**SEMINAL STAIN**

**Introduction:**

Semen & seminal stain The ability to identify traces of body fluid discovered at crime scenes is a very important aspect of forensic investigations. Semen analysis is one of the most popular and informative forensic techniques, it is imperative that any potential body fluid sample is properly collected and not destroyed during the initial identification process. They can also help answer questions regarding the events of a crime. An analytical technique that could identify a particular body fluid rapidly, simply, and non-destructively at the scene of a crime would be a valuable tool for forensic investigators. In addition to blood, semen is one of the most prevalent body fluid found during criminal investigations, especially in cases involving sexual assault. There are currently several tests, both presumptive and confirmatory, that can be used to identify an unknown fluid found at a crime scene to be semen. An alternate light source (ALS) can be used as a finding aid to detect certain biological materials, including semen. Some commercial ALS instruments have been developed such as the Wood’s Lamp Bluemax. The most widely used methods for preservative and confirmatory identification of semen discuss below. Seminal stain is an important biological fluid which requires identification and characterization in the investigation of sexual offences like rape, sodomy, bestiality,etc. Normal semen reference pH range is 7.2 to 7.8. Variations in volume and low pH may be due to congenital abnormalities of the genital tract or to obstruction. A pH of 8.0 or greater can be associated with infection of the prostate, seminal vesicle, or epididymis.

**Examination of semen & seminal stain**

1. **Visual & physical examination**
* **Color**: Thick, yellowish white, glairy, opalescent, secretion having a characteristic odor known as seminal odor.
* **Texture**: On touch, seminal stains are starchy.
* **Appearance**: Garments sent for forensic examination are usually dirty having variety of stains, in natural light some stains are reddish coloured, while others are brown, yellow or faint grey in colour. These are often mixed with stains of blood vaginal discharge, urine and semen, so as to restrict the investigation to seminal stains only, preliminary examination is done under filtered UV light.
1. **Microscopical examination**

Microscopically, detection of spermatozoa is also considered as confirmatory evidence for the presence of semen in a suspected stain. In order to conduct microscopic identification, part of the stained garment should be dissolved in acidulated water in test tube. The test tube should be subjected to ultrasonic oscillation, which can separate the spermatozoa from the dried stain, followed by microscopic examination after fixing the same in dilute sulphuric acid. Since spermatozoa contain gram positive protein, the presence of spermatozoa will be confirmed by microscopic appearance of purple bodies.

1. **Presumptive test**
* **Acid phosphate test:-** The Acid phosphate test is one of the best known and most widely employed techniques for the purpose of identification of semen. This test is adopted because of high acid phosphatase activity of semen. Thus by applying this test a suspected azoospermic or vasectomized person who is a suspect of sexual offence may be identified. Stability of acid phosphatase activity in seminal stain depends on temperature. If the stain is exposed to sunlight the acid phosphatase activity is slowly reduced and if the stain is heated at 60˚C for 5 min. this activity is completely destroy

The enzyme acid phosphatase of semen hydrolyses the calcium alpha-napthyl phosphoric

acid that liberates alpha naphthol. This couple with the age forming material to give a

purple colour.

**Reagent Preparations:**

Buffer

Glacial Acetic acid 1ml

Sodium acetate anhydrous 2gm

Distilled water 100ml

**Step 1 Reagent**

Buffer 50ml

Sodium alpha-naphthyl Phosphate, 0.25% (w/v) 126gm

**Step 2 Reagent**

Buffer 50ml

Naphthanil diazo blue B, 0.5% (w/v) 250gm

Step 1 Reagent and Step 2 Reagent can be made up in bulk and aliquoted into test tubes

and frozen.

When needed, one tube of each reagent can be thawed under warm running water for

use.

**Procedure:**

Place a small piece (2 x 2 mm) of suspected seminal stain

1. Stain material on Whatman filter paper or other suitable test paper. Use proper

standards and controls including positive, negative and unstained controls.

2. Add 1-2 drops of Step 1 Reagent and allow to react for 30 seconds. (No colour

should develop at this stage).

3. Add 1drop of Step 2 Reagent. Record the result after 10 seconds.

 4. A positive reaction is recorded upon rapid development of a purple colour, which is indicative

 of semen.

This is not a confirmatory test for semen.

* **PSA 0r P30 Test**

P30, also known as Prostate Specific Antigen (PSA), is a glycoprotein produced in the prostate gland and is secreted in seminal fluid independently of the production of spermatozoa. The presence of p30 is used as another means of seminal fluid identification, particularly in samples with little or no spermatozoa. While p30 is not restricted to seminal fluid, it’s extremely high concentration in seminal fluid makes it an effective marker to confirm the presence of semen on evidence stains.

**Requirements** : PSA or P30 card and TE Buffer

**Procedure** :

 1. Take a cutting from a suspected semen stain (see chart below for cutting sizes) and place it in an appropriately labeled 1.5 ml or 2.0 ml microcentrifuge tube.

 2. Add an appropriate amount of TE buffer to the samples.

 3. Allow the samples to incubate at room temperature for two hours or overnight at 4°C.

 4. Vortex briefly and centrifuge for 3 minutes. The supernatant is removed for p30 testing and the remaining pellet may be used for slide preparation or in DNA Differential Extraction.

 5. Add 200 µl of each sample’s extract to the sample well of card.

 6. Allow the card to remain at room temperature for 10 minutes.

 7. A positive result may be recorded at any time within the 10 minute period, however an inconclusive or negative result is not confirmed until the full 10 minutes has elapsed.



1. **CONFIRMATORY TEST**
* **Florence test**:- This test was discovered by Dr.Florence in the year 1886. When Florence reagent (PotassiumIodide+Iodine+Water) is applied to the slide it produces rhomboidal shape dark crystals of choline periodide. Similarly, any tissue or biological material containing sufficient high choline concentration would give positive Florence Test. According to Davis and Wilson, if swab is collected within one day of sexual act, choline can be detected. At the same time if swab is taken after 14 hours of intercourse, there is a possibility of negative result.
* **Barberio’s test:-** Barberio’s test was invented by Barberio in the year 1905. When the questioned stain is allowed to react with picric acid it leads to the formation of yellow needle shaped spermine picrate crystals, including the presence of seminal stain.
* **Christmas Tree Stain Test :** The microscopic identification of spermatozoa is a method of confirming the presence of semen in an evidentiary stain. Spermatozoa are identified by either the presence of intact sperm cells displaying a head, midpiece and tail, or sperm heads showing an acrosomal cap. The microscopic examination can be enhanced by staining the slide using a differential stain known as **“Christmas Tree Stain”** which consists of two dyes: **Nuclear Fast Red and picroindigocarmine.**

Sperm heads are usually well differentiated with the acrosome staining significantly less densely than the distal region of the head. Nuclei inside epithelial cells appear pink to purple in color. Sperm tails and epithelial membranes are stained green by the picroindigocarmine.

Sperm cells will stain red with green tails. The sperm head will stain differentially with the acrosomal cap pink and the nuclear material red

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