BODY FLUID STANDARDS

22.1 PURPOSE

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

22.2 RESPONSIBILITY

Forensic Science Examiners (however titled) in the Forensic Biology Unit.

22.3 SAFETY

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

22.4 DEFINITIONS

A. PTT: Purple Top Tube
B. KM: Kastle Meyer Test
C. AP: Acid Phosphatase
D. ALS: Alternate Light Source
E. RSID™: Rapid Stain Identification
F. ABAcard®: Rapid Immunoassay
G. PBS: Phosphate Buffered Saline

22.5 PROCEDURE

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

21.5.1: Materials

A. Body fluid samples (human unless otherwise specified)
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. Centrifuge tubes or test tubes
K. Purple Top Tubes

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

Semen

A. Preparation of semen standards
   1. Aliquot 250µl volumes of neat semen into centrifuge tubes labeled with the sample-type, lot # (date of collection) and preparer's initials.

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

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

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

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

   2. Store in a plastic bag labeled with the sample-type, date/source of neat collection, lot # (date of preparation) and preparer'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 re-package swabs into the paper sleeves and label with the sample-type, source (if available), lot # (date of preparation) and preparer's initials.

Fecal swabs (AP 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 preparer's initials.

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 preparer's initials.

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

C. Dry overnight in the hood. Place sample made on filter paper into a coin envelope or re-package swabs into the paper sleeves and label with the sample-type, source (if available), date of collection, lot # (date of preparation) and preparer's initials.

Acid phosphatase standards

A. Vaginal swabs
   1. Collect semen free vaginal samples (minimum of five days post coital) on swabs.
   2. Dry overnight in hood and re-package the swabs in the paper sleeves.

B. Vaginal/semen mixed swabs
   1. Add 100µl of thawed 1:10 semen to each pre-made vaginal swab or collect post coital (~ 24 hours) vaginal/semen mixed swabs.
   2. Dry overnight in hood and re-package the swabs in the paper sleeves.

C. Semen
   1. Make a stain on filter paper with 1:10 semen.
   2. Dry overnight in hood and place into a coin envelope.

D. Oral swabs
   1. Collect oral sample on swabs.
   2. Dry overnight in hood and re-package the swabs in the paper sleeves.

E. Urine-described on the previous page.

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.

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

Animal standards

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

   Place sample made on cloth swatch or filter paper into a coin envelope or re-package swabs into the paper sleeves and label with the sample-type, lot # (date of collection) and preparer's initials.

B. Commercially available animal sera may be used as positive controls for the corresponding anti-sera. Aliquot 50µl volumes into centrifuge tubes labeled with the sample-type. Store in zip lock bags labeled with the sample-type, lot #, date received and preparer's initials.

22.5.3: Procedure - 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 preparer'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 preparer's initials.

D. Replace the old standards with the new standards in the following Units: Forensic Biology (include crime scene kit), DNA, Latent Prints and other examiners as necessary.

Semen (AP, 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.

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

E. Replace the old standards with the new standards in the Forensic Biology Unit (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 preparer's initials.

D. Replace the old standards with the new standards in the Forensic Biology Unit (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 preparer'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 Unit.

Negative controls

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

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

22.5.4: Procedure - The following standards are stored at room temperature and replaced as needed:

Christmas Tree and Sperm Hy-liter control smears (if made in advance)

A. Collect an epithelial cell (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 preparer's initials and store in a slide box.

Sperm Hy-liter control swabs (made in advance as needed)

A. Spermic semen/epithelial cell (buccal) swabs
Collect epithelial cell (buccal) samples on swabs and add 25ul of thawed, neat semen (spermic) and dry overnight in hood. Re-freeze remaining semen aliquot.

B. Separate spermic semen and epithelial cell (buccal) swabs
   1. Place 25ul of thawed, neat semen (spermic) onto swabs and dry overnight in hood. Re-freeze remaining semen aliquot.

   2. Collect epithelial cell (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 preparer's initials.

22.6 REFERENCES

GL-2 (Safety Manual)