Quadrant II – Transcript and Related Materials

Programme : **Bachelor of Science (Third Year)**

Subject : **Microbiology**

Paper Code : **MID 106**

Paper Title : **Haematology and Clinical Biochemistry**

Unit 2 : **Blood counts**

Module Name : **Determination of haemoglobin - significance, principle and method.**

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Notes

Haemoglobin

Haemoglobin is the main constituent of red blood cell (RBC).It carries out the important function of transportation of O_2 from lungs to various parts of body, and it also transports back CO_2 from body to the lungs. Haemoglobin consists of two components: heme and globin. Haem is combination of iron and protoporphyrin and globin is the protein, formed of amino acid chain.

Clinical Significance of Haemoglobin

1) A decrease in haemoglobin below normal range is an indication of anemia.
2) An increase in haemoglobin concentration occurs in hemoconcentration due to loss of body fluid in severe diarrhea and vomiting.
3) High values are also observed in congenital heart disease (due to reduced oxygen supply) in emphysema and also in polycythemia.
4) Haemoglobin concentration drops during pregnancy due to hemodilution. Children also have low Hb.
5) The Hb concentration is lower in adult female than in adult male.

Decrease or increase in concentration of Hb should be reported as it is a sign of disease.

Normal values	Hb, g/dl
Men	13-18

Women	12-16.5
Children (up to 1 year)	11.0-13.0
Children (10-12 years)	11.5-14.5
Infants (full term cord blood)	13.5-19.5

Determination of Hemoglobin by Sahlis (Acid Hematin) method

Principle

Hemoglobin(Hb) is converted to acid hematin by the action of hydrochloric acid (HCl). Hemoglobin is converted to brown colored acid hematin. The acid hematin solution is further diluted till its colour matches exactly to that of permanent standard comparable tube. The resulting colour after dilution is compared with standard brown glass reference blocks of a Sahli hemoglobinometer.

Specimen

Capillary blood or thoroughly mixed anticoagulated (EDTA or double oxalated) venous bloodThe specimen need not be a fasting sample.

Requirements

1) Sahli hemoglobinometer

It consists of

a) a standard brown glass mounted on a comparator

b) a graduated tube

c) Hb pippete (0.02 ml)

2) 0.1 N hydrochloric acid

3) Distilled water

4) Pasteur pipettes

Procedure

1. By using a pasteur pipette fill the Hb cylinder up to the lowest mark with 0.1 N HCl solution.

2. Add 20 µ liters(0.02 ml) blood with the help of Sahlis pipette. Make sure that there is no air bubble inside the pipette.

3. Mix the blood with HCl, which is already placed in the cylinder. Take care that there is no blood left to the sides of the cylinder. Rinse the pipette twice in the blood solution. Allow it to react for about 10 mins till the solution becomes dark brown in colour.

4. Acid hematin is produced in the cylinder after combination of Hb and acid.

5. Dilute the solution with distilled water by adding few drops at a time carefully and by mixing the reaction mixture, until the colour matches with the standard comparable tube.

6.The matching should be done only against natural light. The level of the fluid is noted at its lower meniscus and the reading corresponding to this level on the scale is recorded in g/dl.

7. Read the Hb concentration directly from the level of the diluted solution.The reading may be in percentage(%) or g/L.

Determination of Hemoglobin by Cyanomethemoglobin method(Colorimetric)

Principle

When blood is mixed with Drabkin's reagent containing potassium cyanide and potassium ferricyanide, haemoglobin reacts with ferricyanide to form methemoglobin which is converted to stable cyanomethemoglobin(HiCN) by the cyanide. The intensity of the colour is proportional to haemoglobin concentration and it is compared with a known cyanomethemoglobin standard at 540 nm.

Requirements

1) Drabkin's reagent

2) Cyanomethemoglobin –standard solution

Composition

a) Potassium ferricyanide: 200 mg

b) Potassium dihydrogen phosphate: 280 mg

c)potassium cyanide : 50mg

d) Distilled water -1000ml

This reagent is stable in apoytehne container at 2-8oC

Standard solution with Hb content 5g, 10g,15 g is recommended.

2) colorimeter with green filter(540nm)

Cyanmethemoglobin(HiCN) standard (Hb standard)

It is commercially available.This standard is directly pipetted in a cuvette and optical density measured at 540 nm.The reading obtained corresponds to 15 g/dl, hemoglobin.The intensity of the colour is proportional to hemoglobin concentration and it is compared with a known cyanmethemoglobin standard at 540 nm.

	Std 5	Std 10	Std 15	Blank
1) Drabkin's reagent, ml	3.34	1.67	0.00	5.0
2) Hb standard, ml	1.66	3.33	5.0	0.0

Procedure

Take two test tubes and label it as 'B'(Blank) and T(Test solution) add 5 ml of Drabkins solution in each test tube. Avoid mouth pipetting as Drabkin solution is poison. Stopper the tube with rubber cap, add 0.02 ml of blood specimen into the tube marked with 'T'. The specimen is taken with the help of Sahlis pipette. Wipe off the tip of the pipette before adding blood into the test tube. Mix the content of the tube and wait for 10 min with the help of blank, Standard(Std), solution, find out absorption of test solution in colorimeter at 540 nm. Hb can be calculated as:

$$\text{Hb conc.} = \text{Absorbance of test solution} \times \frac{\text{Concentration of Std}}{\text{Absorbance of Std}}$$

Prepare a graph by plotting O.D readings on Y-axis and concentration of hemoglobin standards i.e 5.0 g, 10.0 g and 15.0 g on X-axis. A straight line passing through origin indicates agreement with Beers law. This graph can be used as a standard graph for hemoglobin determination.

Precautions

The reagent is poisonous handle carefully. To avoid cyanide, lauryl sulfate has been proposed as a nonhazardous substitute as lauryl sulfate has similar properties to HiCN. Mix anticoagulated blood by swirling properly before pipetting. Adjust carefully the blood column upto the graduation mark and use dry cotton to wipe excess blood on the pipette. If capillary blood is used keep Drabkins reagent ready in a test tube. Collect the free flowing blood into Hb pipette(Sahli pipette), wipe the excess blood and dispense in the reagent. Mix immediately to avoid clotting of the blood.

The hemoglobin content of blood can be determined accurately by using a spectrophotometer as follows

a) Add 0.02 ml of blood in 5.0 ml of Drabkins reagent. Mix well and keepat room temperature for 10 mins.

b) Read absorbance of the reaction mixture at 540nm.

c) Calculate Hb concentration as follows g/dl, Hb

$$= \frac{A^{540}\ \text{HiCN} \times 64500 \times \text{dilution factor(250)}}{44.0 \times d \times 1000 \times 10}$$

A^{540} HiCN – Absorbance of the Drabkin's reagent at 540nm

64500 - Molecular weight of Hb

Specific Gravity method (Qualitative)

Principle

When a drop of whole blood is dropped into a copper sulfate solution with a particular specific gravity, the density of drop is directly proportional to the amount of Hb in that drop.If the drop is denser than the specific gravity of solution, the drop is settled down to the bottom. If not , it will float on the top.

Procedure

Copper sulfate solution of specific gravity 1.055 for male and 1.053 for female is taken in two test tubes. The specific gravity corresponds to minimum Hb level. For both the respective sexes 1.055 specific gravity corresponds to 13 g/dL Hb and specific gravity 1.053 corresponds to 12 g/dL of Hb which is the minimum normal haemoglobin levels of men and women. A drop of blood is collected by skin puncture and taken into long necked pasteur pipette and then immediately drop the blood into proper copper sulfate solution, observe for 15-20 seconds. If the hemoglobin is normal, the drop of blood will sink to the bottom. This indicates that the drop is denser than the specific gravity of the solution If the person is anaemic, the drop will float on the top. This test does not give the exact amount of Hb.This technique is usually applied in blood banking for screening the donor. It is quick and easy, accurate technique.

Gasometric method

In this method, Van Silky apparatus is used for gasometric determination of Hb In this method, blood is first of all saturated with oxygen. Then oxygen is taken off and collected, depending upon the amount of oxygen collected , the amount of Hb can be calculated. For 1 of Hb-1.34 ml, of oxygen is present in blood.

Chemical method

Hb is found out by finding the amount of iron present in blood.The amount of iron is directly proportional to the amount of Hb present in blood.