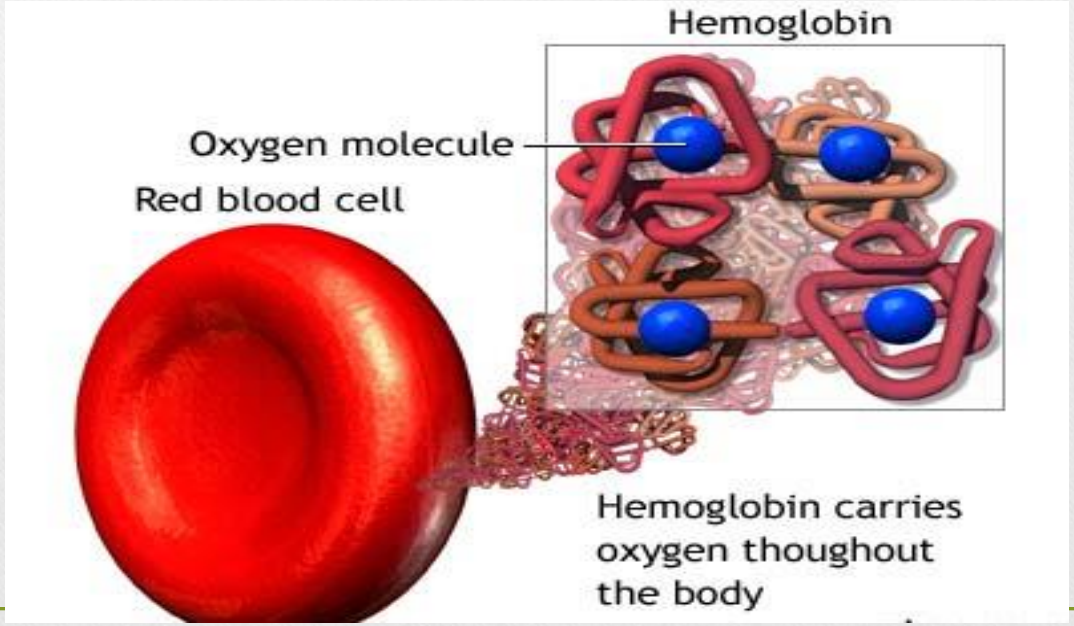

قال الله تعالى
بسم الله الرحمن الرحيم

Topics

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

Hemoglobin (Hb)

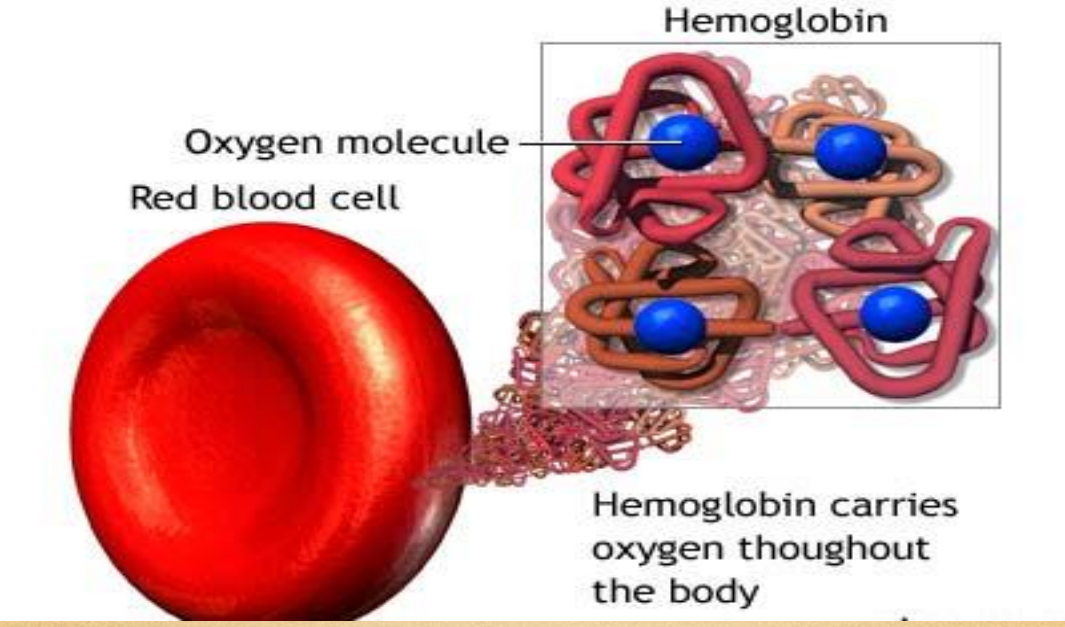
- Red blood cells (RBCs) contain a protein called Hb that carries O2 and CO2.
- Hb is made up of 4 subunits, each of which consists of a heme group attached to a globin polypeptide chain.



Hemoglobin (Hb)

- At the center of the heme is an ferrous iron (Fe2+), which binds to O2

→ each hemoglobin has the ability to transport 4 O2.



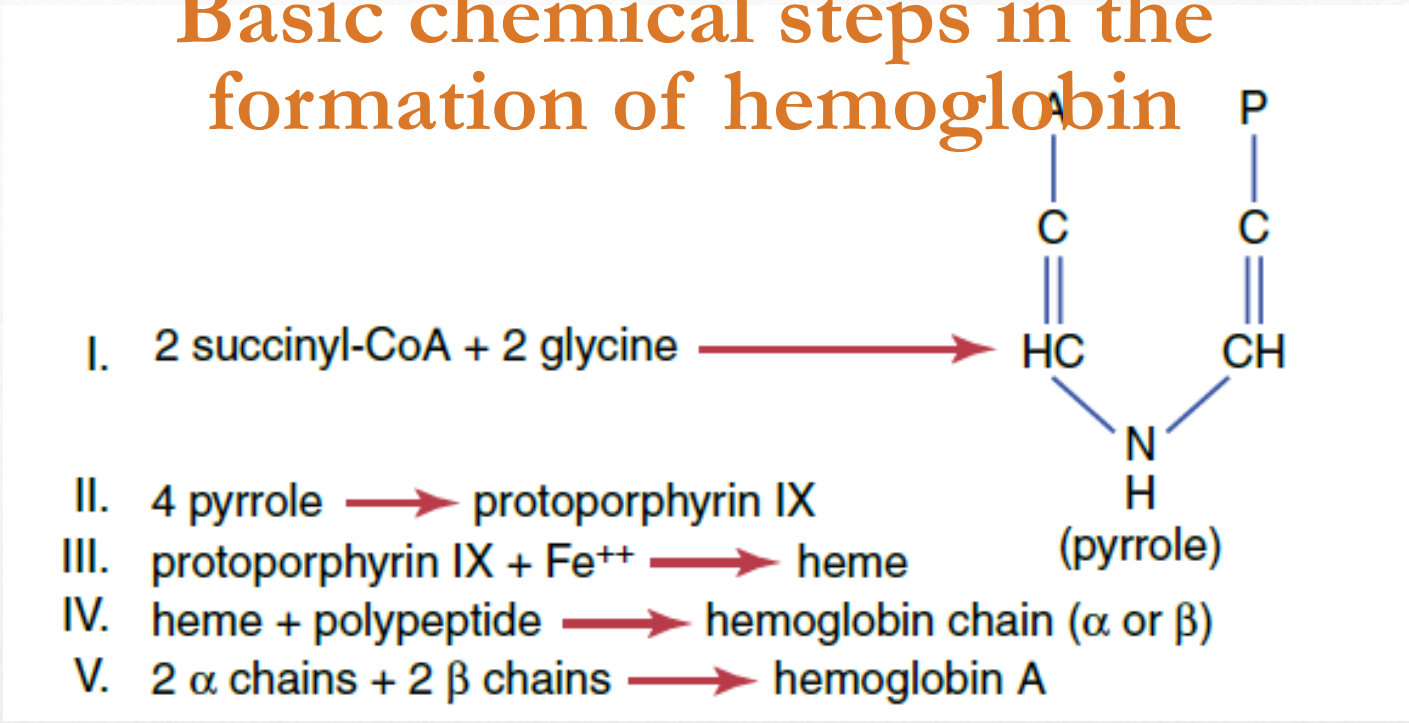
Hemoglobin (Hb)

- 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

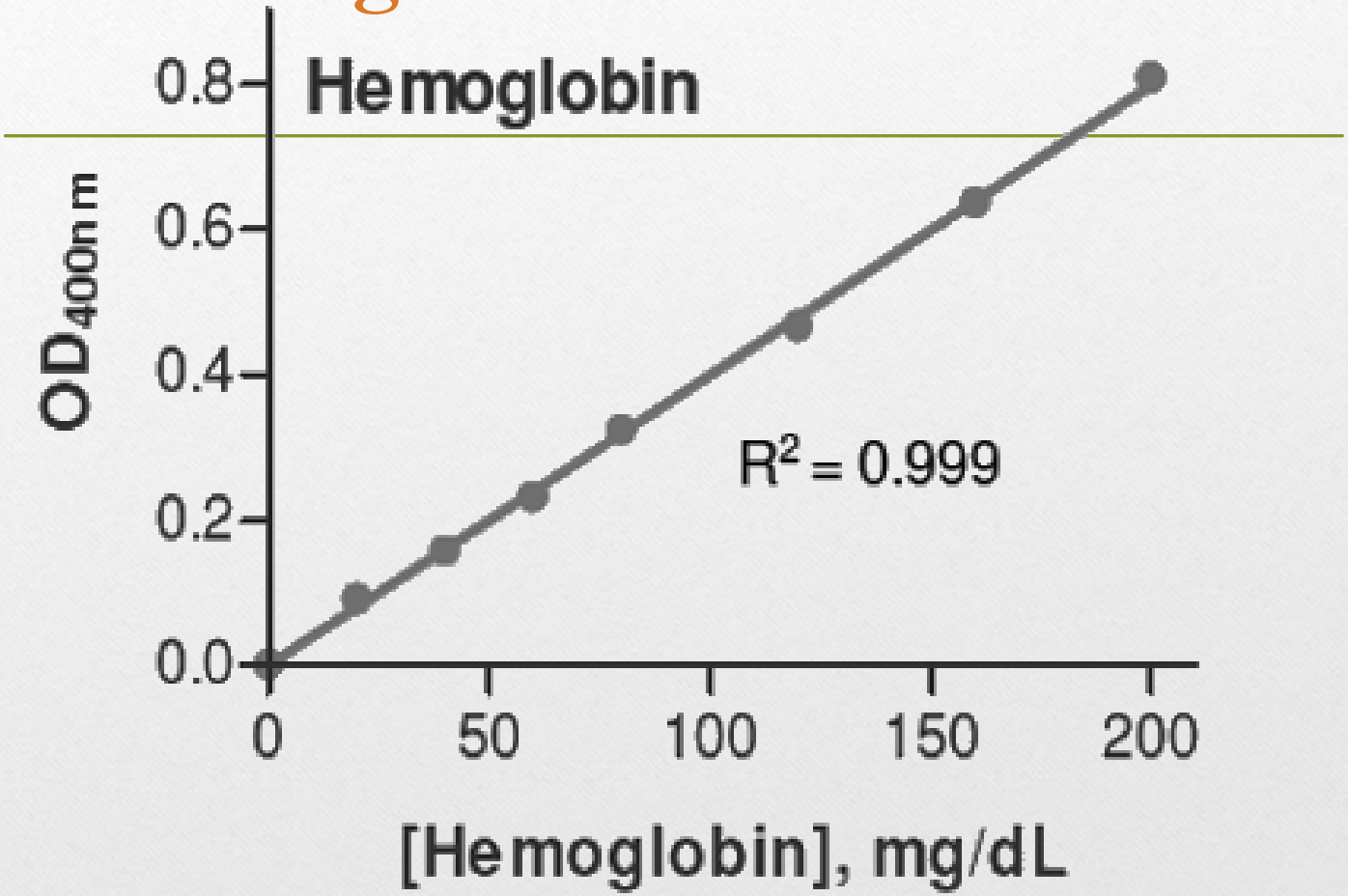
- Blood is mixed with Drabkin's solution that contains:
  - Potassium ferricyanide
  - Potassium cyanide
  - Sodium bicarbonate
- Potassium ferricyanide converts Hb to methemoglobin.
- Methemoglobin combines with potassium cyanide to form cyanmethemoglobin.

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)

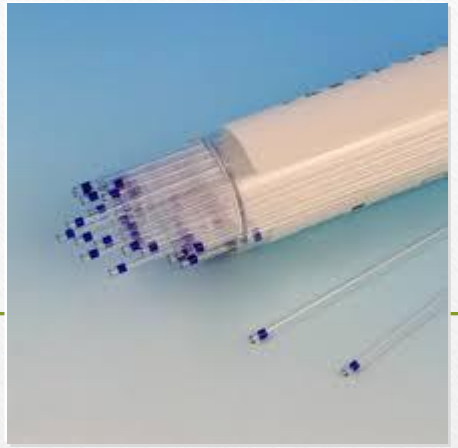
- is a blood test that measures the volume percentage of RBC in a certain blood volume.
- Unit: percentage

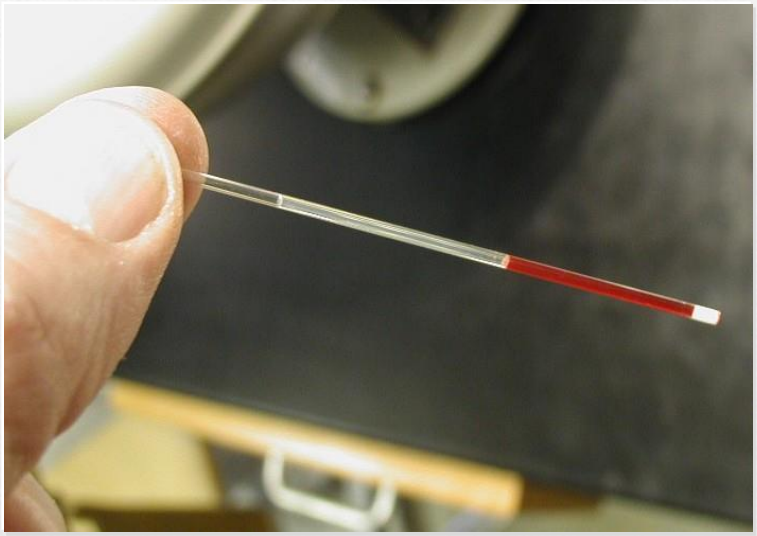
Materials

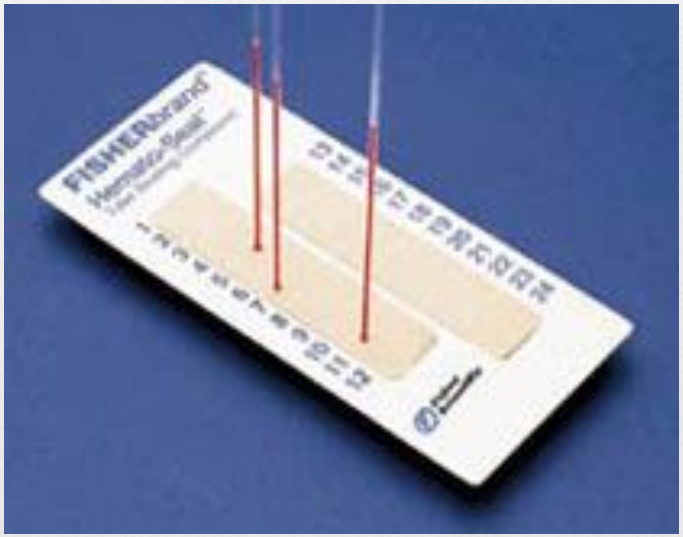
- Blood sample
- Glass capillary tubes
- Critoseal Capillary tube Sealant
- A microhematocrit centrifuge
- Hct ruler

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.

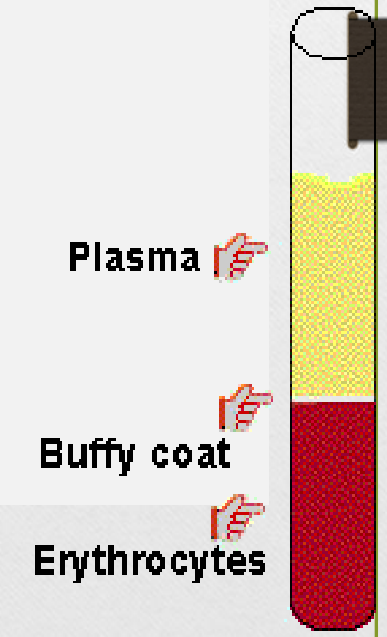




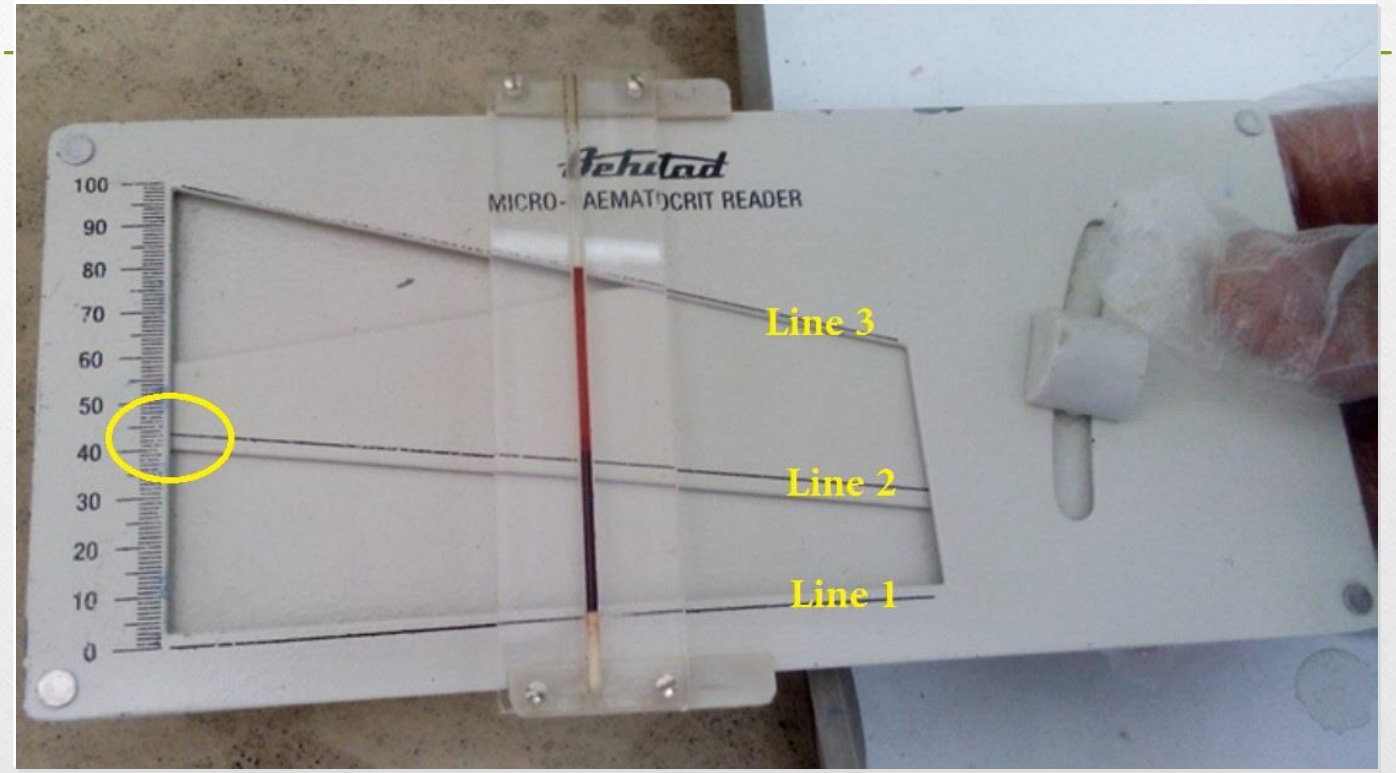

FISHERbrand

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

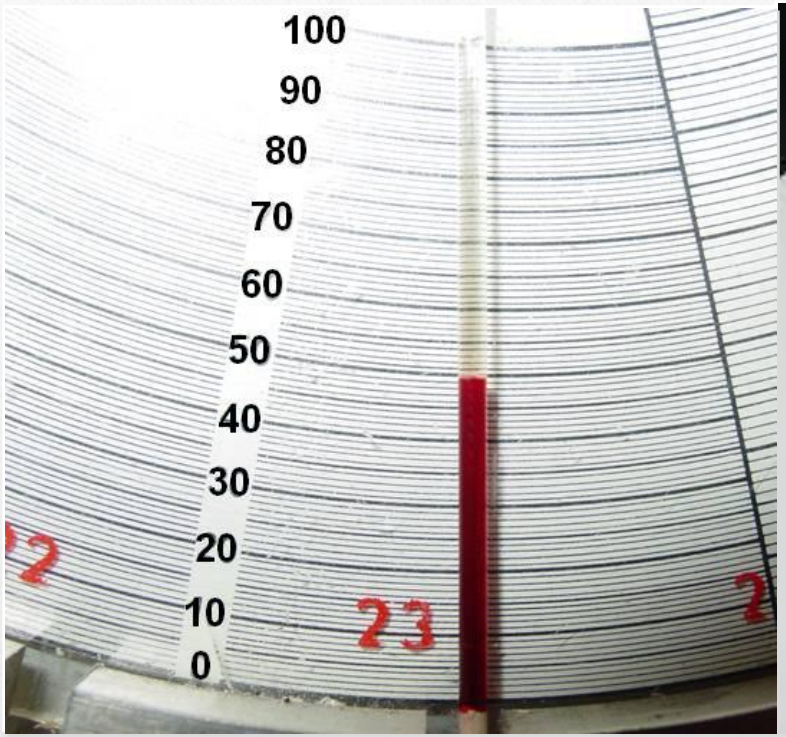
- After centrifugation, blood is divided into three layers: plasma, buffy coat, and RBCs.
- RBCs packed at the bottom form the packed cell volume and the plasma remains above this. In between the RBCs and the plasma, there is a white buffy coat, which is formed by white blood cells and the platelets.

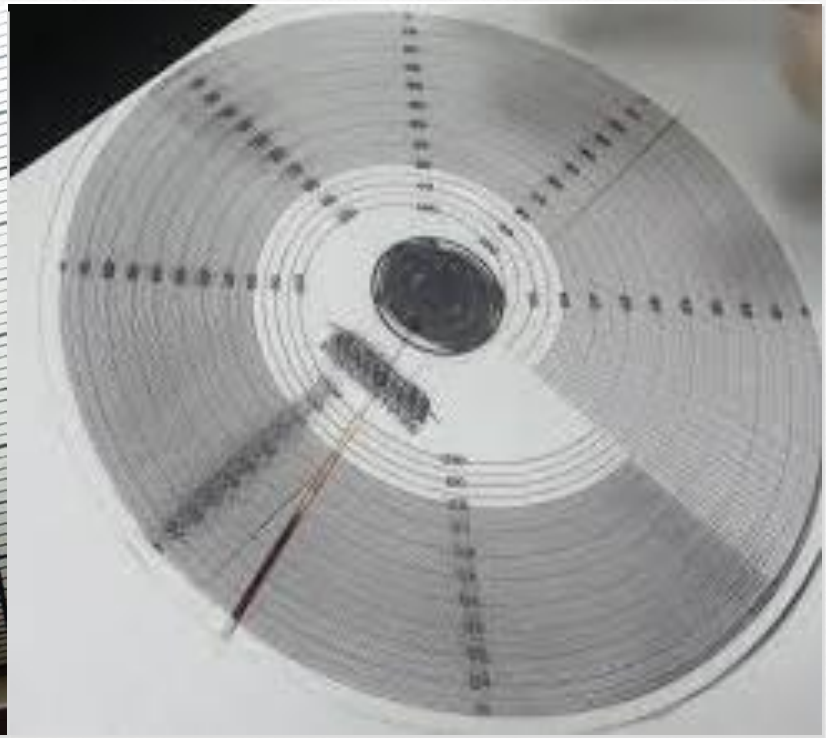


Hct ruler


MICRO- AEMATOCRIT READER
100
90
80
70
60
50
40
30
20
10
0
Line 3
Line 2
Line 1

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

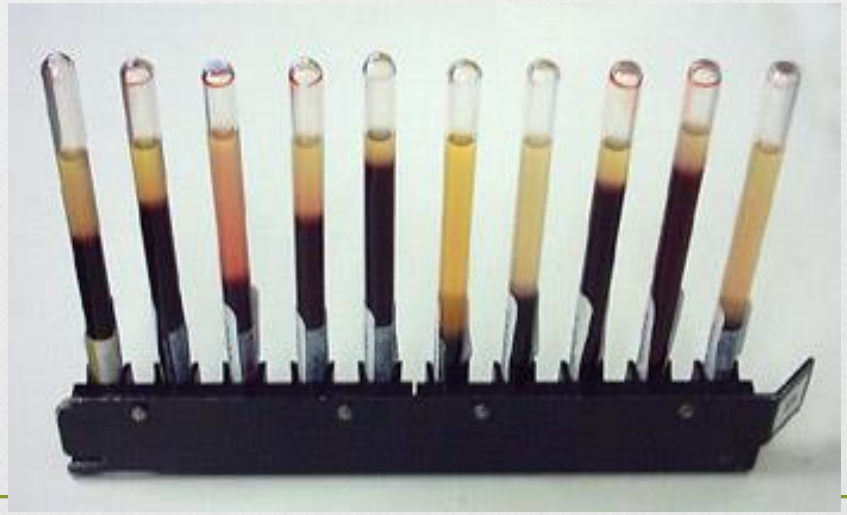
Pregnancy (increases RBCs and plasma. However, plasma volume increases more than RBCs mass)

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)

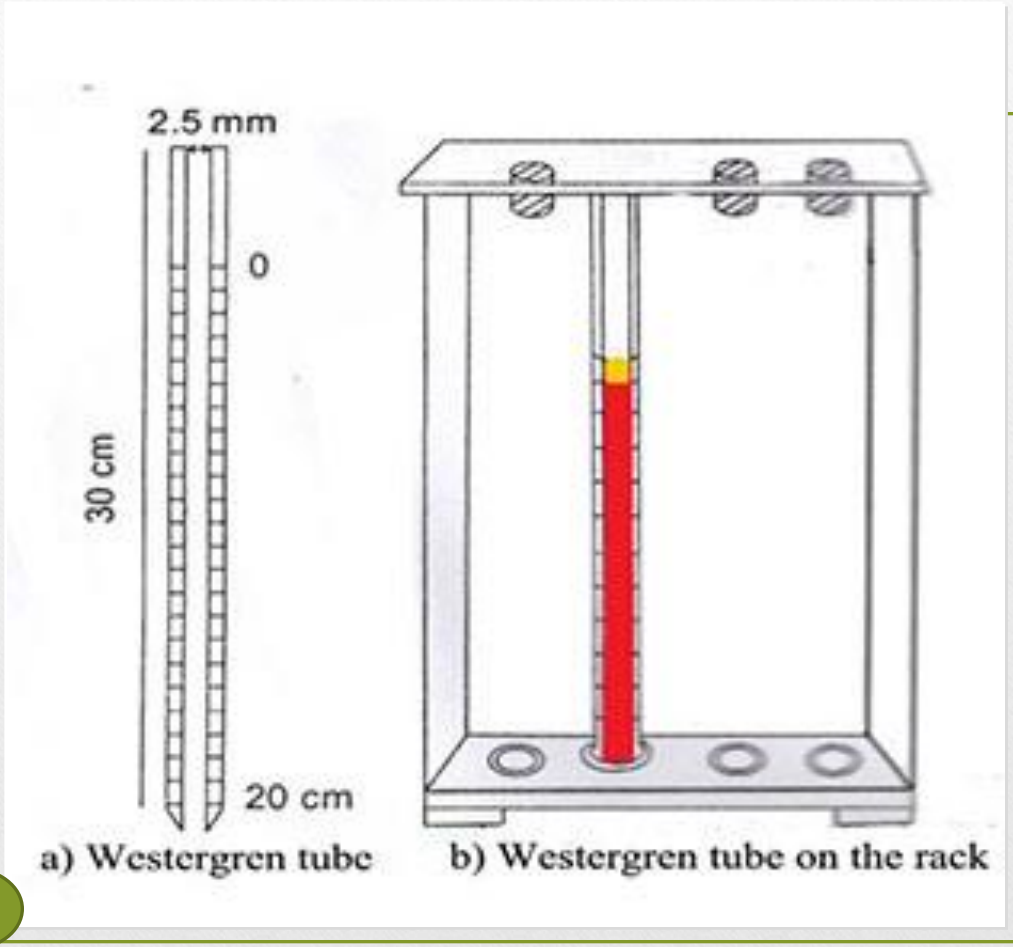
Materials

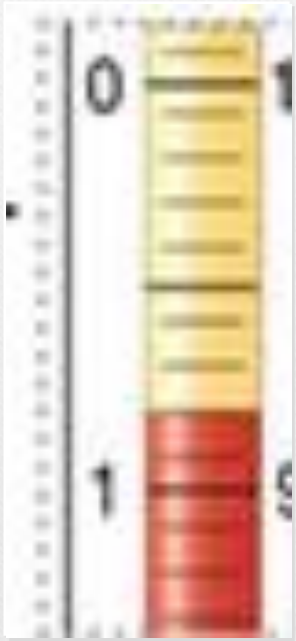
- Westergren ESR pipette
- Sodium Citrate 3.8%
- Timer
- Blood sample
- ESR rack
- syringe & cotton & alcohol

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


2.5 mm
0
30 cm
20 cm
a) Westergren tube
b) Westergren tube on the rack


0
1

Normal Levels

Females h1=10 mm/h

Males h1=5 mm/h

Table 1. Reference values for ESR.

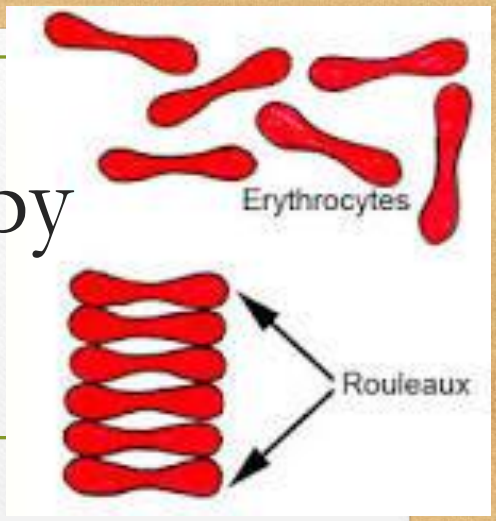
Age	Male	Female
0-50	<15 mm/h	<20 mm/h
51-85	<20 mm/h	<30 mm/h
>85	<30 mm/h	<42 mm/h

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)