Document Title: Acid Phosphatase Reagent QC
Controlled: Yes, with red stamp present
Controlled By: Quality Manager

Prepared By: _____ Date: _____

Approved By: _____ Date: _____

A. PURPOSE:

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

B. RESPONSIBILITY:

Forensic Science Examiners 1 and 2 in the Forensic Biology Section. Ordering information is maintained in a log book in the Forensic Biology Section.

C. SAFETY:

Use appropriate measures for the proper handling of acetic acid according to SOP-GL-2 (Safety Manual) and the Material Safety Data Sheets.

D. PROCEDURE:

1. α-Naphthyl Phosphate Substrate Solution

   a. Materials:

      aa. Acetate buffer (100ml):

Sodium acetate (crystal)	2g (*1.23g if anhydrous)
Distilled water (dH_2O)	100ml

      bb. α-naphthyl phosphate (disodium salt) 0.187g
      cc. Glacial acetic acid
      dd. pH meter
      ee. Plastic tubes (12x75mm) and caps/parafilm
      ff. Test tube racks

   b. Procedure:

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

      bb. Uncover fill hole on electrode of pH meter.

      cc. Turn on pH meter.

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

      ee. Rinse electrode with sample prior to pH measurement.

D. 1. b. ff. Place electrode into sample and wait for "pH" icon to stop flashing, read pH.

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

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

2. Fast Blue B Color Reagent

a. Materials:
   aa. Fast Blue B salt (o-dianisidine diazotate) 2.0g
   bb. Distilled water (dH_2O) 100ml
   cc. Filter paper
   dd. Plastic tubes (12x75mm) and caps
   ee. Test tube racks

b. Procedure:
   aa. Dissolve Fast Blue B salt in dH_2O.

   bb. Filter if necessary.

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

3. Acid Phosphatase Reagent

a. Test each new batch of reagent before use according to SOP-FB-11 (Screening Test for Semen). 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 and make new reagent.

c. If the reagent is suitable for use, record the reagent, lot # (date of preparation) and examiner's
initials on each rack and store in the freezer. AP reagent is suitable for use when non-semen containing body fluids that may contain acid phosphatase activity (i.e. vaginal secretions, fecal material and oral samples) yield weaker/slower results, or negative results, than semen containing body fluids (i.e. semen, semen/vaginal mixture). The PBS blank must yield a negative result. The use of AP reagent and the interpretation of AP results are addressed during training according to SOP-FB-11 (Screening Test for Semen).

d. Discard any frozen aliquots after one (1) year.

E. REFERENCES:

1. Metropolitan Police Forensic Science Laboratory. Biology Methods Manual. 1978, pp.3-17 to 3-20.

2. SOP-GL-2 (Safety Manual).

3. Material Safety Data Sheets.