How Possible is it to Recover Semen DNA from Laundered Cotton and Polyester Fabrics?

Hoda Ahmed Basyoni[1], Karima Mukhtar Mohamed[1], Usama Mohamed El-Barrany[1], Laila Ahmed Rashed[2], Heba Mohamed Aboubakr[1], Heba Abdo Abdel Razik[1]

[1]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Cairo University, Cairo, Egypt,
[2]Department of Medical Biochemistry and Molecular Biology, Medicine, Cairo University, Cairo, Egypt

Abstract

Background: A key element of forensic genetics is the ability to remove DNA from semen stain. Using various laundering conditions to remove semen stain is a routinely faced forensic problem **Objective:** This study was conducted to evaluate the possibility of detection of semen DNA in different types of fabrics after being washed. **Method:** Semen samples were applied to cotton and polyester clothes. After drying, the clothes were submitted to washing in water at room temperature, in water at 50°C temperature and in detergent at room temperature. DNA extraction was done to estimate the quantity of extracted DNA by spectrophotometer. **Results:** Different DNA quantities still could be detected after washing. There was highly statistical significant decrease ($P \leq 0.001$) in the amount of extracted DNA in all group samples (after washing) compared to control group. Washing by detergent proved to have the most destructive effect on the DNA stains on clothes followed by washing by water at 50^0C while washing by water at room temperature showed the highest recovery of DNA in clothes. The amount of the DNA detected in cotton samples before washing was higher than the amount detected in polyester samples before washing.

Key words: *DNA, Semen Stain, Laundering Condition*

Introduction

Forensic analysis plays a crucial role in criminal investigations, especially those related to sexual assault cases[1].

Exchanging evidences due to contact between the perpetrator, victim and scene is imperative to happen in every crime against human as in sexual assaults[2].

Laundering the clothes-containing evidence is usually done by criminals to obscure their involvement in a crime. However, laundered clothes usually remain as a potential source of evidence[3]

Laundering additives include anything used for laundering purposes such as detergents and bleaches[4]

Corresponding author:
Karima Mukhtar Mohamed
Email address: rokamukhtar@gmail.com

The obtained genetic profiles within the semen traces from clothing samples might probably act as an evidence of sexual assault[5]

Material and Methods

This work was conducted in the laboratory of Biochemistry department at Kasr Alainy Cairo University.

Materials

· Fabric supports for semen stains (100% cotton and 100% polyester).

· Products for cleaning (from the market): Detergent ‘’Persil powder’’ **(Table 1).**

Table (1): Composition and manufacture of detergent.

Type	Composition	Manufacture
Persil® (Detergent) (biological)	Sodium perborate and sodium silicate	Henkel (Egypt ltd) Under license of Henkel (Germany)

Tap Water

Methods

1. Sample collection and preparation:

Ø Cotton and polyester fabrics were sourced from a local fabric store. Fabrics were new and unworn and had not been submitted for washing previously. Each fabric was cut into squares (5 ×5cm for each).

Ø Semen samples were collected in plastic tubes from healthy donors after taken their informed consent, the process of semen collection was done in andrology department at Kasr Alainy Cairo University.

Ø 3ml of semen was added to each piece of cloth. The placement of the semen deposit on the clothing was clearly marked with water-insoluble ink to allow it to be easily and specifically targeted. The marked area was 1cm^2

2. Laundering protocol:

Each of cotton and polyester semen-stained pieces of clothes were divided into four groups, every group was presented by15 samples, and were treated as follows:

1. First group: control samples were not submitted for washing.

2. Second group: samples were submitted for washing in water at room temperature (25-30°C).

3. Third group: samples were submitted for washing in water at 50°C temperature.

4. Fourth group: samples were submitted for washing in water and detergent (Detergent= 20 gm in 500 ml tap water) at room temperature (25-30°C).

Clothes' samples of second, third and fourth groups were laundered by hand washing for a duration of 10 min. During washing, the fabrics were well agitated by hand in order to remove the maximum amount of the stain.

After washing, the clothing items were allowed to dry at room temperature for 24 hours and then kept in dry paper envelope.

3- DNA extraction:

Each Fabric (5 ×5cm) was further cut (1 cm^2) at marked area and stored in a 1.5 ml test tube for extraction, Then cloth was soaked with 100 µl of buffer. The substrate was then placed into a spin basket and centrifuged for 15 minutes to obtain the sample[6,7]

4- Quantitation of extracted DNA:

After extraction, quantity of extracted DNA is estimated by Nanodrop® spectrophotometer. Spectrophotometer was used to measure the absorbance of isolated DNA at 260 nm, 280nm and 230nm. The Nanodrop® instrument can measure using only 0.5 -2 ng/µl up to 12,000ng/µl.

Figure (1): Show Nanodrop® spectrophotometer

Statistical Data Analysis

Data were coded and entered using the statistical package SPSS version 25. Data was summarized using mean and standard deviation for quantitative variables. Comparisons between groups were done using one-way analysis of variance (ANOVA) with multiple comparisons post hoc test. Comparisons between cotton and polyester were done using paired t test[8]

Findings

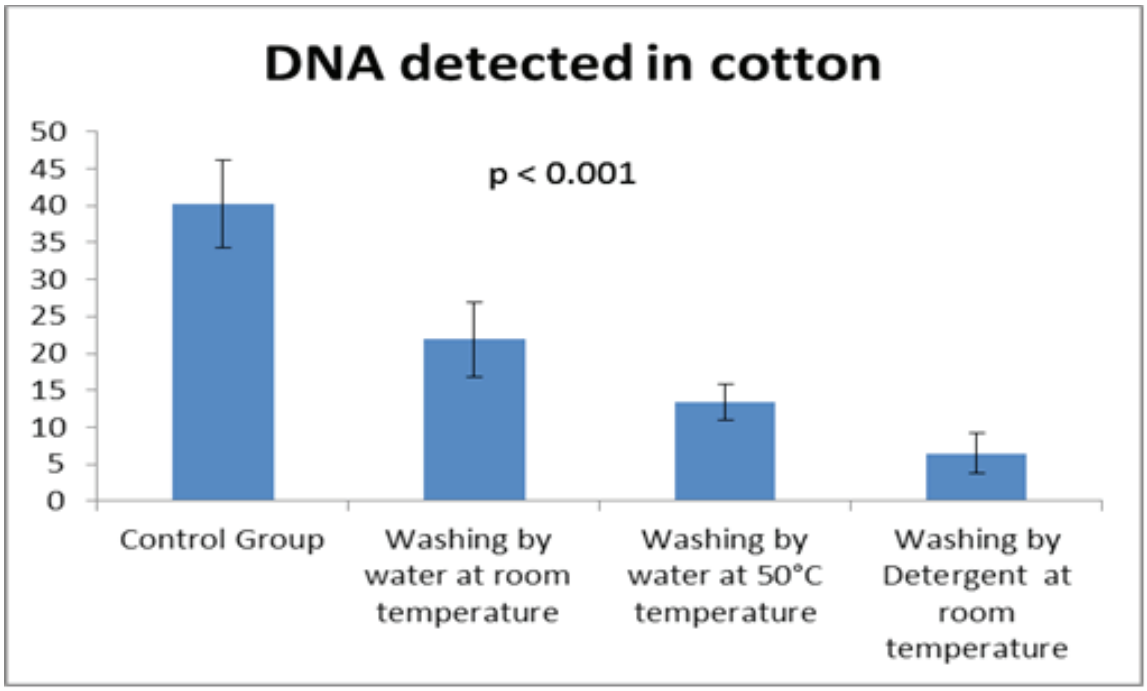

Figure (2): A comparison between the DNA quantities in all cotton samples.

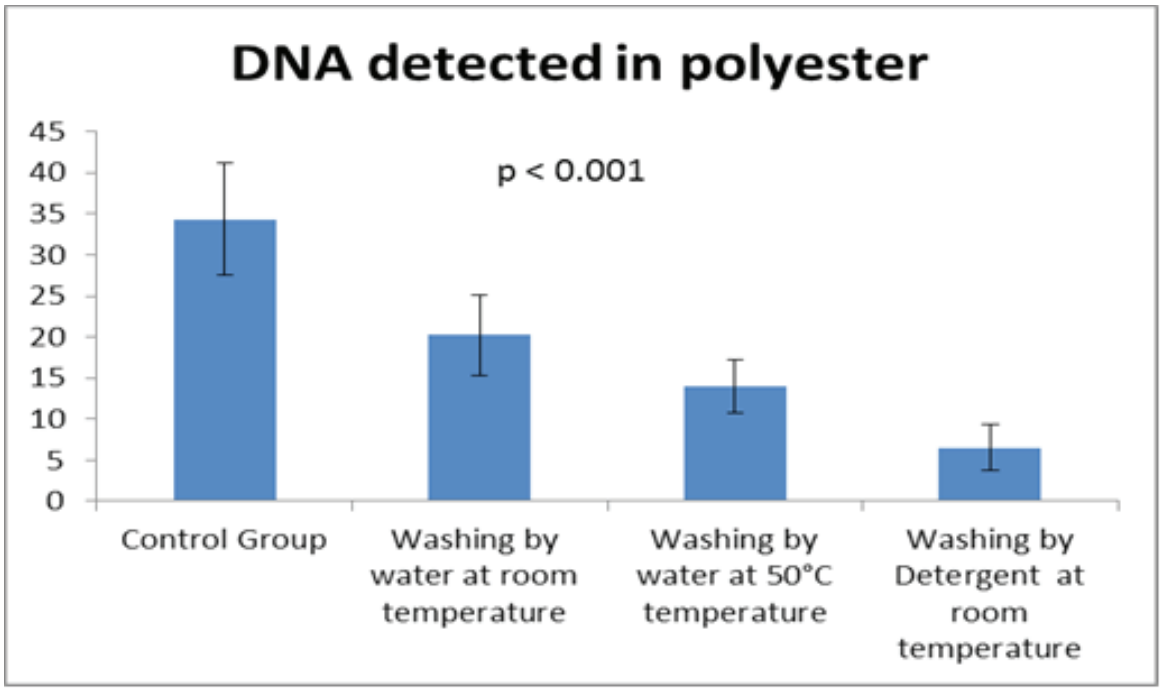

Figure (3): A comparison between the DNA quantities in all polyester samples.

It was observed that DNA still could be detected in both cotton and polyester fabrics after washing in water at room temperature, after washing in water at 50°C temperature, and after washing in detergent at room temperature as shown in **Figure(2,3)**.

There was highly statistical significant decrease ($P \leq 0.001$) in the amount of extracted DNA after washing in water at room temperature, in water at 50°C temperature and in detergent at room temperature but still could be detected compared to control group in both cotton and polyester samples as shown in Table(2).

There was highly statistical significant decrease ($P \leq 0.001$) in the amount of extracted DNA after washing in water at 50°C but it is still could be detected compared to the amount of the DNA extracted after washing in water at room temperature in both cotton and polyester samples as shown in Table(2).

The amount of the DNA extracted from cotton and polyester samples after washing in detergent at room temperature was very minute compared to the amount of the extracted DNA after washing in water at room temperature as shown in Table(2).

Washing in detergent at room temperature proved to have more degrading effect on DNA recovery than washing in water only at 50°C in both cotton and polyester samples as shown in Table(2).

Table (2): Comparsion between the quantity of the extracted DNA (ng/µl) within all studied groups in cotton and polyester fabrics.

	Control Group		Washing in water at room temperature		Washing in water at 50°C temperature		Washing in detergent		P value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
DNA detected in cotton (ng/µl)	40.31	5.93	21.88a	5.05	13.40ab	2.48	6.47abc	2.74	< 0.001
DNA detected in polyester (ng/µl)	34.29	6.81	20.25a	4.92	14.01ab	3.24	6.53abc	2.74	< 0.001

[a]Significant decrease compared to control group.

[b]Significant decrease compared to washing in water at room temperature group.

[c]Significant decrease compared to washing in water at 50°C temperature group.

The amount of the DNA detected in cotton samples in control group was statistically significantly higher than the amount detected in polyester samples in control group (before washing), while, there was no statistical significant difference in the amount of extracted DNA from cotton and polyester fabrics after washing in water at room temperature, after washing in water at 50°C temperature and after washing in detergent at room temperature as **shown in Table (3)**

Table (3): Comparison between cotton and polyester samples regarding the quantities of the extracted DNA within the studied groups.

	DNA detected in cotton (ng/µl)		DNA detected in polyester (ng/µl)		P value
	Mean	Standard Deviation	Mean	Standard Deviation	
Control Group	40.31	5.93	34.29	6.81	0.036
Washing in water at room temperature	21.88	5.05	20.25	4.92	0.339
Washing in water at 50ºC temperature	13.40	2.48	14.01	3.24	0.576
Washing in Detergent	6.47	2.74	6.53	2.74	0.942

Discussion

In the present study it was noticed that DNA is still could be detected after washing in water at room, at 50°C temperature and in detergent at room temperature. This was in accordance with an earlier study by Spector & Von Gemmingen[9] who examined the effect of washing on the persistence of sperms by laundering white cotton undershorts in a washing machine using both cold and warm washes with three different detergent types, This study was concluded that very small amount of semen could be detected after washing.

Furthermore Noël et al.,[10]who stated that after washing once, most semen stains could be detected with Prostatic specific antigen testing and strong fluorescence was showed with Alternate light source , after each wash the fluorescence intensity decreased, semen stains showed weak fluorescence but they could all still be detected even after being washed six times .Also Schlagetter&Glynn[11] noticed that less fluorescence with Alternate Light Source was detected in the washed items than that in the controls items, so the ability of Alternate Light Source to identify washed semen stains was decreased but still could be present.

Previous studies[12,13,14] observed that enough DNA was still could be detected after washing once in majority of cases.

In the present study, the DNA still could be detected after washing by water only at room temperature, Both Joshi et al.,[15] and Crowe et al.,[16]who observed that even after water immersion for up to 72 hours , there is strong

positive results of acid phosphatase (AP).

The results of the present work showed significant decrease in DNA quantities extracted from samples washed in water at 50°C than control samples and those washed in water at room temperature. This was in accordance with Farmen et al.,[17] who found when laundering semen stains at 40°C, the amount of DNA was double the amount recovered from laundered semen stains at 60 °C.

Also Rebecchi et al.[18]who conducted another study on the effect of 21 days of temperature exposure and observed that the DNA stability could markedly affected by high temperatures up to 50°C so decrease its existence.

In addition Al- Kandari et al.,[19] who indicated that at high temperature semen samples appear to be resistant to destruction even after exposure to temperature over 28 days due to the sperm head's structure.

Similary Dissing[20]who studied the effect of the surrounding environment's temperature on DNA stability, observed that after eight months DNA could be amplified at 45°C and 100% humidity, but at 55°C and 100% humidity, it survived for one month only.

In the present work, biological detergent (Persil) proved to have the highest destructive effect, indicated by the marked decrease in the amount of extracted DNA. This coincided with Houston[3] who suggested that diluting laundry additives to the recommended amount by the manufacturer would be more effective at hindering the recovery of DNA than water only.

The present study was also in agreement with Brayley et al.,[21] who reported that there is diminished of DNA recovery when washed with biological detergent compared with non-biological detergent.

The present study showed that the amount of the DNA detected in cotton samples in control group was higher than the amount detected in polyester samples in control group.

The previous finding coincided with Dahi et al.,[22], and Thabet et al.,[23] who reported highest DNA detection from cotton fabric, followed by other fabrics. Also Schlagetter & Glynn[11] who noticed that polyester fabric may not have retained as much of the semen stains during the wash cycles and attributed that to the synthetic nature of polyester which has more uniformity, when it compared to the other fabrics, which were composed of natural fibers as cotton.

Also Nolan et al.,[24] reported that cotton and terry towel showed maximum spermatozoa retention compared to the other fabrics.

Moreover, Schlagetter & Glynn[11] stated that the stain on the wool showed less fluorescence and the semen stain on the cotton fluoresced strongly, while fluorescence in the denim was very difficult to detect. No fluorescence at all was showed on the polyester.

This is mainly attributed to that natural fibers are characterized by having overlapping cuticles which may retain the impacted cellular matters[25].While synthetic fibershave smooth outside which cannot keep cell retained[26].

Also due to the fabric structure as O–H groups of cotton are able of formation of strong hydrogen bonds with nucleic acid chains resulting in strong intermolecular attractions[27]

The previous findings did not coincide with Fiedler et al.,[28] who showed that there was no significant difference in the detectable traces of biological stains between the different types of fabrics.

Conclusion

It is concluded that DNA still could be detected in cotton and polyester after washing in water at room temperature, in water at 50°C temperature and in detergent group at room temperature. Washing in detergent proved to have the most destructive effect on the DNA stains on cloths followed by washing in water at 50^0C while washing in water at room temperature showed the highest recovery of DNA in cloth.

The amount of the DNA detected in cotton samples before washing was higher than the amount detected in polyester samples before washing. So, elimination of semen stain from fabrics is more difficult than generally believed.

Competing Interests: The authors declared that they have no competing interests

Ethics approval: The study work was conducted after the approval of Ethical Committee, Faculty of medicine, Cairo University.

Consent for publication: Consent forms were given and signed by all subjects prior to participation

References

1- Cross M. Detection of Secondary Transfer of Human Spermatozoa between Items of Clothing during a Domestic Washing Machine Cycle using the Quantifiler® Trio DNA Quantification Kit (Doctoral dissertation, Murdoch University). 2017

2- Horswell J. Crime scene investigation. In The Practice Of Crime Scene Investigation. 2004;33-76.

3- Houston EL.The Effects of Various Laundering Factors on the Recoverability of Dna (Doctoral dissertation, Wright State University). 2016.

4- Castelló A, Francés F, Verdú F. DNA evidence uncompromised by active oxygen. Scientific World J. 2010; 10:387-392-

5- Gingras, F., Paquet, C., Bazinet, M., Granger, D., Marcoux-Legault, K., Fiorillo, M. & Jolicoeur, C. Biological and DNA evidence in 1000 sexual assault cases. Forensic Science International: Genetics Supplement Serie. 2009;2(1), 138:140.

6- Spear T, Khoshkebari N. Analysis of old biological samples: A study on the feasibility of obtaining body fluid identification and DNA typing results. California Criminalistics Institute (CCI). Sacramento.2001

7- Choi EH, Lee S.K, Ihm C, Sohn YH. Rapid DNA extraction from dried blood spots on filter paper: potential applications in biobanking. Osong Public Health Res. Perspect.2014; 5(6): 351-357

8- Chan YH. Biostatistics102: Quantitative Data − Parametric & Non-parametric Tests. Singapore Med J.2003;44(8): 391-396.

9- Spector J, Von Gemmingen D. The effect of washing on the detection of blood and seminal stains. Canadian Society of Forensic Science Journal, 1971;4(1): 3-9.

10- Noël S, Lagacé K, Raymond, S, Granger, D, Loyer, M, Bourgoin, S, Séguin D. Repeatedly washed semen stains: Optimal screening and sampling strategies for DNA analysis. Forensic Science International: Genetics. 2019;38: 9-14.

11- Schlagetter, T.G. & Glynn, C.L. The effect of fabric type and laundering conditions on the detection of semen stains. Int Jof Forens Sci.2017; 2(2).

12- Andrews C, Coquoz R. PCR DNA typing of washed stains. In Advances in forensic haemogenetics. 1994;343-345.

13- Kulstein G, Wiegand P.Comprehensive examination of conventional and innovative body fluid identification approaches and DNA profiling of laundered blood-and saliva-stained pieces of cloths. International journal of legal medicine.2018;132(1): 67-81.

14- Hofmann M, Adamec J, Anslinger K, Bayer B, Graw, M, Peschel O, Schulz MM. Detectability of bloodstains after machine washing. International journal of legal medicine.2019; 133(1):3-16.

15- Joshi UN, Subhedar SK., Saraf, DK. Effect of water immersion on seminal stains on cotton cloth. Forensic science international.1981;17(1): 9-

16- Crowe G, Moss D, Elliot D.The effect of laundering on the detection of acid phosphatase and spermatozoa on cotton t-shirts. Canadian Society of Forensic Science Journal.2000; 33(1): 1-5.

17- Farmen RK, Cortez P, Frøyland ES. Spermatozoa recovered on laundered clothing. Forensic Science International: Genetics Supplement Series.2008;1(1): 418-420

18- Rebecchi L, Cesari M, Altiero T, Frigieri A, Guidetti R.. Survival and DNA degradation in anhydrobiotic tardigrades. Journal of Experimental Biology.2009;212(24): 4033-4039

19- Al-Kandari NM., Singh J, Sangar VC. Time-dependent effects of temperature and humidity on quantity of DNA in samples of human saliva, blood and semen in Kuwait. Int. J. Pharm. Sci. Res.2016; 7: 2852-2873.

20- Dissing J, Søndervang A, Lund S. Exploring the limits for the survival of DNA in blood stains. Journal of forensic and legal medicine.2010;17(7): 392-396.

21- Brayley-Morris H, Sorrell A, Revoir AP, Meakin GE, Court DS & Morgan RM.Persistence of DNA from laundered semen stains: implications for child sex trafficking cases. Forensic science international: genetics.2015;19, 165:171.

22- Dahi HF, Ahmed MG, Azza AM, Heba EE, Rustaiyan A, Samiee K, Own FM. Assessment the Effects of Transgenic Egyptian Bt Cotton that

Contain Two Genes Expressing Cry.2013.

23- Thabet HZ, Ghandour NM, & Salama RH. EFFECT OF SOME CLEANING PRODUCTS O BLOOD DNA RETRIEVAL FROM CLOTH.2018.

24- Nolan A, Speers SJ, Murakami J, Chapman B. A pilot study: The effects of repeat washing and fabric type on the detection of seminal fluid and spermatozoa. Forensic science international.2018; 289:51-56.

25- Nolan AN. The detection of spermatozoa on washed forensic exhibits (Doctoral dissertation, Murdoch University). 2016.

26- Tascan M ,Vaughn, EA. Effects of fiber denier, fiber cross-sectional shape and fabric density on acoustical behavior of vertically lapped nonwoven fabrics. Journal of Engineered Fibers and Fabrics.2008;3(2).

27- Edler C, Gehl A, Kohwagner J, Walther M, Krebs O, Augustin, C, Klein A. Blood trace evidence on washed textiles-a systematic approach. International journal of legal medicine.2017;131(4): 1179-1189.

28- Fiedler A, Rehdorf J, Hilbers F, Johrdan L, Stribl C & Benecke M. Detection of Semen(Human and Boar) and Saliva on Fabrics by a Very High Powered UV-/VIS-Light Source. Open Forensic Science Journal.2008;1: 12-15.