

Practical Physiology

Estimation of Hemoglobin

Sahli's Method



5th Practical



Prepared and Presented by:

**Lecturer Dr/ Ayad AbdElSalam
Assist. Lecturer Dr/ Ghadeer Talib**

**Teaching of Practical Physiology
College of Technology & Health Sciences
Radiological Techniques Department**

Hemoglobin (Hb) is the protein contained in red blood cells that is responsible for delivery of oxygen to the tissues. To ensure adequate tissue oxygenation, a sufficient hemoglobin level must be maintained. The amount of hemoglobin in whole blood is expressed in grams per deciliter (g/dl).

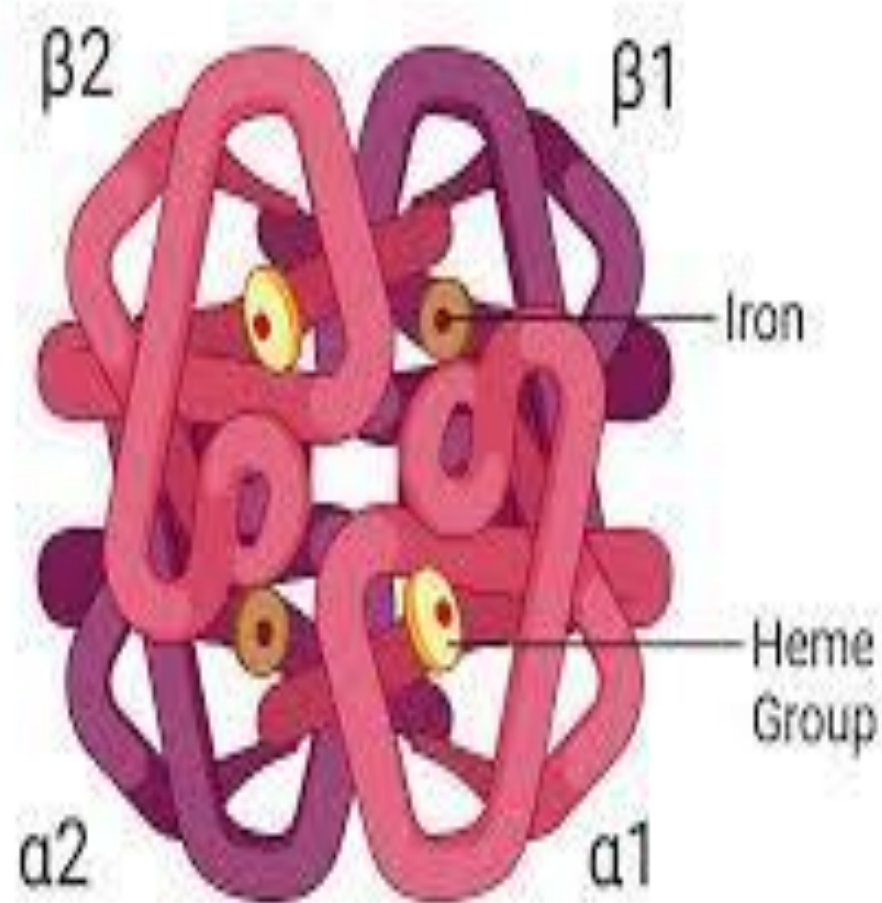
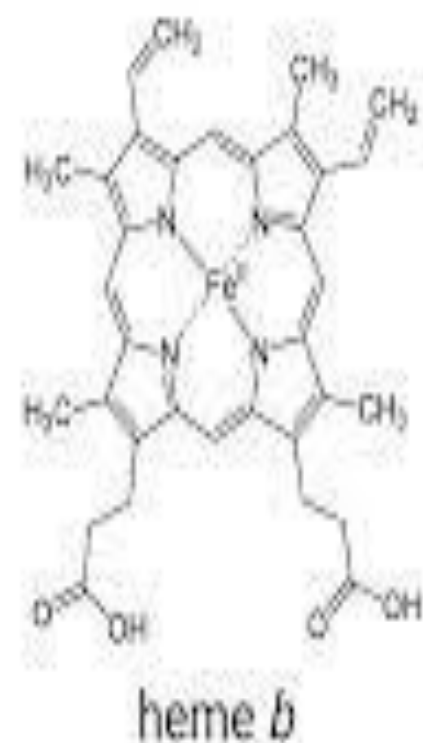
Hemoglobin is the major constituent of the red cell cytoplasm, accounting for approximately 90% of the dry weight of the mature cell. It is comprised of heme and globin.

The Hemoglobin molecule is a tetramer consisting of two pairs of similar polypeptide chains called globin chains. To each of the four chains is attached heme which is a complex of iron in ferrous (Fe^{+2}) form and protoporphyrin.

The major (96%) type of hemoglobin present in adults is called HbA and it has 2 alpha globin chains and 2 beta globin chains ($\alpha_2\beta_2$).

Each haemoglobin molecule carries four molecule of oxygen and each gram of haemoglobin can carry 1.34ml of oxygen.

Hemoglobin



Why do we estimate hemoglobin?

- To detect the oxygen carrying capacity of blood.
- Disease detection, which causes a deficiency or excess of haemoglobin.
- Studying changes in haemoglobin concentration before or after operations and blood transfusions.
- To detect anaemia and its severity and monitor an anaemic patient's response to treatment.
- To check haemoglobin level of blood prior to blood donation.
- To calculate red cell indices.

Sahli's Method or Acid Hematin method

Principle: Hemoglobin is converted to acid hematin by 0.1 N HCl and the resulting brown colour is compared with standard brown glass reference blocks.

The intensity of the brown colour depends on the amount of acid hematin produced, and this is directly proportional to the amount of hemoglobin in the blood sample.

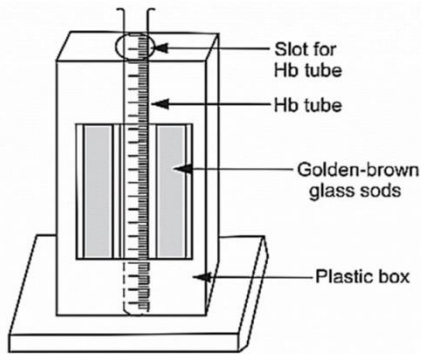
Sahli's Method Procedure

- Place 0.1 N HCl into the Sahli's Hb tube up to the lowest mark.
- Deliver 20 μ l (0.02 ml) of blood from a Hb pipette into the tube thereafter.
- Stir with a glass rod/stirrer and wait for 10 minutes to allow color development.
- Add distilled water drop by drop and stir till color matches with the comparator because 95% of Hb is converted at the end of 10mins and others much later.
- Take the reading at upper meniscus.

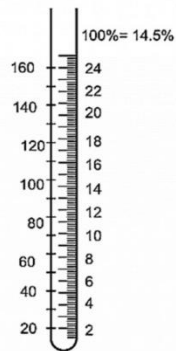




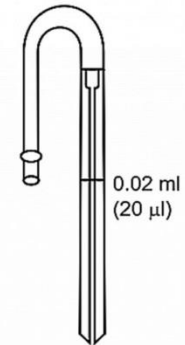
Sahli's Hemoglobinometer Set



Comparator



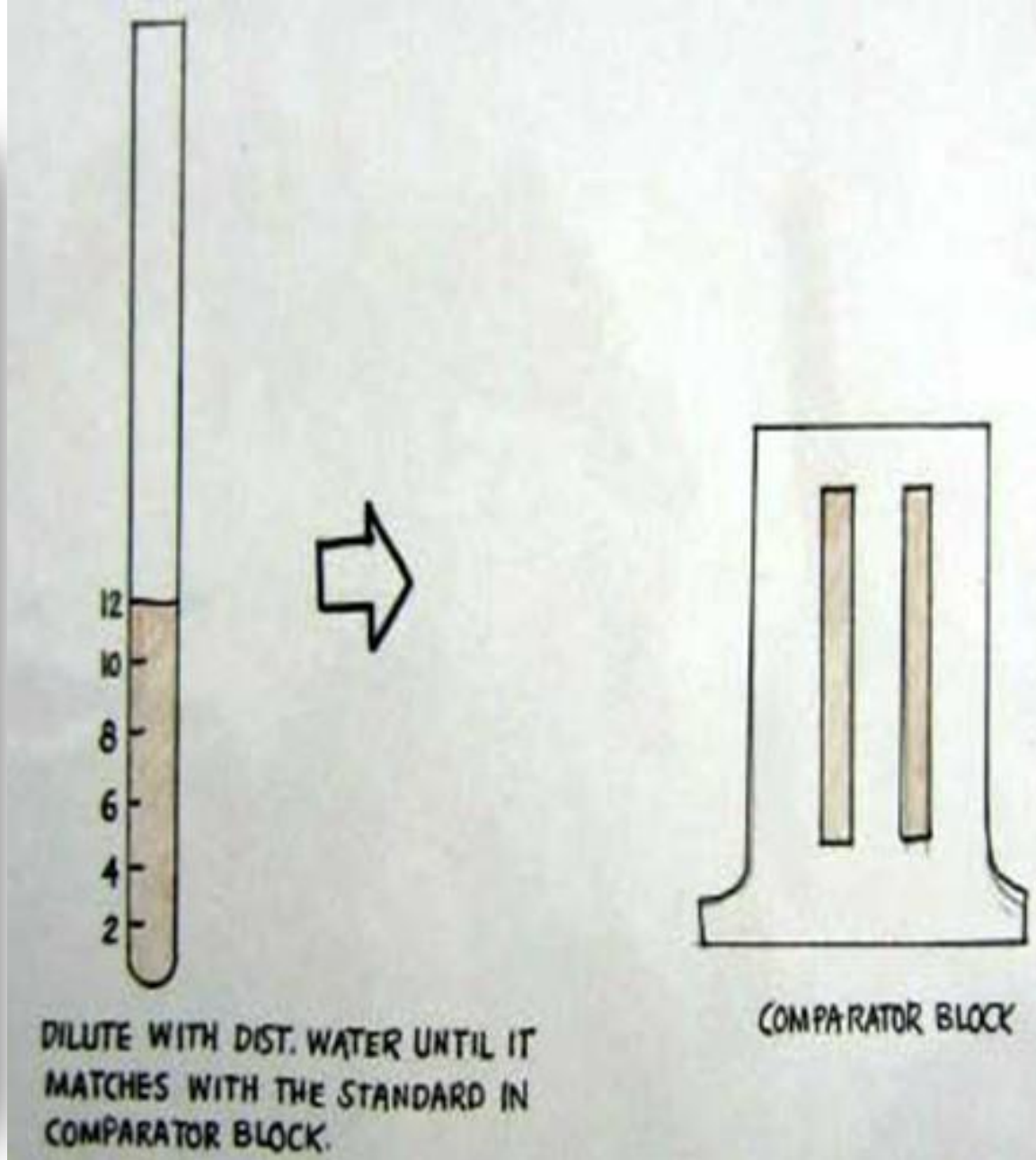
Hemoglobin tube



Hemoglobin pipette



Stirrer



Salhi's Hemoglobinometer

Advantages

- **Simple bedside test.**
- **Reagents and apparatus are cheap.**

Disadvantages

- **There can be visual error.**
- **Comparator can fade over the years.**
- **Color attainment of acid hematin takes long time and also fades quickly.**
- **Source of light (day light or artificial) influences the color comparison.**