ESTIMATION OF HEMOGLOBIN

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ESTIMATION OF HEMOGLOBIN

Introduction

- 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.**

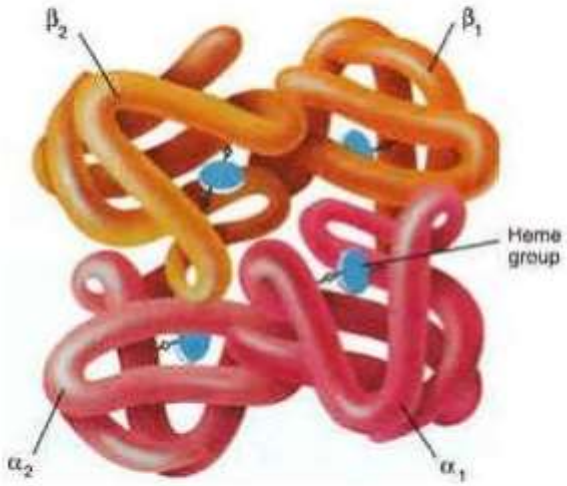
STRUCTURE

Structure of Hemoglobin

- 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 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 (α2β2).

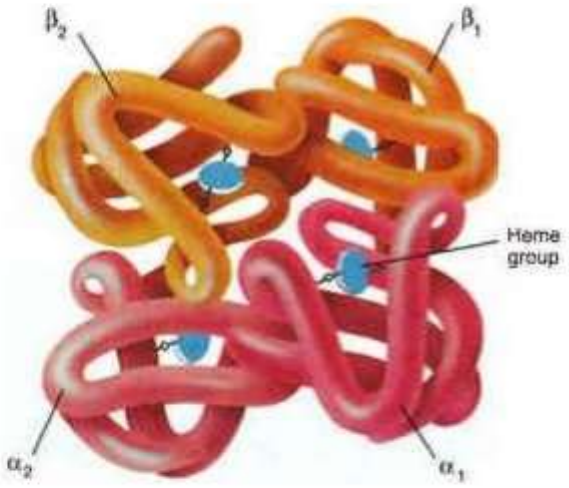


STRUCTURE-hemoglobin

Structure of Hemoglobin

- The gene that codes for
- the formation of α globin chains is located on chromosome 16.
- The gene that codes for the formation of β globin chains is on chromosome 11.

- In adults, a minor amount of HbA2 (α2β2) is also present and constitutes less than 3.5%.

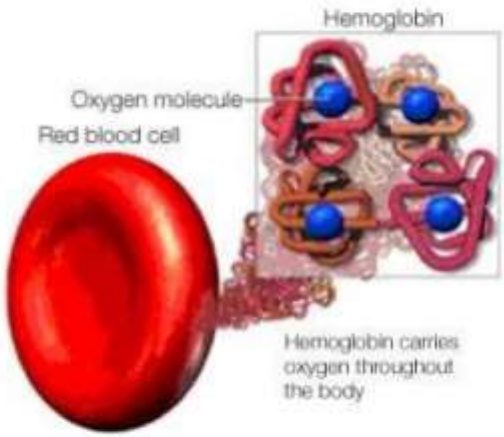


Function of hemoglobin

Function of Hemoglobin

- **Heme has the ability to bind oxygen reversibly and** carry it to tissues.
- It also facilitates the exchange of carbon dioxide between the lungs and tissues.

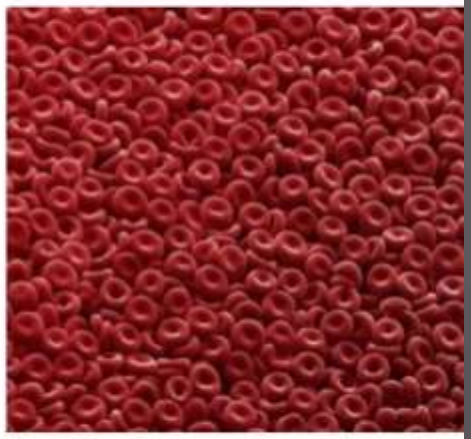
Thus, **hemoglobin functions as the primary medium of exchange of oxygen and carbon dioxide.**



RANGE OF HAEMOGLOBIN

Normal Haemoglobin concentration in humans

- Men - 150 ± 20 g/l
- Women(non pregnant)- 135 ± 15 g/l
- Pregnant women –
  - ✓ 1st trimester 124–135 g/l
  - ✓ 2nd trimester 110–117 g/l
  - ✓ 3rd trimester 106–109 g/l
- Birth- 180 ± 40 g/l



Sahli' s Method for Estimation Of Haemoglobin:

Principle :

- This method depends on converting Hb. Into acid haematin (has a dark brown colour).

- The solution developed is diluted with water and the colour developed after dilution is matched with the colour of the standard tubes.

METHODS FOR ESTIMATION OF HAEMOGLOBIN

A. Visual Methods

A.a. Sahli's method
A.b. Tallquist chart (obsolete)
A.c. WHO Hb Color Scale

B. Photoelectric Method

B.a. Cyanmethemoglobin method
B.b. Oxyhaemoglobin method & Alkaline Hematin method
B.c. Electronic counter method
B.d. Direct reading electronic haemoglobinometer

Hemoglobin Estimation

Sahli's Acid Hematin Method

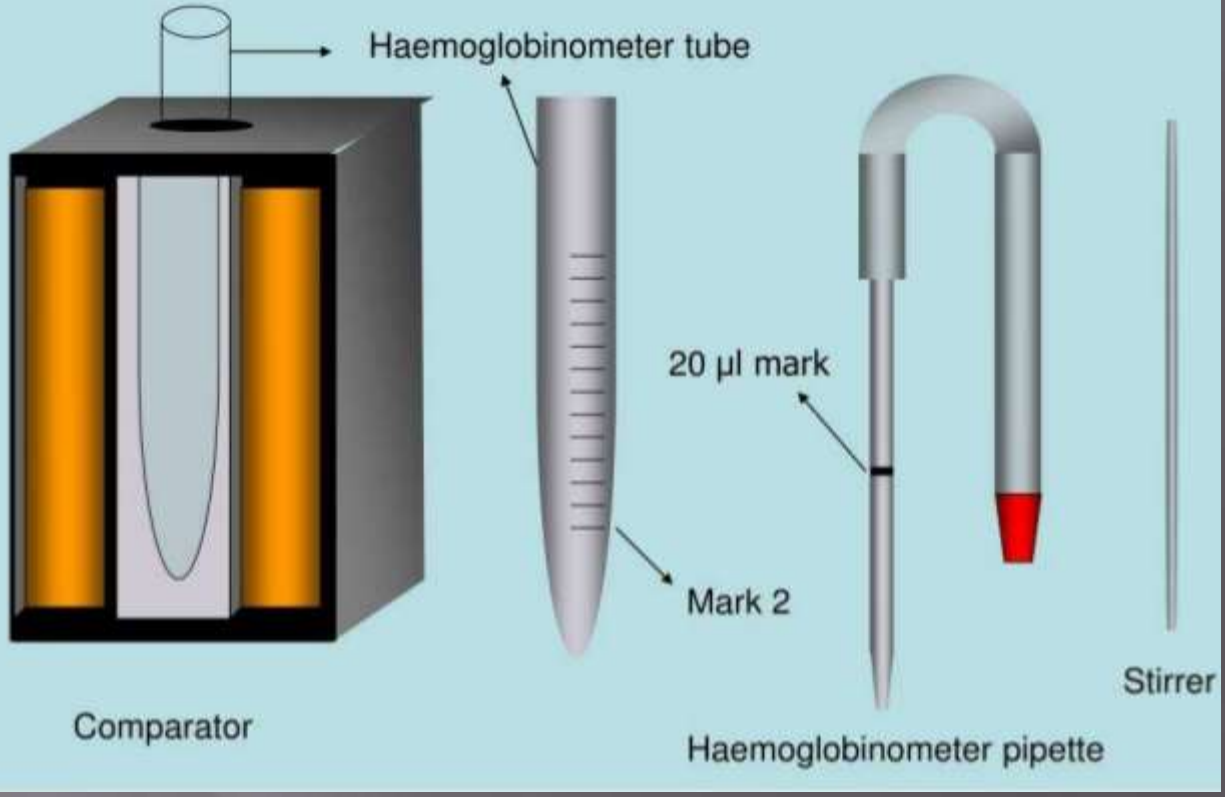
- Equipments-
- Sahli hemoglobinometer
- Sahli pipette(marked at 20 microlite or 0.02 ml)
- Stirrer
- Dropping pipette
- Reagents
- N/10 hydrochloric acid
- Distilled water

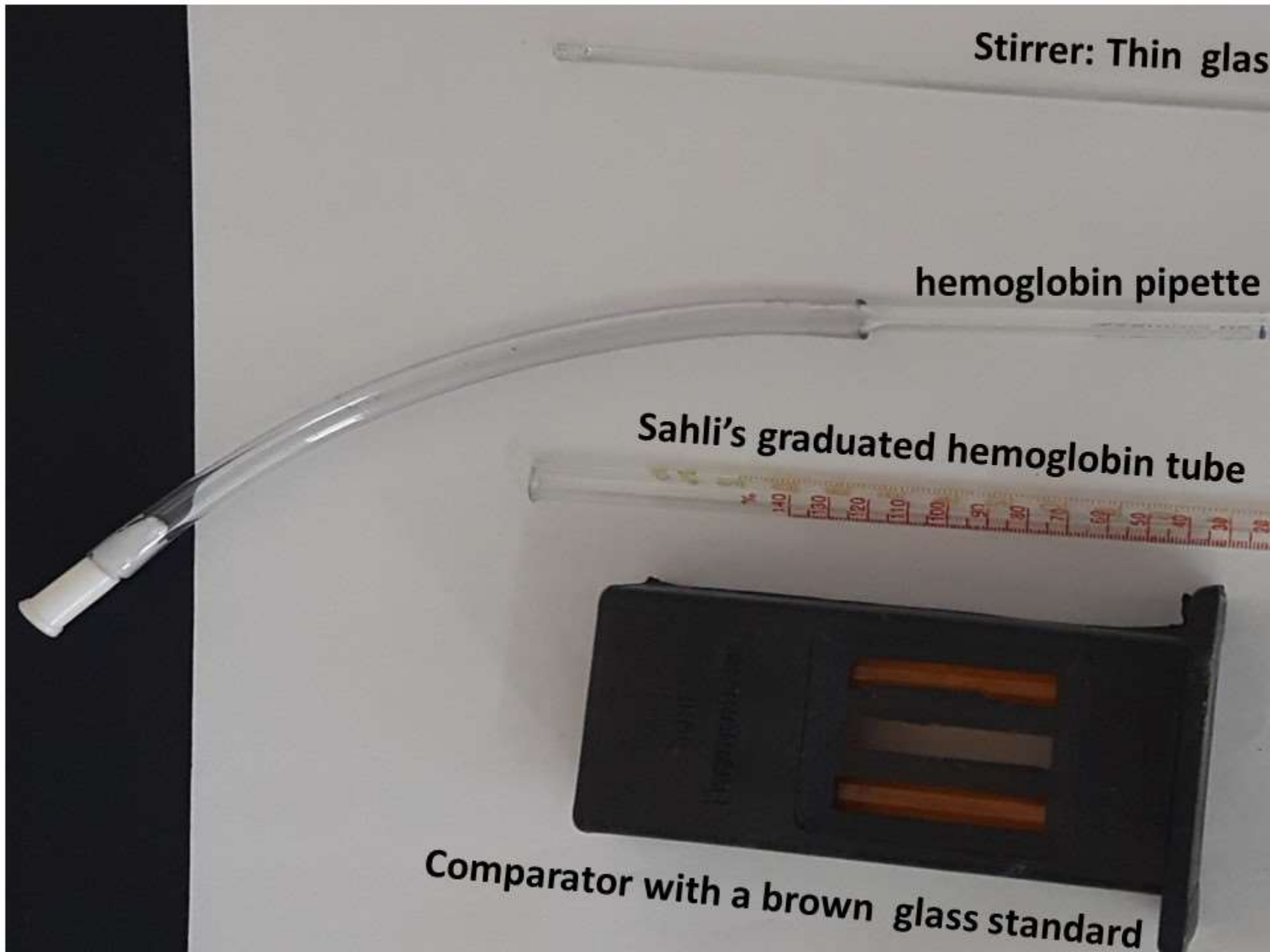
Sahli's Hemoglobinometer

Sahli's Acid Hematin Method

- Place N/10 HCl into Hb tube upto 2 grams.
- Blood sample in Sahli's Hb pipette upto 20 micro litre.
- Add blood sample to acid solution.
- Mix with a stirrer.
- Allow to stand for 10 minutes.
- Add distilled water drop by drop till the colour of the solution matches to brown glass standard.
- Take the reading of the lower meniscus from the graduated tube in grams.

SAHLI'S HAEMOGLOBINOMETER




Stirrer: Thin glas
hemoglobin pipette
Sahli's graduated hemoglobin tube
Comparator with a brown glass standard

Hemoglobin Estimation

- Haemoglobin concentration provides information about the status of anaemia in the population.

Normal adult Hb:

- In male, it is 14-18 g/100ml of blood (dl).
- In female it is 12-16 g/100ml of blood.

Estimation of Hb

Introduction and principle

Hb composed from haem (iron + protoporphyrin) and globin protein. By adding HCl, Hb is converted into colored acid hematine (Sahli method) and the intensity of the color is measured by comparing it with the standard. This could be done visually or spectrophotometrically.

Concentration of Hb is measured either as a percentage of normal or, better in absolute figure (gm/dl)

PRINCIPLE

Commonly used methods
Sahli's Method – A Color Based Method

- Visual method.
- **Principle :**
  - Hb is converted into acid hematin with the action of dilute hydrochloric acid (N/10 HCl).
-
  - The acid hematin is brown in color and its intensity is matched with a standard brown glass comparator in a visual colorimeter called Sahli's colorimeter.

ADVANTAGE

Sahli's Acid Hematin Method

Advantages

- Easy to perform
- Quick
- Inexpensive
- Can be used as a bedside procedure
- Does not require technical expertise

DISADVANTAGE

Sahli's Acid Hematin Method

Disadvantages

- For maximum colour, longer time is required
- Perfect matching with brown glass standard is not possible
- Carboxyhemoglobin,methemoglobin and sulfhemoglobin are not converted to acid hematin
- Developed of colour is slow and acid hematin is not stable
- Source of light will influence the comparison of colours

THANKS