7 Semen Detection

Please refer to SOP #4 (Reagent Quality Control) for quality control testing, storage, labeling, and expiration guidelines, of the Acid Phosphatase Spot Test, Alternate Light Source, SERI Christmas Tree Stain, Phosphate Buffered Saline (1X), and SERATEC® PSA Semiquant test devices.

Many samples submitted to the Houston Police Department Crime Laboratory are tested for the presence of semen. Generally, a sample will be subjected to a presumptive acid phosphatase test, followed by a confirmatory microscopic examination for the presence of spermatozoa. If the sample is not microscopically positive, a third test, the test for the presence of prostate-specific antigen, may be warranted and executed.

7.1 Alternate Light Source

Scope

The Alternate Light Source (ALS) lamps within this lab will be used for detection of forensic semen stains. These lamps provide intense light of specific wavelengths through a hand-held wand.

Some dried semen stains on cloth are detectable visually because their color, off-white or yellow, is different from that of the material on which the semen has been deposited. However, on some substrates, semen stains are not readily visible. Under intense blue light (~450 nm), semen stains typically fluoresce. Blood absorbs light and will not fluoresce; fibers and other bodily fluids can typically fluoresce. Should fluorescence take place, the result only suggests the presence of semen; the test is therefore a presumptive test.

Equipment, Materials, and Reagents

- Alternate light source (ALS) set at 450-455 nm.
- Orange and/or yellow glasses

Procedure

1. Turn on power switch and ensure the fans are working.
2. Wear the proper glasses given and darken the examination room.
3. Turn on lamp switch.
4. Direct the ALS at the evidence samples.
5. Document areas of apparent fluorescence on the evidence.

When ready to shut down the machine, turn off lamp switch and wait for the unit to cool down (~5 minutes). Then turn off the power switch.

Interpretation

Should a stain fluoresce, the result only indicates the possible presence of semen.

Substrate controls may be included in body fluid identification tests as appropriate.

7.2 Acid Phosphatase Spot Test

Scope

Acid phosphatase (AP) is found in relatively large quantities in semen and its presence is indicative of the possible presence of semen in a stain. In the following procedure, acid phosphatase is detected by a color-change reaction. Acid phosphatase liberates the phosphate from α-naphthyl phosphate, and the released naphthol combines with tetrazotized o-dianisidine to form a purple azo dye.

The AP test is semi-quantitative. A stronger reaction is more likely to indicate semen. However, because acid phosphatase occurs in other body fluids, most notably vaginal secretions, this is only a presumptive test. The presence of semen in the sample can subsequently be confirmed by the presence of spermatozoa.

Equipment, Materials, and Reagents

- Sterile diH_2O
- Sterile cotton swab(s) or filter paper
- AP Spot Test reagent
- Timer
- Non-porous surface
- Clips (optional)

AP Procedure for Swabs

Note: Begin at step 1 when it is necessary to transfer the suspected semen sample from an item of evidence to a cotton swab or swabs for ease of testing. Otherwise, begin at step 3 following the procedure below:

1. Moisten cotton swab(s) with sterile diH_2O.
2. Swab the suspected semen stain from the evidence.
3. Moisten filter paper with sterile diH_2O.
4. Press the swabs firmly onto the filter paper.
5. Apply one or two drops of the AP Spot Test reagent to the filter paper.
6. Observe the treated filter paper for up to 60 seconds for a color change.
7. After interpretation, document the test results onto the serology worksheet(s).
8. Discard used filter paper by placing it in the biohazard trash.

AP Mapping Procedure

This procedure can be used when larger items of evidence are presented.

1. Spread the clothing item, bedding, towel, or other item flat onto a non-porous surface (use clips if necessary).
2. Moisten filter paper with sterile diH_2O.

3. Lay the moist filter paper over the item or area of the item to be tested. Press the paper firmly against the item for approximately 30 seconds to 5 minutes to allow for any possible acid phosphatase to transfer to the paper.
4. Mark the position of the paper on the item.
5. Remove the filter paper.
6. Spray or apply drop(s) of AP test reagent to the filter paper.
7. Observe the treated filter paper for up to 60 seconds for a color change.
8. After interpretation, mark the tested areas with results.
9. Discard used filter paper by placing it in the biohazard trash.

Interpretation

Should a color reaction take place within 60 seconds, the result only indicates the possible presence of semen; the test is therefore a presumptive test.

Grade the reaction according to the time it takes for the color to appear as follows:

- 0-15 seconds: 4+
- 16-30 seconds: 3+
- 31-45 seconds: 2+
- 46-60 seconds: 1+
- No change, or color change after more than 60 seconds: negative

A negative result indicates acid phosphatase is absent or below the detection threshold.

Substrate controls may be included in body fluid identification tests as appropriate.

7.3 Body Fluid Extraction

Scope

Possible body fluid stains are removed from the substrate and dissolved in PBS for testing. The extract is used to collect cellular debris from a stain for spermatozoa examination and can also be used for p30 testing.

Microscopic examination for spermatozoa or p30 testing may be skipped if, in the analyst's opinion, consumption for these tests will reduce the potential success of subsequent DNA analysis. Samples that are of limited size may be documented as Quantity Not Sufficient for Further Analysis ("QNS"). Only a presumptive screening result will be reported but DNA analysis may be performed.

Equipment, Materials, and Reagents

- Microcentrifuge tube(s)
- Forceps (optional)
- Scalpel and/or scissors

- Mini-centrifuge
- 1X PBS
- Pipette and pipette tips
- Ultrasonic cleaner

Procedure

1. Add sample to a microcentrifuge tube. The analyst must exercise good judgment regarding the amount of sample to be portioned for stain extraction, providing sufficient sample for DNA analysis. Stain intensity and size are a few characteristics that should be considered. Generally, up to approximately ¼ of a swab or up to approximately (1 cm)^2 of a stain may be consumed for this procedure.
2. Add approximately 200 µl of 1X PBS to the sample. More PBS may be required to ensure the sample is fully submerged.
3. The extract(s) may be placed at room temperature in an ultrasonic cleaner for 5-15 minutes. Alternatively, the extracts may be soaked for 30 minutes - 24 hours in refrigerated storage.
4. Centrifuge the extract(s) briefly.
5. The extract, containing any cellular debris, will be used for microscopic spermatozoa examination and may also be used for p30 testing.
6. Store the extract refrigerated, when necessary, for up to 24 hours. Beyond 24 hours the extract should be stored frozen.

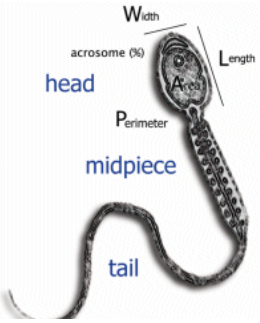
Substrate controls may be included in body fluid identification tests as appropriate.

7.4 Microscopic Spermatozoa Examination

Scope

Spermatozoa (sperm) detected on an evidence sample confirm the presence of semen; the test is therefore a confirmatory test. This procedure uses the SERI Christmas Tree Stain which is a Kernechtrot-Picroindigocarmine differential biological stain that assists in scanning slides for the presence of spermatozoa. The test consists of a two stain method which dyes the tip of sperm heads pink, the bottom of sperm heads red, the tails green or blue and any epithelium green with red nuclei.

Human sperm are flagellated with a total length of about 50 µm. The sperm cell head generally is oval, flattened at the anterior end, with dimensions about 4.6 µm x 2.6 µm x 1.5µm.



Identifying characteristics for spermatozoa:

1. Size (head is approximately 4.6 µm x 2.6 µm x 1.5µm)
2. Morphology (oval to round or teardrop-shaped structure)
3. Acrosomal cap at tip of sperm (unstained or lightly stained)
4. Staining (dark pink to red head, with unstained or lightly stained acrosomal cap; tails green or blue, if present)
5. Presence of a flagella or tail (may not be present)

EQUIPMENT, MATERIALS, AND REAGENTS

- Microscope slide
- Body fluid extract
- Pipette and pipette tips
- Heat block
- Christmas Tree Stain - Solution A and Solution B
- Timer
- Tap water
- 95% ethanol
- Compound microscope
- Immersion oil

Procedure

If slide has already been prepared, proceed to step #4.

1. Pipette 10 µl of the supernatant from the body fluid extract onto a glass slide. Fix smear by heating the slide on the heat block for approximately 5 minutes.
2. Stain the slide.
    a. Cover the sample area on the slide with Solution A for 10 minutes.
    b. Wash with tap water by gentle flooding.
    c. Cover the sample area with Solution B for 15 seconds.
    d. Wash with tap water by gentle flooding.

e. Flood the slide with 95% ethanol and allow the slide to dry for at least 5 minutes on the heat block.

3. Examine the slide for the presence of spermatozoa at 1000X magnification.
4. Document the approximate number of sperm seen on the slide. Once the count exceeds 25 spermatozoa observed, the documented count can be "25+", ">25", or "TNTC". The presence of only one spermatozoon must be verified by a second qualified analyst who must initial and date the examination documentation.
5. If too much cellular material and/or debris is potentially obscuring any spermatozoa on the slide for the analyst to make a determination about the presence or absence of sperm, the following procedure may be performed:
   a. Centrifuge the sample to pellet any cells. Remove and save supernatant for p30 analysis.
   b. Wash the cell pellet 1x with 500µl of sterile diH_2O or 1X PBS, pipetting the pellet up and down or vortexing.
   c. Centrifuge again to pellet any cells.
   d. Re-suspend in 50 to 300µl of sterile diH_2O or 1X PBS, again by pipetting up and down or vortexing.
      a. Use the new suspension to make another slide, repeating steps 2-5. Examination documentation must reflect the creation of multiple slides.

INTERPRETATION

Sperm heads stain red and the tails stain green by this procedure. A minimum of one sperm head should be identified to confirm the presence of semen. A search of the entire slide is required before negative results for spermatozoa can be reported. If the slide contains too much cellular material or debris for the analyst to determine the presence or absence of sperm, the analyst may then report the result as "inconclusive". Alternatively, if sperm-like objects are observed, but one or more of the identifying characteristics are lacking, the slide may be reported as "inconclusive".

It is recommended that "inconclusive" samples proceed to DNA analysis, along with positive samples.

7.5 SERATEC® PSA (p30) Semiquant Test

Scope

The cells that line the ducts of the prostate make a glycoprotein known as p30 or prostate-specific antigen (PSA). The protein is secreted into seminal fluid to a concentration of approximately 0.2 - 3.0 mg/ml, and its detection may indicate the presence of semen. PSA may be found at very low concentrations in vaginal fluid (0.4-1.25 ng/ml) and is therefore a presumptive test.

7 Semen Detection

The SERATEC® PSA Semiquant test is a detection method specifically designed for forensic identification of p30. This test will be used qualitatively only in this laboratory. The test is capable of detecting p30 in a concentration range of at least 2ng/ml - 100μg/ml PSA.

SERATEC® PSA Semiquant test contains two monoclonal murine anti-PSA antibodies as active compounds. One of these antibodies is immobilized at the test region on the membrane. The upstream control region and the region of the internal standard (between control and test region) contain immobilized polyclonal goat anti-mouse antibodies. The amount of antibody at the internal standard is adjusted to a color intensity of the line, which is equal to the color intensity of the test line at a PSA concentration of 4 ng/ml. A glass fiber pad downstream of the membrane is used for sample loading and transmission to a second fiber pad with the dried and gold-labeled second monoclonal murine anti-PSA antibody. PSA at the sample will bind to the remobilized gold-labeled antibody and form a PSA-gold-labeled-anti-PSA-antibody complex.

Through the capillary effect of the membrane, the reaction mixture including the complex mixture is carried upwards with the fluid. In any case, the colored gold-labeled anti-PSA-antibody will bind to the anti-mouse-antibody at the control region and the region of the internal standard, thus developing two red lines (one at the control region and one at the region of the internal standard). These two lines are independent of the existence of PSA in the sample and indicate only the correct execution of the test.

If the sample contains PSA, the PSA-gold-labeled- anti-PSA-antibody complex will bind to the immobilized monoclonal antibody of the test result region that recognizes another epitope on the PSA molecule (sandwich complex). The binding is indicated by the formation of an additional line. Thus a PSA positive sample will show three colored lines in the result window.

Equipment, Materials, and Reagents

1. SERATEC® PSA Semiquant test device – one per sample
2. Body fluid extract
3. Pipettes and pipette tips
4. Timer

Procedure

1. Allow previously extracted sample(s) to warm to room temperature if they have been refrigerated.
2. For each sample, unwrap and label a SERATEC® PSA Semiquant test device.
3. Add 120 μl of the supernatant to the sample well of the device.
4. Results may be read up to 10 minutes from application of the supernatant.
5. Record the SERATEC® PSA Semiquant test results on the Serology Results worksheet.
6. Have a second qualified analyst examine the SERATEC® PSA Semiquant test device and verify the documented test result(s) by initialing and dating the appropriate Serology Results worksheet.

Interpretation

There are three lines that appear in the test device “result window”:

1. **Control "C" line:** the presence of this line confirms the integrity of the test components and the test procedure. It is expected for all tests and must be present. Absence of the "C" line is an invalid result and the test should be repeated if adequate sample remains.
2. **Internal Standard line:** The intensity of this line correlates with a p30 concentration of approximately 4 ng/ml. It is expected for all tests and must be present. The absence of the internal standard line is also an invalid result and the test should be repeated if adequate sample remains.
3. **Test "T" line:** A line indicates the presence of semen and should be documented as a positive result. The absence of a line or a line that appears after 10 minutes is a negative result or p30 concentration is below the detection limit.

Samples that produce a negative result but show strong positive acid phosphatase activity must be diluted to an appropriate dilution and be re-tested to ensure the High Dose Hook Effect is not occurring (see SOP #6.3 ABAcard® HemaTrace® Test Devices).

7.6 Guidelines for Sexual Assault Cases When Contact or Digital Penetration is Alleged

It is not possible to anticipate every situation that may arise or to prescribe a specific course of action for every case; therefore, the examiner must exercise good judgment based on experience and common sense. The following are intended only as guidelines for cases in which digital penetration or other contact may have occurred. Specific case information may suggest a course of action other than what is prescribed below.

Competent (ex: can clearly recall incident):

External swabs with clear notes on why it was collected (ex: "he kissed my breast"):

- Bathed: NTC, add bathed statement in report
- Not bathed: retain

External swabs without clear notes on why it was collected (outer envelope only states "Breast swabs"):

- Press-out; do not retain negatives

Intimate swabs:

Penile and other penetration:

- Within 24 hours, snippet test; retain regardless of semen results
- Beyond 24 hours, press-out; do not retain negatives

Non-penile penetration:

- Within 24 hours, retain
- Beyond 24 hours, NTC

Incompetent (ex: mentally challenged/infants/children):

- Snippet test, retain regardless of semen results

Unconscious (ex: doesn't know what happened, drunk/drugged):

- External swabs:
  - Bathed: NTC, add bathed statement in report
  - Not bathed: retain
- Intimate swabs:
  - Press-out; do not retain negatives

- Unless specifically states a digital/contact situation:
  - Within 24 hours, snippet test; retain regardless of semen results
  - Beyond 24 hours, press-out; do not retain negatives

Any information indicating that semen may be present should always be considered and samples processed accordingly, regardless of above guidelines (ex: "he ejaculated on my neck", shorts, etc.).

7.7 References

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