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Persistence of bloodstains on natural and synthetic clothes at different temperatures using hand-wash and machine-wash with or without detergent

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ABSTRACT

Background: Blood is one of the most significant biological shreds of evidence that helps in investigations. Bloodstains on fabrics can provide important information for the reconstruction of the crime scene. The clothes in the crime scenes are sometimes destroyed and washed by the malefactor using some detergents. This results in complete or partial degradation of bloodstains.
Objective: The impact of detergent, temperature and duration of washing clothes stained with blood on different natural and synthetic fabrics was investigated in this study.
Methods: A separate batch of fabrics was allowed to wash with simple tap water to detect and compare the retention of blood. The effect of machine and hand-washing was also observed. Luminol and KM was employed as presumptive tests while the Takayama crystal test was used as a confirmatory test for the detection of blood.
Results: Findings suggested that the employment of both a detergent and 50°C temperature is more efficient in removing a significant amount of blood as compared to washing only with tap water. In natural fibres, such as cotton, lawn and khaddar retained blood in machine washing without detergent under all temperatures. In synthetic fibres, such as velvet and chiffon, blood was detectable until washed under 50°C with a detergent.
Conclusion: The fabrics obtained from the crime scene which have been washed under 50°C with a detergent may not give positive results for bloodstains with conventionally used methods. Moreover, bloodstains not visible with naked eye can still give positive results with conventionally used testing procedures.

Introduction

Blood is an important evidence often found on the crime scenes involving homicidal and assault cases.[1] Tracing out the major components of blood can be useful for identification and individualization in forensic investigation procedures.[2] A common practice by perpetrators in such cases is the attempt to

hide the blood at the crime scene by trying to remove it from the surfaces, objects and clothing.[1,3] This washing can lead to dilution of the bloodstains and its detection becomes difficult with the naked eye. During crime scene investigations, the primary focus is to find evidences that can help in solving the case.[4,5] Since detection of blood uses chemical methods that involve the reactions between blood proteins such as haemoglobin, the perpetrators often remove the blood by cleaning agents that have to ability to denature haemoglobin.[6]

Enzyme based detergents have been used since the 1960s and have many advantages.[7] These contain proteases which hydrolyse the fragments of blood proteins which are then easily washed off by water.[8, 9] Criminals use such detergents to get rid of bloodstains from clothing items by machine and hand washing.[10,11] The majority of homicidal cases are in rural or countryside area and consist of murders committed with sharp objects which leave blood traces on clothing either by direct splattering or by the passive absorption of the fabric.[12,13] Criminal often remove these stains with detergents which denature protein in the process.[14]

DNA profiling is quite expensive in terms of both time and finance. Thus, it is imperative to confirm the presence of blood on an evidence prior to further downstream processes. Protein and peroxidase stains are the two main types of reagents used for the detection of blood evidence.[15] Peroxidase reagents react with the iron in heme haemoglobin. Heme in hemoglobin catalyses the reaction between the dye used and an oxidizer, producing a quick color change in the presence of blood.[16]

Several different presumptive tests are being used for the detection of blood. Haemoglobin, a major blood protein, catalyses oxidation of chromogenic compound and causes changes in its colour. The solution of Reduced phenolphthalein is known as the Kastle-Meyer reagent 1. The KM test has been reported to have sensitivity of 1:16,384.[17] The KM reagent is most widely used on bloodstains that are latent and the reaction causes it to turn into a pink colour in the presence of blood due to the oxidation of peroxidase activity of haemoglobin.[10,18] Luminol is used for the detection of latent bloodstains especially when it is suspected that the crime scene has been washed.[19,20] Blood diluted up to $1:10^4$ can be detected and in some cases even up to 1: 10^9.[21] The morphology of a bloodstain is influenced by the type of fabric and its textile characteristics, such as fibre length, elasticity and how they are manufactured.[22] The ability of different types of fabrics to retain blood after machine and hand washing has been discussed previously.[11,23]

The focus of this study was to evaluate the retention of bloodstains on various types of natural and artificial commonly worn by the people. This study aimed to create a link between different washing conditions, at different temperature and time to evaluate the persistence of blood stains on various natural and synthetic fibres.

Methods

Fabric collection

10 types of fabrics, 5 artificial and 5 natural fabrics were selected. Artificial fabrics included Tissue, Net, Attal, Velvet and Chiffon while natural fabrics included Lawn, Cotton, Silk, Khaddar and Linen. Only a single type of commercially available detergent was used to wash the bloodstains on 10 different kinds of fabric at different temperatures 30ºC and 40ºC as described previously.[10,24] An additional 50ºC was added to create a link between temperature and the retention ability of fabrics after washing. Positive and negative controls were also prepared for each variable for each fabric, and were treated similarly as the samples.

Sample collection

Blood was collected from a single consenting healthy donor through the venous puncture and was stored in EDTA coated vacutainerr. The diameter of the fabric was approximately 5cm x 5cm for each fabric. 50µl of blood was applied on each fabric cutting which was then allowed to dry at room temperature for 24 hours in a sterile environment to avoid contamination.

Washing of clothes: All the fabrics were washed separately for intervals of 10, 20 and 30 minutes at 30ºC, 40ºC and 50ºC temperature conditions. For detergent-based washing, 2.5 grams of Ariel detergent was used. 3 cloth pieces of the same sample were washed simultaneously in a Samsung washing machine WD90K6410OW for three cycles at a specified temperature. A single cloth piece was taken out after every 10 minutes and the water was changed. Similar steps were repeated for machine washing with tap water. After washing, the fabrics were left to air dry at room temperature.

A separate batch of clothing containing ten types was hand washed with Ariel at room temperature. The fabrics were well agitated to remove the blood stains until no stains were visible with the naked eye. After washing, they were left to air dry for at least 24 hours.

Kastle-Meyer and luminol reagent preparation: The washed fabrics were subjected to Kastle Meyer KM and luminol testing for the detection of blood and the results were noted. Luminol reagent was prepared according to the protocols described previously 25. In luminol testing, the fabrics were subjected to luminol spray. Luminance was an indication of positive results. Positive results were graded as +/- very weak faint blue colour florescence, + weak blue colour florescence, ++ blue colour florescence, +++ Strong blue colour florescence and ++++ very strong blue colour florescence. KM reagent was prepared according to the protocols described previously 26. The pink color was the indication of positive results. Positive results were graded as +/- very faint pink colour, + faint pink colour, ++ Pink colour, +++ Strong pink colour and ++++ Very strong pink colour. Positive and negative controls were run before each test.

Takayama crystal assay reagent preparation: After the detection of blood, the fabrics were subjected to the Takayama crystal assay test for confirmation. Takayama reagent was prepared according to the protocols described previously (27). Visualization of rhombic shaped crystals was the confirmation of the blood.

Results

Effect of Temperature and Detergent washing on Synthetic fabrics

Detection through luminol test: Blood was detected through luminol testing in all the five artificial fabrics (Tissue, Net, Attal, Chiffon and Velvet) in tap water machine washing under the condition of 30ºC, 40ºC and 50ºC. Negative results were obtained for all the fabrics in machine washing with detergent under the condition of 30ºC, 40ºC and 50ºC. However, Velvet showed contrasting results such that blood was detectable by luminol at 30ºC and 40ºC after treatment with detergent (Table 1).

Detection through KM test: Blood was not detected through KM testing in Tissue and Chiffon fabrics in machine washing with tap water at 30ºC, 40ºC and 50ºC temperature. However, Attal fabric showed positive results under all the different temperatures without detergent. For Net and Velvet fabrics, positive results were obtained at 30ºC and 40ºC, however, negative results were obtained at 50ºC without detergent. After machine washing the fabrics with detergent 'Ariel', blood was not detected on any fabrics at any temperature. Moreover, blood was not detected in detergent used hand-washed clothes even at room temperature (Table 1).

Identification through Takayama test: In the microscopic examination, rhombic shaped crystals were observed in all the synthetic fabric types in machine washing, with tap water in the first and second wash under the conditions of 30ºC, 40ºC and 50ºC. Interestingly, blood was not detected in the third wash at these temperatures. No crystals were observed in any of the selected artificial fabric types when machine washed with detergent under any temperature condition. Moreover, there were no crystals observed in hand-washed samples at room temperature (Table 1).

Table 1

			Luminol						KM						Takayama					
			HW*	MW**			MW with D***			HW*	MW**			MW with D***			HW*	MW**			MW with D***	
				1	2	3	1	2	3		1	2	3	1	2	3		1	2	3	1	2	3
		30		+	+	+	-	-	-		-	-	-	-	-	-		++	++	++	-	-	-
		40		+	+	+	-	-	-		-	-	-	-	-	-		++	++	+	-	-	-
		50		+	+	+	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
		RT^	+							-							-						
	NET		Luminol							KM							Takayama						
			HW*	MW **			MW with D***			HW*	MW**			MW with D***			HW*	MW**			MW with D***	
				1	2	3	1	2	3		1	2	3	1	2	3		1	2	3	1	2	3
		30		+++	++	++	-	-	-		+	+	+	-	-	-		+++	+++	+++	-	-	-
		40		+	+	+	-	-	-		++	+	+	-	-	-		+++	+++	++	-	-	-
		50		++	++	++	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
		RT^	+							-							-						
	ATTAL		Luminol							KM							Takayama						
			HW*	MW **			MW with D***			HW*	MW**			MW with D***			HW*	MW**			MW with D***	
				1	2	3	1	2	3		1	2	3	1	2	3		1	2	3	1	2	3
		30		+	+	+	-	-	-		+	+	+	-	-	-		+++	+++	++	-	-	-
		40		+	+	+	-	-	-		+	+	+	-	-	-		++	++	++	-	-	-
		50		+	+	+	-	-	-		++	++	++	-	-	-		-	-	-	-	-	-
		RT^	+							-							-						
	Chiffon		Luminol							KM							Takayama						
			HW*	MW **			MW with D***			HW*	MW**			MW with D***			HW*	MW**			MW with D***	
				1	2	3	1	2	3		1	2	3	1	2	3		1	2	3	1	2	3
		30		+	+	+	-	-	-		-	-	-	-	-	-		+++	+++	+++	-	-	-
		40		+	+	+	-	-	-		-	-	-	-	-	-		+++	+++	++	-	-	-
		50		+	+	+	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
		RT^	+							-							-						
	Velvet		Luminol							KM							Takayama						
			HW*	MW **			MW with D***			HW*	MW**			MW with D***			HW*	MW**			MW with D***	
				1	2	3	1	2	3		1	2	3	1	2	3		1	2	3	1	2	3
		30		+	+	+	+++	++	++		+	+	+	-	-	-		+++	+++	+++	-	-	-
		40		+++	++	+	+	+	+		+	+	+/-	-	-	-		+++	+++	+++	-	-	-
		50		+	+	+	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
		RT^	+							-							-						

SYNTHETIC FABRICS

***HW= Hand wash, **MW= Machine Wash, ***MW with D= Machine Wash with Detergent, ^RT= Room Temperature**

Table 2

			Luminol						KM						Takayama					
NATURAL FABRICS	**Lawn**		**HW***	**MW ****			**MW with D*****			**HW***	**MW****			**MW with D*****			**HW***	**MW****			**MW with D*****	
				1	**2**	**3**	1	2	3		1	2	3					1	2	3	1	2	3
		30		+	+	+	+++	++	++		+	+	+	-	-	-		+++	+++	+++	-	-	-
		40		+	+	+	+++	++	++		+	+	+	-	-	-		+++	+++	+++	-	-	-
		50		+	+	+	+/-	+/-	+/-		+	+	+/-	-	-	-		+	+/-	+/-	-	-	-
		RT^	+							-							-						
	Cotton		**Luminol**							**KM**							**Takayama**						
			HW*	**MW ****			**MW with D*****			**HW***	**MW****			**MW with D*****			**HW***	**MW****			**MW with D*****	
				1	**2**	**3**	1	2	3		1	2	3					1	2	3	1	2	3
		30		++	+++	+++	++++	+++	++		+	+	+	-	-	-		+	+	+	-	-	-
		40		+++	+++	+++	++++	+++	++		+	+	+	-	-	-		+	+	+	-	-	-
		50		+++	+++	+++	+++	+++	++		+	+/-	+/-	-	-	-		+	+	+	-	-	-
		RT^	+							-							-						
	Khaddar																						
			Luminol							**KM**							**Takayama**						
			HW*	**MW ****			**MW with D*****			**HW***	**MW****			**MW with D*****			**HW***	**MW****			**MW with D*****	
				1	**2**	**3**	1	2	3		1	2	3					1	2	3	1	2	3
		30		+	+	+	+++	++	++		+	+	+	+	-	-		++	++	++	-	-	-
		40		++	++	++	+++	++	++		+	+	+	-	-	-		++	++	++	-	-	-
		50		++	++	++	+	+	+		++	++	++	-	-	-		+	-	-	-	-	-
		RT^	+							-							-						
	Linen		**Luminol**							**KM**							**Takayama**						
			HW*	**MW ****			**MW with D*****			**HW***	**MW****			**MW with D*****			**HW***	**MW****			**MW with D*****	
				1	**2**	**3**	1	2	3		1	2	3					1	2	3	1	2	3
		30		+	+	+	-	-	-		+	+	+	-	-	-		+++	+++	+++	-	-	-
		40		+	+	+	-	-	-		+	+	+	-	-	-		++	++	++	-	-	-
		50		+/-	+/-	+/-	-	-	-		+/-	-	-	-	-	-		-	-	-	-	-	-
		RT^	-																				
	Silk		**Luminol**							**KM**							**Takayama**						
			HW*	**MW ****			**MW with D*****			**HW***	**MW****			**MW with D*****			**HW***	**MW****			**MW with D*****	
				1	**2**	**3**	1	2	3		1	2	3	1	2	3		1	2	3	1	2	3
		30		+	+	+	+/-	+/-	+/-		+	+	+	-	-	-		++	++	++	-	-	-
		40		+	+	+	+/-	+/-	+/-		++	++	++	-	-	-		++	++	++	-	-	-
		50		+	+	+	+/-	+/-	+/-		++++	++++	++++	-	-	-		-	-	-	-	-	-
		RT^	-							-							-						

***HW= Hand wash, **MW= Machine Wash, ***MW with D= Machine Wash with Detergent, ^RT= Room Temperature**

Effect of Temperature and Detergent washing on Natural fabrics

Detection through luminol test: Blood was detected through luminol testing on four natural fabrics Lawn, Cotton, Khaddar and Silk at temperature 30ºC, 40ºC and 50ºC in tap water and detergent-based machine washing. A general trend was observed in these fabrics such that after treatment with the detergent, no blood was detected with luminol regardless of temperature conditions. However, Linen showed positive results under the condition of 30ºC, 40ºC and 50ºC in tap water, but detergent-based washing showed negative results under the conditions of 30ºC, 40ºC and 50ºC temperature. Lawn, cotton and khaddar showed positive results in handwashing with Ariel at room temperature, but linen and silk showed negative results (Table 2).

Detection through KM test: Blood was detected through KM testing in the five natural fibers used in this study at 30ºC, 40ºC and 50ºC temperature in tap water and detergent-based machine washing On contrary, Linen showed negative results in the second and third wash at 50ºC in tap water machine washed samples. Blood was not detected after machine washing with detergent except for khaddar, which showed positive results after the first wash at 30ºC. The studied natural fabrics showed negative results after hand washing with Ariel at room temperature (Table 2).

Identification through Takayama test: Microscopic examination of all the five fabrics using Takayama crystal assay showed positive results at temperature 30ºC, 40ºC and 50ºC in tap water machine-washing. Interestingly, khaddar showed negative results in the second and third wash at 50ºC. Linen and silk showed negative at 50ºC. Microscopic examination returned negative results for the studied natural fabrics in machine washing with detergent at temperature 30ºC, 40ºC and 50ºC and hand washing with Ariel at room temperature (Table 2).

Discussion

It was observed from the results that blood is absorbed differently on every fabric. Some fabrics absorb more blood in comparison to other fabrics. They showed a larger diameter of bloodstain when observed after staining. Such kinds of fabrics retained more blood and showed interesting results in the detection. For the clothes having low blood absorption e.g. velvet, the bloodstain appeared as a clot and was easily removed. Almost 50% of the blood is generally removed after machine washing with detergent due to low absorbency as reported in previous studies.[26,28.]

The absorbance of blood is based on the chemistry of fabrics, the weave pattern of the fabrics, chemical composition and the moisture-wicking property of fabrics. Some fabrics retained bloodstains even after machine washing with detergent. The stains were easily visible with the naked eyes. The fabrics used for the study vary in their chemical composition, texture and absorbency.[11] Absorbency is the property that allows the fabrics to absorb stains to varied degrees. After washing with detergent, khaddar and cotton retained a larger amount of blood as compared to other fabrics. As described previously, Silk and Tissue had the least retention of blood after tap-water washing and detergent washing in machine.[29] Linen is a natural fibre obtained from the stalk of the plant. Its moisture-wicking and absorbency properties are very high and it is resistant to many acids.[28]

Cotton is a natural fiber. Its moisture retention and absorbency properties are very high. This might be the reason for a greater amount of blood retention and detectability through presumptive and confirmatory tests on cotton after tap water washing. Silk and Tissue have the least retention of blood after tap-water washing and detergent-washing in machine.[29] This might be due to the low absorbance property of Silk fabric. The bloodstains were effectively removed from the fabrics using Ariel detergent. 50% of the samples showed negative results after 10-15 min washing. Sodium sulfonate “a strong surfactant” and NaOH “a strong alkali” are major components of Ariel, which can effectively remove bloodstains from the fabrics.[25]

Bloodstains were detectable after washing with tap water at different temperatures and the detectability decreases with the increase of temperature. The natural fabrics, Khaddar, Linen and Silk showed negative results when washed under 50ºC temperature condition. This suggests that the employment of 50ºC temperature helped the removal of bloodstain when combined with detergent washing. Blood retention in fabrics could be a useful element when viewed as a shred of evidence. DNA profiling could be achieved from washed clothes even after washing with detergent.

Conclusion

In general, 50ºC temperature, when combined with detergent washing removed blood stains from fabrics since the detergent alone failed to remove blood stains from Velvet and Chiffon clothing at lower temperatures. These findings suggest that fabrics obtained from the crime scene which have been washed under 50ºC with a detergent may not give positive results for bloodstains with conventionally used methods. Moreover, bloodstains not visible with naked eye can still give positive results with conventionally used testing procedures.

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References

1. James SH, Kish PE, Sutton TP. Principles of Bloodstain Pattern Analysis. Principles of Bloodstain Pattern Analysis. CRC Press; 2005.
2. Saferstein R. Criminalistics: An introduction to forensic science. 2004;
3. Pattavina A, Byrne JM, Garcia L. An examination of citizen involvement in crime prevention in high-risk versus low- to moderate-risk neighborhoods. Crime Delinq. 2006;52(2):203–31.
4. Cox M. A Study of the Sensitivity and Specificity of Four Presumptive Tests for Blood. J Forensic Sci. 1991;36(5):13170J.
5. Higaki RS, Philp WMS. A study of the sensitivity, stability and specificity of phenolphthalein as an indicator test for blood. J Can Soc Forensic Sci. 1976;9(3):97–102.
6. Anson ML. The denaturation of proteins by synthetic detergents and bile salts. J Gen Physiol. 1939;23(2):239.
7. Jurado E, Bravo V, Luzón G, Fernández-Serrano M, García-Román M, Altmajer-Vaz D, et al. Hard-surface cleaning using lipases: Enzyme-surfactant interactions and washing tests. J Surfactants Deterg. 2007;10(1):61–70.
8. Banerjee UC, Sani RK, Azmi W, Soni R. Thermostable alkaline protease from Bacillus brevis and its characterization as a laundry detergent additive. Process Biochem. 1999;35(1–2):213–9.
9. Beg QK, Gupta R. Purification and characterization of an oxidation-stable, thiol-dependent serine alkaline protease from Bacillus mojavensis. Enzyme Microb Technol. 2003;32(2):294–304.
10. Edler C, Gehl A, Kohwagner J, Walther M, Krebs O, Augustin C, et al. Erratum to: Blood Trace Evidence on Washed Textiles - a systematic approach. Int J Legal Med. 2017;131(4):1191.
11. Peschel O, Kunz SN, Rothschild MA, Mützel E. Blood stain pattern analysis. Forensic Sci Med Pathol. 2011;7(3):257–70.
12. Peschel O, Ramsthaler F, Rothschild M. Forensische Blutspurenmusteranalyse. Lehmanns Media; 2015. 208 p.
13. McCullers C. Das Herz ist ein einsamer Jäger. Diogenes Verlag AG; 2013.
14. Hartley G, Glynn C. A Comparative Analysis of Commercially Available Protein and Peroxidase Reagents for Blood Detection and Enhancement on Laundered Clothing.
15. Bevel T, Gardner RM. Bloodstain pattern analysis with an introduction to crime scene reconstruction. CRC press; 2008.
16. Casali F, Ciavaglia SA, Gannicliffe C, Lidstone N, Webster LMI. Validation of presumptive tests for non-human blood using Kastle Meyer and Hemastix reagents. Sci Justice. 2020;60(1):30–5.
17. De Almeida JP, Glesse N, Bonorino C. Effect of presumptive tests reagents on human blood confirmatory tests and DNA analysis using real time polymerase chain reaction. Forensic Sci Int. 2011;206(1–

3):58–61.
18. No A. Branch Procedure. Development. 2014;1(11).
19. Barni F, Lewis SW, Berti A, Miskelly GM, Lago G. Forensic application of the luminol reaction as a presumptive test for latent blood detection. Talanta. 2007;72(3):896–913.
20. Pex JO. The Use and Limitations of Luminol in Bloodstain Pattern Analysis. Int Assoc Bloodstain Pattern Anal News. 2005;21(4):11–6.
21. Afreen S %J J of CE. Developing a new combination and proportion of chemicals for the production of laundry detergent at low cost in context of Bangladesh. 2011;26:50–3.
22. Cox M. Effect of Fabric Washing on the Presumptive Identification of Bloodstains. J Forensic Sci. 1990;35(6):12968J.
23. Oldfield C, Morgan RM, Miles HF, French JC. The efficacy of luminol in detecting bloodstains that have been washed with sodium percarbonate and exposed to environmental conditions. Aust J Forensic Sci. 2018;50(4):345–54.
24. Howard D, Chaseling J, Wright K. Detection of blood on clothing laundered with sodium percarbonate. Forensic Sci Int. 2019;302:109885.
25. Jackman DR, Dixon MK. The guide to textiles for interior designers. Portage & Main Pr; 1990.
26. Hatch AL. A Modified Reagent for the Confirmation of Blood. J Forensic Sci. 1993;38(6):13558J.
27. Broadbent AD. Basic principles of textile coloration. Society of Dyers and Colourists; 2001.
28. Castelló A, Francés F, Verdú F. DNA evidence uncompromised by active oxygen. ScientificWorldJournal. 2010;10:387–92.