SCREENING TEST FOR SEMEN (Acid Phosphatase/Brentamine Test)

12.1 PURPOSE

12.1.1: To perform a screening test for the presence of semen in Forensic samples.

A. Theory

Acid phosphatase (AP) is an enzyme present in high concentrations in semen. It is also present in other body fluids but typically at lower concentrations. Used as a color screening test, a positive result indicates that seminal fluid may be present but further testing is required.

This test is conducted utilizing a commercially available premixed powder containing brentamine dye and sodium alpha-naphthyl phosphate. The premixed powder is dissolved in deionized water to make the Brentamine Test Reagent which is applied to the sample.

If acid phosphatase is present, it cleaves the sodium alpha-naphthyl phosphate, releasing sodium phosphate and naphthol. The liberated naphthol couples with the brentamine dye to create a purple azo dye which may be observed as pinkish, pinkish-purple or purple.

B. Limitations

Other body fluids that may contain detectable levels of acid phosphatase include vaginal secretions, fecal material and saliva.

12.1.2: To prepare and conduct quality control testing on the Brentamine Test Reagent.

12.2 RESPONSIBILITY

12.2.1: Test Procedure – Personnel qualified to perform Forensic Biology duties.

12.2.2: Preparation/QC Procedure – Personnel qualified to perform Forensic Biology duties. Ordering information is maintained in a logbook and/or electronically in the Forensic Biology Unit. New chemicals and reagents are purchased according to GL-6 (Purchasing). For additional information, refer to the Biological Inventory located in Appendix 3.

12.3 SAFETY

Use appropriate measures for the proper handling of biohazardous material and the commercially available premixed powder according to GL-2 (Safety Manual) and the Safety Data Sheet.

12.4 DEFINITIONS/ABBREVIATIONS

A. AP: Acid Phosphatase
B. SERI: Serological Research Institute
C. PMR: Premade Reagent (premixed powder)
D. QRW(s): Quality Record Worksheet(s) (Appendix 1)

12.5 TEST PROCEDURE

This test will be performed at the discretion of the examiner, with input from the Unit Lead(s) when applicable, based on the submitting agency requests, case information and the condition of the evidence.

12.5.1: Materials

A. Brentamine Test Reagent (see section 12.6 for daily preparation and QC)
B. dH_2O
C. Cotton swabs or spot plates
D. Light blocking tubes or tubes/aluminum foil
E. Wooden sticks
F. Forceps
G. Scissors/scalpel(s)

12.5.2: Procedure

A. Record the reagent lot number used on the General Reagent Sheet (FBQR-09).

B. Prepare the Brentamine Test Reagent according to section 12.6. Use the appropriate QRW to record the results of the tested controls. If indirect testing will be conducted (see 12.5.2.C.2 below), then the dH_2O used must also be tested and recorded accordingly. Proceed with testing the questioned samples.

1. Aliquots from the prepared stock reagent may be made for use during the day as needed. Label aliquots with reagent lot # (preparation date and preparer's initials).

2. During use, ensure the reagent is protected from light in a light blocking tube or by covering tube with aluminum foil.

3. The daily reagent may be retested with the positive and negative controls within the workday.

C. Samples may be prepared for testing as follows:

1. Direct testing: Cut a piece of fabric, swab or other substrate and place into a spot plate or other appropriate device.

2. Indirect testing: Moisten a cotton swab with dH_2O and swab the questioned stain or area.

3. Direct testing should be considered for stains when possible.
   a. If a limited quantity of sample is suspected, then the non-destructive, indirect testing method may be employed.
   b. If testing is conducted on dark colored cuttings/substrates, then indirect testing should be considered.
   c. Indirect testing is generally used when testing "other areas" of an item.

D. If the sample is determined to be unsuitable for AP testing (including but not limited to the presence of blood-like staining or fecal-type material), record the reason on the appropriate QRW(s). See 12.5.3 below.

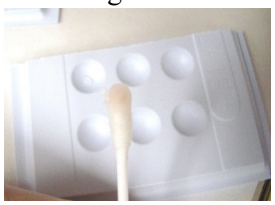
E. For previously tested items (examples: field tested samples, cold case samples):
1. Swabs: See section 1.13.2.1.1 in FB SOP-01 (Evidence Examination and Sample Collection Guidelines).
2. Other evidence: If an area or stain on an item of evidence has previously tested positive, AP screening may be omitted prior to extraction at the discretion of the examiner, with input from the Unit Lead(s) when applicable, based on the submitting agency requests, case information and the condition of the evidence.

F. Test the sample as follows:
1. Add 2 drops of Brentamine Test Reagent to the sample. Ensure the sample is immersed. It may be necessary to use a wooden stick and/or add 1-2 additional drop(s) of reagent.
2. Observe for any color change for up to 3 minutes.
3. Record the results (including any unexpected color change) on the appropriate QRW(s).

12.5.3: Results and Conclusions

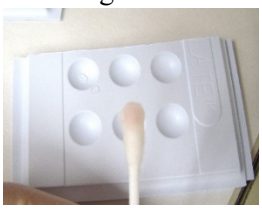
A. Positive
1. Acid phosphatase is detected when a pinkish/pinkish-purple/purple color change is observed. See figures 1, 2 and 3 for examples of varying positive AP results.

Figure 1:



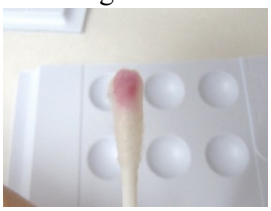
Very light pinkish

Figure 2:



Light pinkish/ pinkish-purple

Figure 3:



Pinkish-purple

2. Positive results may be described and recorded as follows:
↓/vw(+) = very weak positive = very light pinkish/pinkish-purple/purple
w(+) = weak positive = light pinkish/pinkish-purple/purple
(+) = positive = pinkish/pinkish-purple/purple
↑/s(+) = strong positive = dark pinkish/pinkish-purple/purple

The description of a positive result may vary between examiners. This variation is acceptable since all descriptions designate a positive result.

3. Suggested Report Wording:
   a.

Testing Performed	Result	Conclusion
Screening - Semen	Positive	Acid phosphatase detected

   b. *[ ] gave a positive result(s) when tested for the presence of acid phosphatase, a color screening test for semen. [ ] from [ ] was extracted.*

   c. A questioned sample that yields a positive result may be used to make a smear for the identification of spermatozoa according to FB SOP-14 (Identification of Spermatozoa). If a spermatozoon/spermatozoa is/are identified, then eliminate the 'extracted' statement.

B. Negative
1. Acid phosphatase is not detected when no color change is observed.

2. Suggested Report Wording:
   a.

Testing Performed	Result	Conclusion
Screening - Semen	Negative	Acid phosphatase not detected

   b. *[ ] was/were tested for the presence of acid phosphatase, a color screening test for semen. Acid phosphatase was not detected with this test.*

C. Inconclusive
1. If a pinkish/pinkish-purple/purple color change could not be determined after the addition of the AP reagent (including but not limited to the presence of blood-like staining or there is interference from the substrate).

2. Suggested Report Wording:
   a.

Testing Performed	Result	Conclusion
Screening - Semen	Indeterminate	Inconclusive[1]
Comment: [1]Due to an indeterminate result and/or substrate interference, this test was determined to be inconclusive.		

   b. *[ ] was/were tested for the presence of acid phosphatase, a color screening test for semen. Due to an indeterminate result and/or substrate interference, this test was determined to be inconclusive. [ ] from [ ] was extracted.*

3. Record the reason a result is determined to be inconclusive on the appropriate QRW(s).

D. Failed

1. If other than the expected color change is observed (see 12.5.3.A above).

2. Suggested Report Wording:

a.

Testing Performed	Result	Conclusion
Screening - Semen	Failed Test	No conclusion possible

b. *A color screening test(s) for the presence of semen was/were performed on [ ]. Due to the failure of this/these test(s), no conclusion(s) is/are possible.*

3. Record the reason a result is determined to be failed on the appropriate QRW(s).

E. Unsuitable

1. See section 12.5.2.D for additional information.

2. Suggested Report Wording:

Note: The description of the sample will be superscripted since it is unsuitable for the screening test for semen. The sample will continue to extraction and the next serological test as shown in the example below.

a.

Description	Testing Performed	Result	Conclusion
Staining/area(s)[1]	Microscopic - Spermatozoa	Positive	Spermatozoa identified
Comment: [1] This/these staining/area(s) was determined to be unsuitable for the screening test for semen due to [ ]. (see b. below).			

b. *This sample was determined to be unsuitable for the screening test for semen due to [ ]. [ ] = the presence of blood-like staining.*
[ ] = the presence of heavy fecal-type material.

3. Record the reason a sample/item is determined to be unsuitable on the appropriate QRW(s).

F. It should be noted that any result above does not preclude the sample from being extracted, as determined on a case-by-case basis.

12.6 PREPARATION/QC PROCEDURE

12.6.1: Preparation

Materials

A. SERI AP Spot Test PMR 0.13g
B. dH_2O 5ml
C. Controls: positive (known 1:10 semen stain) and negative (blank substrate)
D. Light blocking tubes (~15 ml) or tubes/aluminum foil
E. Wooden applicators
F. Forceps
G. Scissors
H. Test tube racks

This reagent will be prepared at the time of use (daily or as needed) and will be discarded accordingly.

Procedure

A. Dissolve 0.13g PMR in 5ml of dH_2O.

B. Vortex well. A wooden stick may be used to break up any precipitate at the bottom of the tube.

C. Ensure the reagent is protected from light by preparing in a light blocking tube or by covering tube with aluminum foil.

D. The quantity of reagent prepared may be adjusted, according to need.

12.6.2 For daily preparation and QC

A. Test a positive control and a negative control according to the procedure under 12.5 (see 12.5.2.C and 12.5.2.F) prior to testing the questioned sample(s). If indirect testing (12.5.2.C.2) will be conducted, then the dH_2O used must also be tested accordingly.

Record the results according to the Brentamine Test Reagent daily Log Sheet.

B. If the controls do not yield the appropriate results, review the procedure and retest the controls. If the controls still do not yield the appropriate results, then inform the Unit Lead to try to determine the root cause. It may be necessary to prepare and test a new batch of reagent.

C. If the reagent is acceptable for use, record the preparation date and preparer's initials as the lot # on the tube. (i.e. 052318KJL).

The reagent is acceptable for use when a positive result is obtained with the semen control and a negative result is obtained with the blank/negative control.

12.6. 3: For the preparation and QC of newly purchased SERI AP Spot Test PMR

A. Test and record the results of the newly purchased PMR before use according to the test procedure (section 12.5) and the Brentamine Test Reagent Log Sheet. The results are recorded at the first indication of a purple color change and observed for up to 3 minutes.

B. If the appropriate results are not obtained, discard the reagent, review the procedure, make new reagent and retest. If the reagent still does not yield the appropriate results, then inform the Unit Lead to try to determine the root cause.

C. If the reagent is acceptable for use, the PMR may be aliquoted for daily preparation. Record the following on each tube: reagent, PMR lot #, manufacturer's expiration date, quantity of PMR, quantity of dH_2O to add and examiner's initials. Ensure the PMR is protected from light by aliquoting into light blocking tubes or by wrapping tubes/rack with aluminum foil. Store in the freezer.

1. The reagent is acceptable for use when a positive result is obtained with the semen control and a negative result is obtained with the blank/negative control.

2. The use of this reagent and the interpretation of results are addressed during training according to the test procedure and FB SOP-26 (Training Manual and Checklist).

D. Discard any frozen aliquots of PMR according to the manufacturer's expiration date or according to 21.4.3.E in FB SOP-21 (General Chemical and Reagent QC).

Manufacturer's expiration dates with only month and year indicated (i.e. 04/2014) expire the last day of the month noted.

12.7 REFERENCES

A. Connecticut Division of Scientific Services, SERI Brentamine (Acid Phosphatase) Internal Validation, 2018.
B. Li, R., Forensic Biology Second Edition, CRC Press, FL, 2015, Chapter 14: "Identification of Semen", pp. 257 (14.1), 259-264.
C. GL-2 (Safety Manual)
D. GL-6 (Purchasing)
E. Safety Data Sheet