DRY BLOODSTAIN DETECTION ON VARIOUS BLACK FABRIC USING FORENSIC LIGHT SOURCES

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by

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Abbreviations

ALS	Alternative Light Sources
FT-IR	Fourier Transform Infrared
FLS	Forensic Light Sources
IR	Infrared
UV	Ultraviolet

Symbols

℃	Decree Celsius
cm	Centimetre
nm	Nanometre

PENGESANAN KESAN DARAH KERING PADA PELBAGAI KAIN HITAM MENGGUNAKAN SUMBER CAHAYA FORENSIK

ABSTRAK

Darah adalah salah satu tompokan biologi yang paling kerap dan penting digunakan untuk mengenal pasti dan mencirikan penjenayah dan penting dalam mewujudkan hubungan antara pesalah, mangsa, dan jenayah. Bukti darah dan kesan darah dalam bentuk cecair atau kesan yang ditemui di tempat kejadian jenayah dalam kuantiti yang banyak atau dalam jumlah yang sedikit. Kajian ini menyiasat kesan dahah pada fabrik gelap kapas, poliester, dan campuran (kapas/poliester). Kajian ini menyelidik penggunaan Crime lite untuk mengambil gambar kesan darah untuk menyediakan alat bukan invasif alternatif. Kajian ini mengkaji sama ada darah sentiasa menyerap cahaya dalam julat 300 nm hingga 900 nm dan panjang gelombang terbaik untuk memerhatikan darah pada fabrik gelap. Ia juga menyiasat sama ada jenis fabrik menjejaskan keupayaan untuk melihat darah pada fabrik, jika mencuci dan mengeringkan fabrik menjejaskan penggunaan FLS, dan, jika ya, sejauh mana. Titisan darah didepositkan pada tiga fabrik telah difoto dalam kit Crime-lite 2s FLS (Foster dan Freeman) dengan dan tanpa penapis di bawah 400-700nm (cahaya putih), 350nm-380nm (UV), 400nm-430nm (Violet), 420nm-470nm (Biru), dan 445nm-510nm (Biru-hijau). Pemerhatian telah dioptimumkan menggunakan sampel kawalan positif dan cahaya ungu dipilih. Secara keseluruhannya, 1132 gambar telah direkodkan dalam keseluruhan kajian ini. Keputusan menunjukkan bahawa fotografi dengan FLS adalah kaedah tidak merosakkan, bebas bahan kimia dan berkesan untuk pengesanan darah pada fabrik gelap. Tambahan pula, cahaya ungu tanpa penapis merupakan sumber cahaya yang paling berkesan untuk melihat kesan darah pada kain gelap tanpa menggunakan bahan kimia. Menggunakan cahaya ungu, kain yang dibasuh tanpa detergen mengekalkan kesan darah sehingga enam kitaran manakala kain yang dibasuh dengan detergen hanya dapat mengekalkan kesan darah dalam dua kitaran sahaja.

DRY BLOODSTAIN DETECTION ON VARIOUS BLACK FABRIC USING FORENSIC LIGHT SOURCES

ABSTRACT

Blood is one of the most frequent and crucial biological methods used to identify and characterise criminals and regularly used to establishing a link between the offender, the victim, and the crime. Blood and bloodstain evidence in the form of fluids or stains found at the scene of a crime in large quantities or in tiny amounts. This study investigates dried blood stain on the dark fabric of cotton, polyester, and mixed (cotton/polyester). This study looked into using Crime lite to photograph bloodstains to provide an alternative non-invasive tool. This study examined whether blood always absorbed light in the 300nm to 900nm range and the best wavelength for observing blood on black fabrics. It also investigated whether fabric type affected the ability to view blood on fabrics, if washing and drying the fabric affected the use of forensic light source (FLS) and if so, to what extent. Blood drops were deposited on three fabrics were photographed in the Crime-lite 2s FLS kit (Foster and Freeman) with and without a filter under 400-700nm (white light), 350nm-380nm (UV), 400nm-430nm (Violet), 420nm-470nm (Blue), and 445nm-510nm (Blue green). The observation was optimized using positive control samples and violet light was selected. In total, 1132 photographs were in this entire study. Results indicated that photography with FLS was a viable non-destructive, chemical free and effective method for blood detection on dark fabrics. Furthermore, violet light with no filter was the most effective light source for viewing blood stains on dark fabrics without using chemicals. Using the violet light, washed fabric without detergent retain bloodstain for up to six cycles while fabric washed with detergent was only able to retain bloodstain in only two cycles.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Blood is one of the most common and important biological of blood stain used to identify and characterise offenders. The investigation of biological evidence found at the crime scene is essential in establishing a link between the offender, the victim and the crime. Evidence left by the perpetrator at the crime site can be used to investigate what happened. Biological evidence in the form of fluids or stains might be found at the scene of a crime in large quantities or in tiny amounts (Gupta *et al.*, 2016).

The most important evidence in a crime scene investigation is biological evidence including blood, saliva, semen, and urine. The biological evidence can provide valuable important details through DNA evidence that can be used to identify victims and suspects and bloodstain patterns to wite forensic light source that can be used to determine the sequence of events. These biological evidences may be identified using a variety of techniques, which can be further broken down into presumptive testing and confirmatory tests. Confirmatory tests, which provide a definitive identification of the blood of the particular evidence, presumptive tests are only screening tests (Gupta *et al.*, 2016). However, the majority of tests are destructive, destroying any DNA evidence, and certain tests can only be performed in a laboratory. Forensic Light Source (FLS) is one of the most presumptive tests that may be used to determine the majority of biological evidence. FLS can either fluoresce the evidence or increase the contrast of the evidence against the context. Fluorescence occurs when FLS is released to biological evidences such as semen, saliva, and urine, which absorb light at a certain wavelength and subsequently re-emit the absorbed energy as light at a longer wavelength. FLS can also be used to improve the contrast of bloodstains on dark surfaces when the stain is not apparent with the naked eye, such as bloodstains (Virkler & Lednev, 2009).

Among body fluids, detection of blood is most common and usually most visible especially if the blood stain is deposited onto clear substrate or on a high contrast substrate for example on white or light-coloured textile. Blood and other body fluids can link a suspect to a victim, a crime scene, or a weapon in an indisputable chain of proof by identifying a bloodstain pattern, a pool of blood or even a single drop blood. At times, it can also give indication of how the crime progressed.

In human and most animal biological system, blood is a fluid that transports oxygen and nutrients throughout the body while also transporting carbon dioxide and other waste materials to the lungs, kidneys, and liver for disposal. It helps to heal wounds and fights infection. Plasma, a liquid component of blood, makes up around 55% of the total volume (Mcdonald, 2017). White blood cells make about 5% of total blood volume and have a lifespan of 1 to 2 days. They are an essential part of the body's immune system, since they combat illness by attacking bacteria, viruses, and other microorganisms. The quantity of white blood cells (WBCs) in the blood is an important measure of health. Platelets are cell fragments that aid in the coagulation of blood and the prevention of bleeding Platelets are suspended inside the plasma. Equally important is the red blood cells (RBC), RBCs make about 40% of total blood volume and have a lifespan of roughly 120 days. Hemoglobin, a complex molecule that transports oxygen, eliminates carbon dioxide, and gives blood its red hue, is found in RBCs (Gupta *et al.*, 2016).

Hemoglobin's molecular formula is $C_{2952}H_{4664}N_{812}O_{832}S_8Fe_4$. Hemoglobin is the defining characteristic of blood, and forensic blood tests are based on detecting hemoglobin or its constituent parts. Hemoglobin is a protein that transports oxygen between the lungs and all the tissues and cells in the body. Hemoglobin is made up of four protein chains, two alpha chains and two beta chains, each with an iron heme centre that binds oxygen molecules (Franjić, 2019). A forensic scientist will need to decide whether or not it is blood when they discover a suspicious-looking stain at a crime scene the five common sequence of forensic blood examination are usually carried starting with detection of blood stain, blood preliminary test, confirmatory test, species identification and lastly

DNA profiling. This is accomplished by doing a presumptive test, so called because the investigator may assume that the stain is actually blood if the test is positive. The majority of blood presumptive tests indicate a positive result by changing colour when exposed to haemoglobin (Franjić, 2019). Forensic serology approaches rely on chemical or biochemical based biochemical tests, for instance, based on peroxides like activity of the heme group of hemoglobin. The preliminary forensic blood identification tests are actually heme assays. They employ dyes that, when combined with peroxide, change colour in a chemical process known as oxidation. There are protein enzymes in nature that will accelerate this process, but heme will also accelerate it (Merkley *et al.*, 2019).

Blood stained evidence is typically thought of as playing a supportive function in investigation which if relevant and admissible in judicial proceeding, can provide information on what occurred, how it occurred and who had done it. Blood may reveal various things about a crime to an experienced analyst in certain situations. For example, the presence of bloodstains on clothes might suggest that it was worn during the bloodletting and help identify a possible victim or offender (James, 2021). Occasionally, the interpretation of bloodstained-fabric evidence is required to assist in determining the mode of blood deposition. Blood deposition on its surrounding is useful for forensic investigation. Among many, blood deposition on fabric is quite common. When blood deposited and seep into fabric, it interacts. It also depend on the tyle of fabric it I treated with whether it is natural, semi synthetic or fully synthetic (Taylor, *et al.*, 2016). This is referred to as wetting. Immersion, adhesion, spreading, and capillary penetration of a liquid on a fabric surface are the four wetting mechanisms.

Immersion occurs when a piece of fabric is dipped into a pool of blood with enough liquid volume to completely submerge it. The bottom of the cloth will come into contact with the blood first and as the fabric continues to be submerged, blood will spill over and onto the top of the fabric, until all sides of the fabric are in contact with the blood (Castro, 2017). Adhesion is the attraction of two surfaces that have come into touch with each other. This adhesion would be determined by the

qualities of the two surfaces as well as their interaction. For example, if raindrops hit a raincoat, the adhesion is weak and the drips may roll off, if raindrops hit a cotton t-shirt, the adhesion is stronger and the drops will not roll off the fibres. Spreading refers to the flow of blood over fabrics (at least two layers of blood molecules must move over the solid surface. When a sessile blood droplet is placed on a fabric, the blood can spread throughout the fabric surface as the drop base grows larger. The penetration of blood into the fabric surface (also known as 'capillary sorption') is known as capillary penetration. It's the passage of a liquid into the fabric's capillary spaces, which may be thought of as wetting the yarns and fibres and preparing the fabric for wicking (Castro, 2017).

There are various techniques to detect latent blood on substrates, either chemical or non-chemical. Chemiluminescence, using chemicals reagents like Luminol, Fluorescein, and Bluestar, are commonly reported methods. Bluestar is a Luminol derivative that is claimed to be strong and less prone to false positives. These substances cause the blood to glow by interacting with haemoglobin. Luminol and Bluestar emit blue light, but Fluorescein emits a green light that requires a light source to see. These are excellent chemicals to utilise which is very useful in investigation to screen for blood in order to find it and swab it which can be further analysed (James, 2005).

In certain situation, when a chemical is sprayed onto a bloodstain, the stain's morphology changes as a result of the additional liquid. However, when liquid is added to a pattern, the pattern might alter, making it hard to identify the instrument that was used to produce it. If a person's clothes have no spatter stains but have transfer stains, it's conceivable that the person wearing the garments came across the victim and was neither the offender or witness. It's possible that the person wearing the clothes happened upon the victim and was neither the offender nor the witness. The void patterns created by a seat belt can be used to establish who was driving in situations like car accidents. This would be impossible to determine from the blood if the chemical detection means were used and the pattern was destroyed. Consequently, if pattern interpretation is desired, a non-destructive detecting method is required (James, 2021). Furthermore, if the bloodstains can be seen in situ, DNA testing

may be focused on specific spots rather than a whole garment, reducing the chance of a mixed profile (Sterzik & Bohnert, 2016).

The concept of FLS for blood detection is based on the interactions between matter and electromagnetic waves, such as light placed on a surface. Three separate processes—absorption, reflection, and transmission—can occur between the substance and the light source. Some of the light sources used or have been utilised at the crime scene include ultraviolet light, Polilight, Luma lighting, Spectrum 9000, and infrared (W. C. Lee *et al.*, 2013; Sterzik *et al.*, 2016).

If an interaction involving absorption takes place, the substance may release the absorbed energy by light such as fluorescence. Semen and saliva are two bodily fluids that naturally contain fluorescence. Numerous fabrics might fluoresce in the area of tracing, which would enable examiners to differentiate between various fibres (Lee & Khoo, 2010).

The biggest issue with these tests is the sample's destruction. Because the slightest quantity of biological evidence can sometimes break a case, it is critical that these little quantities are evaluated as swiftly as possible at the crime scene using non-destructive technologies. The preservation of DNA evidence is the most crucial reason for these tests to be non-destructive (Virkler & Lednev, 2009).

1.1 Problem Statement

According to current literature, the optimal wavelength for viewing blood with fabric is 410nm, because it absorbs more light and appears considerably darker than the surrounding fabric(Lee *et al.*, 2013; Sterzik *et al.*, 2016). Blood absorbs light from 300nm to 900nm, which is longwave UV (less than 400nm), or UVA (315nm to 400nm), to the visible spectrum (400nm to 700nm), and all the way to what is known as near IR (less than 400nm) (Lee *et al.*, 2013).

In previous years, studies have been conducted on blood detected on fabric using forensic light. The majority of blood pattern analysis (BPA) on fabric research focuses on the interpretation of stain type on fabric (Castro *et al.*, 2013), the effect laundry detergent on stained fabric (Castr *et al.*, 2015), detection on fabric using alternate light source (Schotman *et al.*, 2015) or the trustworthiness of BPA on stained fabric (Taylor *et al.*, 2016). None of the works discussed about the time series studies whether detection of blood is feasible after a few cycles of washing over some period of time.

In certain situation, investigator would need to re-visit the crimes scene to gain more evidence to complete an investigation. fabrics like garment from suspect or bedding sheet that have been washed can potentially have blood residues but detection is difficult or unconfirmed due to the unclear stain appearance. It would be more difficult if the stain is on darker background because visibility is impaired if the stain and the background is very much a like, meaning they have poor contrast against each other. Blood evidence may have been washed prior to that, thus the ability to still recover or even indicate the presence of blood at the crime scene is highly useful.

This research looked into whether blood always absorbs light in the 300nm to 900nm range and to choose the optimised what wavelength for detecting blood on dark fabrics. This study further looked at whether washing the fabrics and drying can actually influence the detection of blood using the optimised light source earlier. The effect of washing cycles were also tested.

1.3 Objectives

The general objective of the study is to determine the feasibility of non-destructive detection of blood stain using forensic light source on dark fabrics under various washing conditions.

1.3.1 Specific Objectives

i. To determine the reactions of crime lite forensic light and the optimum visible wavelength for detection of blood on dark cotton, polyester, and mix cotton and polyester fabrics.

ii. To determine feasibility of blood detection on washed black cotton, polyester, and mix cotton and polyester fabrics before and after a few cycles of washing treatment.

iii. To observe whether detergents and drying method may cause degeneracy on blood fabrics and effect after a few cycles of treatments.

1.4 Research questions

1. Which wavelength (within the visible region) is optimal for detect blood on cotton, polyester, and mix cotton and polyester fabrics?

2. Could the light source still detect blood deposited on washed black fabrics?

3. What effects can be seen after a few washing and drying cycles to the blood stains?

1.5 Significant of Study

The purpose of this research is to demonstrate the use of non-destructive methods for blood stain detection on dark fabrics i.e., natural, synthetic, and mixed fabrics. The use of non-destructive, direct methods without the use of chemical reagents is a green chemistry that could lead to faster, more productive, safe and cost-effective approach. The optimised wavelength within the visible region is proposed so that detection would be maximised, more sensitive and screening time for evidence search can be reduced. The study looked at if washed or unwashed fabrics had an effect on how blood deposited responded to forensic light and if different detergents caused more degradation

on blood fabrics. The study's findings will be beneficial to initial blood evidence detection and collection at crime scene, or the sample collected from the victim or the perpetrator because it is direct simple test and chemical free techniques.

CHAPTER 2

LITERATURE REVIEW

This chapter discusses about the previous works on the development of blood evidence detection using non-destructive technique especially with the use of light source. Additionally, the chapter also incorporate the basic theories related to blood composition, the fibres used and the lighting principles for further understanding.

2.1 Blood Evidence in Forensic Investigation

Blood is the most frequent and vital biological stain that must be recognised and identified when a crime is committed if it is one of the main evidence in the case. Biological evidence collected at the crime scene must be extensively scrutinised and studied in order to establish a link between the culprit, the crime, and the victim. Evidence left at the crime site by the perpetrator can be used to investigate the crime. These biological evidences are found in bulk or trace amounts at the crime scene in the form of fluids or stains. Blood and other body fluids can link a suspect to a victim, a crime scene, or a weapon in a potentially irrefutable chain of proof by identifying them in a single drop (Gupta *et al.*, 2016).

2.1.1 Blood Composition

Blood is a kind of bodily fluid that transports oxygen and nutrients throughout the body while also transporting carbon dioxide and other waste products back to the lungs, kidneys, and liver for disposal. It protects against infection and aids in the healing of wounds. The composition human blood consists of 55% of plasma, 45% of red blood cells, and less than 1 % of white blood cells and platelets. Figure 2.1 illustrates the composition in blood.

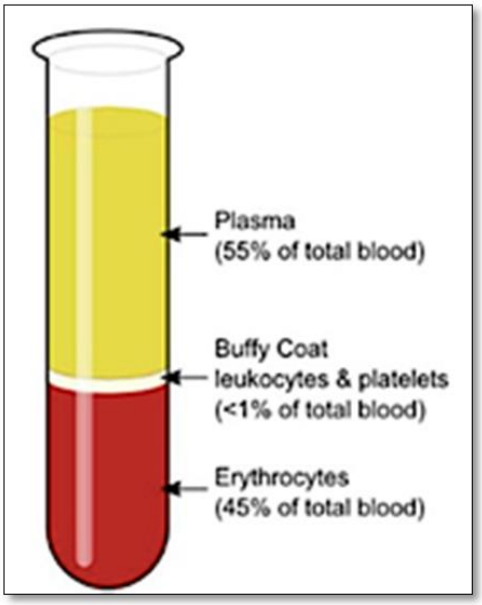

Figure 2. 1:Shows the illustration for blood composition (Kuruc, 2020).

Plasma, a liquid component of blood, accounts for around 55% of the total volume of the blood (Kelly *et al.*, 2018). Red blood cells (RBCs) and white blood cells, as well as cell fragments known as platelets, are suspended inside the plasma.

White blood cells make about 5% of total blood volume and have a lifespan of 1 to 2 days. They are an essential part of the body's immune system, since they combat illness by attacking bacteria, viruses, and other microorganisms. The quantity of white blood cells (WBCs) in the blood is an important measure of health. Platelets are cell fragments that aid in the coagulation of blood and the prevention of bleeding (M. Gupta *et al.*, 2016). To the present time, the use of white blood cell for forensic investigation is not found in any of literature search.

RBCs make about 40% of total blood volume and have a lifespan of roughly 120 days. Haemoglobin, a complex molecule that transports oxygen, eliminates carbon dioxide, and gives

blood its red hue, is found in RBCs. White blood cells make about 5% of total blood volume and have a lifespan of 1 to 2 days. They are an essential part of the body's immune system, since they combat illness by attacking bacteria, viruses, and other microorganisms. The quantity of white blood cells (WBCs) in the blood is an important measure of health. Platelets are cell fragments that aid in the coagulation of blood and the prevention of bleeding (Gupta *et al.*, 2016).

Haemoglobin is a protein found in red blood cells that distributes oxygen throughout the body. It is made up of four globin proteins joined together in four polypeptide chains, two alpha chains and two beta chains Mcdonald (2017). These strands are attached to a heme pigment that is centrally located and contains iron (Figure 2.2).

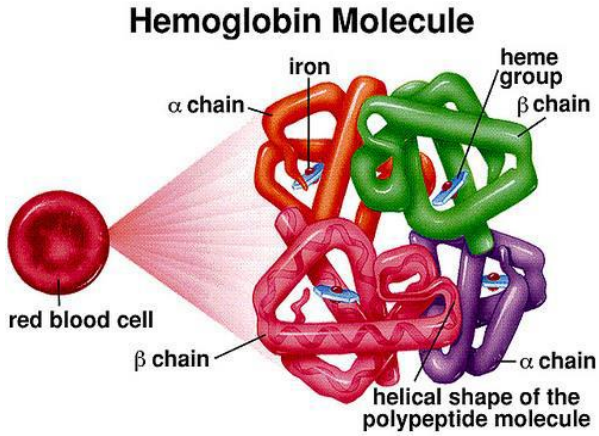

Figure 2. 2: Haemoglobin's molecular structure within a red blood cell, showing the four subunits, each with a haem group, iron atom, and globin chains (Mcdonald, 2017).

The heme component is made up of a ferrous (Fe^{2+}) and four coupled pyrrole molecules called protoporphyrin IX, which is generated when succinyl-CoA attaches to glycine. The iron atom is connected to the globin polypeptide chain by four nitrogen atoms and has two extra links, one to the oxygen molecule and the other to the globin polypeptide chain Marrone and Ballantyne (2009). Under normal conditions, the iron ion present in haemoglobin is ferrous (Fe^{2+}) (Bremmer *et al.*,

2012).The attachment of an oxygen molecule to this structure converts deoxyhaemoglobin to oxyhaemoglobin (Bremmer *et al.*, 2012). Because there are four polypeptide globin chains, each molecule of haemoglobin can transport up to four molecules of oxygen. Carbon dioxide (CO2) and proton concentration (pH) can both affect haemoglobin's affinity for oxygen Bremmer *et al.* (2011); Marrone and Ballantyne (2009). As tissues make and use energy, large amounts of CO_2 and protons are released, raising the pH. When RBCs pass through locations with high CO_2 concentrations, the affinity between oxygen and haemoglobin weakens, and oxygen is liberated (Bremmer *et al.*, 2011).

Blood has always been regarded class evidence under forensic law. In today's world of criminal justice, blood is the most prevalent and crucial evidence. There isn't anything that can replace it. Its presence invariably connects the culprit to the victim. Criminals have tried a variety of inventive methods to hide, clean up, and eliminate blood evidence throughout the years.

2.2 Forensic Examination of Bloodstains

The analysis of bloodstains is a prominent application in forensic sciences during crime scene investigation. Locating blood stain can be challenging especially if it involves minute pin like spatters it may be easily missed or bloodstains on dark clothing especially old stains because blood darken over time. Therefore, the techniques choose to screen for blood is crucial as it can help confirm or exclude the stain as blood or otherwise. There are various techniques to detect blood on fabrics or any other substrate, mainly divided to destructive or non-destructive technique that are chemical methods and forensic light apparatus respectively.

2.2.1 Bloodstain Detection Using Chemicals

Blood may reveal various things about a crime to an experienced analyst in certain situations. The presence of bloodstains on clothes might suggest that it was worn during the bloodletting and help identify a possible victim or offender (James, 2021). Many reagents, such as phenolphthalein and tetramethyl Benzidine, can be used to ritualised suspected bloodstains because they change colour when in contact with peroxides or haemoglobin in the blood (Gupta *et al.*, 2016; Mushtaq *et al.*, 2016). The reagents may give specific reaction which shows emergence of colored reaction or can also luminesces upon reaction with the reagents.

2.2.1.1 Blood Presumptive Test

A presumptive test is commonly performed before a forensic investigator receives a suspicious blood sample. A presumptive test is a qualitative test that validates the presence of a chemical in a sample. Presumptive testing provides an investigator with more information about a questioned stain. An investigator will know that extra tests are needed to identify the stain if a presumptive test yields a positive result. Presumptive tests allow investigators to concentrate on the most important pieces of evidence while avoiding the need to collect non-essential items.

The employed test on the unknown questioned chemical provides a positive result in the form of a colour change. A positive result does not automatically indicate that the sample is blood; rather, it suggests that the stain in question may be blood. Presumptive tests for blood detection in the forensic industry include the Phenolphthalein/Kastle Mayer Test, leuco-malachite green/Hemident, and tetramethylbenzidine/Hemastix.

Other presumptive tests include Luminol, Blue Star, and Fluorescein; however, these are generally used to identify latent blood (Tobe *et al.*, 2007). Chemiluminescence, using chemicals like

Luminol, Fluorescein, and Bluestar, is one of the most used methods (Bluestar is a Luminol derivative that is more strong and less prone to false positives) (Gupta *et al.*, 2016). These substances cause the blood to glow by interacting with haemoglobin. Luminol and Bluestar emit blue light, but Fluorescein emits a green light that requires an ALS to see. These are excellent chemicals to utilise. When a chemical is sprayed onto a bloodstain, the stain's morphology changes because of the additional liquid.

In these presumptive testings, hemoglobin in blood is utilised to respond in presumptive testing. Because these tests are so sensitive, there's a potential an investigator may get a false positive, thus careful attention is needed before one can make final conclusion (Tobe *et al.*, 2007).

2.2.1.1.1 Kastle Mayer Test/ Phenolphthalein Test

The Phenolphthalein Test/Kastle Mayer Test consists of a reagent combination and hydrogen peroxide. The reagent mixture comprises phenolphthalein, potassium hydroxide, deionized water, zinc, and ethanol. The reagent mixture is first applied to a cotton swab that has been rubbed with the questioned biological fluid or to a little amount of the stain. The hydrogen peroxide is then added, and the reaction is observed to see if a colour change occurs. The colour shift is caused by the reduction of hydrogen peroxide, cleavage of oxygen, and oxidation of phenolphthalin to phenolphthalein, as seen in Figure 2.3. A positive presumptive result will be shown by a pink tint, whilst a negative result will not be represented by any colour change (Tobe *et al.*, 2007;Petersen & Kovacs, 2014). When phenolphthalein is oxidised, it gives a pink tint, indicating that the questioned stain is possibly blood. No colour change indicates a poor outcome.

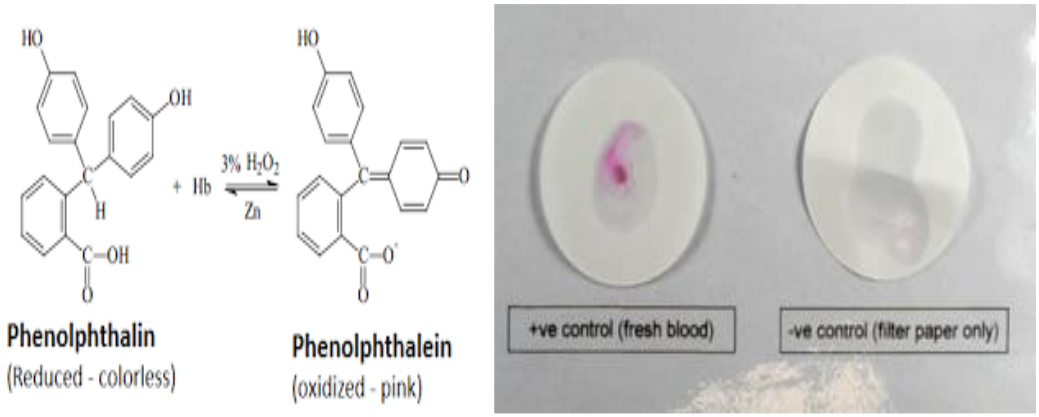

Figure2.3: The reaction of phenolphthalin in the presence of haemoglobin and hydrogen peroxide (Odoardi *et al.*, 2014).

2.2.1.1.2 Hemident Test/Leucomalachite Green

Leucomalachite Green is a reagent consisting of leucomalachite green, glacial acetic acid, distilled water, and zinc dust. The reagent is applied to the stain directly or to a cotton swab containing the reconstituted probable blood stain. After the addition of hydrogen peroxide, the colour shift is visible. If the unexplained stain is blood, a blue-green colour will appear. The blue-green colour is created by the cleavage of oxygen from hydrogen peroxideand the oxidation of leucomalachite green, as seen in Figure 2.4. Hemident is a test kit that detects the presence of blood using leucomalachite green (Petersen & Kovacs, 2014)**.**

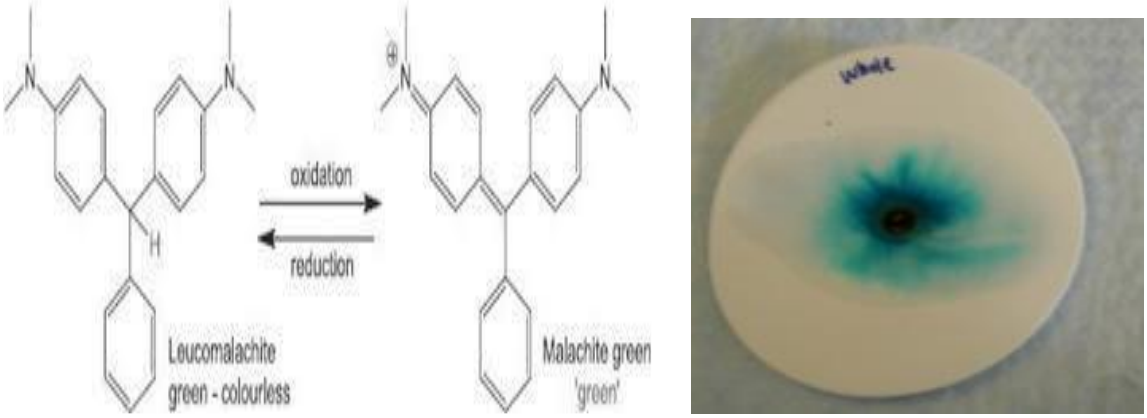

Figure 2.4: Colorless leucomalachite green is oxidised to produce blue-green malachite green, which indicates the presence of blood (Suzanne, 2019).

2.2.1.1.3 Tetramethylbenzidine/Hemastix Test

The Hemastix test consists of a plastic strip with a reagent pad containing 3,3',5,5'-tetramethylbenzidine (TMB) and diisopropylbenzene dihydroperoxide. The test is based on the peroxidase activity of haemoglobin. When heme interacts with the reagent strip, oxygen molecules from hydrogen peroxide are cleaved. As seen in Figure 2.5, this catalytic process will cause the reduced colourless form of TMB to oxidise to the coloured form. As shown in Figure 2.5, a positive result will be a shift in colour from yellow to orange, green, or blue depending on the concentration of blood. A negative result implies that no blood was discovered or that the amount detected was less than the detection limit (Tobe *et al.*, 2007; Petersen & Kovacs, 2014).

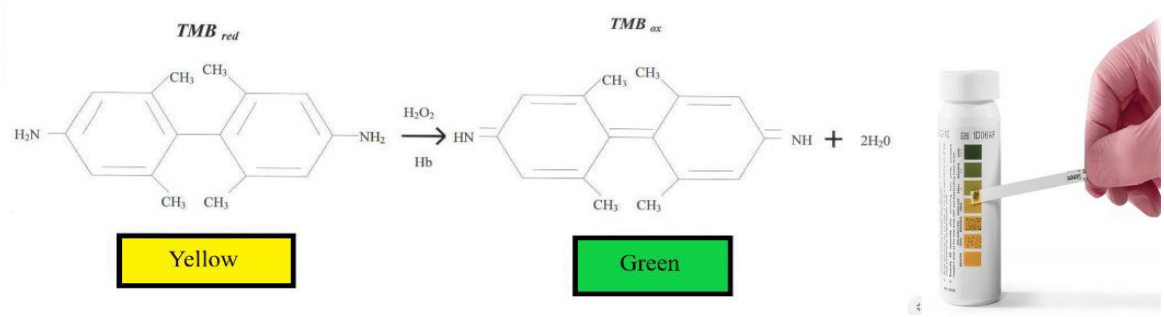

Figure 2.5: TMB catalyst reaction with haemoglobin present. A positive test will be indicated by a colour shift, as shown on the Hemastix test bottle, indicating the presence of blood Suzanne, (2019).

Numerous presumptive blood tests are catalytic, depending on the catalytic action of heme and its derivatives on the destruction of hydrogen peroxide. In these tests, another material is often oxidised in the reaction mixture, causing a colour change or the formation of chemiluminescence (luminol). One of the most used categories of presumptive tests for blood screening is that based on the 3,3',5,5'-tetramethylbenzidine (TMB) colorimetric change in the presence of blood and an oxidant (usually a peroxide) due to the peroxidase-like activity of the heme prosthetic group in the haemoglobin moiety found within red blood cells and its oxidised derivatives, hematin and hemin

(containing Fe3+). The Combur3 Test E, like other catalytic tests for blood detection, detects both the oxidised derivatives of the heme prosthetic group, hematin and hemin moieties (ferric-protoporphyrin group, Fe3+-P) of bloodstains and, to a lesser extent, the heme prosthetic group of fresh blood (ferrous-protoporphyrin group, Fe2+-P). Therefore, the test may detect the presence of both hemolyzed and intact erythrocytes (Vittori *et al.*, 2016).

2.2.1.1.4 Luminol

At crime scenes, one of the presumptive tests used by investigators is luminol. The chemical nature of luminol, its different formulations, and its efficiency in raising blood on diverse substrates have all been studied extensively. It boosts oxidation in an alkaline environment by using the same concept as haemoglobin and its derivatives Virkler and Lednev, (2009). It creates chemiluminescence with a magnificent blue glow when mixed with an oxidising agent such as hydrogen peroxide. At room temperature, Luminol is a yellow crystalline solid. It is photosensitive, flammable, and reacts poorly with strong oxidising, acidic, basic, or reducing chemicals Barni *et al.*, (2007). The stability of light-sensitive Luminol solutions is only 8-12 hours Virkler and Lednev, (2009). Even if the blood has been cleansed or removed, it may be utilised to detect blood traces at a crime scene. The method is based on the iron in haemoglobin and its catalysis in Luminol by hydrogen peroxide. The half-life of luminol fluorescence is 20-40 seconds (Barni *et al.*, 2007).

2.2.1.1.5 Bluestar

Bluestar®, a blood enhancement reagent, is a modified luminol molecule that creates a vivid blue light based on chemiluminescence Blum *et al.*, (2006). Bluestar® tablets (all tablet kit) are frequently diluted in 125 mL deionized water to make a Bluestar® solution. Bluestar®

chemiluminescence does not have the same intensity as Luminol, but it has a longer life span Blum *et al.,* (2006). According to Blum's research (2006), the dilution factor of blood was related to the intensity of light emitted during the reaction. It can detect diluted bloodstains at concentrations of up to 1:1000.

2.2.1.1.6 Fluorescein

Fluorescein is produced by the chemical process of peroxidase-reduction. When fluorescein reacts with decreased colourless haemoglobin and hydrogen peroxide, it swiftly oxidises to fluorescein Cheeseman, (1999). It fluoresces when a yellow or orange filter is employed to activate the reaction product under light with a wavelength between 425 nm and 485 nm. For quite some time, Cheeseman *et al*. (1999) have been researching the efficacy of fluorescein. When fluorescein is applied to non-porous and vertical surfaces, the bloodstain pattern can be altered. Cheeseman, (1999) recommended adding Keltrol RD, a commercial thickener, or xanthan gum, an exo-cellular heteropolysaccharide, to avoid bloodstain washout. Fluorescein has been reported to have a rather limited life duration in its reduced (colourless) form. He also indicated Cheeseman's study the fluorescein reaction lasts for several minutes before the blood spatter pattern disintegrates, allowing photography to capture the stain.

Although there are several advantages to employing the Fluorescein method, there are a few caveats that scientists should be aware of. These same factors can relate to working with Luminol and/or any other presumptive test. Fluorescein must be utilised in a dark environment with an ALS, FLS at 420nm to 485nm, or a powerful UV source (Luminol or Blue Star does not require an external light source). Another caution is the potential for hazardous waste while applying and/or spraying any chemical product. The fluorescing response lasts just five to seven minutes and may not be reproducible with any degree of clarity. However, some research has

indicated that after the treated regions are dry, the Fluorescein and hydrogen peroxide may be reapplied, although the reaction time is reduced. One of the most significant advantages of this approach is its excellent sensitivity and specificity. Another benefit is its ability to diagnose problems quickly. The biggest disadvantage is that not all chemicals fluoresce (Ricardo, 2011).

2.2.1.2 Blood Confirmatory Tests

After a positive presumptive test result, a confirmatory blood test must be performed because confirmatory tests support a presumptive test. These tests are only carried out if presumptive test is positive. Confirmatory tests are more selective and have a lower rate of false positives. Most often, confirmatory tests are done on blood before submitting it to the DNA lab to ensure that it is human. By accurately identifying the specific unknown component, confirmatory tests eliminate the potential of a false positive. Confirmatory tests are more precise in identifying the questioned stain, allowing them to ascertain whether it is blood and whether it belongs to a human. Confirmatory blood tests used in the forensic field include the Takayama and Teichmann tests, Precipitin, ABA Card HemaTrace, HemeSelect, Hexagon Occult Blood Testing Immunocomplex (OBTI), and Rapid Stain Identification-Blood (RSID-Blood) (Odoardi *et al.*, 2014; Stewart *et al.*, 2018).

2.2.1.2.1 Takayama and Teichmann Examinations

Takayama and Teichmann tests detect blood by producing crystals when their specialised reagents react with haemoglobin. The Takayama test generates pink, needle-like hemochromogen crystals when haemoglobin reacts with a reagent made of glucose solution, sodium hydroxide, and pyridine (Figure 2.6). These crystals occur when sodium hydroxide liberates heme, heme iron is

reduced by glucose, and pyridine interacts with the reduced product to produce ferroprotoporphyrin. The formation of homochromogen crystals iobserved under a microscope (Gupta *et al.*, 2016).

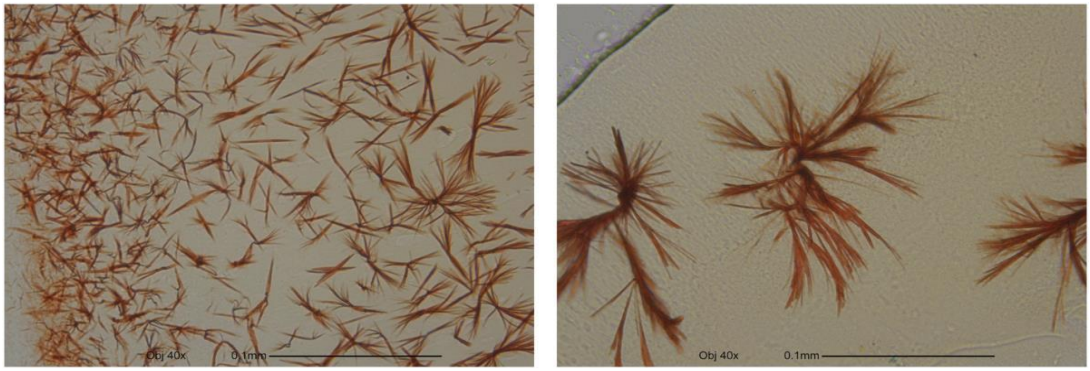

Figure 2.6: Homochromogen crystals generated by the interaction of haemoglobin with the Takayama reagent as seen under a microscope (Stewart *et al.*, 2018).

When haemoglobin combines with a reagent made up of potassium chloride, potassium iodide, and glacial acetic acid, brown ferroprotoprophyrin crystals (Figure 2.7) develop. Hemin crystals, which are iron-containing porphyrins, are formed when the process is heated. The crystals must be examined under a microscope, just like the Takayama test. Despite the fact that the Teichmann and Takayama tests are blood confirming tests, they are not species specific (Gupta *et al.*, 2016).

Regarding the sensitivity and reliability of both microcrystalline tests, the Teichman test is very sensitive to under-and over-heating. On the contrary, the Takayama test is very sensitive in detecting bloodstains where the minimum detection limit is as little as 0.001 ml of blood or 0.1 mg of hemoglobin (WSP, 2021). This results in the Takayama test being capable of providing positive results even for older bloodstains, whereas the old stains could not be detected using the Teichmann test. Furthermore, the Takayama test can also give positive results in blood removed from leather surfaces, which could not be done using the Teichmann test either. Hence, the Takayama test is often viewed as more reliable than the Teichman test. The sensitivity and reliability of the Takayama test is also supported by the study of Samantha & Almalik, (2019), where it was found that it could still

develop haemochromogen crystals for blood despite being exposed to water decomposition media for 30 days. This will be very useful for evidence that has been disposed of into waters, such as murder weapons thrown into a river, for instance. Therefore, the Takayama test is generally preferred over the Teichmann test due to advantages such as less heat sensitivity, ease of usage, and a wider variety of stain compatibility (Virkler & Lednev, 2009).

Figure 2.7: Hemin crystals generated by the interaction of haemoglobin with the Teichmann reagent, as seen under a microscope (Sengupta, 2012).

2.2.1.2.2 Confirmatory Tests for Specific Species

Confirmatory species-specific tests can be performed to determine whether the stain in question is blood and belongs to a human. Some typical species-specific confirmatory tests are Precipitin, ABA Card HemaTrace, HemeSelect, Hexagon OBTI, and RSID-Blood.

A human anti-serum is used in the Precipitin test. Human blood is injected into a rabbit to develop rabbit serum that is sensitive to human blood. The human antiserum is added to the suspect blood and precipitation is observed. If the mixture precipitates, the blood is assumed to be of human origin(Spear & Binkley, 1994; Schotman *et al.*, 2015).

The ABA Card HemaTrace, HemeSelect, Hexagon OBTI and RSIDBlood tests are immunological tests. The ABA Card HemaTrace, HemeSelect, and Hexagon OBTI tests detect human hemoglobin, and the RSIDBlood test detects human glycophorin A (Turrina *et al.*, 2008). These tests use monoclonal antibodies against human hemoglobin. When human hemoglobin reacts with these antibodies, the reaction migrates to the test zone and is indicated by a colored line. The test zone has two columns, one for the test sample and one for the control. A positive test is represented by two lines, one in the control "C" column and one in the test "T" column, and a negative test is represented by a single line in the "C" column, as shown by in Figure 2.8 (Spear and Binkley,1994; Johnston *et al.*, 2008).

Figure 2.8: Positively and negatively findings from an RSIDBlood test. A positive test will result in a line in the control "C" column and a line in the test "T" column, as seen on the left. A negative test is shown by a single line in the C column, as seen on the right (Stewart *et al.*, 2018).

2.2.2 Bloodstain detection using non-chemicals

Blood detection can also be accomplished with forensic light source. The underlying principle of utilising ALS to detect blood is based on how matter interacts with electromagnetic radiation such as light deposited on a surface. Absorption, reflection, and transmission are the three ways matter can interact with the source light. If an absorption contact occurs, the absorbed energy can be dissipated by the material using illumination such as fluorescence. Fluorescence occurs spontaneously in bodily fluids such as sperm and saliva. Many fabrics may glow in the field of tracing, allowing examiners to distinguish between different fibres. Blood absorbs light from 300nm

to 900nm, which is longwave UV (less than 400nm), or UVA (315nm to 400nm), across the visible spectrum (400nm to 700nm), and all the way to what is known as near IR (400nm to 700nm) (700nm to 900nm) (Stoilovic,1991; Lee et al., 2013). Alternative light source (ALS) detection is one of the most basic and non-invasive methods of detecting latent blood at a crime scene. Some of the light sources used or have been utilised at the crime scene include ultraviolet light, Polilight, Luma lighting, Spectrum 9000, and infrared.

2.2.2.1 Non-Chemical Bloodstain Detection-Forensic Light

FLS have been used as an aid to crime scene and exhibit inspection for many years since they can help in the recovery of biological fluids and other traces of evidence. When illuminated with the appropriate wavelength of light, body fluids such as sperm, saliva, and urine absorb light and re-emit it as light at a longer wavelength, a process known as fluorescence. Using the various wavelengths offered by different FLS, Evidence Recovery (ER) searches exhibits for biological stains as one of its primary tasks. The majority of the reported FLS detection techniques were detected with the naked eye. However, despite using the same FLS and goggles, various examiners could see different colours of stains (Lee & Khoo, 2010).

Several forms of forensic light sources have been reported to help in the identification of crime scene evidence using the human eye for direct visualisation. Detection of blood is particularly hard if the stain is latent or if the stain deposited on dark substrates. Thus, non-chemical detection of blood although some may say it is an alternative to the existing blood detection methods, most of the time is actually a complementary approach that is useful for screening of blood stains.

There are several types of forensic light source apparatus for the detection of biological fluids, including blood and other trace evidence. Trace evidence fragments such as hairs, fibres, glass, and paint, tool marks and footwear imprints, drug residues, ballistics, and GSR. Forensic

examinations can benefit from powerful narrowband light that is supplied from this forensic light apparatus.

Multiple wavelength light sources, such as Polilight®,Poliray® and Lumatec Superlite 4005, have been documented in the literature for use in forensic applications (Karchewski *et al.*, 2014).They are made to be portable, has high-intensity, filtered light sources that are widely utilised in crime scenes. There is a lamp (mercury, xenon, or metal halide arc lamp) inside these light sources that produces a wide wavelength of light (UV, visible light, and IR) and a bandpass filter that produces different narrow wavelengths of light. In addition, Vandenberg and Oorschot (2006) reported on a test of the Polilight® model, the PL500, on blood, sperm, urine, and saliva, and found that when seen through appropriate goggles, it exhibits positive detection for all types of tested stains.

Another example is the Crime-lite, which is a handheld, high-intensity alternative light source used primarily by forensic investigators to detect evidence such as fingerprints, bodily fluids and latent evidence from crime scenes. The Crime-lite was one of the first commercially available alternative light sources to benefit from LED technology. LED light sources offer many advantages over previous technologies including durability and lifespan. There are several models of Crime-lite currently available including the Crime-lite 2, these FLS, although they come at a steep price however it is compensated with the green chemical technology which is safer to use, able to use repetitively and more environmental friendly (Karchewski *et al.*, 2014). However, previous research have only examined the efficacy of different wavelengths for crime lite in detecting blood on dark fabrics by diluting blood and then placing it on the test surface (Sterzik *et al.*, 2016; Lee *et al.*, 2013;Vandenberg and Oorschot, 2006).