# Bloodstain Examination

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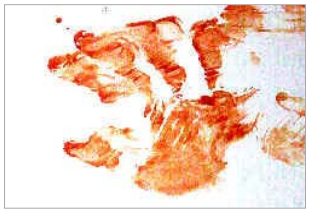
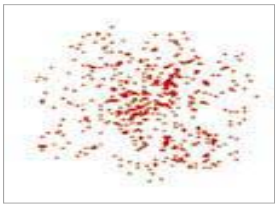
## Bloodstain Pattern Analysis

- **Blood stain Pattern Analysis is defined as the examination of the shapes, locations, and distribution patterns of bloodstains, in order to provide an interpretation of the physical events which gave rise to their origin.**

**Crime scenes that involve bloodshed often contain a wealth of information in the form of bloodstains. The pattern, size, shape, and the location of such stains may be very useful in the reconstruction of the events that occurred.**





## Some information that can be obtained from the examination of bloodstains

- The **direction** a given droplet was traveling at the time of impact.
- Approximate **positions** of the victim, suspect or other objects in the scene.
- The **angle of impact** .
- An estimated **distance** between the target and the point of origin.
- The **type of force** used to create the bloodstain pattern (drip, blow, gunshot, etc).
- The **direction from** which the force was applied.

## Forensic Analysis of Blood Stains:

1. Visual or physical examination of the blood evidence.
2. Presumptive screening test (Is it blood?)
3. Confirmatory test (Seriously, is it blood?)
4. Determine species origin (Is it human or animal blood?)
5. Identify the blood (Whose blood is it?)

## 1- Physical Examination of Blood Stain:

- Shape,
- Size,
- Color,
- Pattern of spatter,
- Age of the stain, antemortem or postmortem stain.
- Determine the angle of impact.

## SHAPE, SIZE AND PATTERN

:

- The type of surface the blood strikes affects the resulting spatter, including the size and appearance of the blood drops
- On clean glass or plastic the droplet will have smooth outside edges.
- On a rough surface,

will produce scalloping on the edges.

- The shape also related to the movement of the animal: standing animal produces circular stain, moveable animal shows pear shaped stain.
- In case of running animal the stain will be pear shape and a small circular spot in front of it.

## Angle of impact:

- Definition: it the angle at which blood strikes a target surface.
- The shape of a blood drop differs according to the angel of impact:
- Round: if it falls straight down at a 90 degree angle(right angle), vertical.

✓ Elliptical: blood droplets elongate (tear drop) as the angle decreases from 90 to 0 degrees (acute angle).

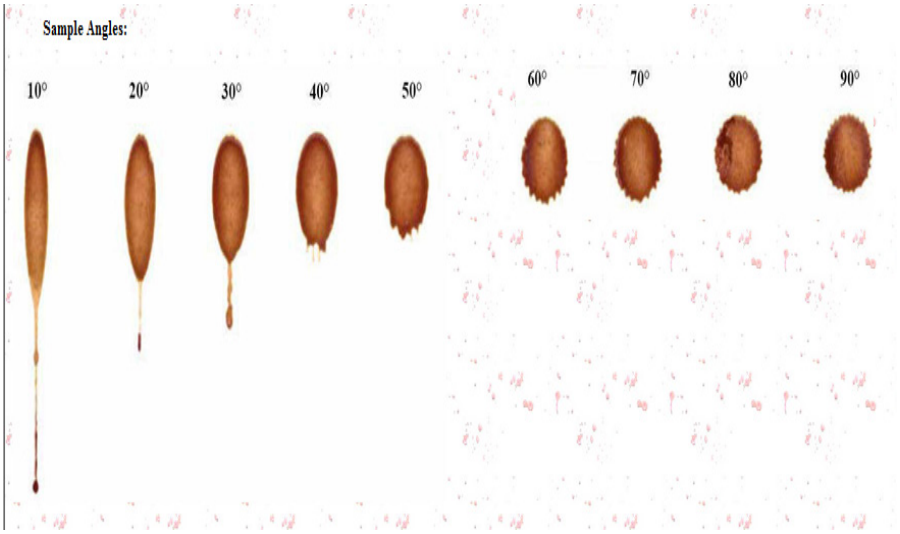
✓ The pointed end of the drop (tail) will indicate the direction of movement.

- **The more acute the angle of impact, the more elongated the stain.**
- **90 degree angles are perfectly round drops with 80 degree angles taking on a more elliptical shape.**
- **At about 30 degrees the stain will begin to produce a tail.**
- **The more acute the angle, the easier it is to determine the direction of travel.**

✓ **from 90 to 0 degrees; the angle can be determined by the following formula:**

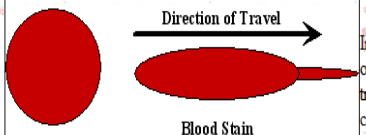
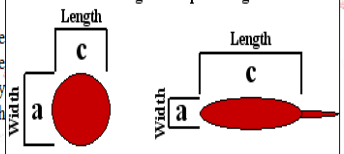
**Impact Angle = sin(-1) (width/length)**

***The shape of a blood drop differs according to the angel of impact***



## Impact Angle = sin(-1) x width/length

Stain Shape, Direction, & Angle of Trajectory:

| Direction of Travel | NOTE: | Angle of Trajectory |
|---|---|---|
|  Blood Stain 90° Angle onto a smooth surface. Blood Stain Acute Angle Note the projection of the stain at one end. That projection shows the direction of travel. | In this lab, you have the option of calculating the trajectory, or you may compare the stains here with the angles we made in class. **In a real crime scene, it would be *calculated*.** | Calculating the Impact Angle  1. Measure the Width (a) 2. Measure the Length (c) 3. Use the following formula $SIN <= \frac{\text{Width (a)}}{\text{Length (c)}}$ |

**TO SOLVE A BLOODSTAIN'S *ANGLE OF IMPACT***

**10** mm Width / **20** mm Length = **0.5** = **SIN** of the Angle

**ARC SIN** of 0.5 = **30**

The *Angle of Impact* is **30°**

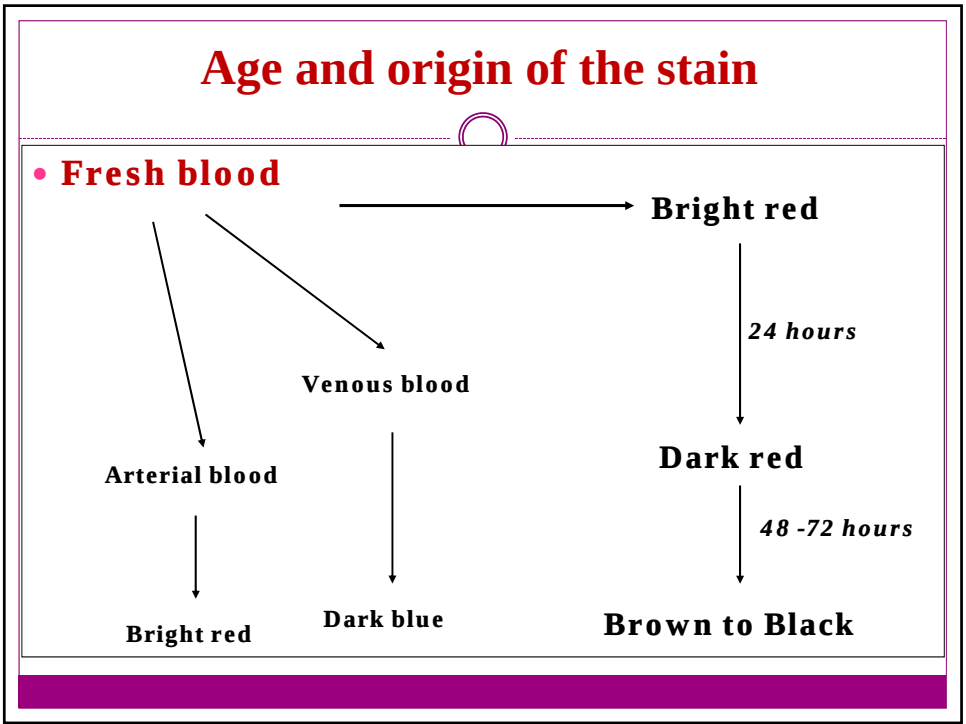
## What else can we get from looking at bloodstain patterns?

- **Direction of travel**

# Color of the stain

- It indicates the age and the origin of the blood stain.
- The fresh blood stain is bright red, moist and sticky.
- In 24 hours, its color changes to reddish brown.
- After 24 hours it becomes dark brown.
- After several weeks it may look black.

# Age and origin of the stain



## WHETHER STAIN IS CAUSED BY ANTE-MORTEM OR POST-MORTEM BLOOD

- Since clotting is observed in ante-mortem blood only, a fibrinous network due to clot formation can be seen in stains due to ante-mortem blood.
- This clot formation and fibrinous network would be absent in stains due to post-mortem blood.

## You have to answer the following questions:

1. **is the stain blood?**
2. **Is it animal or human blood?**
3. **If animals blood, what kind of animal species??**
4. **If it is human's blood, whose blood????**

## Is the stain blood?

**How to Collect the bloodstains from any surface And how to dissolve.**

Identification of blood by chemical tests.

- **All these tests have one thing in common; in one way or another , they all detect hemoglobin.**
- **No other material except blood contains hemoglobin, and if it is certain that hemoglobin is present, then it is certain that the material is blood.**

## Detect the presence of blood

- There are two categories of chemical tests used to detect the presence of blood:

### 1- Preliminary or presumptive tests:

They are generally quick, easy to do, and very sensitive BUT They are NOT specific for blood, as some plant enzymes and vegetable peroxidases cause false positive results.

(preliminary tests, screening tests or field tests)

### 2- Confirmatory or conclusive tests:

A number of different tests have been used to confirm the presence of blood in the tested stain.

## Tests for Identifying Blood

**1. Presumptive Screening Tests for Blood:**

- These tests are based on the presence of peroxidase enzyme activity of haemoglobin which releases nascent oxygen from hydrogen peroxide when added to it. This nascent oxygen changes the colour of the reagent added.
- Good negative tests,
- Some vegetable stains, salivary stain, rust or pus may give a positive test.

## Presumptive or screening Tests:

**- OXIDASE TESTS**

- Benzidine: Positive result = blue color
- **Kastle-Mayer test** (Phenolphthalein): Positive result = **pink**
- Ortho-Tolidine: Positive result = blue
- Tetramethylbenzidine (TMB): Positive result = Green to blue-green.
- Leucomalachite green: Positive result = green.

## Presumptive Test
### - Luminol color test

- A chemical called luminol is sprayed across the scene because it reacts to blood by making it luminescent. It only takes about five seconds.
- Luminol is combined with oxidant and sprayed over the area thought to contain blood.
- Emits a **blue-white** to **yellow green** glow.
- There is one problem with this test: luminal can destroy the properties of the blood that investigators need for further testing.

✓ **Luminol Test:** The luminol reaction is a presumptive test for blood. If the stain is so dilute that it can only be visualized with luminol.

## Fluorescein test:

- **Fluorescein** is a **presumptive test for blood**.
- It is useful in the detection of patterns of older, indistinct or latent bloodstains and in detecting the residue of **blood** remaining after a stain has been cleaned.

- Fluoresces when treated with a UV light.
- No interference with DNA analysis.

## 2- Confirmatory or conclusive tests idea:

**Searching for any component of blood such as:**

- **Cells:** ( shape and diameter of RBCs).
- **Hemoglobin:** (microcrystal test, spectroscopical examination).
- **DNA finger print** in the nucleated part of blood cells (WBCs).

## Confirmatory or conclusive tests

A number of different tests have been used to confirm the presence of blood in the tested stain.

- **Microscopical tests.**
- **Microcrystal tests.**
- **Spectroscopical test.**
- **Immunological or precipitin tests.**

### 1. Microscopic Examination:

**It is very good for fresh blood as it can identify the presence whether that of red blood cells or of white cells, under the microscope. In cases where only stain is present, a stain extract is made and then it is examined under microscope for blood cells.**

- **Shape and diameter of RBCs in different animals**

# Microscopical examination of blood

- **RBCs**

Non nucleated **circular** cells
In all mammals except **camel**

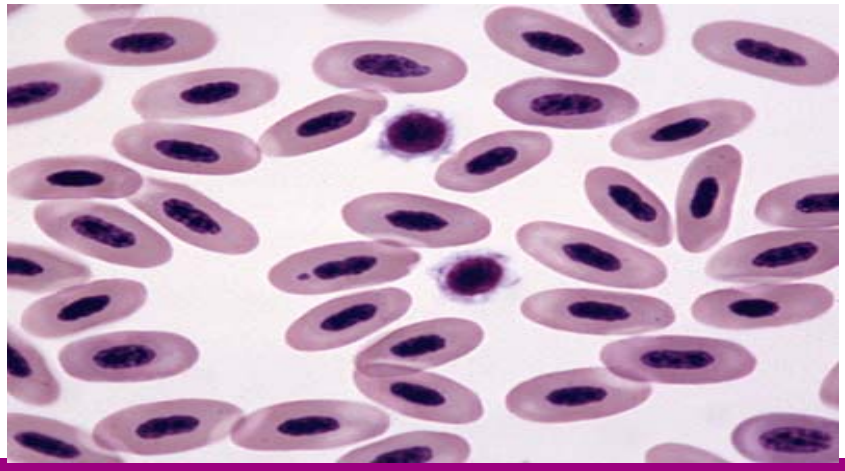
**In camels, non nucleated and oval**

**Oval nucleated cells in Birds, Amphibians, reptiles and Fish**

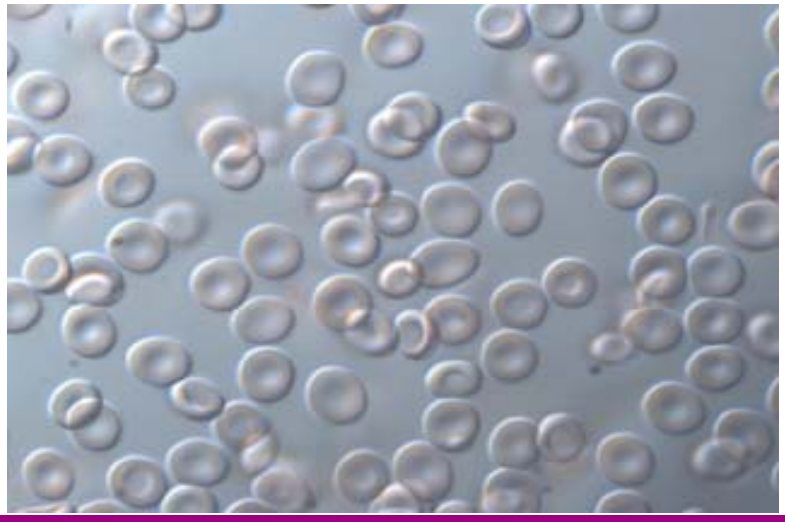
**•Diameter of RBC can be classified into:**

| | | |
|---|---|---|
| **Goat 4.25 µ** | **sheep 4.50 µ** | **Cow 5.50 µ** |
| **Horse 5.70 µ** | **Cat 5.80 µ** | **Pig 6.00 µ** |
| **Dog 7.00 µ** | **Man 7.50 µ** | **Elephant 10.00 µ** |

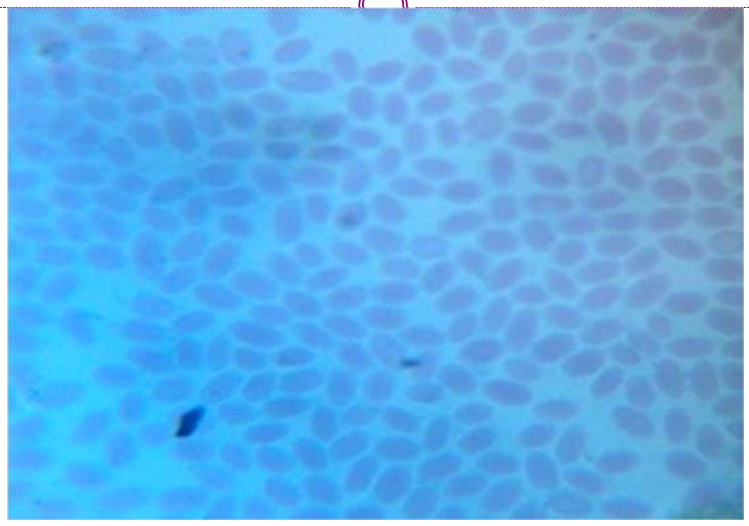
# Oval nucleated cells: birds, fish, amphibians and reptiles.

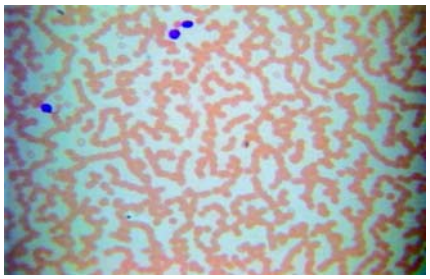


## Circualr non-nucleated cells: all mammals, except camel

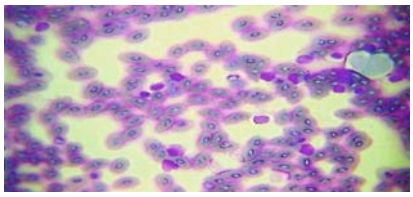


## Camel RBCs: Oval, non nucleated

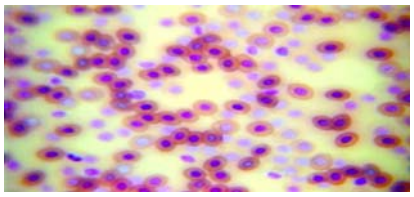




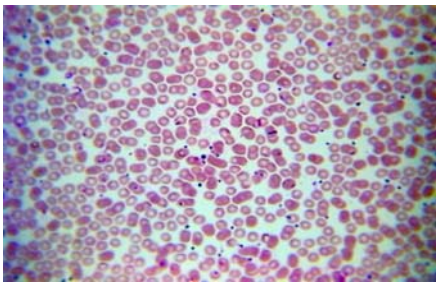
Horse Blood



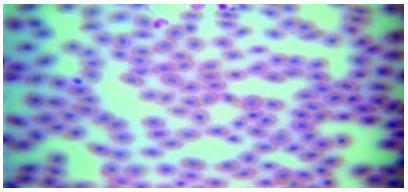
Fish Blood



Frog Blood



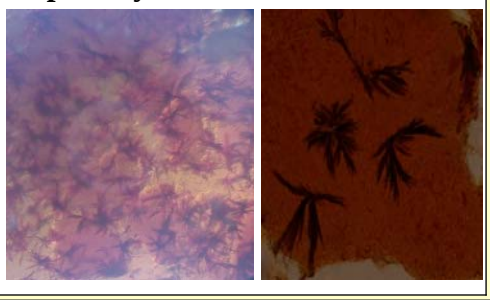
Human Blood



Snake Blood

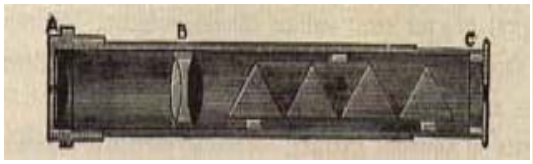
## 2. microcrystal Tests:
## (haemin and hemochromogen)

- **These tests are based on the** property of iron in the haemoglobin to form characteristic colored crystals with certain reagents. These crystals can be easily seen under a microscope.

| **A- haemin crystal test (Teichman), dark brown rhomboid crystals.** | **B - haemochromogen crystal test (Takayama), pink feathery, needle shaped crystals.** |
|---|---|
|  |  |

## 3. Spectroscopical Examination:

- **This test is based on the fact that hemoglobin and its derivatives have characteristic absorption bands in the spectrum.**
- **This is a very reliable test.**
- **In this, absorption spectra of stain is prepared and compared with absorption spectra of haemoglobin and its derivatives.**
- **Oxyhemoglobin, reduced hemoglobin,carboxy hemoglobin and methemoglobin).**



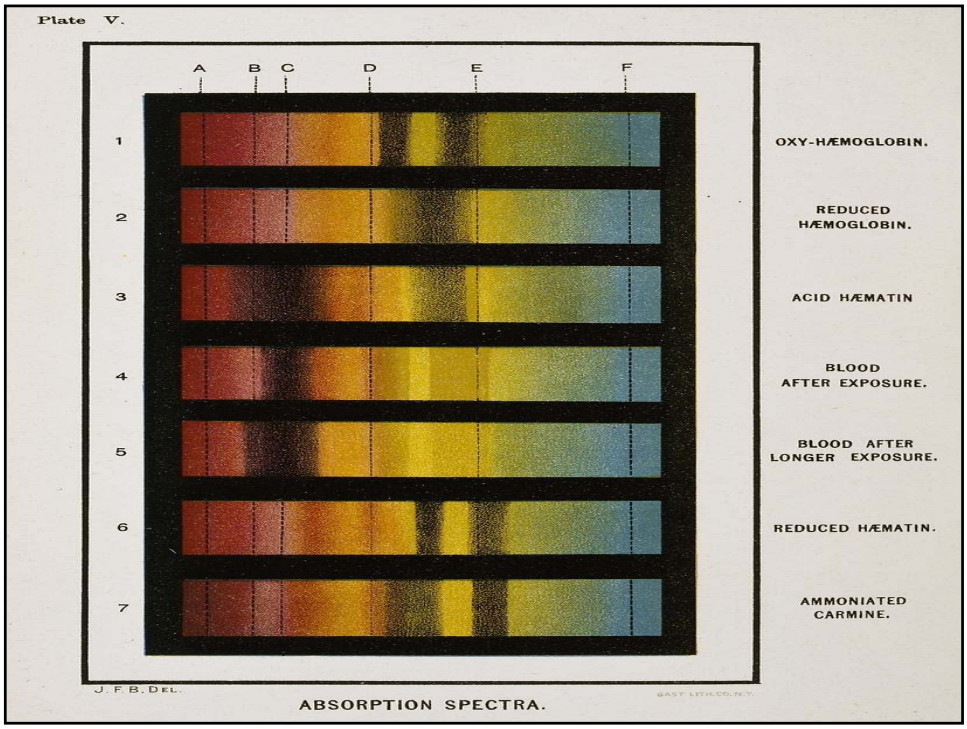

## Advantages of Spectroscopical examination of blood

- It is very simple and easy to carry out.
- It can be done on a very minute amount of blood.
- The used blood can be reused for another chemical tests.
- It can be used for diagnosis of some toxic case such as:

❖ CO ⟶ Carboxy Hb

❖ Sulpha ⟶ Sulph Hb

❖ HCN ⟶ Cyanomet Hb

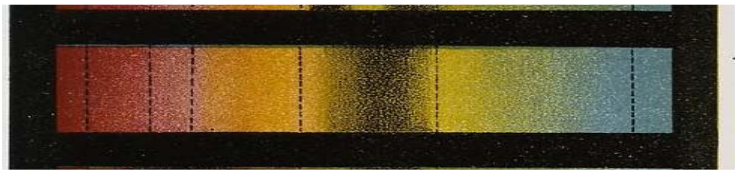



## Oxyhaemoglobin

- Two absorption-bands between D and E, the one nearer the D line being the narrower.
- Add a reducing agent (*ammonium ferrotartrate* reagent) to the solution and again examine spectroscopically.
- Note that the two absorption-bands of oxyHb transformed to one broad band of reduced Hb.

## Reduced Hb

- Note that in place of the two absorption bands of oxyhaemoglobin we now have a single broad band lying almost entirely between D and E. This is the typical spectrum of reduced hemoglobin.



## Carboxy Hb

- Observe that the spectrum of this derivative resembles the spectrum of oxyhaemoglobin in showing two absorption-bands between D and E. The bands of carbon monoxide haemoglobin, however, are somewhat nearer the violet end of the spectrum.
- Add some *ammonium ferrotartrate* reagent which is a reducing agent to the solution and again examine spectroscopically. Note that the position and intensity of the absorption-bands remain unchanged.

## Methaemoglobin and Sulphahemoglobin

- Both give four absorption bands , two of them are the same as those of Oxy Hb and Carboxy Hb.
  The third is to the left of D.
  The fourth is to the right of E
- To differentiate → add reducing agent as ***ammonium sulphide or sodium hydrosulphite or ammonium ferrotartrate*** to the solution and examine again.
- Methemoglobin will be reduced meanwhile no change in case of sulphahemoglobin.

## Serological Characters of blood stains

## SPECIES IDENTIFICATION

- **Once it is identified as a blood stain, next step is to identify whether it is of human or animal origin.**
- **It is done by precipitin test.**

✓ **Precipitin Test:**

✓ **This is a very specific test and quite reliable.**

✓ **It is based on antigen-antibody reaction.**

**Method:**

**1- Preparation of antiserum:**
**Antihuman serum is obtained by injecting human serum in rabbits.**

**2- Stain extract is prepared and treated with anti-human sera.**

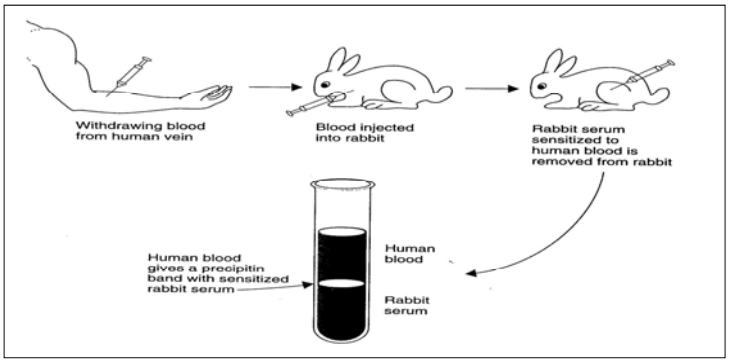
**3- Result: A positive reaction is obtained by precipitation and a ring is observed.**

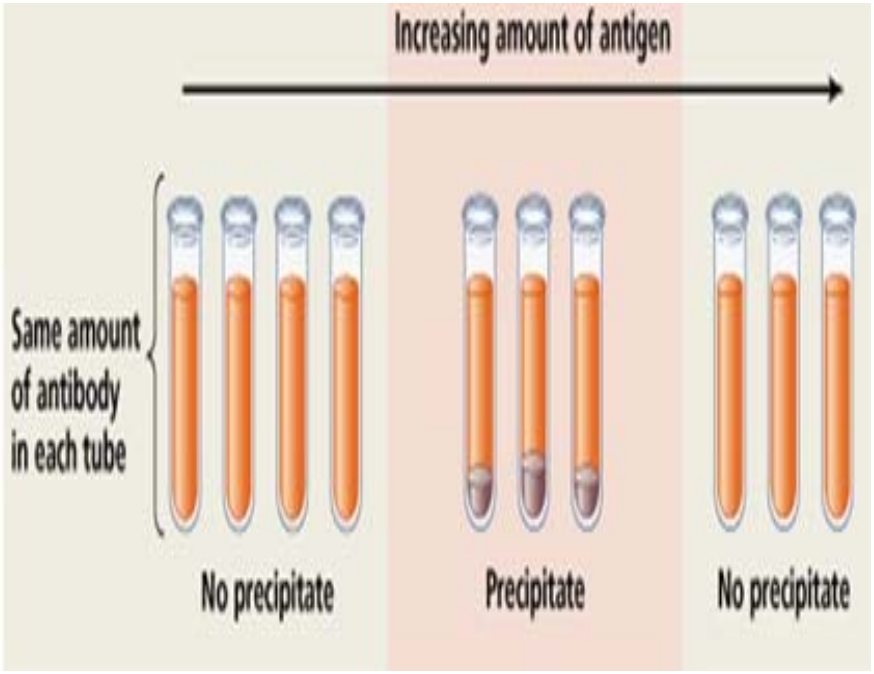
## Human vs Animal Blood

- **Precipitin test—** human blood is injected into a rabbit; antibodies are formed; the rabbit's blood is extracted as an antiserum; the antiserum is added to a blood sample.
- The sample will react with human proteins, if human blood is present.
- This test is very sensitive and requires only a small amount of blood.

## Antiserum

**-When the body is exposed to foreign protein, it develops antibodies.**
**- when this Ab comes into contact with the antigen, Ag-Ab reaction takes place.**



**Problems????**

## Group Reaction in precipitin test:

The antiserum precipitates other sera of related animals as:

- **Antisheep serum precipitates goat serum**
- **Antihorse serum precipitates donkey serum**

✓**How to solve this problem?**

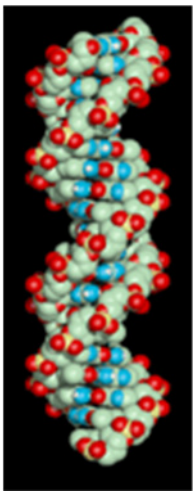
**1- Dilution of the antiserum with normal rabbit's serum.**

**2- Use the related animal in preparation of antiserum.**

**3- precipitation and filtration.**

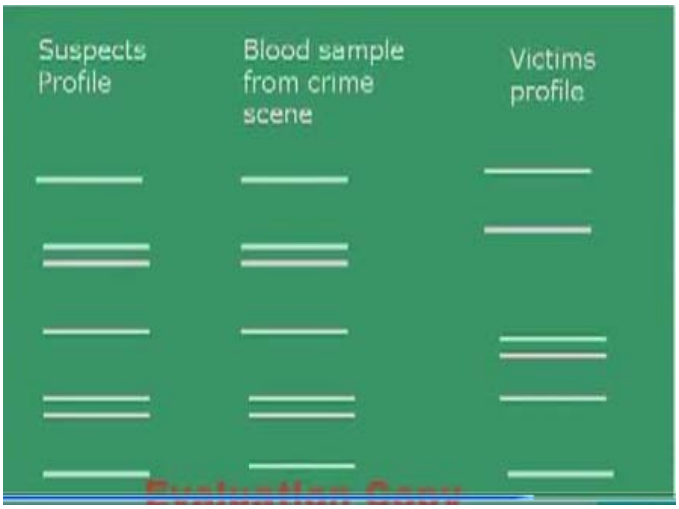
## DNA Fingerprinting

- DNA profiling or typing is sometimes called DNA fingerprinting because it allows the police to identify an individual in the same way as fingerprints do.
- DNA can be extracted from any body fluid (blood, saliva, sweat, nasal mucus,,,etc) or from fragments of a body (hair roots, torn skin or flesh).

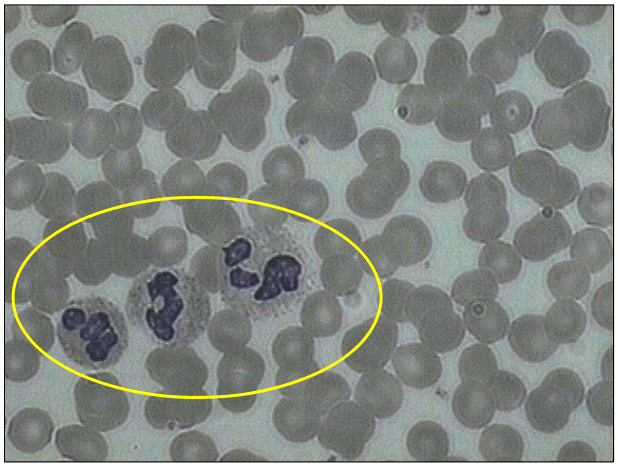


## DNA FINGER PRINT(PROFILING)

**A technique used to distinguish between individuals using samples of their DNA.**




## DNA in the blood stain

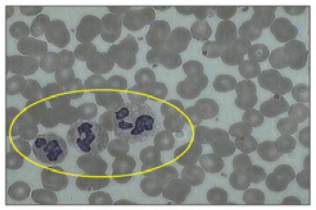


White Blood Cells = DNA in nucleus

## How is DNA Tested:

**1- Isolation of DNA (Extraction ):** This DNA is recovered from the blood using chemicals and enzymes to break and open the cells and release the DNA into a solution.

DNA in the blood stain



White Blood Cells = DNA in nucleus

**2- Amplification of DNA by polymerase chain reaction (PCR) selectively copies the unique parts of DNA.**

## The polymerase chain Reaction PCR (DNA amplification)

**Ability to make millions of copies of specific sequence of DNA in few hours.**

**PCR is an enzymatic process in which specific region of DNA is replicated to yield many copies of particular sequence.**

**Process (Amplification) involves heating and cooling samples in thermal cycling pattern over 30 cycles.**

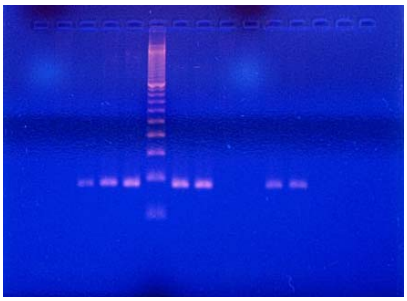
**During each cycle, a copy of the target sequence (amplicon is generated ).**

**The target sequence (amplicon) linked to a radioactive isotope and compared to standard**

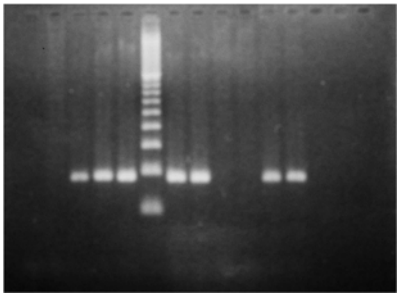
Thermocycler for PCR

**3- Electrophoresis** to separate the DNA bands on agarose gel then matching with reference sample.

**Agarose Gel Electrophoresis**

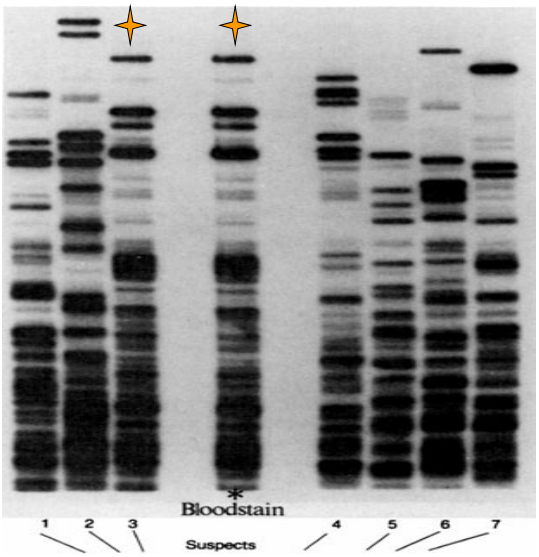


**UV Photograph**



**Polaroid Photograph**

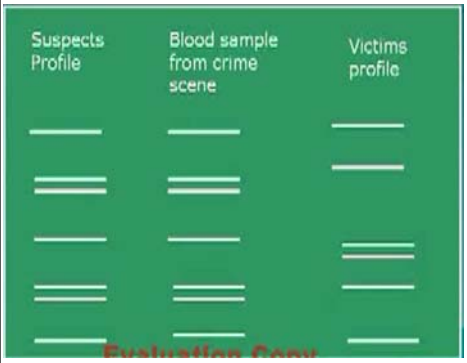
## DNA Fingerprint



## Stage 4:

- The pattern of fragment distribution is then analysed.

**Used to solve crimes**




**Used for paternity testing**