Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Babylon
College of Science
Department of Biology

Detection of Human Blood Stain in crime scene Using Digital Image

A Research Papers

Submitted to the College of Sciences/University of Babylon

in Partial Fulfillment of the Requirements for the Degree of Higher Diploma in Sciences/ Forensic Evidence

By

Basim Abd Alkarim Helal Salih

B.Sc. Biology - 2015 University of kirkuk

Supervised by

prof. dr. Hassan Fadhil Naji Allawi

2021 A.D. 1443 A.H

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

(قل بفضل الله وبرحمته فبذلك فليفرحوا هو خير مما يجمعون)

صدق الله العظيم

سورة يونس الاية ((58))

Certification

I declare that this study was completed under my supervision at the University of Babylon's Department of Biology, College of Science, as part of the degree of higher diploma in forensic evidence.

Supervisor

Prof. Dr. Hassan Fadhil Naji Allawi

Date: / /2021

In light of the available recommendations, I submit this thesis to the examination committee for discussion.

Assistants

Asst.Prof. Dr. Adi Jasem Abd Al-Rezzaq

Head of the Biology Department of the University of Babylon's College of Science

Date: / /2021

Committee Certification

We, the examining committee, certify that we have read the thesis entitled **(Determination of Human Blood Stain in Crime Scene**) and examined the student (**Basim abdalkarim helal halh**) in its contents and that our opinion it is accepted as a thesis for a degree of high diploma in forensic evidences with (**Excellent**)degree.

Signature:
Prof.Dr.Hassan Fadhil Naji Allawi Abdullah
Depart of Biology
College of Science
University of Babylon
Date: / /2021
(Member and Advisor).

Signature:
Prof.Dr.Ali Hmood Mohaisin
Depart of Biology
College of Science
University of Babylon
Date: / /2021
(Chairman)

Signature:
Asst. Prof.Dr. Mona Najah Hassan
Depart of Biology
College of Science
University of Babylon
Date: / /2021
(Member)

Approved by the College Committee of Graduate Studies.

Signature
Prof.Dr. Enass Mohammed Al-rubaie
Dean of College of Science Babylon University
Date: / /2021

Dedication

This thesis is devoted to the quiet evidentiary voice of blood poured by victims of criminal explosions, where the real tales behind many fatalities may go unreported.

Acknowledgements

After "God," who assisted me with my investigation, I'd want to express my gratitude for his support in pursuing my research.

My deepest thanks to my supervisor Prof. **Dr. Hassan fadhil Naji** for his words of wisdom , support, knowledge and experience throughout the duration of this work. He was the best help I could imagine.

I want to thank and all staff of Department of Biology.

I want to thank all staff members of the high diploma program who taught me the advanced concepts of Forensic Science.

I would thank **my mother,father** and my **wife** for all of their support and encouragement over the years. Many thanks to all those whom helped me pursuit this study even with a small help.

Summary

The determination of bloodstain age can link the bloodstain to the crime, exclude a bloodstain as being irrelevant to the crime, approximate the time since the event has occurred, and collaborate eyewitness accounts. However, estimating the age of bloodstain is still a problem in actual forensic science practice. In this study, we used digital image analysis of bloodstains to estimate the time since deposition. This method was performed under different controlled conditions, i.e. with different donors, substrate materials, humidity, light exposure, anticoagulant and temperatures to determine the effects of each factor on the age estimation process. were compared. The environmental effects – temperature, humidity, light exposure, and anticoagulant – on the bloodstain age estimation process were explored. The color values from the digital images were extracted and correlated with time since deposition. Magenta had the highest correlation and was selected for further studies. The Iphone 8plus was the most suitable smartphone as its magenta decreased exponentially with increasing time and had highest repeatability (low variation within and between pictures). Moreover 83% of mock casework samples were correctly classified. No significant within-person and between-person variations was observed. However, the camera, temperature, humidity, and substrate color were influenced the color change of magenta and thus they affected the age determination process. Further improvements to the process could be achieved by including the environmental factor in the prediction equations.Our technique provides a cheap, rapid, easy-to-use, and truly portable alternative to more complicated analysis using specialized equipment, e.g. spectroscopy and HPLC. With proper lighting and controls, the method has the potential to be used in crime scenes directly.

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List of Abbreviation

AFM	Atomic Force Microscopy

CSV	Comma Separated Values
CMYK	Cyan Magenta Yellow and Key
DSLR	Digital Single Lens Reflex
EPA	Enviromental Protection Agency
HSL	Hue Saturation Lightness
HSA	Hue Saturation Value
NPs	Nanoparticles
RGB	Red Green Blue

Chapter One

Interoduction

Introduction

Hairs, saliva, and blood are among the most frequent types of evidence found at a crime scene. Bloodstains are important evidence in violent crimes such as murder, hit-and-run, and assaults (Jerry *et al*. 2011). In forensic science, blood is examined to determine a range of facts, including a narrative of what occurred (blood pattern analysis). While DNA testing may aid At a crime scene, suspects and victims are identified.blood pattern analysis can aid in the chronology of the case (Liu *et al*. 2006). Furthermore, knowing the age of a bloodstain may aid in the connection of a bloodstain to a crime or the exclusion of a bloodstain as innocent. estimate the time since the event happened and integrate eyewitness comments that are unrelated to the crime. Calculating in forensic science practice, the age of a bloodstain, on the other hand, remains a problem. Several methods have been used to determine the age of bloodstains. (Schwarzacher 1930, Miki *et al*. 1987, Inoue et al. 1991, Matsuoka et al. 1995, Anderson *et al*. 2005, Strasser *et al*. 2007) No method has been utilized on a consistent basis. Spectroscopy is the most often used technique. (Schwarzacher 1930, Patterson 1960, Kind *et al*. 1972, Bremmer *et al*. 2010) (Botonjic-Sehic *et al*. 2009, Bremmer *et al*. 2010).However, little study has been done on the effect of substrate color and composition, humidity, and temperature on bloodstain age estimates. On the other hand, Miki et al. Hemoglobin levels were tested by hand. This approach has failed miserably. by determining the parameters of their electron paramagnetic resonance (Miki *et al*. 1987). As a result of these mistakes, efforts are ongoing to enhance procedures and decrease error rates, for example, via the use of high-performance liquid chromatography (Inoue *et al*. 1991). The ratio of hemoglobin alpha chain peak areas to heme protein is utilized to create a connection.

between the age of a bloodstain and this technique. Using an oxygen electrode to measure the quantity of oxy-hemoglobin (HbO2) and measuring the pace of RNA breakdown in old bloodstains are two more methods that have been explored (Matsuoka *et al*. 1995). (Strasser *et al*. 2007; Jiang 2012). All of these methods, however, are time-consuming and require the use of costly, specialized equipment, limiting their usage at crime scenes. Only the most current age estimate method based upon reflectance spectroscopy (Hanson 2010) is portable and quick enough to use on the scene, but it is limited to bloodstains on a white backdrop. Looking for color variations in a blood spot is the most basic method for estimating the age of a bloodstain. Bloodstains fade from crimson to brown and are visible to the naked eye (James 1988). The degradation of hemoglobin is the reason for this change. As blood exits the body, hemoglobin saturates. to completely oxy-Hb with oxygen in the environment (HbO2). In the absence of cytochrome b5 reductase, the autoxidation of HbO2 to met-Hb is not reversed, as it is in vivo. Smith and colleagues (Smith and colleagues, 2004). Patterson et al. utilized this characteristic to determine the age of bloodstains by measuring the hue of the stains (Patterson *et al*. 1960). They also found that environmental variables such as light exposure, temperature, and humidity affect bloodstain reflectance spectra. These color changes are anticipated to be measurable in future research. A picture as a digital file of the bloodstains Digital image analysis in a number of color spaces, such like Red-Green-Blue (RGB) and Hue-Saturation-Value (HSV), may be used to extract the color values of the bloodstains (HSV). This method was used by Thai et al. (1989) to investigate the relationship between peach color change and storage time, as well as a semi-quantitative investigation of amphetamine and methylamphetamine (Choodum 2011). Unlike the other techniques,

digital image analysis just needs a digital camera and a computer, both of which are widely accessible and can therefore be conducted at crime scenes, making it a low-cost, easy, fast, and truly portable method.In this research, we used digital image analysis of bloodstains to determine the time since deposition. This procedure was carried out under a variety of controlled conditions, including various donors, substrate materials, humidity, light exposure, anticoagulant, and temperatures, in order to evaluate the impacts of each component on theage. One of the advantages of the Hema Trace ABA card is that it is primate-specific and does not generate false positive results when used with other peroxidases. The ABA card Hema Trace is far more sensitive, fast, and tolerant to a wide range of evidential circumstances than the Ouchterlony method described above, and it only takes 10 minutes to execute and read the data. According to one study (Binkley, 1994), the antigen saturates the antibody, limiting the formation of the (antibody antigen antibody) sandwich required for positive color detection.This is referred to in forensic science as the (high dose hook effect). The blood used had a hemoglobin level of approximately 13 g/dl and was known human blood. To produce successive dilutions of the known blood, the extraction buffer contained in the ABA card Hema Trace kit was utilized. In the required 150 l, each dilution was administered directly to a Hema trace sample (S) well. The findings were read aloud and discussed 10. Minutes of video were recorded. The Ouchterlony double diffusion plate was utilized on the same serial dilution. Each well received 1: 5 of dilution 1: 16 to 1: 65, 536, and the results were read and recorded after 24 hours (Hochmeisteret *et al*., 1999).

Aim of study

This study focused on determining the age of blood stains using a smartphone camera and digital image processing utilizing the Image color mixer application. The objectives are as follows:

1-The purpose of this investigation was to investigate whether digital image analysis could be used to determine the age of bloodstains.

2-To determine whether individual variations have an impact on color change and, as a consequence, age estimate.

3-Evaluate the impact of temperature and substrate materials on the age estimate of bloodstains using digital image analysis.

Chapter Two

Review of Literatures

2. Review of Literatures

2.1 The composition and function of blood

Humans and other animals have blood as a fluid medium throughout their circulatory systems. This system is made up of the heart, which acts as a muscular pump, and the blood arteries, which assist in circulating blood to different parts of the body. Blood is used for a number of things (Andrew *et al*., 2011).It serves as an internal transportation system, carrying waste for excretion as well as nutrients for metabolism. It also helps with body temperature control, disease resistance, and bodily protection. As a consequence of damage, human blood, like that of other animals, is comprised of 55 percent blood plasma and 45 percent cellular material (i.e. blood cells and platelets). Blood plasma, a light yellow fluid made up of 90% water and 10% dissolved components, contains antibodies, enzymes, hormones, blood proteins, waste products (e.g. carbon dioxide), and nutrients such as amino acids and glucose. Drugs (including alcohol) detected in blood plasma may be tested as part of a criminal investigation (Andrew *et al*., 2011).Human blood is a red, opaque liquid that flows easily but is thicker and viscous than water. The unusual hue is due to hemoglobin, a unique iron-containing protein. Hemoglobin brightens in color when saturated with oxygen (oxyhemoglobin) and darkens when oxygen is removed (deoxyhemoglobin). As a result, blood from a partially deoxygenated patient This vein should be avoided at all costs. Blood from an artery that's been oxygenated is darker. Red blood cells account for roughly 45 percent of blood volume, while white blood cells and platelets account for less than 1%.Plasma is a clear, somewhat sticky, yellowish liquid that makes up the body's fluid component. Following a fatty meal, plasma becomes momentarily turbid within the body, blood is constantly fluid,

and turbulent flow ensures both cells and plasma are mixed fairly evenly, (Kara Rogers, 2011).

Biological Properties of Blood

Like other kinds of connective tissue, blood is a connective tissue made up of cells [formed components] and intercellular material [plasma]. Blood transports oxygen, carbon dioxide, nutrients, hormones, waste products, and heat throughout the body while also acting as a barrier against viruses and other outside elements.The brain, liver, and kidneys all depend on a steady flow of blood to stay alive (Vick 1984; Dailey 2001). Blood makes up 4 to 6 litters in the typical adult human body, accounting for 7 to 8% of total body weight (Dailey 2001). The entire quantity of blood in the circulatory system is referred to as the total blood volume. Blood is a one-of-a-kind biological material as well as a complex biological substance (Wonder, 2001).Despite the fact that the components of blood have been discovered and synthesized, nothing currently available performs as well as blood in terms of maintaining life. One of the features associated with blood's uniqueness is its capacity to break down into its basic components. The capacity to form clots and hemolyze, as well as the deformability of its main cellular portion (Vogel, 1996).

2.1.1Blood Components

Red blood cells, white blood cells, platelets, and plasma are the four main component elements of blood. The entire amount of human blood is made up of formed cellular units suspended in a fluid suspension, as previously stated.Blood that has been centrifuged or left standing separates into its cellular and fluid components. Blood-produced cellular

components account for about 45 percent of total blood volume, with plasma accounting for the remaining 55 percent (Vick 1984; Ganong 1991; Boryczko *et al*., 2003).

2.1.1.1 Red Blood Cell

Red blood cells, also known as red cells, red blood corpuscles, haematids, erythroid cells, or erythrocytes (from the Greek erythros for "red" and kytos for "hollow vessel," with -cyte translated as "cell" in modern usage), are the most common type of blood cell and the vertebrate's primary means of delivering oxygen (O2) to body tissues via blood flow through the circulatory system (Da Costa *et al*., 2016). RBCS receive oxygen from the lungs, or fish gills, and release it into tissues by squeezing it through the body's capillaries. Hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the red color of the cells and blood, is abundant in the cytoplasm of erythrocytes. (Kumar *et al*., 2007).The cell membrane is made up of proteins and lipids, and it provides qualities necessary for physiological cell function like deformability and stability when traveling through the circulatory system, particularly the capillary network. Human mature red blood cells are oval biconcave disks that are flexible. They lack a cell nucleus and most organelles in order to maximize hemoglobin storage capacity; they can be thought of as hemoglobin sacksThe sack is made up of a plasma membrane. Every second,about 2.4 million new erythrocytes are formed in humans. Macrophages recycle the cells after they originate in the bone marrow and circulate throughout the body for around 100-120 days. Red blood cells make up approximately a quarter of all cells in the human body, and each circulation takes around 60 seconds (one minute). Pierigé *et al*., 2008; Blom, 2003).Hemoglobin is the main determinant of blood color in animals. Each molecule contains

four heme groups that interact with other molecules to alter the color of the molecule. Arterial and capillary blood are bright red because oxygen gives the heme group in vertebrates and other hemoglobin-using animals a rich red hue. Deoxygenated blood appears as a deeper red hue in veins after blood donation and when venous blood samples are taken. Because the spectrum of light perceived by hemoglobin in the oxygenated and deoxygenated states varies, this is the case (Kienle *et al*., l996).Carbon monoxide poisoning results in bright crimson blood. Cyanosis is a symptom caused by illnesses that change the heme groups in hemoglobin, which causes the skin to become blue. Met-hemoglobin is formed when heme is oxidized, and it is more brownish and incapable of carrying oxygen. Sulf hemoglobinemia is a rare condition in which arterial hemoglobin is only partially oxygenated and looks dark red with a blue tinge. For a number of reasons, veins near the skin's surface look blue. There are, however, a few things to think about.This shift in color perception is caused by both the skin's light scattering properties and the visual cortex's visual input processing. Due to an accumulation of waste product, skinks of the genus Prasinohaema exhibit green blood. biliverdin, rather than the true color of the venous blood (Austin and Perkins, 2006).

2.1.1.2 White Blood Cell

WBCs, also known as leukocytes or leucocytes, are immune system cells that help defend the body against infectious illness and external invaders. Hematopoietic stem cells, which are multipotent cells found in the bone marrow, are the source of all white blood cells. Leukocytes may be found in thecirculation and lymphatic system, as well as other areas of

the body. White blood cells, unlike nucleated red blood cells (RBCs) and platelets, contain nuclei. White blood cells are divided into many types. White blood cells are divided into neutrophils, eosinophils (acidophiles), basophils, lymphocytes, and monocytes. These categories are identified by their physical and functional characteristics. Monocytes and neutrophils are both phagocytic cells. B cells, T cells, and NK cells are all lymphocytes, and there are numerous subtypes of lymphocytes (LaFleur-Brooks, 2008). Because the number of leukocytes in the blood is usually an indication of illness, the white blood cell count is an important subset of the complete blood count. White cell counts typically range from 4 x 109 to 1.1 x 1010 per liter. In the United States, this is often expressed as 4,000 to 11,000 white blood cells per microliter. In a healthy adult, white blood cells account for around 1% of total blood volume, making them much less than red blood cells. Red blood cells can be seen approximately 40% of the time.However, since immunity is reliant on it, this 1% of blood has a major effect on health. A rise in the number of leukocytes over the maximum limit is referred to as leukocytosis. It's considered normal when it happens as part of a healthy immune response, which happens a lot. It may be abnormal if the condition is malignant or autoimmune. A decrease in white blood cell count below the lower limit is referred to as leukopenia. This is a symptom of an immune system that isn't working properly (Alberts *et al*., 2002).

Types of WBCs

2.1.1.2.1 Neutrophil

Neutrophils are the most common type of white blood cell, accounting for

60–70% of all circulating leukocytes.They are split into two functionally

different subpopulations: neutrophil killers and neutrophil killers (Alberts *et al*., 2002). They protect your body against bacterial and fungal diseases. They are often the first to react to microbial infection, and their activity and death in large numbers result in the production of pus. The most common term for them is polymorphonuclear (PMN) leukocytes, although technically, PMN refers to all granulocytes. They have a nucleus that has three to five lobes that are connected by small strands (Saladin, 2012). As a consequence, the neutrophils seem to have many heads. The grains develop a beautiful violet hue when stained. In wound pus, neutrophils are bacteria-phagocytosing cells that may be detected in large quantities. These cells are unable to replenish their lysosomes (which are needed to breakdown bacteria) after phagocytosing a few infections and perish (Wheater and Stevens, 2002). Neutrophils are the most common cell type in the early stages of acute inflammation. The average lifetime of inactivated human neutrophils in circulation has been estimated to be between 5 and 135 hours using various techniques (Tak *et al*., 2013).

2.1.1.2.2 Eosinophil

Eosinophils account for around 2% to 4% of all WBCs. This number fluctuates during the day, seasonally, and during menstruation. It rises as a result of allergies, parasite infections, collagen diseases, and spleen and central nervous system sickness. They're rare in the blood, but they're abundant in the respiratory, digestive, and urinary systems' mucous membranes. They are experts in parasitic diseases. Eosinophils are the most frequent inflammatory cells in allergic responses. The most frequent causes of eosinophilia include allergies such as asthma, hay fever, and hives, as well as parasite infections. They produce chemicals that kill huge parasites like tapeworms, which are too big for a single

WBC to phagocytize. In general, their nuclei are bi-lobed. The lobes are connected by a thin thread. The cytoplasm is filled with granules that take on a distinctive pink-orange color when stained with eosin (Saladin, 2012).

2.1.1.2.3 Basophil

Basophils are primarily responsible for allergy and antigen responses because they generate the chemical histamine, which causes blood vessels to expand. Because they are the smallest white blood cells (less than 5% of total count) and share physicochemical properties with other blood cells, they are challenging to research (Falcone *et al*., 2000). They've done it. Because basophils produce the chemical histamine, which causes blood vessels to widen, they are mainly responsible for allergy and antigen reactions. They're difficult to study since they're the smallest white blood cells (less than 5% of total count) and share physicochemical characteristics with other blood cells (Falcone *et al*., 2000). They have a blue tint due to many coarse, dark violet granules, making them readily recognizable. The nucleus is bi-or tri-lobed, although it is difficult to detect owing to the vast amounts of granular granules that cover it. They excrete histamine and heparin, two chemicals that assist the body's defenses. Histamine is a substance that causes blood vessels to dilate, increasing blood flow to injured tissue. It also increases the permeability of blood vessels, allowing neutrophils and clotting proteins to penetrate connective tissue more easily. Eosinophils and neutrophils are attracted to an infection site by chemical signals released by basophils. Heparin is an anticoagulant that prevents blood from clotting and increases the movement of white blood cells to a specific location (Saladin, 2012). Many coarse, dark violet granules give them a blue hue, making them easily identifiable. The nucleus is bi-or tri-lobed, but the enormous

quantity of granular granules that cover it makes it impossible to see. They emit two substances that help the body's defenses: histamine and heparin. Histamine causes blood vessels to widen, allowing more blood to flow to damaged tissue. It also improves blood artery permeability, making it easier for neutrophils and clotting proteins to enter connective tissue. Chemical signals produced by basophils attract eosinophils and neutrophils to an infection site. Heparin is an anticoagulant that stops blood from clotting and encourages white blood cells to migrate to a particular area (Saladin, 2012).

2.1.1.2.4 Lymphocytes

Lymphocytes are considerably more common in the lymphatic system than in the blood. Lymphocytes, which comprise B cells and T cells, have a brightly coloured nucleus with an eccentric position and a little quantity of cytoplasm. in the year 2012 (Saladin).

Functions

T cells, which are split into several subgroups, are distinguished by the presence of cyclin Cyclin-Dependent 3. Cytotoxic cells, such as those expressing CD3 and CD, seek for and destroy cells that they perceive to be alien, such as those infected with viruses or cells from another person, such as a donated kidney. T helper cells, which are identified by CD3 and CD4, are required for the most effective generation of particular antibodies by B lymphocytes. In certain cases, however, cytotoxic CD4-bearing cells are clearly visible in response to a range of illnesses. A year ago (Blann and Ahmed). B cells are responsible for the production of antibodies. This is more likely to occur in the United States.

2.1.1.2.5 Monocytes

Monocytes, the most common kind of WBC, perform the same "vacuum cleaner" (phagocytosis) function as neutrophils, but they live much longer because they also convey pathogen pieces to T cells so that they may be identified and destroyed. As a result, an antibody response is induced. Monocytes eventually leave the circulation to become tissue macrophages, which are responsible for cleaning up dead cells and fighting pathogens. Both dead cell debris and aggressive bacteria are too much for neutrophils to handle. Unlike neutrophils, monocytes have the ability to replenish their lysosomal contents and are predicted to have a much longer active life. They have the kidney.

2.1.2.1.6 Platelets

Clot-forming cells are also known as thrombocytes. Platelets are cytoplasm fragments produced by megakaryocytes in the bone marrow that help in the formation of clots. Normal platelets are 1–3 m in diameter and can be scattered or concentrated in the center of cells. Platelets have a 9–12 day lifetime, and the hormone thrombopoietin controls their synthesis (made in the liver). The spleen eliminates platelets that have grown old or damaged. in the year 2013(Agarwal).

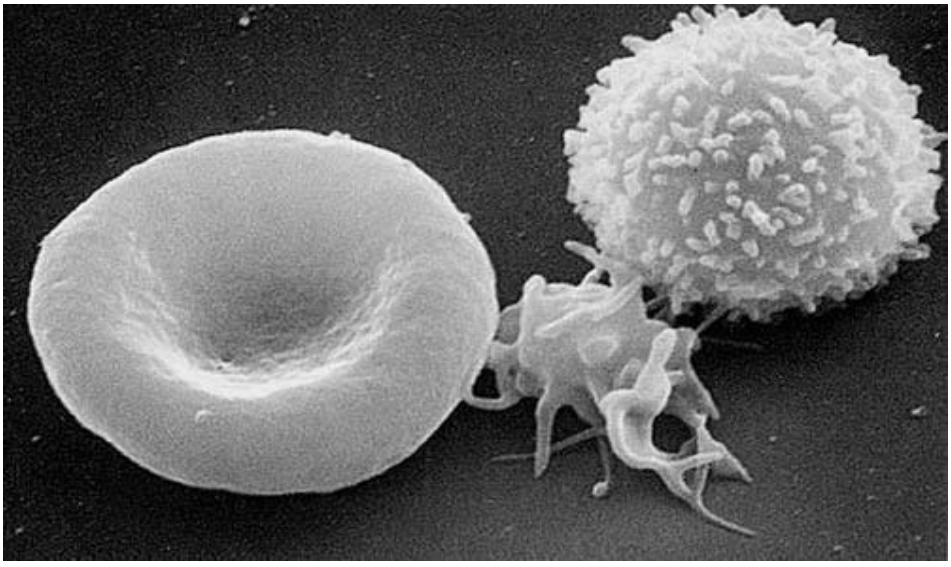


Figure (2.1) RBCs, WBCs, and platelets are blood components that have been formed (Kessel and Kardon, 1979).

2.1.2.1.7 Blood plasma

When the cellular components of whole blood are removed, what is left is blood plasma (Woodcock 1976). Plasma is a straw-colored, semi-viscous aqueous solution made up of 91% water, 1% organic acids, and electrolyte salts, with the remaining 8% consisting of macromolecule proteins such as fibrinogen, globulin, and albumin (Dailey 2001). Albumin is the most significant component of the overall colloid osmotic pressure of plasma proteins, as well as the balance of water metabolism. Fibrinogen, along with other plasma components, is involved in blood clotting and provides groups of carriers for lipids and other water-soluble molecules (Chandran 1992). Normal human plasma volume is around 55% of total blood volume and therefore corresponds to about 5% of body weight or 3.5 liters for a 70kg adult (Ganong 1991). From a rheological standpoint, plasma alone is considered a Newtonian fluid with a viscosity of approximately 1.6 times that of water (Fung 1993; Ciofalo et al. 2002). Only when an anti-coagulant is introduced to plasma does it clot and become a fluid. When the entire blood is allowed to clot and then the clot is removed, the leftover fluid is known as serum. The only difference between serum and plasma is that fibrinogen and a number of other clotting factors have been eliminated (Ganong 1991).

The hematocrit [Hct] or packed cell volume [PCV] is the ratio of the volume of cellular material, mostly RBCs, to the volume of fluid plasma (Vick 1984). PCV is usually reported as a percentage and is an essential indicator of RBC concentration, giving a measure of blood oxygen carrying capability. Adults have a PCV of 38 to 54 percent, whereas babies have a PCV of 55 to 65 percent. A transfusion of RBCs into an adult of average size will raise the PCV by around 3%. Dailey (2001)

defines formalized PCV is the single most significant factor affecting blood viscosity (Bevel and others). Gardne2002;Drochon, 2003; James *et al*., 2005)

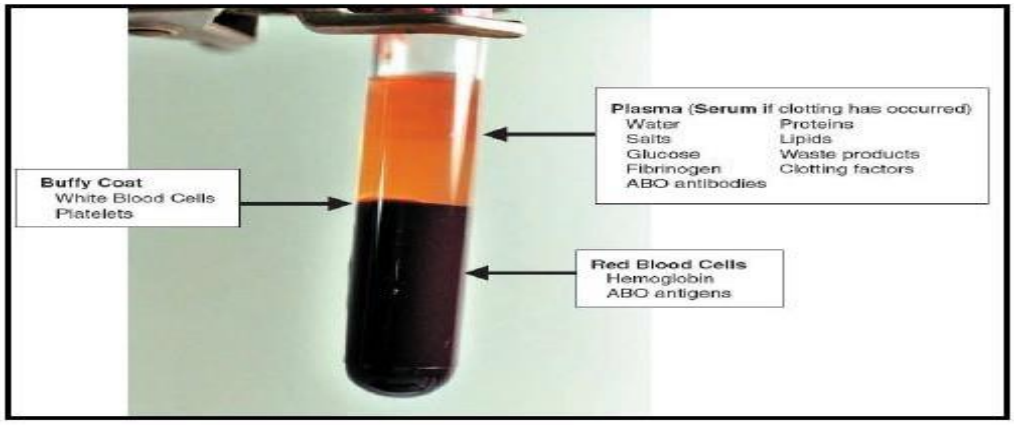


Figure (2.2) Components of whole human blood.

2.2 Physical Properties of Blood

While blood is biologically defined as connective tissue (Dailey 2001), it is also fluid; more specifically, a liquid.A liquid has the capacity to flow freely, hence its shape is bound only by its container. Liquids possess no shape memory and are essentially incompressible like solids (Walker 2000). The characteristics of blood droplet formation and flight and any resulting stains are strongly influenced by a number of physical properties, namely, viscosity, surface tension, and relative density, also known as specific gravity (Raymond.1997). Values for surface tension, specific gravity, and viscosity of water and whole human blood are shown in Table 1.

Table (2.1) Surface tension, specific gravity and viscosity values for water and whole human blood (Giancoli 1991; Wintrobe 1975).

Physical Characteristics	***Water (H2O)***	***Whole Human Blood***
Surface Tension (Nm-1) (37º)	0.072	0.058
Specific Gravity (4ºC)	1.000	1.052 – 1.063
Viscosity (Cp) (37º)	0.695	3.0– 4.7

2.3 Bloodstain Pattern Formation and Description

The formation of an impact spatter pattern needs three precursors, namely: s-an accessible supply of blood in liquid form.

-A transfer of energy into a liquid blood supply to induce the droplet

-like dispersion of its components through the air.

-As well as a nearby surface (s) on which the bloodstains may be placed.

When enough energy is transferred, the physical forces of viscosity and surface tension that hold the blood mass together are overcome (Pizzola *et al*., 1986; Raymond et al., 1996; Gardner 1997), resulting in oscillations and fluid wave fronts within the liquid blood mass (Pizzola *et al*., 1986; Raymond et al., 1996; Gardner 1997) Droplets [impact spatter] of various sizes and speeds detach from the blood source and radiate outward from the region around the energy transfer [impact] point. The deposition of the fluid content of the blood droplets upon contact with a surface creates each unique spattered bloodstain. Individual spattered bloodstains that share a similar force application event and blood source origin are grouped into impact spatter patterns. (Figure 2.3).

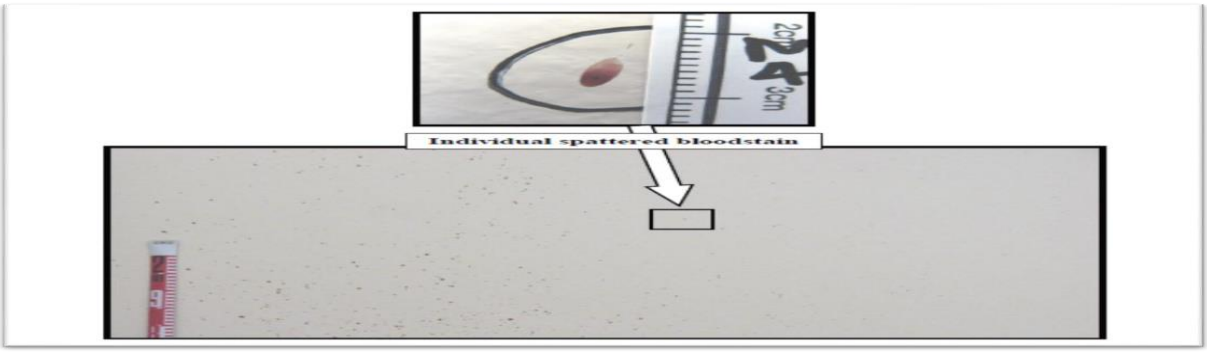


Figure (2.3) Impact spatter pattern (Reynolds M. 2008).

Due to a variety of contributing factors, such as the size and starting velocity of the spatter droplets being a function of the overall energy dynamic inside the impact system, the precise distribution of spattered bloodstains following an impact event may be difficult to anticipate and hard to reproduce (Carter 2001; Raymond *et al*. 2001; Bevel and Gardner 2002; James *et al*. 2005). As a result, the geometric proportions of each individual spattered bloodstain vary depending on the circumstances of the occurrence. The following are some of the factors that may affect the creation and presentation of impact spatter patterns (Raymond *et al*. 2001; Bevel and Gardner 2002; James *et al*. 2005).

- The volume of available blood;
- The magnitude and direction of the impacting force;
- The surface configuration of the implement used to impact the blood source;
- The orientation of the implement at the instant of impact with the blood source;
- The orientation of the blood bearing surface at the instant of impact;
- The orientation of the receiving surface(s) at the instant of spatter

deposition;

- And the movement of the receiving surface(s) at the instant of spatter deposition.

Review the experimental steps

Under controlled circumstances, our method proved very accurate. In actual life, neither the substrate on which the blood will fall nor the surroundings of the crime scene are within one's control. The method suggested in this research was validated via a mock case study. Bloodstains were left on cream leather, fabric, denim, brown flagstone, glossy paper, plastic dishes, shoes, and stainless steel, among other things. These things were strewn around in a room to resemble a normal dwelling. The influences of the environment, such as temperature, light, and humidity, were not taken into account (Appendix B). The data was gathered every 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours for the first 7 days, then once a day for the next 7 days, and once a week for the next 42 days. The results showed that the magenta value varied across all substrates except for bloodstains on fabric (Figures 10 and 11), where the data pattern suggested that bloodstain age could be estimated up to the day the measurement was taken (42 days).The minimal variability between the stains at various time periods for the fabric was most likely due to (1) the white backdrop of the cloth and (2) the fact that the cloth was not directly exposed to sunlight.

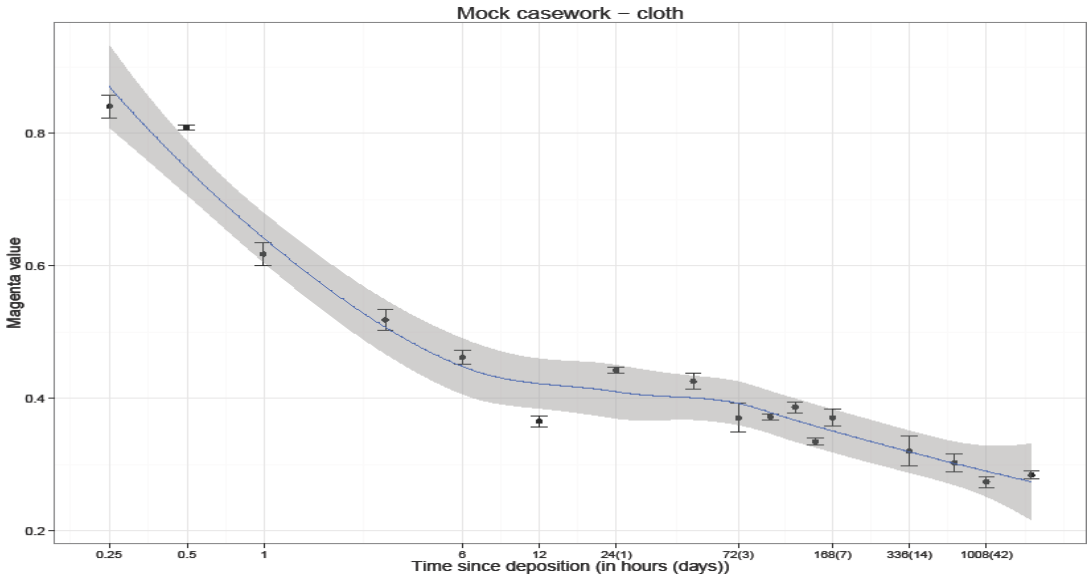


Figure (3.6): Average magenta values from bloodstains placed onto white fabric, with 95% confidence intervals.

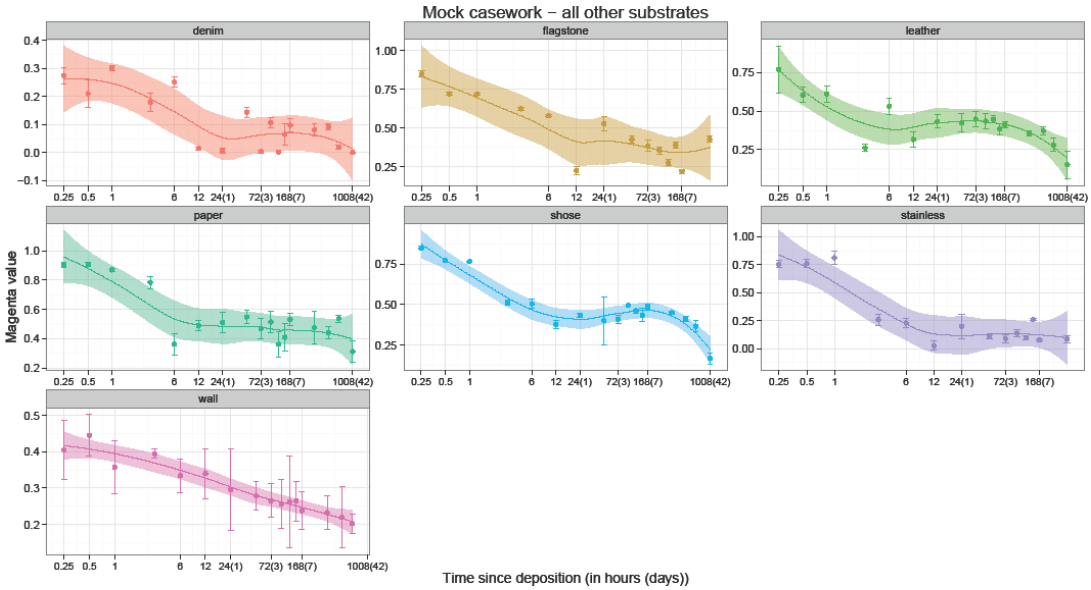


Figure (3.7): Average and 95% confidence ranges of magenta values from bloodstains placed onto different substrates in a mock study.

There was no discernible pattern on the other substrates (3.7) that could be utilized to determine the bloodstain age. This variation may be seen as early as 30 minutes. Each substrate has different variances in M values. The color values achieved were thought to be hampered by

substrate properties, especially the hue of the substrates themselves. When the substrates were dark colors like denim, it was more difficult to measure the change in bloodstain color. Due to the non-porous structure of stainless steel, bloodstains clumped together and had an uneven thickness and color distribution. Because the various substrates were exposed to temperature and humidity in different ways (e.g. substrates that were located closer to the bathroom were probably exposed to higher humidity and vice versa). The denaturation process, such as oxidation of hemoglobin, may be sped up or slowed down by the environment. Before this technique can be used on casework samples, it has to be improved.

Chapter Three

Materials and methods

Materials

3.1. Instrument and Equipment

The Instrument and Equipment used in the present research were listed in table (3-1).

Table (3-1) Instrument and Equipment

Iphone 8 plus	American
Freezer	Germany
Oven	Germany
Magnifying lens	China
Photographic lighting	China
Temperature and humidity sensor	China
EDTA tupe and heparin	jordan

The protocol only needed basic, low-cost photographic equipment. This new approach was simple, quick, and straightforward to deploy. A white photography light with a small desk (3500 cm2 surface area) was illuminated evenly with a Sylvania Osram DULUX S 7-Watt Cool White bulb G32-2 pin base, 500 lumens, 4000K color temperature, (Figure1) with a sensor to measure temperature and humidity and used a magnifying glass to increase focus, An Image J macro was used to do the color analysis, which is a simple script that extracts color values from digital pictures captured using a smartphone camera. The color values were readily measurable, indicating that the color of bloodstains changes over time. The color values were influenced by a number of variables, including the smartphone camera, temperature, humidity, light exposure, enzyme addition, and substrate color.

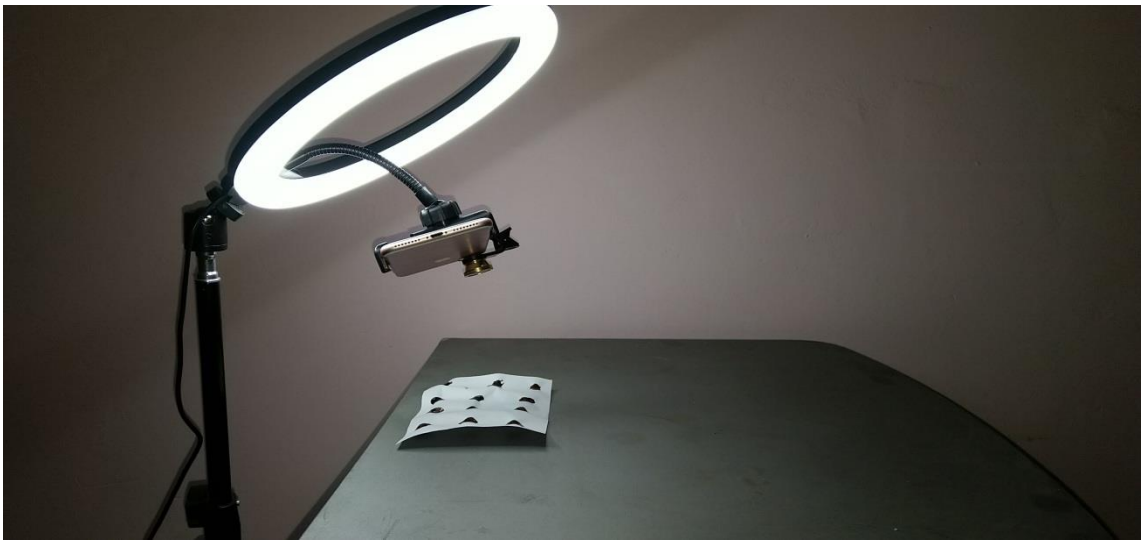


Figure (3-1): A is home-made photographic light system with wooden theater.

3.2 Color value selection

The first experiment was conducted to see whether the color of bloodstains varies with age and, if so, which color changes the most. Four participants, three ladies and one man, provided blood samples. On filter paper, 50 microliters of blood were dropped. Each individual received a total of five bloodstains. Three smartphone cameras (iPhone 8Plus) were utilized to take digital pictures at 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours, then once a day for the next 7 days and once a week for the next 1.5 month (about 42 days). All of the camera settings on the smartphone were set to automatic (white balance, ISO, focusing mode, and metering mode). The smartphone was put on top of a light box that was designed to regulate the quantity of light that entered the room (Figure 1). Each bloodstain was photographed five times. Every digital picture is made up of three 8-bit channels: red, green, and blue. RGB has a range of values from 0 to 255. RGB may be converted to cyan, magenta, yellow, and key (CMYK) color spaces, as well as hue,

saturation, and brightness (HSL). To automate the batch processing, an in-house Image J (http://imagej.nih.gov) macro — a computer script – was used to extract and convert colors (Appendix 1). This allowed for the analysis of a large number of photos and stains at the same time. For additional analysis, the resulting comma-separated-values (CSV) file was imported into Microsoft Excel and the R statistical software.The time since deposition was linearly regressed for each color value from the three color spaces (RGB, CMYK, and HSL). Each relationship's correlation coefficient was then calculated. The correlation coefficient in the statistical linear model is between 0 and 1.0 .The correlation coefficients of highly linked components are close to 1.0. This procedure was carried out in order to find the most accurate predictor of time since deposition. As anticipated, the biphasic change of hemoglobin derivatives was linearized using a base-10 logarithm to linearize the connection between color values and time (in hours) based on fitting a local polynomial regression (LOESS). The color values varied depending on how long it had been since they were deposited. Using linear modeling, M (magenta) and S (saturation) were strongly associated with time after deposition, with R2 values of 0.966 and 0.911 (Table 1). As a result of its strong association with time since deposition, magenta was chosen for future research. The decline in these color values followed a logarithm decay pattern, with a fast decrease at first and a gradual decrease at later time periods (Figure 3). Because the drop in color values in the first hour was even faster than a logarithmic function, the first two time points (15 and 30 minutes) and the time points beyond 6 weeks were omitted from the linear models. Bremmer *et al.* have described the underlying phenomena (Bremmer *et al. 2010*). When blood exits the body, oxyhemoglobin degrades quickly to methemoglobin and hemichrome in the early stages. Because each hemoglobin derivative has a different

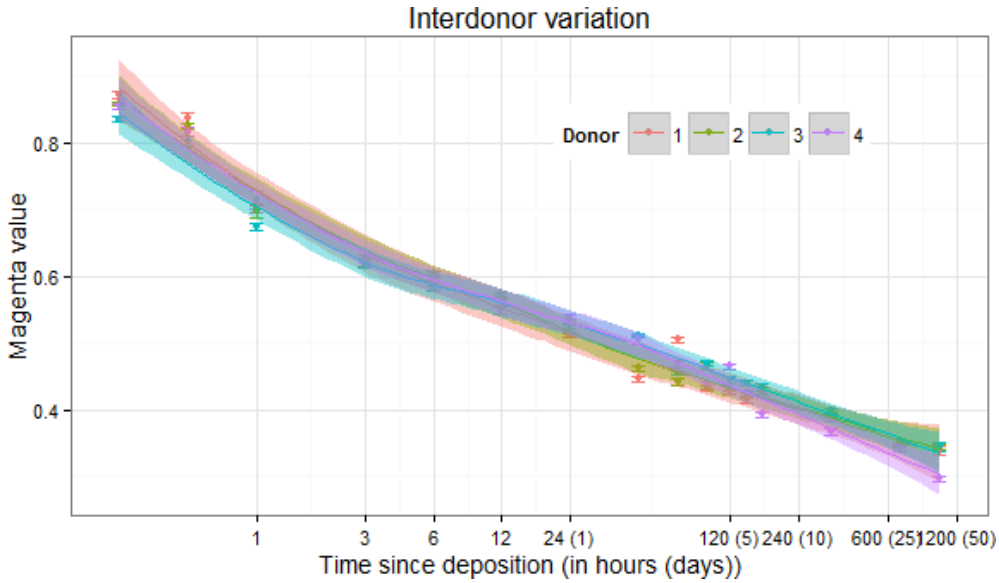
absorption spectrum, the color of the bloodstains is determined by the ratio of three hemoglobin derivatives (oxy-hemoglobin, met-hemoglobin, and hemichrome) in the bloodstain. With time, the proportion of each hemoglobin derivative varies (James *et al*. 1988, Chen and Ikeda-Saito *et al*. 2008, Marrone and Ballantyne 2009). Our findings show that the color shift caused by the denaturation process may be measured using digital image analysis. In RGB terminology, the shift in color of blood from brilliant red to brown is as follows: The difference between red (260,0,0) and brown (170,45,0) is the R channel's reduction and the G channel's rise.

In a regression model, the association coefficient of each color value and the period since deposition

Parameter	**Calibration equation**	**R^2**
R and log time	y = -6.0x+81.6	0.349
G and log time	y = 6.48x+25.6	0.726
B and log time	y = 3.78x+25.6	0.434
C and log time	y = 0x+0	0.000
M and log time	y = -0.119x + 0.688	0.966
Y and log time	y = -0.0843x+0.696	0.896
K and log time	y = 0.0235x+0.680	0.349
H and log time	y = -41.1x+98.9	0.224
S and log time	y = -0.0843x+0.531	0.911
L and log time	y = -0.00392x+0.209	0.026

3.2 Within and between-person variation

person-to- person variation. The volunteers are three females and one male. All were Asian, healthy and non-smoker. Five bloodstains from each person were dropped onto filter paper and kept in a dark room at 25^{o}C. The data were collected at 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, then once a day until 7 days and every week until 42 days. All bloodstains were then analyzed using Iphone 8 Plus. Figure 3 shows the between-person variation with passing time. The main trend observed is the biphasic decrease in magenta value. Only minimal variations were observed within-person, as indicated by the clustering of the magenta values from the five bloodstains of each donor. As for between-person variation, the overlap in the confidence interval of each donor's LOESS fit suggests that there was no person-to-person variation. Previous studies showed similar results with the findings in this study. Anderson *et al.* found an ANOVA value of 0.93 for the ratio (18S: β-actin). Also, for bloodstain age estimation using reflectance spectroscopy, no significant person-to-person variation was found among 12 bloodstains from eight donors (Patterson, 1960). The lack of variation could be explained by the similarity in the amount of hemoglobins, as the volunteers were from the same age group and healthy In summary, the age estimation of bloodstain with digital image analysis has no significant within-person and between-person variations. As such, it could be an appropriate technique to estimate bloodstains age.



Figure(3-2);- Change in magenta values of each donor (N=4) (red represents the first individual, green represents the second, blue represents the third, and purple represents the fourth), as well as 95 percent confidence intervals of magenta values derived from five bloodstains of each donor.

3.3 Age estimation of bloodstains

We assessed prediction accuracy for unknown stains using data from person-to-person variation research. According to a common statistical technique known as one round cross-validation, bloodstains were separated into two subsets (training set and validation set) (Bremmer *et al*. 2010). Data from the training set (70 percent of all data) and the validation set (30 percent of all data) were combined to create a basic linear regression model.was believed to be up to 42 days old. A straightforward method for estimating age using linear regression with magenta value (m) as the predictor for time since deposition in hours (t):

We found that younger bloodstains were more accurate than older bloodstains in our research, which may be explained by the fact that hemoglobin denatures quicker at the start of the aging process (Bremmer *et al*. 2011). (Figure 4). The basic prediction algorithm that simply used magenta values overestimated the actual age of bloodstains. After ten days, the majority of the points displayed are below the line of unity.

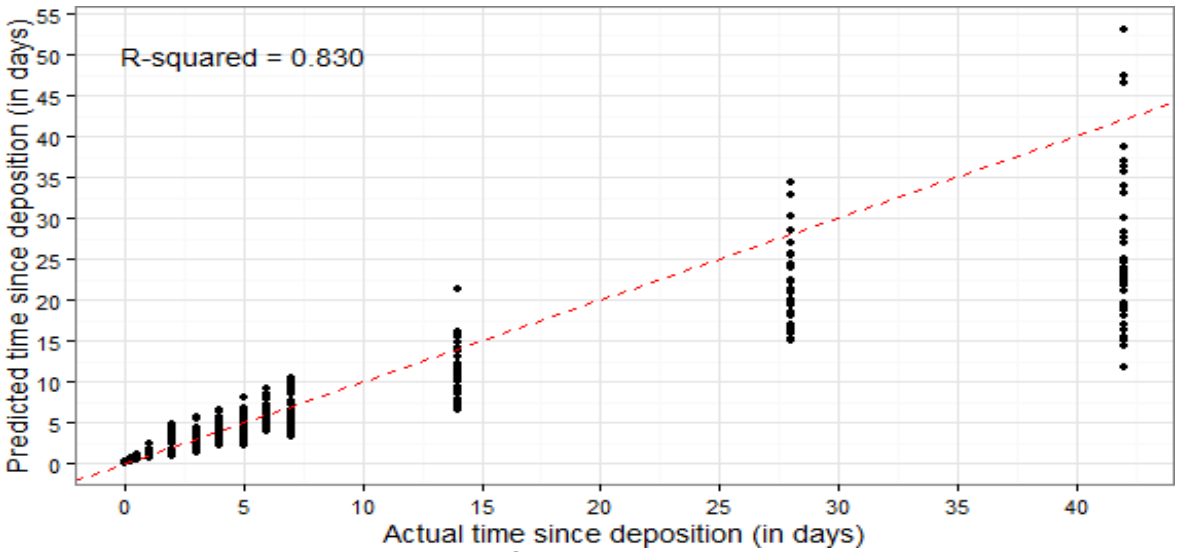


Figure (3-3): Predicted age of bloodstains versus the actual age. Means are shown as black dots. The line of unity is plotted as a dashed red line. The adjusted R- squared of the relationship was 0.830.

As a result, the range of anticipated time since deposition grew as the bloodstain age rose, which is a drawback that must be considered in actual forensic cases. In other words, these findings show that image analysis may be used to estimate age in the near term. Edelman *et al*., who utilized hyperspectral imaging to determine the age of bloodstains, found a similar result. However, Edelman et alapproach .'s is more difficult and complicated than the one suggested here, since the estimate procedure necessitates the use of a specialist instrument. However, additional important variables that influence the hemoglobin degradation process must be studied before image analysis for age estimate of bloodstains may be used in practice. Temperature, humidity, and light

exposure are only a few of these variables. If the impact sizes of these variables could be measured, a smartphone app to assess bloodstain age at a crime scene might be developed.

Chapter Four

Results and Discussion

4. Results and Discussion

4.1 Effects of the environment on the aging process

4.1.1 Temperature

The impact of temperature on digital image analysis was studied. Five bloodstains on filter paper kept at -15 C, 5 C, and 25 C in a dark environment. The data was gathered every 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours for the first seven days, then once a day for the next seven days, and once a week for the last 42 days. For each temperature, the data was displayed as a function of the average magenta value with time since deposition.

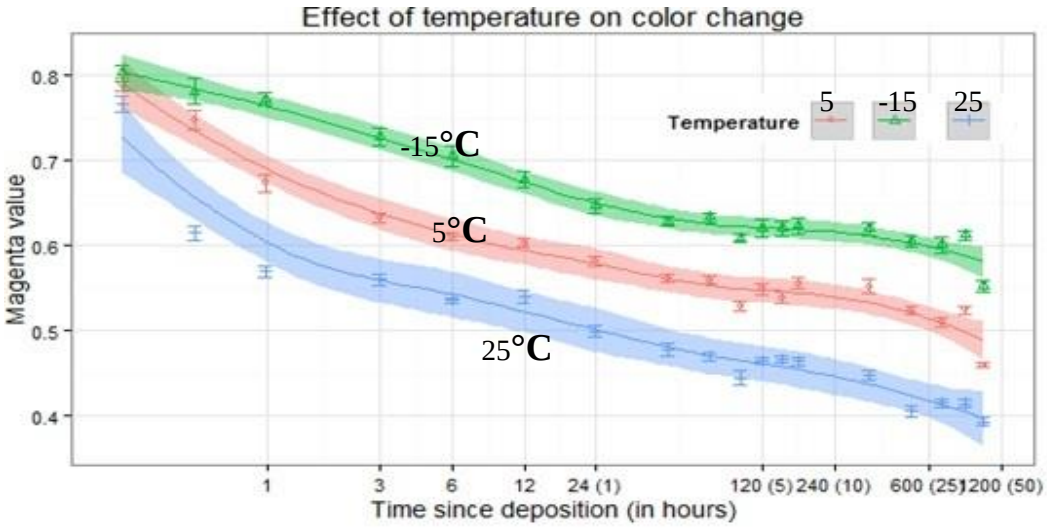


Figure 4.1: Average and 95% confidence ranges of magenta values derived from bloodstains (N= 5 at each humidity level) maintained at -15°C (green), 5°C (red), and 25°C (blue) at different temperatures. The rate of hemoglobin denaturation increased as the temperature rose.

In all temperatures, the early magenta levels (0 hour) were fairly comparable. When the initial measurements were made at 15 minutes, the difference in magenta value became more apparent. The slopes of the

three temperature levels differed, indicating that the rate of change, or slope, of the magenta values rose as the temperature climbed. The slope of the bloodstains was lowest at 15°C and greatest at 25°C. The rate of change for all three temperature levels remained consistent after three hours, as shown by the parallel lines in Figure 5. It may be based on this information.

4.1.2 Humidity

Three bloodstains on filter paper were compared after being kept at three different relative humidity levels: 30%, 50%, and 80% relative humidity. The data was gathered every 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours for the first seven days, then once a day for the next seven days, and once a week for the next 42 days. Figure 6 shows the outcome of plotting average magenta values versus time since deposition. Since the initial measurements, the disparity in magenta values was obvious.

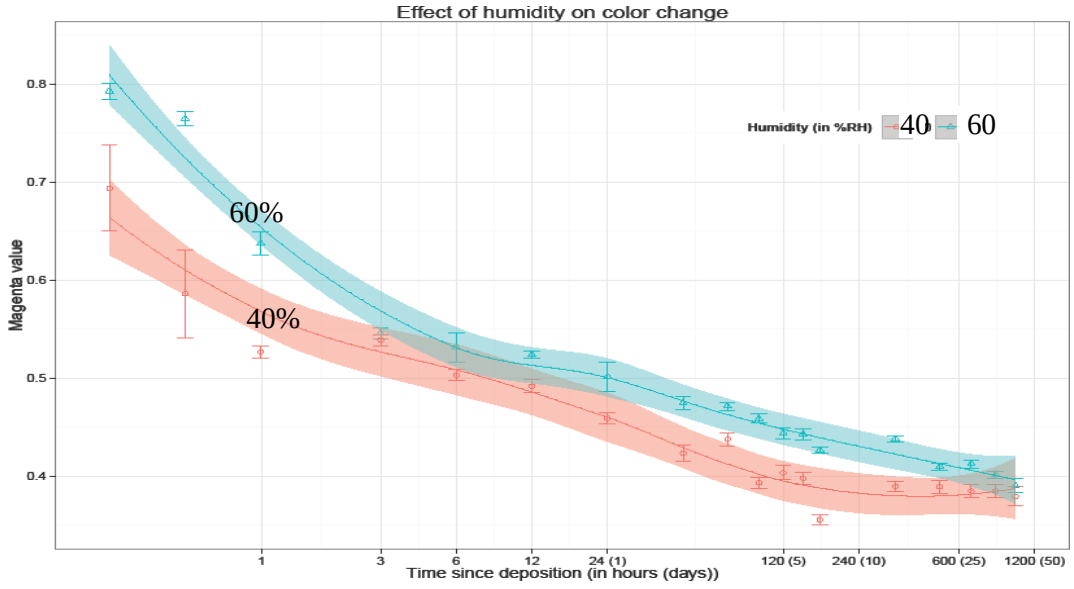


Figure (4.2): Average and 95% confidence intervals for magenta40% (red) and 60% (blue) relative humidity at 35% OC (N = 5 at each humidity level).The color change was delayed by increased humidity.

The magenta value of bloodstains maintained at 40 percent RH declined quicker over time than the magenta value of bloodstains kept at

60 percent RH. This finding is in line with Bremmer *et al*.'s (Bremmer *et al*. 2011) findings on the rate of shift from met-hemoglobin to hemichrome: high humidity accelerated oxidation faster than low humidity. The color shift was observed for two months, and it was discovered that magenta values of both humidity levels fluctuated, which may be due to the smartphone camera's difficulty focusing on old stains. In addition, an in-house humidity chamber made of a foam box, computer fans, and silica gels was used to regulate the humidity level. As a result, between the planned silica gel changes, the humidity level may have varied. Bloodstains kept at 80 percent RH could not be examined due to significant fungal development on the stains.

4.1.3 Light exposure

Three bloodstains were maintained on filter paper in the dark, under fluorescent illumination, and in natural sunshine. The data was gathered every 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours for the first seven days, then once a day for the next seven days, and once a week for the next 42 days. The magenta values' averages were plotted against the period since deposition. When compared to bloodstains maintained in the dark or under fluorescent light, the findings show that exposure to sunshine causes a distinct color change pattern (Figure 7). After 15 minutes, there was virtually little change in magenta levels across the various settings. Bloodstains exposed to sunshine showed lower magenta values after 30 minutes than bloodstains maintained in the dark and subjected to fluorescent light. Bloodstains maintained in the dark and under fluorescent light were unidentifiable after one hour. The hues of the samples exposed to sunlight changed quicker in the early hours (larger drop in magenta value). The rate of

transition from oxy-hemoglobin to met-hemoglobin and met-hemoglobin to hemichrome was thought to be increased by sunshine for two reasons. The temperature of the bloodstains was raised by the sun. Temperature has a positive connection with the rate of color change, according to the prior investigation. Two, light may have accelerated the rate of hemoglobin oxidation (Bremmer *et al*. 2011).

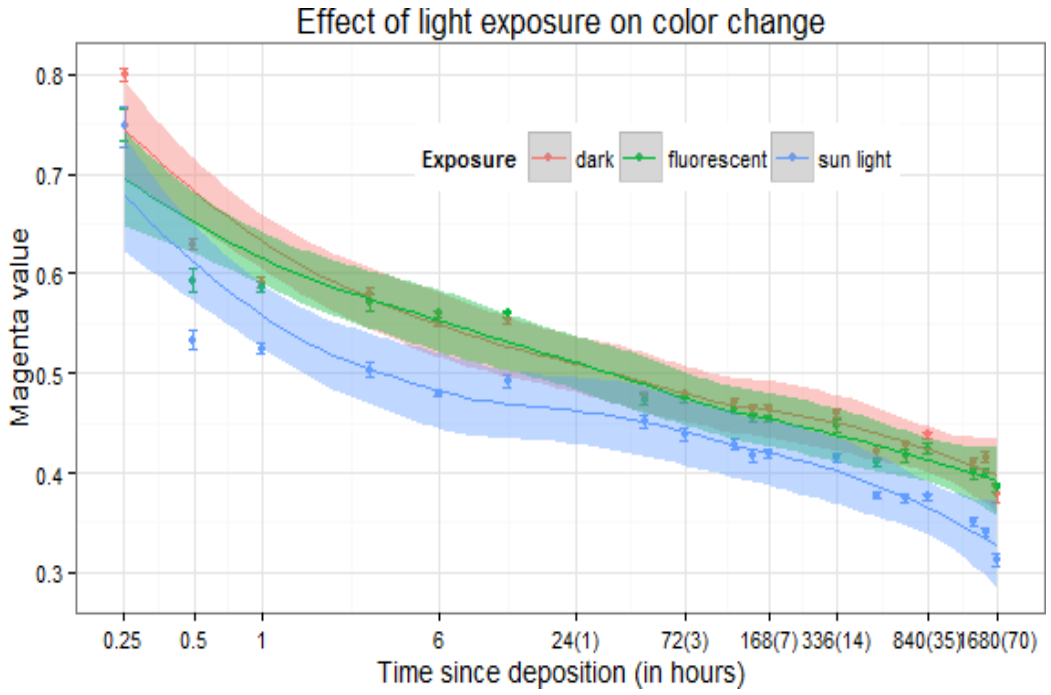


Figure (3.3): Average and 95% confidence ranges of magenta values derived from bloodstains maintained in the dark (red), under fluorescent lighting (green), and in natural sunshine (blue) (N = 5 for each humidity level) (blue). In the dark and under fluorescent light, there was no change in the bloodstains.

4.1.4 Anticoagulant

This experiment compared the change in color of blood with and

without added anticoagulant. Two anticoagulants used to study: EDTA and heparin. All bloodstains were kept at 25^{o}C and the data collected at 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, then once a day until 7 days and every week until 42 days. The averages of magenta value were plotted with time since deposition. The result of the effect of anticoagulant is shown in (3-4).

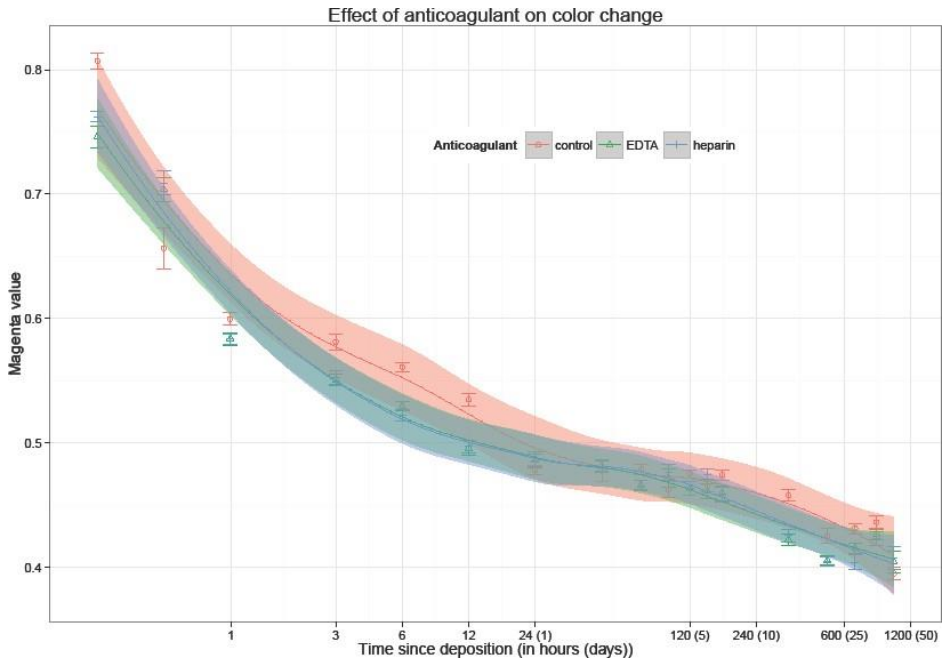


Figure (3.4): Average and 95% confidence interval of magenta values obtained from control bloodstains (red), bloodstains mixed with EDTA (green) and bloodstains mixed with heparin (blue).

Both EDTA and heparin did not affect the magenta value of bloodstains, as shown by the overlapping 95% confidence intervals between control bloodstains and stains mixed with anticoagulants. From Figure 8, only at two time- points (30 minutes and one hour) were slight differences observed. One previous study that investigated anti-coagulant effect on bloodstain color also did not find any influence of anticoagulant on the aging process of bloodstains (Bremmer *et al.* 2010). In general, anticoagulant changes red blood cells shape to spherical. Thus, it was concluded that anti-coagulant did not affect the method for bloodstain age

estimation used in this study. concluded that high temperatures sped up the auto-oxidation of oxyhemoglobin to met-hemoglobin and hemichrome. After 40 days, the data of all temperature levels fluctuated. This time-point seemed to be the limit of bloodstain age that can be analyzed by the technique proposed in this study. The analysis of the effect of temperature was imperative to the bloodstain age estimation process, as the bloodstains in an actual crime scene will also be affected by the ambient temperature at the scene. Knowing the effect of temperature can help a forensic investigator obtain a better estimate of the time since deposition. In Thailand the average temperature is the average temperature in Iraq is 30.6 ± 1.5^{o}C degrees Celsius, this change between seasons on the rate of hemoglobin oxidation.

4.2 The effect of substrate

We have no influence over the location of bloodstains or the kind of substrate on which the blood is discovered at the crime scene. Because the suggested technique relies on the color of the bloodstain, the substrate color may obstruct the measuring procedure. As a result, it is essential to investigate the impact of the substrate. Five bloodstains were dropped on cotton, denim, filter paper, glass, leather, and the wall. All of the bloodstains were kept at a temperature of 25 degrees Celsius in the dark. Data was collected every 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours for the first seven days, then once a day for the next seven days, and once a week for the next 42 days. time since deposition was displayed against the average magenta value. Figure 9 depicts the ability of two substrates (filter paper and cotton) to estimate the age of a bloodstain. Both of the substrates were white. Glass, gypsum walls, and leather, although light in color, reflect more light than filter paper and cotton. As a result, the derived average magenta values were very varied. The measurement of magenta values was further hampered by the somewhat rough surfaces of the gypsum and leather troughs.

Furthermore, bloodstains on denim did not provide enough color diversity. In conclusion, substrate properties influenced the process of measuring bloodstain color and, as a result, the age estimate of bloodstains using digital image analysis. Hanson and Ballantyne's (Hanson *et al*. 2010) technique for removing bloodstains from substrate may be helpful for this procedure.

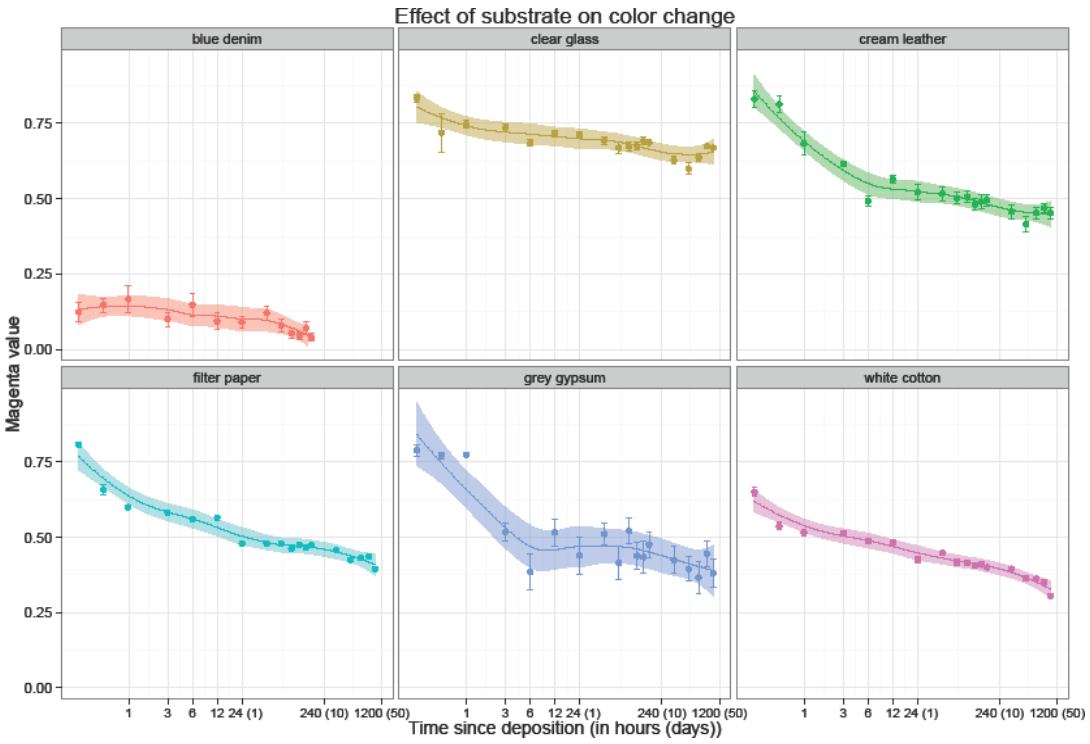


Figure (3.5): Average and 95% confidence intervals of magenta values from bloodstains deposited onto six substrates (blue denim is red line, clear class is brown, leather is green, paper is light blue, gypsum is blue and white cotton is purple).

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الخلاصة

يمكن أن يؤدي تحديد عمر بقعة الدم إلى ربط بقعة الدم بالجريمة ، واستبعاد بقعة الدم على أنها لا علاقة لها بالجريمة ، وتقريب الوقت منذ وقوع الحدث ، وتعاون شاهد عيان. ومع ذلك ، فإن تقدير عمر بقعة الدم لا يزال يمثل مشكلة في الممارسة الفعلية لعلوم الطب الشرعي. في هذه الدراسة ، استخدمنا تحليل الصور الرقمية لبقع الدم لتقدير الوقت منذ الترسب. تم تنفيذ هذه الطريقة في ظل ظروف مختلفة خاضعة للرقابة ، أي مع مانحين مختلفين ، ومواد الركيزة ، والرطوبة ، والتعرض للضوء ، ومضادات التخثر ودرجات الحرارة لتحديد تأثير كل عامل على عملية تقدير العمر. وتمت مقارنة. تم استكشاف الآثار البيئية - درجة الحرارة ، والرطوبة ، والتعرض للضوء ، ومضادات التخثر - على عملية تقدير عمر بقع الدم. تم استخلاص قيم اللون من الصور الرقمية وربطها بالوقت منذ الترسيب. كان لدى اللون الارجواني أعلى ارتباط وتم اختياره لمزيد من الدراسات. كان هاتف Iphone ٨plus هو الهاتف الذكي الذي استخدم حيث انخفض اللون الأرجواني بشكل كبير مع زيادة الوقت ولديه أعلى إمكانية للتكرار (تباين منخفض داخل الصور وبينها). علاوة على ذلك ، تم تصنيف ٨٣٪ من العينات اثناء التجربة بشكل صحيح. لم يلاحظ أي اختلافات كبيرة داخل الشخص وبين الأشخاص. ومع ذلك ، فقد أثرت الكاميرا ودرجة الحرارة والرطوبة ولون الركيزة على تغير اللون الأرجواني وبالتالي أثرت على عملية تحديد العمر. يمكن تحقيق مزيد من التحسينات على العملية من خلال تضمين العامل البيئي في معادلات التنبؤ. توفر تقنيتنا بديلاً رخيصًا وسريعًا وسهل الاستخدام ومحمولًا بالفعل لتحليل أكثر تعقيدًا باستخدام معدات متخصصة ، على سبيل المثال. التحليل الطيفي و HPLC. من خلال الإضاءة والتحكم المناسبين ، يمكن استخدام هذه الطريقة في مسارح الجريمة بشكل مباشر.

وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم
قسم علوم الحياة

الكشف عن بقع دم الأنسان في مسرح الجريمة

رسالة مقدمة الى
مجلس كلية العلوم جامعة بابل كجزء من متطلبات نيل درجة الدبلوم العالي/في العلوم الأدلة الجنائية

من قبل

باسم عبدالكريم هلال صالح
علوم حياة 2015

باشراف

ا.د.حسن فاضل ناجي علاوي

1443ه 2021م