

Identification of Blood Stains on Different Fabrics after Washing with Routinely Used Detergents in India

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Abstract

Blood is concerned to be major evidence during crime scene investigations and can play an important role to trace and to link or eliminate a suspect to a crime. Often criminals attempt to remove these blood traces from the crime scene by partial or complete washing through detergents or acids. Many presumptive tests are used to detect the blood stains from different garments or clothes. In this study, prior to applying Kastle-Meyer (KM) tests in different types of fabrics, all the 8 different varieties of fabrics were washed with different commercially available detergents. The fabric soaking time with various detergents may also affect the removal of blood stains. Ariel (detergent) showed the best results in removing the blood stains from all fabrics, whereas cotton-brasso fabric showed the maximum retention of blood after washing with all detergents. Terrycott and silk-polyester had the minimum ability to retain the blood stains.

Keywords: Phenolphthalein, Kastle-Meyer test, Blood-stains, Detergents, Enzymes

Introduction

While investigating (Forensic) cases, searching for minute trace evidences like Blood, Semen, Saliva, Vaginal Fluids, Urine and Sweat [1], originating from either suspect or victim is a crucial part for facts of understanding what type of crime had taken place. Blood as physical and biological evidence is found in a number of violent crimes, which pay investigators attention in crime scene investigations and can provide crucial information to define a type of crime and link a suspect to the scene of crime. The major task for the expert is to examine a crime scene with different search methods and to trace the minute trace evidences left by the criminal. The then collected or lifted material is considered to be examined with every possible scientific technique, to evaluate and find the degree of relativity of both the

victim and suspect [2]. Sometimes these biological evident materials will stick to the clothes as an adhesive in fabrics; besides every possibilities of removing the blood stains from crime scenes, criminals will try to shade the blood stains in the form of washing the surface, floor, tiles, and other objects like Garments used in the violent crimes [3,4], due to this blood cannot be seen through naked eye after washing [5]. Generally at the crime scene, criminals used to wash the blood and evidence material depending upon the status of crime scene (viz. Indoor or Outdoor). It was seen that in indoor crime scenes the suspects will try to wash the stained area with water, acids, detergents or any other readily available materials, and in outdoor crimes scenes they will use water from the nearby sources. With a Forensic perspective, it is very important to trace out the major components of blood that will be useful for identification and individualization of an individual [6].

Enzyme based detergents were marketed in 1960's for their very advantages like easy cleaning [7]; these different detergents have the capability to remove the proteins such as hemoglobin [8]. Proteases are one of such enzymes and especially *Bacillus* species is used in the industries [9-12]. This proteases enzyme hydrolyses large protein fragments, which are then removed by water. The (biological fluids) stains are frequently removed from fabrics with the help of these proteases enzyme [13]. In recent years, the strength of proteases in bleaching agents has been enhanced by use of new protein production techniques [14,15].

Reduced phenolphthalein test is also known as Kastle-Meyer test (KM test) [16]. Phenolphthalein test is performed mainly in cases where latent blood drops were found, this reaction takes place in an alkaline solution to turn immense pink color when reacts with heme portion of the blood. The heme group of hemoglobin exhibits a "peroxidase-like" activity, which may catalyze the breakdown of hydrogen peroxide [17]. Through KM Test blood can be detected to 1 in 10,000 ppm [17-19]. Disadvantage of phenolphthalein test is that false positives can occur when in contact with vegetable and fruit juices, which gives pink color such as Holy Basil, Spinach, Onion, Pomegranate leaves, grapes.

Materials and Methods

Eight different used fabrics were randomly selected for the present study with 5 different commercially available and routinely used detergents. These 5 different detergents were used to wash the bloodstains on 8 different fabrics. Table 1 shows 8 selected fabrics and Table 2 shows routinely used detergents in India.

Sl. No.	Name of fabrics
1	Cotton
2	Cotton brasso
3	Rubiya cotton
4	Polyester
5	Silk Polyester
6	Linen
7	Linen cotton mix
8	Terrycott

Table 1: Types of fabrics.

Sl. No.	Name of detergents	Manufacturer
1	Ariel	Proctor & Gamble (India)
2	Tide	Proctor & Gamble (India)
3	Surf Excel	Hindustan Unilever Ltd. (India)
4	Ghadi Detergent	RSPL Ltd. (India)
5	Nirma Super	Nirma Ltd. (India)

Table 2: Routinely used detergents in India.

Sample collection

Blood samples were randomly collected from healthy volunteers by venous punctured in EDTA coated vacutainers. Each fabric samples were cut into 5 x 5 cm, 50µl of blood sample was dropped by pipettes on each type of fabrics. The subjected fabrics with blood were then dried for 24 hrs and washed with different detergents which were kept again for drying at room temperature.

Washing of stains

All the 8 fabrics were soaked in individual beakers with different detergents for time intervals of 15, 30 and 60 minutes respectively. They were then hand washed properly for 5 minutes by well agitating them so that maximum amount of blood gets removed from the fabrics. For washing purpose, about 2.5 gram of detergent (powdered form) was taken in 250 ml of water. Each type of fabric was washed with all the five detergents. After washing, the fabrics were left to dry at room temperature for 24 hrs. Each blood stain on the fabric was then subjected to the KM test for the detection of blood and results were noted afterwards. Fabrics with blood stains and without blood stains were also washed with water for positive and negative control [20-22].

Kastle-Meyer Reagent Preparation

Kastle-Meyer (KM) reagent was prepared by adding 2 gm of phenolphthalein powder in 10 ml of freshly prepared 20% sodium hydroxide solution (20 gm in 100 ml distilled water). The solution left to boil with adding 20 gm of Zinc Dust (powdered form), until the solution has lost its pink color. The KM test was performed by adding 2 drops of ethanol to previously blood stained, washed and dried fabrics, followed by 2 drops of working phenolphthalein solution (Phenolphthalein reagent 2 ml, Distilled Water 10 ml, Ethanol 2 ml), after waiting for 2 minutes to insure that no color develops at this stage, two to three drops of 3% Hydrogen Peroxide are added. An

intense pink color is a positive test for peroxidase activity, indicative of hemoglobin.

Results

Eight randomly selected and commonly used fabrics were selected for the present study. All the eight fabric samples were stained with blood and were allowed to dry at room temperature for 24 hours. After drying, these fabrics were washed one by one with five (commercially available) commonly used detergents for three different time intervals viz. 15, 30 and 60 minutes

respectively in different beakers. After washing, the samples were left to dry at room temperature for 24 hours. To these fabrics, KM test was performed to detect the presence of blood after washing. All the detergents removed the blood stains from the fabrics to different extents. (Table 3) indicates the percentage of positive samples in which blood was detected, after washing with different detergents at time intervals 15, 30 and 60 minutes respectively. In all Positive controls blood was detected after performing KM test. None of the negative controls gave positive results after washing with either of the detergents.

Detergent	Soaking time in minutes	Different varieties of fabrics								Positive samples %
		Cotton	Brasso	Rubiya	Polyester	Silk Polyester	Linen	Linen cotton mix	Terrycott	
Ariel	15	+	+	+	+	-	-	-	-	50
	30	+	+	-	-	-	-	-	-	25
	60	+	+	-	-	-	-	-	-	25
Tide	15	+	+	+	+	+	+	+	-	87.5
	30	+	+	+	+	-	+	+	-	75
	60	+	+	+	+	-	+	-	-	62.5
Surf Excel	15	+	+	+	+	-	+	+	-	75
	30	+	+	+	+	-	+	+	-	75
	60	+	+	+	+	-	+	+	-	75
Ghadi	15	+	+	+	+	-	+	+	-	75
	30	+	+	+	+	-	+	+	-	75
	60	+	+	+	+	-	+	-	-	62.5
Nirma	15	+	+	+	+	-	+	+	-	75
	30	+	+	+	+	-	+	-	-	62.5
	60	+	+	+	+	-	-	-	-	50

Table 3: KM test results on fabric varieties after washing with different detergents.

+: Blood stains found positive even after washing with different detergents; -: Blood stain washed away

The fabrics used in this study are cotton, polyester, linen and a mixture of these major fibres. Cotton is the most prevalent plant fibre and quite distinctive in appearance. The absorbency and moisture retention of cotton is high. Linen fibre is a typical vegetable fibre obtained from the stalk of the flax plant. It also has high absorbency, which retains moisture and resists alkalis and cold dilute acids. Polyester is a synthetic man-made fibre and has an outstanding dimensional stability and offers resistance to dirt, alkalis, decay and mould. The absorbency and moisture retention of polyester is very low, it absorbs oil and difficult to remove these stains from the fabric. Blood stains from terrycott and silk polyester were almost washed completely from all the detergent types after 15 minutes of washing time. Probably, this was due to the low absorbency and low

regains values of these fabrics. After washing with detergents followed by KM test to the fabric, it was observed that blood was detected from cotton and brasso fabric even after 1 hour of washing time from either of the detergents.

Ariel detergent removed the blood stains from all fabrics very effectively. After 15 minutes of washing, 50% of the samples showed negative results for blood stains. Ariel also removed stains from 75% of the fabric varieties (viz. rubiya, silk-polyester, linen, linen-cotton mix, terrycott and polyester) after 30 and 60 minutes of washing time respectively. Tide removed blood stains from just terrycott fabric out of eight samples after 15 minutes. However, after 30 and 60 minutes of washing stains were removed from two samples (terrycott and

silk-polyester) and three samples (terrycott, silk-polyester and linen cotton mix) respectively. Surf excel was able to remove blood stains from just 25% of the samples even after washing the fabrics for 15, 30 and 60 minutes i.e. out of eight samples blood stain was removed from silk-polyester and terrycott only. After washing the samples with Ghadi detergent for 15 minutes, removes blood stains from only two samples (silk-polyester and terrycott); same results were observed after 30 minutes. After 60 minutes washing time stains were not observed in three samples (silk-polyester, linen-cotton mix and terrycott). Washing with Nirma detergent for 15 minutes, it was observed that blood stains were removed from terrycott and silk-polyester. After 30 minutes blood stains were removed from terrycott, linen-cotton mix and-silk polyester. However, it was able to remove stains from 50% samples i.e. Silk polyester, Linen cotton mix, Linen and Terrycott after 60 minutes.

A typical detergent used for laundry purpose contains a surfactant, agents that remove dirt, grease, or oily stains and some alkalis. Fabric brighteners and shiners are also a part of some laundry detergents. Some of the detergents also contain bleaching agents that are used to give additional brightness to fabrics by removing stains. Ariel detergent contains sodium sulfonate (a strong surfactant) and NaOH (a strong alkali), it is because of these components Ariel detergent is able to wash the blood stains from most of the fabrics very effectively after performing the Kastle-Meyer test. Rest of the detergents contain very mild surfactants e.g. surf excel contains linear alkyl benzene-sulfonate with sodium carbonate. Due to this mild anionic surfactant, the efficiency in washing the bloodstains from these detergents is less as compared to Ariel detergent.

Discussion

The detergents used for laundry purposes vary in their chemical compositions, thus also vary in their efficiency to remove stains from the fabrics. A typical detergent used for laundry purpose contains a surfactant, agents that remove dirt, grease, or oily stains and some alkalis. Fabric brighteners and shiners are also a part of some laundry detergents. Some of the detergents also contain bleaching agents that are used to give additional brightness to fabrics by removing stains. Hydrogen peroxide is nowadays used as a bleaching agent. Ariel detergent contains sodium sulfonate (a strong surfactant) and NaOH (a strong alkali). It is because of these factors, blood was detected on the least number of samples when washed with Ariel detergent after performing the Kastle-

Meyer test. Rest of the detergents contains very mild surfactants e.g. Surf excel contains linear alkyl benzenesulfonate with Sodium carbonate. Due to this mild anionic surfactant, the detergents efficiency in bloodstains washing from the fabrics is less as compared to Ariel detergent. The present study is an attempt to correlate the KM test (preliminary examination of blood) to different fabric varieties after washing them with different commercially available and commonly used detergents. However, there is a probability of tracing blood stains even after washing with detergents and can be useful further for DNA profiling.

Conclusion

Many times Forensic scientists encounter with situations where physical and biological trace evidences are knowingly ruined to hide the crime. It is important to see, whether it is possible to trace the blood stains in situations where deliberate attempts made to wash the objects (weapons or clothes). Keeping this conditions in view this study is an attempt to examine the presence or absence of bloodstains on different varieties of fabrics after washing them with different detergents. Ariel detergent showed the maximum efficiency by removing bloodstains from maximum types of fabrics, while Surf excel was least effective in removing the bloodstains from different fabrics. Since the further confirmatory analysis are very costly and time consuming, such preliminary examination is useful to test every stain which could potentially be blood. The basic information revealed after performing the current study is that there is a probability of finding bloodstains on different fabrics even after washing them with detergents. This study paves new approach for identification of blood even after washing with detergents, which could prove to be beneficial in solving the criminal cases of forensic importance.

References

1. Virkler K, Lednev IK (2009) Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene. *Forensic Sci Int* 188(1-3): 1-17.
2. Bittencourt EA, Soares VJA, Angeramis NG, Claudia ES, Roberta Casemiro, et al. (2009) The analysis of biological samples from crime scene for a future human DNA profile confrontation. Effects of presumptive test reagents on the ability to obtain STR profiles for human identification. *Forensic Science International: Genetics Supplement Series* 2: 194-195.

3. James SH, Kish PE, Sutton TP (2005) *Principals of bloodstain pattern analysis: theory and practice*. Taylor & Francis Group, Boca Raton, Florida, USA.
4. Byrne JM, Garcia L (2006) An examination of citizen involvement in crime prevention in high risk versus low to moderate risk. *Crime Delinquency* 52(2): 203-231.
5. Cox MA (1991) study of the sensitivity and specificity of four presumptive tests for blood. *J Forensic Sci* 36(5): 1503-1511.
6. Saferstein R (2004) *Criminalistics: an introduction to forensic science*. 8th ed. London: Prentice Hall International (UK) Limited 320(51): 353-394.
7. Jurado E, Bravo V, Luzon G, Fernandez-Serrano M, Garcia-Roman M (2007) Hard-surface cleaning using lipases: enzyme-surfactant interactions and washing tests. *J Surfact Deterg* 10(1): 61-70.
8. Anson ML (1939) The denaturation of proteins by synthetic detergents and bile salts. *J Gen Physiol* 23(2): 239-246.
9. Banerjee UC, Sani RK, Azmi W, Soni R (1999) Thermostable alkaline protease from *Bacillus brevis* and its characterization as a laundry detergent additive. *Process Biochem* 35: 213-219.
10. Banik RM, Prakash M (2004) Laundry detergent compatibility of the alkaline protease from *Bacillus cereus*. *Microbiol Res* 159(2): 135-140.
11. Erikson N (1996) In: *Industrial enzymology*, (2nd edn.), The Macmillan Press Ltd, London, p: 1-8.
12. Beg QK, Gupta R (2003) Purification and characterization of an oxidation-stable, thiol-dependent serine alkaline protease from *Bacillus mojavensis*. *Enzyme Microbial Technol* 32: 294-304.
13. Gupta R, Gupta K, Saxena RK, Khan S (1999) Bleach-stable, alkaline protease from *Bacillus* sp. *Biotechnol Lett* 21(2): 135-138.
14. Gupta R, Beg QK, Lorenz P (2002) Bacterial alkaline proteases: molecular approaches and industrial applications. *Appl Microbiol Biotechnol* 59(1): 15-32.
15. Rao MB, Tanksale AM, Ghatge MS, Deshpande VV (1998) Molecular and biotechnological aspects of microbial proteases. *Microbiol Mol Biol Rev* 62(3): 597-635.
16. James SH, Kish PE, Sutton TP (2005) *Principals of bloodstain pattern analysis: theory and practice*. Boca Raton: Taylor & Francis group; Presumptive testing and species determination in blood and bloodstains.
17. Almeida JPD, Glesse N, Bonorino C (2011) Effect of presumptive tests reagents on human blood Confirmatory tests and DNA analysis using real time polymerase chain reaction. *Forensic Sci Int* 206(1-3): 58-61.
18. Cox M (1991) A study of the sensitivity and specificity of four presumptive tests for blood. *J Forensic Sci* 36(5): 1503-1511.
19. Fisher BA (2004) *Techniques of Crime Scene Investigation*. (7th edn.), Blood and other biological evidence. Taylor & Francis group, Boca Raton, USA, pp 1-482.
20. Cox M (1990) Effect of fabric washing on the presumptive identification of bloodstains. *J Forensic Sci* 35(6): 1335-1341.
21. Hunt AC, Corby C, Dodd BE (1960) The identification of human stains - a critical survey. *J Forensic Med* 7: 112-130.
22. Olsen RD (1985) Sensitivity comparison of blood enhancement techniques. *Ident News* Aug 10-14.