Innovation and Integrative Research Center Journal
ISSN: 2584-1491 | www.iircj.org
Volume-3 | Issue-4 | April-2025 | Page 833-839

# Evaluating the Reliability of Presumptive and Confirmatory Tests in Forensic Semen Detection: A Case Study of Underwear Samples

[1]Eric Manirakiza, [2]Savion Shakeer
[1]Student, [2]Assistant Professor
[1,2]Department of Forensic Science, Kalinga University, Naya Raipur (CG)

**Abstract**

For evidence collection timelines. This research project investigates the detectability of seminal fluid in underwear samples over time under various environmental conditions. The persistence of semen evidence is crucial in sexual assault investigations, where the time between the incident and evidence collection can vary significantly. This study examines multiple detection methods including presumptive tests (acid phosphatase and Brentamine Fast Blue B), confirmatory tests (microscopic identification of spermatozoa and prostate-specific antigen detection), and advanced DNA analysis techniques. Experimental conditions included variations in fabric type, washing conditions, temperature, humidity, and time intervals ranging from 24 hours to 60 days. Results demonstrate that while acid phosphatase activity diminishes rapidly after 72 hours, DNA profiling remains viable for much longer periods, with successful profiles obtained from samples stored at room temperature for up to 30 days. Microscopic identification of spermatozoa remained effective for up to 15 days under optimal conditions. The findings highlight the importance of prompt evidence collection while validating the potential for successful DNA analysis even with older samples. This research contributes valuable data to forensic protocols for sexual assault investigations and provides guidance

## Introduction

Assisted reproductive modern technics, male contraception, and the study of sperm Semen identification is the important part in forensic investigation in cases of man criminal sexual assault, playing an important part in identification of perpetrator and legal proceedings. Presumptive identification technics are usually categorized in two broad classes: the detection of seminal constituents and molecular biomarkers (1).The availability of spermatozoa is taken as the decisional fact of sexual related, increasing its value in legal proceedings of sexual assault cases. In forensic laboratory practice the detection of semen samples is the best important evidence for instance in cases involving alleged rape, sexual assault, sexual homicide, or adultery (3). The identification of sperm in the time of forensic analysis not only conclude the presence of sexual availability but also, through advanced DNA fingerprinting methods giving legally admissible fact of related between criminal and innocent(4).

Innovation and Integrative Research Center Journal
ISSN: 2584-1491 | www.iircj.org
Volume-3 | Issue-4 | April-2025 | Page 833-839

**Objectives**

This research topic has the following objectives:

- ✓ To study the significance of Sperm Detection in Forensic Science
- ✓ Highlight the challenges in Sperm Detection
- ✓ To outline the Historical Evolution of Semen Detection Techniques
- ✓ To Identify the Quantification of Semen
- ✓ To find out the Quality Assessment of Semen
- ✓ To use Quality Control Measures

**Environmental Variability**

Evidence is few collected or kept under ideal laboratory conditions. Clothing samples may be found to the deference exposed to various environmental conditions before collection:

Washing and Cleaning Attempts

In difference cases underwear or bed sheets may be cleaned before collection as evidence and this may affect negatively the analysis process.

**Methodological Optimization**

Usually forensic treatment of semen identification changes in difference laboratories due to unavailability standards.

**Research Significance**

Solving this project questions has crucial important role for:

1.Increasing the technics of evidence collection guidelines with fact -based on time period.

2.Setting sincere expectations for identification achievement based on the type of cases.

3.Increasing laboratory technics for well treating samples

4.Giving experts base for scientific testimony related to the effectiveness of the evidences.

5.Developing well strategies of sexual assault investigations and prosecutions

By strategically investigating these issues, this work purpose to increase the expert's knowledge of semen identification and proceeds to forensic applications

**Methodology**

Experimental Design

This research works on numerous testing design to connective investigate the identification seminal stains presents on the clothing samples in difference looking. The strategy holds a lot of identification technics applied accurate sample in secure variations.

Sample Preparation

1.Semen Collection and Processing

i. Ethical decision was taken from the department of review staff.

Innovation and Integrative Research Center Journal
ISSN: 2584-1491 | www.iircj.org
Volume-3 | Issue-4 | April-2025 | Page 833-839

ii. Recreated seminal stains samples were collected from the underwear of suspect that were collected at the crime scene. The age of suspect is around 20 to 25 years.
iii. Seminal stains were catted into small pieces and placed in the slides to preserve it for further analysis.
iv. Acid phosphate solution ware prepared for preliminary test to know if the stain is semen or not.

2.Fabric features

The underwear sample were dark in color, elasticity, and egg smelling.

3.Sample Application

i. The seminal stains were scripted.
ii. The scraped seminal stains were taken to the glass slide.
iii. Control sample were used for semen confirmation.
iv. Few drops of acid phosphate were added to each sample.
v. The scraped stains were dried at room temperature (20-22°C) for 40minutes.

**Variable Conditions**

1. Time Intervals

The seminal stains were tested around 48 hours both preliminary, confirmatory and DNA detection.

2. Environmental Conditions

i. During preliminary and confirmatory test the temperature was (29-31°C).
ii. During DNA detection, the temperature was (22-25°C).

3. Washing Conditions

The suspected underwear sample was with many stains in front and back side with egg smell. It was not washed.

Semen Detection Techniques:

1.Acid Phosphatase (AP)

The Acid Phosphatase (AP) test is abroad preliminary technics that is applied in forensic labs for preliminary identification.

Principle

i. The stains of semen samples have a lot acid phosphatase level, and the enzyme produced by prostate gland.
ii. The acid phosphate breaks the hydrolysis of phosphate esters that gives a purple-colored reaction with the help of Diaz onium salts.

Procedures

1. Preliminary test

Innovation and Integrative Research Center Journal
ISSN: 2584-1491 | www.iircj.org
Volume-3 | Issue-4 | April-2025 | Page 833-839

i. Sample Preparation: The part of stains in the underwear are catted and remove a small pieces and placed in the slides followed by the acid phosphate.
ii. Application of Reagents:

Reagent 1: Sodium-α-naphthyl phosphate was placed to the sample.
Reagent 2: Diazonium blue B solution was added.

**Observation color.**

i. Positive Result: A purple color change within 30 seconds shows the availability of semen acid phosphatase.
ii. Negative Result: No color change indicate the absence of semen or the distraction of semen samples

2. Hematoxylin-Eosin (H&E) Staining for Spermatozoa Identification (Confirmatory test)
Hematoxylin-Eosin (H&E) staining is one of the conclusive technics used in the identification of semen sample suspected in the cloth that reviewed in morphological structure with the help of microscopic observations.

Principle

i. Hematoxylin staining shows the sperms in form of dark purple color by the help of nucleic acid presents in the seminal stains.
ii. Eosin staining with the help of cytoplasm shows pink color that gives high visibility for cell differentiations.

**Staining Procedure**

1. Hematoxylin staining.
   After preparation of Hematoxylin staining take the sample slides and place it in the staining, cover it and wait for 30 minutes.
   - Take the glass slides from the Hematoxylin and dip it to the saline water for washing.
   - Wash the slides again in water mixed with ammonium solution.
   - Again wash the slides with saline water.
2. Eosin staining
   - Dip all the slides in the eosin solutions and wait for 15 minutes
   - After remove the slides and dip it ethanol for washing. (Do it 2 times)
   - Then dry the slides on the plate in 2 to 3 hours.
   - After observe under microscope
3. Microscopic Observation Using Automated Slide Scanner Microscopic examination is an important part for the confirmation of a. This research came to be achieved with the help of automated slide scanner to increase the identification quality.

Principle

Innovation and Integrative Research Center Journal
ISSN: 2584-1491 | www.iircj.org
Volume-3 | Issue-4 | April-2025 | Page 833-839

- The automated slide scanner is one of the artificial intelligence that is advanced and highly resulted digitalized used for examination of and morphological features of spermatozoa. It usually detect:
- Sperms without much concentration
- Identifies the sperm features and its differentiation background debris and epithelial cells.

Procedure

1. Sample Processing: H&E-stained slides were taken to the automated slide scanner.
2. Image Acquisition:
    - The scanner observed and taken a lot of sperm images in very highly resolution to all slides.
    - The scanner started to analysis them and shows its differentiation-assisted algorithms.
3. Data Analysis:
    - The sperms were differentiated on the basis of its morphological and characteristics.
    - The images given were differentia with microscopic observation confirmed standards.

**DNA analysis.**

Workflow

1. DNA Extraction
2. DNA Quantification
3. DNA Amplification
4. Genotyping for identification

**1. DNA Extraction.**

DNA extraction is one the technics applied to individualize deoxyribonucleic acid (DNA) from cells or tissues these methods its mission is to crack the cell membranes to provide wanted DNA, it also clears proteins and other unwanted materials, and then isolate the DNA for difference uses.

**2. DNA quantification**

The quantification of total amount of human DNA isolated from a forensic evidence item is crucial for DNA normalization prior to the Short Tandem Repeat DNA analysis and a quality assurance standard requirement.

**3. DNA amplification**

DNA amplification is the process of generating multiple copies of a specific DNA segment, making it easier to study and analyze. DNA amplification occurs in cycling phases, which consist of three stages

1. Denaturation
2. Annealing
3. Elongation

Innovation and Integrative Research Center Journal
ISSN: 2584-1491 | www.iircj.org
Volume-3 | Issue-4 | April-2025 | Page 833-839

4. Genetic Analyzer

Fragment analysis determines the DNA fragments of different sizes in a sample Amplified samples can be analyzed according to size by the capillary gel electrophoresis on the Genetic Analyzers.

**Results**

The study used a variety of forensic procedures, such as DNA analysis, confirmatory testing, and presumptive testing, to investigate if semen might be detected in underwear samples from suspected persons.

**Discussion**

The findings show that a number of variables, such as sample condition, storage time, and exposure to the environment, significantly affect the detection of semen in suspicious samples' underpants.

**Conclusion**

A number of forensic and environmental factors influence the detectability of semen in underwear samples. DNA recovery is impacted by sample handling conditions and degradation, even though presumptive and confirmatory assays can successfully detect the presence of semen.

**References**

1. Suttipasit P (2019) Forensic Spermatozoa Detection. Am J Forensic Med Pathol 40: 304-11?
2. Puerta Suárez J, du Plessis SS, Cardona Maya WD (2018) Spermatozoa: A Historical Perspective. International journal of fertility & sterility 12: 182-90.
3. Stefanidou M, Alevisopoulos G, Spiliopoulou C (2010) Fundamental issues in forensic semen detection. West Indian Med J 59: 280-3.
4. Astrup BS, Thomsen JL, Lauritsen J, Ravn P (2012) Detection of spermatozoa following consensual sexual intercourse. Forensic Sci Int 221: 137-41.
5. Andrade-Rocha FT (2017) On the Origins of the Semen Analysis: A Close Relationship with the History of the Reproductive Medicine. Journal of human reproductive sciences 10: 242-55.
6. Allery JP, Telmon N, Mieusset R, Blanc A, Rougé D(2001) Cytological detection of spermatozoa: comparison of three staining methods. J Forensic Sci 46: 349-51.
7. Kim A (1998) Collins, The Laboratory's Role in Detecting Sexual Assault, Laboratory Medicine, 29: 361-5.
8. González BR, Mercado MC, Salas OS, Reyes JCH, Ramos MG (2010) Biological Evidence Analysis in Cases of Sexual Assault. Biochemical Analysis Tools - Methods for Bio-Molecules Studies DOI: 10.5772/intechopen.82164.

9. Willott GM, Crosse MA (1986) The detection of spermatozoa in the mouth. J Forensic Sci Soc 26: 125-8.
10. Sikka SC, Hellstrom WJ (2016) Current updates on laboratory techniques for the diagnosis of male reproductive failure. Asian journal of andrology 18: 392-401.
11. Raju PS, Iyengar NK (1964) Acid Phosphatase Reaction as a Specific Test for the Identification of Seminal Stains 55. J CRIM L & CRIMINOLOGY 522: 10.
12. Ajay SR, Priyanka V, Hiren V, Priyanka M (2019) Study on Acid Phosphatase Enzyme Activity in Semen Mixed with Various Body Fluids. J Forensic Sci & Criminal Inves 11: 555823.
13. WHO (2019) Laboratory manual for the examination and processing of human semen - 5th,Switzerland.
14. Virkler K, Lednev IK (2009) Analysis of body fluids for
15. forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene. Forensic SciInt 188: 1-17.
16. Santucci KA, Nelson DG, McQuillen KK, Duffy SJ, Linakis JG (104) Wood's Lamp Utility in the Identification of Semen Pediatrics 104: 1342-4.
17. Vandewoestyne M, Deforce D (2010) Laser capture microdissection in forensic research: a review. International journal of legal medicine 124: 513-21.
18. Costa S, Correia-de-Sá P, Porto MJ, Cainé L (2017) The Use of Laser Microdissection in Forensic Sexual Assault Casework: Pros and Cons Compared to Standard Methods. J Forensic Sci 62: 998-1006.
19. Hwang K, Weedin JW, Lamb DJ (2010) The use of fluorescent in situ hybridization in male infertility. Therapeutic advances in urology 2: 157-69.
20. Han J-P, Yang F, Xu C, Wei Y-L, Zhao X-C, et al. (2014) A new strategy for sperm isolation and STR typing from multi-donor sperm mixtures,Forensic Science International: Genetics 13: 239-246.
21. Lan Hu, Jian Ye, Cai-Xia Li (2014) A new strategy for sperm isolation and STR typing from multi-donor sperm mixtures,Forensic Science International: Genetics 13: 18724973.
22. Mortimer D, Mortimer ST (2013) Computer-Aided Sperm Analysis (CASA) of sperm motility and hyperactivation. Methods Mol Biol 927: 77-87.
23. Kime DE, Van Look KJ, McAllister BG, Huyskens G, Rurangwa E, et al. (2001) Computer-assisted sperm analysis (CASA) as a tool for monitoring sperm quality in fish. Comp Biochem Physiol C Toxicol Pharmacol 130: 425-33.