SCREENING TEST FOR SEMEN

12.1 PURPOSE

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

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

12.2 RESPONSIBILITY

12.2.1: Forensic Science Examiners 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 in Appendix 2.

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

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 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, determine the root cause and correct.

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

C. Prepare sample for testing as follows:
   1. Moisten a cotton swab with dH_2O and lightly swab the questioned stain or area for testing.
   2. Cut a piece of fabric, swab or other substrate for direct testing without dH_2O.

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

E. A positive AP swab 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 *Suggested Report Statements*

A. Positive
   1. The development of a purple or pink color within 15 seconds indicates a positive result and detects the presence 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 or pink

      Variation between examiners calling a positive result is acceptable since these are not critical designations and will not change downstream testing. The result will still be considered positive.

3. *[ ] (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.

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

[ ] 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).

   [ ] 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.

2. Record the reason a result is determined to be inconclusive on the appropriate Quality Record Worksheet.

D. If an AP swab was used to make a smear, eliminate the 'extracted' statement.

E. 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: Glacial Acetic Acid

A. This chemical is purchased from an outside vendor and is used to prepare acid phosphatase reagent and acetate buffer.

B. Record the date received, date opened and examiner's initials on the bottle.

C. Record the required information on the Chemical Log Sheet.

D. Store glacial acetic acid at room temperature according to the manufacturer's instructions.

E. Place in a dropper bottle labeled with the chemical, lot #, fill date and examiners initials.

F. Replace as needed or according to the manufacturer's expiration date.

12.6.2: 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

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.

12.6.3: Fast Blue B Color Reagent

Materials

A. Fast Blue B salt (o-dianisidine diazotate) 2.0g
B. dH_2O 100ml
C. Filter paper
D. Plastic tubes (12x75mm) and caps
E. Test tube racks

Procedure:

A. Dissolve Fast Blue B salt in dH_2O.
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, determine the root cause and correct.

C. If the reagent is suitable 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 suitable 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 dH_2O blank must yield a negative result.

   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 after one (1) year.

12.7 REFERENCES

A. Blake, E.T. and G.F. Sensabaugh: “Genetic markers In Human Semen: A Review". Journal of Forensic Sciences, Vol. 21, 784-796, 1976.
B. Gaensslen, R. E. , Sourcebook In Forensic Serology, Immunology, and Biochemistry , U.S. Government Printing Office, Washington D.C., 1983, pp. 155-168.
C. Metropolitan Police Forensic Science Laboratory. Biology Methods Manual. 1978, pp.3-17 to 3-20.
D. GL-2 (Safety Manual)
E. GL-6 (Purchasing)
F. Safety Data Sheets