

**Connecticut Department of Public Safety  
Division of Scientific Services  
Forensic Laboratory**

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Document Title: Preparing Body Fluid Standards

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**A. PURPOSE:**

To prepare body fluid standards for the purpose of maintaining quality control of reagents.

**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. RTT: Red 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

**E. PROCEDURE:**

1. Materials
  - a. Body fluid samples
  - b. PBS
  - c. Distilled water (dH<sub>2</sub>O)
  - d. Cloth swatches (white and dark/black)
  - e. Filter paper
  - f. Swabs
  - g. Glass slides
  - h. Coin envelopes
  - i. Micropipet and tips
  - j. Microcentrifuge tubes or test tubes

E. 2. Procedure:

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

Semen

- a. For the preparation of semen standards:
  - aa. Aliquot 250µl volumes of neat semen into microcentrifuge tubes labeled with the sample-type.
  - bb. Store in a zip lock 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. For Christmas Tree Stain:
  - aa. Make a 1:500 dilution of thawed neat semen (spermic) in dH<sub>2</sub>O and aliquot 50µl volumes into microcentrifuge tubes labeled with the sample-type. Re-freeze remaining neat semen aliquot.
  - bb. Store in a zip lock bag labeled with the sample-type, date/source of neat collection, lot # (date of preparation) and examiner's initials.
- c. For p30 Ouchterlony and AP standards:
  - aa. Make a 1:10 dilution of thawed neat semen in dH<sub>2</sub>O and aliquot 50µl volumes into microcentrifuge tubes labeled with the sample-type. Re-freeze remaining neat semen aliquot.
  - bb. Store in a zip lock bag labeled with the sample-type, date/source of neat collection, lot # (date of preparation) and examiner's initials.

Fecal swabs (for AP, p30 ABACard® and Urobilinogen):

- a. Collect fecal material on swabs and dry overnight in hood.
- b. Re-package the swabs in the sleeves that they were removed from and label with the sample-type, source (if available), lot # (date of collection) and examiner's initials.

Breast milk (for AP and 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, date/source of collection, lot # (date of preparation) and examiner's initials.

E. 2. 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 sleeves from which they were removed.
  - 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. Alternative: make a 1:10 dilution of thawed, neat semen in dH<sub>2</sub>O. Re-freeze remaining neat semen aliquot.
    - cc. Dry overnight in hood and re-package the swabs in the sleeves from which they were removed.
  - 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 sleeves from which they were removed
  - e. Urine Stain:
    - aa. Saturate filter paper with urine.
    - bb. Dry overnight in hood and place into a coin envelope.
  - f. Serum:
    - aa. Collect a RTT of blood, centrifuge or store upright to allow serum to separate.
    - bb. Draw off serum and make a stain on a cloth swatch or filter paper.
    - cc. Dry overnight in hood and place into a coin envelope.
  - g. Fecal swabs: described on the previous page
  - h. Breast milk: described on the previous page
- E. 2.
  - i. Negative control: Use blank swabs, filter papers or cloth swatches as needed. Place into coin envelopes.
  - j. 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 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 50ul 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 are maintained at room temperature and replaced annually:***

Blood

- a. For KM, o-Tolidine, Takayama, Ouchterlony, RSID<sup>TM</sup>-Blood and HemaTrace®, crime scene kit:

- aa. Collect one (1) PTT of blood 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.

- bb. Dry overnight in a designated area and cut into thirds.

- cc. Place each into a coin envelope labeled with the sample-type, lot # (date of preparation), expiration date and examiner's initials.

- dd. Distribute to the following sections: Forensic Biology, DNA, Trace and Chemistry (examiners and crime scene kit).

- b. For Collodion and Luminol spray reagents:

- aa. Make a 1:100 dilution of blood from the PTT, in PBS.

- E. 2.
  - b.
    - bb. Saturate a filter paper with the diluted blood and dry overnight in a designated area.

- cc. Place into a coin envelope labeled with the sample-type, Lot # (date of preparation), expiration date and examiner's initials.

- dd. Distribute to the Forensic Biology Section.

- c. For alternate light sources:

- aa. Make blood stains approximately 1" in diameter on black or dark cloth swatches, ensuring that unstained substrate remains around the stain.
- bb. Dry overnight in a designated area.
- cc. Place each into a coin envelope labeled with the sample-type, lot # (date of preparation), expiration date and examiner's initials.
- dd. Distribute to the Forensic Biology Section.
- d. For Latent Print Blood Enhancement Reagents:
  - aa. Label glass slides with the sample-type and lot # (date of preparation).
  - bb. Make fingerprint-type bloodstains from the PTT on the slides.
  - cc. Dry overnight in a designated area.
  - dd. Place into a slide box with a divider rack, separating each slide.
  - ee. Label the box with the sample-type, lot # (date of preparation), expiration date and examiner's initials.
  - ff. Distribute to the Latent Print Section.

Semen

- a. For AP, RSID<sup>TM</sup>-Semen and p30 ABACard<sup>®</sup>:
  - aa. Make a 1:10 dilution of thawed, neat semen in dH<sub>2</sub>O. Re-freeze remaining neat semen aliquot.
  - bb. Saturate each filter paper with approximately 1ml of diluted semen. (set aside extra).
  - cc. Dry overnight in hood and cut into halves.
- E. 2. a. dd. Place each into a coin envelope labeled with the sample-type, lot # (date of preparation), expiration date and examiner's initials.
- ee. Distribute to the Forensic Biology Section (examiners).
- b. For alternate light sources and crime scene kit:
  - aa. Make separate stains approximately 1" in diameter on filter paper with the remaining 1:10 semen, ensuring that unstained substrate remains around each.
  - bb. Dry overnight in hood and cut out stains leaving unstained substrate around each.

- cc. Place each into a coin envelope labeled with the sample-type, lot # (date of preparation), expiration date and examiner's initials.
- dd. Distribute to the Forensic Biology and DNA Sections.

Saliva

- a. For Phadebas<sup>®</sup>:
  - aa. Saturate filter papers with saliva.
  - bb. Dry overnight in hood and cut into halves.
  - cc. Place each into a coin envelope labeled with the sample-type, lot # (date of preparation), expiration date and examiner's initials.
  - dd. Distribute to the Forensic Biology Section (examiners).
- b. For alternate light sources:
  - aa. Make separate saliva stains approximately 1" in diameter on filter paper, ensuring that unstained substrate remains around each.
  - bb. Dry overnight in hood and cut out stains leaving unstained substrate around each.
  - cc. Place each into a coin envelope labeled with the sample-type, lot # (date of preparation), expiration date and examiner's initials.
  - dd. Distribute to the Forensic Biology and DNA Sections.

Urine

- a. For Jaffe and p30 ABACard<sup>®</sup>:
    - aa. Saturate filter papers with female urine.
    - bb. Dry overnight in hood and cut into halves.
    - cc. Place each into a coin envelope labeled with the sample-type, lot # (date of preparation), expiration date and examiner's initials.
    - dd. Distribute to the Forensic Biology Section (examiners).
  - b. For alternate light sources:
    - aa. Make separate urine stains approximately 1" in diameter on filter paper, ensuring that unstained substrate remains around each.
    - bb. Dry overnight in hood and cut out stains leaving unstained substrate around each.
- E. 2.
- a. aa. Saturate filter papers with female urine.
  - bb. Dry overnight in hood and cut into halves.
  - cc. Place each into a coin envelope labeled with the sample-type, lot # (date of preparation), expiration date and examiner's initials.
  - dd. Distribute to the Forensic Biology Section (examiners).
  - b. For alternate light sources:
    - aa. Make separate urine stains approximately 1" in diameter on filter paper, ensuring that unstained substrate remains around each.
    - bb. Dry overnight in hood and cut out stains leaving unstained substrate around each.

- cc. Place each into a coin envelope labeled with the sample-type, lot # (date of preparation), expiration date and examiner's initials.
- dd. Distribute to the Forensic Biology and DNA Sections.

Negative controls:

- a. Place blank filter paper into coin envelopes labeled with the sample-type, lot # (date of preparation), expiration date and examiner's initials.
- b. Distribute to the following sections: Forensic Biology, DNA, Trace and Chemistry (examiners).

*The following is stored at room temperature and replaced as needed:*

Sperm Hy-liter control swabs:

- a. Add 25µl of thawed, neat semen (spermic) to each unstained swab and dry overnight in hood. Re-freeze remaining semen aliquot.
  - b. 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.
3. For all standards prepared, record the appropriate information on the Body Fluid Standard Reagent Log Sheet.

**F. REFERENCES:**

SOP-GL-2 (Safety Manual).