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Persistence of Bloodstains on Washed Fabrics: A Forensic Evaluation Using Kastle-Meyer Test

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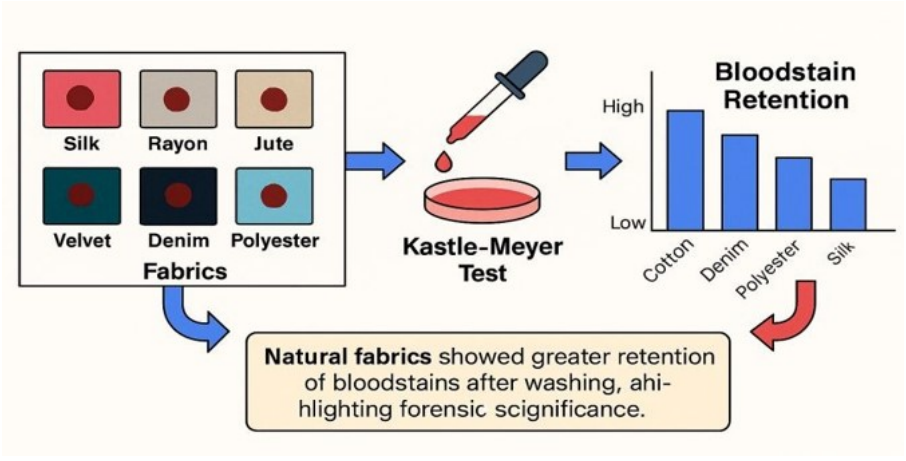
Abstract

Bloodstains can be used as vital physical evidence at crime scenes and implicating evidence for crime scene reconstruction in a variety of criminal inquiries. Because blood readily clings to clothing assailants frequently try to wash away the stain using water or soap. But identifying biological residues on laundered fabric is a long-standing problem in forensic science. This experimental study evaluates bloodstain retention on eight types of fabric (Each fabric type was tested in triplicate) washed through several cycles including fabric constitution as well as stain absorption characteristics and the type of washing plain water compared to detergent. Fresh human blood was deposited on standardized cloths and 10 daily washes were applied either with water or with Rin detergent each time. Stain detection was performed using the Kastle Meyer presumptive test. Cotton consistently retained bloodstains most efficiently even after repeated laundering while synthetic fabrics showed a lower degree of stain resistance. These findings indicate the forensic significance of natural materials and that laundering does not ensure the complete elimination of biological traces. This research highlights the significance of pre-screening tests in forensic science and supports the possibility of recognizing washed evidence during crime scene reconstruction.



Graphical Abstract

Keywords: Bloodstain Retention, Forensic Serology, Washed Evidence, Fabric Analysis, Biological Trace Evidence, Detergent Effect.

List of Abbreviations

KM Test — Kastle Meyer Test; EDTA — Ethylenediaminetetraacetic Acid; LCV — Leuco Crystal Violet; DNA — Deoxyribonucleic Acid; ppm — Parts Per Million; NaOH — Sodium Hydroxide; H_2O_2 — Hydrogen Peroxide; mL — Milliliter; µL — Microliter; g — Gram; RT — Room Temperature

1. Introduction

Biological evidence plays a pivotal role in modern criminal investigations providing critical links between the crime scenes the suspect and the victim. Among all forms of biological evidence blood is the most commonly encountered in cases involving violent crimes such as assaults sexual offenses homicides suicides road traffic accidents and mass disasters [1]. Even trace amounts of blood can yield valuable forensic information assisting in determining the nature of the offense and identifying the individuals involved [2].

Blood evidence is particularly significant when it adheres to porous surfaces such as clothing carpets cushions or other textile materials. Its presence on such substrates can directly associate a suspect with a victim or a crime scene [3]. Consequently, perpetrators often attempt to destroy or conceal this evidence by washing the stained garments believing it will prevent forensic detection and dissociate them from the crime [4]. However, they are often unaware that even washed bloodstains can still be detected through highly sensitive presumptive tests such as luminol and Kastle Meyer which can reveal blood in diluted or laundered conditions [5].

Bloodstain evidence also plays a significant role in medico legal contexts aiding in the reconstruction of events by establishing connections among the crime the offender the victim and the weapon used. Upon washing bloodstains may become invisible to the naked eye however in forensic science detecting such latent bloodstains is crucial for DNA profiling and further evidence examination [6].

The persistence of bloodstains after washing depends on several factors including the fibre composition and weave of the fabric type of detergent duration of soaking and drying conditions such as ambient room temperature or exposure to sunlight [7,8]. Different detergents possess varying abilities to break down proteinaceous substances like haemoglobin. Enzyme based detergents particularly those containing proteases derived from Bacillus species exhibit high efficiency in removing blood due to their strong enzymatic hydrolysis of haemoglobin [9]. These biological detergents accelerate the degradation and removal of blood from textiles.

The Kastle Meyer phenolphthalein test is a reliable presumptive test for detecting invisible bloodstains even after attempts to clean or wash the evidence [5,10]. This test is especially useful when the blood has been subjected to laundering. It operates in an alkaline environment producing a pink coloration upon reaction with the heme group of haemoglobin which mimics peroxidase activity and catalyses the decomposition of hydrogen peroxide [9]. The sensitivity of this test is notable capable of detecting blood concentrations as low as 1 part per million ppm [11]. Bloodstains are frequently visible even after washing but may be altered. However advanced detection modalities including chemiluminescence through luminol and molecular techniques can illuminate these concealed traces which in turn are crucial for forensic analysis [8].

This experiment investigates the persistence of bloodstains on cloth after washing with nothing plain water or a solution of commercial detergent. The objective of the study is to evaluate the reliability of the Kastle Meyer test for detecting such stains

and to improve forensic methods for the detection of latent biological stains on laundered fabrics.

2. Material & Methodology

2.1 Fabric Selection and Classification

The investigation included eight types of textiles frequently encountered in forensic cases and household fabrics. They were classified as natural cotton silk jute, synthetic nylon polyester denim, and semi synthetic rayon velvet fabrics. This selection was made to represent situations where blood may be deposited on clothing or furnishings during a violent act. For standardization every fabric was cut into swatches of identical dimensions to ensure uniform staining and consistent analysis for all samples.

2.2 Blood Sample Collection and Staining Procedure

Fresh human blood was collected from a certified pathology laboratory under standard ethical and biosafety guidelines. To prevent coagulation the samples were preserved with ethylenediaminetetraacetic acid EDTA and stored at 4°C until use. Using a micropipette 50 µL of blood was carefully applied to the center of each fabric swatch. The samples were then left to dry undisturbed at ambient room temperature for 24 hours to allow optimal absorption and adherence of blood to the textile fibers. Each fabric type was tested in triplicate.

The initial appearance of blood droplets on different fabric types is shown in Figure 1 highlighting variation in absorption and spread patterns across natural synthetic and semi synthetic materials.

Figure 1: Visual documentation of human blood droplets applied to various fabric types before washing

2.3 Washing Procedure and Drying Conditions

After drying the stained fabrics were divided into two groups based on the washing treatment to be applied. The first group was washed using plain tap water to simulate basic cleaning attempts while the second group was washed using a commercially available detergent Rin representing a more thorough domestic laundering approach. For detergent based washing 2.5 grams of Rin

powder was dissolved in 250 mL of water and the fabrics were soaked and agitated for five minutes to promote stain removal. All washed fabrics were then dried under natural sunlight to replicate typical household drying practices. To ensure consistency each type of fabric was washed under identical conditions including soaking time agitation intensity and drying duration.

2.4 Control and Repeated Wash Testing

Before beginning the washing cycles a control test was conducted using the Kastle Meyer KM presumptive test for blood detection. One stained and one unstained fabric swatch were tested. As expected, the stained fabric produced an intense pink colour confirming the presence of haemoglobin while the unstained sample remained colourless. After confirming test accuracy each stained fabric swatch underwent a total of ten consecutive washing cycles one per day. Following each wash the samples were tested again using the KM test to evaluate the persistence and detectability of blood over time and across different fabric types.

2.5 Preparation of Kastle Meyer Reagent and Testing Protocol

The Kastle Meyer reagent was freshly prepared by dissolving 2 grams of phenolphthalein in 10 mL of a 20 percent sodium hydroxide solution prepared by dissolving 20 grams of NaOH in 100 mL distilled water. To this solution 20 grams of powdered zinc dust was added and the mixture was gently boiled until it became colourless indicating the reduction of phenolphthalein to its leuco form. The working reagent was prepared by mixing 2 mL of the phenolphthalein stock solution with 10 mL of distilled water and 2 mL of ethanol [12,13].

For the bloodstain detection test each fabric sample was treated with two drops of ethanol on the dried washed area followed by two drops of the working phenolphthalein reagent. After two minutes 2–3 drops of 3 percent H_2O_2 were added to exclude spontaneous colour development. A pink colour change indicated a positive reaction produced by the peroxidase like activity of haemoglobin which catalyses the breakdown of hydrogen peroxide. This test is highly sensitive and can detect blood in concentrations as low as one part per million making it suitable for identifying traces of blood that may remain concealed on washed clothing [14–16].

3. Results

In the current study the persistence of bloodstains on different fabrics after repeated laundering was examined which is highly relevant to forensic investigations where suspects may attempt to remove evidence by washing clothing. Eight common textiles including cotton silk jute rayon velvet polyester nylon and denim were stained with human blood and subjected to ten successive washing cycles using tap water and Rin detergent respectively. Blood detection was carried out using the Kastle Meyer KM test a widely used presumptive method based on the peroxidase like activity of haemoglobin that produces a characteristic pink colour when blood is present.

The results demonstrated clear differences in stain persistence depending on the fabric type. Among all materials cotton showed the highest retention of blood even after repeated washing cycles. This observation can be attributed to the porous and hydrophilic nature of cotton fibres which allow blood to penetrate deeply into the fibre matrix making removal difficult during laundering. Jute and silk also retained detectable traces of blood although their retention capacity was slightly lower than that observed for cotton.

In contrast synthetic fabrics such as polyester and nylon showed significantly lower blood retention. These materials are generally more hydrophobic and tightly woven which limits the absorption of blood and allows stains to be removed more easily during washing. Denim although often considered a synthetic or blended fabric demonstrated moderate stain retention likely due to its dense weave and mixed fibre composition which provides greater absorption compared with other synthetic textiles.

Despite undergoing up to ten washing cycles blood traces were still detectable on several fabric types using the Kastle Meyer test as indicated by the development of a pink colour. This observation highlights the high sensitivity and reliability of presumptive tests such as the Kastle Meyer method for detecting latent bloodstains on laundered clothing. Although washing with detergent showed slightly greater effectiveness in reducing visible stains compared with washing with water alone it did not completely eliminate blood residues particularly on natural fabrics.

Several additional factors influenced the persistence of bloodstains including the chemical composition and structure of the fabric the duration of detergent exposure and the drying conditions. Exposure to sunlight may partially degrade heme molecules however it did not prevent the detection of blood in this study. These findings are consistent with earlier reports indicating that protein-based stains such as blood can remain detectable even after extensive washing.

Although this investigation primarily employed the Kastle Meyer test due to its simplicity low cost and applicability in field conditions it also highlights the importance of advanced forensic techniques including luminol based chemiluminescence and infrared imaging which can further improve the detection of concealed biological traces. Presumptive tests nevertheless remain essential as primary screening tools before more sophisticated confirmatory methods such as DNA profiling are conducted.

The results of the present study reinforce the long-established forensic principle that clothing can retain evidentiary value even after attempts to wash or clean it. Blood residues may persist on textiles particularly on highly absorbent fabrics despite repeated laundering. These findings provide important implications for forensic and legal investigations by demonstrating that washed garments may still contain detectable biological evidence. Such information is valuable for crime scene reconstruction establishing links between suspects and victims and strengthening evidentiary interpretation during criminal investigations. Overall, this study emphasizes the importance of understanding fabric specific interactions with biological evidence and contributes to improving protocols for evidence recovery in forensic science.

As seen in Table 1 bloodstain retention in natural fabrics such as cotton and jute remained high even after laundering with detergent and produced strong to moderate positive results in the Kastle Meyer test KM. In contrast synthetic fabrics showed no visible response and demonstrated low or negligible ability to retain bloodstains.

Table 1: Comparative retention scores of bloodstains across different fabric types after detergent washing.

Fabric Type	Wash Type	KM Test Result	Visual Intensity	Observations
Cotton	Detergent	Positive	Strong	High stain retention after washing
Silk	Detergent	Positive	Moderate	Moderate retention, visible pink reaction
Jute	Detergent	Positive	Moderate	Stain partially retained, moderate reaction
Rayon	Detergent	Weak Positive	Faint	Minimal retention, pale pink reaction
Velvet	Detergent	Weak Positive	Faint	Low retention, weak test result
Polyester	Detergent	Negative	None	No visible reaction, stain removed
Nylon	Detergent	Negative	None	No detectable stain or KM color change

As shown in Figure 2 the percentage retention of bloodstains in natural fabrics such as cotton and jute was higher compared with synthetic fabrics such as polyester and nylon emphasizing the important role of fabric type in stain retention. Although staining became less visible after detergent treatment than after washing with water alone the presence of blood traces was still detected reiterating the forensic value of laundered evidence.

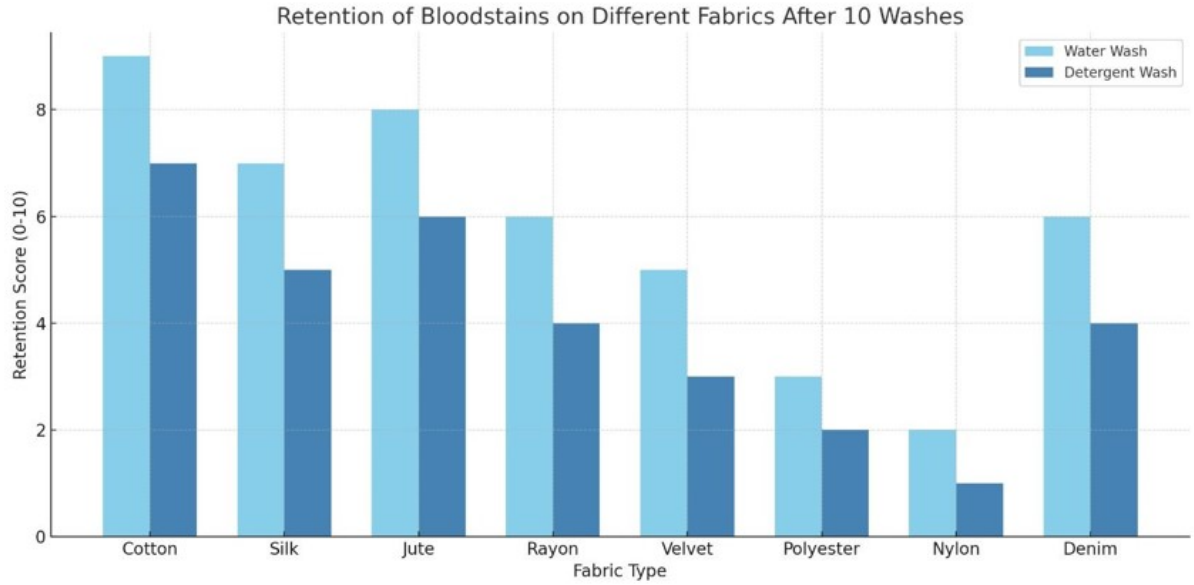


Figure 2: Comparison of bloodstain retention scores on different fabric types after ten washing cycles using tap water and detergent.

4. Discussion

The presence of bloodstains on washed fabrics remains a significant challenge in forensic investigations because laundering does not always remove all traces of biological evidence. This issue is particularly important in forensic science as blood is one of the most common forms of biological evidence encountered at crime scenes. The present study systematically evaluated the persistence of blood residues on different fabric types after repeated washing cycles considering important variables such as fibre composition detergent use and laundering conditions. Consistent with previous research the results indicate that bloodstains tend to persist more strongly on natural fibres particularly cotton and wool compared with synthetic fabrics such as polyester. This behavior can be attributed to the porous and hydrophilic structure of natural fibres which allows deeper penetration and stronger binding of biological fluids within the textile matrix [17].

Several studies have reported that latent or microscopic traces of blood can remain detectable even after multiple washing cycles when sensitive forensic detection methods are used. Techniques such as luminol based chemiluminescence and immunological assays have demonstrated the ability to identify residual blood traces even after extensive laundering [18]. These findings confirm that washed garments may still retain forensic value and can contribute meaningful evidence during investigations.

The effectiveness of stain removal was also influenced by factors such as washing conditions water temperature and the composition of detergents used. Enzyme based detergents particularly those containing proteolytic enzymes are designed to degrade protein-based stains such as haemoglobin and therefore show greater efficiency in removing blood compared with non-enzymatic cleaning agents. Despite this enhanced cleaning ability, the present results indicate that such detergents do not completely eliminate blood residues particularly from absorbent fabrics.

The duration of drying did not significantly influence stain persistence when drying periods varied up to 48 hours among the samples. This suggests that short term ambient drying conditions do not substantially affect the chemical stability of haemoglobin bound within textile fibres. Additionally, the use of enhancement techniques such as luminol [18] and leucocrystal violet LCV can further aid in visualizing stains that may not be visible to the naked eye on laundered clothing thereby improving the reconstruction of crime scenes and interpretation of events [19].

Overall, the findings of this study emphasize the strong persistence of bloodstains on absorbent fabrics and demonstrate that routine laundering does not fully remove biological evidence. These observations highlight the importance of appropriate forensic examination protocols as biological traces may remain detectable even after attempts to clean or conceal evidence. Consequently, this research supports the forensic relevance of laundered clothing as a potential source of evidentiary material and contributes to strengthening the interpretation of biological evidence in criminal investigations and court proceedings [20].

4.1 Limitations of this study

This study has certain limitations that should be considered while interpreting the findings. The experiments were conducted under controlled laboratory conditions, which may not fully replicate real-world scenarios where variables such as washing machine dynamics, varying detergent formulations, water temperature, and environmental exposure can influence stain persistence. The study relied solely on the Kastle–Meyer presumptive test, without the use of confirmatory techniques such as DNA profiling, luminol chemiluminescence, or spectroscopic methods, which could provide more comprehensive validation. Additionally, the assessment of bloodstain retention was primarily qualitative or semi-quantitative, lacking precise instrumental quantification. Variations in fabric weave, thickness, and manufacturing treatments were not extensively controlled, which may also influence absorption and retention behavior. These limitations indicate the need for further studies incorporating larger datasets, advanced analytical techniques, and real-case conditions to strengthen forensic applicability.

5. Conclusion

This study highlights an important forensic observation that bloodstains can persist on certain fabrics even after multiple washing cycles. Natural fibres such as cotton and jute demonstrated greater retention of blood compared with other materials due to their porous and highly absorbent structure. The Kastle Meyer test proved to be an effective presumptive method for detecting these residues even after ten cycles of detergent washing. Several factors including fabric composition washing conditions and absorption characteristics influenced the persistence of blood traces on textiles. The findings reinforce an important forensic principle that laundering does not necessarily eliminate biological evidence. Even when stains are no longer visible to the naked eye detectable traces may still remain on clothing. The study also demonstrates the usefulness of presumptive tests as preliminary screening tools for identifying latent biological stains on washed fabrics. In addition, the results emphasize the importance of fabric analysis in forensic serology and crime scene investigations. Overall, the evidence presented in this work can assist forensic investigators in improving strategies for detecting and preserving biological evidence particularly in cases where attempts have been made to remove stains through laundering.

Statements & Declarations

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Conflicts of Interest

The authors declare that there are no conflicts of interest relevant to this article.

Ethical Approval

Not Applicable

Informed Consent

Not Applicable

Declaration of generative AI and AI-assisted technologies

During the preparation of this work the author(s) used ChatGPT in order to improve the readability and language of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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