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Forensic Examination of Seminal Stains on Different Types of Fabrics After Repeated Wash

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Abstract

In forensic most of the rape cases we encounter cloths of victim, offender and other articles like bedsheets, pillow, sofa, and other objects which may have seminal stains to examine these stains we do acid phosphatase for the preliminary examination of these stains and after that microscopic examination for the confirmation of spermatozoa and further DNA can be extracted from these stains. The evidence submitting for examination in the laboratory has been washed intentionally which resulting in washing of semen from the items of examination. To know the persistence of spermatozoa and acid phosphatase activity 3 different types of fabrics has been repeated wash with normal water and detergent water, acid phosphatase test has been performed on them and microscopy of stains has observed in this experiment.

Keywords: Acid phosphatase, spermatozoa, microscopic, fabrics

1.0 Introduction

In most of the sexual assault cases, evidence given for forensic analysis are regularly examined for seminal fluid. The matter for medical investigations taken from the victim, mainly the genital swabs,

are regularly the main source of associative DNA evidence for suspect identification. [1], During the examination of sexual offences, intimate body swabs, clothing and bedding matters are regularly submitted for examination by forensic scientists. [2]

Seminal fluid is a complex mixture of secretions from 4 urogenital glands in the male, the seminal vesical gland contributes 60%, the prostate gland approximately 30% and the remaining 10% is generated from the epidermis and bulbourethral glands. The average male ejaculates approximately 3.5 millilitres of sperm with each millilitre containing between 10-50 million sperm cells. [3], Brackett (1957) examined the reaction of acid phosphatase test with some very reactive chromogenic components like Diaz-onium ions other aromatic amines like imidazole, pyrroles, indoles, ammonium ion, histidine, hydrazine, hydroxylamine, which is found in commercial douches, suppositories which sometimes give false acid phosphatase reaction is tested in his experiment. [4], Raju *et al.* (1964) analysed the specificity of acid phosphatase test with different vegetables, fruits extract and different biological and physiological fluids from different animals and birds found different colour reactions with different fluids over 100 stains were examined. [5]

Davies Anne (1978) examined semen samples and swabs from laboratory staff and vaginal swabs from cases received in laboratory as evidentiary proof and other extracts from various vegetables and animal semen sample and check the acid phosphatase activity and performed NPP test to determine enzyme activity as units per ml of serum was determined acid phosphatase enzyme activity in different samples also microscopy of these samples for analysis of spermatozoa's and found the results. [6], Joshi U.N., *et al.* (1981) examined the effect of water immersion on seminal stains on cotton cloth, in the experiment they immersed the stained cotton cloth and found that even after 144 hours 6 days immersed the AP activity was still detectable and number of sperm heads was more than 12 per field. [7], Hooft *et al.* (1994) performed two presumptive tests on different body fluids, food and products from daily life which may give false positive results of seminal stains during investigation of seminal stains modified zinc test and acid phosphatase test is performed on these different stains and found out of 1774 semen free samples 46(2.6%) positive results were noted for AP test and 89(5.0%) for modified zinc test. [8]

Kafarowski *et al.* (1996) studied the indirect deposition of spermatozoa on unstained clothes during machine washing from the semen-stained cloths and found that over 50% of the 162 samples excised at least one sperm head was observed and one to two sperm heads were observed in 38% (62/162) of samples three to maximum eight sperm heads were observed in a further 16% of samples (25/162). [9], Allard *et al.* (2007) experiment is carried out in nine laboratories to know the different practices done by them to analyse the semen-stained cloths and swabs in this experiment 11 swabs and 6 cloth pieces stained with different dilutions of semen 1 in 10000 different tests were performed for detection of semen like AP test, microscopic detection of spermatozoa's, PSA test, Choline, Laurell Rocket and DNA. [2] Farmen K. (2007) performed experiments on the machine-washed clothes on 40 degrees Celsius and 60 degrees Celsius and found negative acid phosphatase results in all samples and prostate specific antigen was recovered in 40-degree Celsius samples but not in 60-degree Celsius machine-washed samples and more amount of DNA is recovered from 40-degree Celsius washed samples than 60 degree Celsius samples. [10]

Hellerud *et al.* (2011) examined the samples from the victim genitals were collected for detection of semen in 181 of the 371 cases of alleged rape the swabs were collected from vulva, vagina, and cervix and then AP test were performed, and slides were prepared for microscopic examination and noted the results. [11], Davidson *et al.* (2012) experiment is carried out on behalf of BFF they test

that wetting the test paper only or wetting the semen-stained fabric and the test paper affected detection of semen stains on different fabrics. [12], Lewis *et al.* (2012) analysed the effect on acid phosphatase results by use of make and type of paper. In this research the different type of paper used in acid phosphatase screening is collected from organisation represented in BFF (Body fluid forum) along with details of the make and type then the series of semen dilutions was made and then tested for acid phosphatase and found that most samples of screening paper were able to detect semen dilution of 1 in 40 within 20 minutes. [13], Crowe *et al.* (2013) examined the cotton T-shirts stained with seminal fluid and dry cleaned and some of them is machine washed in cold and hot water then detected by acid phosphatase and spermatozoa detected even after cleaning of stained cloths. [1]

Jobin *et al.*(2013) examined the degree to which a regular machine laundry of semen stained cotton and nylon panties affected semen identification and DNA profiling of the stain in which the all laundered semen stains on cotton panties produced positive result of acid phosphatase test and negative results in case of nylon panties and DNA from laundered semen stains on cotton were reduced to 42.6% of control values, while laundered semen stains on nylon retained less than 1% of DNA detected in controls. [14], Lewis *et al.* (2013) examined to determine whether the direct application of AP reagent to exhibits is alternative to the traditional indirect (blot) testing method used regularly in the laboratory and found that the direct method of acid phosphatase testing was more sensitive than the indirect method on both cotton and synthetic fabric. [15], Spector *et al.* (2013) examined to know the effect of washing on the detection of blood and seminal stains. In which twelve different washing procedures were used on two different type of fabrics cellulose acetate panties and cotton undershorts and performed acid phosphatase test. [16], Nakanishi *et al.* (2014) in this experiment they use five extremely aged samples for the examination ranging from 33-56 years old seminal stains stored at room temperature and performed acid phosphatase test and STR analysis and found that it's possible to analyse extremely aged seminal stains. [17], Singh *et al.* (2015) analysed the variability in sensitivity of acid phosphatase in detection of human seminal stains by modified acid phosphatase test and found that the modified acid phosphatase test can locate a 1/512 serial diluted seminal stain. [18]

2.0 Material and methods:

The semen samples were transferred on various types of fabrics and were checked for AP test and microscopic examinations for seminal stains up to five times of normal washings.

2.1 Preparations and analysis of samples:

2.1.1 Collection of samples

Ten samples each of human seminal fluid from male volunteers who were neither connected in blood relation neither showed hereditary to each other were collected in culture tubes and placed in refrigerator at -4°C for further analysis.

2.1.2 Preparation of acid phosphatase solution

Acid phosphatase solution was prepared by adding 5 ml solution A and 0.5 ml solution B in 44.5 ml of distilled water. Solution A was prepared by dissolving 1 gm Fast Blue B salt in 20 gm sodium acetate (anhydrous) and 10 ml glacial acetic acid in 100 ml of distilled water. Solution B was prepared by dissolving 0.8 gm 1-Naphthyl phosphate sodium salt in 10 ml of distilled water. The prepared solution was kept at 4°C for further use. Yael Herman, Ilan Feine, Ron Gafny (2018) Acid phosphatase test on phadebas® sheets- an optimized method for presumptive saliva and semen detection, Forensic Science International, journal of forensic science international 2018.04.047. [19]

2.2 Preparations and analysis of fabrics with seminal stains after washings:

2.2.1 Collection and preparation of samples for washings

In this study 90 seminal stains were prepared on 90 cloth pieces (30 cotton fabric, 30 woollen fabric and 30 denim fabric) then each type of fabric was washed with normal water washings and detergent water washings for three times. Each type of sample was analysed up to third wash to check the activity of Acid phosphatase test and microscopic examinations.

2.2.3 Analysis of samples by AP test and microscopical examinations

The sensitivity of washed stains was checked with AP solution up to three washings by normal and detergent hand wash. The change in purple colour formed was noted down. Then washed samples were checked for visual examinations based on purple colour formed for Acid phosphatase activity. For microscopic examinations seminal stains were cut to one square cm size, taken in cavity tiles and 3-4 drops of 5% HCl solution was added and kept for 30 minutes. Then the cuttings were teased with forceps and the extract was transferred on microscopic slides. Then slides were kept in room temperature to get air dried. Then dried slides were stained with Eosine–Aniline stain and then seen at 100X10x microscopically.

3.0 Results and discussions

As seen from table number 1 the cotton fabric after the first wash with normal water gave dark purple colour acid phosphatase test reaction, in the second normal water wash of cotton fabric gave the normal purple colour acid phosphatase test reaction and in the third normal water wash of cotton fabric gave light purple colour acid phosphatase test reaction as seen in figure 1 and 2. In figure number 3 1st detergent water wash of cotton fabric gave normal purple colour acid phosphatase test reaction, in second detergent water wash of cotton fabric gave light purple colour acid phosphatase test reaction and in third detergent water wash of cotton fabric gave negative acid phosphatase test reaction. There are several studies showing that spermatozoa from seminal stains persist even after fabric has been washed, Spector (1971) studied the effect of washing on the detection of blood and seminal stains on cellulose acetate fabric panties and cotton fabric undershorts and washed under machine using detergent powder and twelve different washing procedure in which acid phosphatase test gave positive reaction and spermatozoa were found in all washing procedure except in procedure 12 (stained garment soaked overnight in Z detergent and hot wash in Z detergent. [16]



Figure 1: AP test for semen sample (S1) with normal water washes on cotton fabric

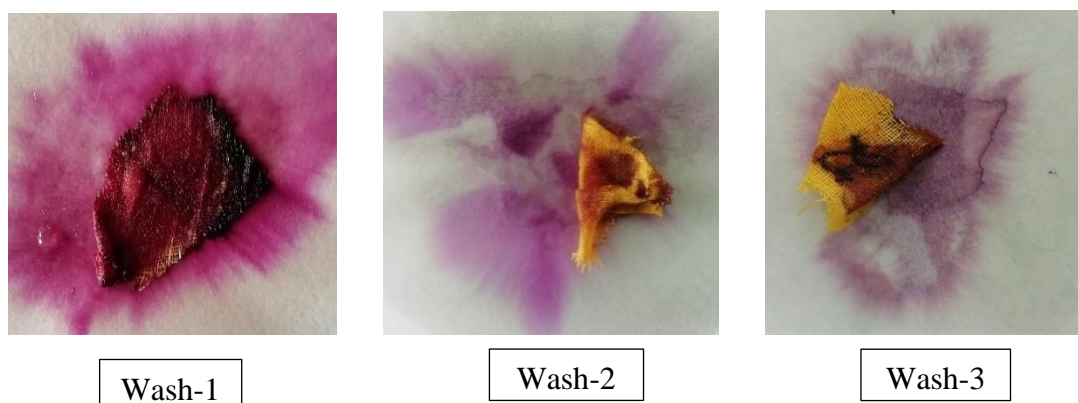


Figure 2: AP test for semen sample (S2) with normal water washes on cotton fabric



Figure 3: AP test for semen sample (S1) with detergent water washes on cotton fabric

As seen in table number 2 the microscopic examination of first normal water wash of cotton fabric 10-12 sperm heads per field were seen, in second normal water wash of cotton fabric 8-10 sperm heads per field were seen and in third normal water wash of cotton fabric 6-8 sperm heads per field were seen. Figure number 4 microscopic examination of first detergent water wash of cotton fabric 8-10 sperm heads per field were seen, in second detergent water wash of cotton fabric 7-8 sperm heads per field were seen and in third detergent water wash of cotton fabric 6-7 sperm heads were seen. Jobin (2003), examined the nine pairs of cotton and nylon panties and stained with semen and washed with detergent and dried, then found that cotton panties have average more sperm heads per field than the nylon panties [14].

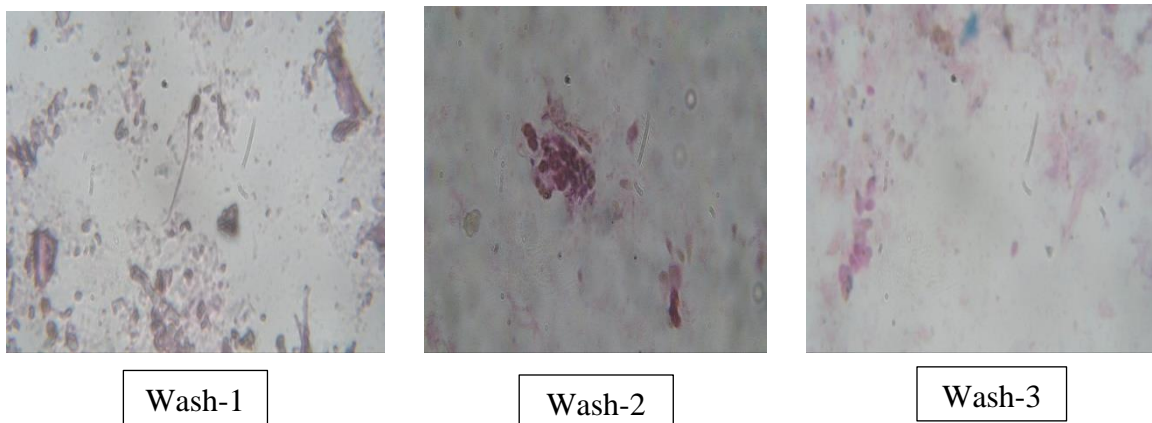


Figure 4: Microscopic view of sample (S1) with detergent water washes on cotton fabric

The first normal water wash of woollen fabric gave normal purple colour acid phosphatase test reaction seen in figure 5, second normal water wash of woollen fabric gave light purple colour acid phosphatase test reaction, and third normal water wash of woollen fabric gave negative acid phosphatase test reaction.



Figure 5: AP test for semen sample (S1) with normal water washes on woollen fabric



Figure 6: AP test for semen sample (S1) with detergent water washes on woollen fabric

As seen in figure 6 first detergent water wash of woollen fabric gave light purple colour acid phosphatase test reaction, the second and third detergent water wash of woollen fabric gave negative acid phosphatase test reaction. Farmen (2008) examined the 30 pairs of cotton underwear's in which

all pairs were machine washed at different temperature 10 at 40 degrees, 10 at 60 degree and 10 at 60 degrees with fabric softener and found that all machine washed gave negative acid phosphatase test result and good amount of DNA was yielded and at 40-degree higher number of spermatozoa was seen. [10]

In microscopic examination of first normal water wash of woollen fabric 8-10 sperm heads per field were seen, in second normal water wash of woollen fabric 7-8 sperm heads per field were seen and in third normal water wash of woollen fabric 5-7 sperm heads per field were seen. In microscopic examination of first detergent water wash of woollen fabric 5-6 sperm heads per field were seen, in second detergent water wash of woollen fabric 4-5 sperm heads per field were seen and third detergent water wash of woollen fabric 3-4 sperm heads were seen. Crowe (2000) examined the 12 cotton t shirts of same colour and style obtained from the same manufacturer and then washed and dry clean with different methods two t-shirts was dry cleaned at three different dry cleaning stores and the other was washed in normal machine washing in cold and hot water, with or without detergent and found the results t-shirts that were dry cleaned gave positive acid phosphatase test reaction and spot cleaned and all machine washed gave negative acid phosphatase test reaction except in cold wash without detergent gave positive AP test reaction. [1]

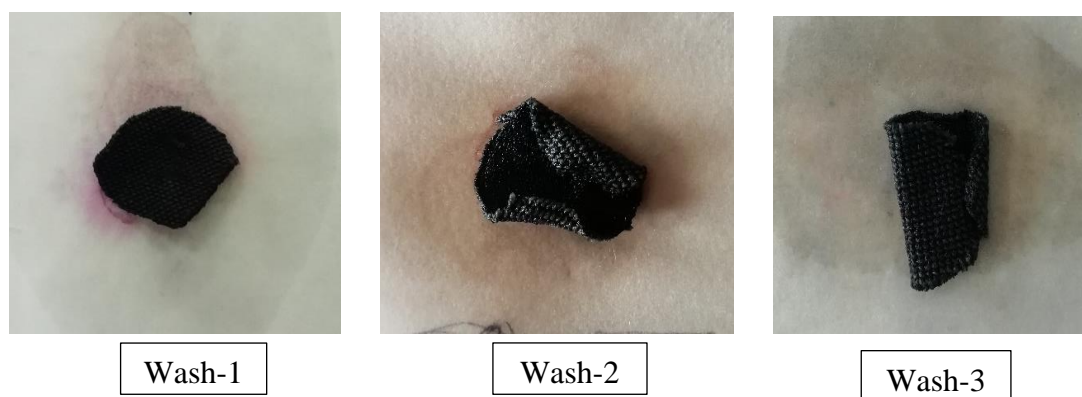


Figure 7: AP test for semen sample (S2) with normal water washes on denim fabric

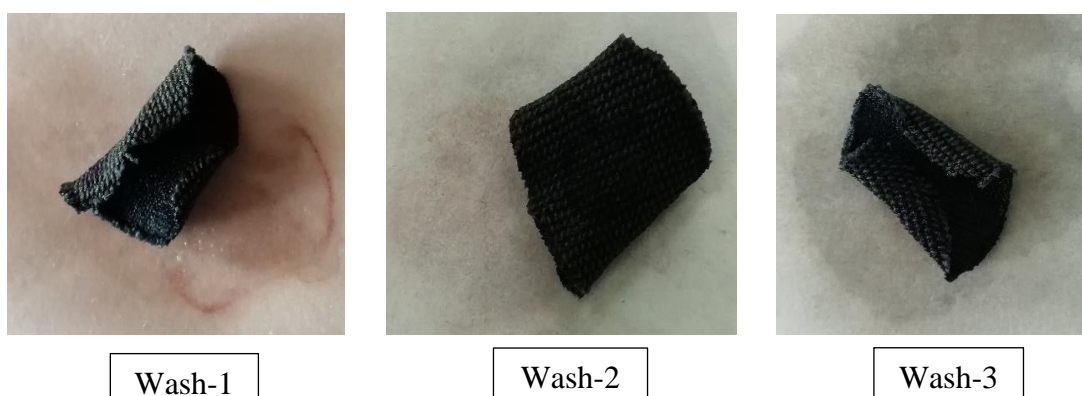


Figure 8: AP test for semen sample (S2) with detergent water washes on denim fabric

As seen in figure 7 normal water wash of denim fabric gave dark purple colour acid phosphatase test reaction, second normal water wash of denim fabric gave normal purple colour acid phosphatase test

reaction, and third normal water wash of denim fabric gave negative acid phosphatase test reaction. As seen in figure 8 detergent water wash of denim fabric gave normal purple colour acid phosphatase test reaction and second and third detergent water wash of denim fabric gave negative acid phosphatase test reaction. Joshi (1981) studied the effect of water immersion seminal stains in which the semen-stained cloths was immersed in water for different time and then examined and found that at 72 hours strong positive results were seen for acid phosphatase test reaction and spermatozoa detection and at 144 hours Acid phosphatase activity decreased and tail less sperm heads were seen. [7]

In microscopic examination of first normal water wash of denim fabric 8-10 sperm heads were seen, in second normal water wash of denim fabric 6-8 sperm heads per field were seen and in third normal water wash of denim fabric 5-6 sperm were seen in figure number 37. In microscopic examination of first detergent water wash of denim fabric 4-5 sperm heads per field were seen, in second detergent water wash of denim fabric 3-4 sperm heads were seen and in third detergent water wash of denim fabric 2-3 sperm heads per field were seen in figure number 9.

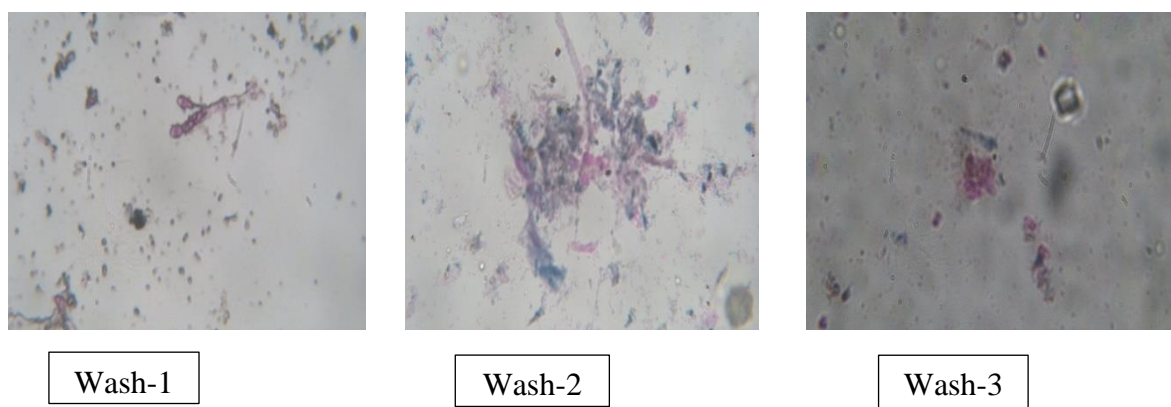


Figure 9: Microscopic view of sample (S1) with detergent water washes on denim fabric

Table 1: Showing the Acid phosphatase results

S.no.	Fabric	Normal water wash			Detergent water wash		
		1 wash	2 wash	3wash	1 wash	2 wash	3wash
1.	Cotton	+++	++	++	++	+	-
	Woollen	++	+	-	+	+	-
	Denim	+++	++	-	+	-	-
2.	Cotton	+++	++	++	++	+	-
	Woollen	++	+	-	+	-	-
	Denim	+	+	-	+	-	-
3.	Cotton	+++	++	++	++	+	+
	Woollen	+++	++	+	++	+	-
	Denim	+	+	+	+	-	-
4.	Cotton	+++	++	++	++	++	+
	Woollen	+	+	-	+	+	-
	Denim	+	+	-	+	-	-
5.	Cotton	+++	++	++	++	++	+

	Woollen	+	+	-	+	-	-
	Denim	+++	-	-	++	-	-

+++ Dark purple colour reaction

++ Normal purple colour reaction

+ Light purple colour reaction

-Negative

Table 2: Showing the microscopic observations

S.no	Fabric	Normal water wash			Detergent water wash		
		1 wash	2 wash	3 wash	1 wash	2 wash	3 wash
		Number of sperma heads per field			Number of sperm heads per field		
1.	Cotton	10-12	8-10	7-8	8-10	7-8	6-7
	Woollen	10-12	8-9	7-8	5-6	4-5	3-4
	Denim	8-10	6-7	5-6	4-5	3-4	2-4
2.	Cotton	10-12	8-9	7-8	5-6	4-5	5-6
	Woollen	6-7	5-6	4-5	5-7	4-5	5-6
	Denim	7-8	5-6	4-5	4-5	4-5	3-4
3.	Cotton	10-12	8-10	7-8	8-9	5-6	6-7
	Woollen	12-13	7-8	7-8	8-9	5-6	6-7
	Denim	10-12	9-10	7-8	8-9	7-8	6-7
4.	Cotton	8-10	7-8	7-8	8-9	5-6	6-7
	Woollen	10-11	7-8	7-8	8-9	7-8	7-8
	Denim	8-9	7-8	5-6	6-7	4-5	4-5
5.	Cotton	8-10	4-5	3-4	8-9	7-8	4-5
	Woollen	10-11	5-7	4-5	8-10	5-6	3-4
	Denim	8-9	6-7	5-6	7-8	6-7	4-5

4.0 Conclusion

The type of fabric affects the detection of acid phosphatase for those fabric which are more absorbent, like cotton, acid phosphatase can be detected after three cycles of normal water wash and detergent water wash and for fabric which is less absorbent than cotton like woollen, acid phosphatase can be detected in case of three cycles of normal water wash but in detergent water can only be detected up to two cycles of detergent water wash and for fabric like denim with very less absorbency acid phosphatase can only be detected after two cycles of normal and detergent water wash. Suggesting that absorbance of seminal fluid into the fabric is needed to somewhat protect the acid phosphatase from being diluted during the wash cycles.

When examining the different fabric types for spermatozoa varying results were found for each fabric like cotton good number of spermatozoa were yielded after three cycles of normal and detergent water wash and woollen yielded good number of spermatozoa in case of normal water wash and less number of spermatozoa in detergent water wash and in denim fabric very less number

of spermatozoa were yielded and in case of detergent water wash only 2-3 spermatozoa heads were seen after three cycles.

It should be noted that three washes should not be considered an end point for detection of acid phosphatase for cotton fabric and detection of spermatozoa on cotton and woollen fabric further examination is required to know the accurate number of washes in which spermatozoa can no longer be detected.

The further study will be done on different fabrics so it can be noted that up to how many washes and different types of washes like detergent washes and machine washes the acid phosphatase and spermatozoa can be detected which will be very helpful in forensic examination of seminal stains on washed samples of different fabrics.

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