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

All prepared body fluid standards will be checked prior to use as a standard. Record the appropriate information on the Body Fluid Standard Reagent Log Sheet.

A. If the appropriate results are not obtained, review the procedure and repeat the test. If the standard still does not yield the appropriate result, then discard and make a new standard. If still unable to obtain the appropriate result, then inform the Unit Lead to try to determine the root cause.

B. If the standard is acceptable for use, follow the packaging and storage instructions for each.

The standard is considered acceptable for use when a positive result is obtained with the body fluid being tested and a negative result is obtained with the blank standard being tested.

21.5.1: Materials

A. Body fluid samples (human unless otherwise specified)
B. PBS
C. dH_2O
D. Cloth swatches
E. Filter paper
F. Swabs

G. Glass slides
H. Coin envelopes
I. Micropipette 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 suggested) 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 suggested) into centrifuge tubes labeled with the sample-type. Re-freeze remaining neat semen aliquot.

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

   2. Make a stain on filter paper with a 1:10 dilution of semen.

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

Urine (AP, 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.

AP 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 a 1:10 dilution of semen (previously described) 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 - previously described

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 - previously described

F. Fecal swabs - previously described

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

Animal standards

A. Collect blood samples from animal sources on 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 suggested) 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®)

A. Collect blood in PTT's and make stains on filter paper. 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. Cut the prepared stains into pieces.

C. 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, DNA, Latent Prints and for other examiners as necessary.

Semen (AP and p30 ABAcard®)

A. Make a 1:10 dilution of neat semen (previously described in section 22.5.2 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. Cut the prepared stains into pieces ensuring no unstained filter paper is included.

D. 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 and for other examiners as necessary.

Saliva (Phadebas®)

A. Saturate filter paper with saliva.

B. Dry overnight in hood. Cut the prepared stains into pieces ensuring no unstained filter paper is included.

C. 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 and for other examiners as necessary.

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

A. Make blood stains approximately 1" in diameter on cloth swatches, ensuring that unstained substrate remains around each 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 ALS Standard Log Sheet.

E. If the appropriate results are not obtained, discard the standard, review the procedure and make a new standard. If still unable to obtain the appropriate result, then inform the Unit Lead to try to determine the root cause.

F. If the standard is acceptable for use, place each into a coin envelope labeled with the sample-type, lot # (date of preparation), control date and preparer's initials.

The standard is considered acceptable for use when it demonstrates fluorescence and/or stain detection under the appropriate alternate light source.

G. Replace the old standards with the new standards in the Forensic Biology Unit and for other examiners as necessary.

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 for other examiners as necessary.

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 micropipette, place approximately 3µl of diluted spermic semen (previously described in section 22.5.2 Semen) onto the smear. Re-freeze the remaining semen aliquot.

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

C. Label the smears with the sample-type, lot # (date of preparation) and preparer's initials.

E. Store in a slide box until use.

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

A. Spermic semen/epithelial cell (buccal) swabs
   1. Collect epithelial cell (buccal) samples on swabs and add 25ul neat spermic semen (previously described in section 22.5.2 Semen).
   2. Dry swabs overnight in hood and re-freeze remaining neat semen aliquot.

B. Separate spermic semen and epithelial cell (buccal) swabs
   1. Place 25ul neat spermic semen (previously described in section 22.5.2 Semen) onto swabs and dry overnight in hood. Re-freeze remaining neat semen aliquot.
   2. Collect epithelial cell (buccal) samples on swabs and dry overnight in hood.

C. Package the prepared 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)