ESTIMATION OF HEMOGLOBIN

Hemoglobin is the major constituent of the red cell cytoplasm, accounting for approximately 90% of the dry weight of the mature cell. 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 form and protoporphyrin.

- **Normal value of Hb**

Males 13.5-18g/dl

Females12 -16 g/dl

- **Significance of Hb estimation**:

Hemoglobin estimation is used as a screening test for detecting anemia.

Various methods are available for estimation of hemoglobin in the laboratory.

I. Methods based on development of color. These are:

- Sahli's or acid hematin method
- Cyanmethemoglobin method
- Oxyhemoglobin method
- Alkaline hematin method

II. Measurement of oxygen combining capacity

III. Measurement of iron content

The commonly used methods are Sahli's/ acid hematin method and Cyanmethemoglobin method.

I.Sahli's/acid hematin Method

❖ Principle:

Blood is mixed with N/10 HCl resulting in the conversion of Hb to acid hematin which is brown in color. The solution is diluted till it's color matches with the brown colored glass of the comparator box. The concentration of Hb is read directly.

❖ Equipment required:

Hemoglobinometer which consists of

- comparator box which has brown colored glass on either side
- Hb pipette which is marked up to 20mm^3(0.02ml blood)
- Tube with marking of Hb on one side
- glass rod
- dropper Reagents required N/10 HCl
- Distilled water

❖ **Sample:** Venous blood collected in EDTA tube

❖ **Procedure:**

1. Add N/10 HCl into the tube up to mark 20%

2. Mix the EDTA sample by gentle inversion and fill the pipette with 20mm^3 blood. Wipe the external surface of the pipette to remove any excess blood.

3. Add the blood into the tube containing HCl. Wash out the contents of the pipette by drawing in and blowing out the acid two to three times. Mix the blood with the acid thoroughly.

4. Allow to stand undisturbed for 10min.

5. Place the hemoglobinometer tube in the comparator and add distilled water to the solution drop by drop stirring with the glass rod till it's color matches with that of the comparator glass. While matching the color, the glass rod must be removed from the solution and held vertically in the tube.

6. Remove the stirrer and take the reading directly by noting the height of the diluted acid hematin and express in g.

❖ **Advantages:**

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

❖ **Disadvantages :**

- Less accurate.
- All hemoglobins (oxyhemoglobin, sulphemoglobin) are not converted to acid hematin and hence the value of Hb obtained is less than the actual value.

- The color of acid hematin develops slowly.
- Color of acid hematin fades with time and dilution must be done exactly after 10 min when the color development is maximum
- Individual variation in matching of color is seen.
- If the matching point is passed, the whole procedure has to be repeated.
- Color of glass in the comparator box tends to fade with time .
- Lack of a true standard.