Estimation Of Hemoglobin (Hemoglobin or Hb or Hgb Test)

- The Hb test measures the level of hemoglobin in the blood.
- Hemoglobin is a protein in red blood cells that carries oxygen from the lungs to the rest of the body. It consists of heme (iron material + pigment) and globin (protein).
- If hemoglobin levels are abnormal, it may be a sign of a blood disorder.
- 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 :
1. Sahli's or acid hematin method
2. Cyanmethemoglobin method
3. Oxyhemoglobin method
4. Alkaline hematin method

II. Measurement of oxygen combining capacity
III. Measurement of iron content

Cyanmethemoglobin Method

This is the internationally recommended method for determining hemoglobin

Principle:

Blood is diluted in a solution containing potassium cyanide and potassium ferricyanide. The latter converts Hb to methemoglobin which is converted to cyanmethemoglobin (HiCN) by **(Drabkin's solution**) potassium cyanide. The absorbance of the solution is then measured in a spectrophotometer at a wavelength of 540nm.

Equipment Require

- Hemoglobin meter
- Hb pipette
- Test Tube or a cuvette

- Stop watch
- Spectrophotometer
- Drabkin's solution

Drabkin's solution pH7.0-7.4 which contains
Potassium cyanide 50 mg
Potassium ferricyanide 200 mg
Potassium dihydrogen phosphate 140 mg
Nonionic detergent 1 ml
Distilled water 1 L

Sample: Venous blood collected in EDTA

PROCEDURE

1. In a test tube, take 5 ml of Drabkin's solution.
2. By pipette, add 20 µl (0.02 ml) of blood to drabkin solution.
3. Stopper the tube, mix by inverting several times, and allow to stand for at least 5 minutes. This time is adequate for conversion of hemoglobin to cyanmethemoglobin.
4. Transfer the test sample to a cuvette.
5. Read the absorbance in a spectrophotometer at 540 nm .
6. Take the absorbance of the standard solution. Absorbance should be read against reagent blank (Drabkins solution).
7. Hemoglobin value is derived from the formula given below.

$$\text{Hemoglobin in gm/dl} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

$$HB = \frac{A\ \text{sample}}{A\ \text{standard}} \times n \quad g/dl$$

n = Concentration of standard

$$HB = \frac{PCV - 2}{3}\ g/dl$$

NORMAL VALUE

Males 15 ± 2 g/dl
Females 13.5± 1.5 g/dl

- Children 1 day may reach 20 g/dl
- Babies 3 months 9.5 - 13.5 g/dl
- Children from 6 months to 5 years 11 g/dl
- Children from 5 to 16 years 11 - 13 g/dl
- Pregnant women not less than 11 g/dl