Australian Journal of Forensic Sciences

ISSN: 0045-0618 (Print) 1834-562X (Online) Journal homepage: http://www.tandfonline.com/loi/tajf20

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To cite this article: Samreen Mushtaq, Nouman Rasool & Sehrish Firiyal (2015): Detection of dry bloodstains on different fabrics after washing with commercially available detergents, Australian Journal of Forensic Sciences, DOI: 10.1080/00450618.2015.1029971

To link to this article: http://dx.doi.org/10.1080/00450618.2015.1029971

Australian Journal of Forensic Sciences, 2015
http://dx.doi.org/10.1080/00450618.2015.1029971

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Detection of dry bloodstains on different fabrics after washing with commercially available detergents

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(*Received 15 November 2014; accepted 11 January 2015*)

Blood is significant evidence that can help an investigator solve a crime. It can link a suspect to a crime and also help in reconstructing the crime scene. Criminals often attempt to eliminate bloodstained evidence at a scene by washing it. These attempts can result in alteration, or partial or complete removal of stained areas. Many presumptive tests are used to detect the bloodstains on clothes. In this study, bloodstained fabrics were washed with commercially available and frequently used detergents. Kastle-Meyer (KM), Leucomalachite green (LMG), Tetramethylbenzidine (TMB) and Hemastix tests were used to detect the presence of blood on these washed fabrics. The Hemastix test was found to be the most sensitive to detecting the washed stains on all cloths. The Leucomalachite green test was found to be the least sensitive. The ability of a fabric to retain blood after washing depends not only upon the chemistry and manufacturing of the fabric but also on the type of detergent. The time of immersion of the fabric with detergent also affects the removal of stains from fabrics. Ariel showed the best results in removing bloodstains from all fabrics. Cotton polyester and khaddar showed the maximum retention of blood after washing with either of the detergents, whereas silk polyester had the minimum ability to hold bloodstains.

Keywords: presumptive tests; Kastle-Meyer; Leucomalachite green; Tetramethylbenzidine; Hemastix; detergents; bloodstains

1. Introduction

Shedding of blood is a frequent occurrence in various crimes, from homicide to assault[1]. It is common that the perpetrator attempts to hide blood at a crime scene by removing and washing surfaces and objects, e.g. clothing containing blood[1,2]. Because of washing, blood can become diluted and hard to see with the naked eye. The main purpose of crime scene investigation is to determine clues that will be helpful in solving the crime[3,4]. Perpetrators may use cleaning agents to remove blood from crime scenes and evidence material. These agents have the ability to denature protein such as haemoglobin[5]. In the 1960s, enzyme-based detergents were marketed for the first time. These detergents attracted interest due to their advantages[6]. Proteases are one of the most significant enzymes in the industry[7]. Proteases hydrolyse fragments of large protein. Large fragments are then removed by water. Stains are therefore removed from fabrics in the presence of these proteases[8]. It is important to mention that in Pakistan

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these detergents are used for machine washing as well as hand washing of clothes. In Pakistan, most of the time, criminals use detergents to clean bloodstains from their clothes by hand washing. Most homicide cases are from the village/countryside area of the country, and invariably, the murders are committed with sharp tools such as hatchets, daggers, and so on. In such a scenario, the clothes of the suspect(s) can contain blood from victim(s). The suspect can attempt to clean the bloodstained clothes at the crime scene with water from rivers or canals by hand washing using detergent. Cox 1990 applied wet blood on fabric and set it to dry for various time periods. These dried stains were washed with a detergent in washing machine and blood was detected with presumptive tests. Our study is conducted to check the ability of some fabrics to retain a bloodstain after hand-washing at room temperature with commercially available and commonly used detergents. Moreover, Cox did not use Hemastix as detection tool for washed blood stains, which is done in our work[9].

DNA testing is a costly technique in terms of money and time. Therefore, it is important to confirm the presence of the right stain prior to sending it for DNA analysis. Presumptive tests of blood are performed to identify the stain in question that whether or not it is blood. In presumptive tests, haemoglobin, present in blood, catalyses the oxidation of a chromogenic compound and changes its colour[10]. The commonly used presumptive tests are the Kastle-Meyer, Leucomalachite green, Tetramethylbenzidine and Hemastix tests.

Reduced phenolphthalein solution is also known as the Kastle-Meyer reagent[10]. The Kastle-Meyer test (KM test) has the ability to detect blood to 1 in 10,000 ppm[3,11,12]. Phenolphthalein is used mostly on latent blood prints and it will cause an alkaline solution to turn pink in the presence of the blood. This is due to its oxidation by the peroxidase-like activity of haemoglobin[12]. The Leucomalachite green test (LMG test) is another presumptive test used for the detection of blood[13]. This reaction is also catalysed by haemoglobin and a green colour is produced. Hydrogen peroxide acts as an oxidizer in this reaction and the reaction is carried out in an acetic acid medium. This was thought to be the most specific but least sensitive of the presumptive blood detection reagents[3,10]. Benzidine had been utilised as a presumptive test but it was found to be cancerous. Tetramethylbenzidine (TMB) was made from benzidine and was thought to be safer as it was not carcinogenic[14]. This reagent is recognised to be exceptionally sensitive but not so specific[3,15,16]. The appearance of a bluish green colour indicates the presence of blood[10]. Hemastix reagent strips are manufactured by Bayer Health Care LLC and work on the peroxidase activity of blood protein haemoglobin. In this reaction, diisopropylbenzene dihydroperoxide acts as a substrate while 3,3, 5,5-tetramethylbenzidine (TMB) behaves as a reporting dye. Many forensics laboratories use Hemastix as a presumptive test for blood. Due to ease of use, portability and sensitivity, police investigators use Hemastix strips for the detection of blood at crime scenes[17].

2. Materials and methods

Twelve different and generally used fabrics were selected on which to conduct this study. Six different commercially available and commonly used brands of detergents were chosen to wash bloodstains on those 12 different types of fabrics. Table 1 shows 12 selected fabrics and Table 2 shows the detergents used in this study.

Table 1. Type of fabrics.

Sr. No	Fabric name	Composition of fabric
1	Cotton	100% cotton
2	Cotton polyester	80% cotton + 20% polyester
3	Keti	65% cotton + 35% polyester
4	Tarawere	80% cotton + 20% linen
5	Wash and wear	75% polyester + 25% cotton
6	Lawn	100% cotton
7	Bosky	100% silk
8	Polyester	100% polyester
9	Khaddar	88% cotton+12% linen
10	Wool	100% wool
11	Silk	100% silk
12	Silk polyester	65% silk + 35% polyester

Table 2. Frequently used washing detergents.

Sr. No.	Name of detergent	Manufacturer
1	Ariel	Procter and Gamble Pakistan
2	Bonus	Colgate-Palmolive Pakistan
3	Surf Excel	Unilever Pakistan Limited
4	Express Power	Colgate-Palmolive Pakistan
5	Sunlight	Unilever Pakistan Limited
6	Rin	Unilever Pakistan Limited

2.1. Sample collection

Before applying blood on fabrics, the fabrics were washed properly with distilled water. Each fabric was cut to a size of 7.6 × 7.6 cm. Blood was taken from three known and healthy volunteers in purple top EDTA vacutainer. Each fabric was stained with 50 µl of blood and left to dry at room temperature for 24 h[18].

2.2. Washing of stains

All fabrics were washed by hand in beakers at room temperature. During washing, the fabrics were well agitated by hand in order to remove the maximum amount of blood, following the conventional methods routinely practised in Pakistan. For washing, 1 g detergent was mixed in 500 mL of water. Fabrics were washed for time periods of 10 min, 20 min and 30 min. One type of fabric was washed with all six brands of detergents. A fabric without bloodstains was also washed with each detergent as a negative control[9]. After washing, fabrics were left to dry at room temperature for 24 h[18]. Each stain on the fabric was subjected to the presumptive test for the detection of blood and the results were noted. A positive control, i.e. a piece of fabric containing a bloodstain, was also washed with distilled water with each batch of washing.

2.3. Reagent preparation

Kastle-Meyer, Leucomalachite green and Tetramethylbenzidine reagents were prepared in the Forensic DNA and Serology Department of Punjab Forensic Science Agency, Lahore, Pakistan. All these reagents were prepared according to the protocols of the

DNA Analyst Training Laboratory Training Manuals of the National Forensic Science Technology Centre (www.nfstc.org). Hemastix strips of Insight Expert manufactured by ACON Laboratories were used.

3. Results

Twelve fabrics were washed with six detergents, one by one, for a period of 10, 20 and 30 min. Kastle-Meyer (KM), Leucomalachite green (LMG), Tetramethylbenzidine (TMB) and Hemastix tests were performed to detect the presence of blood. The detergents removed the bloodstains from the fabrics to various degrees. Table 3 illustrates the washing time (minutes), i.e. time of immersion of the fabric in the detergent, after which no blood was detected. The sign (–) shows that presumptive tests gave positive results for the presence of blood stains on clothes even after 30 min of washing. No blood was detected on any of the negative control fabrics duly washed with either of the detergents. All positive controls gave positive results with all presumptive tests.

Wool is a natural fibre taken from an animal source. It has good, natural wicking properties and will provide insulation even when wet. Cotton is a plant-based fibre and has the ability to retain moisture. The absorbency and moisture retention of wool and cotton are high. Wool is not resistant to alkalis, which show resistance to cleaning agents. In cotton, water can penetrate into pores and can remove the stains from the fabric. Moreover, cotton shows a resistance to alkalis, so alkalis such as bleaching agents can be used to bleach cotton fabrics[20].

Polyester is a synthetic man-made fibre and has outstanding dimensional stability and offers excellent resistance to dirt, alkalis, decay and mould. But it absorb oils and it is difficult to remove these stains. The absorbency and moisture retention of polyester is very low. Polyester also shows resistance to alkalis and bleaches at room temperature. Linen is a natural bast fibre obtained from the stalk of the flax plant. It has high absorbency and moisture retention and shows a resistance to alkalis, solvents, and cold dilute acids[20].

The fabrics used in this study are cotton, polyester wool, linen and a blend of these main fibres in varying compositions (Table 1). Tawera, Lawn, Bosky and silk polyester are less able to retain bloodstains after washing with detergents. This study shows that 50% of bloodstains were removed efficiently from these fabrics after washing with the detergents. On silk and Wash & Wear, 50% of the bloodstains were removed from these fabrics by washing. Probably, this was due to the low absorbency and moisture regain values for these fabrics[19,20]. It is pertinent to mention that the blood was not properly absorbed on Bosky and was found as a clot and dried. This is due to low absorbency of the cloth. The chemistry of fibre and the weaving of the fabric play pivotal roles in the retention of blood[19].

After washing with the detergents, blood was detected on a large number of samples of wool, polyester, Keti and cotton fabrics. It was found that 75–91% of samples showed positive results for the presence of blood. Except for polyester, all other fabrics have high absorbency and moisture regain values[21]. The bloodstains were absorbed in the pores of these fabrics (water wicking) and were not washed away with the detergents (Table 3)

Post-washing detection of blood on khaddar and cotton polyester revealed that all samples were positive for the presence of blood after 30 min of washing. All presumptive tests for blood detected the blood on these samples.

Table 3. Washing time (minutes) after which no bloodstain was detected.

		Cotton	Cotton Polyester	Keti	Tarawera	Wash & wear	Lawn	Bosky	Polyester	Khadder	Wool	Silk	Silk Polyester	Percentage positive sample after 30 min
Ariel	KM	30	–	–	20	30	201	20	20	–	–	20	10	33
	LMG	20	–	20	10	10	20	10	10	–	30	10	10	16
	Hema	–	–	–	–	–	–	–	–	–	–	–	–	100
	TMB	30	–	–	30	30	30	30	30	–	–	30	30	33
Bonus	KM	–	–	–	30	30	–	–	–	–	30	20	20	58
	LMG	–	–	–	10	10	–	10	–	–	–	10	10	58
	Hema	–	–	–	–	–	–	–	–	–	–	–	–	100
	TMB	–	–	–	–	–	–	30	–	–	–	–	–	91
Surf Excel	KM	–	–	–	10	30	–	20	20	–	30	10	20	41
	LMG	20	–	–	10	10	–	10	10	–	–	10	10	50
	Hema	–	–	–	–	–	–	–	–	–	–	–	–	100
	TMB	–	–	–	30	30	–	–	30	–	30	30	30	50
Express Power	KM	–	–	–	30	30	30	10	–	–	30	30	20	41
	LMG	20	–	–	10	10	30	10	–	–	30	–	10	50
	Hema	–	–	–	–	–	–	–	–	–	–	–	–	100
	TMB	–	–	–	–	–	–	30	–	–	–	30	30	75
Rin	KM	–	–	–	30	–	–	10	–	–	–	20	20	33
	LMG	–	–	–	10	10	–	10	–	–	–	10	10	58
	Hema	–	–	–	–	–	–	–	–	–	–	–	–	100
	TMB	–	–	–	–	–	–	–	–	–	–	–	–	100
Sunlight	KM	–	–	–	30	–	–	20	–	–	–	–	20	75
	LMG	–	–	20	10	10	–	10	–	–	–	–	10	58
	Hema	–	–	–	–	–	–	–	–	–	–	–	–	100
	TMB	–	–	–	–	–	–	–	–	–	–	–	–	100
Percentage positive sample after 30 min		79	100	91	41	50	79	41	75	100	75	50	37	

KM-Kastle Mayer Test; LMG- Leucomalachite green Test; Hema- Hemastix; TMB-Tetramethylbenzidine Test; Sign (–) indicates that positive for presence of blood after 30 min of washing.

The laundry detergents have different chemical compositions, thus these detergents vary in their efficiency to remove stains from fabrics. A typical detergent contains a surfactant, agents that remove dirt grease and/or oily stains, and some alkalis. Fabric brighteners and shiners are also part of a few detergents. Some laundry detergents contain bleaching agents that are used to give additional brightness to fabric by removing stains. Hydrogen peroxide is used nowadays as a bleaching agent. A sodium hypochlorite solution cannot be used for wool and polyester, but can be used for cotton[21]. Ariel contains sodium sulphonate (a strong surfactant) along with NaOH (a strong alkali). Due to the presence of these agents, this detergent washed the bloodstains on all fabrics effectively. Blood was detected on the least number of samples when using three presumptive tests, i.e. KM, TMB and LMG tests (Table 3).

Bonus, Surf Excel, Express Power and Rin contain a mild surfactant, e.g. linear alkyle benzenesulfonate along with sodium carbonate. Due to presence of this mild anionic surfactant, the efficiency of these detergents to wash the bloodstains from fabric was less than that of Ariel. Sodium carbonate is used to compete with Ca^{+2} and Mg^{+2} ions present in water and is a well-known agent to remove grease, oil and wine stains[21]. Sodium carboxy methyl cellulose (SCMC) is also part of Surf Excel, Rin and Express Power, and is a well-known agent for removing dirt particles from fabrics. Due to the mild action of these detergents, nearly half of the bloodstains could be removed from fabrics (Table 3).

Sunlight contains alcohol ethyoxalate, a soft surfactant and emulsifier[22]. This detergent also contains a protease enzyme that is known to remove protein-based stains. Sodium carbonate peroxide is a bleaching agent present in this detergent. Blood was detected on the maximum number of samples of bloodstained fabrics when washed with this detergent. This may be due to the soft anionic surfactants, which could not remove the stains properly (Table 3).

4. Discussion

A study was conducted to detect the presence of blood on different white fabrics after washing with six different commercially available detergents. The specific washing technique adopted was the one frequently used in Pakistan for the washing of clothes. After applying blood to the fabric, the diameter of bloodstain was noted. Every fabric showed a different diameter depending on its ability to absorb the blood. Fabric that absorbed more blood showed a larger diameter whereas fabric that absorbed less blood showed a smaller diameter. The fabric with the greater absorption retained more blood and can therefore be more useful for detection of the stain. It can subsequently help in generating the DNA profile. For the cloth with less absorption, e.g. Bosky, blood stays as a clot on the surface and could be removed by scratching. The absorption of blood by the fabrics depends upon chemistry, the weave of the fabric, water wicking and chemical modification. After washing, the visibility of stains was noted. Some stains were still visible to the naked eye and could be seen easily. Bloodstains on some fabrics were not visible after washing. Texture, the nature of the fabric's weave, moisture regain and absorbency allow bloodstains to be absorb into the fabric to variable degrees. Moreover, the visibility of the stain was found to depend on washing time. Stains became less visible when the time given for washing was increased up to 30 min. Cotton polyester and khaddar retained a greater amount of blood even after washing with detergents. Silk polyester and Bosky showed minimum retention of blood after washing[18].

This study has revealed that there is a striking difference in the sensitivities of all four presumptive tests to detect blood on washed fabrics. It was already established that the phenolphthalein test was the most sensitive test for the detection of washed bloodstains on fabric. These stains were washed in a washing machine, not by agitating with hand[9]. In the same study, TMB was found to be the least sensitive for the detection of bloodstains on cloth. In our study, it was revealed that Hemastix (phenolphthalein test) was the most sensitive test; it detected blood on all samples after washing with the detergents. The Hemastix test and the KM test are phenolphthalein tests, but the KM test was less sensitive than Hemastix for the detection of washed bloodstains. The LMG test was significantly less able to detect the washed bloodstains. This test is less sensitive than the phenolphthalein test[23]. The KM and LMG tests could not detect blood in nearly 50% of the washed fabrics (Table 3).

It was established that Ariel removed bloodstains from washed fabrics most effectively compared with other detergents. Sunlight detergent is very poor at removing bloodstains from fabric.

All these fabrics are frequently used in Pakistan in suits. This study revealed that cotton polyester and khaddar retain bloodstains after washing with any of the detergents. With the other types of fabric, bloodstains were washed out to varying degrees. However, the fact that there is a chance of finding bloodstains even after washing with detergent is useful for DNA profiling.

Acknowledgments

Thanks to the administration of Punjab Forensic Science Agency (PFSA), Lahore, Pakistan for providing the facility to conduct this research work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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