documents.

Questions about documents are answered through the application of knowledge, skill, experience, training, or education specific to forensic document examination as well as from a number of other fields, such as the physical sciences. The field of interest may include research into the manufacturing processes and the materials that go into the production of documents, as well as the methods, machines, instruments, and human agencies by which the parts of documents are formed or brought together.

RETIRED

General Equipment Overview:

Equipment used in forensic document examination includes: stereoscopes and other optical aids; photography, photo editing software and other imaging devices, a wide variety of imaging materials adaptable for use with a variety of lighting methods, including the use of the Video Spectral Comparator (VSC) that involves the use of radiant energy in the ultraviolet, visible, infrared, and other regions of the electromagnetic spectrum; as well as the electrostatic detection apparatus (ESDA) or other devices for the detection, or visualization, or both of indentations and other features present in or on paper or similar substrata.

Reporting of Results:

The results of examinations are reported for use by the submitting agency as detailed in GL1 Section 5.10. These results may be later interpreted in the expert testimony/opinions, explaining the bases and reasons for the conclusions, which may be illustrated by the use of demonstrative evidence.

Sources of Error: Not applicable

References:

SWGDOC Standard for Scope of Work of Forensic Document Examiners ver. 2013-1

Purpose: Standard for Minimum Training Requirements for Forensic Document Examiners

This SOP lists the minimum requirements and procedures that should be used for the fundamental training of a forensic document examiner. The procedures outlined here are grounded in the generally accepted body of knowledge and experience in the field of forensic document examination. By following these requirements and procedures, an appropriate trainee can acquire the scientific, technical, and other specialized knowledge, skill, and experience required to reliably perform the work of a forensic document examiner.

Responsibility: Forensic Examiners assigned to the Questioned Documents Unit or performing casework in the Unit

Terminology:
technical visit, *n*—travel for the purpose of obtaining information, knowledge, or training, including interaction with or demonstration by pertinent manufacturers, businesses, and laboratories.

Training Materials and Equipment:
1. The trainee should have access to textbooks, periodicals, papers, and other professional literature.
2. The trainee should have access to equipment appropriate to each area of instruction.

Suggested Requirements for the Trainee:
1. Successfully passed the minimum requirements for Forensic Science Examiner 1 or Connecticut Career Trainee.

Suggested Requirements for the Trainer(s):
1. The principal trainer shall be a forensic document examiner that has been deemed competent in the discipline by the Director or his designee.
2. Have been trained in the topics of instruction as suggested by SWGDOC or other comparable organization.
3. The qualifications of any trainers shall be approved by the Section Lead or the Deputy Director.

Overall Training Program:
1. As a guideline, the training program may take up to 24 months to complete; limited types of analysis may be approved following successful completion of various sections. Training may be completed sooner than 24 months depending on previous training and experience. Refer to training GL-14.
2. Completion of training will depend on the trainee's level of experience and the trainee may be authorized to perform casework in certain areas while continuing training in others.
3. Each area of instruction will have an objective(s) established by the principal trainer. Examination(s) (for example, written test, oral test, practical exercise) will be administered in order to measure the trainee's knowledge and competency in the area.

RETIRED

NOTE —Although attending meetings and presentations or webinars is useful as supplemental training, it does not replace the training outlined in this SOP. However, the principal trainer may grant credit to the trainee for knowledge acquired at such meetings and presentations.

4. Prior experience may be used to successfully complete areas of this training program. If the prior experience is technical in the area of questioned documents, the candidates prior experience will be assessed by the principal trainer and Deputy Director. This assessment may be conducted through interview and a competency test.

5. A training record for each trainee will be maintained and will document the following:
   1) Instruction in each topic area.
   2) A bibliography of relevant literature read.
   3) Examination(s) (for example, written test, oral test, practical exercise).
   4) Case statistics (for example, number, type, items, reports).
   5) Outside training, webinars, technical visits, courses, conferences, or workshops attended.
   6) Research conducted, if applicable
   7) Retraining (if necessary) will be performed under the supervision of the Quality Manager.

RETIRED

6. Training Topics

A formal written training program will include specific topics of instruction. The order in which they are administered is discretionary; however, the amount of time must be adequate to ensure competency in all topic areas. The minimum specific topics to be covered are:

1. Introduction and History of Forensic Document Examination:
   1) Ethical responsibilities and ASCLD Guiding Principles (GL 1.4 and GL 5)
   2) Literature of the field
   3) Evolution of the field
   4) Historical cases
   5) Scientific method
   6) Research methodology
   7) Laboratory General Training, Proficiency testing and Professional Development (GLs 14, 15 and 16)

2. Evidence Handling Procedures:
   1) Introduction to the Laboratory (GLs 1)
   2) Safety and Security Procedures (GL 2 and GL 3)
   3) Introduction to LIMS system (GL 4)

4) Evidence Handling procedures (GL 12 and 13)
4) Laboratory Procedures and protocols (GL 6, 7, 8, 9, 10, 11, 17, 18, 19 and 20)
5) Relationship of forensic document examination to other forensic disciplines.
6) Collection and preservation of Questioned Document evidence
7) Marking and documentation
8) Chain of custody.

3. Examination Procedures:
   1) Procedures and protocols
   2) Theory of individualization
   3) Case organization
   4) Note taking
   5) Conclusions and findings
   6) Report writing

4. Laboratory Instrumentation and Equipment:
   1) Procedures and protocols
   2) Physics of light pertinent to forensic document examination procedures
   3) Microscopy
   4) Measuring systems and devices
   5) Light sources
   6) Electrostatic detection devices
   7) Typewriter examination devices
   8) Computers and peripherals
   9) Software
   10) Other relevant laboratory equipment

5. Paper:
   1) Procedures and protocols
   2) History of paper
   3) Manufacturing processes
   4) Physical properties (for example, light-reactive, watermarks, dimensions, security features)
   5) Physical matches (for example, fibers, tears, edge striations)
   6) Tapes and adhesives
   7) Indentations

6. Writing Instruments and Inks:
   1) Procedures and protocols
   2) History of writing instruments and inks

RETIRED

3) Properties of inks
4) Destructive and nondestructive analyses of inks
5) Writing instrument characteristics
6) Sequence, direction, and pressure of strokes

7. Handwriting (including Cursive or Script Style Writing, Hand Printing, Signatures, Numerals, and Other Written Marks or Signs):
   1) Procedures and protocols
   2) History and theory
   3) Physiology of handwriting and motor control
   4) Handwriting systems
   5) Handwriting comparison process
   6) Handwriting characteristics (individual and class)
   7) Features of handwriting (for example, variation, line quality, skill level)
   8) Distorted handwriting
   9) Factors affecting handwriting (internal and external)
   10) Tracings and simulations
   11) Other handwriting problems

8. Alterations, Obliterations, and Erasures:
   1) Procedures and Protocols
   2) Types of alterations (for example, page substitution, insertion)
   3) Types of obliterations (for example, opaquing fluid, over-writing, chemical)
   4) Types of erasures (physical and chemical)
   5) Detection and decipherment techniques

9. Typewriters:
   1) Procedures and protocols
   2) History of typewriters
   3) Fundamentals of typewriter examination (individualization and comparison)
   4) Typestyle classification
   5) Typing and correction ribbon examinations
   6) Paper fiber transfer

10. Computer Printers:
    1) Procedures and protocols
    2) History of computer printers
    3) Fundamentals of computer printer examinations (individualization and comparison)
    4) Computer printing processes (impact and nonimpact)

RETIRED

5) Font classification

11. Photocopiers:
    1) Procedures and protocols
    2) History of photocopiers
    3) Electrostatic and other imaging processes
    4) Fundamentals of examination (individualization and comparison)
    5) Alteration and manipulation techniques

12. Facsimiles:
    1) Procedures and protocols
    2) History of facsimile machines
    3) Imaging processes
    4) Fundamentals of examination (individualization and comparison)
    5) Alteration and manipulation techniques

13. Printing Processes:
    1) Procedures and protocols
    2) History of printing
    3) Typography
    4) Characteristics of printing processes
    5) Fundamentals of examination (individualization and comparison)
    6) Security features

12. Mechanical Impressions:
    1) Procedures and protocols
    2) History of devices (for example, check writers, rubber and polymer stamps, paper binders, staples, embossing devices, seals and stamped impressions, fasteners, hole punchers)
    3) Fundamentals of examination (individualization and comparison)

13. Charred and Soaked Documents:
    1) Procedures and protocols
    2) Care and preservation
    3) Examination and decipherment

14. Photography and Digital Imaging:
    1) Procedures and protocols
    2) General photography
    3) Document photography
    4) Digital photography

RETIRED

5) Digital imaging techniques
6) Alteration and manipulation techniques
7) Image editing software

15. Miscellaneous Examinations:
    1) Dependent upon the capabilities or requirements of the laboratory

16. Expert Witness and Legal Proceedings:
    1) Procedures and protocols
    2) Terminology
    3) Relevant law
    4) Adjudication systems
    5) Effective communication
    6) Courtroom demeanor
    7) Preparation and use of demonstrative exhibits
    8) Observation of pre-trial conferences and testimony of experts, actual or mock
    9) Participation as an expert witness in mock trials
    10) Understanding of critical challenges to the discipline

17. Practical Experience:
    1) Supervised casework
    2) Training or observation at other forensic document laboratories is recommended
    3) Supplemental education (for example, courses, seminars, technical visits, workshops)

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Required Text Reading:
To develop a contextual understanding, each of the following texts will be read from cover to cover – in the following order. This training will be recorded in the training log. Some assignments that require a written paper will have a specific reading to complete.

Questioned Documents by Albert S. Osborn
The Problem of Proof by Albert S. Osborn
The Mind of the Jury by Albert S. Osborn
Questioned Document Problems by Albert S. Osborn
Suspect Documents by Wilson R. Harrison
Forgery Detection by Wilson R. Harrison
Scientific Examination of Questioned Documents by Ordway Hilton
Typewriting Identification - ID System for Questioned Typewriting by Billy Prior Bates
Forensic Examination of Rubber Stamps by Jan Seaman Kelly
A Selection of International Penmanship Systems by William & Katherine Koppenhaven

Evaluating Evidence by Katherine Mainolfi Koppenhaven
Forensic Signature Examination by Steven A. Slyter
Detecting Forgery - Forensic Investigation of Documents by Joe Nickell
Fundamentals of Criminal Investigation by Charles E. O'Hara & Gregory I. O'Hara
Forensic Science - An Introduction To Criminalistics by P. DeForest, R. Gaensslen & H. Lee
Document Examiner Textbook by Jess E. Dines
Scientific Examination of Documents: Methods and Techniques by David Ellen
Handwriting Identification: Facts and Fundamentals by A. M. Headrick & Roy Huber
Ames on Forgery by D. T. Ames

The following research topics may have a paper and presentation:
1 Forensic Science General
2 The Questioned Document Examiner
3 Laboratory Equipment
4 Theory of Identification
5 Printing Processes
6 Writing Instruments and Inks
7 Paper
8 Handwriting History/General
9 Handwriting – Characteristics
10 Handwriting – Variation
11 Handwriting – Signatures
12 Handwriting – Specimens
13 Handwriting – Comparisons
14 Hand printing and Numerals
15 Typewriting – History/General
16 Typewriting – Examinations
17 Other Mechanical Impressions
18 Office Copiers
19 Miscellaneous Document Problems
20 Historical Cases and Legal Precedence

RETIRED

Authorization for Case Work:
At the end of the training program, a memo/email shall be written to the Deputy Director stating that the analyst has successfully completed their training and has been administered a competency case that evaluated their analytical skills and their ability to clearly communicate their findings in a report.

The Deputy Director may review the training files or simply endorse this memo/email and generate a request to the Quality Section requesting that this analyst be authorized to perform casework in that discipline.

Authorization for Technical Reviews:

The analyst will perform casework and after gaining experience in conducting analysis and reporting findings in a variety of evidence type/conclusions in the discipline, their experience will be evaluated for the ability to conduct technical reviews. This evaluation period may include technical review ghosting with an experienced examiner. Once the analyst has completed the evaluation period, a memo/email shall be written to the Deputy Director stating that the analyst has successfully completed their evaluation period and has been deemed competent to conduct technical reviews in that discipline.

The Deputy Director, after review, may endorse this memo/email and generate a request to the Quality Section requesting that this analyst be authorized to perform technical reviews based upon the analyst's casework experience in that discipline.

Retraining:
In the event that the examiner requires retraining in a specific area, the examiner will be retrained and then administered a competency test in that area prior to resuming casework in that specific area. (GL14)

Sources of Error: Not applicable

References:

SWGDOC Standard for Minimum Training Requirements for Forensic Document Examiners ver. 2013-1

RETIRED

Purpose: Terminology Relating to the Examination of Questioned Documents

This terminology standard includes terms that relate to the examination performed by forensic document examiners. The terms in this Terminology Section refer to typical copybook styles of writing and do not account for exceptional forms.

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit

Terminology:

absent character, *n*—a character or character combination which is present in one body of writing but is not present (for example, does not have a corresponding character) in another body of writing.
alignment, *n*—the adjustment of various mechanisms of a typewriter to ensure the even printing of the characters and their proper positioning relative to the baseline and to the other characters.
aliasing, *n*—see *pixilation*.
alignment defect, *n*—a deviation from the intended appearance or position of a character relative to another character. See impression defect, motion defect. Alignment defects are usually categorized as vertical misalignment (character too high or low relative to the baseline established by the other characters), horizontal misalignment (character too far to the left or right relative to other characters), and twisted or leaning (character leans to the left or to the right); because they are corrected in the alignment process, impression defects are considered as alignment defects.
alteration, n—a modification made to a document by physical, chemical or mechanical means including, but not limited to, obliterations, additions, over writings, or erasures.
apex, *n*—the uppermost point of a character.
ascender, *n*—a stroke that rises above the height of the body of the letter formation.
assisted hand signature, *n*—a signature executed by a writer while the writer's hand, arm, or writing instrument is steadied or stabilized by another.
awkward hand, *n*—see unaccustomed hand.
ball element, *n*—an element used in a single element typewriter in which the fully formed characters are located on the outer surface of a sphere-like device. Frequently called *golf-ball element*.
banding, *n*—uniform density variations or voids in a given color which appear in the direction that the printhead travels.
baseline, *n*—the ruled or imaginary line upon which writing or typewriting appears to rest.
bead defect, *n*—inked or un-inked impression or hole in the paper caused by a contaminant particle encased in plating material located on or adjacent to the printing area of the typeface on a metal coated element.

RETIRED

black write, *n*—process in electrostatic printing in which the photoconductive element is charged with a charge of the same sign as that of the toner. A light beam, used like a "stylus" is used to discharge only those areas that are to receive toner to form the image. In the development process, the charged background areas repel the like charged toner to the discharged areas on the photoconductor.

bleed, *n*—ink feathering of one color into an adjacent color over time.

blemish, *n*—a small extraneous spot found near inked regions of check-writer impressions that is characteristic of machines that use ribbons as their ink source.

bone folder, *n*—a piece of shaped bone or other material, such as plastic or Teflon, typically used by bookbinders to crease paper and to separate pieces of paper that are stuck together.

bridging, *v*—clumping of toner that causes a hollow area in the toner supply that prevents the free flow of toner to the dispenser auger.

carbon paper, *n*—a sheet composed of a supporting substrate on one or both sides of which is a coating containing a transferable (usually colored) material. The coating is of such nature that it will transfer in part or entirely to a copy sheet at the point of pressure contact.

character, *n*—any language symbol (for example, letter, numeral, punctuation mark, or other sign), other symbol or ornament.

character pitch, *n*—the number of characters that can be printed in a horizontal 1 in. (25.4 mm).

character spacing, *n*—the width allotted to each character in a fixed pitch (monospacing) typewriter or to the basic unit in a proportional spacing typewriter; usually expressed in millimeters or as a fraction of an inch. Synonym for horizontal escapement.

characteristic, *n*—a feature, quality, attribute, or property of writing.

check-writer, *n*—a device manually or electrically powered or computer generated, designed to ink, emboss, print, perforate, or shred a monetary value, along with other peripheral information, onto a document.

circularity, *n*—ratio of a single ink dot height divided by its width with 1.0 being a perfect circle.

coalescence, *n*—puddling or pooling of adjacent ink drops on the substrate before they can be dried or absorbed resulting in nonuniformity of color density.

cockle, *n—of paper*, a defective, puckered condition of a paper sheet as a result of non-uniform hygro-expansion which can be related to any non-uniformity in the sheet, including mass distribution and drying stresses.

coincidental peripheral printing, *n*—printing resulting from an impression of unintended printing areas, often on the periphery, of a stamp. This may be due to the manufacturing process or the stamping technique.

comparable, *n/adj*—pertaining to handwritten items that contain the same type(s) of writing and similar characters, words, and combinations. Contemporaneousness and writing instruments may also be factors.

connecting stroke, *n*—a line joining two adjacent characters.

continuous spray, *n*—ink jet technology where drops are generated at a regular unbroken rate. Images are then generated by deflections of the ink droplets after they are charged so they are either intercepted

RETIRED

by a catcher and not permitted to impact the substrate or deflected to intercept the substrate at specific locations.

corona, *n*—device used to place a uniform electrical charge on the surface of a xerographic photoreceptor.

correctable ribbon, *n*—a ribbon that produces an image that is designed to be completely removed from the substrate by means of lift-off.

correction media, *n*—ribbons, tapes, and sheets designed to be struck by the typeface to cover-up or lift-off typed text.

cover-up, *n*—the obliteration of one or more images by means of an opaque material similar in color to the substrate.

cover-up correction, *n*—see overprint correction.

cracking, *n*—condition in which ink that has been absorbed into a substrate causes the coating to shrink to a state much smaller than the original coating dimension causing fractures in the image area.

cross stroke, *n*—a stroke that crosses another portion of the character and is not attached at either end. (For example, the horizontal stroke of the "t").

crossbar, *n*—a stroke that intersects other portions of the character at both ends.

crystallization, *n*—condition in which ink evaporates and forms crystals.

cursive, *n*—a type of writing in which the letters are joined and the writing instrument is not lifted after most strokes.

daisy wheel element (print wheel), *n*—an element used in a typewriter in which the fully formed characters are contained on the ends of finger-like projections radiating out from the center of a disk.

descender, *n*—a stroke that extends below the baseline of the body of the letter formation

dielectric printing process, *n*—nonimpact printing technique in which specially treated paper consisting of a conductive base layer coated with a nonconductive thermoplastic material is used to hold an electric charge usually applied directly by a set of electrode styli. The electric charge corresponds to the latent image of the original. Following the charging step, the paper is imaged by a toner system similar to that of electrostatic copying devices. This technique is sometimes called electrographic, and is currently used on general purpose non impact printers, plotting and facsimile devices.

digital image, n—an image that is stored in numerical form.

digital image processing, n—any activity that transforms a digital image.

direct contact, *n*—two sheets of paper, one on top of the other, with no intervening sheets.

distorted writing, *n*—writing that does not appear to be, but may be natural. This appearance can be due to either voluntary factors (for example, disguise, simulation) or involuntary factors (for example, physical condition of the writer, writing conditions).

drag stroke, *n*—a stroke resulting from incomplete lifting of the pen.

drop on demand (DOD), *n*—ink jet technology where drops are generated as needed to create an image.

dry seal, *n*—a non-inked mechanical device which embosses a design on paper.

dry toner, *n*—material in a dry developer system which when deposited on a substrate by the field of an electrostatic charge pattern, becomes the visible record.

RETIRED

dual-component development, *n*—mixture of dry toner and iron oxide developer that is used for developing electrostatic images in copiers.
electric typewriter, *n*—a typewriter in which an electromechanical device causes the type element to be activated when the keys are struck.
electronic typewriter, *n*—an electric typewriter in which the keyboard input is received by an electronic processor built into the typewriter. This unit then controls the print head and other features to produce the typing action.
electrophotographic printer, *n*—nonimpact printing technique that is similar to the technology used in a typical office copier, which forms a copy by attracting toner particles to a static charge on the surface of a photoconductor, then transferring the toner image to the surface of a sheet of paper. In the normal office copier, the charged image (latent image) of the original document is formed on the photoconductor simply through exposure of the photoconductor to reflected light from the document. In an electrophotographic printer, the image is formed by a light source (laser, LED, LCS, laser diode, or other controlled light source) that erases or discharges a static image charge on the photoconductor according to information being supplied through the input data stream. Each bit of data can be related to a character shape in the memory of the printing system, and in most cases characters are formed by a dot matrix method similar in concept to that of the matrix printer. Paper can be sheet or roll—fed or continuous form.
electrostatic detection device/apparatus (EDD) of (ESDA), n—an instrument that uses electrostatic charge as the mechanism to visualize paper fiber disturbances (for example, indentations, erasures, typewritten material/lift off).
element, *n*—the interchangeable typeface carrier of a single element typewriter. See ball element, daisy wheel element, thimble element.
embossment variation, *n*—non-uniformity of the dry seal impression on the paper stock. It can be caused by the manner of application or by defects in the dry seal.
erasure, n—the area where material has been removed from a document by chemical, abrasive, or other means.
fabric ribbon, *n*—an inked ribbon wherein the substrate is a woven cloth material, such as nylon, cotton, silk, etc.
family *(of type)*, *n*—a class of type designs sharing basic qualities of style and artistic expression that differentiate it from other similar designs.
feathering, *n*—ink spread over substrate causing fuzzy edges, spidery lines and poor print quality.
film, *n*—thin transparent plastic material that covers the item during an examination using an ESDA.
fixed pitch, *adj*—describes a character set in which all character cells are of equal width. (See proportional spacing.)
flashing, *n*—excess material from the molding process.
fluorescence, n—a process by which radiant flux of certain wavelengths is absorbed and reradiated non-thermally at other, usually longer, wavelengths.

RETIRED

fracture pattern, *n*—the spatial arrangement of each complementary edge formation created when a single object is separated into two or more fragments.
full-color copiers, *n—of* ***ink jet technology***, copiers that can reproduce color originals containing gradations of color. They have a minimum of three colored inks (cyan, magenta and yellow).
full-color copiers, *n*—copiers that can reproduce color originals containing gradations of color. Full-color copiers may have up to four individual color developing units containing four different color toners. These colors are frequently cyan, magenta, yellow, and black. The original is scanned by means of an analog system using a series of color filters or by means of a digital scanning process. The full-color copier may require up to four scans to read the original. The copier individually applies one or more color toners to a transfer drum/belt or photoconductor, or both, which is in turn deposited on the paper.
fuser roll, *n*—heated roller that contacts the paper and toner directly and is part of the fuser unit.
glitch, *n*—print defect that displaces the laser scan line so that it appears to start and stop late.
gripper bar, *n*—metal bars used in delivery systems to grasp individual sheets, directing them through the system in a toner device.
guided signature, *n*—a signature executed by a writer while a writer's hand arm, or writing instrument is influenced or controlled by another.
hand printing, *n*—a style of writing in which the letters are not joined and the writing instrument is lifted after most strokes.
handwritten item, *n*—an item bearing something written by hand (for example, cursive writing, hand printing, signatures). NOTE —As used in this standard "handwriting" and "handwritten" are generic terms. Writing is generally, but not invariably, produced using the hand, and may be the result of some other form of direct manipulation of a writing or marking instrument by an individual.
hesitation, *n*—a pause in the writing without the instrument being lifted.
image, *n*—optical counterpart of an object produced by means of an image producing device.
image area, *n*—area on a page occupied by all the printed information, including the space between letters and lines. (See *percent coverage* and *maximum image area*.)
image density, *n*—contrast between image and background as measured by densitometer.
imaging drum, *n*—photoreceptive drum coated with a charge-sensitive material used in the image transfer systems of toner devices.
impact printer, *n*—a printer in which printing is the result of mechanical impacts.
impression, *n*—an image formed by pressure
impression defect, *n*—a deviation from the intended evenness in appearance of a character over the entire impression of the character or relative to the impression of another character. *See* off-foot.
impression format, *n*—the manner in which the paper is embossed or shredded.
indentations, *n*—latent or visible impressions in paper or other media.
indirect contact, *n*—two sheets of paper, one on top of the other, with one or more intervening sheets.
individual prefix, *n*—a prefix especially designed for a particular customer.

RETIRED

individualizing characteristic (writing), *n*—marks or properties that serve to individualize writing. Note: Both class characteristics (marks or properties that associate individuals as members of a group) and individual characteristics (marks or properties that differentiate the individual members in a group) are individualizing characteristics.

individualizing characteristics, *n*—marks or properties that serve to uniquely characterize. Both *class characteristics* (marks or properties that associate individuals as members of a group) and *individual characteristics* (marks or properties that differentiate the individual members in a group) are individualizing characteristics.

indentations, *n*—latent or visible impressions in paper or other media.

infrared (IR), n—referring to radiant flux having wavelengths longer than the wavelengths of light, usually wavelengths from about 760 nm to about 3 mm.

infrared luminescence (IRL), n—the emission of radiant energy during a transition from an excited electronic state of an atom, molecule, or ion to a lower electronic state (fluorescence or phosphorescence, or both), where the spectrum of the excitation source is in the ultraviolet (UV) or visible region of the electromagnetic spectrum, or both, and the spectrum of the emitted energy is in the far red or infrared (IR) region of the electromagnetic spectrum.

ink jet printer, *n*—nonimpact printer in which the characters are formed by projecting droplets of ink onto a substrate.

inked ribbon, *n*—a ribbon composed of a supporting substrate of film, fabric, or paper and a coating or impregnation of a coloring material. The coloring material is of such nature that it will transfer in part or entirely to a copy sheet at the point of pressure contact.

item, *n*—an object or quantity of material on which a set of observations can be made

known, *n/adj*——of established origin associated with the matter under investigation

landscape mode, *adj*—printer output orientation in which printed lines run parallel to the direction of movement of the paper.

laser printer, *n*—nonimpact printer that uses a laser light source driven by digital signals to create images on a photoconductor. (See *electrophotographic printer*.)

lift, *n*—the product of an ESDA examination; a self-adhesive plastic sheet adhering to a film that preserves the results of an ESDA examination.

lift-off, *n*—the removal of one or more images of copy from the substrate by transferring to an intermediate member.

lift-off correction, *n*—the removal of a typed character by re-striking with the same character while interposing an adhesive coated tape or sheet, thereby causing the imprinted character to adhere to the coating and be stripped from the record-medium.

line quality, *n*—the sum total of the attributes of the writing movement (for example, speed, pressure, and skill).

line spacing, *n*—the distance between successive lines of text, usually measured from baseline to baseline, and usually expressed in millimeters or as lines per inch for typewritten text. Synonym for vertical escapement.

RETIRED

liquid ink jet device, *n*—device in which the ink supply is in fluid (for example, solvent or aqueous) form.
liquid toner, *n*—toner material composed of carbon particles or colorants suspended in a liquid carrier.
loop, *n*—a formation that curves and crosses itself.
luminescence, *n*—the emission of radiant energy during a transition from an excited electronic state of an atom, molecule, or ion to a lower electronic state.
manual typewriter, *n*—a typewriter whose operation depends solely upon the mechanical action powered by the operator.
manuscript, *n*—see hand printing.
maximum image area, *n*—portion on a page that can be printed. (See *percentage coverage* and *image area*.)
maximum print position, *n*—rightmost point at which the printer can mark the paper.
model signature, *n*—a signature that is used as a prototype for a simulation or copy, by manual electronic or other means.
monocomponent development, *n*—single component dry toner used for developing electrostatic images in copiers.
monospacing, *n*—see fixed pitch.
motion (as related to typebar typewriters), *n*—the distance traveled by the mechanism for case shifting (usually by the typebar segment or the carriage) and the corresponding separation of the characters on the type slug.
motion defect *(as related to typebar typewriters)*, *n*—a deviation from the intended evenness in appearance of the baseline alignment of unshifted characters relative to shifted characters.
mottling, *n*—non-uniformity of image density which follows patterns in the substrate or by non-uniform ink-substrate interaction.
multi-strike film ribbon, *n*—a ribbon wherein the substrate film such as polyester is coated or impregnated with an ink which allows several different imprints to be made from multiple overstrikes on the same location on the ribbon, and still result in full characters being printed.
natural writing, *n*—any specimen of writing executed without an attempt to control or alter its usual quality of execution.
nonimpact printer, *n*—a printer in which image formation is not the result of mechanical impacts.
nonrecirculating system, *n*—fuser oil application system in which none of the fuser oil that has been removed from the reservoir is returned.
off-foot, *n*—the lack of desired and optimum uniformity of contact between the typeface and the substrate. While the on-feet adjustment of the typewriter evens the impression of the upper and lower portions of all the characters, this term is also applied to uneven impressions that are heavier or lighter on the sides or corners (usually due to misalignment or distortion of individual typebars).
offset, *n*—unintentional transfer of ink (as from a freshly printed substrate).
on-feet, *n*—the desired and optimum uniformity of contact between the typeface and the substrate

RETIRED

on-feet adjustment, *n*—the positioning and setting of various mechanisms of a typewriter to ensure the even printing of the upper and lower portions of the characters.
opacity, *n*—the property of paper that prevents the transmission of light.
original typed text, *n*—typed text imprinted onto the surface of a record-medium as the result of the impact of a type-face striking directly or through a ribbon.
overprint correction, *n*—the removal of a typed character from the text by re-striking with the same character while interposing a tape or sheet coated with an opaque coating material, thereby causing the imprinted character to be covered by the coating.
overtoning, *n*—any of the conditions occurring in the developing unit when the toner concentration is too high.
paper fiber impression, *n*—the imprint of a paper fiber in the ribbon substrate.
parylene processing, *n*—the deposition of a clear polymer coating on a document(s) within a vacuum chamber to strengthen and stabilize the document(s).
patching, *n*—retouching a portion of a written stroke.
payee perforator, *n*—an optional device on a check-writer that perforates or shreds a pattern above the numeric impression region for the purpose of protecting the payee entry from alteration.
pen lift, *n*—an interruption in a stroke caused by removing the writing document from the writing surface.
pen position, *n*—the relationship between the writing instrument and the writing surface.
pen pressure, *n*—the force with which the writing instrument contacts the paper.
percent coverage, *n*—ratio of the area actually covered by the ink (or print material) to the area of the page times one hundred. (See *image area* and *maximum image area*.)
perforation, *n*—penetration through the document.
picker bar, *n*—metal bars used in the delivery system to remove individual sheets of paper from the photoconductive drum in a toner device.
piezoelectric, *n*—ink jet technology where the electrically stimulated deformation of a crystal causes the expulsion of the droplets from the ink chamber.
pitting, *n*—small defects in the surface of the photoreceptor that produce spots or voids on the printout.
pixilation, *n*—stair-stepped or jagged effect resulting from analog to digital conversion.
platen, *n*—a bar-shaped object that pushes the paper stock against the typeface and provides the pressure necessary to obtain an impression.
polyester film encapsulation, *n*—a process whereby a document is sealed between two sheets of polyester film to preserve, stabilize, and facilitate handling.
portrait mode, *adj*—printer output orientation in which print lines run perpendicular to the direction of movement of the paper.
prefix, *n*—the portion of the check-writer impression located immediately to the left of the numeric value.
primary indentations, *n*—impressions caused by the act of writing or other dynamic actions.

RETIRED

printer, *n*—an output unit that produces durable hard-copy record of data in the form of a sequence of discrete graphic characters belonging to a predetermined character set.
printer output area, *n*—maximum area on the page to which the printer will print.
print head, *n*—printing device of an ink jet printing system.
printing element, *n*—the parts of the total check-writer impression that are not parts of the prefix which may encompass the currency type, decimal points, and commas.
printing module, *n*—those components in the laser printer that together drive the laser scanner, create the image on the page, deliver the page to the stacker.
proportional spacing, *n*—a system of printing where the character spacing is set in accordance with the character width. See fixed pitch.
questioned, *n/adj*——associated with the matter under investigation about which there is some question, including, but not limited to, whether the Questioned and Known items have a common origin
range of variation, *n*—the accumulation of deviations among repetitions of respective handwriting characteristics that are demonstrated in the writing habits of an individual. (see *variation*)
raster output scanner, *n*—output peripheral, either stand alone or within a printer, that converts computer data into a bit mapped image, which is sent to the host for storage or a printer for output.
rebound, *n*—a double impression of a typed character, the second lighter than and overlapping the first.
record medium, *n*—a piece of material, usually paper, on which an image is recorded.
retrace, *n*—a stroke written back over the preceding stroke in the reverse direction.
ribbon shift, *v*—the movement of a multi-colored inking ribbon allowing for a change in color to manifest itself in an impressed character.
rubber stamp, *n*—any of a wide variety of hand printing devices made of many materials not necessarily rubber. Syn.—*hand stamp, cachet.*
satellite, *n*—extraneous or undesirable ink droplets. (See also *spatter*, *spray*)
secondary impression(s), *n*—fiber disturbances caused by contact with the embossed side of indentations and not caused by the act of writing.
segment, *n*—a single device on which is forged or attached a set of numerals or symbols which can be set by the operator in establishing an impression value. On some machines a different segment is used for each digit.
side lighting, n—illumination from a light source that is at a low angle of incidence, or even parallel, to the surface of the item. May also be referred to as ***oblique lighting***.
significant difference, *n*—an individualizing characteristic that is structurally divergent between handwritten items, that is outside the range of variation of the writer, and that cannot be reasonably explained.
significant similarity, *n*—an individualizing characteristic in common between two or more handwritten items.
single element typewriter, *n*—a typewriter that generates text via interchangeable “elements” that each contain a full set of characters.

RETIRED

single-strike film ribbon, *n*—an inked ribbon wherein the substrate is a plastic film material such as polyethylene, where each area of the ribbon is capable of producing only one image.
single-strike paper ribbon, *n*—an inked ribbon wherein the substrate is paper, where each area of the ribbon is capable of producing only one image.
slit glass, *n*—alternate scanning surface found in some digital photocopiers used in conjunction with an automatic document feeder.
smudge, *n*—tendency of an image to smear or streak onto an adjacent area when rubbed; involves the redisposition of abraded material.
spatte*r*, *n*—type of extraneous or undesirable ink droplet originating when a portion of an ink droplet strikes the intended area and is deflected to an unintended area.
spray, *n*—type of extraneous or undesirable ink dot near the printed zones which originate from the printhead.
submersion, *v*—the placement of a document(s) into an appropriate liquid to facilitate cleaning, unfolding, or separation of the document(s).
sufficient quantity, *n*—that amount of writing required to assess the writer's range of variation, based on the writing examined.
thermal imaging transfer ribbon, *n*—plastic film or other material, upon which a dye or pigmented coating is applied; imaging results when a thermal printhead transfers the coating onto a suitable substrate or receptor media.
thermal impulse, *n*—ink jet technology where the rapid expansion of a bubble in the ink created by localized electrical heating expels the droplets from the ink chamber.
thimble element, *n*—an element used in a typewriter in which the fully formed characters are located on the ends of finger-like devices that are similar to a daisy wheel except that the device is formed to produce a cup-like or thimble structure.
thread count, *n*—the total number of warp and filling threads in one square inch of fabric.
transmitted light, n—illumination that passes through a document.
type element, *n*—see element
type of writing, *n*—refers to hand printing, cursive writing, numerals, symbols, or combinations thereof, and signatures.
type slug, *n*—the block (usually metal) attached to the end of the typebar that bears the typeface.
type bar, *n*—a bar, mounted on a typewriter, that holds a type slug(s).***ultraviolet*** (UV), n—referring to radiant flux having wavelengths shorter than the wavelengths of light, usually wavelengths from about 10 to 380 nm. Long-wave UV usually refers to the spectral range of UV-A, with wavelengths from about 315 to 380 nm. Short-wave UV usually refers to the spectral range of UV-C, with wavelengths from 100 to 280 nm.
typeface, *n*—the portion of the element or type slug that projects from the body and contacts the surface of the substrate to form the character.
typeface defect, *n*—deviation from the intended appearance of a character due to physical damage to the typeface or its malformation in manufacture.

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typestyle, *n*—a particular variant of a type design.
typestyle classification scheme, *n*—a hierarchical taxonomic schematic, key, or computer database that can be used to determine the source of a particular typestyle.
typestyle library, *n*—an organized collection of reference samples of typestyles and related materials. Reference materials can also include information such as typestyle catalogs, treatises relative to typography and the design of typestyles used on typewriters and other printing systems, typewriters, type slugs, type elements, actual strike-ups, and instruction and repair manuals.
typewriter, *n*—a self-contained machine for character-by-character direct writing by means of keyboard-operated typefaces.
ultraviolet *(UV)*, *n*—referring to radiant flux having wavelengths shorter than the wavelengths of light, usually wavelengths from about 10 nm to 380 nm.
unaccustomed hand, *n*—the opposite hand (or other body part) from that normally used for writing.
variation, *n*—those deviations among repetitions of the same handwriting characteristic(s) that are normally demonstrated in the habits of each writer. Since variation is an integral part of natural writing, no two writings of the same material by the same writer are identical in every detail. Within a writer's range of variation, there are handwriting habits and patterns that are repetitive and similar in nature. These repetitive features give handwriting a distinctive individuality for examination purposes. Variation can be influenced by internal factors such as illness, medication, intentional distortion, etc. and external factors such as writing conditions and writing instrument, etc. *n*—imprecise duplication in multiple impressions from the same machine.
VSC, n – equipment that provides multi-spectral examinations of documents are made under UV, visible and near IR illumination with live on-screen images
watermark, *n*—a localized modification of the formation and/or opacity of a sheet of paper so that a pattern, design, or word group can be seen in the dry sheet when viewed using side lighting or transmitted light.
white write, *n*—process in electrostatic printing where the photoconductive element is charged with a charge of the opposite sign as that of the toner. A light beam, acting like a "charge eraser" is used to discharge all areas of the photoconductor that are not to receive toner to form the image. The toner is attracted to the remaining charged areas of the photoconductor when the latent electrostatic image is developed.

RETIRED

Sources of Error: Not applicable

References:
SWGDOC Terminology Relating to the Examination of Questioned Documents ver. 2013-1

Purpose: Terminology for Expressing Conclusions of Forensic Document Examiners

This SOP provides the terminology intended to assist forensic document examiners in expressing conclusions or opinions based on their examinations.

The following terms are based on the report of a committee of the Questioned Document Section of the American Academy of Forensic Sciences that was adopted as the recommended guidelines in reports and testimony by the Questioned Document Section of the American Academy of Forensic Sciences and the American Board of Forensic Document Examiners.

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Responsibility: Forensic Science Examiners assigned to the Questioned Document Unit or conducting casework in the Unit.

Overview of Reporting of Conclusions and Opinions:

Document examiners begin examinations from a point of neutrality. There are an infinite number of gradations of opinion toward identification or toward elimination. It is in those cases wherein the opinion is less than definite that careful attention is especially needed in the choice of language used to convey the weight of the evidence.

When the opinion is less than definite, there is usually a necessity for additional comments, consisting of such things as reasons for qualification (if the available evidence allows that determination), suggestions for remedies (if any are Known), and any other comments that will shed more light on the report. The report should stand alone with no extra explanations necessary.

The expressions used to differentiate the gradations of opinions should not be considered as strongly defined "categories". These expressions should be guidelines without sharply defined boundaries. To avoid the possibility of misinterpretation of a term where the expert is not present to explain the guidelines in this standard, the appropriate definition(s) could be quoted in or appended to reports.

The material that follows deals with handwriting but forensic document examiners may apply this terminology to other examinations within the scope of their work.

Reporting Terminology:

Identification/Common Authorship (definite conclusion of identity)—This is the highest degree of confidence expressed by document examiners in handwriting comparisons. The examiner has no reservations whatever, and although prohibited from using the word "fact," the examiner is certain, based on evidence contained in the handwriting, that the writer of the Known material actually wrote the

writing in question. The term "identification" is used when the lead examiner has physically observed the subject authoring the handwriting exemplar. The term "common authorship" is used when the examiner relies on the agency surety of the exemplars author.

Example — It is the opinion of the undersigned examiners that the Questioned handwritten entries appearing in Submission 001 and the Known handwritten entries [reportedly] authored by John Doe appearing in Submission 002 share common authorship.

Strong probability (highly probable, very probable)—the evidence is very persuasive, yet some critical feature or quality is missing so that an *identification* is not in order; however, the examiner is virtually certain that the Questioned and Known writings were written by the same individual.

Example — It is the opinion of the undersigned examiners that it is highly probable that the Questioned handwritten entries appearing in Submission 001 and the Known handwritten entries [reportedly] authored by John Doe appearing in Submission 002 share common authorship.

Probable—the evidence contained in the handwriting points rather strongly toward the Questioned and Known writings having been written by the same individual; however, it falls short of the" virtually certain" degree of confidence.

Example — It is the opinion of the undersigned examiners that it is probable that the Questioned handwritten entries appearing in Submission 001 and the Known handwritten entries [reportedly] authored by John Doe appearing in Submission 002 share common authorship.

Indications (evidence to suggest)—a body of writing has few features which are of significance for handwriting comparison purposes, but those features are in agreement with another body of writing.

Example — It is the opinion of the undersigned examiners that there are indications that the Questioned handwritten entries appearing in Submission 001 and the Known handwritten entries [reportedly] authored by John Doe appearing in Submission 002 share common authorship, but the evidence falls far short of that necessary to support a definite conclusion.

No conclusion (totally inconclusive, indeterminable)—This is the zero point of the confidence scale. It is used when there are significantly limiting factors, such as disguise in the Questioned and/or Known writing or a lack of comparable writing, and the examiner does not have even a leaning one way or another.

Example — It is the opinion of the undersigned examiners that no conclusion could be reached as to whether the Questioned handwritten entries appearing in Submission 001 and the Known handwritten entries [reportedly] authored by John Doe appearing in Submission 002 share common authorship.

Indications did not—this carries the same weight as the indications term that is, it is a very weak opinion.

RETIRED

Example — It is the opinion of the undersigned examiners that there are indications that the Questioned handwritten entries appearing in Submission 001 and the Known handwritten entries [reportedly] authored by John Doe appearing in Submission 002 do not share common authorship, but the evidence falls far short of that necessary to support a definite conclusion.

probably did not—the evidence points rather strongly against the Questioned and Known writings having been written by the same individual, but, as in the probable range above, the evidence is not quite up to the "virtually certain" range.

Example — It is the opinion of the undersigned examiners that it is probable that the Questioned handwritten entries appearing in Submission 001 and the Known handwritten entries [reportedly] authored by John Doe appearing in Submission 002 do not share common authorship.

strong probability did not—this carries the same weight as strong probability on the identification side of the scale; that is, the examiner is virtually certain that the Questioned and Known writings were not written by the same individual.

Example — It is the opinion of the undersigned examiners that it is highly probable that the Questioned handwritten entries appearing in Submission 001 and the Known handwritten entries [reportedly] authored by John Doe appearing in Submission 002 do not share common authorship.

elimination—this, like the *definite conclusion of identity*, is the highest degree of confidence expressed by the document examiner in handwriting comparisons. By using this expression the examiner denotes no doubt in his opinion that the Questioned and Known writings were not written by the same individual. Eliminations are often difficult determinations to make in handwriting examinations, especially when only requested exemplars are available, and extreme care should be used in arriving at this conclusion.

Example — It is the opinion of the undersigned examiners that the Questioned handwritten entries appearing in Submission 001 and the Known handwritten entries [reportedly] authored by John Doe appearing in Submission 002 do not share common authorship.

RETIRED

Sources of Error: Not applicable

References:

SWGDOC Standard Terminology for Expressing Conclusions of Forensic Document Examiners ver. 2013-1

Purpose: Procedure for Examination of Handwritten Items

The purpose of this procedure is to provide guidance for the examinations and comparisons involving handwritten items and related procedures. These procedures would be applicable whether the examination and comparison is of Questioned and Known evidence items or of exclusively Questioned evidence items. These procedures include evaluation of the sufficiency of the material provided (Questioned, or Known, or both) for examination. The methods used in a case will depend upon the nature of the material available for examination.

Items submitted for examination may have inherent limitations that can interfere with the methods in this procedure. These limitations should be noted and recorded on worksheet (Quality Record DOC-2). These limitations may be due to the submission of non-original documents, limited quality or quantity, or condition of the items submitted for examination. Other limitations can come from the quantity or comparability of the writing submitted, and include absent characters, dissimilarities, or limited individualizing characteristics. Consideration should be given to the possibility that various forms of simulations, imitations, and duplications of handwriting can be generated by computer and other means.

RETIRED

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit.

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources
   Lighting may include the use of transmitted, side or vertical lighting to improve the ability to view fine details.
2. Stereomicroscopes with fiber-optic lighting
3. Scanners
4. Cameras

Procedure:

The Questioned Document Unit will usually be the first Unit to receive document-type evidence for analysis. In the event that another Unit examined this evidence first, it should be understood that there may be analysis methods that may limit the examination of handwriting on these documents. Prior handling, testing, or chemical processing (for example, for latent prints) or swabbing of a document may interfere with the ability of the examiner to see certain characteristics.

1. Beginning of examination: Refer to QD SOP-3 for guidance on transferring, LIMS documentation and evidence identification.

2. All examination steps, relevant observations, and results shall be documented on QR-DOC-2 and/or case notes. If the examiner notes that the submitted evidence is of limited quality or quantity, the examiner or Case Management Unit may need to contact the submitting agency to acquire more evidence. If it is noted that the evidence received has been handled or chemically treated that limits or prevents the examination, the submitting agency may be contacted to communicate this information. (see GL20)

3. During the examination procedure, the examiner may use different types of lighting and magnification (such as stereomicroscopes) to examine the documents and the writing present on the documents. The documents may be scanned or photographed to preserve the writing and for incorporation into the case notes.

4. Determine whether the examination is a comparison of Questioned writing to Known writing or a comparison of Questioned writing to Questioned writing.

5. At various points in these procedures, if the examiner determines that a particular feature is not present or that an item is lacking in quality or comparability may indicate that the examiner should discontinue or limit the procedure(s). It is at the discretion of the examiner to discontinue the procedure at that point and report accordingly or to continue with the applicable procedures to the extent possible. The reasons for such a decision shall be documented on Quality Record DOC-2 and/or in the case notes.

RETIRED

<u>Questioned Writing:</u>

1. Determine whether the Questioned writing is original writing. If it is not original writing, request the original.

   If the original documents are not available, evaluate the quality of the best available reproduction to determine whether the significant details of the writing have been reproduced with sufficient clarity for comparison purposes and proceed to the extent possible. If the writing has not been reproduced with sufficient clarity for comparison purposes, discontinue these procedures and report accordingly.

2. Determine whether the Questioned writing appears to be distorted. If it appears to be distorted, determine whether it is possible to establish that the apparently distorted writing is natural writing.

If it is not natural writing, or if it is not possible to establish whether the apparently distorted writing is natural writing, determine whether the apparently distorted writing is suitable for comparison and proceed to the extent possible. If the available Questioned writing is not suitable for comparison, discontinue these procedures and report accordingly.

3. Evaluate the Questioned writing for the following:
   1) ***Type of Writing***—If there is more than one type of writing within the Questioned writing, separate the Questioned writing into groups of single types of writing.
   2) ***Internal Consistency***—If there are inconsistencies within any one of the groups created (for example, suggestive of multiple writers), divide the group(s) into subgroups, each one of which is consistent.
   3) Determine the natural range of variation of the writing for each group or sub-group of the Questioned writing.
   4) Determine presence or absence of individualizing characteristics.

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Known Writing:

1. Determine whether the Known writing is original writing. If it is not original writing, request the original.

   If the original documents are not available, evaluate the quality of the best available reproduction to determine whether the significant details of the writing have been reproduced with sufficient clarity for comparison purposes and proceed to the extent possible. If the writing has not been reproduced with sufficient clarity for comparison purposes, discontinue these procedures and report accordingly.

2. Determine whether the Known writing appears to be distorted. If it appears to be distorted, determine whether it is possible to establish that the apparently distorted writing is natural writing.

   If it is not natural writing, or if it is not possible to establish whether the apparently distorted writing is natural writing, determine whether the apparently distorted writing is suitable for comparison and proceed to the extent possible. It should be determined whether additional Known writing would be of assistance, and if so, it should be requested. If the available Known writing is not suitable for comparison, discontinue these procedures and report accordingly.

3. Evaluate the Known writing for the following:
   1) ***Type of Writing***—If there is more than one type of writing within the Known writing, separate the Known writing into groups of single types of writing.

2) ***Internal Consistency***—If there are unresolved inconsistencies within any of the groups (for example, suggestive of multiple writers), contact the submitter for authentication. If any inconsistencies are not resolved to the examiner's satisfaction, discontinue these procedures for the affected group(s), and report accordingly.

4. Determine the natural range of variation of the writing for each group of the Known writing

5. Determine presence or absence of individualizing characteristics

<u>Comparison of Questioned to Known or Questioned to Questioned:</u>

1. Evaluate the comparability of the bodies of writing {Questioned writing to Known writing (K to Q) or Questioned writing to Questioned writing (Q to Q)}.

2. If the bodies of writing are not comparable, discontinue comparison and request comparable Known writing, if appropriate or available. The submitting agency may be contacted and referred to try to get a handwriting exemplar. This exemplar form (QR – QD HE) may be sent to the agency or downloaded from the DSS website.

   If comparable Known writing is made available or is comparable, continue to step 3. If comparable Known writing is not made available, discontinue examination and report accordingly.

3. Conduct a side-by-side comparison of comparable portions of the bodies of writing. Side-by-side comparisons may include the use of printed materials or use of computers and software.

4. Determine whether there are differences, absent characters, and similarities. Evaluate their significance individually and in combination.

5. Determine if there is a sufficient quantity of writing (Questioned writing, or Known writing, or both). If writing (Questioned writing, or Known writing, or both) is not sufficient in quantity for elimination or identification, continue the comparison to the extent possible. When appropriate, request more Known writing. If more Known writing is made available, evaluate the comparability of the bodies of writing and continue if appropriate.

6. Analyze, compare, and evaluate the individualizing characteristics and other potentially significant features present in the comparable portions of the bodies of writing.

   Among the features to be considered are elements of the writing such as abbreviation; alignment; arrangement, formatting, and positioning; capitalization; connectedness and disconnectedness;

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cross strokes and dots, diacritics and punctuation; direction of strokes; disguise; embellishments; formation; freedom of execution; handedness; legibility; line quality; method of production; pen hold and pen position; overall pressure and patterns of pressure emphasis; proportion; simplification; size; skill; slant or slope; spacing; speed; initial, connecting, and terminal strokes; system; tremor; type of writing; and range of variation. Other features such as lifts, stops and hesitations of the writing instrument; patching and retouching; slow, drawn quality of the line; unnatural tremor; and guide lines of various forms should be evaluated when present. Potential limiting factors such as age; illness or injury; medication, drugs or alcohol (intoxication or withdrawal); awkward writing position; cold or heat; fatigue; haste or carelessness; nervousness; nature of the document, use of the unaccustomed hand; deliberate attempt at disguise or auto-forgery should be considered.

7. Evaluate the similarities, differences, and limitations. Determine their significance individually and in combination. Note all similarities, differences and limitations in the case notes.

8. Form a conclusion based on results of the above analyses, comparisons, and evaluations.

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Reporting Conclusions and Opinions:
The conclusion(s) or opinion(s) resulting from the procedures in this standard may be reached once sufficient examinations have been conducted. The number and nature of the necessary examinations is dependent on the question at hand. The bases and reasons for the conclusion(s), or opinion(s), should be included in the examiner's documentation and reported as suggested in QD SOP-7.

Sources of Error: not applicable

References:
SWGDOC Terminology for Expressing Conclusions of Forensic Document Examiners
SWGDOC Terminology Relating to the Examination of Questioned Documents
SWGDOC Standard for Examination of Handwritten Items

Purpose: Procedure for Examination of Altered Documents

This procedure provides guidance for examinations involving altered documents that are of Questioned nature. These procedures include evaluation of the sufficiency of the material available for examination.

Items submitted for examination may have inherent limitations that can interfere with the methods in this procedure. These limitations should be noted and recorded on worksheet and/or in case notes. (Quality Record DOC-2). Limitations can be due to submission of non-original documents, limited comparability, or condition of the items submitted for examination (for example, items that are stained, soiled, water-damaged, charred, or shredded).

Responsibility: Forensic Science Examiners assigned to the Unit or conducting casework in the Unit.

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources
   Lighting may include the use of transmitted, side or vertical lighting to improve the ability to view fine details.
2. Stereomicroscopes with fiber-optic lighting
3. Video Spectral Comparators
4. Electrostatic Detection Apparatus
5. Measuring devices
3. Scanners
4. Cameras

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Procedure:

The Questioned Document Unit will usually be the first Unit to receive document-type evidence for analysis. In the event that another Unit examined this evidence first, it should be understood that there may be analysis methods that may limit the examination of handwriting on these documents. Prior handling, testing, or chemical processing (for example, for latent prints) or swabbing of a document may interfere with the ability of the examiner to see certain characteristics.

1. Beginning of examination: Refer to QD SOP-3 for guidance on transferring, LIMS documentation and evidence identification.

2. All examination steps, relevant observations, and results shall be documented on QR-DOC-2 and/or case notes. If the examiner notes that the submitted evidence is of limited quality or quantity, the examiner or Case Management Unit may need to contact the submitting agency to acquire more evidence. If it is noted that the evidence received has been handled or chemically

treated that limits or prevents the examination, the submitting agency may be contacted to communicate this information. (see GL20)

3. During the examination procedure, the examiner may use different types of lighting and magnification (such as stereomicroscopes) to examine the documents and any alterations present on the documents. The documents may be scanned or photographed to preserve the alterations or other noted features and for incorporation into the case notes.

4. At various points in these procedures, if the examiner determines that a particular feature is not present or that an item is lacking in quality or comparability may indicate that the examiner should discontinue or limit the procedure(s). It is at the discretion of the examiner to discontinue the procedure at that point and report accordingly or to continue with the applicable procedures to the extent possible. The reasons for such a decision shall be documented on Quality Record DOC-2 and/or in the case notes.

5. Examine the document for the presence of characteristics indicative of alterations. These can include, but are not limited to, the following:
   1) Overwriting,
   2) Characteristics of multiple writing instruments,
   3) Crowded or awkward placement of writing and/or printed text,
   4) Paper fiber disturbance,
   5) Use of different fonts, sizes, and/or styles,
   6) Area(s) of discoloration,
   7) Presence of an obscuring substance,
   8) Smearing,
   9) Uneven margins,
   10) Different printing processes,
   11) Irregular spacing and alignment, both vertical and horizontal,
   12) Differences in fastening and binding mark,
   13) Inconsistent handwriting features,
   14) Unusual sequence of line intersections contrary to what may be claimed, and
   15) Variations in paper characteristics.

   Care must be taken in the evaluation of the following characteristics present on the documents since these characteristics may occur in the normal preparation, handling, and storage of the document.

<u>Non-Destructive Examination Methods</u>

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1. Non-destructive procedures shall be performed when applicable depending on the evidence presented.

2. Examine the document macroscopically, or microscopically, or both.

3. Examine the document using various lighting techniques, such as side lighting and transmitted lighting.

4. Examine the document using visualizing techniques such as UV, IR, and IRL.

    1) If the examiner is using the VSC4, a control shall be created and examined using the VSC4, the result of the control check will be recorded on the day of examination. The control is created by using two different brand type pens of the same color ink. The expected result should be that the two inks fluoresce at different wavelengths. If the expected result is achieved, this will be marked off on DOC-2 as the control was okay.

        If the control does not function correctly, the created control will be evaluated if it was made correctly. If the control was made correctly, the VSC-4 equipment shall be taken out of service pending repair and re-validated before use.

    2) If the examiner is using the VSC8000, the examiner will run control checks on the days of use using the provided Standards. The examiner will indicate on DOC-2 if the control checks performed as expected. See QD SOP-9 Appendix 1 for additional guidance on maintenance schedule and control checks.

        The VSC8000 has the ability to measure items in a document. The calibration of the measurement function should be done on days of use and it will be calibrated to a NIST measuring device (ruler).

        If the control checks do not function correctly, the VSC8000 shall be taken out of service pending repair and re-validated before use.

5. Document any alterations with notes and photographs. Measurements of alterations or other significant features will be taken and added to the case notes.

6. The document should be processed with the ESDA.

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1) When using the ESDA, a control shall be created and run on the day of use with each case. The control is made by folding a piece of paper in half and writing on the exterior side of the fold. Indented writing will be located on this document when unfolded. A successful control check will be the development of this indented writing on the document. If the expected result is achieved, this will be marked off on DOC-2 as the control was okay.

2) After completion of the ESDA method on the evidence, any further characteristics/features that were developed should be documented with imaging techniques and in the case notes.

If the control check does not function correctly, the ESDA shall be taken out of service pending repair and re-validated before use.

7. Analyze, compare, and evaluate the findings.

8. Determine the need for destructive examinations. If further processing is not needed, the examiner may stop at this step and proceed to evaluate the conclusion(s), and generate a report based on their findings and opinions.

RETIRED

Destructive Examination Methods

1. Destructive examination methods should only be commenced once it is determined that no testing by other Units of the Laboratory is needed. If testing is needed by the other Units, the evidence should be processed by those Units first and then returned to the Questioned Document Unit to complete the analysis.

2. Depending on the destructive examination method that might be used, a consumption issue may be present if an arrest has been made in the case. Case Management will be asked to follow up with the submitting agency to determine if an arrest has been made in the case. If an arrest has been made, Case Management shall be notified to process a "Consumption Notification" and no testing can be completed until a response has been received or the consumption notification grace period has passed. If no arrest has been made, destructive examination methods may proceed.

3. Prior to using these techniques, the item(s) should be appropriately documented on QR-DOC-2. These destructive techniques need not be performed in the order given.

4. Where an obscuring substance is present, use a solvent may be used to make the paper translucent for visualization of any obscured entry(s). Exposure to these chemicals should be limited since over exposure may affect the obscuring substance and substrate. The solvent used will be documented on the worksheet and if possible, an area away from the alteration should be tested with the solvent prior to proceeding with using on the questioned area.

5. Physically remove (for example, abrade, scrape, or peel) the obscuring substance from the document.

6. Chemical ink examinations may be referred to the Chemistry Section.

7. Analyze, compare, and evaluate the findings.

8. If further processing is not needed, the examiner may stop at this step and proceed to evaluate the conclusion(s), and generate a report based on their findings and opinions.

Report:

1. Once the examinations and evaluations have been completed, reports may include one or more of the following types of conclusion(s), opinion(s), and other finding(s):
   1) Whether alterations were observed.
   2) Whether any of the altered entries were decipherable.
   3) The text or description of altered entries.
   4) Method or sequence of alterations.
   5) Images of alterations and original entries.
   6) Other information about the alterations.
2. Images may be inserted into the report to provide clarification or information to the submitting agency.

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Sources of Error: Not applicable

References: SWGDOC Standard for Examination of Altered Documents ver. 2013-1

Appendix 1: VSC8000

Days of Use:

Using the provided "Standards Document", a control check will be done on the days of use. The Standards Document has the following images which will be used for control checks:

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EXHIBIT TWO

EXHIBIT THREE

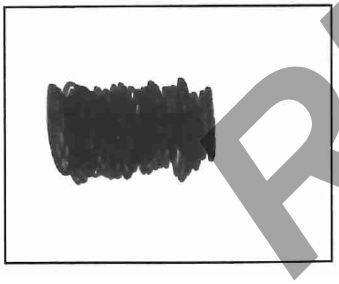

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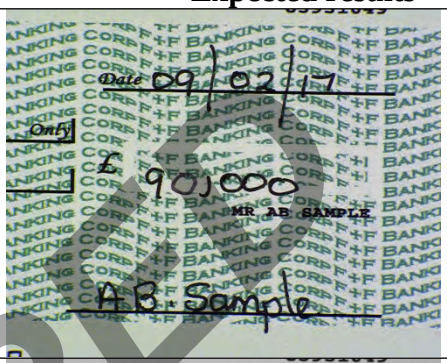
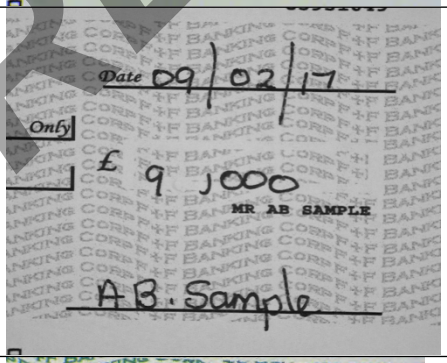
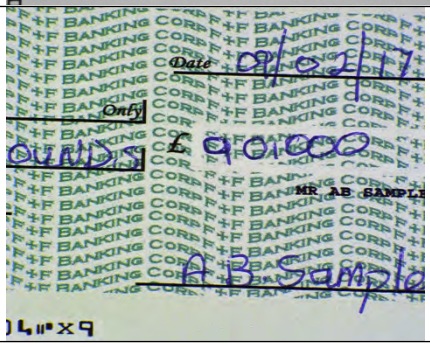
Exhibit Four – indented writing with the word "Test"

Control Check performed on Days of Use:

Exhibit Number	Setting/lighting to use	Expected results
1	viewed under flood light	
1	viewed under flood light using the 725 nm filter (IR)	
2	viewed under flood light	

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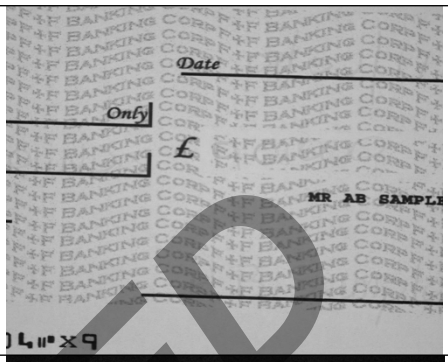
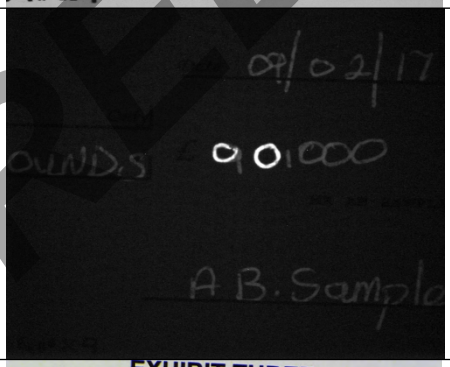
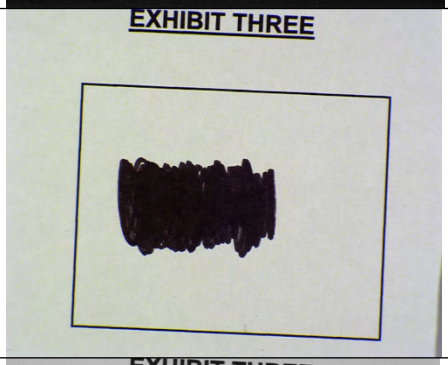
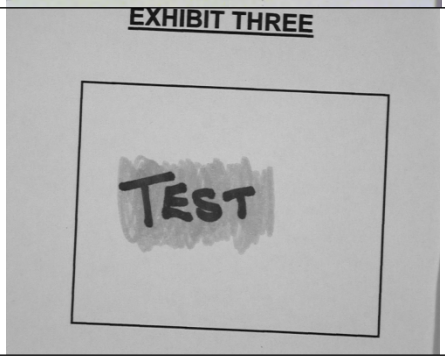
2	viewed under flood light using the 725 nm filter (IR)	
2	viewed under spot light (605-730 nm) using the 830 nm filter (IRL)	
3	viewed under flood light	
3	viewed under flood light using the 725 nm filter (IR)	

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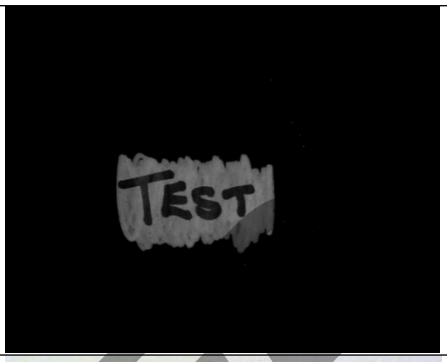
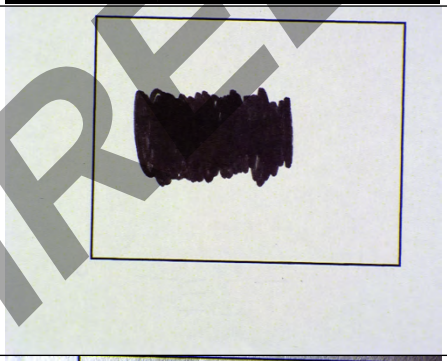
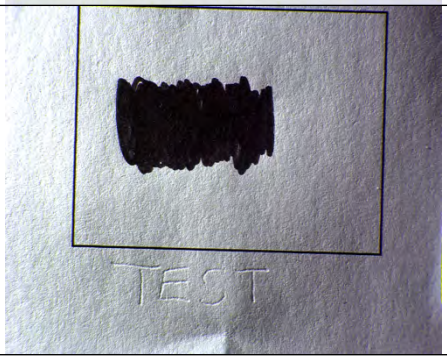
3	viewed under spot light (605-730 nm) using the 830 nm filter (IRL)	
4	is viewed under flood light	
4	viewed under side light	

RETIRED

On days of use, the spectrometer will also be checked using the white standard on the Didymium Oxide Filter (VSC Calibrator). The expected reading should be a straight line along the base of the graph indicating 0% transmittance.

After running all the control checks, the VSC-8000 controls okay box will be checked off on QD DOC-2.

Twice a Year:

- A full diagnostic scan will be conducted and the results will be printed and placed into a logbook. The date, initials of the examiner conducting the check and the results will be indicated on the QR-VSC8000 Check sheet.
- White balance will be conducted using a white sheet of paper. The date, initials of the examiner conducting the check and the results will be indicated on the QR-VSC8000 Check sheet.
- Spectrometer will be checked using the didymium oxide filter (VSC Calibrator) and the white square standard. The date, initials of the examiner conducting the check and the results will be indicated on the QR-VSC8000 Check sheet.
- The UV light ((365 nm – blue, 312 nm – red, 254 nm – yellow) and the anti-stokes (green) will be checked using the VSC Calibrator. The date, initials of the examiner conducting the check and the results will be indicated on the QR-VSC8000 Check sheet.

RETIRED

Biannual	Setting/lighting to use	Expected results
Diagnostic Scan	Auto run that will check the various functions of the instrument including: motor, lamp, filter of camera and spot.	Pass
White Balance	Calibration using a white paper.	Calibration successful
Spectrometer Check	Spectrometer reading of didymium oxide filter WCT-DD-02c	Expected results should appear similar to Figure 1 (below)
Spectrometer Check white standard	Spectrometer reading of didymium oxide filter white standard	Expected result should be a line along the base of the graph
UV Light Check	365 nm	Blue fluorescence
UV Light Check	312 nm	Red fluorescence
UV Light Check	254 nm	Yellow fluorescence
Anti-Stokes	Anti-stoke setting	Green fluorescence

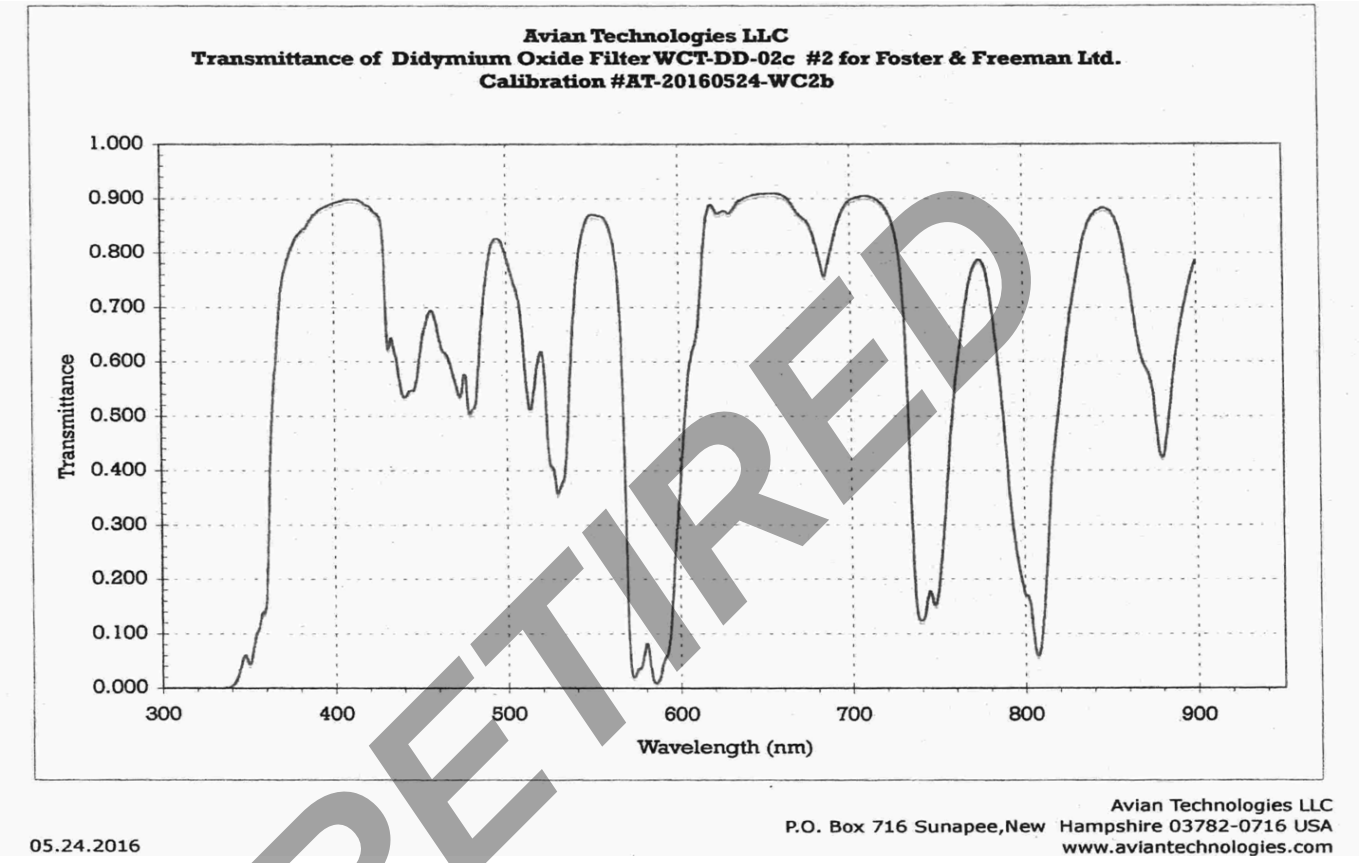


Figure 1: Spectrometer expected results

RETIRED

Purpose: Procedure for Indentation Examinations

This SOP provides guidance for forensic document examiners regarding the examinations and comparisons involving visualization and recording of indentations. When sheets of paper are in direct or indirect contact with one another, impressions on the top sheet can produce indentations on the sheet(s) below. These procedures are essentially non-destructive in nature; however, pencil writing and single-strike ribbon typing can be partially lifted from the document by ESDA. Although this effect can be minimal, adequate documentation of the evidence should precede ESDA processes.
Paper fiber disturbances caused by erasures or present in torn paper edges may also be visualized using these procedures. Electrostatic detection device examinations may be useful in developing other types of impressions on paper items (for example, typewritten material, shoeprints and latent prints).

RETIRED

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources
   Lighting may include the use of transmitted, side or vertical lighting to improve the ability to view fine details.
2. Stereomicroscopes with fiber-optic lighting
3. Electrostatic Detection Apparatus
4. Measuring devices
5. Scanners
6. Cameras

Procedure:

1. Examine subject document(s) using oblique illumination and photograph any visible indented impressions and/or image. In some instances, the use of side lighting in a room with subdued light may provide better visualization of indentations.

   Document any indentations observed on the QR DOC-2 or QR-IM1 (imprint examination) and/or in case notes. If indentations are not observed, document the lack of visible indentations.

2. Determine whether the item is suitable for ESDA examination. The size, shape, density or condition of an item may make it unsuitable for the ESDA portion of the procedure (for example,

some book covers, large file folders and items that have been wet or damaged after indentations were made).

Certain items submitted for examination may have inherent limitations that can interfere with the procedures in this standard. Limitations should be noted and recorded on the QR DOC-2 and/or in the case notes.

Items should be handled as little as possible prior to ESDA examination to prevent contamination.

3. A complete examination involves the use of both the optical and ESDA portions of the procedure. All indentations may not be revealed if the optical and ESDA portions of the procedure are not conducted.

   The results of prior storage, handling, chemical testing for latent prints may interfere with the ESDA results. Indentation examinations should be conducted prior to any chemical processing. Items should be handled appropriately to avoid compromising subsequent examinations (for example, with clean gloves).

4. Prior to beginning the ESDA process, the unit will be checked to ensure that it is operating correctly. Check the condition of the cascade toner beads utilized in ESDA examinations. They can deteriorate with use, affecting the quality of the developed ESDA image. The ESDA shall be operated and maintained utilizing the instructions provided in the operating manual. Damaged ESDA equipment shall be taken out of service pending repair and re-validated before use.

5. Pre-treat documents in a humidifier chamber for approximately 2 minutes prior to processing with (ESDA). Record humidity level on worksheets (QD DOC-2 or QR-IM2). Distilled water shall be utilized in the humidifier chamber.

6. Prior to processing the evidence with the ESDA, run a control. This control will consist of a piece of paper that has been folded over and written on one side. The control is considered positive when the indented writing is identified on the opposite/folded side of the paper. If the control indentation is not successfully visualized, the problem shall be corrected before any further indentation examinations are conducted with that instrument.

   The result of this control check will be recorded in the log book located in the Unit. The control paper will be placed into the case jacket.

7. Process subject document(s) on the Electrostatic Detection Apparatus according to manufacturer's recommended procedures. If indented markings of value are noted, a plastic overlay lift may be made. If indented markings of no value are noted (i.e. consistent with writing on evidence packaging), this indented writing may be documented with photography and a note may be placed in the case notes/worksheet.

RETIRED

8. All lifts whether negative or positive shall be appropriately documented, scanned/photocopied, sub-itemized in JusticeTrax. These plastic overlay lifts are treated as evidence and returned to the submitting agency at the completion of the case.

   It should be noted that repeated processing with the ESDA can lead to the development of degraded images.

Reporting of Results:
The basis and reason for the conclusion(s), opinion(s), or finding(s) should appear in the examiner's documentation and may also appear in the report. In some cases, it might be helpful for the submitting agency to have an image embedded in the report of the indented writing.

Once examinations and evaluations have been completed, reports may include the following types of conclusion(s), opinion(s), or finding(s):

1) Whether indentations were observed.
2) Whether decipherable indentations were or not observed.
3) The text of deciphered indentations.
4) Information as to the source of indentations.

References:
SWGDOC Standard for Indentation Examinations ver. 2013-1
ESDA 2 Electrostatic Detection Apparatus User Manual 14-06

RETIRED

Purpose: Procedure for Physical Match of Paper Cuts, Tears, and Perforations in Forensic Document Examinations

This procedure provides guidance that should be used by forensic document examiners for examinations and comparisons to determine whether or not two or more paper fragments were at one time joined to form a single piece of paper. These procedures are applicable whether the examination(s) and comparison(s) is of Questioned and Known items or of exclusively Questioned items. The examination may not be limited to, physical match examinations of paper items. The physical matching or realignment of items of evidence may occur in two or three dimensions.

Limitations can be due to limited quantity of the evidence presented, or comparability, or condition of the items submitted for examination. The condition of a paper sample may make it unsuitable for some types of examinations (for example, items that are water soaked, stained, soiled, charred, or finely shredded paper). Such features are taken into account in these examinations.

The results of prior storage, handling, testing, or chemical processing (for example, for latent prints) can interfere with the examination of certain characteristics in conducting these physical matches. Whenever possible, document examinations should be conducted prior to any chemical processing. Items should be handled appropriately to avoid compromising subsequent examinations.

RETIRED

Responsibility: Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources. Lighting may include the use of transmitted, side or vertical lighting to improve the ability to view fine details.
2. Stereomicroscopes with fiber-optic lighting
3. Measuring devices
4. Scanners
5. Cameras

Procedure:

At various points in these procedures, a determination that a particular feature is not present or that an item of evidence is lacking in quality or comparability may indicate that the examiner should

discontinue or limit the procedure(s). It is at the discretion of the examiner to discontinue the procedure at that point and report accordingly or to continue with the applicable procedures to the extent possible. The reasons for such a decision shall be documented in the case notes.

1. Beginning of examination: Refer to QD SOP-3 for guidance on transferring, LIMS documentation and evidence identification.

2. During the examination procedure, the examiner may use different types of lighting and magnification (such as stereomicroscopes) to examine the documents. The light sources should be of sufficient intensity to allow fine detail to be distinguished. Natural light, incandescent or fluorescent sources, or fiber optic lighting systems are generally utilized. Transmitted lighting, side lighting, and vertical incident lighting can also be useful.

3. The examiner may have to use other apparatuses to hold the documents in place for the examination of the physical matches. These apparatuses can include clamps, clips, temporary adhesives, glass plates and other materials that will not adversely affect the specimen(s).

4. The evidence may be scanned or photographed to preserve the details observed and for incorporation into the case notes.

5. Determine whether or not the specimens are broken or separated.

6. Determine whether or not the specimens are suitable to be physically realigned.

7. Evaluate the specimens for individualizing characteristics.

8. Conduct a side-by-side comparison of the specimens using the following steps:
   1) Visual inspection.
   2) Manual alignment.
   3) Edge-to-edge realignment.
   4) Surface markings.
   5) Measurements and pattern count.

9. Evaluate similarities, differences, and limitations. Determine their significance individually and in combination. Reach a conclusion and report accordingly.

RETIRED

Reporting of Results

1. The basis and reasons for the conclusion(s), opinion(s), or finding(s) should be included in the examiner's documentation QR DOC-2, case notes and may also be included in the report.

2. Once examinations and comparisons have been completed, reports may include, but are not limited to, the following types of conclusions and other findings:
   1) The paper fragments are consistent with having been at one time joined to form a single piece of paper.
   2) Although class similarities were observed, there were insufficient individual features to determine whether or not the paper fragments were at one time joined to form a single piece of paper.
   3) The paper samples did not originate from a single piece of paper.

3. As a result of the reconstruction of the paper fragments, additional examinations (for example, latent prints or indentations) may be appropriate. The report may also include information such as the visible text, indentations, and contaminants observed following reconstruction.

Sources of Error: NA

References:
SWGDOC Standard for Physical Match of Paper Cuts, Tears, and Perforations in Forensic Document Examinations ver. 2013-1

RETIRED

Purpose: Procedure for Examination of Rubber Stamp Impressions

These procedures focus on the methods that should be used by forensic document examiners for examinations and comparisons involving rubber stamps and their impressions. These procedures should assist the forensic document examiner to reliably reach an opinion concerning whether two or more impressions have a common origin or if a rubber stamp impression was created by a specific rubber stamp. The particular methods used in a given case will depend upon the nature and sufficiency of the material available for examination. These procedures may not cover all aspects of particularly unusual or uncommon examinations.

Some evidence items submitted for examination may have inherent limitations that can interfere with the procedures in this standard. Limitations should be noted and recorded.

Limitations can be due to submission of non-original documents, limited quantity or comparability, or condition of the items submitted for examination (for example, impressions made with over-inked or inadequately inked stamps, partially imprinted impressions, or variations in surface texture).

Prior storage, handling, testing, or chemical processing (for example, for latent prints) can interfere with the examination of certain characteristics. Whenever possible, document examinations should be conducted prior to any chemical processing. Items should be handled appropriately to avoid compromising subsequent examinations.

Consideration should be given to the possibility that a rubber stamp can be manufactured which duplicates the impressions of another stamp, and that various forms of simulations, imitations, and duplicates of rubber stamps or rubber stamp impressions can be generated by computer and other means.

RETIRED

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources or fiber optic lighting systems. Lighting may include the use of transmitted, side or vertical lighting to improve the ability to view fine details.
2. Stereomicroscopes with fiber-optic lighting
3. Electrostatic Detection Apparatus

4. Measuring devices
5. Scanners
6. Cameras
7. A stamp pad, stamp pad ink and adequate smooth (bond) paper or other suitable substrate to collect specimens from the rubber stamp if available.

Procedure:

RETIRED

1. Examine subject document(s) using oblique illumination and photograph any visible indented impressions and/or image. In some instances, the use of side lighting in a room with subdued light may provide better visualization of indentations.

   Document any indentations observed on the QR DOC-2 or case notes. If indentations are not observed, document the lack of visible indentations.

   At various points in these procedures, a determination that a particular feature is not present or that an item is lacking in quality or comparability may indicate that the examiner should discontinue or limit the procedure(s). It is at the discretion of the examiner to discontinue the procedure at that point and report accordingly or to continue with the applicable procedures to the extent possible. The reasons for such a decision shall be documented on the QR DOC-2 or case notes.

2. Determine whether the submitted Questioned impression(s) were produced by a rubber stamp. If not a rubber stamp impression (original or copy), discontinue examination and report accordingly.

   Examination of the original is preferable, and consideration should be given to obtaining the original, if not submitted.

3. Determine whether the examination is a comparison of Questioned impressions; a comparison of a Questioned impression(s) with a Known impression(s); or a comparison of a Questioned impression(s) with a rubber stamp(s).

4. Determine whether the submitted Questioned impression(s) is suitable for comparison. If it is not suitable for comparison, discontinue the procedure and report accordingly. Factors that affect the suitability include clarity, detail, degree of inking or condition of the document.

   Limited sufficiency and comparability of the impressions can be a restrictive factor in an examination and its conclusions but does not necessarily require the discontinuation of the examination.

5. If a Known document(s) is submitted, determine whether the Known document(s) is suitable for examination, or comparison, or both. If it is not suitable, discontinue the procedure and report accordingly in the case notes and in the report. Factors that affect the suitability include clarity, detail, or condition of the document.

6. If the original is not submitted, evaluate the quality of the best available reproduction to determine whether significant details have been reproduced with sufficient clarity for comparison purposes and proceed to the extent possible. If the reproduction is not of sufficient clarity for comparison purposes, discontinue these procedures and report accordingly.

7. If a rubber stamp(s) is submitted, its condition should be noted (for example, clean, dirty, inked, worn, damaged). When applicable, class characteristics (for example, typeface design and size) should be noted in the case notes. Consideration may be given to sampling ink from the stamp prior to taking exemplars. If samples of ink are required, the examiner should consult with the Deputy Director of the Chemistry Section to determine the best sampling method.

8. When producing an exemplar, note any visible features that reproduce on the impression. Prepare appropriate specimens, as needed.

9. Determine if any of the Known specimen impressions are suitable for comparison. If none of the Known specimen impressions are suitable for comparison and no others are obtained, discontinue these procedures and report accordingly.

10. If the Known specimens are suitable for comparison, conduct a side-by-side comparison of the Questioned impressions, or the Questioned impression to the Known impressions and/or to the rubber stamp(s).

11. Compare class characteristics (for example, size, type style, text, shape). If different, discontinue and report accordingly.

12. If the class characteristics lead the examiner to continue with the analysis, compare individualizing characteristics in common such as wear and damage defects, reproducible blemishes, impression voids, improper and extraneous inking, or coincidental peripheral printing.

13. Evaluate similarities, differences, and limitations. Determine their significance individually and in combination. Once all the evaluations are completed, the examiner should have enough information to reach a conclusion and report accordingly.

RETIRED

14. The examiner may report the following conclusions:
    1) ***Identification***—When the examination reveals no significant, inexplicable differences between two or more items, and there is agreement in all individualizing characteristics, an identification is appropriate. This conclusion is used when there are no differences and no limitations associated with absent characters; and any possibility of a duplicate rubber stamp can be eliminated.

    2) ***Elimination***—If significant, inexplicable differences between two or more items are found at any level of the analyses, an elimination is appropriate.

    3) ***Qualified Opinions***—When there are limiting factors and the examination reveals similarities or differences of limited significance between two or more items, the use of qualified opinions can be appropriate (that is, the impressions or observed features contain limited similarities or differences; or limitations associated with absent characters, individualizing characteristics, or distorted impressions are present; or limitations associated with the possibility of the existence of a duplicate rubber stamp; or a combination of these). This opinion requires explanation of the limiting factors in the report.

    4) ***No Conclusion/Inconclusive***—When there are significant limiting factors, and the examination reveals no significant differences, a report that no conclusion can be reached is appropriate (that is, the impressions or observed features contain insufficient significant similarities and insufficient differences). This opinion requires explanation of the limiting factors.

RETIRED

Sources of Error: Not applicable

References:

SWGDOC Standard for Examination of Rubber Stamp Impressions ver. 2013-1

Purpose: Procedure for Examination of Dry Seal Impressions

This procedure provides guidance that should be used by forensic document examiners (Standard for Scope of Work of Forensic Document Examiners) for examinations and comparisons involving dry seal devices and their impressions. By following these procedures, a forensic document examiner can reliably reach an opinion concerning whether two or more dry seal impressions have a common origin, or if a dry seal impression was created by a specific dry seal device.

Evidence submitted for examination may have inherent limitations that can interfere with the methods listed in this procedure. Any limitations should be noted and recorded on the QR DOC-2 or case notes. Limitations can be due to submission of non-original documents, limited quantity or comparability, or condition of the items submitted for examination (for example, distorted impressions, partially imprinted impressions, or variations in surface texture).

Improper prior storage and handling, previous testing, or chemical processing (for example, for latent prints) can interfere with the examination of certain characteristics. The effects can include, but are not limited to, flattening of the embossment or impression, partial destruction of the paper, and stains. Whenever possible, document examinations should be conducted prior to any chemical processing. Items should be handled appropriately to avoid compromising subsequent examinations.

When conducting this type of examination, the examiner should consider the possibility that a dry seal device can be manufactured which duplicates the impressions of another dry seal.

RETIRED

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources
   Lighting may include the use of transmitted, side or vertical lighting to improve the ability to view fine details.
2. Stereomicroscopes with fiber-optic lighting
3. Electrostatic Detection Apparatus
4. Measuring devices
5. Scanners
6. Cameras

Procedure:

1. Examine subject document(s) using oblique illumination and photograph any visible indented impressions and/or image. In some instances, the use of side lighting in a room with subdued light may provide better visualization of indentations.

   Document any indentations observed on the QR DOC-2. If indentations are not observed, document the lack of visible indentations.

   At various points in these procedures, a determination that a particular feature is not present or that an item is lacking in quality or comparability can indicate that the examiner should discontinue or limit the procedure(s). It is at the discretion of the examiner to discontinue the procedure at that point and report accordingly or to continue with the applicable procedures to the extent possible. Document the reasons for such a decision and report this accordingly.

2. Determine whether the submitted Questioned impression(s) were produced by a dry seal device. If the Questioned impression was not created by a dry seal device, discontinue examination and report accordingly.

3. Determine whether the examination is a comparison of Questioned impressions; a comparison of a Questioned impression(s) with a Known impression(s); or a comparison of a Questioned impression(s) with a dry seal device.

4. Determine whether the submitted Questioned impression(s) is suitable for comparison. If it is not suitable for comparison, discontinue the procedure and report accordingly. Factors that affect the suitability include clarity, detail, degree of embossing or condition of the document. Submission of the original is usually necessary for the examination of individualizing characteristics.

5. If a Known document(s) is submitted, determine whether the Known document(s) is suitable for examination, or comparison, or both. If it is not suitable, discontinue the procedure and report accordingly. Factors that affect the suitability include clarity, detail, or condition of the document.

6. If the original is not submitted, evaluate the quality of the best available reproduction to determine whether significant details have been reproduced with sufficient clarity for comparison purposes and proceed to the extent possible. If the reproduction is not of sufficient clarity for comparison purposes, discontinue these procedures and report accordingly.

RETIRED

7. If a dry seal device(s) is submitted, its condition should be noted (for example, clean, dirty, worn, damaged).
   1) Note, when applicable, class characteristics (for example, typeface design and size).
   2) Note any visible features that reproduced on the impression.
   3) Prepare appropriate specimens, as needed.

8. Determine if any of the Known exemplar impressions are suitable for comparison. If none of the Known exemplar impressions are suitable for comparison and no others are obtained, discontinue these procedures and report accordingly.

9. Conduct a side-by-side comparison of the Questioned impressions, or the Questioned impression to the Known impressions and/or to the dry seal device(s).

10. Compare class characteristics (for example, impression format, typeface design, other present designs and relative sizes). If different, discontinue and report accordingly.

11. Compare individualizing characteristics in common such as wear and damage defects, embossment variation patterns.

12. Evaluate similarities, differences, and limitations. Determine their significance individually and in combination. Once the evaluation has been completed, the examiner should be able to reach a conclusion and report accordingly.

13. Once examinations and evaluations have been completed, reports may include the following types of conclusion(s), opinion(s), or finding(s):
   1) ***Identification***—When the examination reveals no significant, inexplicable differences between two or more items, and there is agreement in all individualizing characteristics, an identification is appropriate (that is, compared impressions or compared impression and dry seal contain substantial significant similarities; there are no significant, inexplicable differences; and no limitations associated with absent characteristics; and any possibility of a duplicate dry seal can be eliminated).
   2) ***Elimination***—If significant, inexplicable differences between two or more items are found at any level of the analyses, an elimination is appropriate (that is, the impressions contain substantial significant differences). There may be similarities, but the presence of differences is used to reach the elimination conclusion.
   3) ***Qualified Opinions***—When there are limiting factors and the examination reveals similarities or differences of limited significance between two or more items, the use of qualified opinions can be appropriate (that is, the impressions or observed features contain limited similarities or differences; or limitations associated with absent characters,

RETIRED

individualizing characteristics, or distorted impressions are present; or a combination of these). Qualified opinions require explanation of the limiting factors.

4) ***No Conclusion/Inconclusive***—When there are significant limiting factors, and the examination reveals no significant similarities or significant differences, a report that no conclusion can be reached is appropriate (that is, the impressions or observed features contain insufficient significant similarities and insufficient differences). This opinion requires explanation of the limiting factors in the report and case notes.

Sources of Error: Not applicable

References:

SWGDOC Standard for Examination of Dry Seal Impressions ver. 2013-1

RETIRED

Purpose: Standard for Preservation of Liquid Soaked Documents

This procedure provided guidance that should be used by forensic document for the preservation of liquid soaked documents. These procedures include evaluation of the sufficiency of the material requiring preservation. The particular methods employed in a given case will depend upon the nature of the material requiring preservation. This procedure does not cover all the steps that might be necessary to preserve unusual or uncommon liquid soaked documents. Consultation with a document conservationist, archivist, or related material expert, as well as reference materials, may be necessary.

Due to the extensive time required for some of these procedures and the type of procedures chosen can conflict with investigative needs and other forensic examinations. The examiner should communicate with the stakeholder of the time that will be needed for the completion of the preservation methods. Additionally, if other testing is needed, the examiner will discuss the preservation techniques with the Deputy Director and technical responsibility of the other disciplines involved to determine the best course of examination.

RETIRED

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit.

Safety:

The examiner should take necessary precautions and wear protective equipment when handling liquid soaked documents in case the liquid may be a hazardous material.

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources
   LED or fluorescent sources, or fiber optic lighting systems may be utilized. Transmitted lighting, side lighting, and vertical incident lighting have been found useful in a variety of situations.
2. Stereomicroscopes with fiber-optic lighting
3. Trays, tanks, and pliable screening.
4. Bone folder or similar device.
5. Polyester film or other encapsulation material, as required.
6. Imaging or other equipment for recording observations, as required.
7. Other apparatus, such as humidity chamber, laboratory oven, and chemicals, as appropriate.
8. Measuring devices

9. Scanners
10. Cameras

Procedure:

1. Examine the evidence and detail all relevant observations with notes and photography. Images should be made to document the initial condition of the evidence and subsequently as needed.
2. Evaluate the documents for the following:
    1) The nature and condition of the documents.
    2) The nature of the liquid(s).
    3) The extent of the effect from the liquid(s).

3. Determine the appropriate procedures to optimize preservation of the document(s).
    1) For wet, single-page document(s):
        a. Select a suitable method, such as submersion or drying, to unfold the document(s), if necessary.
        b. Select a suitable method, such as air drying, freeze drying, or pressing, and dry the document(s).
    2) For wet, multi-page document(s):
    Determine if the wet pages can be separated or unfolded without additional damage. This can be accomplished by submerging the document(s) in an appropriate liquid, such as water or mineral spirits. If the pages cannot be separated or unfolded, select a suitable drying process, such as air drying, freeze drying, or pressing.
    3) For dried document(s):
    Attempt to separate, if necessary, and flatten the pages using appropriate equipment, such as bone folders, picks, probes, and tweezers. Prior to or during the attempt to separate and flatten the document(s), it may be necessary to re-humidify or re-submerge the document(s). Re-humidification with appropriate fluids may be accomplished with an atomizer, humidity chamber, or both. When re-submerging the document(s), an appropriate container and screen should be utilized.
    4) For documents received frozen:
    Freeze dry the document(s) and treat as if it was a dry document. If time does not permit, thaw the document(s) and treat as wet document(s).

4. Encapsulate the document(s) upon completion, with materials such as polyester film or glass.

RETIRED

Sources of Error: Not applicable

References:

SWGDOC Standard for Preservation of Liquid Soaked Documents ver. 2013-1

RETIRED

Purpose: Procedure for Examination of Fracture Patterns and Paper Fiber Impressions on Single-Strike Film Ribbons and Typed Text

This procedure provides guidance that should be used by forensic document examiners for examinations and comparisons involving single-strike film ribbons with typed text and related procedures. These procedures are applicable whether the examination and comparison is of Questioned and Known items or of exclusively Questioned items. This type of evidence may include the examination of lift-off and cover-up correction tapes and sheets, carbon paper and carbon copies or of documents produced with certain non-impact printing devices (for example, printing devices using a thermal imaging transfer ribbon).

By following these procedures, a forensic document examiner can reliably reach an opinion concerning whether a particular single-strike ribbon or lift-off correction tape was used in the preparation of a specific typed text. The reaching of an opinion may be affected by interferences such submission of non-original documents, limited quantity or comparability, or condition of the items submitted for examination (for example, the condition of the ribbon or the paper, or both). The nature of the paper can affect the quality and quantity of fiber impression(s) as well as ink transfer and retention. Results may also be affected by inappropriate prior storage and handling of the evidence items. Previous testing or chemical processing (for example, for latent prints) can interfere with the examination of certain characteristics.

RETIRED

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources. Lighting may include the use of transmitted, side or vertical lighting to improve the ability to view fine details.
2. Stereomicroscopes with fiber-optic lighting
3. Scanners
4. Cameras

Procedure:

The Questioned Document Unit will usually be the first Unit to receive evidence for analysis. In the event that another Unit examined this evidence first, it should be understood that there may be analysis methods that may limit the examination of fracture patterns and paper fiber impressions on this evidence. Prior handling, testing, or chemical processing (for example, for latent prints) or swabbing of this evidence may interfere with the ability of the examiner to see certain characteristics.

1. Beginning of examination: Refer to QD SOP-3 for guidance on transferring, LIMS documentation and evidence identification.

2. During the examination procedure, the examiner may use different types of lighting and magnification (such as stereomicroscopes) to examine the evidence. Natural light, incandescent sources, fiber optic light guides and sources are generally utilized. Transmitted lighting, side lighting, and vertical incident lighting may be utilized to visualize the detail. The documents may be scanned or photographed to preserve the detail that was located and for incorporation into the case notes.

   All observations are recorded on the QR DOC-2 or the case notes associated with the examinations.

3. The following procedures should be performed when applicable. These procedures should be performed in the order given. If at any point during the examination, a determination that a particular feature is not present or that an item is lacking in quality or comparability may indicate that the examiner should discontinue or limit the procedure(s). It is at the discretion of the examiner to discontinue the procedure at that point and report accordingly or to continue with the applicable procedures to the extent possible. The reasons for such a decision shall be documented.

4. Determine whether any original typed text is present. If no original typed text is present, a paper fiber examination is not applicable. When the non-original typed text is of sufficient quality, a limited fracture pattern comparison of gross features may be possible.

5. Determine the type of ribbon used to prepare the typed text on the document. If fabric or multi-strike film ribbon, discontinue these procedures and report accordingly.

6. Determine, if possible, whether the ribbon type is consistent with the original typed text (for example, lift-off compatible or permanent).

RETIRED

7. Determine if the type style on the document is present on the ribbon. A ribbon can contain more than one style of type, if more than one type of print is present, note the differences and record this information with photographs.

8. Determine whether the text on the document is present on the ribbon. This can be determined by visual inspection.

9. Determine whether the text on the ribbon and the text on the document match in all details, including errors and corrections.

10. Determine whether the fracture pattern of characters on the ribbon match those of corresponding characters on the document.

11. Determine whether there are areas of un-transferred ink within the void area of a character on the ribbon that match a void within the outline of the corresponding character on the document.

12. Determine whether there are impression(s) of paper fibers within the void area of a character on the ribbon that match paper fibers within the inked area of a corresponding character on the document.

13. Evaluate fracture pattern and paper fiber matches and discrepancies, and any limitations. Determine their significance individually and in combination.

RETIRED

Reporting of Results:

The conclusion(s), opinion(s), or findings resulting from the procedures in this standard may be reached once sufficient examinations have been conducted. The basis and reasons for the conclusion(s), opinion(s), or finding(s) should appear in the examiner's documentation and may also appear in the report. The following conclusions may be concluded at the completion of the examination:

1) ***Identification***—When the examination reveals no significant, inexplicable differences between the ribbon and the typed text, and there is significant agreement in all individualizing characteristics, an identification is appropriate.
2) ***Elimination***—If significant, inexplicable differences between the ribbon and typed text are found at any level of the analyses, an elimination is appropriate.
3) ***Qualified Opinions***—When there are limiting factors and the examination reveals similarities or differences of limited significance between the ribbon and typed text, the use of qualified opinions can be appropriate. Agreement of typestyle(s) or relevant text, or both shall be reported.

4) ***No Conclusion/Inconclusive***—When there are significant limiting factors, a report that no conclusion can be reached is appropriate. This opinion requires explanation of the limiting factors.

Sources of Error: Not applicable

References:

SWGDOC Standard for Examination of Fracture Patterns and Paper Fiber Impressions on Single-Strike Film Ribbons and Typed Text ver. 2013-1

RETIRED

Purpose: Procedure for Examination of Fracture Patterns and Paper Fiber Impressions on Single-Strike Film Ribbons and Typed Text

This procedure provides guidance that should be used by forensic document examiners for examinations and comparisons involving single-strike film ribbons with typed text and related procedures. These procedures are applicable whether the examination and comparison is of Questioned and Known items or of exclusively Questioned items. This type of evidence may include the examination of lift-off and cover-up correction tapes and sheets, carbon paper and carbon copies or of documents produced with certain non-impact printing devices (for example, printing devices using a thermal imaging transfer ribbon).

By following these procedures, a forensic document examiner can reliably reach an opinion concerning whether a particular single-strike ribbon or lift-off correction tape was used in the preparation of a specific typed text. The reaching of an opinion may be affected by interferences such submission of non-original documents, limited quantity or comparability, or condition of the items submitted for examination (for example, the condition of the ribbon or the paper, or both). The nature of the paper can affect the quality and quantity of fiber impression(s) as well as ink transfer and retention. Results may also be affected by inappropriate prior storage and handling of the evidence items. Previous testing or chemical processing (for example, for latent prints) can interfere with the examination of certain characteristics.

RETIRED

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources. Lighting may include the use of transmitted, side or vertical lighting to improve the ability to view fine details.
2. Stereomicroscopes with fiber-optic lighting
3. Scanners
4. Cameras

Procedure:

The Questioned Document Unit will usually be the first Unit to receive evidence for analysis. In the event that another Unit examined this evidence first, it should be understood that there may be analysis methods that may limit the examination of fracture patterns and paper fiber impressions on this evidence. Prior handling, testing, or chemical processing (for example, for latent prints) or swabbing of this evidence may interfere with the ability of the examiner to see certain characteristics.

1. Beginning of examination: Refer to QD SOP-3 for guidance on transferring, LIMS documentation and evidence identification.

2. During the examination procedure, the examiner may use different types of lighting and magnification (such as stereomicroscopes) to examine the evidence. Natural light, incandescent sources, fiber optic light guides and sources are generally utilized. Transmitted lighting, side lighting, and vertical incident lighting may be utilized to visualize the detail. The documents may be scanned or photographed to preserve the detail that was located and for incorporation into the case notes.

   All observations are recorded on the QR DOC-2 or the case notes associated with the examinations.

3. The following procedures should be performed when applicable. These procedures should be performed in the order given. If at any point during the examination, a determination that a particular feature is not present or that an item is lacking in quality or comparability may indicate that the examiner should discontinue or limit the procedure(s). It is at the discretion of the examiner to discontinue the procedure at that point and report accordingly or to continue with the applicable procedures to the extent possible. The reasons for such a decision shall be documented.

4. Determine whether any original typed text is present. If no original typed text is present, a paper fiber examination is not applicable. When the non-original typed text is of sufficient quality, a limited fracture pattern comparison of gross features may be possible.

5. Determine the type of ribbon used to prepare the typed text on the document. If fabric or multi-strike film ribbon, discontinue these procedures and report accordingly.

6. Determine, if possible, whether the ribbon type is consistent with the original typed text (for example, lift-off compatible or permanent).

RETIRED

7. Determine if the type style on the document is present on the ribbon. A ribbon can contain more than one style of type, if more than one type of print is present, note the differences and record this information with photographs.

8. Determine whether the text on the document is present on the ribbon. This can be determined by visual inspection.

9. Determine whether the text on the ribbon and the text on the document match in all details, including errors and corrections.

10. Determine whether the fracture pattern of characters on the ribbon match those of corresponding characters on the document.

11. Determine whether there are areas of un-transferred ink within the void area of a character on the ribbon that match a void within the outline of the corresponding character on the document.

12. Determine whether there are impression(s) of paper fibers within the void area of a character on the ribbon that match paper fibers within the inked area of a corresponding character on the document.

13. Evaluate fracture pattern and paper fiber matches and discrepancies, and any limitations. Determine their significance individually and in combination.

RETIRED

Reporting of Results:

The conclusion(s), opinion(s), or findings resulting from the procedures in this standard may be reached once sufficient examinations have been conducted. The basis and reasons for the conclusion(s), opinion(s), or finding(s) should appear in the examiner's documentation and may also appear in the report. The following conclusions may be concluded at the completion of the examination:

1) ***Identification***—When the examination reveals no significant, inexplicable differences between the ribbon and the typed text, and there is significant agreement in all individualizing characteristics, an identification is appropriate.
2) ***Elimination***—If significant, inexplicable differences between the ribbon and typed text are found at any level of the analyses, an elimination is appropriate.
3) ***Qualified Opinions***—When there are limiting factors and the examination reveals similarities or differences of limited significance between the ribbon and typed text, the use of qualified opinions can be appropriate. Agreement of typestyle(s) or relevant text, or both shall be reported.

4) ***No Conclusion/Inconclusive***—When there are significant limiting factors, a report that no conclusion can be reached is appropriate. This opinion requires explanation of the limiting factors.

Sources of Error: Not applicable

References:

SWGDOC Standard for Examination of Fracture Patterns and Paper Fiber Impressions on Single-Strike Film Ribbons and Typed Text ver. 2013-1

RETIRED

Purpose: Procedure for Non-destructive Examination of Paper

These procedures provide guidance to forensic document examiners for the nondestructive examinations of paper. These procedures are applicable whether the examination is of Questioned and Known items or of exclusively Questioned items. By following these procedures, a forensic document examiner can reliably evaluate the physical similarities or differences between papers that can lead to a determination as to whether papers originated from the same source.

Certain items of evidence submitted for examination may have inherent limitations that can interfere with these procedures. Limitations should be noted and recorded on the QR DOC-2 or case notes. The condition of a paper sample may make it unsuitable for some types of examinations (for example, item(s) that are water soaked, stained, soiled, charred, or finely shredded). Additionally, storage conditions such as exposure to light, heat, or moisture can affect the appearance of paper during certain tests.

It should be noted that in the paper manufacturing process, reams of paper and other paper products can be comprised of sheets from one or more rolls of paper. Differences in paper characteristics may be present in individual sheets from the same ream or product and, therefore, must be considered when assessing color, thickness, UV fluorescence, IRL, opacity, surface texture and printed material.

RETIRED

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources
   Lighting may include the use of transmitted, side or vertical lighting to improve the ability to view fine details.
2. Stereomicroscopes with fiber-optic lighting
3. Scanners
4. Cameras
5. Measuring devices - Micrometer capable of measuring in increments of 0.02 mm or 0.001 inch. Ruler measuring at least 300 mm long, marked in increments of 0.5 mm or less, or measuring at least 12 in. long, marked in increments of 1⁄64 in. or less.
6. Scale capable of measuring 0.001 g.

7. IR image conversion device or system with appropriate light sources and filters for use in IR and IRL examinations.
8. ESDA device
9. UV light source

Procedure:

The Questioned Document Unit will usually be the first Unit to receive document-type evidence for analysis. In the event that another Unit examined this evidence first, it should be understood that there may be analysis methods that may limit the examination on these documents. Prior handling, testing, or chemical processing (for example, for latent prints) or swabbing of a document may interfere with the ability of the examiner to see certain characteristics.

1. Beginning of examination: Refer to QD SOP-3 for guidance on transferring, LIMS documentation and evidence identification.

2. All examination steps, relevant observations, and results shall be documented on QR-DOC-2 and/or case notes. If the examiner notes that the submitted evidence is of limited quality or quantity, the examiner or Case Management Unit may need to contact the submitting agency to acquire more evidence. If it is noted that the evidence received has been handled or chemically treated that limits or prevents the examination, the submitting agency may be contacted to communicate this information. (see GL20)

3. During the examination procedure, the examiner may use different types of lighting and magnification (such as stereomicroscopes) to examine the documents and the writing present on the documents. The documents may be scanned or photographed to preserve the writing and for incorporation into the case notes.

4. At various points in these procedures, a determination that a particular feature is not present or that an item is lacking in quality or comparability may indicate that the examiner should discontinue or limit the procedure(s). It is at the discretion of the examiner to discontinue the procedure at that point and report accordingly or to continue with the applicable procedures to the extent possible. The reasons for such a decision shall be documented.

5. Determine whether the examination is a comparison of Questioned paper sample(s) or a comparison of a Questioned paper sample(s) with a Known paper sample(s).

RETIRED

6. Determine whether the submitted paper samples are suitable for comparison. If not suitable for comparison, discontinue the procedure and report accordingly.

7. Examine the paper samples with transmitted light. Record any watermarks, if present. When identifying a manufacturer or dating a paper sample by the use of a watermark, refer to laboratory and published industry resources. If necessary, contact the appropriate paper manufacturer for further information.

8. Examine the color of the paper samples. Determine the significance of any differences observed.

9. Measure the thickness of the paper samples with a micrometer. An averaging of measurements made at the center and opposite edges of each paper sample, is recommended.

10. Examine the paper samples for UV fluorescence and IRL.

11. Examine the samples for chemical or other contamination, alterations, and carbonless paper transfers.

12. Examine the relative opacity of the paper samples.

13. Examine the surface texture of the paper samples (for example, smoothness, patterns).

14. Measure the paper samples with a ruler, recording length and width measurements.

15. Measure the weight of the paper sample. The relative basis weight can be compared by dividing the weight of the paper by its area.

16. Examine corners of the paper samples and evaluate angles (for example, squared, curved, rough finish).

17. Examine edges of the paper samples with magnification, or UV sources, or both for remnants of binding, adhesives, or padding material.

18. Examine edges of the paper samples for manufacturing markings (for example, cut marks, striations or coloration). Evaluate for proper orientation of each page with all other pages.

19. Examine paper samples with lines or other printed material with appropriate instruments capable of magnification, IR, IRL, and UV examinations. Measure line length, spacing, and other printed material. Examine for broken or deformed patterns.

RETIRED

20. Examine the paper samples for the presence of security features (for example, planchettes or security fibers).

21. Examine the samples for carbonless paper chemicals and form printing image quality that can indicate a carbonless system.

22. Locate and record any trace materials (for example, opaqueing solution, correction strips, tape, or other materials) on the paper samples.

23. Examine the paper samples for surface damage due to abrasions, handling, storage, or other physical changes. If folds, creases, crimp markings, fiber disturbances, or other relevant characteristics, are located on any sample, determine the significance as they relate to other samples.

24. Examine the paper samples for size and spacing of staples and staple holes. If the pages of the documents are stapled together, determine any pattern similarities or differences between the number and pattern of staple holes present.

    Prior to the removal of any staples, record the position of the staple holes relative to the existing staple(s). Coordination with the submitter of the evidence may be needed before removing any staples.

25. Examine the paper samples for perforations, hole punches, or other torn portions.

26. Examine the surfaces of the paper for indentations such as handwriting, clipboard marks, paper clip impressions, and other extraneous markings.

27. Evaluate similarities, differences, and limitations in the documents examined. Determine their significance individually and in combination and reach a conclusion. The basis for the examiners opinions should be located in the case notes and also expressed in the final report.

28. Once examinations and evaluations have been completed, reports may include, but are not limited to, the following types of conclusion(s), opinion(s), or finding(s):
    1) Evidence such as indentations, contaminants, physical similarities, etc., associates the paper evidence samples as being attached, handled by, or originating from the same source.

RETIRED

2) The paper samples originate from or share the same manufacturer source (mill, post-mill processing, binding, printing, trimming, packaging and distribution processes) or post-manufacturer source (consumer or user level).

3) The paper samples can neither be associated nor disassociated as originating from or sharing the same source.

4) The paper samples did not originate from or share the same source.

5) Evidence such as indentations, contaminants, physical similarities, etc., associates the paper samples as being attached, handled by, or originating from the same source.

RETIRED

Sources of Error: Not applicable

References:

SWGDOC Standard for Non-destructive Examination of Paper ver. 2013-1

Purpose: Procedure for Examination of Documents Produced with Liquid Ink Jet Technology

This procedure provides guidance be used by forensic document examiners the for examination of documents produced with liquid inkjet technology and related procedures. These procedures are applicable whether the examination is of a Questioned and Known item(s) or of exclusively Questioned item(s). These procedures include evaluation of the sufficiency of the material available for examination.
The particular methods employed in a given case will depend upon the nature and sufficiency of the material available for examination. These methods are applicable to examinations involving copiers, printers, facsimile devices, and multifunction devices using ink jet technology.

By following these procedures, a forensic document examiner can reliably reach an opinion concerning whether two or more documents produced with ink jet technology are from the same device, whether a particular device created the document, or the determination of the make or model of a device.

Items submitted for examination may have inherent limitations that can interfere with these procedures. Limitations should be noted and recorded on the QR DOC-2 or the case notes. Limitations can be due to the generation of the document(s), limited quantity or comparability, or condition of the items submitted for examination.

The results of improper prior storage, handling, testing, or chemical processing (for example, for latent prints) may interfere with the ability of the examiner to see certain characteristics. The effects can include, but are not limited to, partial destruction of the substrate, stains, and deterioration of the ink. Whenever possible, document examinations should be conducted prior to any chemical processing. Items should be handled appropriately to avoid compromising subsequent examinations.

Consideration should be given to the possibility that various forms of manipulation and duplication of ink jet-produced items can be generated by computer, scanner, digital camera, graphic pad or other means.

Additionally, it should be noted that commercially some ink supply units are interchangeable between different brands or models of machines. Due to the trend to become more recycle friendly, some ink units are refillable and ink from suppliers other than the original manufacturer may be used.

Some multi-function devices utilizing toner technology can operate in either printing or copying mode, at different resolutions and can produce both multi-color (for example, CYMK) black or monochrome (for example, one color black). These various outputs from one machine have many significant differences among them.

RETIRED

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit.

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources
   Lighting may include the use of transmitted, side or vertical lighting to improve the ability to view fine details.
2. Stereomicroscopes with fiber-optic lighting
3. Scanners
4. Cameras
5. Rulers in metric, U.S. customary units, printers' measure, and desktop publishing units.
6. VSC

RETIRED

Procedure:

The Questioned Document Unit will usually be the first Unit to receive document-type evidence for analysis. In the event that another Unit examined this evidence first, it should be understood that there may be analysis methods that may limit the examination of these documents. Prior handling, testing, or chemical processing (for example, for latent prints) or swabbing of a document may interfere with the ability of the examiner to see certain characteristics.

1. Beginning of examination: Refer to QD SOP-3 for guidance on transferring, LIMS documentation and evidence identification.

2. All examination steps, relevant observations, and results shall be documented on QR-DOC-2 and/or case notes. If the examiner notes that the submitted evidence is of limited quality or quantity, the examiner or Case Management Unit may need to contact the submitting agency to acquire more evidence. If it is noted that the evidence received has been handled or chemically treated that limits or prevents the examination, the submitting agency may be contacted to communicate this information. (see GL20)

3. During the examination procedure, the examiner may use different types of lighting and magnification (such as stereomicroscopes) to examine the documents and the writing present on

the documents. The documents may be scanned or photographed to preserve the writing and for incorporation into the case notes.

4. At various points in these procedures, a determination that a particular feature is not present or that an item is lacking in quality or comparability may indicate that the examiner should discontinue or limit the procedure(s). It is at the discretion of the examiner to discontinue the procedure at that point and report accordingly or to continue with the applicable procedures to the extent possible. The reasons for such a decision shall be documented in QR DOC-2 or the case notes.

5. Determine whether the submitted Questioned document(s) was produced with liquid ink jet technology. If not, discontinue examination and report accordingly.

6. Determine whether the examination is comparison of a Questioned document(s) to a Known document(s), a comparison of exclusively Questioned documents, or is another type of examination of a Questioned item(s) (e.g., to determine date limitations or class of machine).

7. Determine whether the Questioned document(s) is suitable for examination, or comparison, or both. If it is not suitable, discontinue the procedure and report accordingly. Factors that affect the suitability include clarity, detail, or condition of the document.

8. If a Known document(s) is submitted, determine whether the Known document(s) is suitable for examination, or comparison, or both. If it is not suitable, discontinue the procedure and report accordingly. Factors that affect the suitability include clarity, detail, or condition of the document.

9. If the original is not submitted, evaluate the quality of the best available reproduction to determine whether significant details have been reproduced with sufficient clarity for comparison purposes and proceed to the extent possible. If the reproduction is not of sufficient clarity for comparison purposes, discontinue these procedures and report accordingly.

10. If a device is examined, its condition should be noted. Note damage to easily accessible internal components of the device such as the print head or paper transport mechanism. If possible, the submitting agency should try to obtain the service records and pertinent other information noted and recorded. Note the capabilities, features, and settings of any variable features on each device examined. If the device has internal memory, retain or recover any stored information.

    If the device has an internal storage/memory, the examiner should contact the supervisor of the Computer Crimes and Electronic Evidence Unit to discuss the best course of action to take regarding the examination.

RETIRED

11. Prepare appropriate exemplars with the submitted device, taking into consideration the features of the device and possible chemical ink examinations.

    The examiner should take into consideration that visible external components of the device such as the platen, slit glass, collators, and cover/automatic document feeder that may contain physical evidence, obstructions, debris, correction fluid, marks, or scratches. Before taking exemplars, consideration must be given to the possible destruction or loss of physical evidence within the device (for example, fragments torn from the Questioned document).

12. If none of the exemplars produced by the submitted device are suitable for comparison and no others are obtained, discontinue these procedures and report accordingly.

13. Examine the Questioned item(s), or the Questioned and Known items. The type of substrate used in an ink jet printer may affect the appearance of the ink such as banding, circularity, feathering, bleed, mottling, offset, spatter or satellite droplets.

14. Examination(s) for indentations (QD SOP-10) may be performed for the purpose of visualizing indented writing or physical characteristics such as marks from the paper transport mechanism.

15. Various illumination techniques (color filtering, infrared, or ultraviolet) may be used to provide additional information such as security features or stains.

16. Examination(s) for alterations (QD SOP-9) may be performed.

17. Identification of the typestyle(s) may provide useful information (for example, dating information).

    Compare class characteristics (for example, paper supply system, ink type, marks caused by mechanics, color capability). If significant unexplainable differences exist, discontinue and report accordingly.

18. If possible, classify the device used to produce a Questioned document(s). When identifying a manufacturer of a Questioned item(s), refer to published industry resources. If necessary, contact the device manufacturer or distributor for further information.

19. Compare individualizing characteristics such as wear and damage defects, misalignments, reproducible marks, banding, voids, and improper or extraneous ink transfer. Perform and note measurements as needed.

RETIRED

Successive copying on the same machine will make marks slightly out of register. Doubling or tripling of a pattern of dots or marks indicates, respectively, two or three generations of copies on the same machine. Copying on more than one device may bear the distinctive marks of all machines.

Care must be taken in the evaluation of characteristics as some may be caused by factors external to the print device (for example, artifacts from or manipulation of the source computer file) or characteristics common to a particular model of machine.

20. Evaluate similarities, differences, and limitations. Determine their significance individually and in combination. Conclusion(s), opinion(s), or findings resulting from these may be reached once sufficient examinations and information has been gathered.

21. Once examinations and evaluations have been completed, reports may include the following types of conclusion(s), opinion(s), or finding(s):
    1) ***Identification***—When the examination reveals no significant differences between two or more items and there is agreement in significant individualizing characteristics, an identification is appropriate. There may be limitations.
    2) ***Elimination***—If significant differences between two or more items are found at any level of the analyses, an elimination may be appropriate. There may be limitations. There may be similarities.
    3) ***Qualified Opinions***—When there are limiting factors and the examination reveals similarities or differences of limited significance between two or more items, the use of qualified opinions can be appropriate. This opinion requires explanation of the limiting factors.
    4) ***No Conclusion/Inconclusive***—When there are significant limiting factors, a report that no conclusion can be reached is appropriate. This opinion requires explanation of the limiting factors.

RETIRED

Sources of Error: Not applicable

References:

SWGDOC Standard for Examination of Documents Produced with Liquid Ink Jet Technology ver. 2013-1

Purpose: Procedure for Examination of Documents Produced with Toner Technology

This procedure provides guidance that should be used by forensic document examiners for examinations of documents produced with toner technology, and related procedures. These procedures are applicable whether the examination is of Questioned and Known item(s) or of exclusively Questioned item(s). These procedures include evaluation of the sufficiency of the material available for examination. These methods are applicable to examinations involving photocopiers, printers, facsimile devices, and multifunction devices using toner technology.

By following these procedures, a forensic document examiner can reliably reach an opinion concerning whether two or more documents produced with toner technology are from the same device, whether a particular device created the document, or the determination of the make or model of a device.

Items submitted for examination may have inherent limitations that can interfere with the procedures in this standard. Any limitations should be noted and recorded on the QR DOC-2 or case notes. Limitations can be due to the generation of the document(s) limited quantity or comparability, or condition of the items submitted for examination.

The results of improper prior storage, handling, testing, or chemical processing (for example, for latent prints) may interfere with the ability of the examiner to see certain characteristics. The effects can include, but are not limited to, partial destruction of the paper, stains, and deterioration of the toner. Whenever possible, document examinations should be conducted prior to any chemical processing. Items should be handled appropriately to avoid compromising subsequent examinations.

Consideration should be given to the possibility that various forms of manipulation and duplication of toner-produced items can be generated by computer or other means. Some toner supply units are interchangeable between different brands or models of machines. Some toner units are refillable and toner from suppliers other than the original manufacturer may be used.

Additionally, some multifunction devices using toner technology can operate in either printing or copying mode, at different resolutions and can produce both multi-color (for example, CYMK) black or monochrome (for example, one color black). These various outputs from one machine have many significant differences among them.

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit.

RETIRED

Equipment:

1. Lighting (natural, fluorescent) and alternate light sources
   Lighting may include the use of transmitted, side or vertical lighting to improve the ability to view fine details.
2. Stereomicroscopes with fiber-optic lighting
3. Scanners
4. Cameras
5. Rulers in S.I., U.S. Customary Units, printers' measure, and desktop publishing units

Procedure:

The Questioned Document Unit will usually be the first Unit to receive document-type evidence for analysis. In the event that another Unit examined this evidence first, it should be understood that there may be analysis methods that may limit the examination on these documents. Prior handling, testing, or chemical processing (for example, for latent prints) or swabbing of a document may interfere with the ability of the examiner to see certain characteristics.

1. Beginning of examination: Refer to QD SOP-3 for guidance on transferring, LIMS documentation and evidence identification.

2. All examination steps, relevant observations, and results shall be documented on QR-DOC-2 and/or case notes. If the examiner notes that the submitted evidence is of limited quality or quantity, the examiner or Case Management Unit may need to contact the submitting agency to acquire more evidence. If it is noted that the evidence received has been handled or chemically treated that limits or prevents the examination, the submitting agency may be contacted to communicate this information. (see GL20)

3. During the examination procedure, the examiner may use different types of lighting and magnification (such as stereomicroscopes) to examine the documents and the writing present on the documents. The documents may be scanned or photographed to preserve the writing and for incorporation into the case notes.

4. At various points in these procedures, a determination that a particular feature is not present or that an item is lacking in quality or comparability may indicate that the examiner should discontinue or limit the procedure(s). It is at the discretion of the examiner to discontinue the procedure at that point and report accordingly or to continue with the applicable procedures to the extent possible. The reasons for such a decision shall be documented in the QR DOC-2 or case notes.

RETIRED

5. Determine whether the submitted Questioned document(s) was produced with toner technology. If not, discontinue examination and report accordingly.

6. Determine whether the examination is a comparison of a Questioned document(s) to a Known document(s), a comparison of a Questioned document(s) to a Questioned document(s), or is another type of examination of a Questioned document(s) (for example, to determine date limitations or class of machine).

7. Determine whether the Questioned document(s) is suitable for examination, or comparison, or both. If it is not suitable, discontinue the procedure and report accordingly. Factors that affect the suitability include clarity, detail, or condition of the document.

8. If a Known document(s) is submitted, determine whether the Known document(s) is suitable for examination, or comparison, or both. If it is not suitable, discontinue the procedure and report accordingly. Factors that affect the suitability include clarity, detail, or condition of the document.

9. If the original is not submitted, evaluate the quality of the best available reproduction to determine whether significant details have been reproduced with sufficient clarity for comparison purposes and proceed to the extent possible. If the reproduction is not of sufficient clarity for comparison purposes, discontinue these procedures and report accordingly.

10. If a device is examined, its condition should be noted. Note damage to easily accessible internal components of the device such as the fuser rollers or imaging drum. If possible, the submitting agency should try to obtain the service records and pertinent other information noted and recorded. Note the capabilities, features, and settings of any variable features on each device examined. If the device has internal memory, retain or recover any stored information.

    If the device has an internal storage/memory, the examiner should contact the supervisor of the Computer Crimes and Electronic Evidence Unit to discuss the best course of action to take regarding the examination.

11. Prepare appropriate exemplars with the submitted device, taking into consideration the features of the device and possible chemical ink examinations.

    The examiner should take into consideration that visible external components of the device such as the platen, slit glass, collators, and cover/automatic document feeder that may contain physical evidence, obstructions, debris, correction fluid, marks, or scratches. Before taking exemplars,

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consideration must be given to the possible destruction or loss of physical evidence within the device (for example, fragments torn from the Questioned document).

12. Prepare appropriate exemplars, taking into consideration the features of the device and possible chemical toner examinations.

13. If none of the exemplars are suitable for comparison and no others are obtained, discontinue these procedures and report accordingly.

14. Examine the Questioned item(s), or the Questioned and Known item(s).

15. Examination(s) for indentations (QD SOP-10) may be performed for the purpose of visualizing indented writing or physical characteristics such as marks from the paper transport mechanism.

16. Various illumination techniques (color filtered, infrared, or ultraviolet) may be used to provide additional information, such as security features or stains.

17. Examination(s) for alterations (QD SOP-9) may be performed.

18. Identification of the typestyle(s) may provide useful information (for example, dating information).

    Compare class characteristics (for example, paper type, paper supply system, toner type, marks caused by mechanics, color capability). If significant unexplainable differences exist, discontinue and report accordingly.

19. If possible, classify the device used to produce a Questioned document(s). When identifying a manufacturer of a Questioned item(s), refer to published industry resources. If necessary, contact the device manufacturer or distributor for further information.

20. Compare individualizing characteristics such as security features, wear and damage defects, misalignments, reproducible marks, voids, and improper or extraneous toner transfer. Perform and note critical measurements, where needed.

    Marks may not appear on every successive page but will often appear in the same position relative to one or more edges of the sheet (assuming the same paper orientation). Two or more marks with a similar cause usually maintain a fixed spatial relation to each other and/or to the image area of the copy.

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Successive copying on the same machine can make marks slightly out of register. Doubling or tripling of a pattern of dots or marks indicates, respectively, two or three generations of copies on the same machine. Copies from more than one device will usually bear the distinctive marks of each machine.

21 Evaluate similarities, differences, and limitations. Determine their significance individually and in combination. Conclusion(s), opinion(s), or findings resulting from these may be reached once sufficient examinations and information has been gathered.

22. Once examinations and evaluations have been completed, reports may include the following types of conclusion(s), opinion(s), or finding(s):
    1) ***Identification***—When the examination reveals no significant differences between two or more items and there is agreement in significant individualizing characteristics, an identification is appropriate. There may be limitations.
    2) ***Elimination***—If significant differences between two or more items are found at any level of the analyses, an elimination may be appropriate. There may be limitations. There may be similarities.
    3) ***Qualified Opinions***—When there are limiting factors and the examination reveals similarities or differences of limited significance between two or more items, the use of qualified opinions can be appropriate. This opinion requires explanation of the limiting factors.
    4) ***No Conclusion/Inconclusive***—When there are significant limiting factors, a report that no conclusion can be reached is appropriate. This opinion requires explanation of the limiting factors.

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Sources of Error: Not applicable

References:

SWGDOC Standard for Examination of Documents Produced with Toner Technology ver. 2013-1

Purpose: Procedure for the Validation of a New Method or Technology

Responsibility:

Forensic Science Examiners assigned to the Questioned Documents Unit or performing casework in the Unit.

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PROCEDURE:

1. Before a new method, software or technology is introduced, a validation of the method will be performed. A plan of validation will be proposed to the Quality Section and Deputy Director prior to conducting the validation. Guidance on the validation plan can be found in GL-22 and the form needed to present the plan is labeled "Laboratory Method Validation Summary Form" and is located in Qualtrax under the General Laboratory Forms tab.

2. All methods used to fulfill customer requests deemed suitable by the section supervisor (their designee or technical leader are approved by the Director prior to use. This includes the range and accuracy of the results obtained being relevant to the customer's needs. All methods are based on standard methods described and developed by national organizations – eg. SWGDOC.

   In the case of a slight variation for a current method, the Unit will treat is as a deviation of testing and have the procedure approved by the technical responsibility, Deputy Director and Director. A deviation workflow is completed and must be approved prior to the deviation taking place.

3. Any new method, software or technology that is to be added to routine casework must be validated according to the appropriate parameters. All documentation of the new method validation shall be saved in the Deputy Director's Office. If possible, the reliability of the new method shall be compared to published results and become part of the validation file.

4. Any changes to an existing method must be validated as above.

Sources of Error: Not applicable

References:
GL-1, GL-22
SWGDOC

Purpose: Reference Materials

References Book:

Ames on Forgery by D. T. Ames

A Selection of International Penmanship Systems by William & Katherine Koppenhaven

Detecting Forgery - Forensic Investigation of Documents by Joe Nickell

Document Examiner Textbook by Jess E. Dines

Evaluating Evidence by Katherine Mainolfi Koppenhaven

Forensic Examination of Rubber Stamps by Jan Seaman Kelly

Forensic Science - An Introduction To Criminalistics by P. DeForest, R. Gaensslen & H. Lee

Forensic Signature Examination by Steven A. Slyter

Forgery Detection by Wilson R. Harrison

Fundamentals of Criminal Investigation by Charles E. O'Hara & Gregory I. O'Hara

Handwriting Identification: Facts and Fundamentals by A. M. Headrick & Roy Huber

Q.D. Examiner's Occult Guide by Dennis Mooney, Paige Doherty and Trent Eichhorn

Questioned Documents by Albert S. Osborn

Questioned Document Problems by Albert S. Osborn

Scientific Examination of Documents: Methods and Techniques by David Ellen

Scientific Examination of Questioned Documents by Ordway Hilton

Security Threat Groups - Symbols & Terminology Manual by U.S. Department of Justice, Federal Bureau of Prisons

Suspect Documents by Wilson R. Harrison

The Mind of the Jury by Albert S. Osborn

The Problem of Proof by Albert S. Osborn

Typewriting Identification - ID System for Questioned Typewriting by Billy Prior Bates

20th Century Handwriting Systems by Albert Sommerford

Journals and Papers:

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American Bar Association Journal

Canadian Society of Forensic Science Journal

Federal Bureau of Identification Law Enforcement Bulletin

International Journal of Forensic Documents Examiners

Journal of the American Society of Questioned Document Examiners

Journal of Criminal Law, Criminology and Police Science

Journal of Forensic Identification

Journal of Forensic Science

Various papers prepared for presentation at American Academy of Forensic Science Conferences

Various papers prepared for presentation at American Society of Questioned Document Examiners Conferences

Various papers prepared for presentation at the International Association for Identification Conferences

Various papers prepared for presentation at the Northeastern Association of Forensic Scientists

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