Determination of hemoglobin concentration (Hb)

Hemoglobin (Hb) is the red pigmented protein found in the erythrocytes. Hb is synthesized in the immature erythrocytes in bone marrow. The protein part, known as globin is colorless and consists of four subunits (four peptide chains, two alpha and two beta), plus four non-protein red pigments called heme. Each Hb molecule can carry up four oxygen molecules. Function of Hb is the transport of oxygen and carbon dioxide, and to aid in acid-base regulation. Normal values of Hb are:

Males 14-18 g/dl.

Females 12-16 g/dl.

Children 10-14 g/dl.

Methods of Hb Determination:

1- Oxyhemoglobin

2- Cyanomethemoglobin (Colorimetric method)

3- Acid Hematin (Sahli method)

4- Direct matching

Colorimetric Method assay principle:

Hb is oxidized by ferricyanide which is converted into soluble pigment Cyanomethemoglobin by cyanide using Drabkin's diluent solution. (1g Sodium bicarbonate, 50mg Potassium cyanide (KCN), 200 mg Potassium ferricyanide (K3FeCN6) complete to 1000 ml distilled water). Obtain 10 µl blood sample and mix well with 5ml Drabkin reagent, incubate for 5 min and measure absorbance of specimen against reagent blank.

Sahli method assay principle:

The intensity of red pigment is directly proportional to amount of hemoglobin present. Hemoglobin in blood can exists as a mixture of HBO_2 and a very small amount of methemoglobin, sulph-hemoglobin and carboxy hemoglobin ($HbCo_2$). Since each one of these has a different color and optical properties, conversion of all these pigments into a standard form is necessary before the total hemoglobin can be measured colorimetrically.

In this method all the hemoglobin is converted to acid haematin (Sahli; King). The intensity of the color is measured by comparing it with hemoglobin concentration expressed either as a percentage of normal in absolute figures (grams % or millimoles per liter). Provided the value of the standard for normality used is given, the Sahli apparatus is calibrated in percentage, 14 grams hemoglobin being taken as 100% of normal.

Apparatus

The sahli hemoglobinometer is the most practical for routine clinical use.

1- A colored standard, either sealed tube containing a suspension of acid haematin or, a tinted glass having the same color as standard haematin solution.

2- A small tube calibrated in percentage values. This tube has a capacity of 2 ml. to the mark 100. A value of 100% in this procedure corresponds to 14 g. haemoglobin per 100ml blood. A capillary pipette to contain $20mm^3$ / or (20 µl) of blood by finger puncture technique.

Procedure:

1- Be sure your apparatus is clean and dry.

2- Add N/10 HCl to the mark 10 in the graduated open tube.

3- From a freely flowing finger puncture (or from well mixed oxalated /or heparinized blood) fill the capillary pipette to the mark 20. Do not squeeze hard the finger.

4- Thoroughly, wipe the blood adhering to the outside of the capillary pipette.

5- Expel the blood from the pipette under acid solution in the tube. Wash the pipette and mix the blood with the acid by sucking up the mixture and expelling it again a number of times. Do not mix by tipping the tube over your finger.

6- Let mixture stand in the tube for 10 minutes. After this most of the hemoglobin will be converted to acid haematin.

7- Dilute the blood mixture with distilled water until the color exactly matches that of the standard tube. Use diffuse sunlight, not artificial light. When nearing the end point add one drop at a time tacking a reading and mixing well with a thin glass rod after each drop.

8- Read the level of the tube. It is always a good habit to put an extra drop of the acid after taking the final reading and noting that the color is in fact just too light.

9- Calculate the hemoglobin in grams.

10- One can take an extra reading to calculate a mean value, or drink a liter of water and repeat the assay to see the difference in readings.