Medical laboratories instruments

Haemoglobin Estimation

(Measurement of Hb concentration by Sahli method)

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Laboratory: 8

First stage

Hemoglobin

It is the main constituent of RBCs/which gives the blood its characteristic red color.

Normal level:

Women: 12 to 16 g/dl

Men: 13 to 18 g/dl

Children: 11 to 16 g/dl

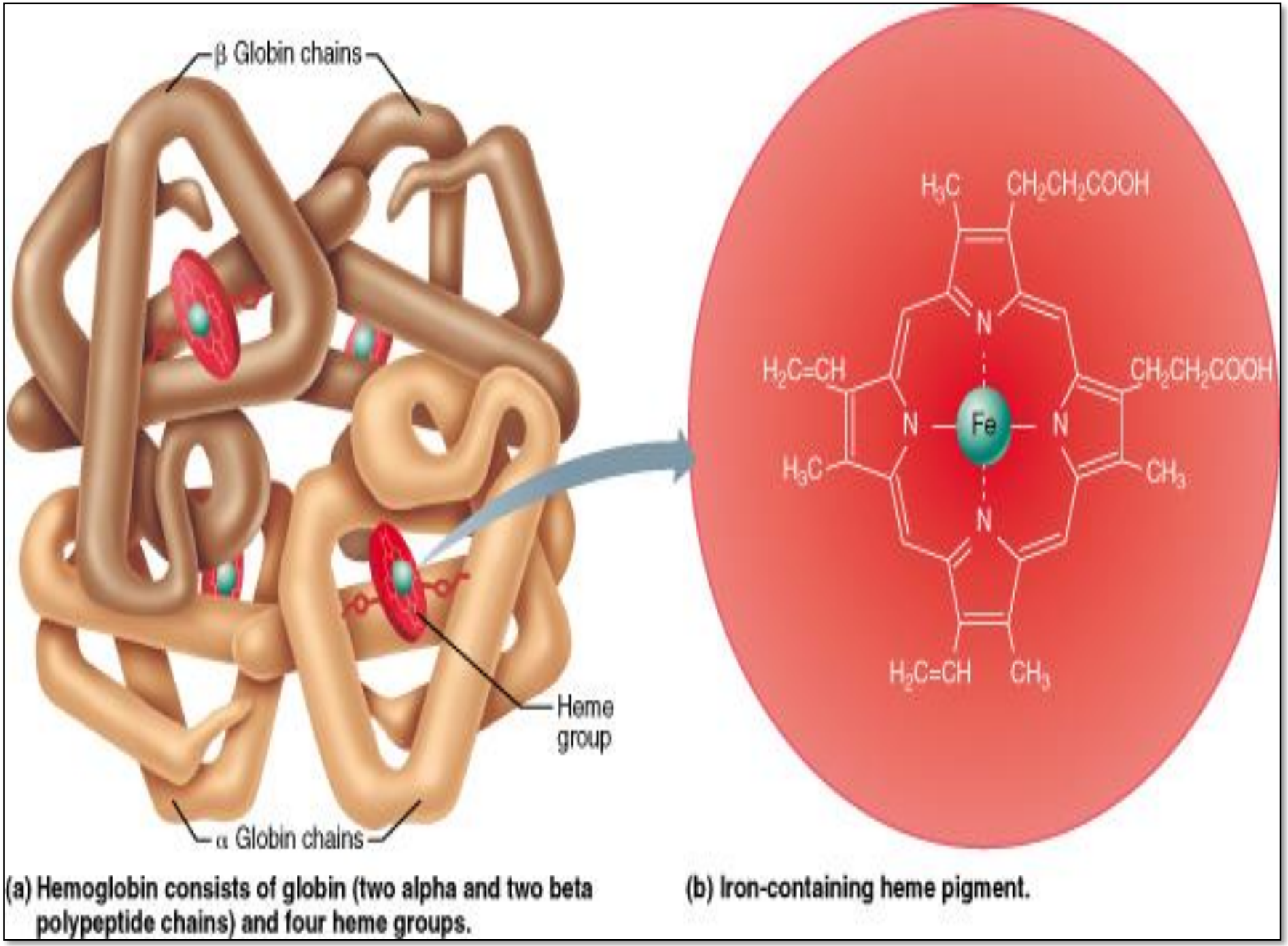
Pregnant women: 11 to 12 g/dl

HB consists of two parts:

1- Pigment heme (iron containing pigment), which constitutes (4%)

2- Protein globin (96%)

Hemoglobin consists of 4 heme groups, each is protein chain (2 pairs of polypeptides in each), 2 of those protein chains form hemoglobin molecule.



(a) Hemoglobin consists of globin (two alpha and two beta polypeptide chains) and four heme groups.

(b) Iron-containing heme pigment.

Hb can be measured by any of the following methods

a) Colorimetric method – based on color

b) Physical method – based on specific gravity

c) Chemical method – based on iron content of Hb.

d) Gasometric method – based on oxygen combining capacity of Hb.

Significance of Hb estimation

- Decrease in the haemoglobin below the normal range is an indication of anemia.
- Causes for increase in the haemoglobin concentration:

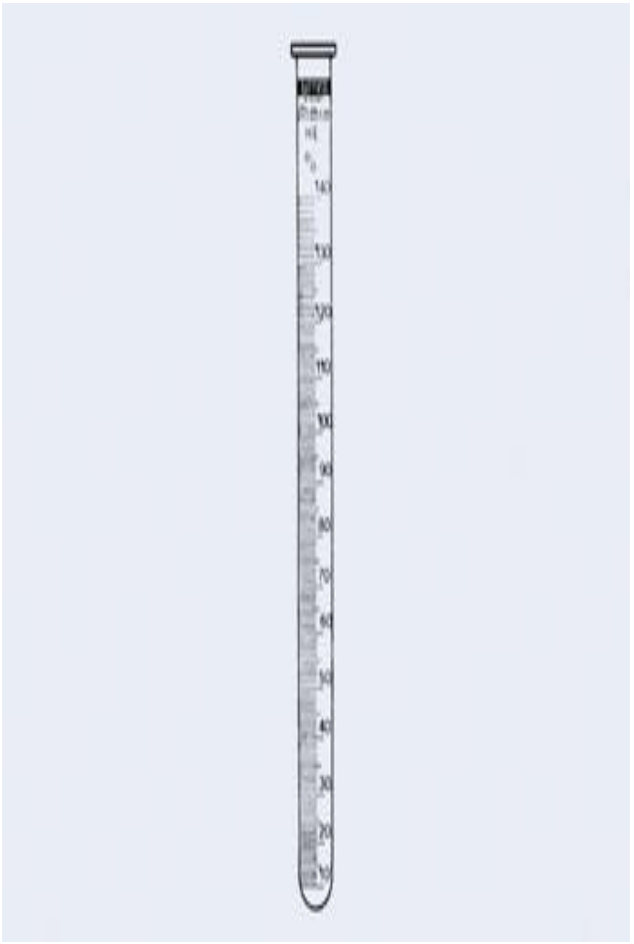
- Hypoxic states
- Increased secretion of erythropoetin
- Polycythemia vera

Sahli´s Kit (Haemometer)

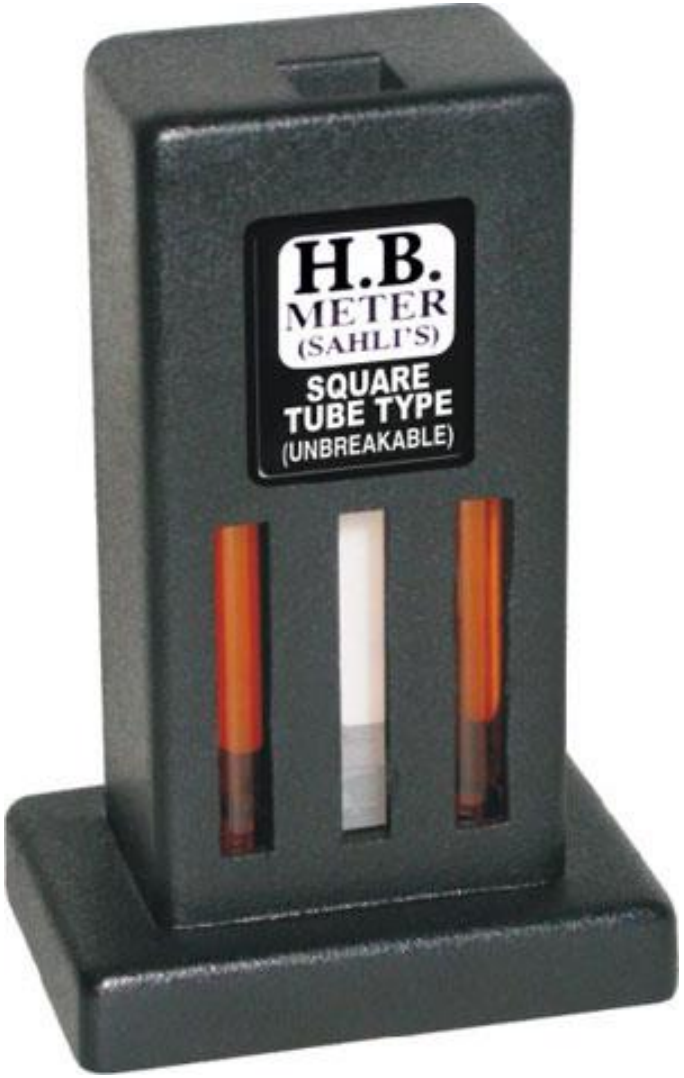
Dropper

Sahli's paipette

Hemometer comparative tubes with double scales (g%) & (percent of normal)



Color comparator



Principle Sahli method:

- In this method, the blood Hb is converted to brownish hematin compound by the action of HCl. Each type of Hb must be converted to standard form and color which is and hematin.
- The higher the Hb conc. The intense the color of hematin will be, the intensity of the color is measured comparing it with standard solution of Hb.

Procedure

- Place 5 drops of (0.1N HCl) in the bottom of graduated Sahli tube. This amount should fill the tube to around the (10%) mark on the red scale (red calibration)
- To this add blood sucked till the specific mark (20 µl = 0.02ml) on the Hb pipette. Do not allow air to enter the pipette column
- Insert the tip of the pipette beneath the surface of Sahli tube and gently blow out the blood
- Mix the blood with 0.1N HCl, and by sucking the mixture and expelling it again, and then let the tube stand for 10 min (Hb converts to hematin)
- Add DW to the hematin solution, drop by drop (stir after each addition) until its color matches the color of the standard color

Thank You