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<tr>
<th>Principal Investigator</th>
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<tr>
<td>Dr. A.K. Gupta</td>
<td>Dr. G.S. Sodhi</td>
<td>Dr. (Mrs.) Vimal Rakh</td>
</tr>
<tr>
<td>Professor and Head, Department of Forensic Science</td>
<td>Associate Professor, Forensic Science Unit, Department of Chemistry, SGTB Khalsa College, University of Delhi</td>
<td>Deputy Director, Centre for e-Learning and Assistant Professor, Department of Chemistry, SGTB Khalsa College, University of Delhi</td>
</tr>
<tr>
<td>Sam Higginbottom Institute of Agriculture, Technology &amp; Sciences, SHIATS, Allahabad</td>
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<tr>
<th>Paper Coordinator</th>
<th>Author</th>
<th>Reviewer</th>
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<tbody>
<tr>
<td>Dr. A.K. Gupta</td>
<td>Dr. Munish Mishra</td>
<td>Dr. M. S. Rao</td>
</tr>
<tr>
<td>Professor and Head, Department of Forensic Science</td>
<td>Assistant Professor, Department of Forensic Science, Sam Higginbottom Institute of Agriculture, Technology &amp; Sciences</td>
<td>Ex-Chief Forensic Scientist, MHA, GOI, Hon Advisor GFS University, Gandhinagar</td>
</tr>
<tr>
<td>Sam Higginbottom Institute of Agriculture, Technology &amp; Sciences, SHIATS, Allahabad</td>
<td>Mr. Rajeev Kumar</td>
<td></td>
</tr>
<tr>
<td>Senior Scientific Assistant (Chemistry), Forensic Science Laboratory, GNCT, Delhi</td>
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Anchor Institute: SGTB Khalsa College, University of Delhi
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Learning outcomes

After studying this module, you shall be able to know-

- What is the blood and composition of blood
- How is blood examined
- What is source of blood
- How will you know that examined sample is blood of human or animal
- What is the age of blood stain
- What is the blood group
- What are the confirmatory test of blood

2. Introduction

2.1 Blood-
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, etc at crime scene in form of valuable evidence.

Blood stains are of two type-
1-Visible blood stain
2-Invisible blood stain

2.2 Component of blood-
The blood, which constitute around 1/13th of the body weight consist of medium plasma and suspended cells like-

![Diagram of blood components](Source-www.netwellness.org)
1. **RBC (Erythrocytes)** - Red blood corpuscles containing hemoglobin are responsible for carrying oxygen from the lungs to various parts of the body. They are formed in the bone marrow.

2. **WBC (Leukocytes)** - White blood corpuscles containing antibodies that fight foreign bodies, which cause infections and disturb the immune system.

3. **Platelets (Thrombocytes)** - Platelets are blood cells that help in blood clotting.

4. **Plasma** - Plasma is the yellowish, liquid portion of the blood that contains electrolytes, nutrients, proteins and vitamins.

### 2.3 Major Functions of blood are-

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

### 3. Examination of blood

#### 3.1 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 230-269nm wavelength.)

#### 3.2 Presumptive Screening test -

The blood stain obtain from suspected area, contaminated with material should be tested for positive blood stain. Presumptive tests produce a color reaction or release of light, in presence of catalytic property of blood.

### 3.3 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. The sensitivity of phenolphthalein test is about 1:5 lakhs.

**Reagent -**

**Stock Solution**

| Phenolphthalein | 2.0 g |
| Potassium Hydroxide | 20.0 g |
| Distilled Water | 100 ml |
Zinc Dust 20.0g

Working Solution:

1: Ethanol 10 ml
2: Phenolphthalein Stock 2 ml
   Distilled Water, 10 ml
   Ethanol 2 ml
3: 3% Hydrogen Peroxide 10 ml

Reagent Preparation:-

The reagent are formed by adding phenolphthalein (2g), potassium hydroxide (20g) distilled water (100ml). Mix, add Zn powder, A few boiling chips 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 or in other words, it is responsible for colour obtained in the 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 hemoglobin.

3.4 Tetra methyl Benzidine (TMB) Test-

Reagent -

Acetate Buffer
- Sodium acetate 5.0g
- Glacial Acetic Acid 13 ml
- Distilled Water 57.0.0 ml

Working Solution
- TMB solution 1.5g
- Acetate Buffer 20.0 ml

Reagent Preparation-

Take 1.5gm of benzidine and 13ml of glacial acetic acid and57ml 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 hemoglobin.

3.5 Luminol test-
Luminol reagent-
- Sodium perborate: 0.7g
- 3-Aminophthal hydrazide: 0.1g
- Sodium bicarbonate: 5.0g

Reagent Preparation-
The reagent are formed by taking 0.7g of sodium perborate and 0.1g of 3-aminophthalhydrazide is mixed with 0.5g of sodium bicarbonate.

Procedure:
Take the suspected blood stain and add few amount of Luminol reagent appearance of fluorescent color indicate positive test of blood.

3.6 Leucomalachite Green test-
Reagent-
- Leucomalachite green: 0.1g
- Sodium perborate: 3.2g
- Glacial acetic acid 65%(Solution): 66ml
- Distilled water: 33ml

Reagent Preparation-
The reagent of Leucomalachite green test is formed by the combination of Leucomalachite green (0.1g), sodium perborate (3.2g), and Glacial acetic acid (66ml) in 33ml distilled water. If the color appear green, it confirms the presence of blood.
Procedure-
Add Leucomalachite green solution on the stain. If indicate green colour appears it indicate the the presence of blood.

4. Confirmatory test of blood:

4.1 Takayama test - It is also known as Haemochromogen test.

Takayama reagent –
Saturated solution of glucose 3ml
Pyridine solution 3ml
10%NaOH (sodium hydroxide solution) 3ml
Glacial Acetic Acid 7ml

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 with a cover slip. Add a drop of Takayama Reagent and allow to flow under the cover slip. Warm 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 hemoglobin.

4.2 Teichmann’s test-
Teichmann’s reagent-
KCl, KBr and KI 0.1 g each
Glacial Acetic Acid 100ml.

Reagent preparation-
For the Teichmann’s test, the reagent are formed by the combination KCl, KBr and KI at about 0.1g each in 100ml of Glacial acetic acid. The reagent react with hemoglobin and give 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 rhombohedron shaped crystals of ferroprotoporphyin chloride is a positive reaction for haeme.

5. Microscopic test-

Microscopic Method of examination is having paramount importance relating to identification of blood stain-
1. Determination of the species of origin by nucleated RBC’s along with the cell structure.
2. Sex determination by examining chromatin bodies in Leucocytes.
3. Detection of blood related pathological condition.

6. Spectroscopic Method-

In this method identification of haemoglobin and its derivatives is done by characteristics absorption band, when viewed through microscope.

Test-
A small portion of the suspected stain (as small as 2mm) is put in 0.5% Potassium cyanide solution for 15 min rest and then filterate. The filtered thus obtained is taken in 1cm cell and passed a U-V light in a spectrometer, the absorption observed at from 300 - 600 micron. The absorption maximum at 422 milli micron obtained that the presence of cyclohaemoglobin.

7. Detection of species origin of blood-

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.
The species specific proteins from the bloodstain or tissue are extracted in normal saline (8.5 g of sodium chloride in one liter of distilled water) or 5% ammonia solution.
The following method are applied to detect the species of origin-

7.1 Precipitin tube method-
Take six precipitin tubes (number can vary on the number of antisera used) and place them vertically in a precipitin tube stand and label. Put a drop of the bloodstain/tissue extract in the tubes. Carefully add one drop of antiserum for species origin (anti-Human serum, anti-Fowl serum, anti-Dog serum, anti-Cow Serum, anti-Goat serum, etc.) along the walls of tube. Leave undisturbed for 30 minutes at room temperature. Carefully examine the white ring at the interface of two solutions. If ppt is formed, it belongs to that specific anti-serum.

7.2 Double diffusion method-
In this method both of the reactants, antigen and antibody diffuse towards each other in agar gel plate, and when an antigen combines with its specific antibody at optimum proportions, precipitin are formed. Fill the central well with tissue extract and peripheral wells with different anti sera for species origin like (anti-Human serum, anti-Fowl serum, anti-Dog serum, anti-Cow Serum,
anti-Goat serum, etc.). Cover the Petri dish and keep gel in a moist chamber for overnight. Examine the gel for the presence of precipitin band form

7.3 Crossover Electrophoresis –
In a buffered gel, the stain extract (antigen) is placed in the cathodic well and the anti serum in the anodic one. When electric current is applied the globulin antibodies migrate cathodically because of electro endosmosis. The other serum proteins migrate anodically.

- A precipitin reaction occur midway between the paired well , When an antigen combines with its specific antibody.
- Fill the cathodic side well of the pair with stain extract and anodic well with species specific antiserum (anti-Human serum, anti-Fowl serum, anti-Dog serum, anti-Cow Serum, anti-Goat serum, etc.).
- Place the slide on the electrophoresis chamber. The stain extract should be nearest to the cathod and the antiserum nearest to the anode and Connect the gel to tank buffer chambers by two pieces of filter papers on each side. Run electrophoresis for 20 minutes at 150 volts. Record the conditions.
- After that electrophoresis, switch off the power supply and remove the slide and then observe the slide with the aid of a lamp. A fine white line of precipitate between a pair of wells is a positive reaction.

8. Age of blood stain-
Age of blood stain can be calculated by colour and nature of the stain –
In Fresh blood stain observed 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.

9. Detection of blood group-
After determining the origin of blood. It is also examined by prosecution to mention blood group. The modern serological techniques have divided blood in two three constituent classes for the discrimination of human blood, A,B and O.

9.1 The blood grouping and typing antigen-
Although several type of antigen have been discovered, in so far as dried blood stain are concerned, only ABO,MN and Rh antigenic system have been found of practical utility in the crime work.

9.2 The Polymorphic Enzymes-
These enzyme are located in red blood cells, which are responsible to catalyze biochemical reactions in body. The enzyme which have same function to perform but differ in physical-chemical properties are called as isoenzymes. Many such isoenzyme have been identified in dried blood stains using electrophoresis techniques. Few example of these isoenzyme are phosphoglucomutase (PGM), Adenylate kinase (AK), Erythrocyte acid phosphatase (EAP) and esterase D(EAD).
PGM system which has three common varients -
(a) PGM-1
(b) PGM2-1
(c) PGM-2 have been found very stable.

9.3 Rh system-

This system is however not found useful in the analysis of dried blood stain. It is more often used in disputed paternity cases in which whole blood analysis is done.

10. Factor affecting the detection of blood group from bloodstains -
Following factors are responsible for detection of blood group from stains
1. Putrefaction
2. Thermal condition.

11. Summary

- The blood is important evidence found at crime scene that leads to further investigation of various crimes.
- Blood examination has an important role in solving suicidal, accidental and homicidal cases.
- Various tests are performed to identify and confirm blood stains of human or animal origin.
- Identification of paternity and maternity cases by blood grouping.
- Species and origin of blood detection given indication of various animals.
- Determination of age of blood stains.