SCREENING TEST FOR SEMEN (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 other body fluids, but is present in semen at higher 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. The formation of a purple color indicates the presence of acid phosphatase.

B. Limitations

Vaginal secretions, fecal material and other body fluids contain detectable levels of acid phosphatase.

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

12.2 RESPONSIBILITY

12.2.1: Forensic Science Examiners (however titled) from the Division of Scientific Services who have been trained in the discipline of semen screening and in the discipline of extracting samples for semen according to FB SOP-26 (Training Manual and Checklist).

12.2.2: Forensic Science Examiners in the Forensic Biology Section. Ordering information is maintained in a log book in the Forensic Biology Section. New chemicals and reagents are purchased according to GL-6 (Purchasing). For additional information, refer to the Biological Inventory (Appendix 3) in the FB folder on the shared drive.

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

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), 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

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 work day.

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.

D. 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 1-2 additional drop(s) of reagent.
2. Observe for any color change for up to 3 minutes.
3. Record the results on the appropriate QRW.

E. 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).

12.5.3: Results and Conclusions

A. Positive
1. The development of a purple color within 3 minutes indicates a positive result and the detection of acid phosphatase activity.

2. Positive results may be designated and recorded as follows:
↓/vw(+) = very weak positive = very light purple
w(+) = weak positive = light purple
(+) = positive = purple
↑/s(+) = strong positive = dark 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. *[ ] (each) consisted of [#] swabs. These swabs gave positive results when tested for the presence of acid phosphatase, a color screening test for semen. One (1) swab/a portion of one (1) swab from [ ] was extracted.*

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

c. If a positive AP questioned sample was used to make a smear and a spermatozoon/spermatozoa was/were identified (see section 12.5.2.E above and FB SOP-14 Identification of Spermatozoa) then eliminate the 'extracted' statement.

B. Negative

1. No color change indicates a negative result and no acid phosphatase activity detected.

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 purple color change could not be determined after the addition of the Brentamine Test Reagent (i.e. when blood is present or there is interference from the substrate).

2. Suggested Report Wording:

a.

Testing Performed	Result	Conclusion
Screening - Semen	Indeterminate	Inconclusive[1]

Appendix:
[1]Due to indeterminate results and/or substrate interference, this/these test(s) was/were determined to be inconclusive.

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

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

D. It should be noted that any result above does not preclude the sample from being extracted.

12.6 PREPARATION/QC PROCEDURE

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

12.6.1: Preparation

Materials

A. SERI AP Spot Test PMR 0.26g
B. dH_2O 10ml

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. 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 0.26g PMR in 10ml 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.D) 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 (i.e. 0.13g or 0.26g), quantity of dH_2O to add (i.e. 5ml or 10 ml), 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 other body fluids that may contain acid phosphatase activity (i.e. vaginal secretions, fecal material and oral samples) yield weaker/slower results or negative results, as compared to semen and semen/other body fluid mixtures (i.e. semen/vaginal secretions mixture).

2. The blank/negative controls must yield negative results.

3. 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).

12.7 REFERENCES

A. Gaensslen, R. E., Sourcebook In Forensic Serology, Immunology, and Biochemistry , U.S. Government Printing Office, Washington D.C., 1983, pp. 155-168.
B. Saferstein, R., Forensic Science Handbook Volume II, Prentice Hall, Englewood Cliffs, NJ, 1988, Chapter 7, "The Identification and Individualization of Semen Stains", pp. 348-363.
C. Connecticut Division of Scientific Services, SERI Brentamine (Acid Phosphatase) Internal Validation, 2018.
D. Li, R., Forensic Biology, CRC Press, Boca Raton, FL, 2015, pp. 259-264.
E. GL-2 (Safety Manual)
F. GL-6 (Purchasing)
G. Safety Data Sheet