**\_ Lecture Four : Hb estimation by different methods**

**Manual Haemoglobin (Hb) Estimation : *The Cyanmethaemoglobin Method***

**Introduction:**

Measurement of Hb concentration in whole blood is a basic screen for anaemia or for polycythemia. There are many methods for the Hb estimation, but the best recommended method is the Cyanmethaemoglobin method.

**The advantage** :

standardized and possessing stable solutions.

Venous or capillary blood collected in EDTA. Alternatively, free flowing capillary blood may be added directly to the diluting fluid and measured.

**Equipments:**

- Spectrophotometer.

- Automatic pipettes.

- Racks.

**Disposable materials:**

- Drabkin's solution.

- Plastic or glass tubes.

- Blue tips.

- Yellow tips.

**Principle:**

Blood is diluted in a solution containing potassium cyanide potassium ferri-cyanide (Drabkin's solution), Hb is oxidized to methaemoglobin by potassium ferri-cyanide, methaemoglobin in turn combines with potassium cyanide to form cyanmethaemoglobin . The absorbance of the solution is measures in a spectrophotometer at wave length 540 nm against Drabkin's solution as a blank. The result is (calculated from formula provided below) expressed in gm/liter or mg/dl.

**Method:**

1. Pipette 4 ml of Drabkin's solution in a tube.

2. Pipette exactly 0.02 ml (20μl) of well mixed blood using a pipette.

3. Clean outside of the pipette and wash out the blood in the tube containing diluent (dilution=1/200).

4. Mix and leave for 5-10 minutes for reaction to be completed.

5. Read absorbance in the spectrophotometer at wavelength 540nm.

**Notes on this technique:**

1. The blood sample must be properly mixed before taking the sample, and if refrigerated, allow to worm.

2. Care must be taken when handling potassium cyanide.

3. Using clean tubes and pipettes.

**Comments:**

The cyanmethaemoglobin is a reference method for Hb estimation: because:

1. All types and compounds of Hb except sulphaemoglobin are estimated.

2. Highly reliable, and stable standard are available.

**Normal Range:**

* Adult males: 14-18 g/dl
* Adult females: 12- 16 g/dl
* Children: 11-14 g/dl.
* Newborn infants: 13.0-20 g/dl

**Calculation:**

 Absorption of sample

Hb(g/dl)= ---------------------------------- X concentration of standard

 Absorption of standard