

Lab no 6**Haemoglobin [Hb]**

Haemoglobin is the main constituent of the red blood cells and carry out the important function of transportation of oxygen from lungs to various parts of the body. To lesser extent it transport back carbon dioxide from the body to the lungs.

When fully saturated each gram of haemoglobin holds approximately

1.34ml of oxygen. The red cell mass of on adult contains approximately

600gm of haemoglobin, capable of carrying 800ml of oxygen.

The major portion of normal haemoglobin in adult blood is termed *haemoglobin A*. The globin portion of each *haemoglobin A* molecule is composed of two alpha chain containing 141 amino acids and two beta chains, made up of 146 amino acids. The molecules is ellipsoidal, with four heme groups [responsible for oxygen transport] at the surface .

Each heme group is associated with one molecule of oxygen, haemoglobin is referred to as oxyhaemoglobin [HbO_2]. In both haemoglobin and oxyhaemoglobin, iron remain in ferrous state [Fe^{+2}].

When iron oxidized to ferric [Fe^{+3}], methemoglobin [hemoglobin, Hi] is formed, and the molecule loses it's capacity to carry oxygen or carbon dioxide.

Haemoglobin determination using Cyanmethemoglobin method.**Principle:**

The whole blood is added to cyanmethemoglobin [HiCN] reagent. The pottasium ferricyanide in the reagent converts the haemoglobin iron from the ferrous state [Fe^{++}] to the ferric state [Fe^{+++}] to form methemoglobin [Hi] which then combines with potassium cyanide to form stable pigments, cyanmethemoglobin [HiCN].

Hi = haemoglobin = haemoglobin in which the iron has been oxidized to the ferric state.

HiCN = hemoglobin cyanide = Hi which has been banded to the cyanide ions.

The non ionic detergent present in the reagent improves the lysis of the red blood cell and decreases the amount of turbidity resulting from abnormal proteins. Such as lipoprotein.

The color intensity of this mixture is measured in a spectrophotometer at wavelength of 540 nm.

The intensity of absorbance of the cyanomethemoglobin is proportional to haemoglobin concentration.

Reagent:

- Potassium phosphate. - Sodium chloride.
- Potassium ferri-cyanide. - Detergent.
- Potassium cyanide.

Precaution: "toxic"!

Extreme care should be taken with this reagent as it contains cyanide Which is poisonous. Do not pipette by mouth..

The undiluted reagent is stable up to the expiry date specified when stored at 2-8 c⁰ in dark bottle.

Procedure:

- Wave length ----- 540nm.
- Temperature ----- 25/30/37 c⁰.
- Cuvette ----- 1cm Light path.

Working reagent	5.0 ml
Blood sample	20μ

Mix, wait for 3 min.

Read the Abs. of sample. After setting the spectrophotometer to 0.00 by using blank soln. [working reagent only].

Calculation:

- ⇒ Use the following standard curve to determine the conc. of blood sample.
- ⇒ Conc. [gldl] = 35.8 x A⁰.
- ⇒ Conc. [mmol/L]= 22.9 x A⁰.

Reference value:

Male ----- 14-18 grldl [8.7-11.2mmol /L].

Female ----- 12-16grldl [7.5-9.9mmol /L].

Hemoglobin Standard curve (experimental done)

