

# EXAMINATION OF BLOOD

## SEQUENCE:

- Red stain is blood or not
  - Screening
  - Confirmatory
- If blood is confirmed
  - Animal
  - Human
    - Male or Female
  - DNA Profiling & Blood Grouping

## STAIN IS BLOOD OR NOT:

- |  |  |
|--|--|
| <ul style="list-style-type: none"><li>➤ Screening</li><li>a) Physical<ul style="list-style-type: none"><li>• Screening by naked eye</li><li>• UV lamp</li></ul></li><li>b) Chemical<ul style="list-style-type: none"><li>• Benzidine test</li><li>• Phenolphthalein test</li></ul></li></ul> | <p><b>Confirmatory</b></p> <ul style="list-style-type: none"><li>a) Spectroscopy</li><li>b) Takayama test &amp; Teichmann's test</li></ul> |
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## A-PHYSICAL EXAMINATION:

- Naked eye examination: Visit to the scene of crime provides valuable clues.
  - 1) Clothing
  - 2) Size, shape height and direction of blood fall
  - 3) Character of stain from
    - Living or dead body
    - Artery or vein
    - Victim or assailant
    - Infant or adult
    - Male or female

### Chromosomal Investigation:

Barr and Davidson bodies are small condensation of nuclear and chromatin material, which stain more deeply than the rest. Barr body varies from 20 - 80 in females (chromatin +ve) and 0-4 in males (chromatin -ve). Barr body is present in epithelial cells of buccal mucosa while Davidson body is present in WBCs of the females.

- 4) Age of stain (with color changes of blood)
- 5) Condition of body
- 6) Other causes of blood stain (menstrual blood, hematemesis, abortion and stain due to bugs and insects bites)
- 7) Stains due to red paint, fruit juices, leaves juices, vegetable juices, which resembles the blood the stain.

To differentiate them, 2 screening tests may be performed on crime scene.

- Ammonia (NH<sub>3</sub>) turns vegetable and fruit stain green, Nitric acid turns aniline dyes yellow but blood remains unchanged.
- Alkaline solution of aminophthalic acid hydrazide is mixed with H<sub>2</sub>O<sub>2</sub> and sprayed on suspected area, the area will luminescent if stain is due to blood.



INSPECTION:

➤ Blood Drop Fall

When blood drop falls vertically downward from 2-3cm height, to a flat surface the shape of stain will be irregular circle.



Blood Drop

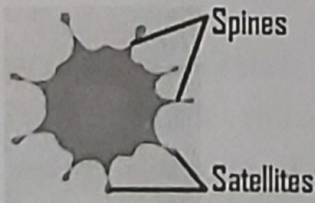
Vertically downward



Irregular Circle

➤ More height

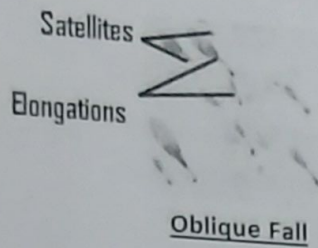
When the blood drop falls from more height (6-8cm), then it will show projections



With Projections

➤ Oblique fall

If blood drop is falling obliquely at flat surface, it will produced an elongated stain, resembling exclamation mark.



➤ Age of stain (with colour changes on white cloth)

- Fresh blood
  - Bright red because of hb
- Brownish red (24-48 hours)
  - Hb → Met Hb
- Dark brown /Black
  - More than 48 hours
  - Hb → Met Hb → Haematin

▪ Examination under UV lamp:

Under UV lamp followed by palpation and smelling of stain.

It is used to detect;

- **Stain on dark fabric**                      **Washed out stain**  
 On dark fabrics the stains are difficult to locate & may easily escaped on examination by naked eye.
- **On white fabric, look**  
 light yellowish.



## A) CHEMICAL TESTS:

- BENZIDINE TEST
- PHENOLPHTHALEIN

### PRINCIPLE:

These tests detect the presence of Hb (a peroxidase which in the presence of H<sub>2</sub>O<sub>2</sub> oxidizes colourless bases into coloured salts). The value of these tests lies in negativity.

- ❖ A-ve result will exclude the presence of blood.
- ❖ A+ve result given by many other substances in addition to blood

For Example: Plant juices, oxidizing agents (most common), pus saliva and mucous.

- **BENZIDINE TEST:**

**Composition:**

10% Solution of benzidine in glacial acetic acid and H<sub>2</sub>O<sub>2</sub>

**Logistics involved:**

benzidine reagent, suspected samples, filter papers and test tubes.

**Procedure:**

Place 1 drop of suspected sample in a tube, then add 1 drop of benzidine reagent and add a drop of 3% H<sub>2</sub>O<sub>2</sub>.

**Result:**

Immediate **bluish green color** will appear which indicates that the test is +ve. This test is highly sensitive test and positive with dilution of 1 part of blood in 500,000 parts.

- **PHENOLPHTHALINE TEST:**

**Composition:**

1-2 Grams of phenolphthalein, 20-25g of KOH, 100ml of distilled water, then add 15 grams of powdered zinc. This test is also very sensitive & give results in 1 part of blood with 500,000 parts.

**Logistics involved:**

Phenolphthalein reagents, suspected sample, filter papers and test tube.

**Procedure:**

Place 2-3 drops of suspected sample in a test tube then add 1 drop, of phenolphthalein reagent and then add 1 drop of 3% H<sub>2</sub>O<sub>2</sub>.

**Result:**

Immediate **pink or purple color** will indicate that test is +ve.



## CONFIRMATORY TEST:

### A-Spectroscopic Examination:

#### Logistics Involved:

- Suspected sample
- Micro spectroscope
- Oxidizing agents
- Reducing agents
- Micro test tube

#### Findings:

##### 1) Oxy Hb:

If the sample contains fresh oxygenated blood that is oxy Hb, two dark absorption bands will be seen between D and E lines. The one nearer the D line is about half the breadth of other one.

##### 2) Reduced Hb:

Addition of reducing agents like ammonium sulphide will cause these two bands to merge into one broad band of reduced Hb, between D and E line. An oxidizing agent will reverse the effect.

##### 3) Haemochromogen:

The addition of an alkali to Hb will form haemochromogen which will present two absorption bands. One darker band is almost midway between D and E line and the other fainter one lies in the green part of spectrum away from D line.

Note: if these three spectra are obtained from same stain, so it is conclusive proof of the presence of Hb and so of blood.

##### 4) Met-Hb:

If the stain is old, a certain amount of met-Hb will have been formed. It may occur *In-vitro* due to many substances and *In-vivo* as a result of poisoning by certain substances such as nitrates. This spectrum shows four bands, two bands, between D and E line, one in the red area and the fourth one in the green area of spectrum. The fourth band is the faintest one.

##### 5) Carboxy-Hb:

It is found in CO poisoning and it will show absorption spectrum much similar to oxy-Hb except that it is unaffected by the reducing agent.

## B-CONFIRMATORY OR MICROCHEMICAL TESTS.

### 1)TAKAYAMA/HAEMOCHROMOGEN CRYSTAL TEST:

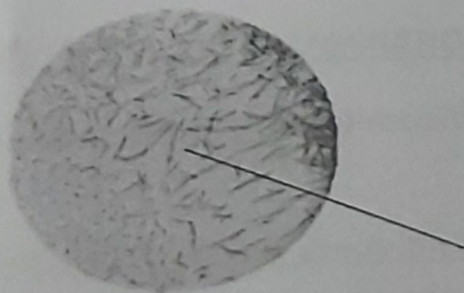
#### Logistics Involved:

- 10% NaOH 3ml
- Saturated solution of glucose 3ml
- Distilled water 7ml
- Pyridine 3ml
- Cover slips
- Burner
- Microscope
- Glass slide

Procedure: Take a drop of suspected sample on glass slide add 2-3 drops of Takayama's reagent. Cover it with cover slip and wait for 5-10mins. Then observe the slide under microscope.

Result: If results are + ve then Needle shaped (feather shaped)

crystals of salmon pink (orange pink) color are seen under microscope



Haemochromogen, Needle shaped,

(Salmon pink coloured) crystals



## 2) TEICHMANN'S / HAEMIN CRYSTAL TEST:

### Logistics Involved:

- Glacial acetic acid mixed with NaCl crystals
- Microscope
- Glass slides
- Cover slips
- Burner

**Procedure:** Take a drop of suspected sample on glass slide. Add 2-3 drops of Teichmann's reagent, then cover it with cover slip, heat it for few minutes wait for 5-10 mins and then observe the slide under microscope.

**Result:** Brown colour rhomboid shape crystals are seen which indicate test is +ve



Haemin Chloride, Rhomboid Shaped (Brown coloured) crystals

## SPECIES SPECIFIC EXAMINATION

### 1) Immunological Test (Precipitin Test)

#### Antigen – antibody precipitin test:

It is the test used to determine if the blood is human or not.

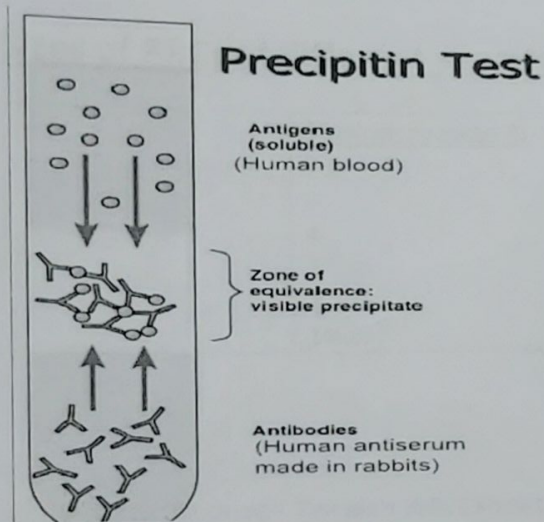
#### Principle:

This test depends upon the fact that when a foreign protein (agent) like human blood in small doses is injected into an animal (e.g. rabbit) repeatedly, in response to which antibodies are formed that are known as **precipitinogen**.



**Procedure:**

- Take a test tube, add small amount of anti-human serum in suspected sample of blood.
- If sample is positive for human blood then it will react with human proteins and a cloudy precipitate will form where the two layers meet. It represents the formation of antigen-antibody complex.

**Microscopic Examination:****Principle:**

It is based on the microscopic morphology of RBC. The fluid used should be isotonic with serum to avoid destruction of cells. Red blood cells in old stains are generally altered or destroyed and cannot be recognized.

**Apparatus:**

- Glass slides
- Cover slips
- Microscope
- GIEMSA stain

**Procedure:** Put a drop of blood sample in a middle of glass slide. Take another glass slide and slide it on the blood drop, make a thin layer of blood smear. Dry it and then dip it in GIEMSA stain for half an hour. Dry it again and wash it under clean tap water. Then finely examine the slide under microscope.

### Details of findings :

The RBCs of mammals are circular, biconcave and non-nucleated with the exception of camel. The RBCs in them are oval and non-nucleated. In birds, reptiles, fish and other amphibians RBCs are oval and nucleated.

### Sizes of RBCs of different species:

<u>Species:</u>	<u>Size(micrometer):</u>
Human	7.5
Cat	6
Goat & sheep	4
Deer	2
Frog	22x15
Pigeon	14x6

### WBC's Morphology:

WBCs help in identification of the sex of the source. Female's WBCs shows a drum-stick like structure, this extension of chromatin material is known as **Davidson's body**. This structure is absent in WBCs of males.

### SEROLOGICAL EXAMINATION (BLOOD GROUPING):

#### Mendelian Laws Of Inheritance:

- ❖ A blood group gene (antigen) cannot appear in a child, unless present in one or both parents.
- ❖ If one of the parents is homozygous for a particular blood group gene (antigen) it must appear in the blood of the child.

#### Logistics involved:

- 5% RBCs suspension
- Anti- sera A, B and D
- Glass slides
- Test tubes
- Centrifuge machine
- Microscope

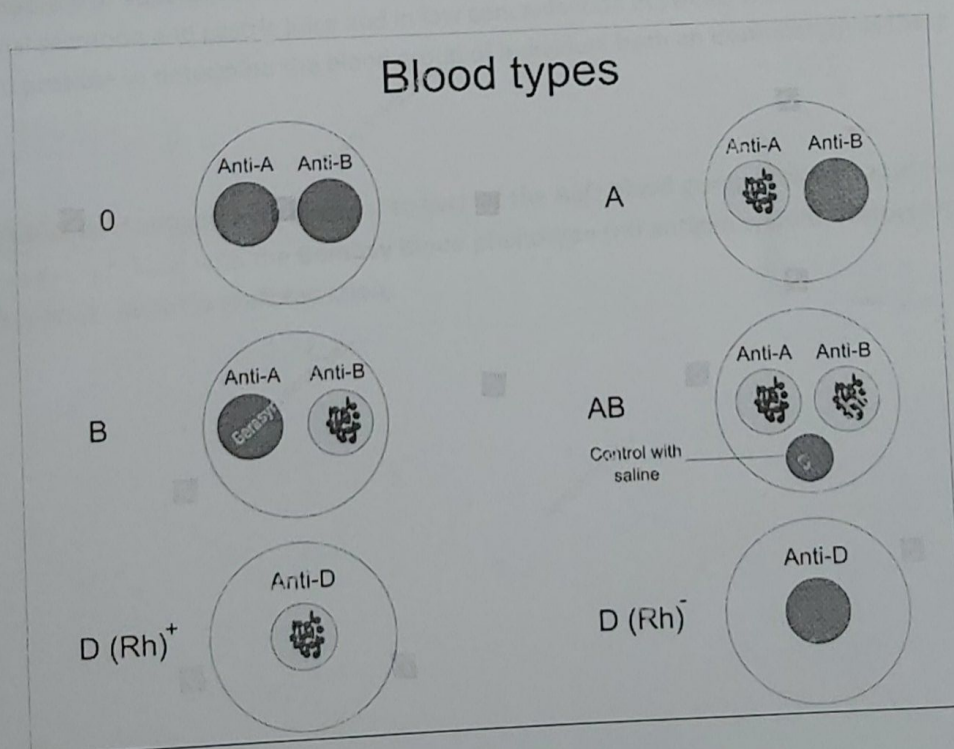


### Procedure:

- Prepare a 5% RBCs suspension with the help of centrifuge machine. In this way, separation of blood cells and plasma will take place.
- Take 3 glass slides and label them as A, B and D.
- In each glass slide add a drop of blood suspension (RBCs).
- Put a drop of Anti-sera A and B on a glass slide A and B respectively and add Anti-sera D on the glass slide D, then finally observe the findings (agglutination).

### Result:

- 1) If agglutination is present on glass slide A with anti-sera A, then blood group is **A**.
- 2) If agglutination is present on glass slide B with anti-sera B, then blood group is **B**.
- 3) If agglutination is present on glass slide C with both anti-sera A&B, then blood group is **AB**.
- 4) If no agglutination present on glass slide E with both anti-sera A&B, then blood group is **O**.
- 5) If agglutination is present on glass slide D with anti-sera D, then blood group is **RH+VE** and if absent then **RH-VE**.



## Blood Grouping:

Blood Group	On RBCs	In Plasma
	<b>Antigen (agglutinogen)</b>	<b>Anti-Body (agglutinin)</b>
A	A	Anti - B
B	B	Anti - A
AB	AB	None
O	H (substance)	Anti-A, Anti-B, (Both)

### ABO – SYSTEM:

An integral part of ABO system is secretory system . Approximately 80% of the population secretes ABO blood group substances in most of cells and fluids of the body. These individuals are known as **secretors**. The rest 20% who secrete very little known as **non- blood group secretors**. All secretors also secrete a "**substance H**". ABH group specific substances are found in high conc.in saliva, semen, vaginal secretion and gastric juice and in low concentration in sweat, tears and urine. It is therefore, often possible to determine the blood group of individual from an examination of these secretions.

### NOTE:

**SUBSTANCE H**, H antigen is a precursor to each of the ABO blood group antigens, apparently present in all people except those with the **Bombay Blood phenotype** (Hh antigen system) Histocompatibility antigen, a major factor in graft rejection.