TRACE EVIDENCE COLLECTION / HAIR-LIKE FIBER EXAMINATION

19.1 PURPOSE

A. To properly collect trace material, more specifically, hair-like fibers.
B. To identify human hair(s) and prepare the root/root-type structure for DNA testing.

19.2 RESPONSIBILITY

Personnel qualified to perform Forensic Biology duties.

19.3 SAFETY

The appropriate measures for the proper handling of biohazardous materials, sharp instruments and chemicals will be used according to GL-2 (Safety Manual).

19.4 DEFINITIONS/ABBREVIATIONS

A. OTM: Other Trace Material
B. QRW(s): Quality Record Worksheet(s) (Appendix 1)
C. LIMS: Laboratory Information Management System
D. HLF(s): Hair-Like Fiber(s)

19.5 PROCEDURE: General Guidelines

Refer to FB SOP-01 (Evidence Examination and Sample Collection Guidelines) for instruction on cleaning utensils and equipment in laboratory areas.

A. Questioned trace evidence may be collected in the Forensic Biology Unit or in other units of the Laboratory as necessary and transferred to the Forensic Biology Unit for examination.

B. Trace evidence will be collected, examined and retained in such a manner as to prevent loss.

C. For homicide cases:
   1. Generally, hair-like fibers and other trace material will be collected, packaged, labeled appropriately (see 19.6) and returned to the submission/item packaging.
   2. The collected/returned trace material will not be sub-itemized in LIMS.
   3. If the trace material is retained, see 19.7.
   4. The presence or absence of hair-like fibers and/or other trace material will be documented on the appropriate QRW(s).

D. For sexual assault cases:
   1. If present, hair-like fibers will be collected (see 19.6 and 19.7), retained (see FB SOP-02: Sexual Assault Evidence Collection Kit Examination) and documented on the appropriate QRW(s). The absence of hair-like fibers will also be documented, though typically not for Sexual Assault Evidence Collection Kits.
   2. The presence or absence of trace material, other than hair-like fibers, will also be documented on the appropriate QRW(s).

E. For other cases:
   1. Generally, hair-like fibers and other trace material will not be collected/returned or collected/retained, therefore, no sub-itemization is necessary.
      a. If the trace material needs to be collected, packaged and returned, see 19.5.C.
      b. If the trace material needs to be retained, see 19.7.
   2. Hair-like-fiber(s) observed on fire debris evidence with an Accelerant Detection request will be collected, packaged, sub-itemized and barcoded.
      a. The sub-item will not be containerized.
      b. The packaged/barcoded hair-like-fibers will be attached to the exterior of the submission packaging for subsequent transfer(s)/return.
   3. The presence or absence of hair-like fibers and/or other trace material will be documented on the appropriate QRW(s).

F. All trace material/HLFs, regardless of disposition, will be considered (and reported out as) not examined unless a specific request has been made at the time of examination.

G. See 19.12 for report writing.

19.6 PROCEDURE: Trace Evidence Collection and Packaging (when necessary)

A. Trace material may be packaged in layers, with the primary packaging (paper fold, adhesive paper, glassine envelope, for example) containing the collected material and the secondary packaging (envelope, sheet protector, for example) containing the primary packaging.

B. If the sample is to be retained, create the sub-item in LIMS.

The sub-itemized trace material being retained will immediately be held in a secure location. If transferred to a temporary storage location other than one's evidence locker, the transfer must be documented in LIMS. Evidence transferred into a temporary storage location does not need to be verified.

The evidence will be verified upon transfer to the appropriate secure long-term storage location,. (see 19.7)

19.6.1 Materials:

Below is a list of common trace material collection and packaging supplies, however, other suitable collection and packaging materials may be used, as appropriate.

A. For collection:
   1. Forceps
   2. Adhesive paper
   3. Lint roller
   4. Scraper (metal or plastic)

B. For packaging:
   1. Paper for paper fold (see paper fold addendum)
   2. Sheet protectors
   3. Bench paper
   4. Trace tins
   5. Envelopes (glassine/paper)
   6. Glass microscope slides and cover slips

19.6.2 Collection by Picking

A. Pick visible hair-like fibers/OTM from the submission/item with forceps.

B. Place the collection into appropriate primary packaging. Label this packaging with the Lab#, item#, description and initials. Place the primary packaging in a secondary package and label it similarly to the primary packaging.

C. The collected/retained trace material will be verified according to 19.7.

19.6.3 Collection by Lifting

A. Adhesive paper can be used to collect hair-like fibers/OTM from a smaller submission/item.
   1. The note may be folded upon itself. Place into the appropriate primary packaging.
   2. Label this packaging with the Lab#, item#, description and initials.
   3. Place the primary packaging in a secondary package and label it similarly to the primary packaging.
   4. The collected/retained trace material will be verified according to 19.7.

B. A standard lint roller may be used to collect hair-like fibers/OTM from a larger submission/item.
   1. Remove the cover and set aside.
   2. Discard the topmost layer of roller sheets.
   3. Roll the evidence, collecting the topmost removable trace material/debris.
   4. When the tackiness of the roller sheet diminishes, tear off the sheet and continue rolling the submission/item with the next sheet on the roll. Repeat as necessary recording the number of sheets used.
   5. Place the used roller sheet(s) in a sheet protector, maximum two per protector. Sheets must be separate and apart from each other. Heat seal between the two roller sheets in the sheet protector and the sheet protector opening. Initial the heat seal(s). Label the sheet protector with the Lab#, item#, description and initials.
   6. The collected/retained trace material will be verified according to 19.7.

19.6.4 Collection by Scraping

A. In a contained area, hold the evidence above clean bench paper and scrape the trace evidence from the submission onto the paper.

B. Use the scraper edge to gather the trace material into a pile on the clean paper. A paper fold may be made from this paper by cutting the paper around the piled trace material into a manageable size with a scalpel. Other types of primary packaging may be used as well.

C. Label the primary packaging with the Lab#, item#, description and initials. Place the primary packaging in a secondary package and label it similarly to the primary packaging.

D. The collected/retained trace material will be verified according to 19.7.

19.7 PROCEDURE: Verification and Retention of Trace Samples

A. Trace retained from evidence will be sub-itemized in LIMS according to GL-4 (LIMS).

B. The type(s) of trace evidence noted and retained will be documented on the appropriate QRW(s). (For example: hair-like fibers, debris, mineral matter/material, other trace material.)

C. Each trace sub-item will be electronically transferred in LIMS to the appropriate storage location. Locations include, but are not limited, to 'Trace Storage – retained trace' or 'Freezer Storage'.

D. The LIMS transfer sheet will be printed and a second analyst (however titled) will verify the labeling of the primary packaging, and secondary packaging (if present) against the appropriate transfer sheet. Due to the potential for sample loss, the second analyst will not open the primary packaging/collected trace material packet to view the contents for verification purposes.

1. If correct, the second analyst will initial and date the LIMS transfer sheet.
2. If the second analyst discovers a discrepancy, then the appropriate correction(s) will be performed.
   - The Unit Lead will be informed and the root cause will be determined and corrected if possible. Further appropriate action may be taken by the Unit Lead.
3. The initialed and dated transfer sheet (or copy) will be maintained in the appropriate case jacket(s).

E. Once verified, the envelope (or similar) containing the primary packaging with retained trace will be sealed and the seal initialed.

F. Place the sub-item in the appropriate storage location designated on the LIMS transfer sheet.

1. For LIMS 'Trace Storage – retained trace' samples:
   Place each sealed trace material sub-item in a larger manila envelope labeled with the Lab ID#, initials and each sub-item # contained within. Seal with tape and initial the seal.

2. For LIMS 'Freezer storage' samples:
   When the presence of blood or other body fluid-like material is suspected, place each sealed sub-item in a plastic bag, label the plastic bag with the Lab ID#, heat seal and initial the seal.

19.8 PROCEDURE: Examining Questioned Trace Evidence Collection for Hair-Like Fibers (HLFs).

A. Record the lot numbers of all reagents/solutions used on the appropriate QRW(s).

B. Digital images of each step of the examination and each hair-like fiber examined may be useful in determining the appropriate human hairs to forward for DNA testing.

19.8.1: Materials:

A. Forceps
B. Adhesive paper
C. Stereoscope
D. Glass microscope slides
E. Cover slips
F. dH_2O or other appropriate mounting medium
G. Sterile disposable pipettes
H. Compound microscope
I. Digital imaging device
J. Disinfecting solution
K. Paper folds
L. Envelopes (glassine/paper)

19.8.2: Procedure:

A. Examine the trace evidence collection macroscopically, and stereoscopically when necessary, using forceps to separate out any hair-like fibers (HLFs).
   1. Capture digital images as applicable. A scale will be included.
   2. Number each hair-like fiber. (i.e. HLF #1, HLF #2, HLF #3, etc).
   3. Record the approximate length (in centimeters), color and texture on the appropriate QRW(s).
   4. Return any remaining portion of questioned trace material to its packaging. Some trace evidence collections may consist of only hair-like fibers.

B. Prepare each HLF for microscopic examination:
   1. The microscopic examination of each HLF will be completed before proceeding to the next HLF.
   2. HLF(s) to be mounted:
      a. Choose the appropriate glass microscope slide and cover slip based on the length and morphology of the hair-like fiber evidence and parameters of the microscope.

b. Label the glass microscope slide with the Lab#, item#, HLF# and initials.
c. Temporarily mount the HLF in dH_2O, or other appropriate medium. Each HLF should be mounted separately, each with its own cover slip. The ends should be easily located.
d. Once prepared, a photograph may be captured of the mounted HLF with a cm scale.

3. Hair-like fiber(s) previously mounted on glass microscope slides with a permanent mounting medium:
   a. Using a marker, draw a dot at one end of each HLF (root/root-like structure end if present/discernible). Place a number next to each dot, thus assigning each hair-like fiber a HLF#.
   b. Record color, texture and approximate length (in centimeters), of each HLF on the appropriate QRW(s).
   c. Photograph the mounted HLF(s) with a cm scale.

C. Microscopic examination for each HLF:
1. The mounted HLF will be examined with a compound microscope, generally at ~100x magnification.
   a. The examiner will determine if the HLF is a hair and whether the hair is of human or animal origin.
   b. If human, the presence or absence of a root/root-type structure will be determined.
   c. If a root/root-type structure is present, the presence or absence of tissue-like material will be determined.

   Note: Since the presence of debris is commonly observed on HLF(s) during microscopical exam and is not the purpose of the exam, noting its presence is not necessary.
2. The examiner will document observations of each HLF# that support the identification conclusion (i.e. human hair, animal hair and/or other trace material) on the appropriate QRW(s) including:
   a. The presence or absence of a cuticle, cortex and medulla
   b. The presence of a root/root-type structure (i.e. hair) or the absence of a root/root-type structure (i.e. hair fragment)
   c. The presence or absence of tissue-like material on the root/root-type structure for each human hair
   d. Additional examples of hair documentation may include descriptions of the root/root-type structure, color, cortex, pigment, cuticle (scale), medulla, tip, and/or gross morphological features.
3. Photo-documentation:
   a. Digital images of the root/root-type structure/tissue-like material, mid-section/ shaft and tip will be taken under a 5x, 10x and/or 20x ocular.
      i. The ends of fragments may also be photographed.

ii. If images are captured utilizing an objective other than 10x, it may be useful to include the objective in the file name.

b. Images may be captured utilizing the Leica Application Suite (LAS EZ) capture software as follows:

i. Turn on the microscope light and place the mounted sample on the stage.

ii. To ensure a white background, the day light filter (DLF) toggle should be forward (towards operator), and the N16 toggle should be back (towards microscope body).

iii. Open the LAS EZ program.

- In the options drop down, select preferences.
- Under the save images field select the ellipsis icon (…) to choose or create a folder in which to save your images.
- Images are captured under the Acquire tab:
  - The images may be captured using either the manual (M icon) or the automatic (shutter icon) settings. With either option, the image capture settings can be adjusted in the exposure adjust area.
  - A white balance (shaded square icon) may be performed on the image.
  - Once the desired image is in focus on the screen, select the acquire image button.
  - The captured images can be viewed under the browse tab. The images can be renamed (right click thumbnail image and select rename current image).

iv. Captured images are saved to a designated folder on the shared drive.

4. Images should be organized in the case file in a manner that can be easily followed by the case reviewers. See suggested below:

a. Image of mounted HLF #1

b. Stereoscopic image of HLF #1 (if captured)

c. Microscopic image(s) of HLF #1 (i.e. root/root-type structure/tissue-like material, mid-section/shaft and tip)

d. Repeat for HLFs #2, #3, #4, etc.

D. A second qualified examiner will confirm the identification conclusion.

1. They may observe the actual HLF or may review the image(s) and description(s) (i.e. during the Technical Review).
2. They will initial and date the appropriate QRW(s) to document the confirmation.

E. The examiner will choose the most suitable human hairs for DNA testing (see 19.9).

F. Microscopically examined HLFs not chosen for DNA testing:
   1. Each microscopically examined HLF will be packaged separately for retention.
      a. Each temporarily mounted HLF may remain on the glass microscope slide by allowing the mounting medium to evaporate and securing the cover slip with clear tape.
      b. Alternatively, each HLF may be removed from its mounting and placed in a separate package (typically adhesive paper and/or a glassine envelope or a paper fold), sealed and labeled with the Lab#, item#, HLF# and initials.
   2. Permanently mounted HLFs/trace materials may remain on the glass microscope slide.
   3. Place the primary packaging in a secondary package and label it similarly to the primary packaging.
   4. Document on the appropriate QRW(s) how these HLFs/trace materials are re-packaged. (Example: item #001-002 (trace material from hat) retained as 10 glass microscope slides plus the original one white paper fold).
   5. The re-packaged retained trace material will be re-verified prior to long-term storage to ensure the accuracy of the new package labeling.

19.9 PROCEDURE: Preparing Hairs for DNA Testing

19.9.1: Materials:

A. Sterile microcentrifuge tube(s)
B. Pipette/tips
C. Scalpel(s)
D. Forceps
E. Adhesive paper
F. dH_2O
G. HistoClear
H. Diamond/carbon scribe
I. KimWipes
J. Hood
K. Stereoscope
L. Envelopes (glassine/paper)

19.9.2: Root/root-type structure preparation:

A. The examiner will choose the most suitable human hairs for DNA testing. These human hairs may possess the greatest amount of tissue-like material on the root/root-type structure and/or possess macroscopic morphological characteristics of interest.

B. Generally, only the chosen human hair(s) will be sub-itemized (see 19.11).

C. Removing the hair from its mounting:

1. For temporarily mounted hairs:
   a. Slide the cover slip off the microscope slide.
   b. If previously wet mounted, dry the slide/hair with a clean KimWipe.
2. For permanently mounted hairs:
   a. Under a hood using a diamond/carbon scribe, gently apply enough pressure along the length of the hair to crack the cover slip. Avoid applying pressure directly above the hair.
   b. Apply just enough HistoClear to fill the crack and allow time for the medium to soften.
   c. Using forceps, remove any necessary cover slip fragments and gently pry the hair out of the softened medium.
   d. Remove any remaining medium from the hair by soaking it in additional HistoClear on a clean glass microscope slide and dabbing it with a KimWipe.
   e. If the hair is removed in its entirety, the examiner will confirm the length, in centimeters.
   f. Alternatively, only the root/root-type structure may be removed (see 19.9.2.D for guidance). The remaining shaft will be appropriately documented and labeled.

D. Excising the root/root-type structure (with tissue-like material) from the hair:
1. Label a sterile microcentrifuge tube with the Lab#, item # and examiner's initials (see 19.11) and add 25µl of dH_2O.
2. Using forceps, place the hair on a sheet of adhesive paper.
3. When possible, approximately 0.5cm is typically the total length of the root/root-type structure and adjacent hair shaft forwarded to DNA.
4. Using forceps to gently hold the portion to be forwarded, use a scalpel to cut this portion from the remining shaft.
5. Place the root/root-type structure into the prepared tube, ensuring it is transferred from the forceps into the tube/dH_2O.
6. If there is no consumption issue, the sample will be forwarded for DNA testing without verification at this time. These samples will be electronically transferred in LIMS, physically placed into a designated storage location for pending DNA extractions and added to the appropriate DNA batch spreadsheet.
7. If there is a consumption issue, the sample(s) will be placed in a plastic bag labeled with the Lab#. These samples will be verified and the bag heat sealed/initialed (see 19.7). These samples will be electronically transferred in LIMS and physically placed in a designated storage location.
8. Remaining hair shafts will be packaged accordingly, verified (see 19.7), electronically transferred in LIMS and physically placed in a designated storage location.

19.10 PROCEDURE: Examination of Known Hair Samples

A. Known hair samples may be examined when deemed appropriate for the case scenario or when a biological known is needed for DNA comparison purposes.

B. Examining the microscopic morphology of the contents of the known hair sample is not necessary to designate the contents as human hairs. The use of the submitting agency's labeling is appropriate to use in quotes to describe the sample, for example: "known head hair".

C. Collecting human hair roots for DNA comparison purposes:

1. Document the contents:
   a. Photo-document the entire contents of the known hair sample.
   b. Note the approximate number of hairs and their macroscopic morphological characteristics such as length, color and texture on the appropriate QRW(s).
   c. See 19.8.2.B.3 for hairs previously mounted with a permanent mounting medium.
2. Examine the root/root-type structure ends of the hairs stereoscopically. It may be possible to identify tissue-like material. If tissue-like material cannot be discerned, then the hairs may be temporarily mounted on a glass microscope slide for viewing under higher magnification.
3. Choose approximately five to ten hairs with tissue-like material and photo-document the root portions at the appropriate magnification.
4. See 19.9.2.C for removing hairs from their mounting.
5. The examiner will excise the root/root-type structure portions of the hairs (see 19.9.2.D), placing them all into one labeled tube.
6. Sub-itemize in LIMS (see 19.11) with description "known head hair - person's name" (or similar).
7. If there is no consumption issue, the sample will be forwarded for DNA testing without verification at this time. These samples will be electronically transferred in LIMS, physically placed into a designated storage location for pending DNA extractions and added to the appropriate DNA batch spreadsheet.
8. If there is a consumption issue, the sample will be placed in a plastic bag labeled with the Lab#. These samples will be verified and the bag heat sealed/initialed (see 19.7). These samples will be electronically transferred in LIMS and physically placed in a designated storage location.
9. Remaining hair shafts being retained will be packaged accordingly, verified (see 19.7), electronically transferred in LIMS and physically placed in a designated storage location.

19.11 PROCEDURE: Itemizing

A. A trace collection is given a sub-item number according to GL-4. Trace material removed from the collection and further examined, but not chosen for DNA testing, will remain part of the trace collection and labeled in a manner consistent with the collection, regardless of how packaged.

B. Human hairs that are chosen for DNA testing from the trace collection are each further sub-itemized according to GL-4.

1. The remaining shaft will be retained and remain sub-itemized accordingly.
2. The root/root-type structure removed for DNA testing will be additionally sub-itemized.
3. If the entire hair is forwarded to DNA, it will remain sub-itemized accordingly.

C. **Example**:

#001 #1 Envelope with "Hairs found on broken window"
#001-001 Hair-like fibers - "broken window"
#001-001-01 Human hair - "broken window"
#001-001-01-01 Root portion of human hair - "broken window"
#001-001-02 Human hair - "broken window"

D. Multiple known hairs will be sub-itemized accordingly as one sample.

Example:

#002 #2 Envelope with "Head Hairs - J. Smith"
#002-001 Known hair sample - "J. Smith"
#002-001-01 Known hair sample - "J. Smith"
#002-001-01-01 Root portions of known hair sample - "J. Smith"

19.12 PROCEDURE: Report Writing:

A. General Guidelines (Homicides and Sexual Assault cases):

1. If hair-like fibers are present, their collection and disposition will be specified in the Forensic Biology or DNA report.
2. The presence of trace material (i.e. not collected) will be specified in the Forensic Biology or DNA report.
3. Only when a trace examination is requested on the Request for Analysis form (SOP-ER-02:1) will the absence of trace material be included in the report.

B. General Guidelines (Other Cases):

1. The presence of hair-like fibers and/or other trace material will be specified in the Forensic Biology or DNA report.
2. Only when a trace examination is requested on the Request for Analysis form (SOP-ER-02:1) will the absence of trace material be included in the report.

C. Suggested Report Wording:

1. Examination Method
   a. All examinations were conducted macroscopically unless otherwise noted.
   b. All examinations were conducted microscopically and/or macroscopically.
   c. All examinations were conducted macroscopically and microscopically.

2. Trace/Hair-like fiber Collection/Disposition
   a. Hair-like fiber(s) and other trace material were noted on/in [ ].
   b. No hair-like fiber(s) was/were noted on/in [ ].
   c. No trace material was noted on/in [ ].
   d. Trace material/hair-like fiber(s) was/were collected/removed from [ ] and returned to the submission/item packaging.
   e. Trace material/hair-like fiber(s), designated as item #[ ], was/were retained at the Laboratory.
   f. Other trace material/Debris was observed on/in [ ].

3. Hair Identification/Disposition
   a. A competent hair examiner from the Laboratory or another ASCLD/ANAB accredited laboratory may act as the Technical Reviewer of a report which includes hair identifications.
   b. <u>Statement format:</u>
      - *Human hair(s) was/were observed in item #[ ].*
      - *No tissue-like material was observed on the root(s)/root-type structure(s) of this/these hair(s).*
      - *A human hair fragment was observed in item #[ ].*
      - *Tissue-like material was noted on the root(s)/root-type structure(s) of some of these human hairs.*
      - *The root(s)/root-type structure(s) from this/these human hairs was/were forwarded to the DNA Unit for analysis.*
      - *The remaining shaft(s) of this/these human hair(s) was/were retained at the Laboratory.*
      - *Animal hair(s) was/were observed in item #[ ].*
      - *Animal hair fragment(s) was/were observed in item #[ ].*
      - *This/these hair-like fibers demonstrated insufficient characteristics for further identification.*

c. Table format:

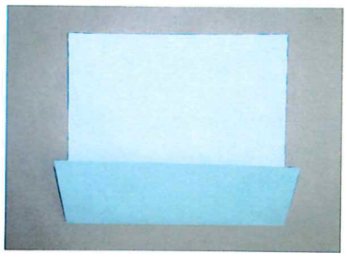
Description	**Conclusion**	**Tissue-like material at root/root-type structure**	**Disposition**
Hair-like fiber	Human hair	Yes	Root portion/Root-type structure to DNA as # xxx-xxx-xx
			Remaining shaft retained
Hair-like fiber	Human hair	No	Retained
Hair-like fiber	Human hair fragment	Not applicable	Retained
Hair-like fiber	Animal hair	Not applicable	Retained
Hair-like fiber	Animal hair fragment	Not applicable	Retained
Hair-like fiber	Other trace material/Fiber-like material	Not applicable	Returned to submission packaging
Hair-like fiber	Insufficient characteristics for further identification	Not applicable	Returned to item packaging

19.13 REFERENCES:

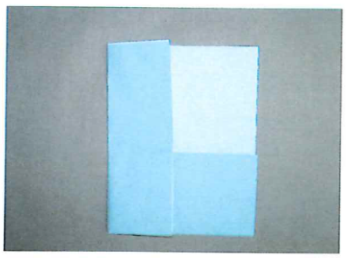
1. Li, Richard, Forensic Biology, Second Edition, CRC Press, 2015. Chapter 4: Sources of Biological Evidence, pp. 89-92 and pp. 93-95 (Figures 4.20-4.24).
2. Hicks, J., Microscopy of Hairs: A Practical Guide and Manual, FBI Laboratory, 1977.
3. Saferstein, R., Forensic Science Handbook, Prentice Hall, 1982. Chapter 5: The Forensic Identification of and Association of Human Hair, pp. 185-221.
4. Saferstein, R., Forensic Science Handbook, Prentice Hall, 1982. Chapter 9: Foundations of Forensic Microscopy, pp. 417-528.
5. Leica Application Suite EZ Capture Software version 3.0.0
6. GL-2 (Safety Manual)
7. GL-4 (LIMS)

FB SOP-19 Trace Evidence Collection / Hair-like Fiber Examination - Addendum

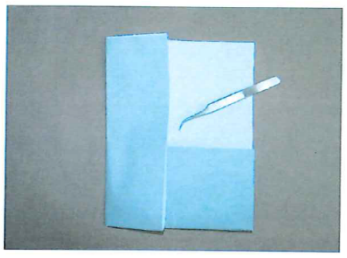
1. Fold 1/3rd of Width



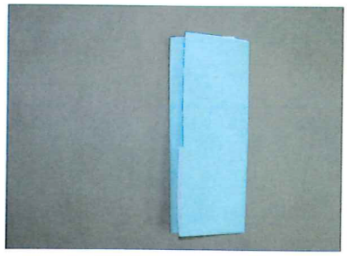
2. Fold 1/3rd of Length

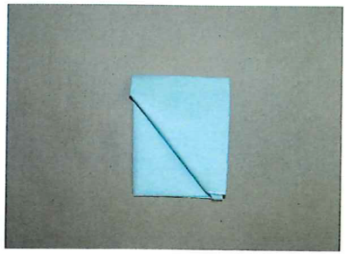


3. Place Trace in Pocket



4. Fold Remaining 1/3rd of Length



5. Fold in Half

6. Fold Corners Under