HLS MODULE
PHYSIOLOGY LAB 1
HEMATOLOGICAL TESTS

BY

Dr. Fatma Farrag Ali

Associate Professor of Medical Physiology

Faculty of Medicine-Mutah University

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1- Determination of Hemoglobin (Hb) Content

Apparatus: **Sahli apparatus (hemoglobinometer).**

Principle:

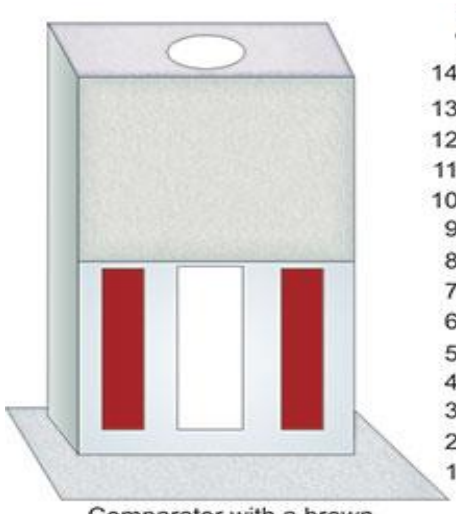
This methods depends on measurement of the Hb content (or its % of normal) by matching the blood color with a standard solution that has the color of normal blood (containing 15 gm Hb%).

Sahli apparatus consists of:

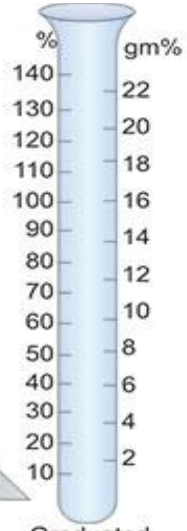
1. **A pipette** to measure **20 cu mm (µl) of blood**.
2. **A central graduated diluting tube**; two graduations are present:

- Yellow colored graduation indicating amount of Hb in g/100 ml blood.
- Red colored graduation indicating Hb% of normal in blood (the normal is 100% = 15 gm/100 ml blood).

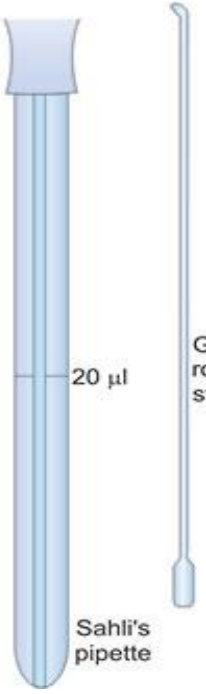
3. **Two lateral colored standard tubes**.



Comparator with a brown glass standard



Graduated hemoglobin tube



Sahli's pipette

Glass rod or stirrer

Method:

1. **0.1N HCl** is added to the diluting tube up to the mark **10%**.
2. Sterilize the finger with alcohol; allow drying and making a pin prick.
3. Suck blood with the micropipette up to the mark **20 cu mm (0.02 ml; 20 µl)**.
4. Evacuates the micropipette into the diluting tube to which 0.1N HCL was previously added. **HCl converts Hb to acid hematin (brownish)**.
5. Distilled water is then added drop by drop to the sample till its color becomes the same as that of the standard tubes.
6. The Hb content (in gm) and its % of normal can then be directly obtained from gradations on the dilution tube.

RESULTS

The amount of hemoglobin in:

1. Normal adult male: is 14-17 gm %. (average: 15 gm %)
2. Normal adult female: is 12-15.5 gm%. (average: 13.5 gm %)
3. Newborn : is 19-20 gm%.

Clinical significance:

Diagnosis of anemia or polycythemia.

Estimation of Haemoglobin (Hb)

2- Measurement of Hematocrit value (Hct)
or
Packed Cell Volume (PCV)

Hematocrit value (Hct)

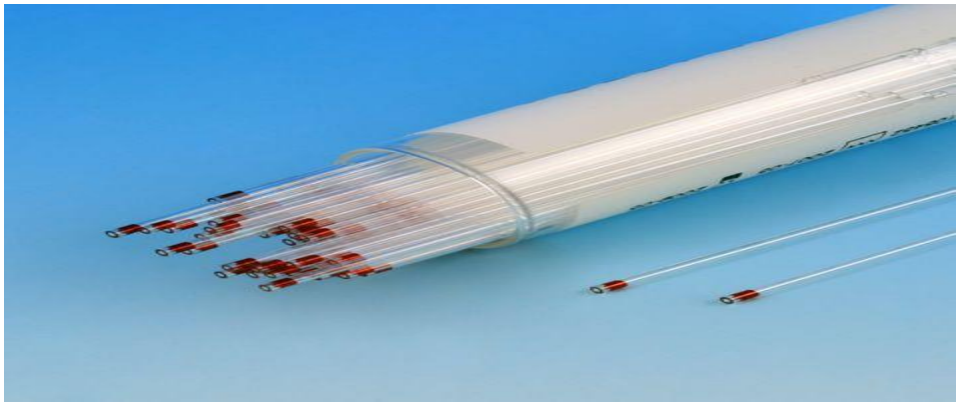
Definition: Percentage ratio of RBCs volume to total blood volume.

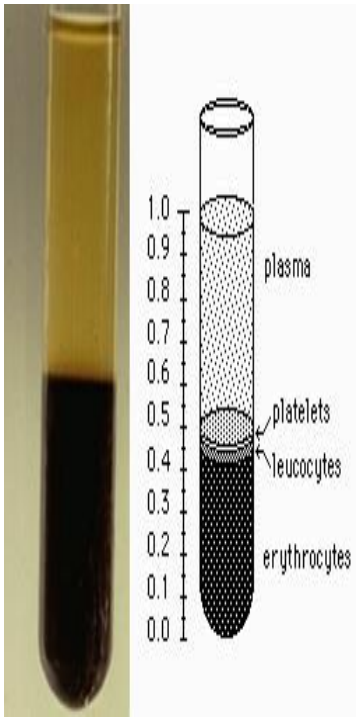
OR the volume of packed RBCs (PCV) in 100 ml Blood.

So, it is also called **PCV**.

$$\text{Hct (PCV)} = \frac{\text{Packed cell volume}}{\text{Total blood volume}} \times 100$$

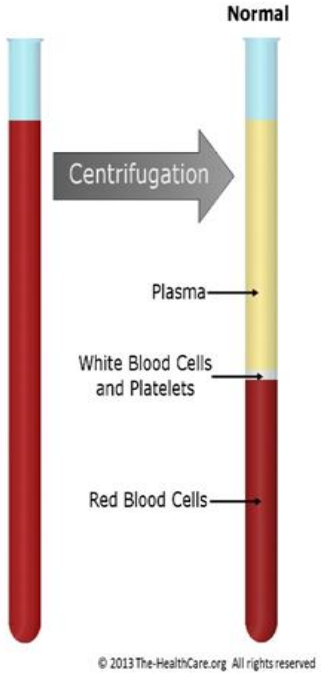
Method of estimation: graduated heparinized capillary tube.





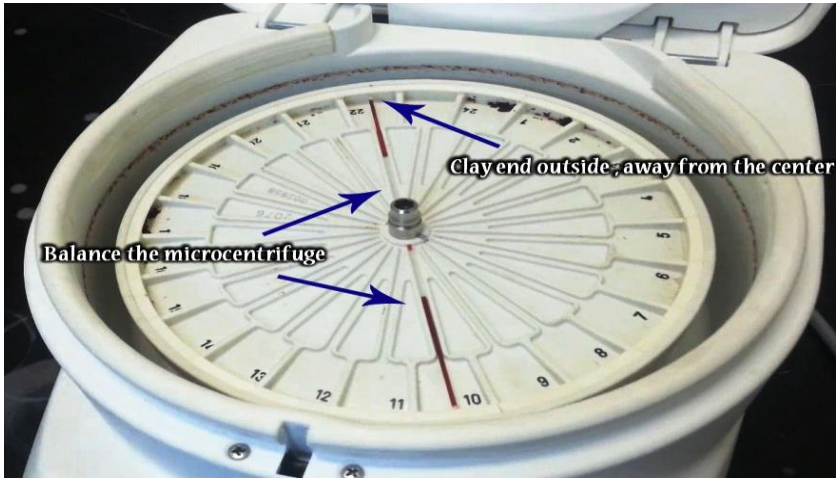
Principle:

- Separation of blood cells from plasma by centrifugation depending on differences in specific gravity.
- RBCs have specific gravity 1090 will sink into the bottom with WBCs and platelets (specific gravity 1060) and plasma (1025-1030) on top.

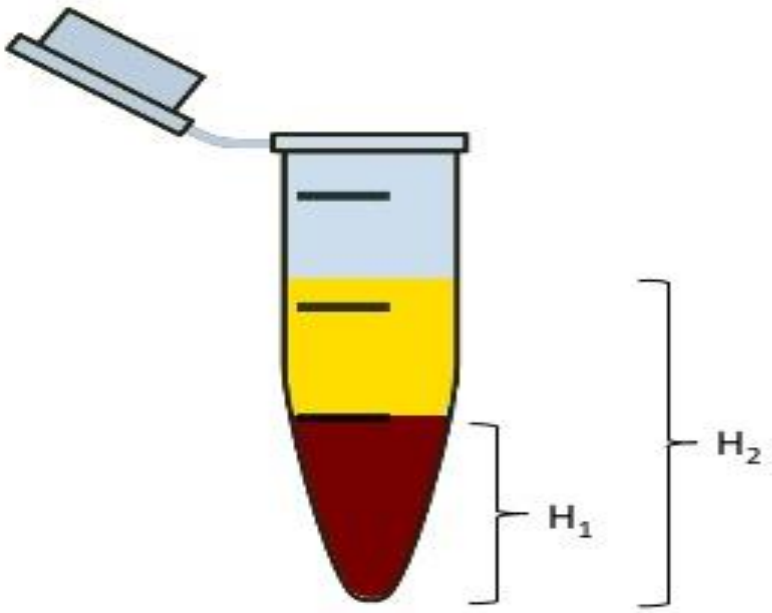


Steps of measurement of Hct (PCV)

- A blood sample from the subject is placed in a graduated heparinized capillary tube and is then centrifuged.
- The red cells are packed in the bottom of the tube while the clear plasma remains above and the white cells and platelets form a small buffy layer just above the red cell column.
- Hct is then calculated by dividing the red blood cells column by the total blood column and multiplying by 100.



Hematocrit Determination



Where:

- H_1 = height of the RBC column
- H_2 = height of the RBC + height of the plasma column
- Calculate Hc% (hematocrit) value

Calculation:

$$HCT = \frac{\text{Length of column of RBC}}{\text{Total length of blood component}} \times 100$$

RESULTS

Normally, Hct is about :

- **45%** for adult male.
- **35%** for adult female.
- **60%** for newborn (due to polycythemia).

Changes in Hct

Hct is affected by changes in red cell volume relative to the plasma volume.

Decreases in:

1- Anemia

2- Hydration

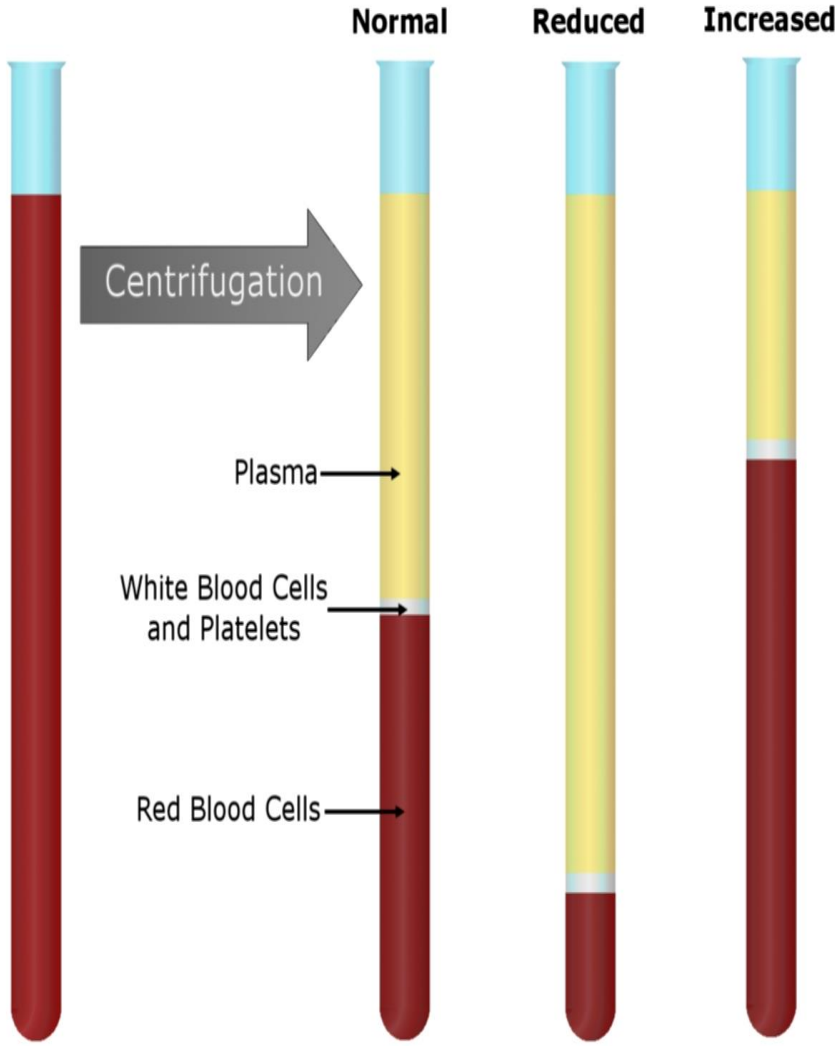
3- After fluid transfusion

Increases in:

1- Polycythemia

2- Dehydration

3- Loss of plasma as in burns



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Hct measurement video

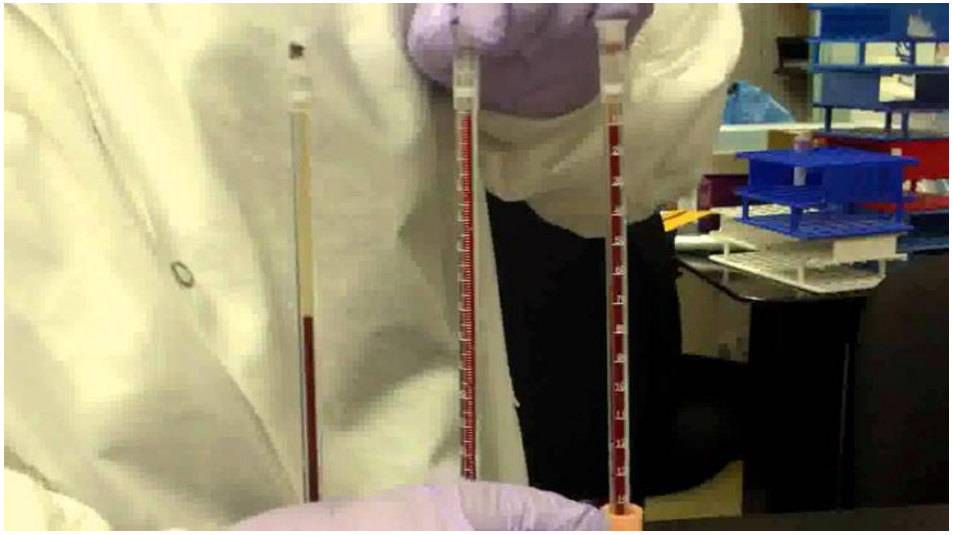
3- Measurement of Erythrocyte Sedimentation Rate (ESR)

Definition

It is the distance sedimented by R.B.Cs in **mm** when put in a vertical stationary tube at the end of 1 hour.

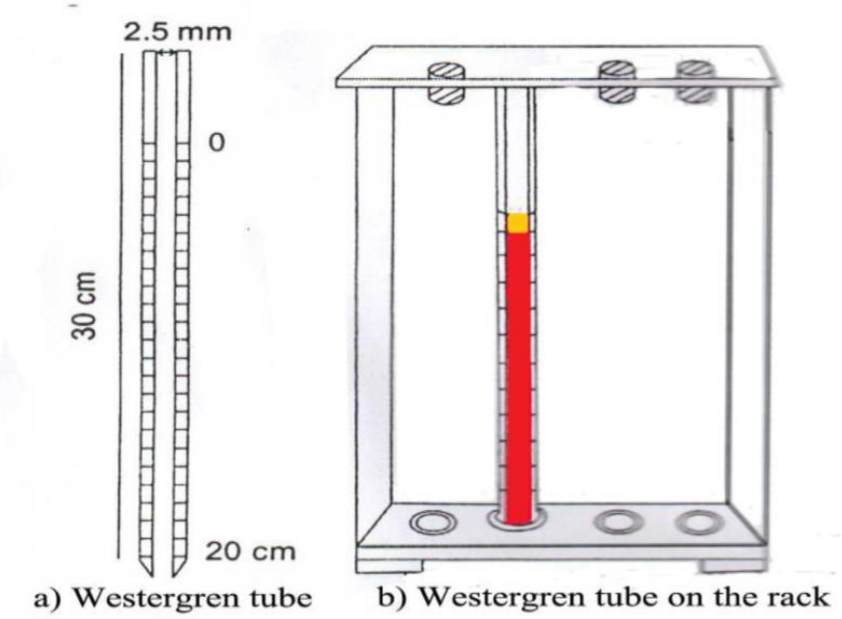
Method of ESR determination

- Westergren tube



WESTERGREN TUBE

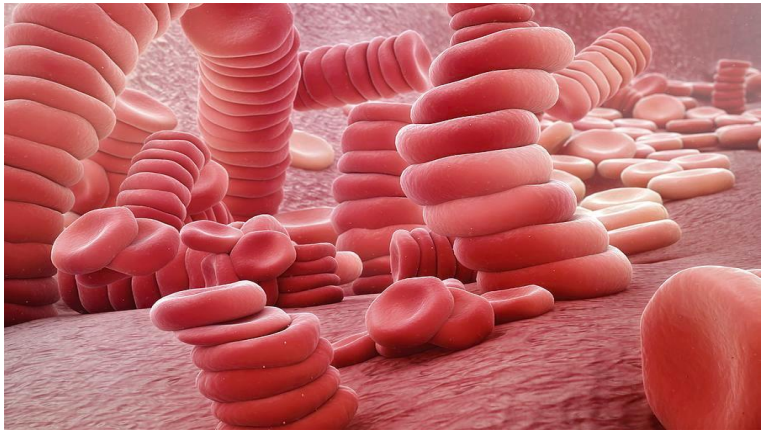
- ✓ The Westergren tube is open at both ends.
- ✓ It is 30 cm in length and 2.5 mm in diameter.
- ✓ The lower 20 cm are marked with 0 at the top and 20 at the bottom.



Principle:

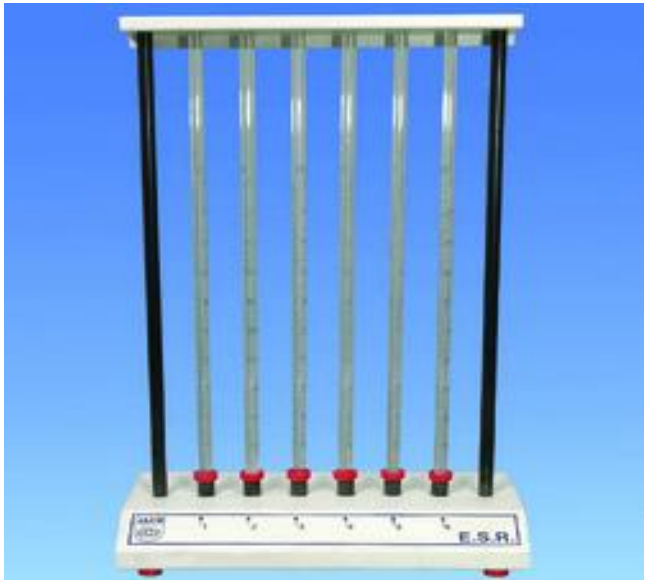
- ✓ When anticoagulated blood is allowed to stand in a narrow vertical glass tube, undisturbed for a period of time, the RBCs settle out from the plasma.
- ✓ The rate at which they settle is measured as the number of millimeters (mm) of clear plasma present at the top of the column after one hour (mm/hr).
- ✓ RBCs tend to sediment towards the bottom because of:

1. RBCs density is greater than that of plasma.
2. RBCs tend to aggregate forming rouleaux shapes in which the ratio of mass to surface area increases and being heavier than plasma, they sediment to the bottom more rapidly.



REQUIREMENTS :

- Anticoagulated blood (0.5 ml of 3.8% sodium citrate solution + 2 ml blood): 1 part citrate to 4 parts of blood (anticoagulant / blood ratio: 1:4).
- Westergren tube.
- Westergren stand.
- Rubber bulb (sucker).



Procedure:

- Mix the anticoagulated blood.
- Draw the blood into Westergren tube up to 0 mark with the help of rubber bulb.
- Set the tube upright (vertical position) in the stand.
- Leave the tube undisturbed, the RBCs settle down leaving a clear plasma column above.
- At the end of 1 hour, read the result.

Normal values of ESR:

Sex	1st hour
Male	5 mm/hr
Female	8 mm/hr

Factors that affect ESR:

Factors that increase ESR:

1. **Physiological :** as during **pregnancy** and **menstruation, after exercise and after meals**.
2. **Pathological:** ESR generally increases in cases of **inflammation and tissue destruction** due to increased fibrinogen or globulins e.g.

- Acute & chronic infection: tonsillitis, appendicitis and tuberculosis.
- Myocardial infarction.
- Degenerative & neoplastic diseases
- Rheumatoid arthritis.

Factors that decrease ESR:

Pathological decrease in: polycythemia.

N.B.: In anemia, ESR increases except in iron deficiency anemia and spherocytosis (ESR decreases due to reduction of intrinsic ability of RBCs to sediment).

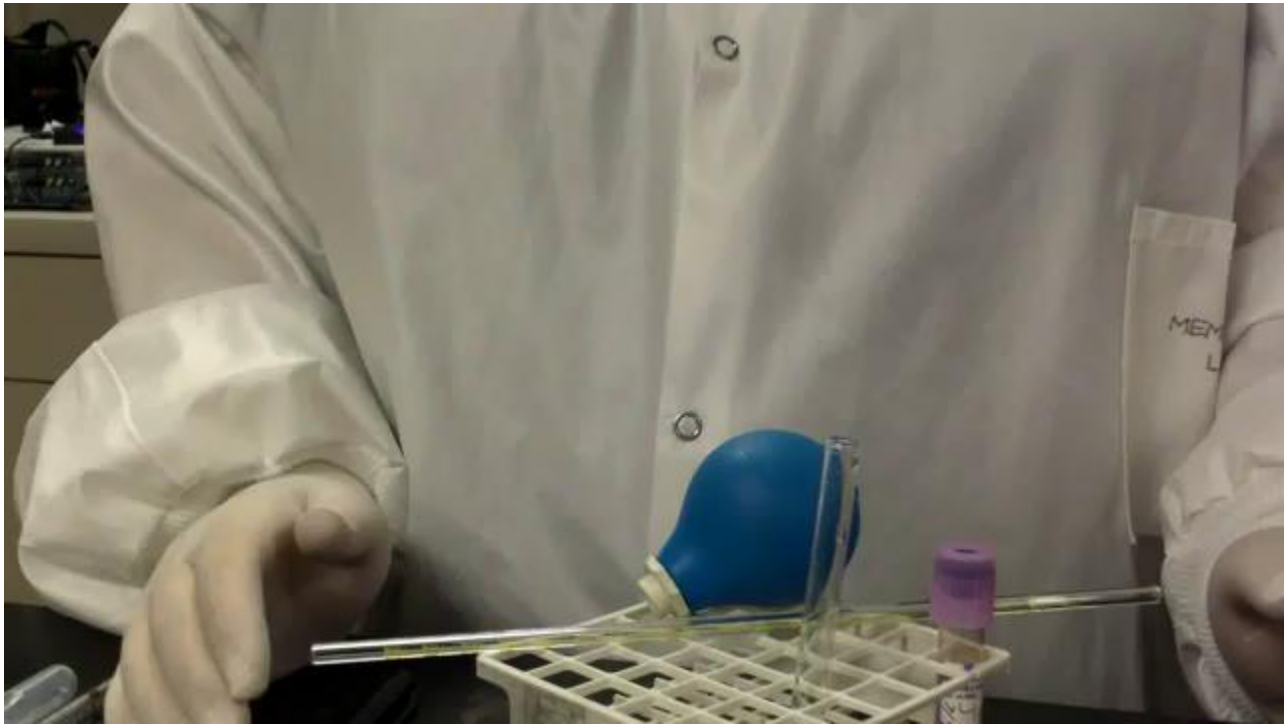
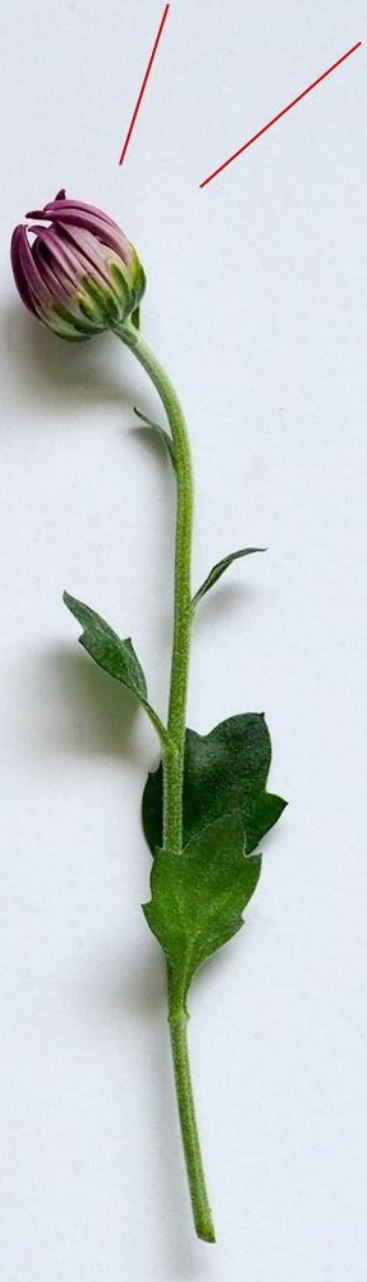
Clinical importance of ESR estimation:

The erythrocyte sedimentation rate (ESR) is a **non-specific test**. **WHY?**

- It is raised in a wide range of infectious, inflammatory, degenerative, and malignant conditions associated with changes in plasma proteins, particularly increases in fibrinogen and immunoglobulins.

ESR is not a diagnostic test of any particular disease but used to indicate:

- ✓ The presence of tissue damage.
- ✓ The severity of the disease (ESR is increased above **100 mm/hr** in rheumatic fever, tuberculosis (T.B.) and malignancy where tissue damage is high).
- ✓ The **follow up** (the activity of disease and its response to treatment **(prognostic test)**.



THANK YOU