

Significance of Blood and its Identification: A Perspective in Forensic Investigation

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ABSTRACT

The recognition and individualization of blood and other biological evidence is a significant aspect in any crime scene investigation. Blood found at crime scene, can be a valuable evidence in many criminal activities involving physical violence such as murder, assaults, rape, etc. Blood is a connective tissue consisting of plasma and formed elements in the cardiovascular system. 'Heme' is the major component of the blood, taking part in the identification and confirmation of a stain as a blood stain. Presumptive test methods are by no means conclusive by themselves therefore, further analysis is required to be done by confirmatory test methods. Takayama and Teichmann crystal tests are routinely performed as confirmatory test in forensic laboratories.

INTRODUCTION

Among the investigations which fall under the purview of forensic science, the recognition of blood stain forms one of the most important problems which needs utmost attention and solution. Blood stains are significant evidence used in many forensic investigations. It links victim to his/her suspect via Locard's exchange principle (Li, 2015). Blood is a common and important

finding at crime scene, used to determine and individualize criminal suspected of committing some kind of crime. Blood may be present either in the form of liquid or in dry state as well as stains on any object. It may or may not be visible to naked eyes. After detecting stain at the crime scene, a technique for blood stain identification is required to prove the presence of blood in the stain.

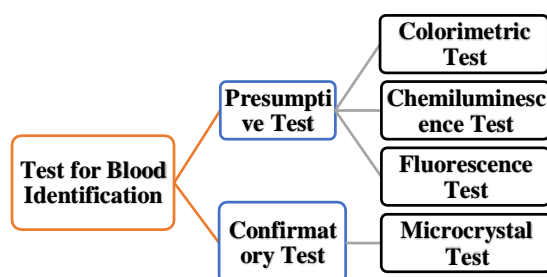
The most important query that arises firstly in a crime scene investigation is, “whether the stained material is blood or not?”. The stains of some other substances such as rust stain, vegetable stain, synthetic dye stain, mineral stain and spot of grease, resin and tar stain shows resemblance to the blood stains. Although, these stains do not give positive result further in forensic examination. However, identification of suspected stain is done by some presumptive and confirmatory tests. In this context, blood becomes an important part of crime scene investigation as there is no other alternative for it, whether for medical or forensic purposes.

Blood

Blood is a specialized colloidal mixture and a fluid connective tissue that helps in circulating nutrients, oxygen and hormones to different parts of human body (Martini *et al.*, 2018). It mainly consists of two components - cells or formed elements and plasma. It consists of dissolved protein and other solute in watery fluid. Formed elements include RBCs (erythrocytes), WBCs (leucocytes), and platelets (thrombocytes). Blood is slightly alkaline in nature having pH range of about 7.35-7.45. It is more viscous than water. As like most of the liquids, blood do not have its own shape; it adjusts to its surrounding. Blood droplets tends to be in round and circular shape as every liquid have the tendency to reduce its surface tension as much as possible (Peschel *et al.*, 2011). Blood is red in color due to iron containing complex protein named as hemoglobin. Hemoglobin is a globular protein and a tetramer consisting of two alpha and two beta chains. It helps in the transportation of oxygen and carbon dioxide throughout the body. It consists of two components heme and globin. Heme is the non-protein pigment consisting of protoporphyrin ring and iron. Globin is the protein which is attached to the heme. Heme is the most important component of the blood, taking part in the identification and confirmation of a stain as a blood stain.

In this article, the identification of blood stain by various methods will be discussed with reference to forensic investigations. Presumptive and confirmatory test methods are helpful in this way for the recognition of blood stain.

Test for Identification of Blood



Presumptive Tests for blood

Presumptive tests are those tests which determine the sample, whether it is blood or not. These tests are based on the oxidation - reduction reaction principle and the reaction are catalyzed by heme moiety of blood hemoglobin. In this reaction, colorless substrate is oxidized by heme catalyst of the blood and changes it into colored product. Presumptive test can detect very minute amount of the blood in the sample with 10^{-5} - 10^{-6} - fold dilutions (li.,2015).

Colorimetric Test

In colorimetric test, small amount of blood in the sample is determined with the aid of color reagent. Most commonly used color reagents are Benzidine, Phenolphthalin and Leucomalachite green. Detailed procedure and properties of these tests are as following:

- **Benzidine Test:** Benzidine is an organic compound which was used as an intermediate in dye manufacturing. In suspected sample, add one drop of benzidine solution and then add one drop of hydrogen peroxide and observe the color change. If it is blood, then it will give blue to dark blue color.

Benzidine is considered as carcinogenic and is known to give false-positive result. In spite of its several drawbacks, it has been routinely used in many forensic labs to identify the blood in the suspected sample.

- **Phenolphthalin Test:** This test is also known as Kastle-Mayer test. Phenolphthalin is a reduced form of phenolphthalein which is used as an indicator and dyes in titration of minerals and organic acid as well as alkalis. In suspected sample, add one to two drops of ethanol, two drops of phenolphthalin solution and one to two drops of hydrogen peroxide, and then observe the color change. Positive test for blood will give pink color.
- **Leucomalachite Green Test:** Triphenylmethane dye is known as malachite green. Leuco base in the malachite green makes it colorless compound. Apply one to two drops of leucomalachite green in the suspected blood stain and then add one to two drops of hydrogen peroxide. Note any immediate blue-green color change. If no color change is observed, then it shows negative result.

Chemiluminescence Test

In chemiluminescence test, emission of light is observed as a result of chemical reaction. Luminol is used as a chemiluminescence reagent to identify blood at the crime scene. Before using this test, it should be noted that the background of the suspected area must be in dark. Oxidation of luminol is catalyzed by heme of the blood in the presence of oxidant, produce light. Color of light and pattern of the impression is immediately observed and noted before it fades away. Latent impression of blood like bloody fingerprint and footprint which is not visible by naked eyes can also be identified by this method.

Fluorescence Test

In fluorescence test, fluorescein is used as reagent to identify the presence of blood and blood stain pattern at the crime scene. When fluorescein is sprayed over suspected area then it is catalyzed and oxidized by heme and shows fluorescence. By using alternate light source, fluorescent area can be observed when exposed to light in the range of 425-485 nm. If blood is present, then fluorescein emits intense yellow-green fluorescence light.

Disadvantages of Presumptive Test

Presumptive tests are not used for the confirmation of blood because other substances also show false-positive reaction. Strong oxidant like few metal salts, household cleaners, hair-coloring products also show false-positive reaction with presumptive test reagents even in the absence of heme of the blood. Few plants like horseradish contains peroxidase which also catalyze oxidation reaction and show false-positive result. Method of presumptive tests is by no means conclusive by themselves, and further analysis remains essential.

Confirmatory Tests for Blood

Two major tests, Takayama crystal Test and Teichmann crystal test are routinely used in forensic laboratories as confirmatory test. These are microcrystal tests in which blood stain is treated with chemicals and characteristic colored crystals are formed (Gaensslen *et al.*, 1983). Morphologies of these crystals are observed by using a microscope.

Takayama Crystal Test

In this test suspected blood stain is treated with glucose and pyridine under alkaline condition to form microcrystals of pyridine ferroprotoporphyrin.

Reagent preparation

Glucose solution (100g/100mL) - 3mL, 10% Sodium Hydroxide - 3mL, Pyridine - 3mL,

Distilled Water - 7mL. Mix all these reagents and store in closed bottle.

Procedure

- Place sample to be tested on microscopic slide and cover with a coverslip.
- Add a drop of Takayama reagent and allow to flow over coverslip.
- Heat the slide gently on a hot plate at 65°C for 10-20 seconds.
- Allow to cool at room temperature and observe under high power microscope.
- If red-brown needle shaped crystals are observed, then result is positive.

- If prismatic brown colored rhombohedral shape crystals are seen on microscope, then the result is positive.



Takayama test showing Positive result



Teichmann test showing positive result

Teichmann Crystal Test

When suspected blood stains are treated with acetic acid, salt and heated for few seconds, prismatic brown-colored crystals of hematin chloride are formed.

Reagent Preparation

Potassium Chloride or Potassium Bromide - 0.1g, Potassium iodide - 0.1g, Glacial acetic acid -100mL. Mix all these reagents and store in closed bottle.

Procedure

- Place Sample to be tested on a microscopic slide and cover with a coverslip.
- Add one to two drops of reagent and allow to flow over coverslip.
- Heat the slide gently on a hot plate at 65°C for 10-20 seconds.
- Allow slide to cool at room temperature and observe under microscope.

CONCLUSION

Crime is spread all over the world, and in this scenario, precise forensic examination becomes very crucial and important in crime scene investigation. In forensic investigation, blood is quite common trace evidence in solving cases to find the perpetrator and providing justice to the victim. Crime where violent act takes place, trace amount of blood may be transferred to and from the individuals. Blood can be frequently found in cases like firearms injury, road traffic accident, sexual assault cases etc. where detection, collection, identification and preservation of the blood sample should be done by forensic expert. In the detection of blood, presumptive test gives easy and rapid result and have less accuracy whilst confirmatory test gives more accurate and slow result. Therefore, presumptive tests may be

used at crime scene whilst confirmatory test requires a forensic lab with sufficient equipment and chemicals.

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