SCREENING TEST FOR SEMEN

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 two solutions in succession to render a result:

First, a clear solution of alpha-naphthyl phosphate is applied to a sample of the questioned stain. If AP is present, it splits the alpha-naphthyl phosphate into a-naphthol and an inorganic phosphate.

Then, an amber colored solution of Fast Blue B is added. Any liberated a-naphthol produced from the first reaction will bind to the Fast Blue B to form an a-naphthol/Fast Blue B complex. This complex is visualized as a pink to dark purple precipitate, indicating the presence of AP.

B. Limitations

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

12.1.3: To prepare reagents for the screening test for semen and to perform quality control on prepared reagents.

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 of acetic acid according to GL-2 (Safety Manual) and the Safety Data Sheets.

12.4 DEFINITIONS

AP: Acid Phosphatase

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. Acid Phosphatase Reagent
   1. α-naphthyl phosphate substrate solution
   2. Fast Blue B color solution
B. Controls: positive (known 1:10 semen stain) and negative (blank substrate)
C. dH_2O
D. Cotton swabs or spot plates
E. Aluminum foil

12.5.2: Procedure

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

B. Test a positive control and a negative control according to the following procedure (steps 12.5.2C -12.5.2F) prior to testing the questioned sample(s). If using dH_2O for the testing of questioned samples, the dH_2O must be tested with the positive and negative controls.

   1. If the controls yield the appropriate results, record on the appropriate Quality Record Worksheet (Appendix 1) and test the questioned samples.

   2. 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.

   3. If necessary, the reagent may be retested with the controls within the workday.

   4. During use, the reagent should be protected from light by covering with aluminum foil.

C. Samples may be prepared for testing as follows:
   1. Indirect testing: Moisten a cotton swab with dH_2O and lightly swab the questioned stain or area.
   2. Direct testing: Cut a piece of fabric, swab or other substrate and place into a spot plate.

D. Test the sample as follows:
   1. Add 1-2 drops of α-naphthyl phosphate substrate solution to the sample. Wait 10 seconds.
   2. Add 1-2 drops of Fast Blue B color solution to the sample/α-naphthyl phosphate solution.
   3. Observe for any color change within 15 seconds.
   4. Record the results on the appropriate Quality Record Worksheet (Appendix 1).
   5. Discard any unused reagent daily.

E. A questioned sample that yields a positive AP 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 or pink color within 15 seconds 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 or pink
w(+) = weak positive = light purple or pink
(+) = positive = purple or pink
↑/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 or pink color change could not be determined after the addition of Fast Blue B (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 Quality Record Worksheet (Appendix 1).

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: α-Naphthyl Phosphate Substrate Solution

Materials

A. Acetate buffer (100ml):

Sodium acetate - crystal	2g (1.23g if anhydrous)
dH_2O	100ml

B. α-naphthyl phosphate (disodium salt) 0.187g
C. Glacial acetic acid
D. pH meter
E. Plastic tubes (12x75mm) and caps/parafilm
F. Test tube racks

The control date will be one (1) year from the date of preparation.

Procedure:

A. Dissolve α-naphthyl phosphate in 100ml of acetate buffer.

B. Uncover fill hole on electrode of pH meter.

C. Turn on pH meter.

D. Rinse electrode with dH_2O and gently blot with kimwipe (don't rub or wipe).

E. Rinse electrode with sample prior to pH measurement.

F. Place electrode into sample and wait for "pH" icon to stop flashing, read pH.

G. Bring to pH 5 by adding glacial acetic acid drop-wise, allow to thoroughly mix and monitor the pH before adding another drop.

H. Aliquot 1.0 or 1.5ml volumes into appropriately labeled plastic tubes in test tube racks and cover.

12.6.2: Fast Blue B Color Reagent

Materials

A. Fast Blue B salt (o-dianisidine diazotate) 2.0g (20% dye) or .42g (95% dye)
B. dH_2O 100ml
C. Filter paper
D. Plastic tubes (12x75mm) and caps
E. Test tube racks
F. Aluminum foil

The control date will be one (1) year from the date of preparation.

Procedure:

A. Dissolve Fast Blue B salt in dH_2O (protect from light by covering with aluminum foil).
B. Filter if necessary.
C. Aliquot 1.0 or 1.5 ml volumes into appropriately labeled plastic tubes in test tube racks and cover.

12.6.4: Acid Phosphatase Reagent

A. Test each new batch of reagent before use according to the test procedure. The results are recorded at the first indication of a pink or purple color change and observed for 15 seconds.

Record the results according to the Acid Phosphatase Reagent Log Sheet.

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, record the reagent, lot # (date of preparation), control date and examiner's initials on each rack and store in the freezer.

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

D. Discard any frozen aliquots on the control date.

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. GL-2 (Safety Manual)
C. GL-6 (Purchasing)
D. Safety Data Sheets