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Detection of Seminal Material on Hand-Washed Textile Evidence

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ABSTRACT

Background. In sexual assault cases, a medical examination of the victim is often not conducted timely. This leaves the only chance to spot the ejaculate of the assailant on the victim's clothing. Washing the victim's clothes before the detection of semen in the forensic laboratory is a challenging task. Therefore, the current study aimed to examine the persistence of seminal material on seven different types of hand-washed fabrics using five laundry detergents.

Methods. The presence of seminal material on washed fabrics was determined using an alternate light source, acid phosphatase test, p30 test, and sperm head count.

Results. The study demonstrated that presumptive testing was not positive for most washed fabrics. A fairly large number of spermatozoa retained on a few fabrics even after 20 minutes of washing. The cotton yarn fabrics and tight weaving with warf and weft more than 100x100/inch could retain more sperms. The DNA was also isolated from sample and quantified using a Quantifiler Duo DNA quantification kit.

Conclusion. Good quality and quantity of human DNA were obtained from most of the washed fabrics, which could successfully generate the STR profile of the donor. The current study recommended using hand-washed textile items for forensic analysis in sexual assault cases.

Keywords: detergents, DNA quantity, semen detection, sexual assault evidence, sperm count, washed fabrics

Highlights

- Fabric type and detergent composition significantly influence the retention of seminal material, i.e. finer weaves like cotton exhibiting better sperm retention post-washing.
- Despite washing, high-quality human genomic DNA was successfully extracted from several fabrics, enabling the generation of STR profiles, thereby validating the forensic potential of hand-washed clothing.
- Presumptive tests for semen, such as the acid phosphatase (AP) and PSA tests, showed limited success in detecting seminal material on washed fabrics.

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1. INTRODUCTION

The detection of biological fluid of human origin from crime scene samples is the first crucial step towards the establishment of the identity of a suspect [1]. The high concentration of acid phosphatase's presence of spermine, flavins, and spermatozoa in semen makes it distinct from other body fluids [2]. Approximately, 50-200 million sperms/ml (~5ml on average) are produced in a single ejaculation [3]. Therefore, it could be the most potential and abundantly available biological fluid in sexual assault cases. Sometimes, it is difficult to find semen on intimate samples due to washing of the genital area and urination before the medical examination. In such scenario, clothes may contain potential semen stains. Routinely, forensic laboratories do not accept washed clothes, although many reports have been published regarding spermatozoa being found in machine-washed clothes [1, 4].

Many factors contribute to delayed medicolegal examination including shame, social pressure, poverty, and inadequate awareness [5, 8]. This delay destroys potential biological evidence on or inside the victim's body [6]. While due to lack of proper knowledge, the victim's clothing is washed, leaving little chance of recovering the assailant's DNA [7, 9, 10] .

The degree of washing of a stain and persistence of seminal material on fabrics depends upon the physical and chemical nature of the fabric and detergent. It also depends upon the soaking time and tumbling during washing [6, 11, 12]. Handwashing is quite common in different countries, especially in poor areas. Many studies have been conducted on the extraction of semen from machine-washed clothes, however, scarce literature is available on hand-washed clothes. This study aimed to investigate the detection of seminal material on hand-washed fabrics with different detergents.

The current study primarily focused on washing semen-stained cloth pieces, detection of seminal fluid with presumptive testing (ALS, AP, PSA), and confirmation/recovery of spermatozoa from pieces of clothes (KPIC).

2. METHODOLOGY

2.1. Semen Sample Collection

Approximately, 35ml semen was collected in intervals from a healthy male donor with a sperm count of 30 million/ml. A total of 25µl seminal material was spotted on each fabric type of approximately 2cm^2 cuttings and was then left to dry at room temperature for 24 hours.

2.2. Fabrics and Detergents

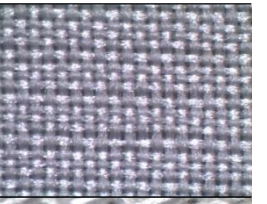
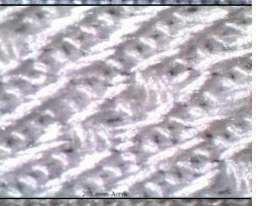
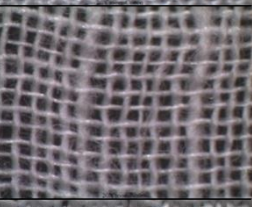
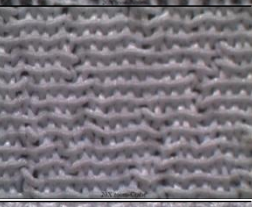
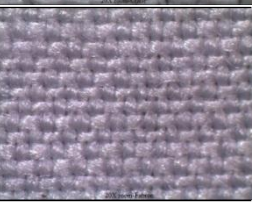
Seven different fabrics, pre-treated with boiling distilled water, commonly used for manufacturing garments of both genders were selected for this study. The general properties of fabrics are shown in Table 1.

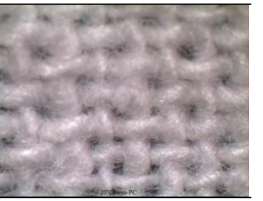
Detergents, such as Ariel (Procter and Gamble), Brite (Colgate-Palmolive), Bonus (Colgate-Palmolive), Sunlight (Unilever Limited), and Surf Excel (Unilever) were used to wash semen-stained fabrics. All these detergents are basic in nature, with a pH between 10-11.

Three semen-stained pieces of each fabric were soaked in a 2% solution of each detergent for three different periods, for instance, 05, 10, and 20 minutes with continuous manual agitation, to mimic routine hand-washing practices. After completing the soaking period, these cloth pieces were rinsed with plenty of distilled

water thrice with vigorous agitation. Distilled water washing in a similar manner was considered a negative control.

Table 1. Physical Parameters of various fabric types and retention of sperm after detergent washing

Sr. No.	Fabric Name	Warf/Weft	Common Products	Thread Name/Gauge	Average Sperm count	Microscopic Image
1	Staple	120x96/inch^2	Shorts, Leggings	Staple/40x40	1682	
2	Acrylic	52x46/inch^2	Socks, Sweaters	Acrylic/40.2x 30.1	891	
3	Cotton	150x96/inch^2	Shirts, Trousers	Pure Cotton/52x52	4166	
4	Vale	80x70/inch^2	Bra, Panties	Pure Cotton/40x40	499	
5	Crabe	200x80/inch^2	[1]Dopatta, Bedding	Polyester/46x 46	596	
6	Fabron	114x78/inch^2	Blouse, [1]Sarrhi	Polyester Viscose /30x30	3038	

Sr. No.	Fabric Name	Warf/Weft	Common Products	Thread Name/Gauge	Average Sperm count	Microscopic Image
7	Poly cotton	44x34/inch^2	Bed sheets, Curtain	Pure Cotton/16x16	3760	

*1 Female specific clothing

2.3. Serological Examination

After washing, all fabrics were air-dried for two hours at room temperature and subjected to Alternate Light Source (ALS) in order to locate semen stain, and an acid phosphatase test was performed [13, 14]. Furthermore, a PSA test was also performed on all fabrics to detect p30 proteins. Microscopy examination was done using the Christmas tree staining method to detect the presence of sperm heads, and the score was calculated [15, 16].

2.4. Isolation and Quantification of DNA

Quantifiler Duo DNA quantification kit (Thermo Scientific, USA) was used to quantify human/human male DNA according to the manufacturer's instructions. DNA from all fabric samples was isolated with an organic extraction protocol. The isolated DNA was dissolved in TE-4 buffer and stored at -20C° for further processing[17].

3. RESULTS

In the current study, pieces of seven fabrics used in the clothing of both genders were stained with semen. The fabrics used in the study had different textures and compositions of yarn, interlaced at a right angle with variable numbers of warf and weft per inch. The yarns used to manufacture these fabrics included linen, polyacrylonitrile, cotton, polyester, and polyester viscose, which confer different water absorbance and retention capabilities to fabrics (Table 1). The verification of each test result was confirmed with positive and negative samples to determine the efficiency and contamination of chemicals, respectively (Figure-1).

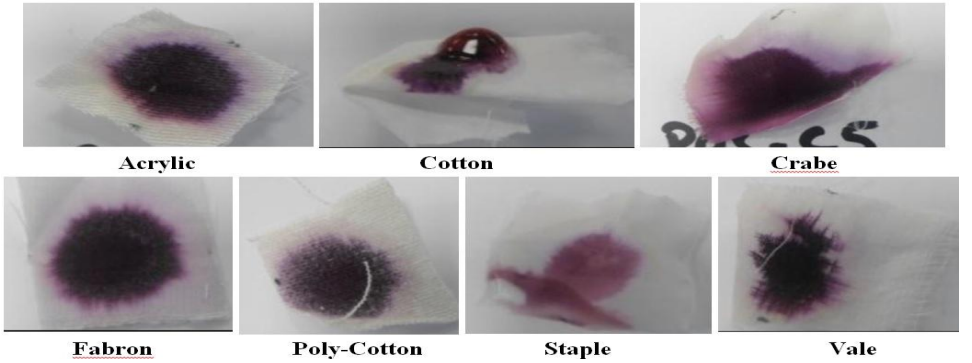


Figure 1. Acid Phosphatase Test Results on Positive Controls of Different Fabrics

No stain was observed with the naked eye on any piece of fabric after washing with any detergent. The fluorescence was observed on almost all fabrics soaked for 10 minutes in detergents, while no fluorescence was observed after 20 minutes of soaking. A slight fluorescence was observed on acrylic, cotton, vale, fabron, and poly cotton even after 20 minutes of washing.

AP test was negative for all fabrics and washed for any time interval. A slight violet color appeared on cotton after 5 minutes of washing with Arial and on Fabron after 5 and 10 minutes of washing with Arial. This color intensified after 5 minutes. AP test was positive for all fabrics washed with distilled with water for any time interval.

PSA test was negative for all fabrics, indicating that detergents successfully removed or destroyed prostate-specific antigens on each fabric treated with detergents. PSA and AP were protein in nature and usually lost their activity even after trivial washing.

Spermatozoa are hard cells that persist on fabric even after washing with detergents. Different fabrics showed different capacities to retain spermatozoa when soaked in different detergents. High sperm counts, that is, up to a few hundred, were observed on fabrics washed with Bright and Sunlight. Surf Excel and Sunlight removed spermatozoa from fabrics in a better way though, sperm heads recovered after washing with these detergents were enough to get DNA for STR profiling.

The nature of fabric and detergent contributed to the holding of spermatozoa by fabrics. Staple, cotton, fabron, and poly cotton showed a good ability to seize sperms after washing. Except poly cotton, these fabrics have a high number of warf and weft per inch^2 as well as high gauge compared to other fabrics. Furthermore, it was also observed that soaking fabrics for 5 minutes removed the spermatozoa more effectively as compared to soaking fabrics for 20 minutes in detergent in staple, acrylic, and cotton. These fabrics retained more sperm heads when washed with Arial, Brite, and Bonus for 20 minutes as compared to 5- or 10-minute washing. Results suggested that the detergent might help attach spermatozoa with the fabric yarn due to opposite charges.

The genomic DNA was isolated from washed pieces of fabric using organic extraction methods and quantified using Quantifiler® Duo DNA Quantification Kit. A high concentration of human genomic DNA was obtained from staple, cotton, fabron, and poly cotton. The quantity and quality of isolated human DNA were so good that STR profiles could easily be generated. A low quantity of human genomic DNA was obtained from fabrics washed with distilled water, Surf Excel, and Bonus, that is, 0.19ng from crabe, 0.15 from cotton, and 0.02 from poly cotton, when these fabrics were washed for 20 minutes. Except for a few fabrics, a sufficient amount of DNA was obtained from all others, which could be used as STR profile of the donor.

4. DISCUSSION

Many reports are present on the persistence of seminal material after laundering clothes in washing machines with or without using detergents [5, 17]. The spermatozoa yield depends upon the type of yarn, weaving, nature of detergent, and time of washing [18].

Karadayi et al. reported that semen stains could be visible on clothes washed at 30ºC and 60ºC in washing machines

[19]. No semen stain was visible with the naked eye on any fabric post-washing, however, slight fluorescence was observed on a few washed fabrics under ALS. Cotton florescence was more intense than on the fleece, nylon, and polyester fabrics [10, 20]. The nature and color of material on which semen is deposited also play an important role in developing photoluminescence [21].

Proteins are more sensitive to washing conditions and lose most enzyme activity, making it difficult to identify the stains on the basis of presumptive tests. The PSA test was negative for all fabrics in this study, while in previous studies PSA test was found to be positive on machine-washed clothes [1, 12, 22]. Cotton, vale, and poly cotton gave light color for the AP test, while the AP test was negative on all other fabrics. Cotton yarn was the major constituent of these fabrics having negative charges, which may help phosphatases to establish a bond with yarn and provide positive results for AP[19]. Few researchers have suggested monitoring change in color up to 10-16 minutes, as the appearance of violet color may take a little longer than recommended time [15, 18].

Spermatozoa are known to stay on laundered clothes and many factors, such as washing time, nature of cloth and detergent, as well as temperature play vital roles in the attachment of sperm with cloth [20]. Moreover, the chemical nature of detergent (acidic or basic nature), ionic strength, and addition of any additive (ionic charge) may affect sperm recovery form washed evidence items [6, 7]. This study detected a sperm range from few to many spermatozoa (Table-I) on fabrics after washing with different detergents. Some detergents helped bind sperm with the fabrics. The warf and weft/inch [2] of staple, cotton, crabe, and fabron were higher than other fabrics used in the study. Therefore, there was a high probability of the entrapment of more sperm heads on these fabrics. The ability of a fabric to retain water, weaving properties, gauge, surface, and type of yarns also contributes to the retention of sperm on fabric [12]. The recovery of spermatozoa is low from machine-washed cloths as compared to hand-washed clothes [13, 20].

The genomic DNA isolated from sperm heads obtained from hand-washed clothes was of good quality and quantity. A low yield of DNA was obtained from fabrics washed with water. An insufficient amount of DNA was obtained from acrylic and poly cotton after washing with water for 10 and 20 minutes. From the rest of the fabrics, enough DNA was obtained, which could be used to generate an STR profile. Beckwith *et al.* reported that a good quantity of human DNA could be obtained from clothes washed with tape, river, and seawater after soaking up to 96 hours [20]. These results are consistent with the findings of Beckwith *et al.* 2008, who reported that tap water did not completely remove sperm from clothes, so it yielded a good amount of human genomic DNA [23].

4.1. Conclusion

Washed cloth items can be a useful piece of evidence to detect seminal material. In Pakistan, forensic laboratories often implement stringent policies regarding the submission of biological samples, particularly when such evidence has been exposed to water or other chemicals. Unfortunately, these policies frequently result in the rejection of evidence that has been subjected to washing, leading to the loss of critical forensic information. Given the potential significance of washed clothing in the

investigation of sexual crimes, it is essential that forensic laboratories reassess and modify their current policies. By doing so, they could better utilize this type of evidence, ensuring that vital forensic data is not discarded prematurely, thereby enhancing the accuracy and comprehensiveness of criminal investigations.

CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

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No funding has been received for this research.

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