BLOODSTAIN PATTERN ANALYSIS: EXAMINING THE EFFECT OF WIND BLOW ON PASSIVE BLOODSTAIN PATTERN DROPPED FROM DIFFERENT HEIGHTS

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BLOODSTAIN PATTERN ANALYSIS: EXAMINING THE EFFECT OF WIND BLOW ON PASSIVE BLOODSTAIN PATTERN DROPPED FROM DIFFERENT HEIGHTS

by

CHIA SHENG

Thesis submitted in partial fulfilment of the requirements for the degree of Bachelor of Science in Forensic Science with Honours

February 2025

CERTIFICATE

This is to certify that the thesis entitled BLOODSTAIN PATTERN ANALYSIS: EXAMINING THE EFFECT OF WIND BLOW ON PASSIVE BLOODSTAIN PATTERN DROPPED FROM DIFFERENT HEIGHTS is bona fide record of research work done by Mr. CHIA SHENG during the period from October 2024 to February 2025 under my supervision. I have read this thesis and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis to be submitted in partial fulfilment for the degree of Bachelor of Science in Forensic Science with Honours in School of Health Sciences, Universiti Sains Malaysia. This thesis has not been submitted previously for the award of any other degree, diploma, or fellowship and represents the candidate's independent work.

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… …………

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DECLARATION

I, CHIA SHENG, hereby declare that the thesis entitled BLOODSTAIN PATTERN ANALYSIS: EXAMINING THE EFFECT OF WIND BLOW ON PASSIVE BLOODSTAIN PATTERN DROPPED FROM DIFFERENT HEIGHTS submitted to Universiti Sains Malaysia in partial fulfilment for the degree of Bachelor of Science in Forensic Science with Honours, is the result of my own research, unless otherwise mentioned and officially acknowledged. I further declare that this work complies with all ethical guidelines and academic integrity policies of the school and has not been previously or concurrently submitted in its entirety for any other degree at Universiti Sains Malaysia or any other institution. I allow Universiti Sains Malaysia to utilise the thesis for teaching, research and promotion.

………………………………

(CHIA SHENG)

Date: 26 February 2025

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LIST OF SYMBOLS

℃	Degree Celsius
°	Angle (degree)
™	Trademark
®	Registered trademark
cP	Centipoise
%	Percentage
m	Metre
cm	Centimetre
mm	Millimetre
m/s	Metre per second
m/s^2	Metre per second squared
mg	Milligram
K_2EDTA	Ethylenediaminetetraacetic acid dipotassium salt dihydrate
$\pm$	Plus minus
"	Inches
ft	Feet
v	Velocity
g	Gravitational acceleration
h	Height of fall
µL	Microlitre
g/mL	Gram per millilitre
β	Beta coefficient

LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
BPA	Bloodstain pattern analysis
SWGSTAIN	Scientific Working Group on Bloodstain Pattern Analysis
IABPA	International Association of Bloodstain Pattern Analysts
FBI	Federal Bureau of Investigation
RBC	Red blood cell
PCV	Packed cell volume
AOO	Area of origin
AOC	Area of convergence
MLR	Multiple linear regression
3D	Three-dimensional
AI	Artificial intelligence

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ANALISIS CORAK KESAN DARAH: MENGKAJI KESAN TIUPAN ANGIN TERHADAP CORAK KESAN DARAH PASIF YANG DIJATUHKAN DARI KETINGGIAN BERBEZA

ABSTRAK

Analisis corak kesan darah memainkan peranan penting dalam penyiasatan tempat kejadian jenayah dengan membina semula peristiwa yang melibatkan darah serta memberikan maklumat tentang penyerang, mangsa, dan sifat jenayah tersebut. Corak kesan darah pasif membolehkan anggaran ketinggian sumber darah dan hal ini berpotensi untuk dikaitkan dengan ketinggian penyerang atau mangsa. Namun, jenayah yang melibatkan pendarahan di persekitaran luar boleh dipengaruhi oleh faktor luaran yang tidak terkawal dan tidak dapat diramalkan seperti tiupan angin dan hal ini boleh mengganggu tingkah laku biasa darah yang meninggalkan badan seseorang, dan menyukarkan tafsiran yang tepat terhadap bukti kesan darah. Tambahan pula, bilangan kajian yang dijalankan terhadap kesan tiupan angin ke atas corak kesan darah pasif adalah terhad, terutamanya apabila digabungkan dengan ketinggian sumber darah yang berbeza. Kajian ini bertujuan untuk mengkaji kesan kelajuan angin dan ketinggian jatuh titisan darah terhadap peralihan kedudukan dan ciri fizikal kesan titisan darah pasif, serta bagaimana pendedahan berpanjangan kepada tiupan angin mempengaruhi penampilan fizikal kesan darah tersebut. Dalam kajian ini, satu titisan darah telah dilepaskan daripada hujung pipet secara perlahan-lahan menggunakan mikropipet ke atas jubin lantai putih yang licin bagi setiap gabungan kategori kelajuan angin dan ketinggian jatuh. Data yang diperoleh telah dianalisis secara statistik menggunakan regresi linear berganda. Penemuan menunjukkan bahawa kelajuan angin dan kesan gabungannya dengan ketinggian jatuh menyebabkan

peningkatan peralihan kedudukan mendatar (*x*) sehingga 15.70 cm tetapi tidak memberi kesan yang signifikan terhadap peralihan kedudukan menegak (*y*). Pengecutan yang diperhatikan pada kebanyakan kesan darah memadamkan ciri fizikal asalnya. Oleh itu, tiada hubungan ditemui antara kelajuan angin dan ketinggian jatuh dengan lebar, panjang, nisbah lebar kepada panjang, bilangan duri, bilangan kesan satelit, sudut impak, dan sudut gelinciran. Bentuk kesan darah didapati tidak banyak berubah dari sifat sferikalnya, dan tiada arah tuju yang jelas ditunjukkan pada kesan darah apabila titisan darah jatuh secara pasif dari ketinggian 60 cm di bawah pengaruh berbagai kategori kelajuan angin. Kesimpulannya, kajian ini menunjukkan kesan titisan darah pasif yang tidak dapat diramalkan dan mudah terdistorsi di persekitaran luar, serta mencadangkan supaya penyiasat memberi pertimbangan yang teliti terhadap kesan tiupan angin dan ketinggian jatuh titisan darah ketika menangani bukti kesan darah di tempat kejadian jenayah, terutamanya persekitaran luar yang sebenar.

BLOODSTAIN PATTERN ANALYSIS: EXAMINING THE EFFECT OF WIND BLOW ON PASSIVE BLOODSTAIN PATTERN DROPPED FROM DIFFERENT HEIGHTS

ABSTRACT

Bloodstain pattern analysis serves vitally in crime scene investigation by reconstructing the bloodshed events and providing information on the assailant, victim and crime nature. Passive bloodstain pattern enables the estimations of blood source's height which could potentially correlate with the height of assailant or victim. However, a bloodletting crime occurring at outdoor environment introduces uncontrollable and unpredictable external factors such as wind blow which can interfere with the usual behaviour of blood leaving the body, hindering the correct interpretation of bloodstain evidence. Additionally, limited research has been done on the effect of wind blow on passive bloodstain pattern, especially when incorporating with different heights of blood source. This study aims to examine the effects of wind velocity and height of blood drop fall on the position shift and physical characteristics of passive blood drop stain, as well as how prolonged exposure to wind blow affect the stain's physical appearance. In this study, a single drop of blood was gently dispensed from the pipette tip using a micropipette onto a smooth white floor tile under each combination of wind velocity category and fall height. The data obtained were statistically analysed using multiple linear regression. The findings showed that wind velocity and its combined effect with fall height caused increase in the horizontal (x) position shift for up to 15.70 cm but had no significant effect on the vertical (y) position shift. The shrinkage observed on majority of the bloodstains obliterated their original physical characteristics, hence no relationship was found between wind velocity and

height of fall with width, length, width-to-length ratio, number of spines, number of satellite stains, angle of impact, and glancing angle. The bloodstain's shape was found to be not distorted from sphericity much and no directionality was revealed on the stain when blood drop fell passively from 60 cm height across different categories of wind velocity. To conclude, this study demonstrates the unpredictable and distortable passive blood drop stain at outdoor environment and suggests careful consideration of wind blow and height of blood drop fall effects by the investigators when dealing with bloodstain evidence, especially at actual outdoor crime scenes.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

In a crime scene especially those involving violence crime, blood evidence is often found at the site regardless of the presence or absence of victim's body. The presence of blood evidence could indicate the occurrence of bloodshed events and may provide much information related to the crime to the investigators. Typically, the first clue that comes into mind of the forensic investigators which they try to extract from the blood is deoxyribonucleic acid (DNA) evidence. This is particularly true as it can give a big lead in the investigation by possibly suggesting the identity of the victim and/or assailant, genetical characteristics of the victim and/or assailant, and the minimum number of persons present during the crime. In certain instances, the blood sample may be the only source to obtain DNA evidence from the crime scene.

However, it will be an overlook of evidence if the investigators merely extract DNA information from the blood left at the crime scene. A thorough investigation should also analyse the blood evidence physically, or in other words, investigate the morphology of bloodstains formed by the blood and the patterns generated. Collectively, this refers to bloodstain pattern analysis (BPA). BPA, also called blood spatter analysis, is a discipline in forensic science that reconstruct the bloodshed events through examination of bloodstain pattern, including the individual stains that form the pattern.

Although the discipline of BPA has been started and used informally for centuries, the first modern and systematic study only came in 1895 published by Dr. Eduard Piotrowski of the University of Kraków in Poland, titled "On the formation, form, direction, and spreading of blood stains after blunt trauma to the head."

Nonetheless, it was until 1955 when BPA evidence was recognised by the American legal system where the American court first accepted affidavit submitted by Dr. Paul L. Kirk from the University of California at Berkeley on his BPA findings in the case of *State of Ohio v. Samuel Sheppard* (Court of Appeals of Ohio, 1955). Since then, more research on BPA have been done which led to increasingly more recognition and uses of BPA evidence in legal systems.

BPA requires the knowledge of four areas, particularly biology (understanding biological components of blood), chemistry (application of chemicals to detect trace or latent bloodstains), physics (understanding the trajectory of blood droplet flight path), as well as mathematics (determining the area of origin of blood source using trigonometric functions) to interpret and deduce conclusions from the bloodstains. It is important in crime scene investigation in terms of giving clues on the types of trauma suffered, the way or direction the wound is inflicted, types of weapon used, number of blows struck, number and sequence of bloodshed events, positions of victim and assailant during the assault, and many more (Freeman and McManus, 2022; Global Forensic and Justice Centre, 2013). By reconstructing the crime scene and events through BPA, police officers can corroborate statements from witness and thus aid in the criminal investigation process.

The analysis of bloodstain ranges from examination of the overall pattern observed for determination of types of bloodshed event to close-up inspection on individual bloodstain for calculation of blood origin three-dimensional location (area of origin). Various mechanisms have been proposed to explain the formations of different types of bloodstain patterns (listed in Chapter 2.3.2) found at crime scene. Looking at the passive blood drop stain, it yields as important information as those from larger bloodstain pattern. Hence, passively dropped bloodstain required thorough study as well to increase understanding on the various bloodstain distributions at crime scene and their interpretations.

Passive blood drop commonly occurs when there is wound inflicted and bleeding, where the blood drops passively (only due to gravity without any external force) from the body of the victim such as fingertip, or from the weapon used by the assailant like knife tip, forming a single bloodstain. According to Scientific Working Group on Bloodstain Pattern Analysis (SWGSTAIN (2009)), this kind of bloodstain is termed drip stain. But when multiple blood drops are continuously dripping into the same spot forming slight spatter, it is termed as drip pattern. A passively dropped bloodstain could tell the height of the blood source based on its physical characteristics (for example size and shape), which could possibly correlate with the height of the assailant and/or victim (Leonova et al., 2019). As investigators could estimate the height of the blood source, the postures (standing, sitting, kneeling or lying down) of the victim during generation of the passive blood drop could also be determined when combined with other evidence or statements from witnesses (Hahn, 2023). Furthermore, a person who moves while blood is continuously dripping may form drip trail giving the investigators indication on the movement and direction of the person (National Science Exploration Competition (Taiwan), 2021; “Bloodstain Pattern Analysis”, 2013). However, the bloodstain is affected by a number of factors, including the presence and velocity of air current or wind. Thus, it requires vast number of studies.

This study focuses on investigating the effect of wind blow on passive blood drop stain. Wind, or air current, is an uncontrollable environmental variable that may introduce uncertainties or cause alterations to the crime scene and evidence during or post-crime to an unknown extent. This is especially true at outdoor crime scene, which makes investigation and analysis of evidence, including bloodstain pattern, harder for the investigators. Hence, more research is needed to examine the degree and pattern of wind blow effect on different bloodstain patterns with different parameters. This will enhance knowledge and understanding of bloodstain pattern analysts when dealing with similar issue in actual crime scene.

The establishment of International Association of Bloodstain Pattern Analysts (IABPA) in 1983 has become the primary organisation for the BPA community which includes experts from different areas such as law enforcement agencies, private sectors, laboratories and academia from different countries to communicate, collaborate and work in the discipline of BPA. IABPA fosters the development and research on the science of BPA while standardising the scientific techniques involved in the examination. In addition, SWGSTAIN was established in March 2002 by Federal Bureau of Investigation (FBI), in which a list of recommended terms regarding the discipline of BPA has been published in 2009.

1.2 Problem statement

In real case scenario, a crime scene can possibly happen at indoor or outdoor. In comparison, outdoor crime scene has more uncontrollable external factors mainly originated from the environmental conditions, such as wind blow. The influence of wind blow may bring changes to the bloodstain patterns and cause uncertainties in examining them.

Spivey (2016) found that the presence and intensities of wind up to 17 km/h could affect individual blood droplets flight path (distribution) and directionality in impact spatter pattern, leading to the alteration of overall pattern appearance and area of origin (AOO) determination. Leonova et al. (2019) also reported that the greater the height of blood drop fall, the larger the diameter of bloodstain. The questions aroused here are: a) How does wind blow (and its velocity) affect passive bloodstain pattern? b) Does the effect of wind blow dependent on the height of blood drop fall?

In an outdoor bloodshed event, it is undeniable that there could be many different scenarios, conditions and factors at the crime scene having the potential to influence how the blood leaving a body behaves and how it forms a bloodstain pattern, whether a crime scene investigator aware of it or not. Though these variables may be insignificant on their own, combination of them could lead to pivotal change in the interpretation of bloodstain evidence. To take real life scenario as example, imagine situation A where one lane is closed down on a highway during non-peak hours, and situation B where slightly higher-than-normal traffic volume appeared on a highway during peak hours. These two situations may only cause slight or moderate delay on their own, but if both occur at the same time (one lane is closed on a highway during peak hours), this could lead to severe congestion and turns minor issues into a significant traffic jam. The same goes to BPA where two influential factors occurring together could lead to a synergistic effect towards the bloodstain evidence. Hence, this shifts the attention to studying wind blow and fall height effects together, as wind is a natural phenomenon (ENESSERE Clean Energy Company, n.d.) that commonly occurs yet uncontrollable and unpredictable, while variation of body height in the population gives possibilities of blood drop falling at different heights from the perpetrator and/or victim, thus their individual and combined effects need to be investigated thoroughly.

There are limited research studies concerning the influence of wind blow and height of blood drop fall on passive bloodstain pattern. The related studies found were from Kabaliuk (2014) and Többen (2021), who investigated the effect of wind blow on the trajectory (fall path) of passive blood drop falling from different heights. However, both research findings were contradicting to each other. The former study stated that wind blowing at blood drop falling from greater height causes increased displacement from its original trajectory while the latter found the opposite. It is necessary for further clarification on the interaction and relationship between these two variables to understand if their effect on the bloodstain is dependent on each other. Moreover, the specific wind velocities studied by Kabaliuk (2014) and Többen (2021) were equal or above 5 m/s. The effect of a slower wind blow (below 5 m/s) is unknown, leaving rooms for future research to investigate on. In addition, their studies focused on the trajectory of passive blood drop, but the characteristics of the bloodstain itself such as size and shape were not being considered. This is a potential area for future research in the field of BPA.

This study aims to investigate the effect of relatively slower wind blow on passive bloodstain pattern dropped from different heights. Table fans are used to generate different velocities of wind blow, while blood drop is released slowly and gently from micropipette at different heights under the influence of wind blow. The position shift from centre point and physical characteristics of bloodstain are measured to assess the effect of wind blow and height of fall. The physical appearance of bloodstain exposing to constant wind blow over a period of time is also studied to understand how wind alters a bloodstain after depositing on a surface. The outcomes will help investigators to improve crime scene reconstruction accuracy by considering the combination effect of wind blow and height of fall.

1.3 Objectives

The objectives of the study have been developed as follows:

1.3.1 General objective

To investigate the effect of wind blow on passive bloodstain pattern dropped from different heights.

1.3.2 Specific objectives

i. To determine the position shifting of passive blood drop stain in relation to wind velocity and height of fall.

ii. To determine the physical characteristics of passive blood drop stain in relation to wind velocity and height of fall.

iii. To investigate the effect of prolonged exposure to wind blow on the physical appearance of bloodstain.

1.4 Hypothesis

Hypotheses are formulated as below:

(1) H_0: There is no relation between wind velocity and height of fall with the position shifting of passive blood drop stain.

(1) H_A: There is a relation between wind velocity and height of fall with the position shifting of passive blood drop stain.

(2) H_0: There is no relation between wind velocity and height of fall with the physical characteristics of passive blood drop stain.

(2) H_A: There is a relation between wind velocity and height of fall with the physical characteristics of passive blood drop stain.

(3) H_0: Prolonged exposure to wind blow has no effect on the physical appearance of bloodstain.

(3) H_A: Prolonged exposure to wind blow has an effect on the physical appearance of bloodstain.

1.5 Significance of the study

The influence of wind blows on BPA including passive blood drop stain cannot be neglected. Better understanding on the influence of wind blow on passive blood drop stain allows investigators to more accurately reconstruct the crime scene and extract information regarding the assailant and/or victim in cases where crimes occurred in windy condition at outdoor. For instance, investigators could more accurately determine the approximate height of the blood source and subsequently that of the assailant or victim and their postures (standing, sitting, kneeling or lying down) by considering the degree of wind blow effect at the time of crime when doing bloodstain measurement.

The findings from this study can help prevent misinterpretation and misclassification of bloodstains by the investigators at an outdoor crime scene. As individual bloodstains may be susceptible to alterations under the influence of wind blow, this introduces higher ambiguity on the identification and classification of the stains, where incorrect analysis may overlook important clues at the crime scene. Correct recognition of different bloodstain types is crucial in correlating with each event and possible source that is responsible for the bloodstains.

From the recommended guidelines for basic BPA course requirements established by IABPA Education Committee (2019), one of the contents to be delivered is the "Limitations of determining distance fallen for individual droplets". As this study manipulates the wind velocity and distance fallen for passive blood drop, it could benefit the BPA instructors when developing teaching content for the topic mentioned above. It contributes to the expansion of understanding on the topic in the BPA community.

1.6 Scope of the study

This study is limited to examining the effect of cross wind (wind blowing perpendicular to the fall path of the blood drop) at velocities not exceeding 4 m/s on passive bloodstain pattern, particularly passive drop. The wind velocities are set based on the Malaysia's average annual wind velocity, 1.8 m/s, measured at 2 m height above ground from 1989-2008 (20 years) according to data provided by Malaysian Department of Meteorology, reported by Asia Wind Energy Association (n.d.). Five measurements of heights of blood drop fall are selected to investigate the wind blowing effect, which are 30, 60, 90, 120, and 150 cm. These heights represent different positions of stabbing using knife (will be explain further in section 3.3.3) by a Malaysian average-height male adult (165-170 cm).

Measurements and documentations on the horizontal and vertical positions of the bloodstain from the centre point, and the bloodstain's size, shape, edge characteristics, angle of impact, and directionality once the bloodstain dried are performed to evaluate the wind blowing effect on its position shift and physical characteristics. The bloodstain's physical appearance at different times after formation (immediately, 5 minutes, 30 minutes, 1 hour, 3 hours) under constant wind blow are also observed and documented in this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In BPA, the most fundamental yet core element is the blood. A thorough understanding and knowledge on the blood and its behaviour are a must to have before a crime scene investigator or bloodstain pattern analyst can conduct an analysis on the bloodstains and pattern. Researchers have been contributing to the wide field of BPA tirelessly by exploring into multiple key areas over the years to answer the problems and issues faced in real-life cases. These have gradually established BPA as a well-recognised discipline in forensic science.

This chapter encompasses information and findings obtained in BPA-related research, starting from a broad discussion on how blood as the essential tissue for survival in human's body behaves, narrowing into the role of bloodstains and pattern in a crime scene investigation, and finally focusing specifically on the characteristics and influencing factors of a single bloodstain.

2.2 Understanding blood

Blood is an essential component in the body for the survival of humans and animals. In humans, about 8% of total body weight of an average adult is make up of blood, corresponds to approximate 4-5 litres and 5-6 litres of blood for female and male respectively (Singh et al., 2021). However, each individual differs in the blood volume and percentage in body by age, height, weight, and sex. Blood plays an important role in the body by transporting oxygen and nutrients to each cell, and carry carbon dioxide and wastes away from them to excrete out of the body. It is considered as a liquid connective tissue that fills the cardiovascular system as it connects every system of the human body (Guyton and Hall, 2000).

2.2.1 Physical properties of blood

Looking at the compositions of whole blood, it comprises of about 55% of plasma and 45% of cellular components (red blood cells, white blood cells, and platelets) with red blood cells being the major ones constituting 99% of the cellular components (Spivey, 2016). All these components make up the physical properties of blood, and thus changes in the blood components and percentages could affect blood's physical properties. Analysing bloodstain requires studies of the blood's physical properties to understand its behaviour and tendency of following certain pattern from leaving the body to impacting a surface.

2.2.1(a) Viscosity

The definition of viscosity given by James et al. (n.d.) is the resistance to change of form or flow. Different literatures have reported different ranges of values for normal human blood viscosity, from 3.5 up to 6 cP or mPa·s (Dintenfass, 1985; Nader et al., 2019). In contrast to water, blood is a shear thinning or pseudoplastic non-Newtonian fluid, which means that its viscosity decreases when shear rate increases (Nader et al., 2019; Spivey, 2016). As different types of blood vessels possess different shear rates ranging from few s^{-1} to more than 1000 s^{-1}, blood viscosity differs in large arteries, veins, and microcirculation (Connes et al., 2016), and it can go up to 60 cP or more at a shear rate of 0.1 s^{-1}. Therefore, no specific value can be given to normal blood viscosity.

Many in-dept studies have been done on the determinants of blood viscosity. These determinants are haematocrit, red blood cell (RBC) deformability, red blood cell

(RBC) aggregation, plasma viscosity, and temperature. Haematocrit, also referred as packed cell volume (PCV), is the volume percentage of RBC in the blood. Research findings showed that higher value of haematocrit causes higher blood viscosity. Cokelet and Meiselman (2007) and Lowe and Barbenel (1988) reported that the impact of haematocrit on blood viscosity is more pronounced at lower shear rate compared to higher shear rate.

On the other hand, the increase of RBC deformation and aggregation (which have higher tendencies of occurrence at respective high and low shear rates) have lowering and rising effect respectively on the blood viscosity in general (Kabaliuk, 2014; Nader et al., 2019). Among the cellular components of blood, RBCs are the contributing factor to blood viscosity or blood flow but not quite much for white blood cells and platelets, mostly due to their relatively low quantities (James et al., 2005; Kabaliuk, 2014). As for blood plasma, which is a Newtonian fluid where its viscosity is independent of the shear rate, contributes to the whole blood viscosity as well. Normal plasma viscosity at 37 ℃ is around 1.15-1.35 cP (Dintenfass, 1985; Késmárky et al., 2008; Lowe and Barbenel, 1988), with the rising in plasma viscosity leads to increase of whole blood viscosity.

Temperature, particularly body temperature, is believed to have impact on blood viscosity. The blood viscosity is found to increase with decreasing temperature, and vice versa (Attinger et al., 2013; James et al., 2005; Shiri et al., 2019). A study conducted by Buono et al. (2016) showed that an increase of rectal temperature from 37.11 ± 0.35 to 38.76 ± 0.13 ℃ after 2 hours of moderate-intensity exercise in the heat caused significant decrease of blood viscosity by 7% (from 3.97 to 3.69 cP at 300 s^{-1}). However, when this exercise-induced hyperthermia effect was combined with the effect of exercise-induced haemoconcentration (which increase the blood viscosity), there was little overall change in blood viscosity before and after exercise. Buono et al. (2016) predicted that despite of the exercise-induced haemoconcentration, the effect of exercise-induced hyperthermia combined with increasing shear rate could lower blood viscosity by 31% below the pre-exercise levels.

Apart from the internal and less-controllable changes in the body, the impact of external activity such as exercise (slightly discussed above) on blood viscosity was well-studied by many researchers. A review paper published by Nader et al. (2019) concluded that acute cycling exercise in healthy individuals leads to increase in blood viscosity by increasing haematocrit, plasma viscosity, and decreasing RBC deformability. Acute running exercise does not change blood viscosity, and may tend to decrease. As for chronic (long-term) or regular exercise, it decreases blood viscosity by increasing RBC deformability and decreasing haematocrit. The decrease in haematocrit is due to repeated plasma volume expansion (every time after exercise) that results in chronic "autohemodilution". This reduces plasma viscosity which in turn decreases blood viscosity.

2.2.1(b) Surface tension

Surface tension is the force that pulls molecules on a liquid surface towards its interior, causing reduction of surface area and penetration resistance (James et al., n.d.). It can also be seen as the molecular cohesion forces acting on the liquid surface, minimising the exposed surface area to achieve the most stable condition possible. Surface tension is an important physical property in blood that has vital importance to BPA, as it is responsible for maintaining the spherical shape of blood drop during flight by counteracting phenomenon such as in-flight oscillations. This in turn has influence on the bloodstain’s size and shape, and essentially affects the angle of impact and AOO calculation since blood drop is assumed to be spherical upon impacting a surface. Concentration of anticoagulant and the temperature are believed to have the tendency to affect whole blood surface tension (Kabaliuk, 2014).

2.2.1(c) Relative density

According to Giancoli (2005), relative density is the ratio of a substance's density to the water's density at 4℃. Substances denser than water (0.998 g/mL at room temperature) will have relative density greater than 1, whereas those that are less dense than water will have relative density smaller than 1. It is a physical property to be determined in blood drop study as it may affect blood dynamics and blood drop in-flight behaviour. The relative density of blood is approximately 1.06 g/mL (denser than water) due to the RBCs in blood (James et al., 2005; Ostadfar, 2016).

2.2.2 Blood dynamics

Depending on the physical properties, blood behaves and travels in certain ways and patterns upon leaving the body to produce stains. It is important to understand the blood's motion and behaviour to aid in BPA especially when analysing individual stains. The following sub-sections focus on the dynamics of passive blood drop. Although certain similarities are found with pure water droplet due to approximate 50% of blood is made up of water, differences still exist upon close observations. Further details of blood dynamics will be explained below.

2.2.2(a) Formation of blood droplet

When an object picks up blood for example from stabbing or bludgeoning, or a body part bleeds, the blood will flow to the lowest point of the object or body part due to gravity. As blood continues to flow and accumulates at the lowest point, a liquid (blood) mass adhering to the dripping surface due to interfacial tension force starts to grow, forming a hanging or pendant drop. When the weight of the drop surpasses the interfacial tension force because of further accumulation of blood, the drop elongates and forms a neck that connects to the volume of liquid (blood) adhering to the dripping surface (residual liquid) (Kabaliuk, 2014). Up to this stage, no notable difference can be observed between blood and water drop.

From the study of Kabaliuk (2014), a slightly different and more variable passive blood drop formation was observed starting at this stage as compared to water droplet. As the neck continued to elongate by gravitational force in passive blood drop, a non-uniform (stepped) axisymmetric-shaped ligament, or bridge, with multiple liquid bulges (satellite beads) was formed (Figure 2.1a). This differs from the uniform axisymmetric cylindrical ligament observed in water.

Over time, the blood ligament became thinner and a few phases of fragmentation occurred. Kabaliuk (2014) observed that the ligament frequently ruptured anywhere in its lower part first and left a fragment attached to the primary drop. This fragment is likely to merge with the primary drop during the fall. Sometimes, it ruptured in its lower end without leaving any visible fragment attached to the primary drop. There were also some cases where the ligaments broke first at the middle or upper end. Hence, ligament could break in different ways during the detachment of primary drop as shown in Figure 2.1b, f and g.

The ligament was then ruptured near the residual liquid, and the fragment attached to the residual liquid was drawn back into it by surface tension. The remaining ligament may contract vertically into one or further break up along its length to form more than one accompanying (satellite) drops (Figure 2.1c - e). These accompanying drops may collide and bounce off from each other. Thin lines were observed at the points of rupture before ligament breakup.

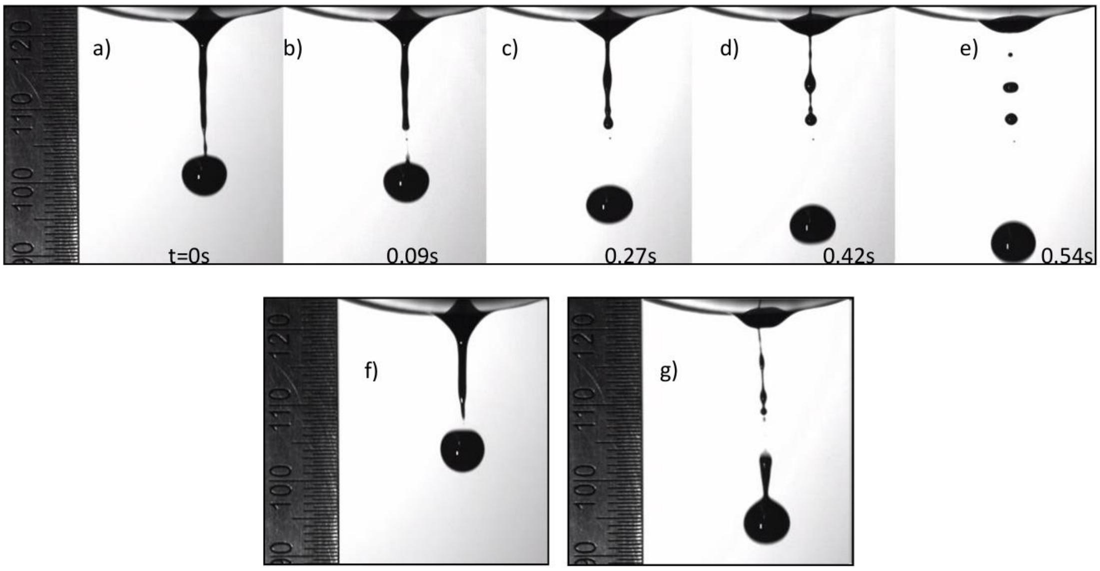

Figure 2.1 Close-up images of porcine blood dripping from a stainless-steel cylinder with H (mean curvature of the object surface) = 0.013 mm^{-1} and 2.4 µm surface roughness. (b), (f) and (g) are the variants observed during the detachment of primary drop (Kabaliuk, 2014).

2.2.2(b) Flight of blood droplet

Once a blood drop is formed, it undergoes microscopic changes on its body due to internal and external forces. These changes include shape oscillations, drop deformation (or distortion from sphericity), drop disintegration, or in-flight drop evaporation. When a blood drop detaches from the ligament, it is usually in a non-spherical shape, mostly prolate shape, depending on the dripping object. As it falls, surface tension pulls the drop horizontally and vertically, causing it to oscillate in oblate and prolate shapes as shown in Figure 2.2 (Kabaliuk, 2014; Raymond et al., 1996a). This is because the surface tension force tends to act in a way that minimises the surface area of the drop, hence shaping it towards spherical shape to obtain the smallest possible surface area. Over time, the amplitude of oscillations is damped by the viscosity of the blood and settle into sphere.

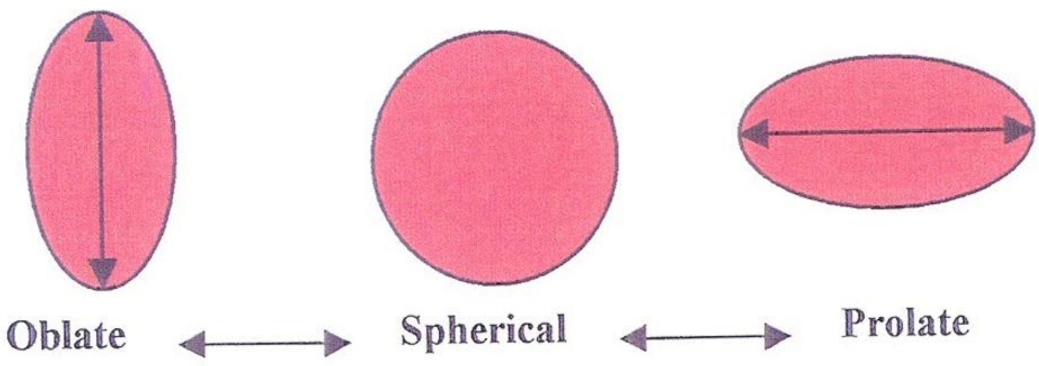

Figure 2.2 A blood droplet oscillates between these three phases in flight (Raymond et al., 1996a).

There are various factors affecting drop oscillation, including drop size, viscosity, velocity, drop liquid and surrounding media physical properties, initial condition of drop distortion, and Weber numbers of flight (Kabaliuk, 2014). Specifically on drop size, Gardner (2014) and Raymond et al. (1996a) found that smaller blood drop size has shorter period of oscillation, although it is for projected or impact blood drop. Similarly, observations from Kabaliuk (2014) showed that larger blood drops falling from at least 1.5 m of distance passively have lower frequencies but longer period of oscillations compared to smaller drops.

The other findings of Kabaliuk (2014) also showed that the blood drop oscillations decay time (time required for the oscillation to decay to 1% of its initial amplitude) decreases when the blood drop size decreases and/or when viscosity increases. The blood drop oscillations decay distance (distance travelled required for the oscillation to decay to 1% of its initial amplitude) also decreases when drop diameter and/or velocity decrease, and/or when viscosity increases.

However, one of Kabaliuk's experiments showed that blood drop passively fell from a hose connector (6.3 mm outer tube diameter) producing 5.34 mm of primary drop diameter still showed some oscillations after 1.5 m of fall. On the other hand, Raymond et al. (1996a) who also conducted blood droplet oscillation study found that a blood droplet with radius of 1 mm (diameter of 2 mm), which forms an approximate 4 to 5 mm radius bloodstain, has an oscillating life span of around 7 cm in free fall. It is understood that the drop size (or volume) and height of fall play important roles in determining whether oscillation would still persist upon impacting the receiving surface. This can be crucial during investigation as James et al. (2005) suggested that blood droplet which impacts the surface at 90° during the prolate phase of oscillation could produce bloodstain similar to that of a spherical blood droplet impacting an angular surface (elliptical bloodstain). Therefore, crime scene investigators who misidentified it as impact spatter (due to its elliptical shape) and used it to calculate AOO may cause incorrect AOO determination.

In the case of an impact spatter pattern, if looking at the oscillation factor alone, investigators are recommended to select bloodstains that are smaller and further away from the probable blood source, if possible, when calculating AOO to minimise the effect of blood drop oscillation on bloodstain's dimension (Gardner, 2014). This contradicts the findings of de Bruin et al. (2011) which stated that larger and nearer (to the probable blood source) bloodstains are better in determining AOO, most likely due to other external factors. It is believed that the effects of oscillations (and distortion from sphericity) are negligibly small on drop fall velocity, distance and time (Kabaliuk, 2014).

2.2.2(c) Deposition of blood droplet on surface

The deposition of blood droplet on a surface has been studied by Bevel and Gardner (2008), who proposed four distinct impacting phases:

1. **Contact and Collapse:** Upon contacting the surface, the blood droplet begins to collapse from the bottom upward (portion contacting the surface starts to collapse while the portions above remain intact). As it progresses, the blood is pushed outwards and forms an outwardly-growing rim around the collapsing droplet. In the case of an acute angle, the blood is pushed in the direction of blood drop travel and the rim is also formed following that direction.
2. **Displacement:** Majority of the blood has displaced to the edge or rim. Dimples and spines start to form at the edge of the stain, which may result in satellite stains later. Overall size of bloodstain is formed. Similarly, acute angle will cause dimples and spines to be formed more on the front edge (the side opposite the angle of impact).
3. **Dispersion:** Blood is forced into the boundary rim and protrudes upwards and outwards (blossom effect). For an acute angle, the blossom effect is at the front edge of the stain. While surface tension still holding the body and the protruded blood together, satellite spatters begin to form.
4. **Retraction:** Surface tension pulls the blood back into the boundary. The spines between the parent stain and the satellite spatters become narrow as blood is pulled into one or another. Satellite spatters that possess sufficient momentum or energy can break off completely and form separate stains.

2.3 Identifying bloodstain patterns

This section discusses about the patterns formed when viewing multiple individual bloodstains as a group that originated from the same event. Bloodstain patterns give an overall idea on the crime activity occurred at or after the time of crime.

2.3.1 Classification of bloodstain pattern

Classification of bloodstain pattern is an important aspect in BPA as it helps narrowing down the scope of analysis, identifying the probable cause of the bloodstain, as well as aiding in presentation of evidence in court. There are two common central aspects in the classification methods developed since the inception of BPA, which are i) the same basic types of bloodstain pattern are considered in most of the methods, and ii) either directly observable characteristics or the creation mechanism of bloodstains and pattern are used as terms to describe and eventually classify bloodstain patterns (Bevel and Gardner, 2008). These can be seen upon close inspection on the classification methods developed by different researchers.

Hook et al. (2024) have effectively summarised ten classification methods from 14 sources according to chronological order, as listed below:

i. Classification by velocity

ii. Classification by the movement of blood

iii. Classification by size

iv. Spatter, non-spatter, and composite bloodstains

v. Passive, transfer, projected, and miscellaneous bloodstains

vi. Active, passive, and transfer bloodstains

vii. Passive, spatter, and altered bloodstains

viii. Taxonomic classification systems

ix. Classification based on SWGSTAIN terminology
x. Classification by energy

Some of these methods are contributed by more than one author or publication, and some consist of revised or derived versions. Generally, "passive, transfer, projected/impacted/active" are employed or can be seen (as part or whole) in majority of the classification methods.

For classification by velocity (mostly on the velocity of the impacting object instead of the blood drop flight), it can only be identified via stain size (since velocity cannot be observed), but the sizes of the stains may not be consistent throughout the pattern, hence leading to disagreement and difficulty in classifying bloodstain patterns. Combining with other factors of confusion and ambiguity, this classification method has been abandoned by the BPA discipline due to its considerable limitations (Liu et al., 2020; Morris, 2022; Wonder, 2001). Apart from velocity, classification by energy, movement of blood, and based on SWGSTAIN terminology are also unsuitable for casework.

Throughout the review process, Hook et al. (2024) found that all ten methods face the common issue of indistinguishable features within the bloodstain patterns, making classification complex and introduce ambiguity. They also noticed that the creation mechanistic terminologies (such as spatter, projected, transfer etc.) are too heavily depended on in current methodologies. This could introduce issues of contextual bias and subjective judgments because it assumes or identifies the cause of a bloodstain pattern before thorough analysis is carried out. Further research should focus on forming terminologies based on directly observable characteristics of the bloodstain patterns to developed unambiguous and clear criteria for bloodstain patterns classification.

2.3.2 Types of bloodstain pattern

Various types of bloodstain pattern can be encountered in a crime scene. In Table 2.1, some examples of these patterns are classified using the frequently employed classification method of "passive, transfer, and projected (or similar terms)". Patterns that do not fall in any of these categories are classified as "miscellaneous" (Bevel and Gardner, 2001).

Table 2.1 Types of bloodstain pattern in the classes of "passive, transfer, projected, and miscellaneous".

Classification	Types of bloodstain pattern
Passive	Passive drop Drip Drip trail Flow Pool
Transfer	Pattern transfer Wipe Swipe
Projected	Gunshot impact Sharp force impact Blunt force impact Cast-off Arterial spurt (or gush) Expectorate (or expirated)
Miscellaneous	Void Fly spot

2.4 Physical characteristics of individual bloodstain

An individual bloodstain, regardless of dripping passively from an object or found within an impact spatter pattern, possesses certain physical characteristics that are useful for providing information during BPA. This section covers these physical characteristics and the forensic application of individual bloodstain.

2.4.1 Shape

Generally, blood droplet that strikes a smooth receiving surface at 90° will form a nearly circular stain and those that strike at lower angle will form elliptical shape. As the impact angle decreases (more acute angle), the bloodstain formed is more elongated (Drazdik et al., 2024). However, in real crime cases, investigators may not always be able to get these well-defined bloodstains. This is because the shape, contour or appearance of a bloodstain can be influenced by many factors, including angle of impact, velocity of impact, and types of receiving surface.

An issue that is being encountered in the crime scene is that the bloodstains found are in irregular shape, making the interpretation of bloodstains and back-tracking of the blood drop trajectory harder for investigators. Study from Shiri et al. (2019) demonstrated that surface coatings or residues on a smooth, non-absorbent and non-porous surface (in this case refers to glass surface) could cause passive blood drop stain to shrink (retract) after depositing on the surface, which might lead to irregular-shaped bloodstain. In an extreme weather condition such as snowing in certain countries, the bloodstains formed on snow surface were found to exhibit irregular shape due to diffusion and spreading through the surrounding snow (Plante et al., 2021). This scenario is possible as crime involving bloodletting event may happen at outdoor during winter season in those countries.

In most cases, a bloodstain may have consisted of additional features on its edge and around it, particularly spine and satellite stain, respectively (Figure 2.3). Spine, or sometimes used interchangeably with “spike” although it is less specific and formal, is the spoke or ray emanating out from the edge of a bloodstain, usually results from a blood drop impacting a non-smooth surface (Global Forensic and Justice Centre, 2013). As for satellite stain, based on the definition given by SWGSTAIN (2009), is the smaller bloodstain that is created during the formation of the parent stain as a result of blood impacting a surface. In situation where the blood impacts the surface at or around 90°, the spines and satellite stains can be distributed quite evenly around the circular bloodstain. When the impact angle is small, spines and satellite stains can be shifted towards the side of the elliptical bloodstain opposite the impact angle, indicating the direction of travel. The more elliptical the stain, the more definite the directionality is. The spine that shows directionality in this kind of elliptical stain can be referred as “tail” as well. In some instances where the impact angle is less acute, “scallops” pattern may be observed (Figure 2.3).

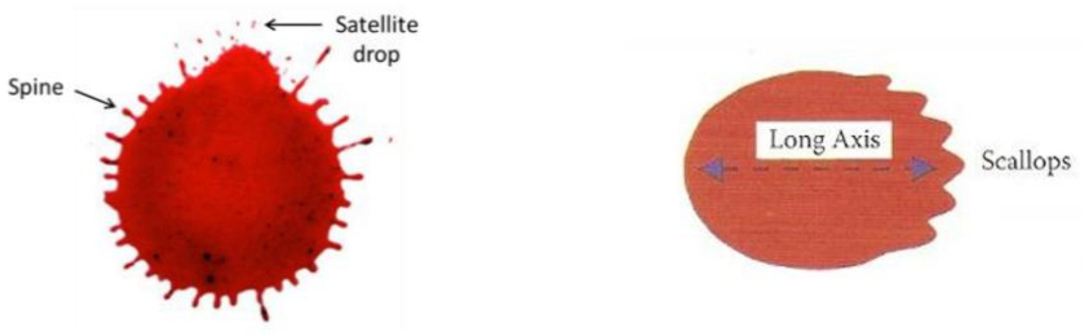

Figure 2.3 Diagram on the left shows the spine and satellite stain (or drop) of a bloodstain (Neitzel and Smith, 2017). Diagram on the right showed the scallops of a bloodstain.