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Comparing the Effectiveness of Kastle-Meyer and Luminol in Detecting Bloodstains on Laundered Fabrics

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Abstract
Most cleaning agents can visibly remove bloodstains from substrates like fabric, but sensitive preliminary tests can still detect the presence of blood in such cases. There have been many studies conducted to compare the efficiency of preliminary tests to detect blood from fabric washed with detergents that are commonly available in the market. This study aims to compare the effectiveness of Kastle-Meyer and Luminol in detecting bloodstains on laundered fabrics. The research involved conducting controlled experiments where bloodstains were intentionally applied to various types of fabrics (Cotton, Linen, Polyester, Rayon, and Satin Polyester) and dried for 12h and 24h. Subsequently, the fabrics were laundered using standard procedures with and without laundry agents (Ariel detergent powder, Sodium Tetraborate – Borax powder and Sodium hypochlorite – Bleaching solution) to simulate real-life scenarios. The results were then analyzed to determine the accuracy and reliability of each method in detecting blood traces post-laundering. The outcome is that Kastle-Meyer gives better results with stains that were washed earlier but significantly shows a reduction in performance when the drying period is increased. Whereas Luminol gives stable performance for stains dried for longer. The findings of this study provide valuable insights into the most effective method for detecting bloodstains on laundered fabrics, which can have significant implications for forensic investigations and criminal cases.

Keywords: Kastle-Meyer, Luminol, Bloodstains, laundered fabrics, laundry agents, Presumptive tests.

1. Introduction

Blood is one of the most common evidence found in any case involving major bodily injury. Apart from providing DNA evidence, blood on immovable permanent surfaces like floors and walls help confirm the scene of crime, the type of wound, and the sequence of events, while blood on transferable objects like clothes and weapons help link the objects to the crime scene and the source of blood. Fabrics are found almost everywhere, which includes and is not limited to clothes that were worn by victims, suspects, or witnesses, curtains, furniture covers, etc. Hence, the chances of blood getting on any kind of fabric are higher. Perpetrators, who try to avoid legal action, usually will not have the time to clear the evidence at in the crime scene as their primary concern will be fleeing the scene without getting caught. So, they try to remove any evidence linking them to the crime scene or the victim by creating a fake alibi, cleaning, or discarding any belongings they possess that could be traced back to the scene or the victim. If the

perpetrator does not have any prior relation to the victim or the scene, they could travel and discard the evidence at an unsuspecting location. But in cases where the perpetrators know the victim beforehand or have connection to the scene, they are confined to a particular location to avoid appearing suspicious to the investigators and resort to cleaning the evidence to remove any traces of the crime. The formation of bloodstain on fabric depends on the properties of both the fabric and the blood [1]. The perpetrator may try to wash it off using water and any washing agent if available until the stain becomes invisible to the naked eye. But the diluted blood absorbed by the fabric can still be detected using chemical tests [2]. This depends on the fabric composition, the test used, and whether detergent was used [3].

1.1. Presumptive Tests

Preliminary / Presumptive tests are rapid chemical screening tests that are done at the scene to indicate the possibility of the presence of blood [4]. Further confirmatory tests are done to confirm this possibility. Hemoglobin (Hb) ($C_{2952}H_{4664}N_{812}O_{832}S_8Fe_4$) not only transports oxygen and gives red pigment to the blood but also exhibits peroxidase-like activity by catalyzing redox reactions. A general redox reaction catalyzed by peroxidase:

$$RH_2 + H_2O_2 \Rightarrow R + 2H_2O$$

Here, RH_2 is an organic molecule that loses two electrons and two protons to become R and H_2O_2 is an oxidant that gains two electrons to become H_2O. In color reactions, the product R has color, whereas RH_2 is colorless. Hemoglobin catalyzes this reaction. Color change presumptive tests include the Kastle-Meyer (KM) or phenolphthalein test, Tetramethyl Benzidine (TMB) test, Leucomalachite green (LMG) test and Leucocrystal violet (LCV) test. In luminescent reactions, the chemiluminescent reagent glows when oxidized and is performed in a darkroom. This includes the Luminol test and Fluorescein test.

1.1.1. Kastle-Meyer Test

Kastle-Meyer (KM), also known as Phenolphthalein test, is the most sensitive test that can detect blood diluted up to 1:10,000 ppm on cotton fabric [5]. It was named after Joseph Hoeing Kastle and Erich Meyer, who contributed to its discovery [6]. The reagent is prepared by reducing the pink color phenolphthalein (C20H14O4) to a colorless phenolphthalin in an alkaline medium using zinc dust.

$$\text{Phenolphthalin (Colorless)} + H_2O_2 + Hb^{4+} \Rightarrow \text{Phenolphthalein (Pink)} + H_2O + Hb^{3+}$$

Hb loses electron by catalyzing the conversion of H_2O_2 to water and oxygen. In turn, Phenolphthalin (RH_2) oxidizes back to the pink color phenolphthalein (R) by donating electrons to Hb. Any color change after 10s of adding H_2O_2 is not considered because it occurs naturally and is not catalyzed by Hb [5]. The test reduces the amount of extractible high-molecular Deoxyribonucleic Acid (DNA) from the stain [7].

1.1.2. Luminol Test

3-aminophthalhydrazide ($C_8H_7N_3O_2$), also known as Luminol, is a chemical that produces light when mixed with an oxidizing agent [8]. Light produced due to a chemical reaction is called Chemiluminescence (CL). The preparation of the test reagent involves activation of luminol with an oxidant in an alkaline medium. There are two luminol preparation formulae that are commonly used, the Grodsky formulation

which uses sodium perborate as the oxidant, and the Weber formulation uses hydrogen peroxide [4]. The oxidant present in the alkaline reagent causes oxidation of luminol under the catalytic activity of Hemoglobin [8]. In this multistep oxidation process, an excited intermediate of luminol (3-aminophthalate) is produced which emits light until it returns to the ground state. The test must be conducted in darkness to observe the CL. Just like KM, luminol also gives false positives to certain peroxidase-like substances like bleach [9]. Studies show that luminol does not destroy DNA evidence [7].

1.2. Blood

Blood is a fluid connective tissue. It serves numerous functions, such as respiration, excretion, transportation of hormones and enzymes, regulation of temperature, acid-base balance, and water balance, etc. [10]. It consists of a cellular (solid) part and a plasma (liquid) part.

1.2.1. Hematological Parameters of Human and Pig Blood

M.A. Raymond, et al. (1996) states that the viscosity of pig blood is about 20% higher than human blood [11]. Table 1 shows the reference ranges of some hematological parameters of human blood and pig blood [12, 13]. It can be noted that the hemoglobin count and other such parameters in pig blood and human blood are almost similar.

Table 1. Hematological parameters of human and pig blood with their reference ranges

Hematological Parameters	Reference Ranges		
	Human Blood		Pig Blood
Packed Cell Volume / Hematocrit	Male	42%–50%	32–50 %
	Female	37%–47%	
Hemoglobin	Male	14–18 g/dL	10–16 g/dL
	Female	12–16 g/dL	
Erythrocytes	4.2-5.9 × 1012/L		5–8 × 1012/L
Reticulocytes	0.5%–1.5% of red cells		0–1.0 % of red cells
Mean Corpuscular Volume	80–98 fL		50–68 fL
Mean Corpuscular Hemoglobin	28–32 pg		17–21 pg
Mean Corpuscular Hemoglobin Concentration	33–36 g/dL		30–34 g/dL
Platelets	150-350 × 109/L		200–500 × 109/L
Leucocytes	4-10 × 109/L		11–22 × 109/L
Plasma Proteins	6.0-7.8 × 10 g/L		6–8 × 10 g/L
Plasma Fibrinogen	200–400 mg/dL		200–400 mg/dL

1.3. Fibers

A fiber is any substance, natural or man-made, that can be processed into fabric [14]. Fabric is a flexible planar substance constructed from solutions, fibers, yarns, or fabrics in any combination. The raw materials are either woven, knit, or mechanically processed into fabric. There are two categories of fibers:
Natural fibers: These are taken from plants, animals, or mineral sources in their fiber form. They can be absorbent like cotton or warm and bulky like wool.
Artificial fibers: These are chemicals obtained from manufacturing facilities and then processes into fiber form. Fibers completely made up of synthetic materials are called Synthetic fibers, which includes polyester, nylon, spandex, etc. Certain fibers obtained from plants are not considered natural because they are not produced naturally in fiber form but are processed into fiber by manufacturing facilities. They are called Semi-Synthetic fibers. Their properties include stretchy, strong, and abrasion resistant. A fine example of this kind of fiber is rayon.

1.4. Bloodstain Formation on Fabric

Factors that influence blood drop formation are blood viscosity, surface tension, cohesion forces, and gravity [15]. A falling blood drop maintains its spherical shape due to its cohesive nature. This is also influenced by air resistance and gravity. When the blood drop hits the surface, it loses its spherical shape and disperses as the surface tension breaks. The shape of the bloodstain depends on the surface texture. The formation of bloodstain on fabric is through the process of wetting and wicking [1]. Wetting is the stage where the blood (liquid) interacts with the fabric (solid). Immersion, Adhesion, Spreading, and Capillary penetration are four mechanisms of wetting. Either one or more or all the mechanisms can occur simultaneously. After the interaction, the liquid starts spreading through the fabric in the longitudinal (length and width) and transverse (thickness) plane, which is called Wicking. Four categories of wicking are:

- Wicking without diffusion. (e.g., Polyester).
- Wicking with diffusion. (e.g., Cotton).
- Wicking with adsorption. (e.g., Fabrics that are washed with fabric softener).
- All three occur simultaneously. (e.g., a polyester-cotton-blended fabric dried with a fabric softener).

Not all the blood deposited on the fabric is wicked because the viscosity of blood increases due to water evaporation during wicking [16]. According to Therese de Castro (2017), factors that affect the formation of bloodstains on fabric include:

- Fiber content of the fabric = cotton, wool.
- Fabric structure = woven, knit.
- Properties of fabric surface = roughness, compliance, thickness, absorbency.
- Prior laundering of the fabric = new fabrics, laundered fabric
- Treatment applied to the fabric = finishing agents.

When the fabric is examined, the stain pattern may not resemble the initial stain pattern, leading to misinterpretation of stain velocity and angle of impact [16]. This is because of the wicking process, the angle of the fabric during the wetting and wicking process, and the surface that backs the fabric.

1.5. Laundry Detergents

Detergents are amphiphilic structured compounds with a hydrophilic head in the outer surface and hydrophobic tail in the inner surface forming a micelle [17]. This micelle form helps them to dissolve in water. Since they decrease surface tension, they are called surfactants. The hydrophobic part sticks to the soil in the fabric and is incorporated into the micelle. Then the hydrophilic part repels it from the fabric and dissolves the insoluble substance in the water [18]. There are several ingredients that make laundry detergents. Each ingredient has a unique role in cleaning, and not all ingredients are related to stain removal. While ingredients like surfactants and builders help remove stains, ingredients like zeolite and alkaline agents aid to enhance their performance, fragrances and colorants enrich the quality of the clothes, and processing aids and opacifiers make the product commercially attractive. The addition of protease to detergent removes blood effectively as it hydrolyses the peptide bonds in the protein present in blood [19].

1.6. DIY Laundry Agents

Stain removal is not just detergent and water in most households, especially when it comes to tough stains like blood. In such cases, the laundry will take a DIY turn. Ingredients readily available in the house are used to remove or boost the performance of detergent in removing the stains. Criminals trying to cover up their crime may not have time for these DIY hacks, but if these hacks can boost the detergent's performance by reducing the need to wash the clothes multiple times, it saves their time. Some of the common household ingredients used in laundry include distilled white vinegar, washing soda, lemon juice, rubbing alcohol, baking soda, hydrogen peroxide, and borax (sodium tetraborate) [20]. Not much is known about the influence of these ingredients on the chemical screening tests for blood except H_2O_2. The use of borax as a laundry booster in many households has been in practice for a very long time [21]. It improves the effectiveness of laundry agents by:

- Increasing the pH of water,
- Emulsifying and dispersing oils,
- Neutralizing calcium and other metals,
- Softening hard water,
- Increasing particulate surface charge to prevent redeposition of dirt,
- Removing stains by oxidizing them, and
- Stabilizing enzymes in biological stains.

1.7. Forensic Analysis of Laundered Bloodstains

Bloodstains on fabrics can be removed by water at a certain temperature and pressure [22]. But the older the stain is, the harder it is to remove it with only water. Even if the stain becomes invisible to the naked eye, especially man-made fabrics, the preliminary tests can still detect the blood absorbed into the material. This holds true for both absorbent and non-absorbent fabrics [23, 24, 25]. Machine washing removes stain effectively but it does not decrease the detectability of blood unless the washing temperature is higher [22, 26, 27].

Natural fabrics are more absorbent than man-made and blended fabrics, and so they retain more blood, making it difficult to completely wash off the stain [23, 24, 25, 28]. The use of detergents interferes with presumptive tests depending on variables like ingredients present in them, soaking period, number of washes, bloodstain drying duration, type of fabric, etc. Presence of oxygen bleaching ingredients in detergents produce false negative results in KM and Luminol, but it does not impede the DNA analysis

[29]. Testing after a few days reduces the interference of bleach, thus increases the chances of an accurate result [30]. The temperature and duration of drying bloodstains before washing also impacts the presumptive tests. Drying bloodstains at high temperatures makes them easier to detect using luminol [22, 31]. Increasing the bloodstain drying duration increases the chances of detectability of blood [22, 32].

There are multiple factors that interfere in the analysis of laundered bloodstains. Studying and understanding these factors help improve the tests and hence, devise a better analytical method that combats these interferences. The present study compares the two most used presumptive tests – KM and Luminol. KM is the best for detecting diluted bloodstains because of its high sensitivity, and Luminol is known for its high efficiency in detecting latent bloodstains. The tests' efficiency is compared with respect to various types of fabrics dried at varying duration and washed with different laundry agents.

2. Literature Review

2.1. Laundry Agents

Studies that focused on the effect of different detergents on the performance of preliminary tests suggest detergents like Ariel, which contains strong surfactants, and detergents used in combination with active oxygen agents like sodium percarbonate are effective in reducing the detectability of blood on fabrics.

In the study by Varsha Singh, et al. (2022), 4 types of absorbent fabrics were stained with blood, dried under atmospheric conditions for 4-5 hours and laundered using 4 different washing agents. Then the samples were tested using preliminary tests (TMB test and Luminol test) and a confirmatory test (Takayama test) repeatedly at regular intervals until they showed negative result. Cotton retained blood much better than other fabrics. Dettol soap did not remove bloodstains effectively, leading to maximum positive results. Ezee liquid detergent showed the least number of positive results followed by Ariel detergent and Surf Excel detergent.

Samreen Mushtaq, et al. (2016) analyzed the efficiency of preliminary tests to detect blood on fabrics washed with commercially available detergents. The study involved 12 different types of fabrics and 6 detergents that are commercially available in Pakistan. It focused mainly on how different detergents and the duration of washing interfere with different tests. It concluded that Hemastix is more sensitive in detecting blood on laundered fabrics compared to KM, LMG, and TMB tests. It also concluded that cotton, polyester, and khaddar retain blood more compared to tarawera, bosky, and silk polyester. Among the detergents, Ariel was better at removing bloodstain from fabrics.

Diane Howard, et al. (2019) analyzed the performances of TMB reagent, Luminol, Bluestar® Magnum, ABAcard® Hematrace® and Rapid Stain Identification Series (RSID^{TM}-Blood) to detect blood on cotton fabrics laundered with sodium percarbonate. Cotton washed with and without sodium percarbonate in both cold and hot temperatures were used as samples. Vanish Oxi-Action manufacturer's instructions were used as reference. The study concluded that RSID^{TM}-Blood is effective in detecting blood on all fabrics washed with sodium percarbonate at all temperatures, showing an 86% chance of returning positive if blood is present. It also concluded that TMB, Bluestar® Magnum and Hematrace® have only a 24% chance of returning positive when sodium percarbonate is present, while Luminol has 61%, but it reduced to 39% with increase in temperature.

The study by I. Arjun Rao, et al. (2016) analyzed the efficiency of the KM test to detect blood in fabrics washed with detergents. It experimented with 8 different fabrics and 5 detergents commercially available in India. It focused on how different detergents and washing time interfered with the test. It concluded that the KM test is effective in detecting blood on laundered cotton and brasso, whereas detergents were able

to completely remove traces of blood from terrycot and silk polyester. Ariel was better at removing bloodstains from fabric compared to others.

2.2. Type of Fabric

The type of fabric and its blood retaining ability also plays a major role in the detectability of blood from laundered fabrics. Studies show that blood deposited on man-made fabrics like nylon and polyester is hard to detect after washing because of their poor retaining ability, whereas natural fabrics like cotton are good at retaining blood traces even after washing. Light colored fabrics show strong fluorescence on the Luminol test compared to dark colored fabrics because they absorb some of the fluorescence. The weave pattern and the density of fabric also impacts the blood retaining ability of fabrics after washing.

The study of Carolin Edler, et al. (2017) analyzed the efficiency of the Luminol test in detecting blood on 4 types of fabrics laundered using different washing and drying techniques. It concluded that luminol can effectively detect blood on all types of washed fabrics except microfleece. There was no difference in the results between fabrics directly air-dried after washing and fabrics put on spin dry before air-drying. The intensity of CL decreases with an increase in washing temperature and is less intense in blended fabric and microfleece, which is owed to their less density compared to cotton jersey and cotton denim. It also stated that almost all samples showed a complete DNA profile or individual alleles.

The study by Sharifah Mastura Syed Mohd Daud, et al. (2019) analyzed the efficiency of the KM test to detect blood on 6 types of fabrics washed with normal water and detergents commonly available in Malaysia. The bloodstained samples were soaked for 20-, 40-, and 60-minutes duration in detergent water before washing in the machine. Washing fabrics with only normal water did not remove the stain, therefore showing an intense pink color. Fabrics soaked for 60 minutes produced low intense results, removing bloodstains effectively. Cotton and spandex gave 100% positive results on all 4 detergents used, and polyester gave only 25% positive results. Fabrics laundered with Dynamo detergent gave the least positive results due to the presence of strong surfactants in it.

2.3. Laundry Methods

According to some studies, the detectability of blood on laundered fabrics also depends on different laundry methods, temperature of water, and drying techniques. Washing fabrics at high temperatures reduced the detection of blood effectively compared to washing at lower temperatures, especially when sodium percarbonate is used with detergents because it improves the performance of detergents. Fabrics laundered for more cycles gave less intense or even negative results.

In the study by Saritha D'Souza, et al. (2020), 5 types of natural fibers and 5 types of man-made fibers, stained with goat blood, were laundered, and tested both dry and wet using Benzidine test for consecutive days. The intensity of the test decreased with each wash. Samples laundered with detergents showed positive results for a smaller number of days compared to samples laundered without detergent. As natural fabrics are absorbent, they retain blood much longer than man-made fabrics.

Thomas W. Adair, et al. (2005) analyzed the effectiveness of the Luminol test and Leuco-Crystal Violet (LCV) test to detect blood on 100% cotton T-shirts after washing and to discern the pattern. Whole horse blood was used for the study and deposited as footwear impression, projected bloodstain pattern using a syringe, and misted bloodstain pattern using aerosol spray in 10 T-shirts. One unstained T-shirt is used as a control sample. First 5 T-shirts were laundered without dry cycles like T-shirt #1 – 1 wash, T-shirt #2 – 2 washes without dry cycle .... T-shirt #5 – 5 washes without dry cycle. And the next 5 T-shirts were

laundered with dry cycles in between washes like T-shirt #6 – 1 wash and dry cycle, T-shirt #7 – 2 alternating washes and dry cycles .... T-shirt #10 – 5 alternating washes with 5 dry cycles. All samples showed positive for both Luminol and LCV tests except the control sample. LCV did not show any discernable pattern whereas Luminol showed clear and discernable projected bloodstain pattern in all 10 samples and discernable footwear impression pattern on 3 samples.

2.4. Bloodstain Drying Temperature and Duration

The impact of temperature and duration from the deposition of blood on fabric to washing on the detectability of blood is also analyzed in many studies. Studies suggest that bloodstained fabrics left to dry at high temperature show more positive results compared to drying at room temperature. Most studies are not conclusive on the effect of lag period before washing because of interference of other parameters such as washing temperature, washing methods, and storage conditions.

Michaela Hofmann, et al. (2019) tested the performance of the Luminol test to detect blood on cotton and polyester fabrics of both white and black color laundered using 2 different washing machines under varying conditions. Combur® rapid test was used to confirm the results of Luminol test. The study conducted 6 experiments to analyze each condition separately. The pre-treatment of fabrics (cold water, aspirin, stain remover) or the lack thereof showed no significant impact on the results. The blood detectability was higher at lower temperatures. White fabrics showed clear luminescence, whereas black fabrics absorbed the luminescence partially. Both powder and liquid detergent removed bloodstains effectively, but the new model washing machine performed well compared to the old one. All bloodstained fabrics dried in a climate chamber at 40°C showed positive compared to drying at room temperature before washing. This is in accordance with the study on the detectability of blood inside motor vehicles using luminol, where it is suggested that high temperature converts hemoglobin into methemoglobin, therefore increasing the detectability of blood [31]. Fresh or less than days old bloodstains showed weak CL compared to bloodstains dried for 3 or more days [22].

Dilara Öner Kaya, et al. (2023) analyzed the efficiency of FLS system (Forenscope-Mobile Multispectral UV-VIS-IR Imaging Systems®) and SERATEC® HemDirect hemoglobin test to detect blood on 5 types of bloodstained fabrics laundered at 4 different temperatures after drying for 3 different intervals. The fabrics were of 4 different colors – 2 light tones and 2 dark tones and were laundered in a Bosch Maxx 8 machine using enzyme-based liquid laundry agent. 62%, 72%, and 81% of fabrics laundered after 24h, 1 week, and 1 month lag periods respectively yielded stain images. FLS image quality and detection rate decreased with the increase in washing temperature, and it produced weak results in dark-colored fabrics. HemDirect test gave strong positive results for all fabrics laundered at 15°C without detergent and for cotton, denim and fleece fabrics that were laundered after 1 month lag period at 30°C whereas velvet gave weak positive results. Fabrics laundered at a temperature higher than 60°C showed negative results regardless of lag period.

In the study by Ivan Stojanović (2019), Hemastix test strips and Bluestar forensic tablets were used to detect blood from cotton laundered under different conditions. Bloodstained samples were left to dry at 6 different intervals – 6h, 24h, 72h, 10 days and 30 days, before washing using 4 different methods – Laundry by machine at 95°C with Ariel detergent, Laundry by machine at 60°C with Ariel detergent, laundry by hand at 30°C with Ariel detergent and laundry by hand at 30°C without Ariel detergent. All positive controls gave positive results in both tests. Washing by hand is not sufficient to remove visible bloodstain from cotton compared to machine washing. All samples laundered by hand, regardless of the

drying period before laundering and laundry methods, gave positive results for blood if present, and all samples laundered by machines gave negative results regardless of the drying interval.

In the study by C. Oldfield, et al. (2018), the performance of Luminol is assessed by testing on Carpet and Denim stained with fresh porcine blood and laundered with and without sodium percarbonate. The study conducted 4 experiments to analyze each condition separately. Bloodstained denims were laundered using biological and non-biological detergents with and without sodium percarbonate at 10°C, 40°C and 60°C. Sodium percarbonate at higher temperature increased the effectiveness of detergents to remove blood traces, whereas the absence of sodium percarbonate showed 100% positive results. When bloodstained carpets were laundered using neutral detergent with and without sodium percarbonate at 10°C and 40°C, all samples produced positive results. Both denim and carpet were left to dry for 0, 1, 4, 7 and 14 days at either indoor laboratory conditions or outdoor environmental conditions before subjecting to the above-mentioned respective laundry conditions. Samples left to dry in environmental conditions showed significant. Reduction in blood detection. The drying period before washing did not show any noticeable change in results.

2.5. Presumptive Tests

The sensitivity of the preliminary test used to detect blood on laundered fabrics also plays a major role. Both luminol and KM tests can detect up to 1:100,000 dilution of blood [7]. Ana Castelló, et al. (2002) concluded that luminol is sensitive up to blood dilution of 1:300,000. It gave low CL and negative results on bloodstained fabrics laundered at high temperature but gave intense CL on bloodstains dried for more than 3 to 4 days before washing [26, 34]. K. Gautam, et al. (2023) states that the LMG test gives less false-positive results compared to other preliminary tests, and KM tested with Bleach gives negative results. Hemastix, RSIDTM-Blood and KM were also considered to perform good compared to TMB, Bluestar® Magnum and Hematrace®.

In the study conducted by Monika Gupta, et al. (2016), Cotton (absorbent) and nylon (non-absorbent) clothes were stained with blood, dried at room temperature for 2 to 3 days, and laundered by hand with and without detergent. Then the samples were subjected to preliminary tests (TMB, Luminol), Confirmatory test (Teichmann's) and blood grouping test (mixed agglutination method) after each wash till the 10th wash. In both TMB and Luminol, only nylon gave negative results after the 4th wash, the rest gave positive results. Teichmann's gave negative results for everything except control sample. Both clothes gave positive agglutination results except nylon after the 5th wash.

The study of Tuğba Ünsal Sapan, et al. (2021) tested the ability of the luminol solution and 2 DNA extraction methods to produce accurate results on cotton and nylon fabrics laundered at different temperatures using the same detergent. The luminol test was assessed using the luminol score scheme used by Michaela Hofmann, et al. (2019) and concluded that cotton and nylon showed high visibility after washing at low temperatures but decreased with high temperature. It also concluded that QIAamp DNA Mini Kit yielded low level profile for both fabrics laundered at 40°C and 60°C and Phenol Chloroform Isoamyl Organic isolation method did not yield profile at any level for fabrics laundered at 90°C.

In the study by Hiroaki Nakanishi, et al. (2020), 100% cotton and 100% polyester T-shirts were stained with blood and laundered using either liquid handwash, dishwashing liquid, laundry detergent liquid, soap bar, or normal water. There is no specific drying or washing period. The washed samples and unwashed positive control samples were then subjected to preliminary tests, DNA/RNA extraction, m-RNA detection and STR typing. The luminol test, LMG test, and anti-human Hb immunochromatography test

were used for preliminary testing. Luminol showed positive results for all positive samples, LMG too showed positive results except for polyester fabrics laundered with laundry detergent and soap. Though the Anti-human Hb test was supposed to be sensitive, all samples gave negative results except one sample (out of 4) from each fabric laundered with water. The study concludes that washing blood off fabric even without detergent significantly reduces Hb to an undetectable rate. HBB and 18S genes were detected in all samples in m-RNA detection. DNA extracted from all washed fabrics was significantly less compared to unwashed positive control.

2.6. Blood

Preliminary tests are not specific to human blood. So, blood obtained from other mammals such as goat, horse, and pig were used in some studies [27, 36, 37]. It is also important to note that most studies use blood mixed with anticoagulants.

3. Materials and Methodology

3.1. Aim

To analyze and compare the performances of Kastle-Meyer and Luminol tests in detecting bloodstains on laundered fabrics.

3.2. Objectives

- To assess the detectability of blood using KM and Luminol with respect to different types of fabrics.
- To understand the effect of laundry agents on KM and Luminol tests.
- To observe how varying bloodstain drying period before washing affects its detectability by both tests.

3.3. Need & Significance

Blood is very important in reconstructing crime scenes and identifying individuals involved in the crime. Even if the perpetrator flees the scene without leaving any evidence, blood on his/her clothes will link them to the crime scene. In most cases, washing the blood off the cloth will not hinder the presumptive tests from detecting it. But there are certain factors that affect the detectability of bloodstains on laundered fabrics, such as the type and thickness of the fabric on which the blood was deposited, the age of the bloodstain before washing, chemical composition of the laundry agent used, time lapsed between washing and test conducted, etc. Hence, it is important to assess the sensitivity of presumptive tests to detect blood from different fabrics laundered with different agents.

3.4. Variables

3.4.1. Independent Variables

- Type of fabric
- Bloodstain drying period on fabric
- Laundry agent used

3.4.2. Dependent Variables

- Visibility of bloodstain after washing
- Result of the tests
- Intensity of the tests

3.5. Laboratory

The experiments were conducted in the Laboratory of Centre of Excellence in Digital Forensics (CoEDF) institution located at Perungudi, Chennai.

3.6. Samples

The samples used in this study are listed in Table 2. All the fabrics were bought in a local textile shop. Pig blood was bought from a nearby slaughter house. Ariel was bought from a grocery store, and the chemicals were bought from a local shop. Images of the samples are attached in the annexure.

Table 2. Samples list with symbols

Fabric Samples	
Category	**Fabric**
Natural Fabric	Cotton (C)
	Linen (L)
Semi-Synthetic Fabric	Rayon (R)
Synthetic Fabric	Polyester (P)
	Satin Polyester (S)
Blood Sample	
Pig blood was used to simulate human blood. The blood was used as soon as it was collected from the slaughter-house without any anticoagulants.	
Laundry Agents	
Agent	**Concentration**
Normal Water (1)	100ml water
Ariel Detergent Powder (2)	One tbsp to a liter of water. 100ml/sample
Sodium Tetraborate (3)	One tbsp to a liter of water. 100ml/sample
Sodium Hypochlorite (4)	1% Solution

3.7. Presumptive Tests

3.7.1. Kastle-Meyer Test

Reagent Preparation

- Stock solution

1. Phenolphthalein powder – 2.00g
2. Potassium Hydroxide – 20.00g
3. Distilled water – 100ml
4. Zinc dust – 20.00g

Boiled for 3 hours and cooled down.

- Working solution

1. Phenolphthalein stock solution – 2ml
2. Distilled water – 10ml

3. Ethanol – 2ml

Solution a – Ethanol

Solution b – Phenolphthalein working solution

Solution c – 3% Hydrogen Peroxide

Method

A drop or two of solutions a and b were added. After making sure no color change had occurred, solution c was added. An intense pink reaction indicates positive for blood, and no color change indicates negative for blood.

3.7.2. Luminol Test

Reagent Preparation

- Luminol powder – 0.02g
- Sodium Carbonate – 1g
- 3% Hydrogen Peroxide – 100ml

The solution was mixed with a stirrer and transferred to a clean spray bottle.

Method

The solution was sprayed onto each sample and observed for CL in a dark room. The appearance of CL indicates positive for blood, and no reaction indicates negative for blood.

3.8. Procedure

Step 1: A total of 120 samples were divided into 3 groups – 0, 12h and 24h as shown in Table 3. The control group samples were laundered by hand and tested without any blood. The experimental samples were pinned to polystyrene sheets and labeled.

Table 3. Sample Groups

Sample Groups		Fabrics	Washing Agents	Presumptive Tests
Control Group	40 Negative control samples (0)	Each sample is cut into an 8x8cm dimension. Each group has 8 samples from each fabric	The 8 samples were divided into 4 for washing - 2 for each agent	One sample was tested using KM and the other using Luminol
Experimental Groups	40 samples dried for 12h before washing (12h)			
	40 samples dried for 24h before washing (24h)			

Step 2: Three drops (0.15ml appx) of blood were dropped on each experimental sample with a plastic pipette and were left to dry in a closed room without any direct sunlight

Step 3: After drying, the samples were soaked in washing solutions for an hour. Then the samples were laundered by hand and air-dried.

Step 4: Once dried, the visibility of stains in each sample was observed and rated using the visibility scheme by Carolin Edler, et al. (2017):

0 - No visibility

1 - Faint visibility: only sketchy visibility of the bloodstain, no discernible outline

2 - Moderate visibility: well noticeable bloodstain, Distinct outline, Minor coloring of the bloodstain
3 - Clear visibility: clearly discernible bloodstain, Intense coloring of the bloodstain

Step 5: The samples were tested for blood using either KM or luminol. The intensity of KM reaction was rated using the following scheme:
0 – No color reaction
1 – Mild pink reaction
2 – Moderate pink reaction
3 – Intense pink reaction

Similarly, evaluation scheme by Michaela Hofmann, et al. (2019) was used to rate the CL of the Luminol reaction:
0 - no trace of luminescence.
1 - slight, very weak luminescence.
2 - clear luminescence.
3 - very bright, long-lasting luminescence, often with visible primary traces.

Step 6: The observed results were noted and tabulated.

3.9. Statistical Analysis

Microsoft Excel was used to prepare tables and charts.

4. Results and Discussions

4.1. Results

The visibility of the stains on laundered fabrics was noted before the tests were conducted. This is to relate the performance of the tests with both visible and invisible stains. Table 4 and 5 presents the bloodstain visibility rate of laundered samples [23].

Table 4. Visibility rate for K-12h and L-12h Samples

Type Of Fabric	Laundry Agent used							
	Normal Water (1)		Ariel Powder (2)		Sodium Tetraborate (3)		Sodium Hypochlorite (4)	
	K-12h	L-12h	K-12h	L-12h	K-12h	L-12h	K-12h	L-12h
Cotton (C)	1	1	0	0	1	0	0	0
Linen (L)	3	3	1	1	2	2	0	0
Polyester (P)	2	3	0	0	2	2	0	0
Rayon (R)	3	3	1	1	1	2	0	0
Satin (S)	0	0	0	0	0	0	0	0

Table 5. Visibility rate for K-24h and L-24h Samples

Type Of Fabric	Laundry Agent used							
	Normal Water (1)		Ariel Powder (2)		Sodium Tetraborate (3)		Sodium Hypochlorite (4)	
	K-24h	L-24h	K-24h	L-24h	K-24h	L-24h	K-24h	L-24h
Cotton (C)	2	3	0	0	1	1	0	0
Linen (L)	3	3	1	1	2	2	0	0
Polyester (P)	2	3	0	0	2	1	0	0
Rayon (R)	3	3	1	1	2	2	0	0
Satin (S)	1	1	0	0	1	1	0	0

4.1.1. Kastle-Meyer Test

All negative control samples tested using KM gave negative results. The result and the intensity of the test were noted for all samples. The time taken to react in all samples was instant (less than 2 seconds) without any delay. The pink reaction faded back to colorless in cotton, satin, and polyester, in that order within 5 - 10 minutes approximately, whereas the reaction in rayon and linen remained unchanged. The results of the K-12h and K-24h samples are shown in Table 6 using positive (+) and negative (-) symbols. Table 7 shows the intensity rate of K-12h and K-24h samples.

Table 6. Result of KM test for K-12h and K-24h Samples

Type Of Fabric	Laundry Agent used							
	Normal Water (1)		Ariel Powder (2)		Sodium Tetraborate (3)		Sodium Hypochlorite (4)	
	K-12h	K-24h	K-12h	K-24h	K-12h	K-24h	K-12h	K-24h
Cotton (C)	+	+	+	-	+	+	-	-
Linen (L)	+	+	+	+	+	+	-	-
Polyester (P)	+	+	+	-	+	+	+	-
Rayon (R)	+	+	+	+	+	+	-	-
Satin (S)	+	+	+	+	+	+	-	-

Table 7. Intensity rate of KM reaction for K-12h and K-24h Samples

Type Of Fabric	Laundry Agent Used							
	Normal Water (1)		Ariel Powder (2)		Sodium Tetraborate (3)		Sodium Hypochlorite (4)	
	K-12h	K-24h	K-12h	K-24h	K-12h	K-24h	K-12h	K-24h
Cotton (C)	3	3	2	0	3	3	0	0

Linen (L)	3	3	2	3	3	3	0	0
Polyester (P)	3	3	1	0	3	3	1	0
Rayon (R)	3	3	2	2	3	2	0	0
Satin (S)	2	3	1	1	2	2	0	0

4.1.2. Luminol Test

All the Negative control samples tested using luminol gave negative results. In all the samples, the time taken to react was less than one second (appx) and lasted for less than 3 seconds, while some showed long-luminescence lasting for 10-15 seconds. The results of the test and the intensity of the test were noted. Table 8 show the results of L-12h and L-24h. Table 9 shows the intensity rate of CL [22].

Table 8. Results of Luminol test for L-12h and L-24h Samples

Type Of Fabric	**Laundry Agent Used**							
	Normal Water (1)		**Ariel Powder (2)**		**Sodium Tetraborate (3)**		**Sodium Hypochlorite (4)**	
	L-12h	**L-24h**	**L-12h**	**L-24h**	**L-12h**	**L-24h**	**L-12h**	**L-24h**
Cotton (C)	+	+	+	-	+	+	-	-
Linen (L)	+	+	+	+	+	+	-	-
Polyester (P)	+	+	-	-	+	+	-	-
Rayon (R)	+	+	+	+	+	+	-	-
Satin (S)	+	+	+	+	+	+	-	-

Table 9. Intensity rate of Luminol reaction for L-12h and L-24h Samples

Type Of Fabric	**Laundry Agent Used**							
	Normal Water (1)		**Ariel Powder (2)**		**Sodium Tetraborate (3)**		**Sodium Hypochlorite (4)**	
	L-12h	**L-24h**	**L-12h**	**L-24h**	**L-12h**	**L-24h**	**L-12h**	**L-24h**
Cotton (C)	1	3	1	0	2	1	0	0
Linen (L)	3	3	1	3	3	3	0	0
Polyester (P)	3	3	0	0	3	3	0	0
Rayon (R)	1	2	3	2	2	2	0	0
Satin (S)	2	3	3	1	2	2	0	0

4.2. Discussions

4.2.1. Influence of Laundry Agents

Laundry agents used in this study range from top-brand detergent bought from a grocery store to a broad-spectrum disinfectant bought from a chemical store. These variety of agents were selected to incorporate a wider area of possible scenarios. Studying the samples washed only in water helps primarily focus on

the influence of laundry agents on the preliminary tests by eliminating the influence of other factors such as water temperature, water hardness, etc. Water alone does not remove bloodstains effectively and thus the results were straightforward [24, 38]. As expected, it had a comparatively higher stain visibility rate as shown in Figure 1 after washing, and both tests yielded 100% positive results with high intensity.

Samreen Mushtaq, et al. (2016) and I. Arjun Rao, et al. (2016) described in their respective studies that samples washed with Ariel gave the least number of positive results for KM when compared to other commercially available detergents as it contains sodium sulphonate - strong surfactant and NaOH - strong alkali. It also contains sodium hypochlorite and percarbonate in small quantities. It did remove stains effectively, and the tests yielded 75% positive results as shown in Figure 2. Cotton and polyester samples laundered with Ariel after 12 hours gave negative results for both tests. The intensity of the tests was also slightly reduced when laundered with Ariel, as shown in Figures 3 and 4.

Sodium Tetraborate (Borax) was only slightly better than water in removing bloodstains. Before the experiment, it was unclear whether the ingredient would affect the preliminary tests as there were no prior studies to refer to. It did not interfere much with both the test reactions, leading to 100% positive results. Since it is more commonly used as a laundry booster, it should be checked in future studies whether the chemical affects the preliminary tests when combined with the ingredients in the laundry agents.

Compared to the above three, test results produced by fabrics laundered with 1% Sodium Hypochlorite were drastically different. The tests gave 5% positive results as only KM detected blood on one polyester sample with an intensity rate of 1. The tests were done within 24hrs of washing the samples. Luminol test could have given more positive results if the test was done after a certain period as suggested by Ana Castelló, et al. (2009). Bleach did not produce any false-positives on the negative controls.

Figure 1. Bloodstain visibility rate after washing with respect to Laundry Agents

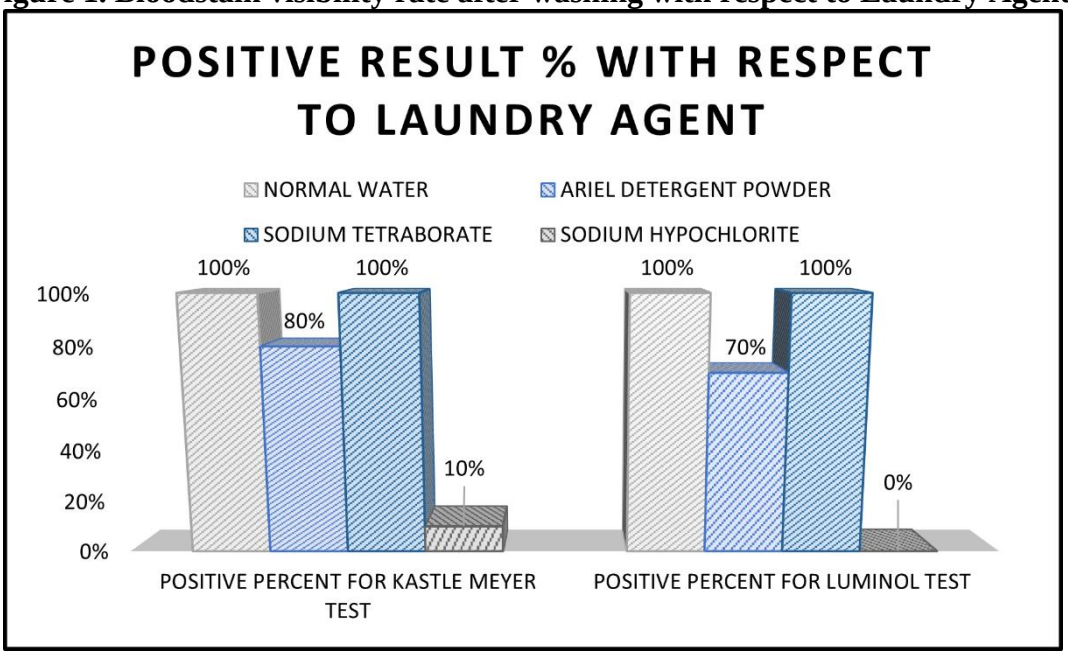


Figure 2. Positive result % of both tests with respect to Laundry Agents

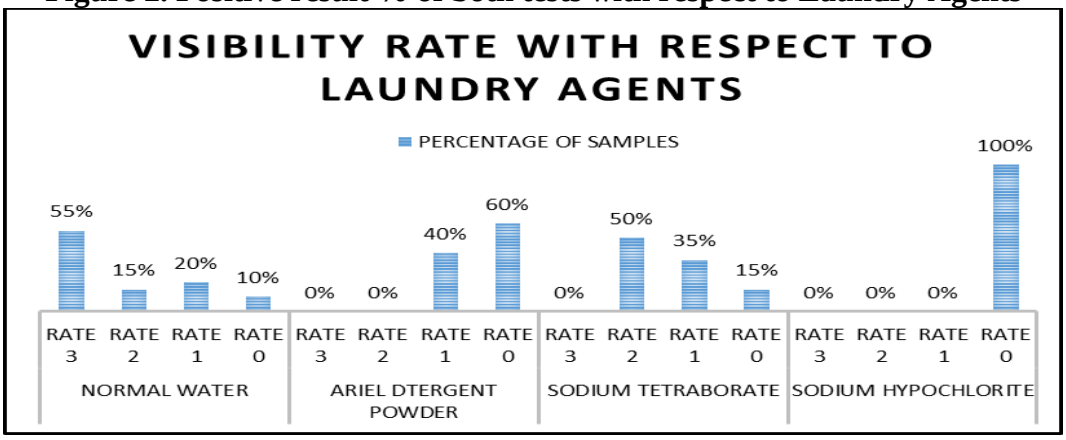


Figure 3. KM Intensity Rate with respect to Laundry Agents

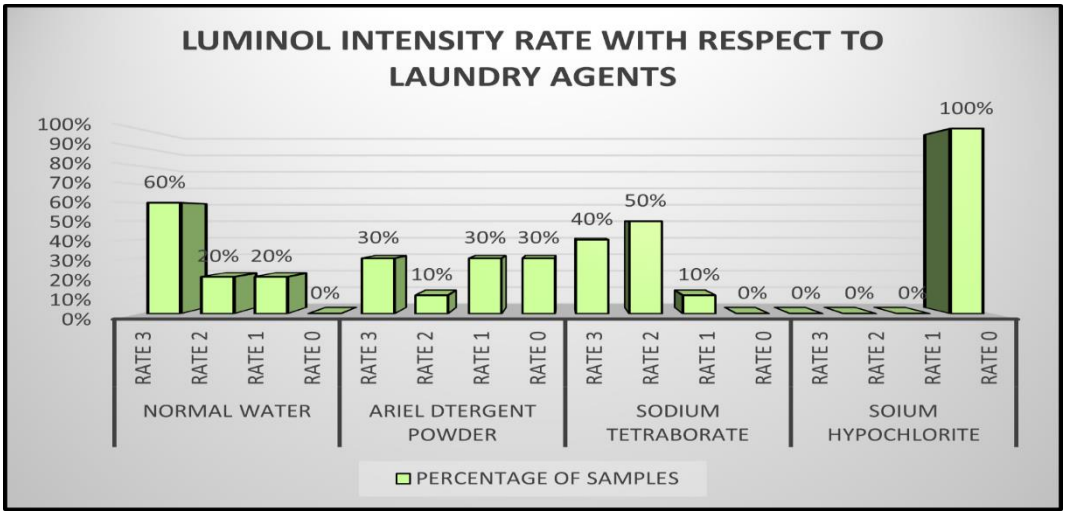
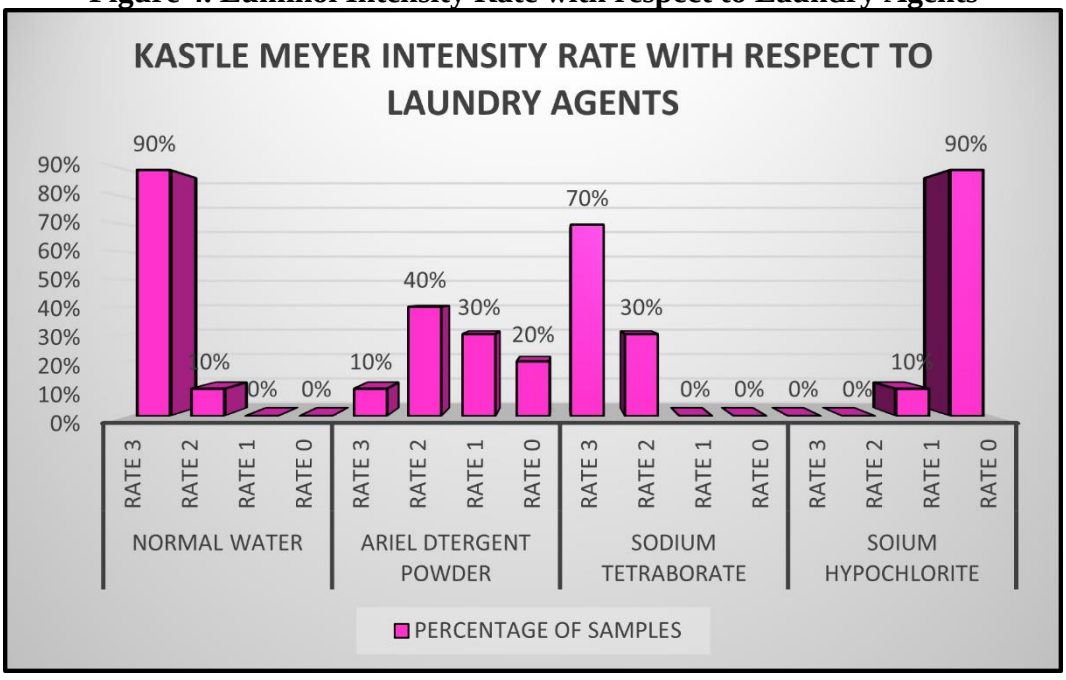


Figure 4. Luminol Intensity Rate with respect to Laundry Agents



4.2.2. Influence of Bloodstain drying period before washing

The two bloodstain drying periods observed in this study (12h and 24h) are short because of the notion that perpetrators often try to cover up their crime within 24hrs. The stains became slightly more visible in samples washed after 24hrs as shown in Figure 5. The efficiency of KM was high in 12h dried samples which dropped significantly in 24h dried samples as shown in Figure 6. Even with unwashed bloodstains, the speed and intensity of the KM reaction decreases when the stain has aged for more than a week [41]. Since both the drying periods are still within 24hrs, there is not much difference in the intensity of KM reactions as shown in Figure 7. Michaela Hofmann, et al. (2019) and Dilara Öner Kaya, et al. (2023) have concluded in their respective studies that luminol becomes more effective as the bloodstain drying period increases. This holds true as shown in Figure 8, unlike KM, most positive reactions in 12h dried samples still gave positive reactions when the drying period was increased. According to Ana Castelló et al. (2009), luminol can overcome the interference of bleach on porous surface, if tested a few days later after laundry. This can be tested for different fabrics in future studies. Cotton laundered by Ariel gave negative result for both test when dried for 24h which was unexpected [38]. The intensity rate of most positive reactions was strong for both tests regardless of the bloodstain drying period.

Figure 5. Bloodstain Visibility Rate after washing with respect to drying period

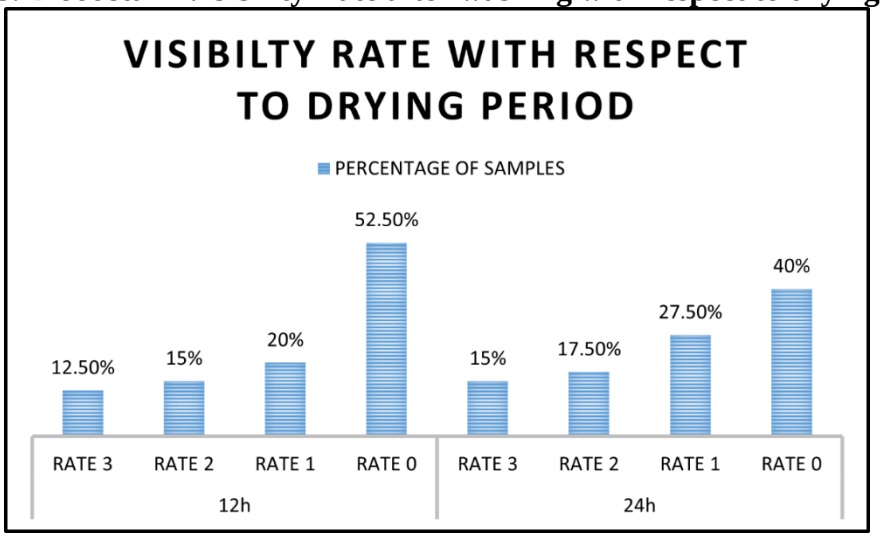


Figure 6. Positive Result % of both tests with respect to drying period

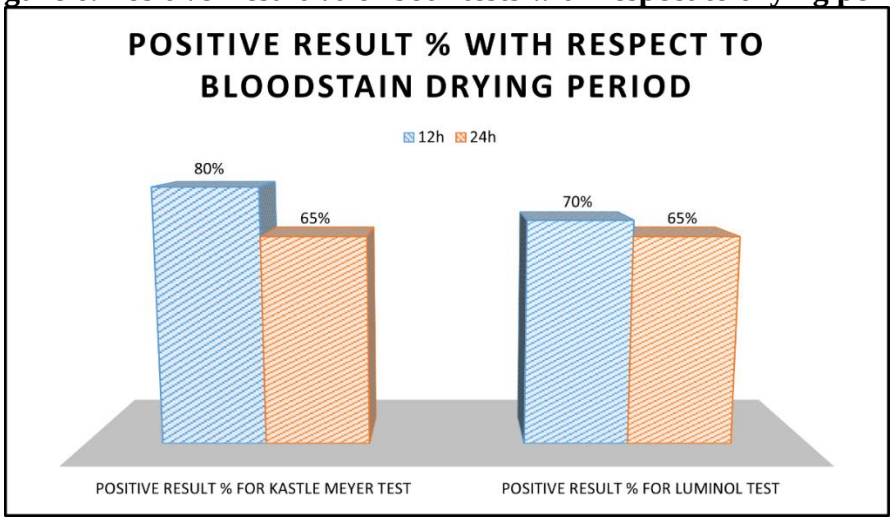


Figure 7. KM Intensity Rate with respect to Drying period

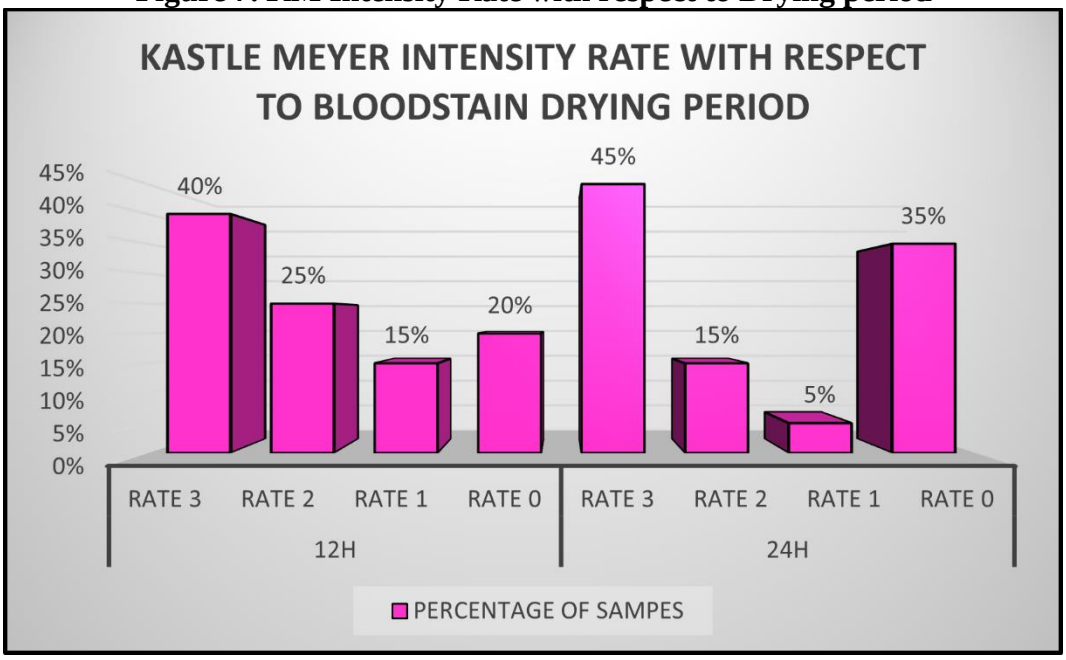
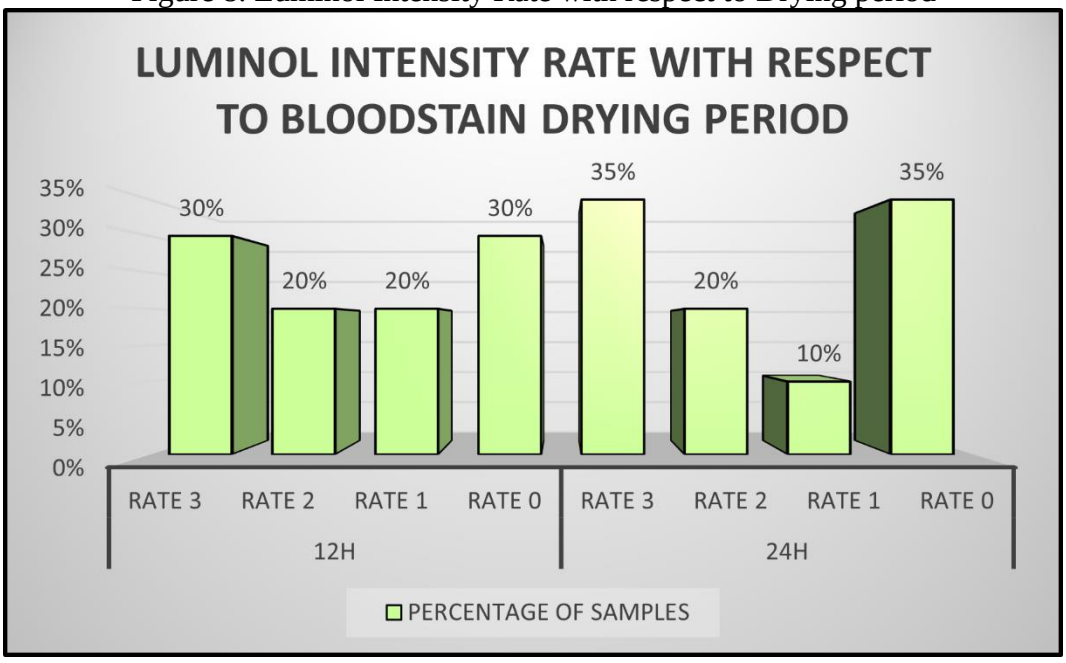


Figure 8. Luminol Intensity Rate with respect to Drying period



4.2.3. Influence of Type of Fabric

For this study, two natural fabrics - Cotton and Linen, two synthetic fabrics – Polyester and Satin Polyester and a semi-synthetic fabric – Rayon were selected. Cotton, Polyester, and Linen were plain weave materials, and Rayon was twill weave material. The fabric order in which the rate and extent of wicking increased as did the diameter of bloodstain:

Polyester → Cotton → Linen → Rayon → Satin

Blood drop on cotton and polyester took longer to absorb and formed clot on the surface that could be scrapped off. The fabric order in which second light-colored layer formed around the initial stain on the fabrics went from undistinguishable to more prominent:

Satin → Rayon → Linen → Cotton → Polyester

The washed stains on rayon and linen were more visible, except when laundered with bleach, compared to other fabrics as shown in Figure 9. While blood easily washes off synthetic fibers, cotton with its high moisture retaining properties did not absorb blood effectively and had low to none stain visibility. This could be because the finishing agents in the newly bought fabric did not loosen up even after soaking it overnight before the experiment and hence decreased the blood absorption rate [1]. The low stain visibility rate of stain polyester samples did not affect the tests' performance. Both KM and luminol on cotton, linen, rayon, and satin gave an equal number of positives. But luminol's performance was significantly reduced on polyester samples as shown in Figure 10. KM positive reactions on cotton, linen, and polyester were more intense, whereas the intensity rate on rayon and satin was more distributed, as shown in Figure 11. As for luminol positive reactions, CL on linen and polyester were stronger followed by rayon and satin, as shown in Figure 12. CL on most cotton samples was very weak.

Figure 9. Bloodstain Visibility Rate after washing with respect to Type of Fabric

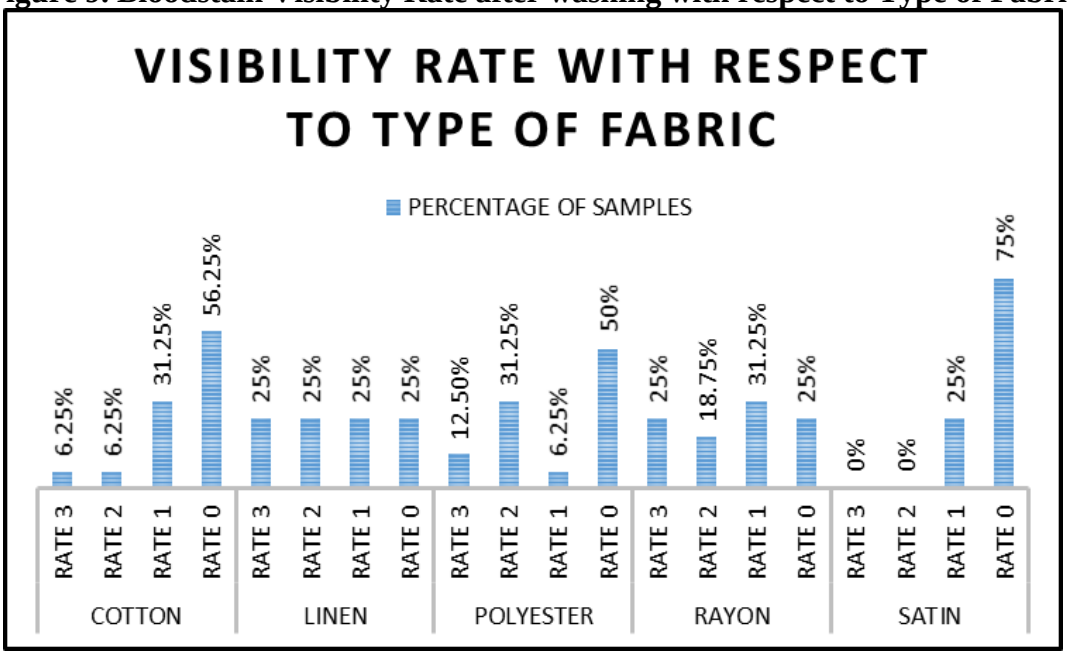


Figure 10. Positive Results % with respect to Type of Fabric

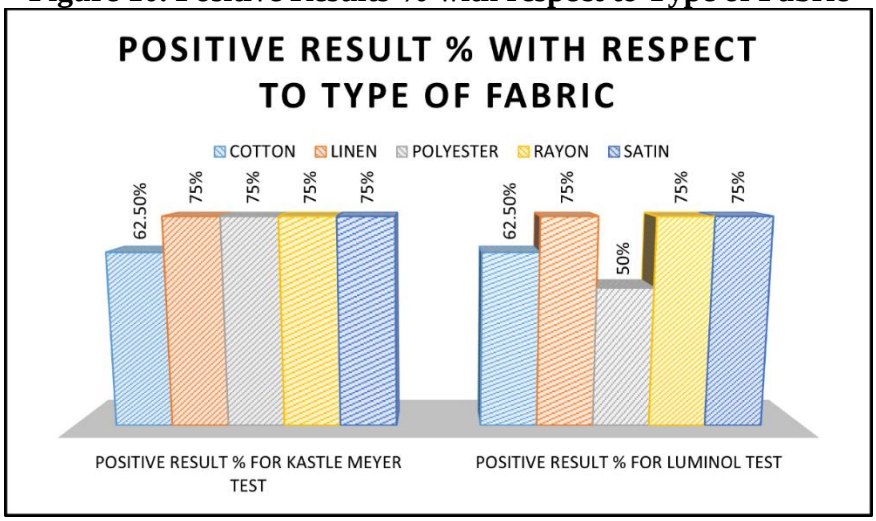


Figure 11. KM Intensity Rate with respect to Type of Fabric

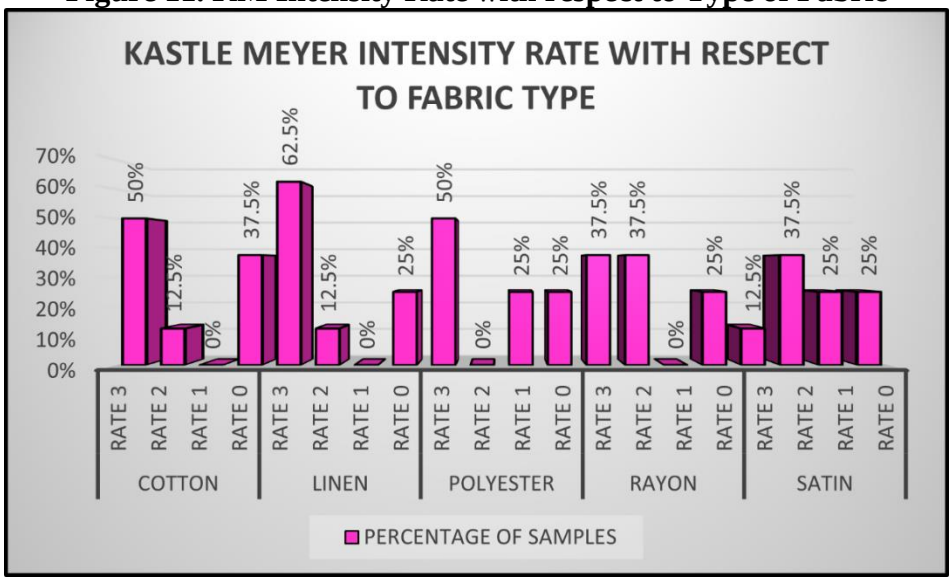
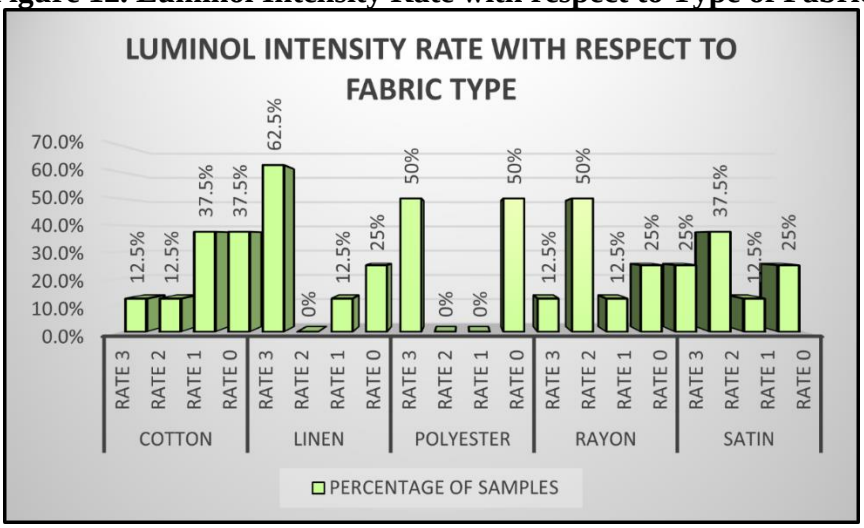


Figure 12. Luminol Intensity Rate with respect to Type of Fabric



5. Summary and Conclusion

5.1. Summary

The study was conducted to compare the efficiency of Kastle-Meyer and Luminol tests to detect bloodstains on laundered fabrics. For this, five types of fabrics were stained with pig blood and dried for 12h and 24h before laundering with different laundry agents. The stain visibility after washing was rated before testing the samples with either KM or luminol. The result and intensity of the tests were noted. Based on the results, it is inferred that KM is more efficient in detecting bloodstains when the drying period is less, and luminol gives better performance with bloodstains that are dried longer. But both are affected by the interference of bleaching agents.

5.2. Conclusion

Conclusions drawn from the findings of the study include:

- Kastle-Meyer is a better choice if the evidence was suspected to have laundered in less than a day.
- Results indicate that luminol might give better results if the tests were delayed.
- Both the tests were effective in detecting blood on samples laundered with diluted Borax (100% detectability), indicating that the activity of borax does not interfere with the reaction between the tests and the hemoglobin present in the sample.
- Bleaching agent, both concentrated (95% negative results) and the small amounts present in Ariel detergent (25% negative results), have proved to significantly affect the results of luminol and Kastle-Meyer tests when the tests were done within a day of laundry.
- The performances of both the tests were good on satin polyester samples (75% detectability), even if the fabric had the least bloodstain visibility rate among the other fabric samples because of its non-absorbent nature.
- Increasing the bloodstain drying period increases the visibility of the bloodstains (47.5% to 60%) but it decreases the detectability (75% to 65%) of the bloodstains.

5.3. Limitations

The current study only focuses on two preliminary tests and assesses their performances with respect to a few varieties of fabrics and laundry agents. There are still many types of fabrics of varying properties and DIY laundry ingredients out there that can be used to explore more possibilities for bloodstain detection on laundered fabrics. The time limit in the study restricts further evaluation of the detectability of bleached bloodstains by Kastle-Meyer and Luminol after a certain period. Apart from these two tests, there are several advanced tests that can be studied in relation to detection of bloodstain on laundered fabrics.

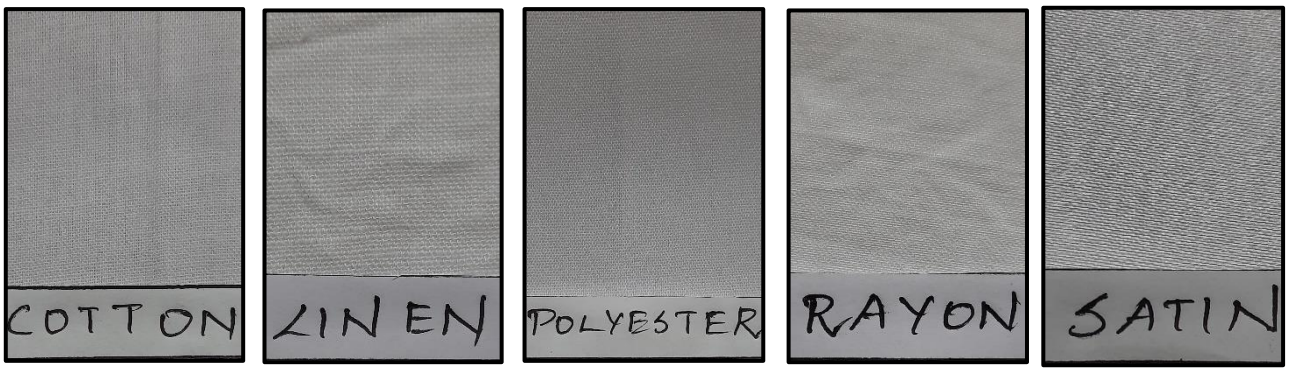
5.4. Implications

Luminol is the first choice for presumptive testing for latent bloodstains. But it may not be effective in detecting bloodstains on fabrics laundered recently. This false negative result could affect an entire investigation. It is not always possible for the analysts to know when and how exactly the fabric could have been washed. The findings of the current study suggest that it is important to use another presumptive test that shows more potential in detecting bloodstains on freshly laundered fabrics in addition to luminol testing. This prevents the elimination of crucial evidence and saves time and cost involved in the investigation.

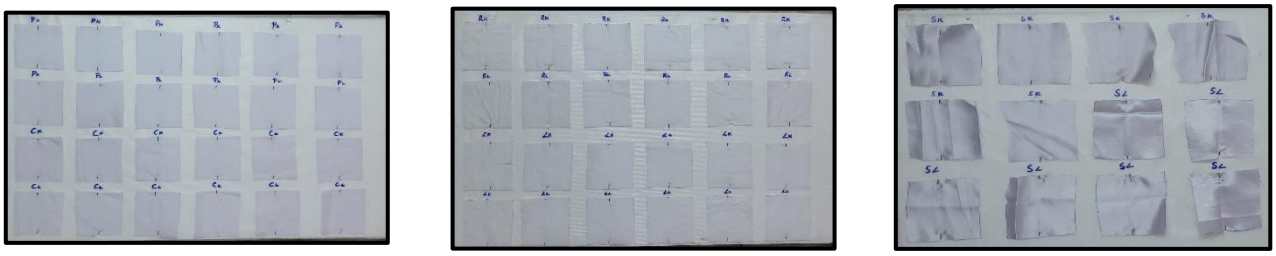
5.5. Recommendations

Since the current study is conducted with pig blood, it must be further evaluated with human blood. Future studies can explore the ways to overcome the bleach interference on both tests and thus increase their reliability and accuracy. The sample size can be extended as there are various types of fabrics and DIY laundry ingredients out there that need to be studied in relation to presumptive testing. Since borax is known for its laundry boosting properties, it could be tested in combination with detergent to analyze whether it interferes with the preliminary tests when combined with other active ingredients or in a more concentrated form. There are limited studies on the effect of duration between washing blood off fabrics and testing on the performance of the preliminary tests. In the current study, the samples were tested in less than 24 hours after washing. Future studies can extend the period between laundering the samples and testing them using Kastle-Meyer and Luminol for more than 24 hours, and so on, and compare the findings with the data of the present study.

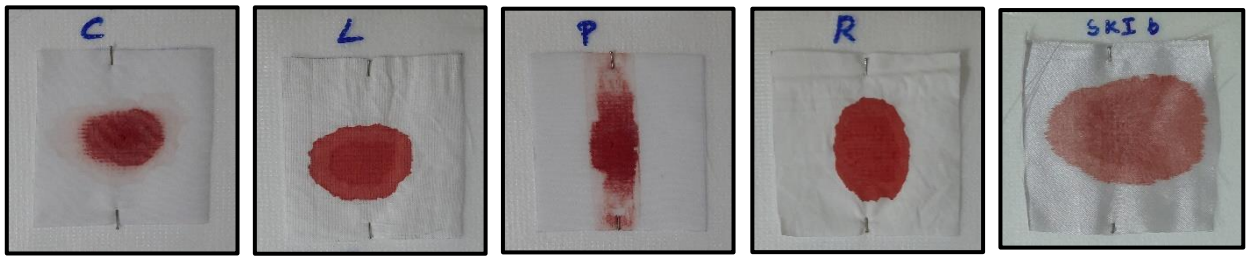
6. Appendix



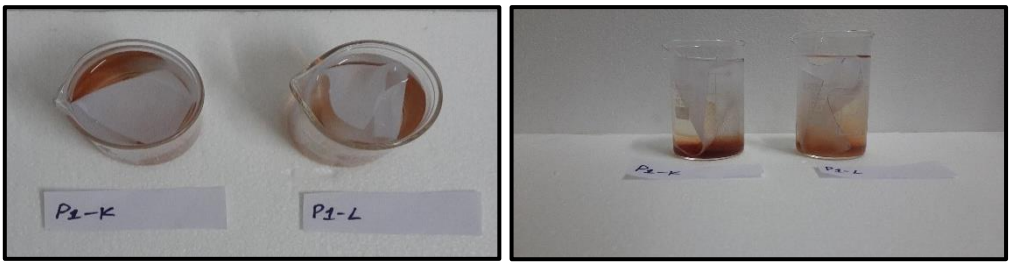
Images of Fabric Samples



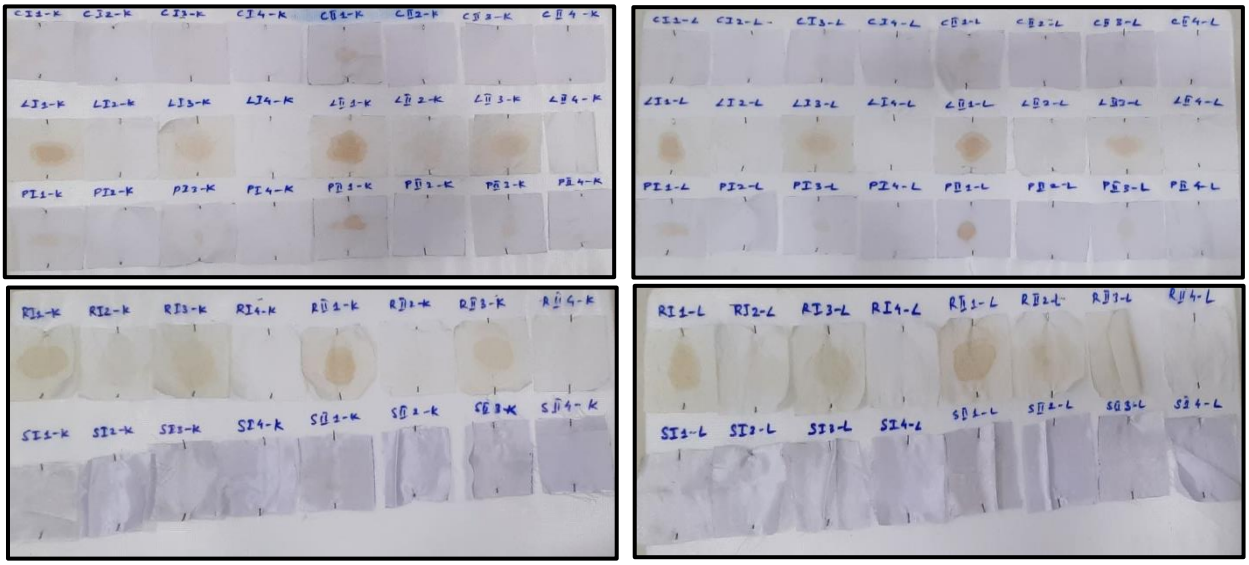
Images of Sample Preparation



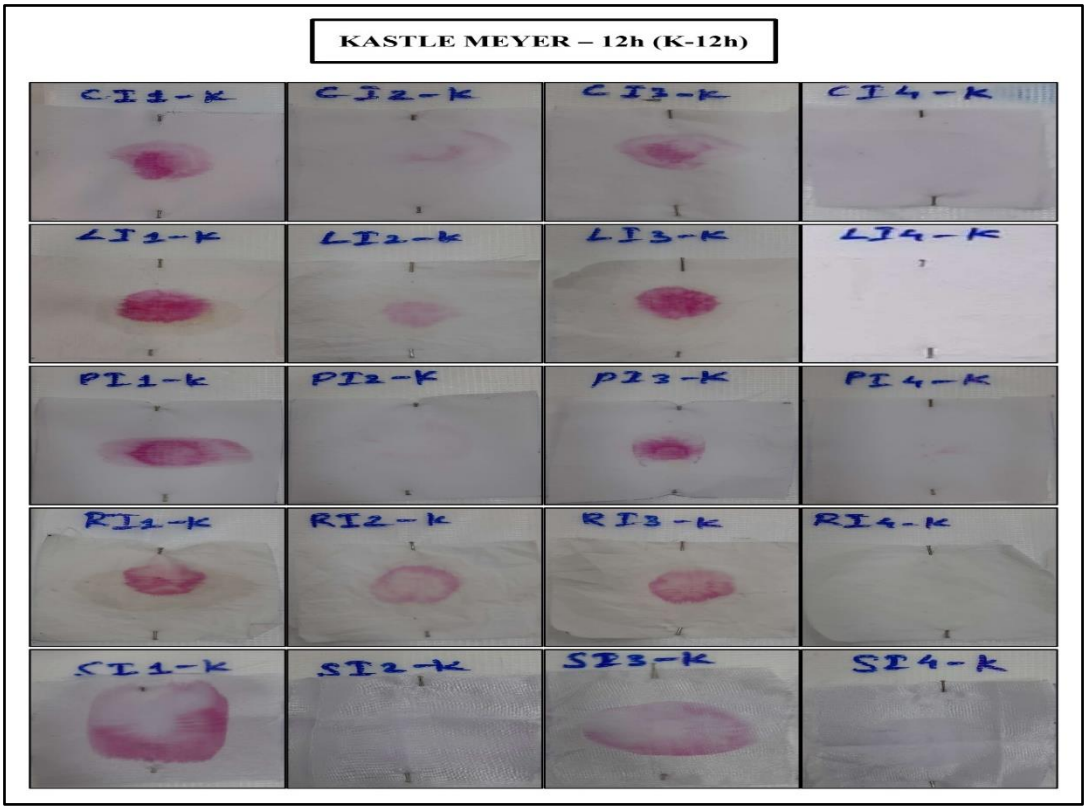
Images of Blood Deposited on Fabrics



Images of Soaking Samples in Washing Solutions

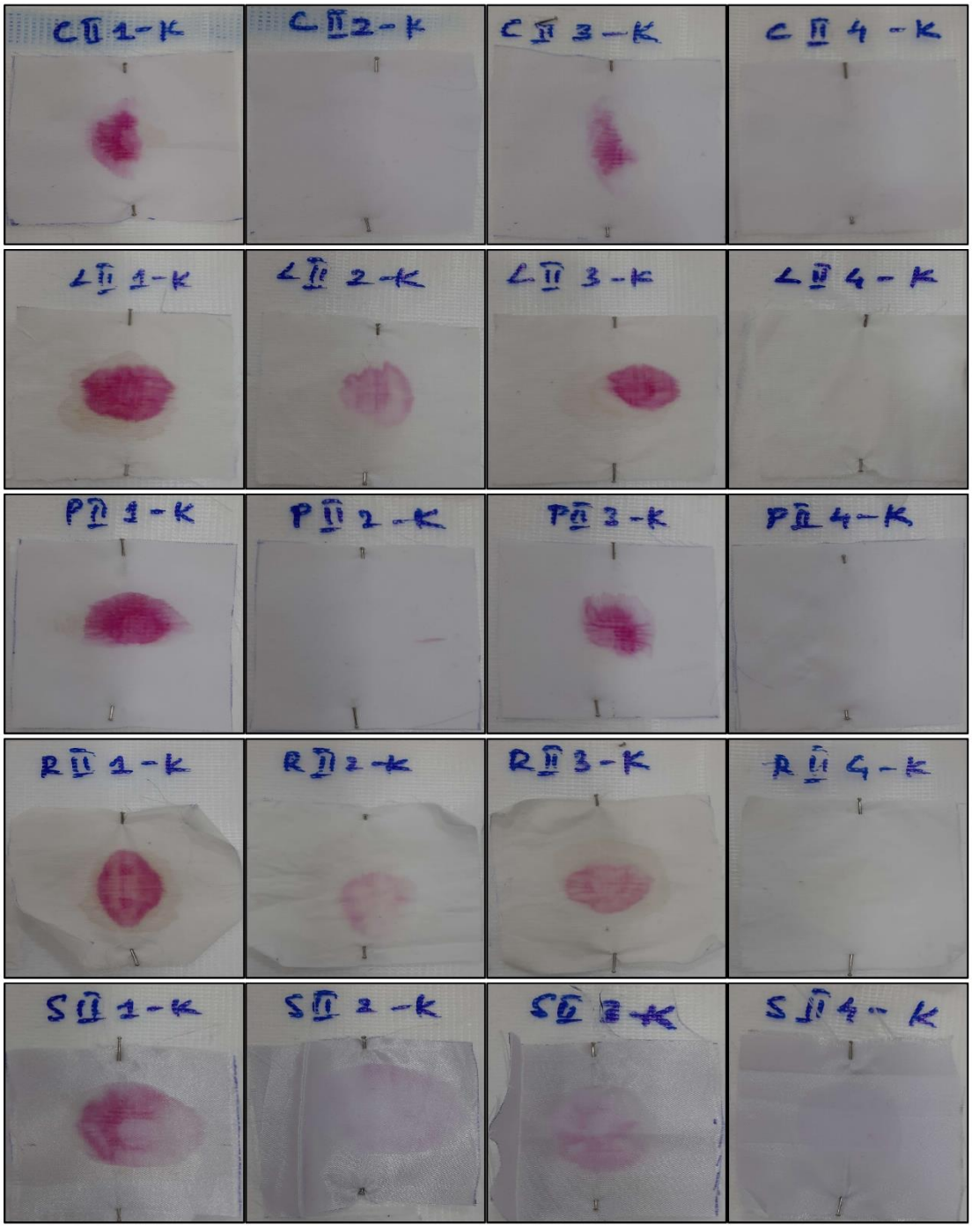


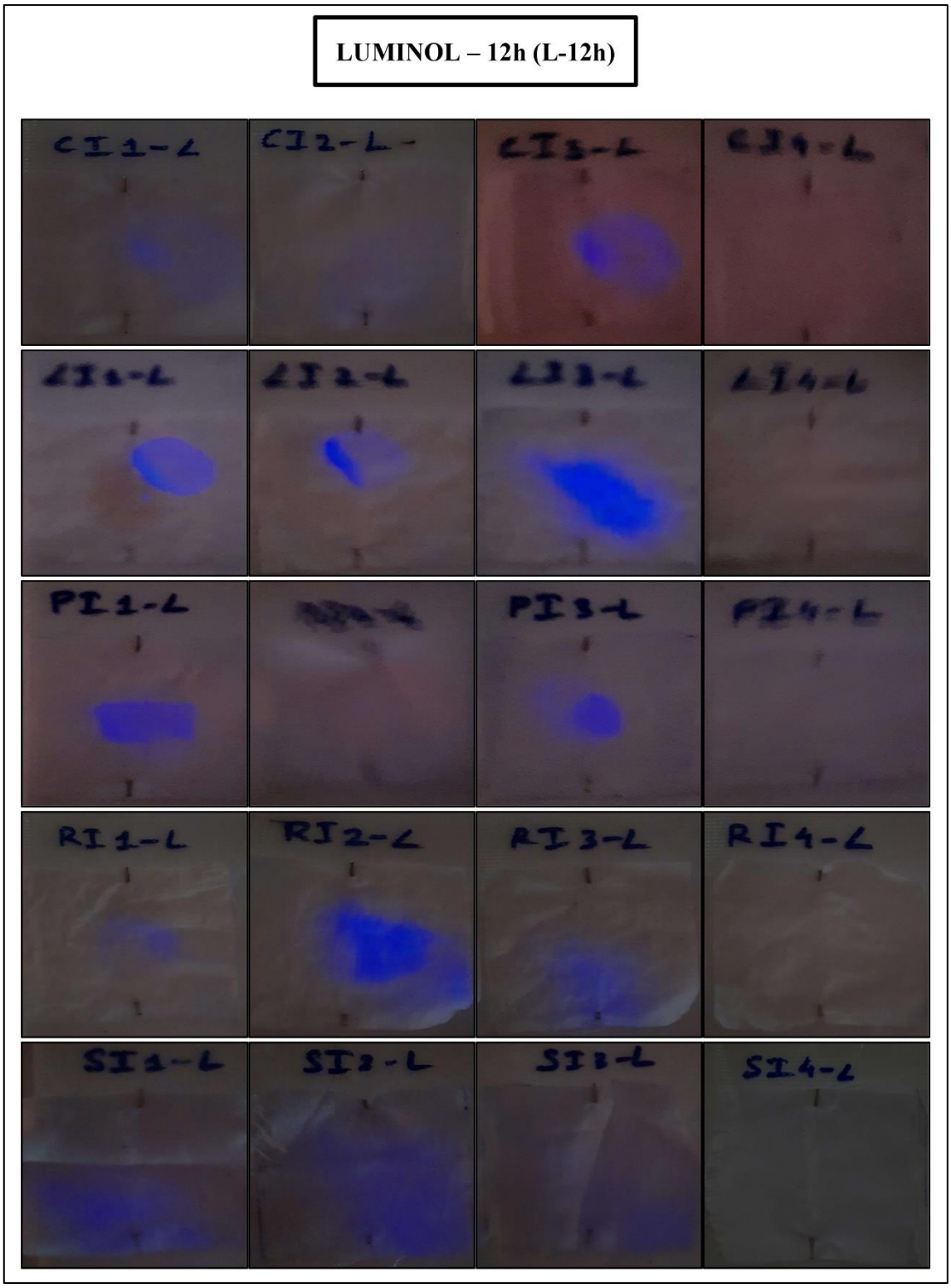
Images of Laundered Bloodstain Samples

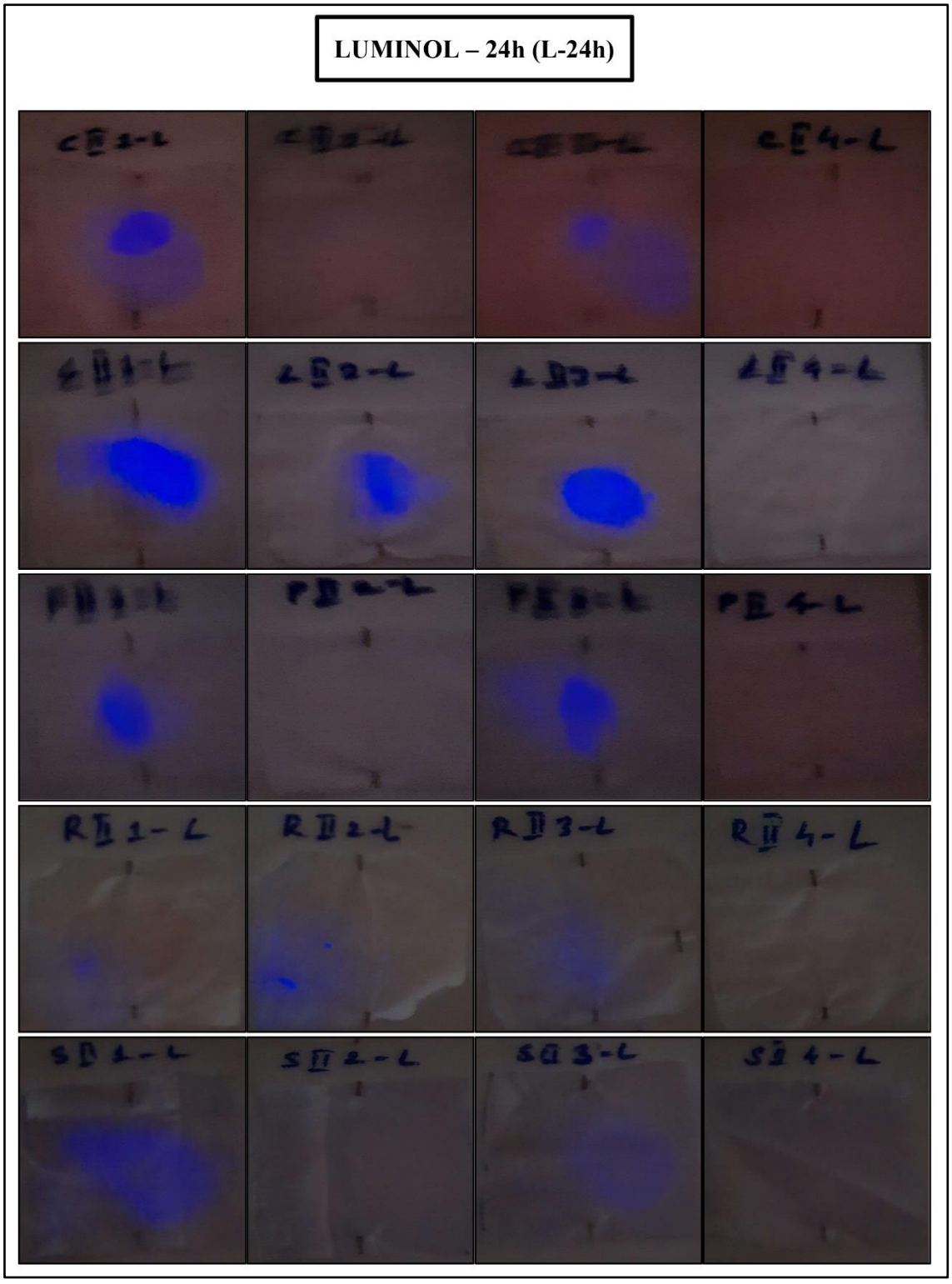


Images of Tested Samples

KASTLE MEYER – 24h (K-24h)




LUMINOL – 12h (L-12h)
CI1-L
CI2-L
CI3-L
CI4-L
LI1-L
LI2-L
LI3-L
LI4-L
PI1-L
PI3-L
PI4-L
RI1-L
RI2-L
RI3-L
RI4-L
SI1-L
SI3-L
SI3-L
SI4-L



7. Reference

1. Therese de Castro., "Forensic Interpretation of Bloodstains on Fabrics", Forensic Textile Science, Woodhead Publishing, 2017, 127-167.
2. Hiroaki Nakanishi, Takeshi Ohmori, Katsumi Yoneyama, Masaaki Hara, Aya Takada, Kazuyuki Saito, "Bloodstain Examination and DNA Typing from Hand-washed Bloodstains on Clothes", Legal Medicine, 2020, 47, 101758.

3. Milton Cox, "Effect of Fabric Washing on the Presumptive Identification of Bloodstains", Journal of Forensic Sciences, 1990, 35(6), 1335-1341.
4. Wendy J. Koen, Chris Halkides, Kimberly Lott, "Presumptive and Confirmatory Blood Testing", Forensic Science Reform: Protecting the Innocent, 2017, 239-69.
5. Milton Cox, "A Study of the Sensitivity and Specificity of Four Presumptive Tests for Blood", Journal of Forensic Sciences, 1991, 36(5), 1503-1511.
6. Robert E. Gaensslen, "Sourcebook in Forensic Serology, Immunology, and Biochemistry", US Department of Justice, National Institute of Justice Washington, DC, 1983, 628.
7. Shanan S. Tobe, Nigel Watson, Niamh Nic Daéid, "Evaluation of Six Presumptive Tests for Blood, their Specificity, Sensitivity, and Effect on High Molecular-Weight DNA", Journal of Forensic Sciences, 2007, 52(1), 102-109.
8. Filippo Barni, Simon W. Lewis, Andrea Berti, Gordon M. Miskelly, Giampietro Lago, "Forensic Application of the luminol Reaction as a Presumptive test for Latent Blood Detection", Talanta, 2007, 72(3), 896-913.
9. Anders Nilsson, "The Forensic Luminol Test for Blood: Unwanted Interference and the Effect on Subsequent Analysis", Linköping University, the Swedish National Laboratory of Forensic Science, 2006.
10. K. Sembulingam, Prema Sembulingam, "Essentials of Medical Physiology", Jaypee Brothers Medical Publishers, 2012, p. 58-60.
11. M.A. Raymond, E.R. Smith, J. Liesegang, "The Physical Properties of Blood - Forensic Considerations", Science and Justice: Journal of the Forensic Science Society, 1996, 36(3), 153-160.
12. Abimbola Farinde, "Lab Values, Normal Adult: Laboratory Reference Ranges in Healthy Adults", EMedicine, 2021. https://emedicine.medscape.com/article/2172316-overview?form=fpf
13. Susan E. Aiello, Michael A. Moses, "The Merck Veterinary Manual 11th Edition", Merck and company, 2016.
14. Sara J. Kadolph, Anna Langford, "Textiles", 2002.
15. Richard Li, "Forensic Biology", Chemical Rubber Company (CRC) Press, 2015, p. 35-37. https://doi.org/10.1201/b18209
16. Stephen Michielsen, Michael Taylor, Namrata Parekh, Feng Ji, "Bloodstain Patterns on Textile Surfaces: A Fundamental Analysis", National Institute of Justice, US Department of Justice, 2015.
17. Judith M. Neugebauer, "Detergents: An Overview", Methods in Enzymology, 1990, 182, 239-253.
18. Divya Bajpai, V.K. Tyagi, "Laundry Detergents: An Overview", Journal of Oleo Science, 2007, 56(7), 327-340.
19. Lee Xin Tian, Lee Te Chuan, Mohamad Ali Selimin, "Effect of Protease in Commercialized Detergent Powder on Blood Removal Efficiency", Research in Management of Technology and Business, 2022, 3(1), 266-274.
20. Kathy Brown, "Homemade House Cleaning Recipes: Easy to Make Laundry Detergent, Dish Washer, Cleaners, Fabric Softener, Stain Remover and Many More for a Cost-Effective Living", PublishDrive, 2015.
21. U.S. Borax, "Borates in Detergency", 2021. https://www.borax.com/BoraxCorp/media/Borax-Main/Resources/Brochures/borates-detergency.pdf

22. Michaela Hofmann, Jiri Adamec, Katja Anslinger, Birgit Bayer, Matthias Graw, Oliver Peschel & Martin M. Schulz, "Detectability of Bloodstains after Machine Washing", International Journal of Legal Medicine, 2019, 133, 3-16.
23. Carolin Edler, A. Gehl, J. Kohwagner, M. Walther, O. Krebs, C. Augustin & A. Klein, "Blood Trace Evidence on Washed Textiles - A Systematic Approach", International Journal of Legal Medicine, 2017, 131, 1179-1189.
24. Sharifah Mastura Syed Mohd Daud, Saranya Sundram, "Identification of Bloodstains on Different Fabrics after Washing with Commonly used Detergent in Malaysia", Journal of Management and Science, 2019, 17(1), 9-9.
25. Monika Gupta, Vaibhav Saran, Munish Kr. Mishra, A.K. Gupta, "Examination of Traces of Blood Stains on Different Fabrics after Washing", International Journal of All Research Education and Scientific Methods, 2016, 4(6), 204-209.
26. Tuğba Ünsal Sapan, Işıl Tuna Erdoğan & Sevil Atasoy, "Human Identification from Washed Blood Stains", Bulletin of the National Research Centre, 2021, 45, 1-13.
27. C. Oldfield, R. M. Morgan, H. F. Miles & J. C. French, "The Efficacy of Luminol in Detecting Bloodstains that have been Washed with Sodium Percarbonate and Exposed to Environmental Conditions", Australian Journal of Forensic Sciences, 2018, 50(4), 345-354.
28. Samreen Mushtaq, Nouman Rasool, Sehrish Firiyal, "Detection of Dry Bloodstains on Different Fabrics after Washing with Commercially Available Detergents", Australian Journal of Forensic Sciences, 2016, 48(1), 87-94.
29. Ana Castelló, Francesc Francés, Fernando Verdú, "DNA Evidence Uncompromised by Active Oxygen", The Scientific World Journal, 2010, 10, 387-392.
30. Ana Castelló, Francesc Francés, Fernando Verdú, "Bleach Interference in Forensic Luminol Tests on Porous Surfaces: More about the Drying Time Effect", Talanta, 2009, 77(4), 1555-1557.
31. T.I. Quickenden, C.P. Ennis, J.I. Creamer, "The Forensic use of Luminol Chemiluminescence to Detect Traces of Blood inside Motor Vehicles", Luminescence: The Journal of Biological and Chemical Luminescence, 2004, 19(5), 271-277.
32. Dilara Öner Kaya, Şükriye Karadayi, Beytullah Karadayi, Gürsel Çetin, "Evaluation of the Detectability of Different ages of Bloodstains on Fabrics in Different Washing Conditions and at Various Wavelengths", Journal of Forensic and Legal Medicine, 2023, 94, 102486.
33. Varsha Singh, Shruti Rajwar, Suchi Gupta, "Detection of the Bloodstain on Different Fabrics after Washing with Commercial Detergent", Indian Journal of Natural Sciences, 2022, 13(72), 42126-42135.
34. Diane Howard, Janet Chaseling, Kirsty Wright, "Detection of Blood on Clothing Laundered with Sodium Percarbonate", Forensic Science International, 2019, 302, 109885.
35. I. Arjun Rao, P. Ashish, "Identification of Bloodstains on Different Fabrics after Washing with Routinely used Detergents in India", Australian Journal of Forensic Sciences, 2016, 1(1), 1-5.
36. Saritha D'Souza, Pooja V. Menon, "Efficacy of Benzidine Test in the Identification of Blood Stains found on Different Fabrics after Washing for Consecutive Days", Journal of Forensic Research and Criminal Investigation, 2020, 1(1), 13-18.
37. Thomas W. Adair, Rebecca L. Shaw, "Enhancement of Bloodstains on Washed Clothing using Luminol and LCV Reagents", Journal of Bloodstain Pattern Analysis, 2005, 21(4), 4-10.

38. Ivan Stojanović, "Detection of Bloodstains on Cotton Fabric after Washing", Acta Medica Medianae, 2019, 58(1), 24-27.
39. Ana Castelló, Francesc Francés, Fernando Verdú, "Accuracy, Reliability, and Safety of Luminol in Bloodstain Investigation", Canadian Society of Forensic Science Journal, 2002, 35(3), 113-121.
40. K. Gautam, G. Soumya, R. Joshi, R. Rawal, "Estimating the Presence of Blood with Reference to Anti-Forensic - A Comparative Study", Austin Journal of Forensic Science and Criminology, 2023, 10(1), 1093.
41. Hemant Kumar, "Sensitivity and Specificity Comparison of Presumptive Tests for Blood", International Journal of Creative Research Thoughts, 2022, 10(2), a296-a308.