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Importance of Blood In Criminal Investigations

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ABSTRACT

Blood is one of the most important biological traces that are often found on the crime scene. Due to valuable information it contains, it is considered to be a very important forensic tool. Analysis of different aspects of bloodstains can contribute to clarify the circumstances under which some violent crimes have been committed. Such crucial information can point criminal investigation in the right direction and help solve the crime. In some cases it can also help with legal determination of criminal offence which can lead to more accurate and more appropriate punishment for the perpetrator. It is very important to determine the sequence of events during the commitment of a violent crime involving blood. Analysis of different aspects of bloodstains includes appropriate methods from natural sciences, particularly methods in molecular biology and also from mathematics, physics and chemistry. Proper knowledge enables interpretation of results and makes it possible to get closer to the truth, solve that particular crime and bring the perpetrator to justice. After determining that it is blood by using serological tests, DNA profiles which account for the donors of different bloodstains are obtained. For the answers about sequence of events and mechanisms of creation of specific groups of bloodstains on the crime scene, investigation is pointed towards morphological analysis of bloodstains.

KEYWORDS: Blood, DNA, Origin, Patterns, ABO blood grouping.

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1. INTRODUCTION

Blood is a fluid medium, which in humans and in other vertebrates, is found within the cardiovascular system. This system consists of the heart, which performs as a muscular pump and the blood vessels which serve to circulate the blood to different parts of the body. Blood has numerous functions. It acts as an internal transport system. It also plays an important role in maintaining body temperature, defending against infection and protecting the body from the consequences of injury.

Human blood, in common with that of other mammals, consists of 55 per cent (by volume) blood plasma and 45 per cent (by volume) cellular material (i.e. blood cells and platelets). Blood plasma is a pale yellow fluid composed of approximately 90 per cent water and 10 per cent dissolved materials, including antibodies, enzymes, hormones, blood proteins, waste products (e.g. carbon dioxide) and nutrients such as amino acids and glucose. Substances, for example drugs (including alcohol) can also be found in blood plasma and may be tested for as part of a criminal investigation. Blood serum is blood plasma minus its protein content. This clear liquid is exuded when whole blood or plasma is clotted. The clotting process involves the use of blood proteins, such as fibrinogen, which as a result are removed from the plasma, thus producing the serum.

The cellular components of blood may be divided into the three main types listed below:

- **Erythrocytes (Red Blood Cells) -** These are the commonest type of blood cell and account for over **44** per cent of the total blood volume. They contain haemoglobin, an iron-containing protein, responsible for the carriage of oxygen (and carbon dioxide) in the blood. In contrast to most other mammalian cells, erythrocytes lack nuclei.
- **Leucocytes (White Blood Cells) -** These cells together with thrombocytes constitute **less than 1** per cent of the total blood volume. White blood cells are involved in protecting the body from infection. They may be further subdivided into phagocytes and

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lymphocytes, which are responsible for the capture and ingestion of foreign substances (such as bacteria) and the production of antibodies respectively.

- **Thrombocytes (platelets) -** These are non-nucleated cell fragments, which are formed from the fragmentation of very large cells called megakaryocytes in the bone marrow. Thrombocytes are involved in the process of blood clotting.
- **Plasma**- Plasma is the yellowish, liquid portion of the blood that contains electrolytes, nutrients, proteins and vitamins. It accounts for 55 per cent of the total blood volume.

Major Functions of Blood are:

- Transportation.
- Maintain body temperature
- Control pH (acid base balance)
- Removal of toxins from the body (Excretion)

2. BLOODSTAINS

Blood is one of the most important physical evidence which is frequently encountered at the crime scene as a pool of blood, droplet, stains etc. It can be found in almost every type of criminal activity involving physical violence like murder, assaults, rape. Forensic scientists are called upon so as to examine items of physical evidence, clothing, vehicle etc.

3. DOCUMENTATION AND COLLECTION OF BLOOD STAINS

Documentation of bloodstain evidence will most typically be carried out using photography, including photographs of the wider scene along with close-up images of particular bloodstains. A scale or any other forms of measuring instruments may be placed in the photograph in order to give the precise size of a bloodstain.

Blood collection techniques:

- **Wet Blood**: Wet blood from a stain is absorbed through a pool into sterile pure cotton swab and is then dried with a flux of cold air using a hair drier.

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- **Dry Blood Stains:** Dried blood specimens is scrapped with a clean sterile scalpel from permanent surface into a paper envelope. Blood is absorbed from dried stain by transferring it to saline wet sterile pure cotton swab. Now, air-dry and then pack it in a paper bag. The stains are air dried on cloths and are packed thoroughly. Blood stain patterns are not to be disturbed because they may create additional stain pattern during drying and packaging process.
- **Blood in Snow or Water:** Suspected blood in snow or water is collected immediately to avoid further dilution in a clean and air tight container. Evidence is refrigerated and submitted to the laboratory as soon as possible in ice box. Absorb the suspected liquid blood onto a clean cotton cloth or swab. Air-dry the cloth or swab and pack in clean paper or an envelope. Plastic containers should not be used.
- **Buccal (Oral) Swabs:** Use a clean swab or gauze piece to collect buccal (oral) sample. Rub the inside surfaces of cheeks thoroughly. Air dry the swabs with cold flux of air and pack in a clean paper envelope.

4. AGE OF BLOOD STAIN

Age of blood stain can be calculated by colour and nature of the stain. The Fresh blood stain is bright red in colour, moist and sticky. Within 24 hours, the stain turns reddish brown in colour. After 24 hours, the stain turns dark brown and finally black. The methodology for the age estimation of bloodstains needs further measurements and development as the current aging measurements have been made under controlled environmental conditions to allow the underlying chemical change to be accurately studied. However, the aging process is likely to depend on environmental variables such as temperature, humidity and light intensity, as well as physical variables such as substrate. Given that these variables will be uncontrolled at crime scenes, it will be necessary to make further measurements to investigate the effects of these variables based on the process of ageing. Therefore based on the possible dependencies it must be possible to create bloodstain aging model that will take into account the actual or estimated environmental and physical conditions at the crime scene.

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5. EXAMINATION OF BLOOD

The bloodstains are subjected to examination to answer one or more of the following questions:

- Whether the stain is blood or any other substance?
- If it is blood, is it of animal or human origin?
- If it is of animal, which species is it?
- If human, what is the blood group?

i. PHYSICAL EXAMINATION

In the natural light, the blood stains appear as brown, reddish brown stains, clot or crystals of reddish brown color. If the stain are clear and visible then, examined under UV light at 230- 269 nm wavelength.

ii. PRESUMPTIVE TEST FOR BLOOD

At the crime scene, presumptive tests may be used to detect the presence of blood that might otherwise be overlooked, either because it occurs in minute amounts or because it merges well with its background. In some cases, attempts may have been made to clean up the blood at a crime scene prior to the arrival of the investigating authorities. Presumptive tests may also be employed to indicate whether a particular stain is probably composed of blood (and not of some other substance, such as ink or rust) before other, more complicated, blood-specific tests are carried out. The blood stain obtain from suspected area should be tested for **positive blood stain**. This test is done to know whether the stain is really a Blood or some other substance.

a) Phenolphthalein Test (Kastel Meyer Test)

Phenolphthalein is reduced by Zn powder in a strongly alkaline medium. If this reduced phenolphthalein is oxidized by oxygen liberated by the action of peroxides on hydrogen per oxide (H_2O_2), then a pink or purple color is obtained, if the stain of blood.

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Reagent Preparation: The reagent are formed by adding phenolphthalein (2g), potassium hydroxide (20g), distilled water (100ml). Mix well and now add Zn powder and boil under reflux 2-3 hours until the stock solution is formed. Cool and decant into a bottle containing some zinc to keep in the reduced form. Now add ethanol (10ml), phenolphthalein stock solution (2ml), distilled water (10ml), again ethanol (2ml) and 3% hydrogen peroxide (10ml) as working solution. Hydrogen peroxide is used in every colour reaction. If the colour is obtained pink so, it confirms the presence of blood.

Procedure: A small cutting, swab or extract of the suspected bloodstain is placed on filter paper or spot test paper. Two or three drops of ethanol are placed on the stain. Two drops of working phenolphthalein solution are added to the stain. After waiting to insure that no color develops at this stage, two or three drops of 3% Hydrogen peroxide are added. An intense pink color indicates the positive test for peroxides activity and indicates the presence of haemoglobin.

b) Tetra Methyl Benzidine (Tmb) Test

The test is based on the presence of haemoglobin in blood. Peroxidase enzyme in blood speed up the oxidation of benzedine with the formation of blue-green color.

Reagents

Reagent Preparation: Take 1.5gm of benzidine and 13ml of glacial acetic acid and 57ml of distilled water .After shaking benzidine solution is ready for test.

Procedure: Place cutting or swabbing of the stain on filter paper or spot test paper. A drop of TMB Solution is placed on the stain, followed by a drop of 3% Hydrogen Peroxide and mix with glass rod. Appearance of immediate blue-green color is a positive test for peroxides activity i.e indicative of presence of haemoglobin

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iii. CONFIRMATORY TEST FOR BLOOD

With this test, the identification of blood can be made more specific.

a. Takayama Test (Haemochromogen test.)

Reagent preparation: Taken the 3ml of saturated solution of glucose, 3ml of pyridine solution and 3ml of NaOH solution along with 7ml of Glacial acetic acid.

Procedure: Place the material to be tested on a microscopic slide and cover it with a cover slip. Add a drop of Takayama Reagent and allow to flow under the cover slip. Warm the slide gently on a hot plate at 65°C for 10-20 seconds. Allow to cool and observe under microscope at 100X. The appearance of pink needle shaped crystals of pyridine Haemochromogen (Pyridineferroprotoporphyrin) is positive reaction for haeme and confirms the presence of haemoglobin.

b. Teichmann's Test

Reagent preparation: For the Teichmann's test, the reagent are formed by the combination of KCl, KBr and KI at about 0.1g each in 100ml of Glacial acetic acid. The reagent reacts with haemoglobin and gives brownish rhombic crystal, Confirms the presence of blood.

Procedure: Place material to be tested on a microscopic slide and cover with a cover slip. Let the reagent flow under the cover slip. Warm the slide gently on a hot plate at 65°C for 10-20 seconds. Allow to cool and observe under microscope at 100X. The appearance of brown rhombic shaped crystals of ferroprotoporphyrin chloride is a positive reaction for haeme.

iv. DETECTION OF SPECIES ORIGIN FROM BLOOD

The precipitin test for species of origin is based on antigen–antibody complex formation, which produces a clearly visible, cloudy precipitate. This serological test was developed by the German

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biologist Paul Uhlenhuth in 1901. In his experiments, he injected rabbits with protein extracted from the egg of a chicken and afterwards harvested the rabbit's serum. He then introduced this antiserum into the white of a chicken's egg and observed the formation of a cloudy precipitate (precipitin). This work was further developed to produce antisera capable of identifying (by the formation of a precipitate) the blood protein of humans and a number of other different animals.

The **precipitin test** may be applied to bloodstains in a number of different ways. For example, it may be conducted in a capillary tube, with a layer of human antiserum (i.e. serum containing antibodies specific for human antigens) overlain by a layer containing an extract of the bloodstain under investigation. The formation of a cloudy precipitate at the interface between the two layers indicates a positive result for human blood. In another method, known as **cross-over electrophoresis**, a gel-coated slide containing twin wells is used. A liquid extract of the bloodstain is placed in one depression, while human antiserum is placed in the other. The application of an electric current to the slide induces the antibodies (from the antiserum) and the antigens (from the blood sample) to move towards each other. If a line of precipitation forms where the two meet, then the bloodstain is human in origin

Clearly, human antiserum is used first to determine whether the blood sample is human in origin. However, if the result is negative and if it is deemed necessary to search further for the species of origin, the precipitin test can be repeated using antiserum prepared for other animals. The precipitin test is highly sensitive, needing only tiny samples of blood. Furthermore, it has been found to be effective for testing dried bloodstains more than a decade old.

The biological evidence has been identified necessarily to determine and confirm whether it is of human origin or not. If it is non-human origin, then to which species it belongs to the species specific proteins in the bloodstains or other body tissues may be identified with the help of species specific antibodies.

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6. BLOOD GROUP SYSTEM

Karl Landsteiner, an Austrian scientist discovered the ABO blood group system in the year 1900. In his experiments, he mixed different blood types and noted that the plasma from certain blood type produced agglutinates or formed clusters which were caused by the absence of molecules on red blood cells and resulting in antibodies to defeat that molecule. He then made a note of the agglutination and divided the blood types into 4 different groups. For the discovery of ABO blood group, he was awarded the Nobel Prize. The blood grouping system plays a very important role during the blood transfusion. If another blood type is introduced into our body, our immune system recognizes it as foreign and attacks it, resulting in a transfusion reaction. Mismatches with the ABO and Rh blood types result in most serious and life-threatening transfusion reactions. Hence, it is advisable to have the blood group checked before the blood transfusion.

The ABO blood group system consists of 4 types of blood group – A, B, AB, and O and is mainly based on the antigens and antibodies on red blood cells and in the plasma. Both antigens and antibodies are protein molecules in which antigens are present on the surface of Red Blood Cells and antibodies are present in the plasma which is involved in defending mechanisms. The basis of ABO grouping is of two antigens- Antigen A and Antigen B. The ABO grouping system is classified into four types based on the presence or absence of antigens on red blood cells surface and plasma antibodies.

- Group A – contains antigen A and antibody B.
- Group B – contains antigen B and antibody A.
- Group AB – contains both A and B antigen and **no** antibodies (neither A nor B).
- Group O – contains **no** antigen (neither A nor B) but contains both antibodies A and B.

The ABO group system is important during blood donation or blood transfusion as mismatching of blood group can lead to clumping of red blood cells causing various disorders. It is important for the blood cells to match while transfusing i.e. donor-recipient compatibility is necessary. For example, a person of blood group A can receive blood either from group A or O as there are no

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antibodies for A and O in blood group A. The individuals of blood group O are called as universal donors, whereas individuals of blood group AB are universal recipients.

7. BLOODSTAIN PATTERN ANALYSIS

Much of the physical evidence present at crime scenes can be used to help establish the identity of the individual(s) involved. For example, fingerprints, footwear impressions and trace materials such as hairs and other fibres can all be used to connect an individual, or individuals, with a particular crime scene, though with varying degrees of certainty. In contrast, the analysis of bloodstain patterns, a form of physical evidence frequently found at violent crime scenes may provide valuable information about what occurred during the course of a crime and the order in which these events took place. It may therefore play a pivotal role in crime scene reconstruction. The interpretation of bloodstain patterns requires particular expertise, which is acquired to a large extent, through direct experience.

When it is considered that adult human males contain approximately 5–6 litres of blood and adult human females about 4–5 litres, it is not surprising that, in many instances of violent crime, copious amounts of blood are found at the scene. If the crime is committed indoors, the floors, walls and even ceilings may all show evidence of bloodstains. This type of evidence may occur in several rooms within a house and therefore the search of the scene, carried out with the aid of a good light source, should be both extensive and thorough. As with all types of physical evidence, it is essential that all bloodstains present at a crime scene are recorded by an appropriate combination of notes, sketches, photographs and/or video footage before they are disturbed by the investigators.

Often found at the scenes of violent crimes, the analysis of bloodstains can provide vital clues as to the occurrence of events. The successful interpretation of bloodstain patterns may provide clues as to the nature of the offence, the possible sequence of events, any disturbance to the scene that may have occurred, and even the position of individuals and objects during the

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incident. It may prove beneficial in refuting or corroborating eyewitness accounts. The appearance of a bloodstain can depend on a number of factors, including the velocity at which it was travelling, distance travelled, the amount of blood, the angle of impact, and the type of target onto which it lands.

Single Drop

- These bloodstains typically refer to blood drops that have fallen vertically, whether it be from an injured person or another object, and landed onto another surface.
- As a blood drop falls perpendicular to a surface it maintains a spherical form until impacting.
- The size and appearance of this stain will depend on a number of factors.
- The volume of a single drop of blood will vary depending on the quantity of blood present and the surface area available from which the drop is falling
- The height from which the blood falls will affect the size of the stain, with greater heights tending to result in larger bloodstains.
- The nature of the target can alter the appearance of the stain. For instance, a rough target surface can result in increased distortion to the stain and even satellite stains, which are additional stains radiating outwards. A drop of blood falling into an existing bloodstain will result in a drip pattern.

Impact Spatter

- This type of bloodstain is the result of a forceful impact between an object and wet blood, causing the blood to break into smaller droplets.
- A greater force will typically produce smaller droplets, with the density of blood drops decreasing moving further away from the initial blood source.
- The study of impact spatter may provide insight to the relative position of individuals and objects during an incident and the nature of the incident.

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Cast-Off Stain

- Cast-off bloodstains occur when centrifugal force causes blood drops to fall from a bloodied object in motion.
- In this instance, the blood flung from a blood-stained object, such as a weapon, may produce characteristic patterns of numerous individual blood drops forming a curved or straight line.
- If an object is repeatedly moved, each subsequent swing will result in less cast-off as less blood remains on the object.
- Bloodstains produced in this fashion can be particularly difficult to interpret as there is a great deal of possible variation in patterns produced.
- However depending on the nature of the motion of the bloodied object, cast-off blood will at least produce relatively linear stains.

Transfer Bloodstains

- Transfer or contact stains result when a bloodied surface comes into contact with another surface, transferring blood to the secondary target.
- The study of this type of bloodstain can prove particularly beneficial in establishing a sequence of events at the incident scene and tracing the movement of objects or individuals.
- Similarly, such bloodstains may be left by the hands of an individual, thus opening the possibility of fingerprint evidence.

Pool Stains

- Pooling bloodstains refer to the accumulation of blood on a particular surface, generally from a prolonged bleeding wound or accumulation of arterial blood.
- If a body is not present at the incident scene, it may even be possible to roughly estimate whether the victim is likely to be dead or alive based on how much blood they have lost.

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Point of Origin – Directionality and Angle of Impact

- In the reconstruction of an incident scene involving bloodstains, it is often beneficial to establish the point of origin of bloodstains, based on directionality and angle of impact.
- The examination of certain bloodstains may allow the determination of the direction of travel of blood as it impacts the target. Whereas, a drop landing perpendicular to a surface (depending on the type of surface) will tend to produce a more circular pattern than those landing at an angle producing an elongated stain.
- The tapered end of this stain will generally point in the direction in which the droplet was travelling. Small amounts of blood may break away from the parent stain entirely. These are known as satellite stains.
- It is possible to estimate area of origin purely through visual observation of bloodstain patterns.
- Depending on the type of bloodstain pattern, it may be possible to establish the angle at which a blood droplet hit a target, referred to as the angle of impact.
- If the angle of impact of multiple bloodstains is established, it may be possible to determine the area of convergence (the point where lines of travel from multiple stains meet) through stringing techniques and establish the area of origin.

8. CONCLUSION

Analysis of morphological aspects of blood is very important during criminal investigation of violent crimes involving blood traces, particularly violent crimes but also other types of criminal events where blood is one of the resulting evidence on the crime scene, such as traffic accidents or property crime. After determining whose is it, by analyzing all measurable characteristics of bloodstains left on the crime scene it can be determined was it a murder or a suicide, was the injury deliberate or was it the result of helping the victim, what was the sequence and the dynamics of events during the commission of the crime, approximately how long did it take to commit the crime and how much time has elapsed after the formation of those bloodstains, what are subsequent events that took place after the crime was committed, and which was the direction

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of movement of persons involved after the crime was committed. Unfortunately, because of the predominance of DNA analysis which is most often used method of analyzing blood traces, analysis of morphological aspects of bloodstains is in most countries still not very developed and not widely used. It is also valuable to stress the importance of further education and specialization of forensic experts as well as all other involved in crime scene processing to produce knowledge and capacity of viewing bloodstains not only as DNA containing traces but also as a source of other valuable information that can help solve very serious violent crimes.

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