1.5 Semen and Seminal Fluid Detection

Semen is comprised of seminal fluid and spermatozoa (sperm cells). The biochemical and immunological tests identify the seminal fluid component only. Microscopic analysis and the identification of sperm cells are confirmatory for the presence of semen.

1.5.1 Alternate Light Source (ALS)/Ultraviolet (UV) Light

1.5.1.1

Emitted fluorescence may aid in the location of stains believed to contain seminal fluid. The fluorescence helps merely to localize areas for more detailed examination; it does not have any value by itself in the identification of any specific body fluid stain.

1.5.1.2

Lack of fluorescence does not prevent additional testing from being performed, if deemed necessary.

1.5.1.3

Screening items such as clothing or bedding for the presence of seminal fluid stains may be facilitated by the use of an alternate light source (ALS). Alternate light sources include a UV light (sometimes referred to as a "Wood's Lamp" by Forensic Nurses), the Omnichrome, Mini-Crimescope MCS-400, or Foster+Freeman Crime-Lite® 82S, to name a few. Users must read the directions accompanying each ALS in order to learn the best combination of wavelengths and filters, to avoid damaging the instrument during start up and shut down, and to protect their eyes from the powerful light. The use of appropriate goggles (dependent on the ALS) helps to make the reaction detectable to the eye, while simultaneously protecting the eyes from the light source. If proper eye protection is not worn, permanent damage to the eye may occur.

The principle behind the light sources is that biological fluids may react to light between 450 and 455 nm wavelengths, while wearing the appropriate filter glasses or goggles. The reaction may either appear as a light stain against a dark background, or in some circumstances, the stain appears darker against a light background. The reaction must be interpreted with caution since other substances (such as, but not limited to, urine, saliva, makeup, yogurt, cleaners, bleach alternatives, etc.) may also react to an ALS. Samples exhibiting a reaction to an ALS require further examination to indicate the presence of seminal fluid or semen. ALS shall not be the sole determination of the absence of semen and/or seminal fluid on items where a mixed body fluid stain is a potential (i.e. blood semen/seminal fluid mixtures). It should also be noted that stains may not fluoresce on certain fabrics (ex.) black fabric and red fabrics).

1.5.1.4 References

The Use of Polilight in the Detection of Seminal Fluid, Saliva, and Bloodstains and Comparison with Conventional Chemical-Based Screening Tests, Vandenberg et al, Journal of Forensic Science Vol. 51 #2, March 2006

Virginia Division of Forensic Science, Forensic Biology Procedure Manual (Book 2 of 2), Case Approach and Identification of Biological Substances: Other Biological Samples, Effective Date 3-14-06, p 6 of 9.

Foster+Freeman Crime-Lite® 82S: Narrowband Light Sources (Visible Light) User Guide

1.5.2 Qualitative Acid Phosphatase

1.5.2.1

The Acid Phosphatase test is a preliminary chemical test used to screen stains for the presence of seminal fluid. This test is based on the detection of acid phosphatase, a major component of seminal fluid. In the presence of acid phosphatase, the α-naphthol diazotizes with the dye to yield a colored azo-dye.

1.5.2.2 Safety Considerations

Glacial acetic acid - CAUTION - Corrosive. Flammable.

Sodium Acetate - CAUTION - Irritant.

α Naphthyl Phosphate - CAUTION - Irritant. Emits toxic fumes under fire conditions.

Diazo Blue B - CAUTION - Highly Toxic. Emits toxic fumes under fire conditions.

1.5.2.3 Reagents

Buffer
2 g Sodium Acetate
0.5 mL Glacial Acetic Acid
QS to 100 mL with dH_2O

Reagent 1
α Napthyl Phosphate (Calcium, Sodium or Potassium)
Buffer
Prepare a solution of α Napthyl Phosphate (Calcium, Sodium or Potassium) - with a concentration of approximately 1 mg of α Napthyl Phosphate in 1 mL of buffer
Make daily or freeze and protect from light

Reagent 2
Diazo Blue B
Buffer
Prepare a solution of Diazo Blue B - with a concentration of approximately 1 mg of Diazo Blue B in 1 mL of buffer. Make daily or freeze and protect from light.

1.5.2.4 Controls

Positive and negative controls shall be used for function verification. These will be run at the beginning of processing for the day. The positive control shall be a known seminal fluid sample. The control results and the date run shall be recorded.

1.5.2.5 Procedure

Moisten filter paper/swab with distilled water. Press the filter paper against the suspected stain or gently rub the stained area with the moistened swab. Alternatively, a small piece of the stain/swab can be removed and the reagents directly applied.

Add Reagent 1 (clear solution) followed by Reagent 2 (colored solution) in a 1:1 ratio.

Observe for a color reaction within 30 seconds.

1.5.2.6 Results and Interpretation

Positive reaction: Purple color
Negative reaction: No color development or pink/slow color development
Inconclusive reaction: Development of a color other than that specified for a positive or negative reaction or a failure of the controls.

1.5.2.7 References

Kind, S.S., The Acid Phosphatase Test, Methods of Forensic Science, Volume Three, 1964.

Kirk, Paul, Identification of Miscellaneous Physiological Fluids, Crime Investigation, 1953.

Metropolitan Police Forensic Science Laboratory Biology Methods Manual, 1978, Chapter 3, p.3-18.

1.5.3 Microscopic Identification Of Spermatozoa (Christmas Tree Stain)

1.5.3.1

The nuclear material in sperm heads and epithelial cells are stained red by the Kernechtrot's solution and the protein material in the epithelial membranes and the sperm tails are stained green by the Picroindigocarmine solution.

1.5.3.2 Safety Considerations

Saturated picric acid - CAUTION - Explosive when dry. DO NOT PURCHASE DRY PRODUCT!!!

1.5.3.3 Reagents

Kernechtrot Stain (red)
5 g Aluminum Sulfate

100 mL Distilled Water
0.1 g Nuclear Fast Red
Heat the water to boiling. Remove from heat. Add reagents and stir. Filter.

Picroindigocarmine Stain (green)
300 mL saturated picric acid
1 g of Indigo Carmine dye
Dissolve 1 g of Indigo carmine dye in the picric acid solution. Swirl to mix. Filter.

Alternatively, the premade reagents (Kernechtrot and Picroindigocarmine) may be purchased from an approved vendor.

1.5.3.4 Controls

A positive control shall be used for function verification. This will be carried out for each lot of stain that is generated or purchased. The function verification shall be repeated monthly for the duration of the lot of stain. A slide shall be prepared of buccal cells mixed with human semen and staining the slides as outlined in procedure 1.5.3.5. The presence of appropriately stained cellular material as outlined in 1.5.3.1 shall be noted in the documentation. If the lot of stain is divided or shipped separately, each aliquot of stain must be verified.

1.5.3.5 Procedure

Cover smear with Kernechtrot's stain (red) for a minimum of 10 minutes.

Wash away stain thoroughly with distilled water.

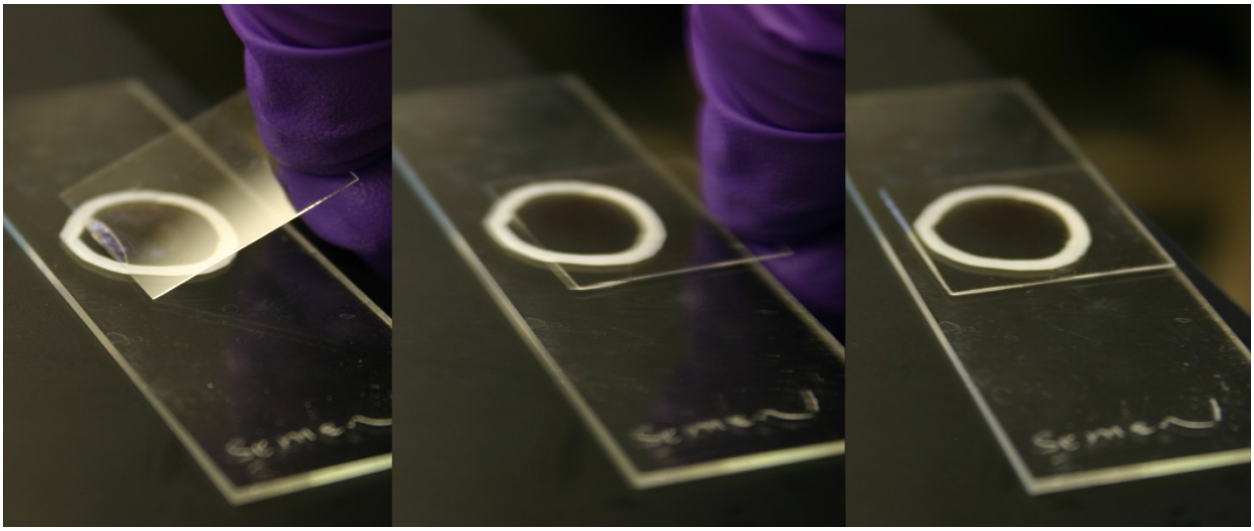
Cover smear with Picroindigocarmine stain (green) for approximately 15 seconds.

Wash stain off with ethyl alcohol.

Allow smears to dry.

Observe using a microscope. All stained smears must be initially viewed using a standard laboratory microscope. This initial microscopic examination is not intended to be an exhaustive search for spermatozoa covering a significant portion of the stained smear. Rather, it is intended to be a brief scan of the stained smear to identify readily observable spermatozoa. If spermatozoa are observed during this initial review, completion of the microscopic examination may follow procedure 1.5.3.6. If spermatozoa are not observed during this initial review, completion of the microscopic examination may follow procedure 1.5.3.6.

If viewing the slide at larger magnifications (100X) place a drop of immersion oil on the slide. Holding the cover slip at an angle, bring the edge up to the oil and after making contact with the oil slowly lower the cover slip. As the cover slip is almost all the way down, slide it slowly so it is not hanging over the slide. This technique reduces the formation of bubbles when lowering the slip down onto the drop parallel to the slide.



1.5.3.6 Spermatozoa Documentation and Results-Manual Microscopic Exam

Scan the slide for the presence of spermatozoa and/or cellular material.

Without phase microscopy: Spermatozoa heads are neon-like pink/red with pale pink (almost colorless) acrosomal caps, blue-green necks/midpieces, and green tail. Epithelial cells appear bright blue with red to purple nuclei.

With phase microscopy: Spermatozoa heads are neon-like pink/red with darker pink/purple acrosomal caps and green tails. Epithelial cells and most bacteria stain green with some of the nuclei pink/red; however, these are shaped differently than spermatozoa. Yeast cells take on the same color as spermatozoa but are shaped differently.

Document the approximate average number of spermatozoa observed/field or slide. All estimates for spermatozoa quantitation shall be conducted at a 200x total magnification or higher.

Examples:
1-5 sperm/field
1 sperm/5 fields
5 sperm/slide

Document if spermatozoa are intact and any other helpful information about the slide such as bacteria or white blood cells present.

The observation of one or more spermatozoa heads is confirmatory for the presence of semen. If only 1 spermatozoa or spermatozoa head is observed, there must be documented confirmation of its presence by a second qualified examiner.

1.5.3.7 References

Allery et al, Cytological Detection of Spermatozoa: Comparison of Three Staining Methods, Journal of Forensic Science Volume 46, #2, 2001.

Oppitz E., Eine neue Farbemthode zum Nachweis der Spermien bei Sittlichkeitsdelikten. Arch Kriminol 1969; p. 144, 145-148.

1.5.4 ABAcard p30 - Immunological Test for Seminal Fluid

1.5.4.1

Prostate specific antigen (PSA) is a glycoprotein produced in the prostate and secreted into seminal fluid. PSA is found in high levels in seminal fluid but can also be found in other body fluids. Therefore, it is used to indicate the presence of seminal fluid.

Caution: The PSA level for healthy individuals has been reported to be approximately 4.0 ng/mL of liquid urine, significantly below the detection level of this test. However, urine PSA levels may vary significantly and in extreme cases exceed the detection level of this test in presumably healthy male individuals.

1.5.4.2 Materials

OneStep ABAcard PSA (p30) from Abacus Diagnostics

1.5.4.3 Controls

Positive and negative controls shall be used for function verification. These will be run prior to the use of the kit lot. Future shipments of the same lot number will still need controls run for function verification to account for potential differences in shipping conditions. The positive control shall be a known seminal fluid sample. The control results and the date run shall be recorded.

1.5.4.4 Procedure

Extract a portion of the stain in 300 µl of dH_2O or HEPES buffered saline for 2 hours at room temperature.

Centrifuge for 3 minutes (this supernatant can be stored at 2-8 C but must be brought to room temperature before proceeding with the test).

Remove the test card from the package and label the card for identification purposes.

Add 200 µl of extract to the sample well 'S' of the test card.

Read results at 10 minutes.

1.5.4.5 Results and Interpretation

Positive reaction: If there are two pink lines, one each in the test area 'T' and in the control area 'C'.
Negative reaction: If there is only one pink line in the control area 'C'. Note: If preliminary test (i.e. acid phosphatase) results are a strong positive, there may be the presence of "high dose hook effect". The test may be repeated using a higher sample dilution.
Invalid reaction: If there is no pink line in the control area 'C'. (The test should be repeated if determined to be invalid.)

1.5.4.6 References

Benton, K.A., Donahue, J.A., and Valadez, Jr., M. Analysis of the ABAcard OneStep p30 Test For Use in the Forensic Laboratory Presented at the 1998 Spring Meeting of SWAFS.

Hochmeister, M., et al, Evaluation of Prostate-Specific Antigen (PSA) Membrane Test Assays for the Forensic Identification of Seminal Fluid, J. For. Sci. 1999, Vol 44, pp 1057-1060.

Sokoll, L.J., Chan, D.W. Prostate-Specific Antigen: its discovery and biochemical characteristics Urologic Clinics of North America v24(2), p. 253-9, 1997

Annbruster, D.A. Prostate-Specific Antigen: Biochemistry, Analytical Methods, and Clinical Application Clinical Chemistry v39(2), p. 181-95, 1993

Engelmann, U.H., Schramek, P., Tomamichel, G., Deindl, F., Senge, T.H. Vasectomy Reversal in Central Europe: results of a questionnaire of urologists in Austria, Germany, and Switzerland. J Urology v143(1), p. 64-7, 1990

Graves, H.C.B., Sensabaugh, G.F., Blake, E.T., Postcoital Detection of a Male-Specific Semen Protein: application to the investigation of rape New. Engl. J. Med. V312(6), p. 338-43, 1985

Sensabaugh, G.F. Isolation and Characterization of a Semen-Specific Protein from Human Seminal Plasma: a potential new marker for semen identification. J. For. Sci. v23(1), p. 106-15, 1978

Spear, T.F. et al, "The Evaluation of ABAcard® p30 Test for the Identification of Semen":, CAC News, 1st Quarter 2001, pp 22 - 24.

Abacus Diagnostics ABAcard® p30 Test for the Forensic Identification of Semen Technical Information Sheet.

1.5.5 Seratec® PSA Semiquant - Immunological Test for Seminal Fluid

1.5.5.1

Prostate specific antigen (PSA) is a glycoprotein produced in the prostate and secreted into seminal fluid. PSA is found in high levels of seminal fluid but can also be found in other body fluids. Therefore, it is used to indicate the presence of seminal fluid.

Caution: The PSA level for healthy individuals has been reported to be approximately 4.0 ng/mL of liquid urine, significantly below the detection level of this test. However, urine PSA levels may vary significantly and in extreme cases exceed the detection level of this test in presumably healthy male individuals.

1.5.5.2 Materials

Seratec® PSA Semiquant test from Seratec Diagnostica®

1.5.5.3 Controls

Positive and negative controls shall be used for function verification. These will be run prior to the use of the kit lot. Future shipments of the same lot# will still need controls run for function verification to account for potential differences in shipping conditions. The positive control shall be a known seminal fluid sample. The control results and the date run shall be recorded.

1.5.5.4 Procedure

Extract a portion of the stain or swab in 750 µl dH_2O or standard buffer solutions at a neutral pH range (Tris buffered saline) can be used.

Incubate extract for 2 hours at 4°C.

Centrifuge for approximately 3 minutes. Bring supernatant to room temperature.

Remove test from protective pouch and label device for identification purposes.

Add approximately 200 µl, or 5 drops, of sample to the test well.

Read results at 10 minutes.

1.5.5.5 Results and Interpretation

The test area contains three lines:

Line1: Labeled "T" on test device is the test result
Line 2: Internal standard (correlates with a concentration of approximately 4ng/mL PSA)
Line 3: Labeled "C" on test device is the test control

Positive reaction: Line 1 (result), line 2 (internal standard) and line 3 (control) all appear.
Negative reaction: Line 2 (internal standard) and line 3 (control) appear. Note: If preliminary test (i.e. acid phosphatase) results are a strong positive, there may be the presence of "high dose hook effect". This test may be repeated test using a higher sample dilution.
Invalid reaction: Line 2 (internal standard) and/or line 3 (control) are not detectable. (The test should be repeated if determined to be invalid.)

1.5.5.6 References

Hochmeister et al. (1999) Evaluation of Prostate-Specific (PSA) Membrane Test Assays for the Forensic Identification of Seminal Fluid: J Forensic Sci Vol 44: 1057-1060.

Sato et al. (2002) Use of the "SMITEST" PSA Card to Identify the Presence of Prostate-Specific Antigen in Semen and Male Urine. Forensic Sci Int 127: 71-74.

Laux et al. (2003) Forensic Detection of Semen II. Comparison of the Abacus Diagnostic OneStep ABAcard p30 Test and the Seratec PSA Semiquant Kit for the Determination of the Presence of Semen in Forensic Cases. MAFS Newsletter Fall 2003: 11-18.

Simich et al. (1999) Validation of the Use of a Commercially Available Kit for the Identification of Prostate Specific Antigen (PSA) in Semen Stains. J. Forensic Sci Vol 44: 1229-1231.

Seretec Diagnostica Seratec® PSA Semiquant Technical Information Sheet