

قَالَ اللَّهُ تَعَالَى

إِنَّمَا أَمْرُهُ إِذَا أَرَادَ شَيْءًا أَن يَقُولَ لَهُ كُنْ فَيَكُونُ

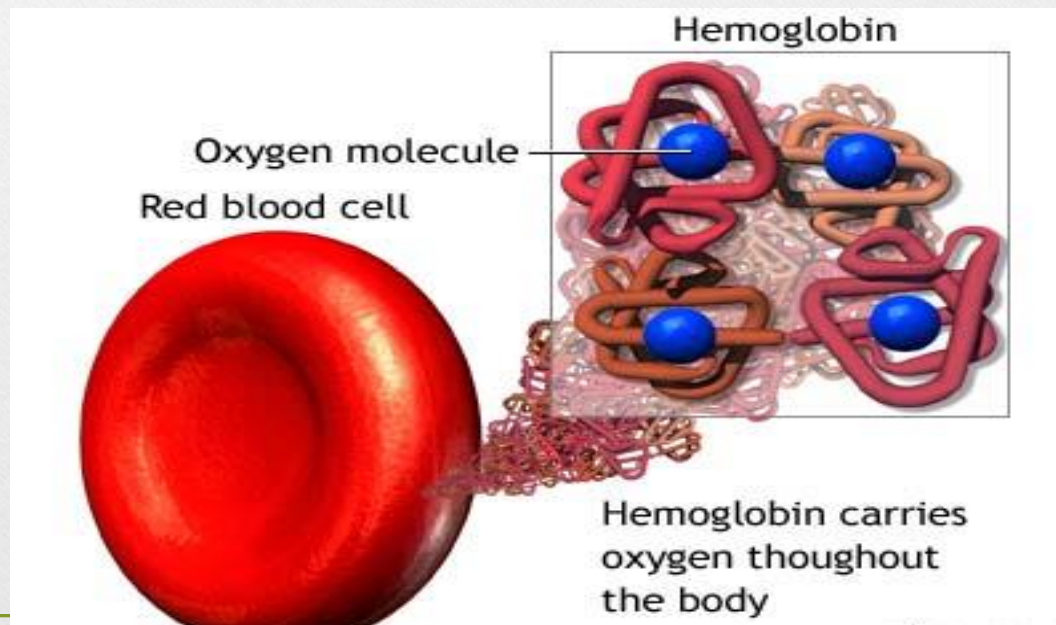
Topics

- Hemoglobin measurement (Hb)
- Hematocrit measurement (Hct)
- Measurement of erythrocyte sedimentation rate (ESR)



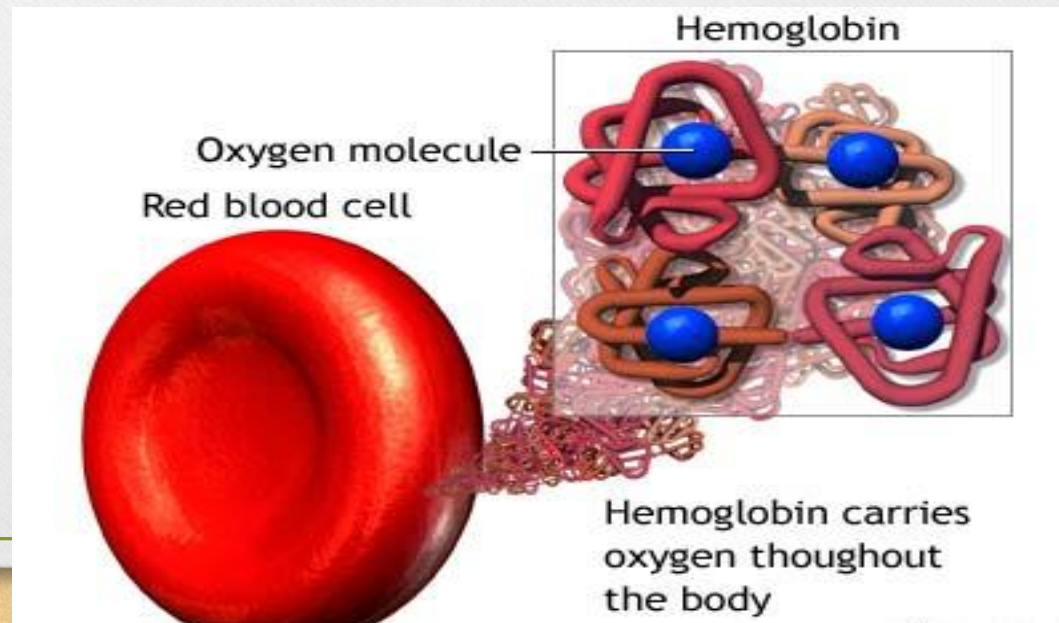
Hemoglobin (Hb)

- Red blood cells (RBCs) contain a protein called Hb that carries O₂ and CO₂.
- Hb is made up of 4 subunits, each of which consists of a heme group attached to a globin polypeptide chain.



Hemoglobin (Hb)

- At the center of the heme is an ferrous iron (Fe^{2+}), which binds to O_2
- each hemoglobin has the ability to transport 4 O_2 .



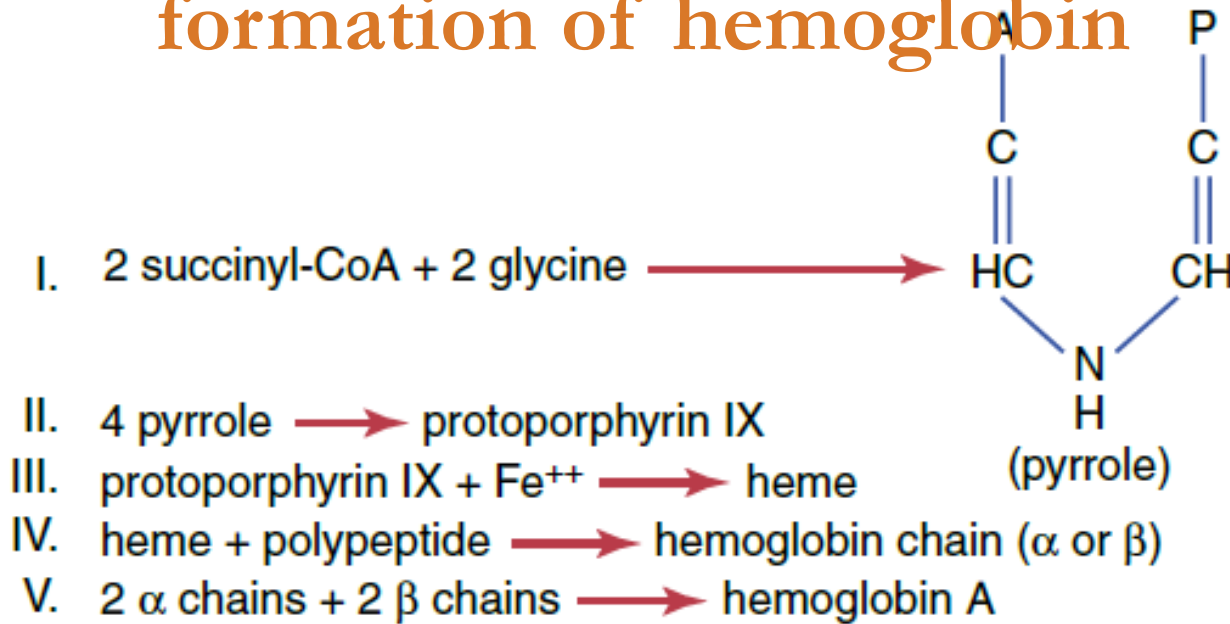
Hemoglobin (Hb)

- In 100 ml of blood, there is about 15 g of Hb.
- Each gram of hemoglobin can combine with 1.34 ml of oxygen.
- Therefore, 100 ml of blood has the capacity to carry 20 ml of oxygen by hemoglobin.

Hb formation

- begins in **proerythroblast** stage in the bone marrow and continues into the **reticulocyte** stage. So, when reticulocytes enter into the blood, they continue to form Hb for another day.

Basic chemical steps in the formation of hemoglobin



- Hemoglobin A (common form of hemoglobin in adults), is a combination of 2 α chains and 2 β chains.
- Hemoglobin F (in the fetus) contains 2 α chains and 2 γ chains.
- Hemoglobin F binds to oxygen more strongly than Hemoglobin A, enabling the transfer of oxygen from mother to fetus prenatally.

Normal Hemoglobin Levels and Ranges

- Males: 15-18 g/dl
- Females: 13-16 g/dl
- Infants: 18-20 g/dl

Hemoglobinometry

(Measurement of the Hb content in blood)

by Cyanmethemoglobin Method

Cyanmethemoglobin Method

Materials

- ☐ Drabkin's solution
- ☐ Micropipette
- ☐ Cuvettes
- ☐ Spectrophotometer

Cyanmethemoglobin Method

- Blood is mixed with **Drabkin's** solution that contains:
 - ☐ Potassium ferricyanide
 - ☐ Potassium cyanide
 - ☐ Sodium bicarbonate
- Potassium ferricyanide converts Hb to methemoglobin.
- Methemoglobin combines with potassium cyanide to form cyanmethemoglobin.

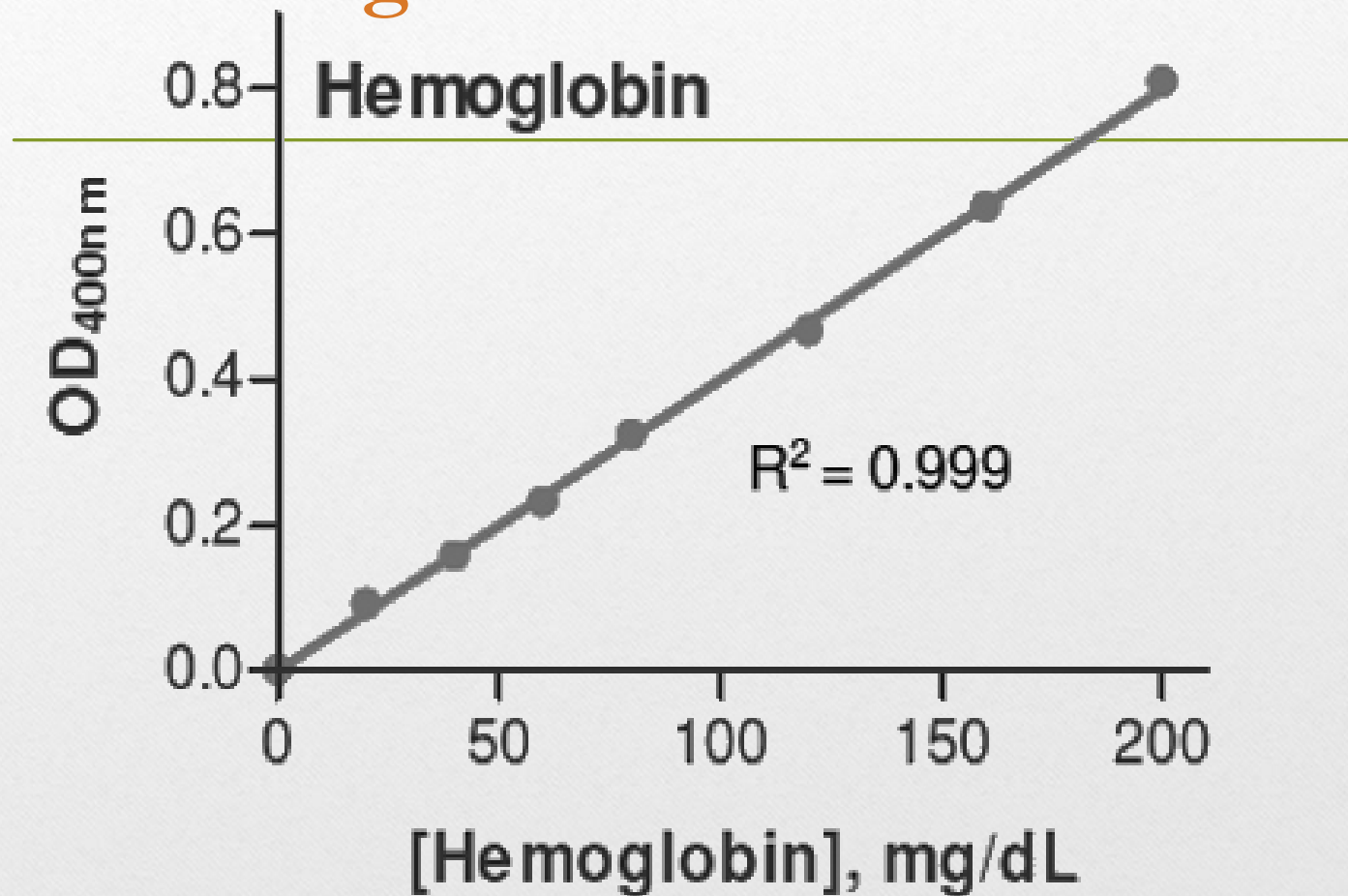
Cyanmethemoglobin Method

- Take 5 ml of Drabkin's solution in 2 tubes (control and test).
- Mix the blood sample by gentle inversion
- Take 20 microliters of blood using a micropipette. Wipe the outer surface of tip to remove excess blood. Add this blood to test tube.
- Cover the end of the tube with parafilm.
- Place the tube in a dark place for 5 to 10 minutes.
- Set the spectrophotometer at a wavelength of 540 nm.
- The test tube contains Drabkin's solution and blood. You need to measure the OD of blood. So, adjust the OD of the control tube (Drabkin's solution) at 0.
- Measure the absorbance of test tube in the spectrophotometer.

Spectrophotometer



Estimate the hemoglobin concentration using the standard curve



Hematocrit (Hct) or PCV (packed cell volume)

- is a blood test that measures the volume percentage of RBC in a certain blood volume.
- Unit: percentage

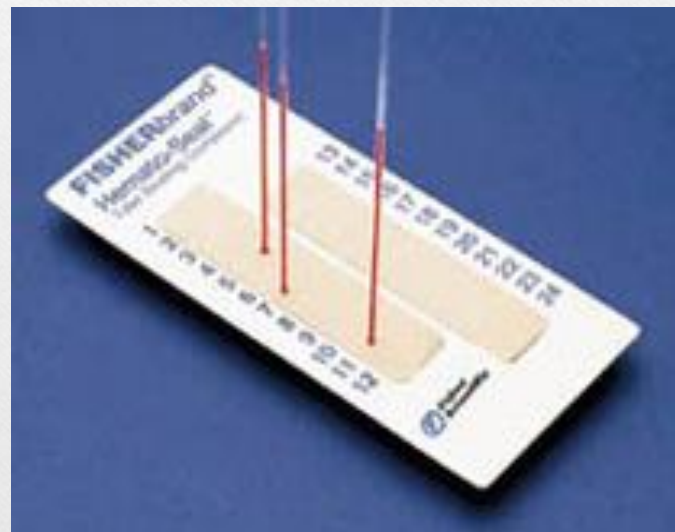
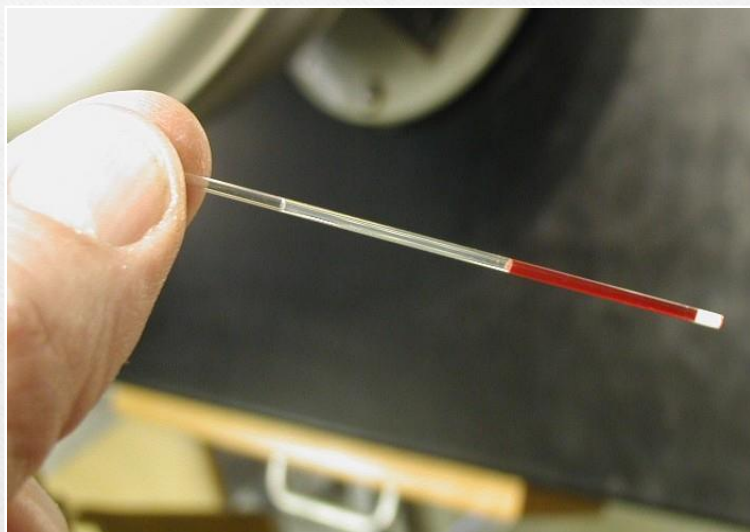
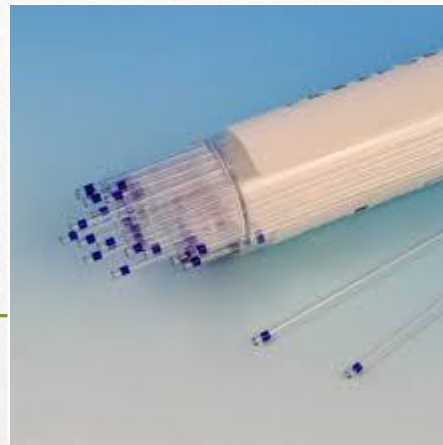
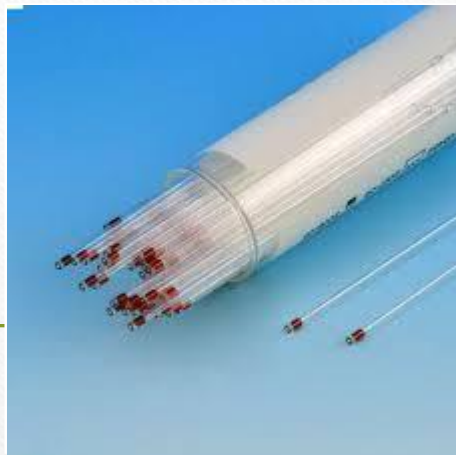
Materials

- Blood sample
- Glass capillary tubes
- Critoseal Capillary tube Sealant
- A microhematocrit centrifuge
- Hct ruler



Methods

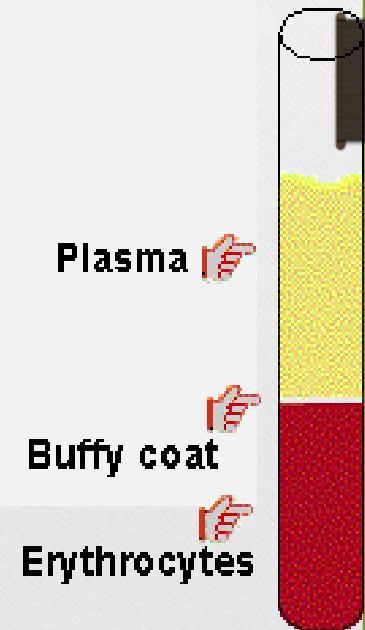
- Invert the blood vial 3 to 4 times.
- Insert the capillary tube into the blood.
- Capillary tube is filled by capillary force. Allow the 3/4 of tube to fill with blood.
- Seal one end of the tube with capillary tube sealant.
- Place the tube in the centrifuge, sealed end outward.
- After five minutes of centrifugation, the Hct can be measured by ruler.



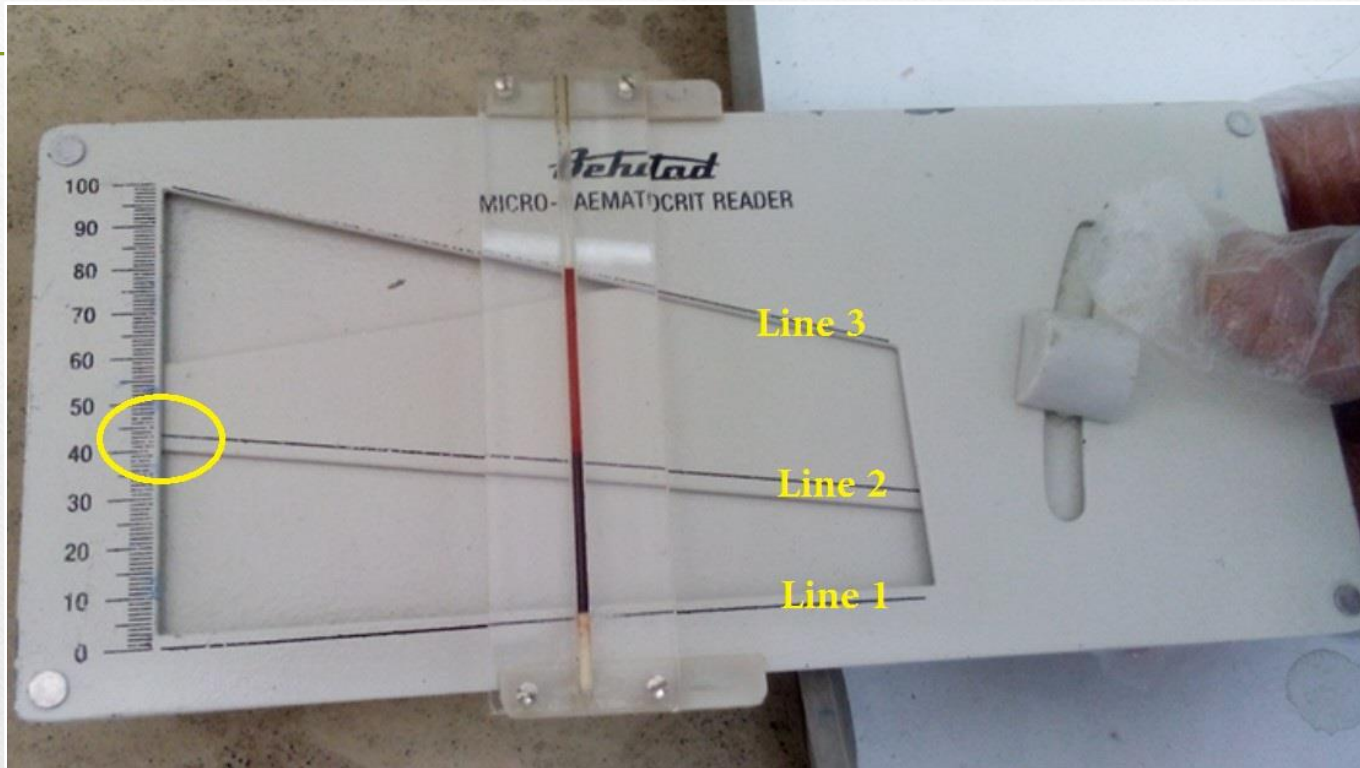
In order to balance centrifuge, place tubes directly opposite each other



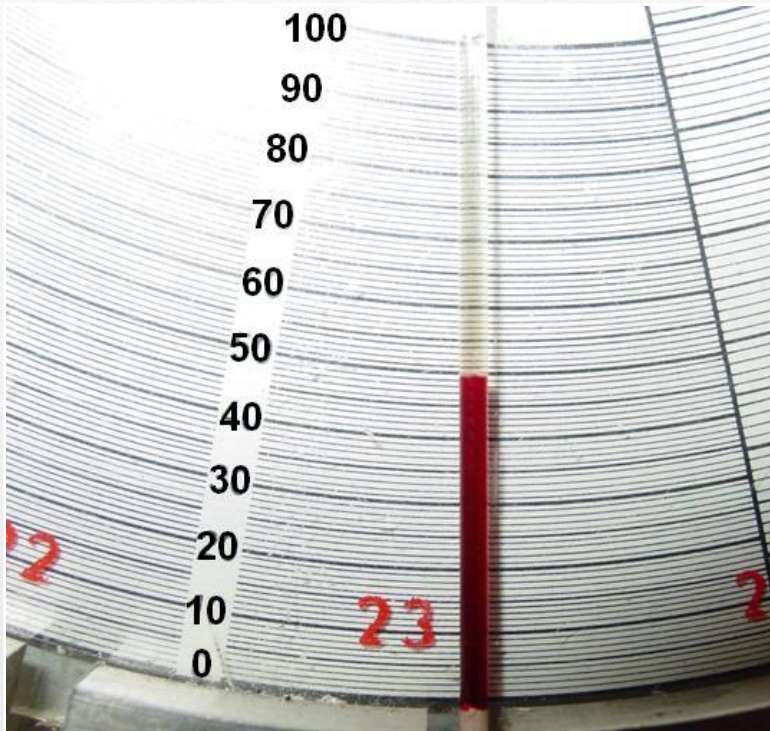
- After centrifugation, blood is divided into three layers: plasma, buffy coat, and RBCs.
- RBCs packed at the bottom form the packed cell volume and the plasma remains above this. In between the RBCs and the plasma, there is a white buffy coat, which is formed by white blood cells and the platelets.



Hct ruler



Hct ruler



Normal Hct Levels

- Males: 42% to 52%
- Females: 37% to 47%.
- 1-12 Months: 36% to 40%
- Newborns: to 60%

Hct changes

Hct measures the volume of packed RBCs relative to whole blood.
Therefore:

❖ **An increased Hct may be due to:**

Dehydration (which reduces plasma volume)

Polycythemia (that is an increased number of RBCs)

❖ **A decreased Hct may be due to:**

Anemia

Pregnancy (increases RBCs and plasma. However, plasma volume increases more than RBCs mass)

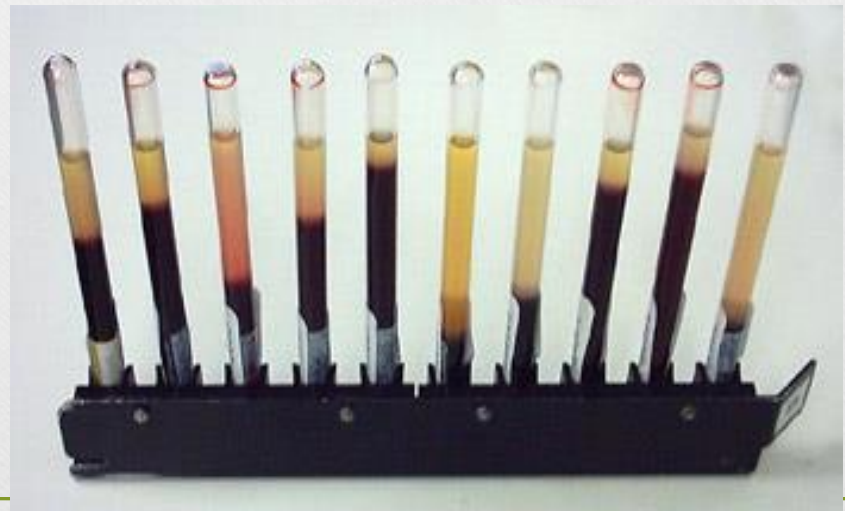
Over hydration

Kidney failure (causes anemia due to erythropoietin deficiency).

ESR

(Erythrocyte Sedimentation Rate)

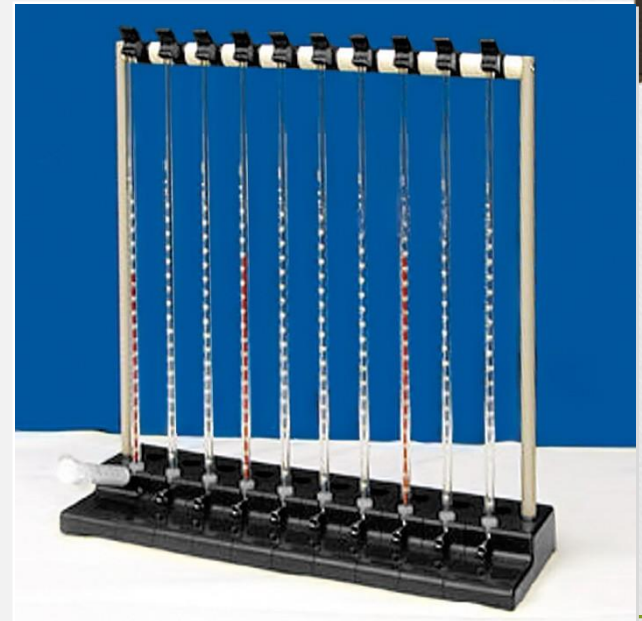
- RBCs sedimentation rate per time unit
- Unit: mm/hr
- The ESR is affected by many factors. So, it is a non-specific test.



Erythrocyte sedimentation rate (Westergren technique)

Materials

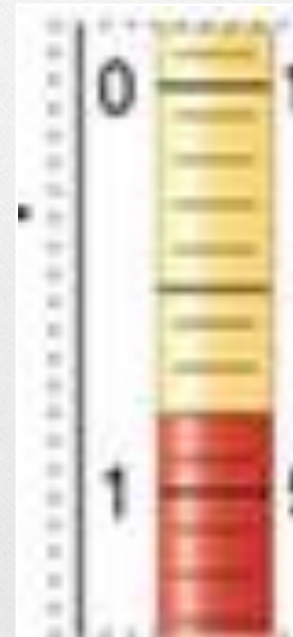
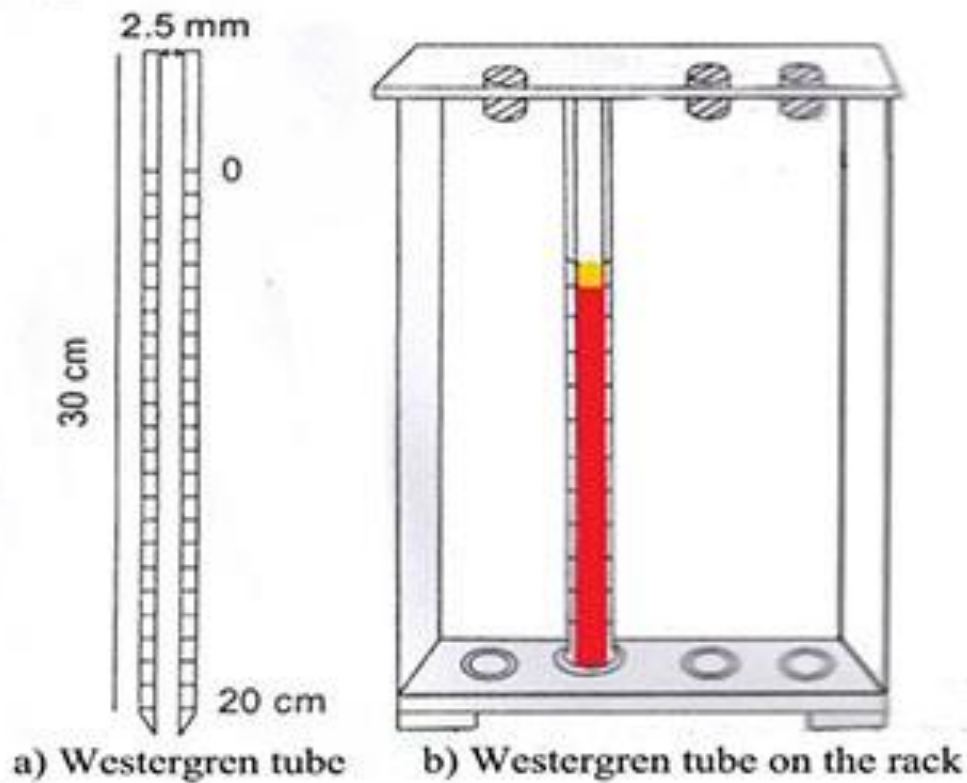
- Westergren ESR pipette
- Sodium Citrate 3.8%
- Timer
- Blood sample
- ESR rack
- syringe & cotton & alcohol



Procedure

- Add 0.4 ml of sodium citrate to 1.6 ml of blood.
- Mix gently without shaking and put in the graded tube (Westergren pipette)
- Leave pipette stand vertically on the stand for 1 hour.
- Read the ESR without moving it.

ESR reading



Normal Levels

Females $h_1 = 10 \text{ mm/h}$

Males $h_1 = 5 \text{ mm/h}$

Table 1. Reference values for ESR.

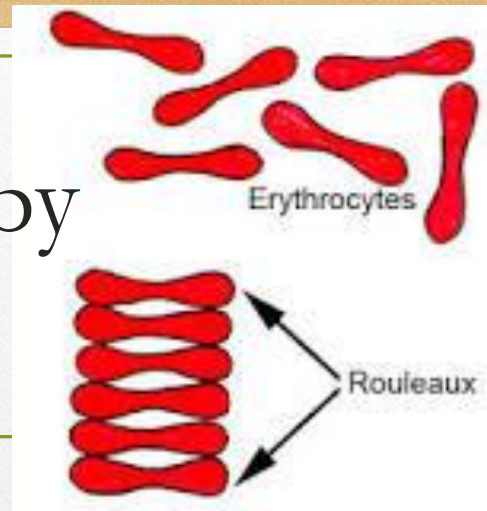
Age	Male	Female
0-50	<15 mm/h	<20 mm/h
51-85	<20 mm/h	<30 mm/h
>85	<30 mm/h	<42 mm/h

Source: Sox H.C., Liang M.H. The erythrocyte sedimentation rate: guidelines for rational use. Ann Int Med 1986;104:515-23.

Factors affecting ESR

- Shape of RBCs
- Number (increasing the number of RBCs decreases ESR)
- Size
- Plasma protein (with effect on Zeta potential)
- Albumin ↓
- Immunoglobulin, fibrinogen ↑

Plasma proteins change ESR by affecting **zeta potential**



- A group of RBCs that are clumped together will form a stack called a rouleau.
- Rouleaux formation allows the RBCs to settle at a faster rate → increases the ESR.
- The membranes of RBCs have a negative charge, named as zeta potential. This potential causes RBCs to repel each other (pushes RBCs apart from each other).
- Fibrinogen and immunoglobulins with positive charges reduce zeta potential. Therefore, they increase rouleaux formation and ESR.
- Albumin has negative charge and decrease ESR by increasing zeta potential.

ESR is not a specific test because many factors affect it:

Physiological factors:

- ❖ Age Infants ↓
- ❖ Gender..... Women ↑ (ESR is higher in females than in male)
- ❖ Altitude ↓ (living in altitude increases RBCs count due to erythropoietin secretion)
- ❖ Pregnancy ↑

Pathological factors that influence ESR

❖ Increasing factors

- Acute infections
- Lung diseases
- Bone diseases
- Some anemias
- Autoimmune disorders
- Some cancers

❖ Lowering factors

- • Lack of fibrinogen in plasma
- Polycythemia
- Some anemias
- (Spherocytosis, sickle cell disease: abnormal shapes of RBCs impair rouleaux formation)