International Journal of Forensic Science and Legal Medicine

Vol. 6, Issue - 1, January-June 2026

DETERMINATION THE SENSITIVITY OF SEMEN SAMPLE AT DIFFERENT TIME INTERVALS ON DIFFERENT SURFACES

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ABSTRACT

The study investigates the tenacity and detectability of semen on different surfaces over a 28-day period using acid phosphatase (AP) as a presumptive test and Florence test as confirmatory test. Ten surfaces including cotton, clothes, glass, wood, tiles, condoms, soil, dry leaves, stones, and paper, were inoculated with semen samples and tested periodically. Results indicated that cotton and clothes retained positive results for semen detection throughout the study, while glass, tiles, and paper showed a detection window of up to seven days. Wooden surfaces showed detectability for up to four days, with shorter periods observed for dry leaves, condoms, stones, and soil. The study explains the importance of surface type and time interval in semen detection for forensic investigations, providing valuable insights for improving investigative procedures in criminal justice system.

Keywords: Detectability, Presumptive Test, Confirmatory Test, Semen, etc

INTRODUCTION

Identification of body fluids, including blood, semen, saliva, and the like, is essential to forensic science since it is often the first step in a criminal investigation and is used as evidence in court. Because many body fluid stains are invisible or only present in tiny amounts or mixtures, they can be challenging to diagnose. Identification tests for body fluid testing were based on chemical or enzymatic assays, which were usually presumptive in character and restricted in specificity or sensitivity. Many of the initial assays consumed biological material that was already restricted and were not compatible with DNA profiling, a crucial step in many different types of studies[1].

Seminal stain is an essential biological fluid that must be detected and characterized in order to investigate sexual offenses such as rape, sodomy, bestiality, etc. Semen evidence is a reliable indicator that can help identify the specific sexual assault and facilitate the examination of the crime scene. Given the daily increase in sexual assault instances, semen should always be detected, even if it is present in minute amounts and the stain is old[2]. A joint endeavour of forensic biology and serology has been the study of semen stains. For crime scene investigations, fundamental semen and seminal stain examination might yield crucial information, especially in cases of rape.

Differentiating various body fluids is an important first step because the liquid idea itself teaches[3]. Criminal investigations, particularly those involving allegations of sexual assault, require forensic semen analysis. The fluid produced by the male reproductive system consists of other substances in addition to spermatozoa. Taking a sample of the victim's biological material or searching for semen stains on clothing in the hopes of linking them to a suspect is one of the primary objectives of the forensic laboratory in

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these kinds of cases[4].

When sexual offenses are suspected to have been committed, finding semen on intimate swabs and/or clothes or bedding items is often essential. Acid phosphatase (AP), an enzyme soluble in water, is abundant in semen. With a wealth of documented results, AP screening is the most often used test in the forensic science field as a presumptive test for the detection of semen stains[5-7]. Sample screening processes sometimes involve the use of acid phosphatase (AP) overlay, UV light, other light sources, and P-30 (prostate specific antigen, or PSA). All of these methods are presumptive assays for semen identification. Light-based methods are unable to differentiate between different cell types and may result in a higher proportion of false positives since they identify nucleic material in all cell types. Because vaginal fluid and bacteria contain low levels of AP activity, this test is not the best sperm-specific one. While P-30 is a more specific biomarker than AP, it may not be a rigorous confirming test for seminal fluid because certain guys can also have this biomarker in their urine, mainly when they are experiencing prostate issues[8-16]. Important details on four sperm motility and viability, production, male genital tract patency, secretions from accessory organs, emission, and ejaculation are provided by routine semen analysis. Even though it provides useful This test is not a fertility test; rather, it provides information for the initial assessment of the infertile man[17]. It doesn't say anything about the spermatozoon's mobility or capacity to fertilize an ovum. moving on to the further stages of development that are necessary to finish the process of fertilization. It is crucial to realize that even though there may be a connection between the 10 In terms of test results and "fertility," the assay does not evaluate fertility in isolation [18–19]. The Ain Shams University hospitals of andrology and dermatology conducted a study on male clients who came to the reproductive clinic. These men were sent to the lab to have their semen examined as part of the evaluation process for male infertility. Extensive guidelines were supplied prior to the sample collection. Among them was avoiding coitus for a period of three to four days. Masturbating into sterile bottles with large mouths was the method used to collect aseptic samples.

MATERIAL AND METHODS

Sample collection: Semen sample was collected in a 4-5 ml sterile container and preserved at 4°C temperature before the experiment.

MATERIAL REQUIREDS

Reagents used: Glacial Acetic Acid, Sodium Acetate Anhydrous, Distilled Water, Sodium Alpha-Naphthyl Phosphate, 0.25%(W/V), Potassium Iodide (Ki), Iodine, Distilled Water, Normal Saline.

Equipment's: Chemical Reagents, Microscope, Thermometer, Centrifuge, & PH Meter etc.

Biological Sample: Semen

Presumptive Analysis

Acid Phosphate Test- Sodium alpha naphthyl phosphate method

Reagent Preparations: Buffer -Glacial Acetic acid, Sodium acetate anhydrous, Distilled water,

Step 1 Reagent- Buffer, Sodium, alpha-naphthyl Phosphate,0.25%(w/v). Step 2 Reagent-Buffer, NaphthanildiazoblueB,0.5% (w/v),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: Put a little (2 x 2 mm) piece of suspected seminar stain substance on a piece of Whatman filter paper or another appropriate test paper. Employ appropriate benchmarks and safeguards, such as unstained, negative, and positive controls; refer to below. Give the Step 1 Reagent a drop or two, then wait 30 seconds for it to respond. (No hue ought to develop at this point). Use one drop of the Step 2 Reagent. After ten seconds, note the outcome. A favorable response is noted when a purple color develops quickly, which is suggestive of semen. This is not a semen confirmation test[24].

Confirmatory Test

Florence test-

Preparation of Florence Reagent: by mixing distilled water with potassium iodide (KI) and iodine. **Procedure:** Place one or two drops of the stain's aqueous extract onto a slide. Add a drop of Florence reagent from the sides and allow it to mix slowly under a cover slip. Examine under a microscope with magnifications of 10X and 40X. The Florence test for semen presence is indicated by the presence of dark brown rhombic crystals of choline periodide.

Procedure For Test

The semen was applied to various surfaces with the help of a dropper and the sample was kept for 28 days. The dried menstrual blood was moistened with normal saline on day 1, day 2, day 3, day 4, day 5, day 6, day 7, day 14, day 21 and day 28 and sample was collected from the surface with the help of earbuds and confirmatory and presumptive tests were done. All the samples were inoculated with semen and were placed inside a room and were named and numbered according to the study ranging from day 1 to day 28 as shown in the figure.



Figure 1: All prepared surfaces

E. Surface preparation of samples

Cotton- 20 drops of semen sample each for presumptive and confirmatory test respectively was inoculated on the cotton surface and were labelled from day 1 to day 28.

Clothes- 20 drops of semen sample each for presumptive and confirmatory test respectively was inoculated on the clothes surface and were labelled from day 1 to day 28.

Glass- 20 drops of semen sample each for presumptive and confirmatory test respectively was inoculated on the glass surface and were labelled from day 1 to day 28.

Wooden- 20 drops of semen sample each for presumptive and confirmatory test respectively was inoculated on the wooden surface and were labelled from day 1 to day 28.

Tile- 20 drops of semen sample each for presumptive and confirmatory test respectively was inoculated on the tile surface and were labelled from day 1 to day 28.

Condom- 20 drops of semen sample each for presumptive and confirmatory test respectively was inoculated on the condom surface and were labelled from day 1 to day 28.

Soil- 20 drops of semen sample each for presumptive and confirmatory test respectively was inoculated on the soil surface and were labelled from day 1 to day 28.

Dry Leaf- 20 drops of semen sample each for presumptive and confirmatory test respectively was inoculated on the dry leaf surface and were labelled from day 1 to day 28.

Stone- 20 drops of semen sample each for presumptive and confirmatory test respectively was inoculated on the stone surface and were labelled from day 1 to day 28.

Paper- 20 drops of semen sample each for presumptive and confirmatory test respectively was inoculated on the paper surface and were labelled from day 1 to day 28.

RESULT & DISCUSSION

1. Presumptive- **Acid phosphatase test (**AP test)
2. Confirmatory- Florence Test

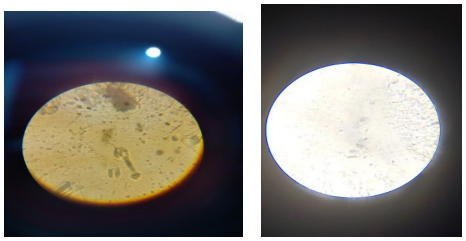


Figure 2: Positive & Negative Results Image of Florence Test

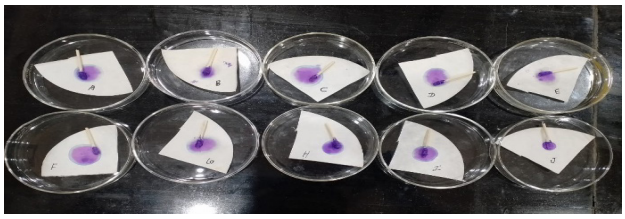


Figure 3: Test results on Day 1

On day 1, semen samples were evaluated at all 10 surfaces, and the results from both presumptive and confirmatory tests indicated positive detection of semen on all surfaces.

On days 2, semen samples were evaluated on all 10 surfaces, and the results from both presumptive and confirmatory tests indicated positive detection of semen on surfaces such as cotton, clothes, glass, wooden surfaces, tiles, condoms, dry leaves, stones, and paper. And gave a negative result for soil.

On days 3, semen samples were evaluated on all 10 surfaces, and the results from both presumptive and confirmatory tests indicated positive detection of semen on surfaces such as cotton, clothes, glass, wooden surfaces, tiles, dry leaves, and paper. And gave a negative result for soil, condoms and stones. According to Rodriguez[20], the detection of semen using the acid phosphatase (AP) test on condoms can vary, but generally, the AP test can detect semen for up to 24-48 hours after deposition. This is due to the enzyme's stability and the protective nature of the condom material, which limits exposure to degrading environmental factors.

On days 4, semen samples were evaluated on all 10 surfaces, and the results from both presumptive and confirmatory tests indicated positive detection of

Table 2: Table showing results at all surfaces

S.No.	Sample	Surface	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 14		Day 21		Day 28	
			RESULT		RESULT		RESULT		RESULT		RESULT		RESULT		RESULT		RESULT		RESULT		RESULT	
			P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C
A	Semen	Cotton	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B	Semen	Cloth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C	Semen	Glass	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
D	Semen	Wooden	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
E	Semen	Tiles	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
F	Semen	Condom	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G	Semen	Soil	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H	Semen	Dry leaf	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
I	Semen	Stone	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J	Semen	Paper	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-

semen on surfaces such as cotton, clothes, glass, wooden surfaces, tiles, dry leaves, and paper. And gave a negative result for soil condoms and stones.

On days 5, semen samples were evaluated on all 10 surfaces, and the results from both presumptive and confirmatory tests indicated positive detection of semen on surfaces such as cotton, clothes, glass, tiles, and paper. And gave a negative result for soil condoms, dry leaves, wooden surfaces, and stones.

On days 6, semen samples were evaluated on all 10 surfaces, and the results from both presumptive and confirmatory tests indicated positive detection of semen on surfaces such as cotton, clothes, glass, tiles, and paper. And gave a negative result for soil condoms, dry leaves, wooden surfaces, and stones.

On days 7, semen samples were evaluated on all 10 surfaces, and the results from both presumptive and confirmatory tests indicated positive detection of semen on surfaces such as cotton, clothes, glass, tiles, and paper. And gave a negative result for soil condoms, dry leaves, wooden surfaces, and stones.

On days 14, semen samples were evaluated on all 10 surfaces, and the results from both presumptive and confirmatory tests indicated positive detection of semen on surfaces such as cotton and clothes. And gave a negative result for glass, tiles, paper soil condoms, dry leaves, wooden surfaces, and stones.

On days 21, semen samples were evaluated on all 10 surfaces, and the results from both presumptive and confirmatory tests indicated positive detection of semen on surfaces such as cotton and clothes. And gave a negative result for glass, tiles, paper soil condoms, dry leaves, wooden surfaces, and stones. Whereas, according to Sato[21], the duration for which semen can give a positive acid phosphatase (AP) test on glass is typically within 24 to 48 hours. According to [22], semen can give a positive acid phosphatase test on wood for up to 72 hours under optimal conditions.

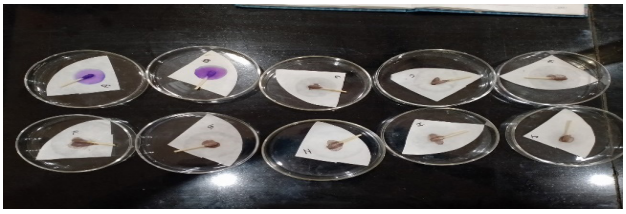


Figure 4: Test results on Day 28

On days 28, semen samples were evaluated on all 10 surfaces, and the results from both presumptive and confirmatory tests indicated positive detection of semen on surfaces such as cotton and clothes. And gave a negative result for glass, tiles, paper soil condoms, dry leaves, wooden surfaces, and stones. The acid phosphatase test can detect semen on cotton for up to 14 days, depending on various conditions such as the size of the fabric sample and the amount of semen present as indicated in the study of Sapan[23].

CONCLUSION

The goals of our study assessing semen sensitivity on various surfaces and time intervals and evaluating the validity of presumptive and confirmatory tests in identifying semen samples—were satisfactorily met. The findings unequivocally show how semen's permanence and detectability vary over time on various surfaces. Paper, glass, and tile surfaces demonstrated a narrower detection window of seven days, but cotton and fabric surfaces yielded positive results for the maximum time of 28 days. The longest period of detection was four days for wood, three days for dry leaf, two days for condom and stone, and one day for soil. These results highlight how crucial it is to take surface type and time intervals into account when performing semen detection-related forensic investigations.

These kinds of findings are essential for improving the accuracy of criminal investigations and fortifying forensic processes. All things considered, our research offers insightful information about how to

best optimize forensic processes for semen detection, offering useful advice to law enforcement and forensic professionals. The pursuit of justice can be aided by investigators who improve the efficacy and efficiency of semen detection by taking into consideration surface characteristics and time frames during the collection and examination of evidence.

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