Document Title: Preparing Body Fluid Standards
Controlled: Yes, with red stamp present
Controlled By: Quality Section

Prepared By: _____ Date: _____

Approved By: _____ Date: _____

A. PURPOSE:

To prepare body fluid standards for the purpose of maintaining quality control of reagents and alternate light sources.

B. RESPONSIBILITY:

Forensic Science Examiners 1 and 2 in the Forensic Biology Section.

C. SAFETY:

Use appropriate measures for the proper handling of biohazardous materials according to the SOP-GL-2 (Safety Manual).

D. DEFINITIONS:

1. PTT: Purple Top Tube
2. KM: Kastle Meyer Test
3. AP: Acid Phosphatase
4. ALS: Alternate Light Source
5. RSID: Rapid Stain Identification
6. ABAcard: Rapid Immunoassay
7. PBS: Phosphate Buffered Saline
8. dH_2O: Distilled water

E. PROCEDURE:

1. Materials
   a. Body fluid samples
   b. PBS
   c. dH_2O
   d. Cloth swatches (white and black)
   e. Filter paper
   f. Swabs
   g. Glass slides
   h. Coin envelopes
   i. Micropipet and tips
   j. Microcentrifuge tubes or test tubes
   k. Purple Top Tubes

E. 2. Procedure:

The following standards are stored in the freezer and replaced as needed:

Semen

a. Preparation of semen standards:
   aa. Aliquot 250µl volumes of neat semen into microcentrifuge tubes labeled with the sample-type.

   bb. Store in a ziplock bag labeled with the sample-type, source (if available) and/or lot # (date of collection) and examiner's initials. (Note on the label if the sample is aspermic).

b. Christmas Tree and Sperm Hy-liter Stain Standards:
   aa. Make a dilution (1:250 suggested) of thawed neat spermic semen in dH_2O and aliquot into microcentrifuge tubes labeled with the sample-type. Re-freeze remaining neat semen aliquot.

   bb. Store in a ziplock bag labeled with the sample-type, date/source of neat collection, lot # (date of preparation) and examiner's initials.

c. AP standards:
   aa. Make a 1:10 dilution of thawed neat semen in dH_2O and aliquot 50µl volumes into microcentrifuge tubes labeled with the sample-type. Re-freeze remaining neat semen aliquot.

   bb. Store in a ziplock bag labeled with the sample-type, date/source of neat collection, lot # (date of preparation) and examiner's initials.

Urine (RSID™-Urine and p30 ABAcard®)

a. Saturate filter paper or swabs with neat female urine and dry overnight in the hood.

b. Place sample made on filter paper into a coin envelope or repackage swabs into the paper sleeves and label with the sample-type, source (if available), lot # (date of preparation) and examiner's initials.

Fecal swabs (AP, p30 ABAcard® and Urobilinogen):

a. Collect fecal material on swabs and dry overnight in hood.

b. Re-package the swabs in the paper sleeves and label with the sample-type, source (if available), lot # (date of collection) and examiner's initials.

E. 2. Breast milk (p30 ABAcard®):

a. Store liquid breast milk in the freezer. Label with the sample-type, source (if available),

lot # (date of collection) and examiner's initials.

b. Thaw as needed and make a stain of the breast milk sample on filter paper. Re-freeze remaining sample.

c. Dry overnight in hood and place into a coin envelope labeled with the sample-type, source (if available), date of collection, lot # (date of preparation) and examiner's initials.

Acid phosphatase standards:

a. Vaginal swabs:
   aa. Collect semen free vaginal samples (minimum of five days post coital) on swabs.

   bb. Dry overnight in hood and re-package the swabs in the paper sleeves.

b. Vaginal/semen mixed swabs:
   aa. Add 100µl of thawed 1:10 semen to each pre-made vaginal swab *or* collect post coital (~ 24 hours) vaginal/semen mixed swabs.

   bb. Dry overnight in hood and re-package the swabs in the paper sleeves.

c. Semen:
   aa. Make a stain on filter paper with 1:10 semen.

   bb. Dry overnight in hood and place into a coin envelope.

d. Oral swabs:
   aa. Collect oral sample on swabs.

   bb. Dry overnight in hood and re-package the swabs in the paper sleeves.

e. Urine Stain:
   aa. Saturate filter paper with urine.

   bb. Dry overnight in hood and place into a coin envelope.

f. Fecal swabs: described on the previous page

g. Negative control: Use blank swabs, filter papers or cloth swatches as needed. Place into coin envelopes.

E. 2. h. Label each acid phosphatase standard with the sample-type, source (if available), lot # (date of collection/preparation) and examiner's initials. Store together in a manila envelope labeled as 'AP' Standards.

Animal standards:

a. Collect blood samples from animal sources on white cloth swatches or filter paper and dry overnight in a designated area.

Place into coin envelopes labeled with the sample-type, lot # (date of collection) and examiner's initials.

b. Commercially available animal sera may be used as positive controls for the corresponding anti-sera. Aliquot 50µl volumes into microcentrifuge tubes labeled with the sample-type.

Store in zip lock bags labeled with the sample-type, lot #, date received and examiner's initials.

The following standards are maintained at room temperature and replaced annually *(one (1) set of the expired standards are retained for research purposes and the remainder are discarded)**:***

Blood (KM, o-Tolidine, Takayama, Ouchterlony, RSID™-Blood and HemaTrace®, crime scene kit):

a. Collect blood in PTT's and make stains on filter papers.

Refrigerate any remaining blood in the PTT labeled with the sample-type, source (if available), lot # (date of collection) and examiner's initials. Replace as needed.

b. Dry overnight in a designated area.

c. Cut and place each piece of stain into a coin envelope labeled with the sample-type, lot # (date of preparation), control date and examiner's initials.

d. Replace the old standards with the new standards in the following sections: Forensic Biology, DNA, Trace (examiners and crime scene kit) and other examiners as necessary.

Semen (AP, RSID™-Semen, p30 ABAcard® and crime scene kit):

a. Make a 1:10 dilution of thawed, neat semen in dH_2O. Re-freeze remaining neat semen aliquot.

b. Saturate each filter paper with approximately 1ml of the 1:10 dilution of semen.

c. Dry overnight in hood.

E. 2. d. Cut and place each piece of stain into a coin envelope labeled with the sample-type, lot # (date of preparation), control date and examiner's initials.

e. Replace the old standards with the new standards in the Forensic Biology Section (examiners and crime scene kit).

Saliva (Phadebas®):

a. Saturate filter papers with saliva.

b. Dry overnight in hood.

c. Cut and place each piece of stain into a coin envelope labeled with the sample-type, lot # (date of preparation), control date and examiner's initials.

d. Replace the old standards with the new standards in the Forensic Biology Section (examiners).

Blood, Semen, Saliva, Urine (alternate light sources):

a. Make blood stains approximately 1" in diameter on black cloth swatches, ensuring that unstained substrate remains around the stain.

b. Make separate saliva, urine and 1:10 semen stains approximately 1" in diameter on filter paper, ensuring that unstained substrate remains around each stain.

c. Dry overnight in a designated area. Cut out the stains made on filter paper leaving unstained substrate around each.

d. Check the new standard with the appropriate alternate light source(s) before use and record the results on the Body Fluid Standard Log Sheet.

e. If appropriate for use, place each into a coin envelope labeled with the sample-type, lot # (date of preparation), control date and examiner's initials.

f. If the appropriate results are not obtained, discard the standard, review the procedure and make a new standard.

g. Replace the old standards with the new standards in the Forensic Biology Section.

Negative controls:

a. Place blank filter paper into coin envelopes labeled with the sample-type, lot # (date of preparation), control date and examiner's initials.

b. Replace the old standards with the new standards in the following sections: Forensic Biology, DNA and Trace (examiners).

E. 2. ***The following standards are stored at room temperature and replaced as needed:***

Christmas Tree and Sperm Hy-liter control smears (may be made in advance as follows):

a. Collect a buccal sample on a swab and form a smear onto a glass slide.

b. With a micropipet, place approximately 3µl of thawed diluted spermic semen onto the smear. Re-freeze the remaining semen aliquot.

c. Dry the positive control smear at room temperature or 37°C (do not apply open flame heat to the Sperm Hy-liter control smears).

d. Label the smears with the sample-type, lot # (date of preparation) and examiner's initials and store in a slide box.

Sperm Hy-liter control swabs

a. Buccal/spermic semen swabs
Collect buccal samples on swabs and add 25µl of thawed, neat semen (spermic) and dry overnight in hood. Re-freeze remaining semen aliquot.

b. Separate buccal and spermic semen swabs
aa. Place 25µl of thawed, neat semen (spermic) onto swabs and dry overnight in hood. Re-freeze remaining semen aliquot.

bb. Collect buccal samples on swabs and dry overnight in hood.

c. Package the swabs in separate coin envelopes and label with the sample-type, lot # (date of preparation) and examiner's initials.

The following standard is prepared for the LP Section as needed:

Latent Print Blood Enhancement Reagents:

a. Label glass slides with the sample-type and lot # (date of preparation).

b. Make fingerprint-type bloodstains from the PTT on the slides and dry overnight in a designated area.

c. Forward to the LP Section for use.

3. For all standards prepared, record the appropriate information on the Body Fluid Standard Reagent Log Sheet.

F. REFERENCES:

SOP-GL-2 (Safety Manual).