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.

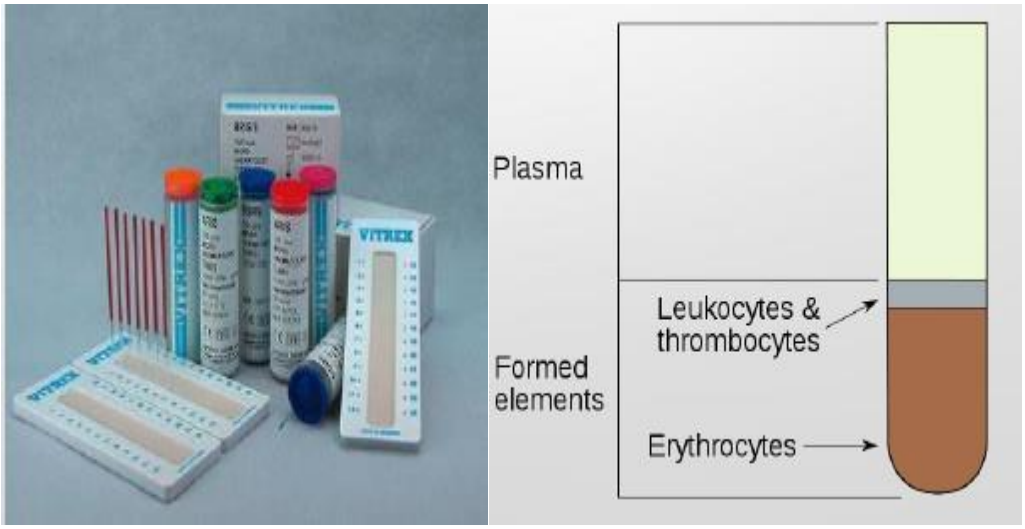
Normal Range:

- 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:

$$\text{Hb(g/dl)} = \frac{\text{Absorption of sample}}{\text{Absorption of standard}} \times \text{concentration of standard}$$

Lecture five : Haematocrit (Packed Cell Volume – PCV) Determination



The haematocrit (PCV) is the percent of the packed red cells in a volume of whole blood. The hematocrit may also be referred to as Packed Cell Volume (PCV) or erythrocyte volume fraction (EVF). It reflects the combination of total number of RBCS , and the volume that they occupy in plasma . When accurate measurements of Red cell count and Hb concentration are available, the absolute values can be calculated

Note: Remember the Hematocrit is a reflection of the RBC concentration, not the RBC mass.

It is a screening test for anemia or polycythemia. In comparison, hemoglobin estimation is less accurate, and RBC count far less accurate.

Principle:

A volume of anticoagulated blood is placed in a glass tube which is centrifuged so the blood will be separated into three layers: Red cells, Buffy coat (WBC and platelets) and plasma. Ideally there should be complete separation of the three layers.

Haematocrit is the ratio of the height of red cells column to that of the whole blood in the tube.

The two methods of direct measurement of the PCV which are in current use are:

1. Macro-method using Wintrobe tubes.

2. Micro-method using capillary tubes.

3. Electronic cell counting

The more popular one is the micro-method, as it has the advantage of short time of centrifugation and better packing of the red cells.

Micro-Haematocrit Method:

Test sample:

Heparin or EDTA venous or capillary blood.

Equipments:

- Micro-haematocrite centrifuge.
- Plastic sealer or Bunsen burner.

Disposable materials:

- Capillary tubes 75 mm long and internal diameter of 1 mm.

Method:

1. The blood sample should be used as fresh as possible, and well mixed.
2. Using the capillary action, allow blood to enter the tube stopping at 10-15 mm from one end. Wipe the outside of the tube.
3. Seal the dry end by pushing into the plasticine two or three times.

4. If heat sealing is used rotate the dry end of the tube in a fine Bunsen Burner flame.

5. Place the tube into one of the centrifuge plate slots, with the sealed end against the rubber gasket of the centrifuge plate.

6. Keep a record of the patient number against centrifuge plate number.

7. Centrifuge for five minutes.

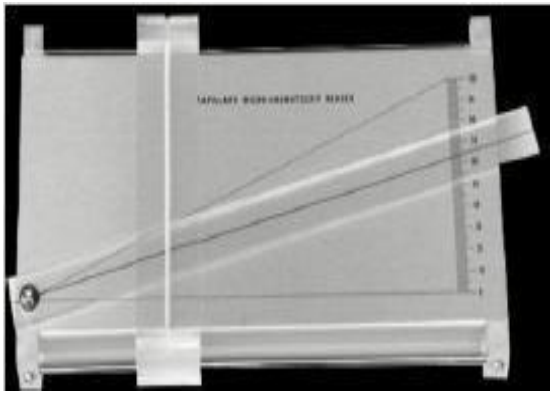
8. Read the PCV in the micro haematocrit reader.

9. Unit: the haematocrit result is expressed in percentage.

Note: It is preferable to perform the test in duplicate.

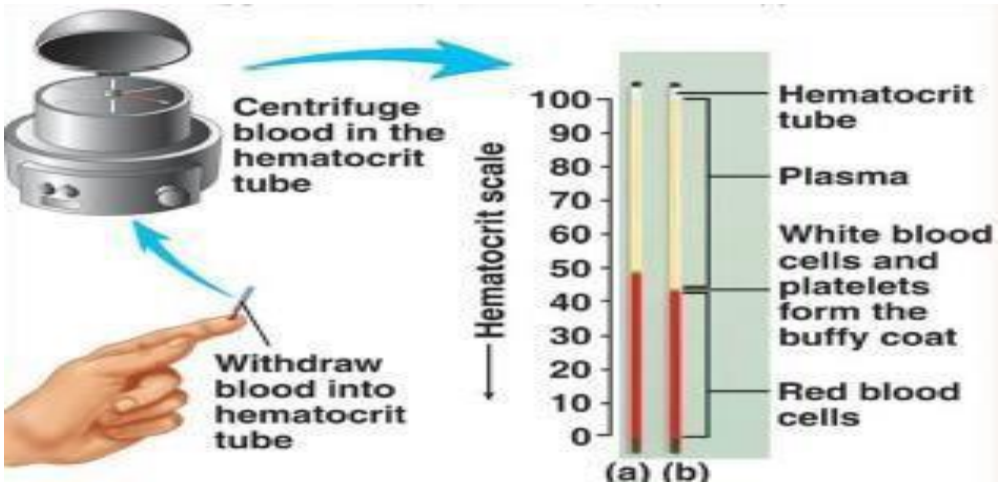
Normal ranges: The normal values of PCV vary according to the age and sex of the individuals. The normal ranges are

- Adult males = 40% - 52%.
- Adult females = 37% - 47%.
- Pregnant =30%–46%
- Neonates = 40%–68%
- 3 months = 29%–54%
- 1–2 years = 35%–44%



Microhaematocrit reader.

Microhematocrite Centrifuge



Results extraction:

Hct% = {Height of RBCs (mm) / Height of RBCs and plasma (mm)} ×100

For example, if the height of packed red cells is 45 mm, then = 45/ 100 ×100 = 45 percent.

It also means that out of 100 volumes (or parts) of blood 45 volumes (or parts) are red cells and 55 volumes (or parts) are plasma. Thus, out of 1 liter of blood, 450 ml are red cells and 550 ml are plasma.

Importance of the PCV : It is so important for the following reasons

- Simple , Accurate Reliable
- Screening for large clinic population

PCV-Low

1. **in pregnancy** Cause is a hemodilution the RBCs are "diluted

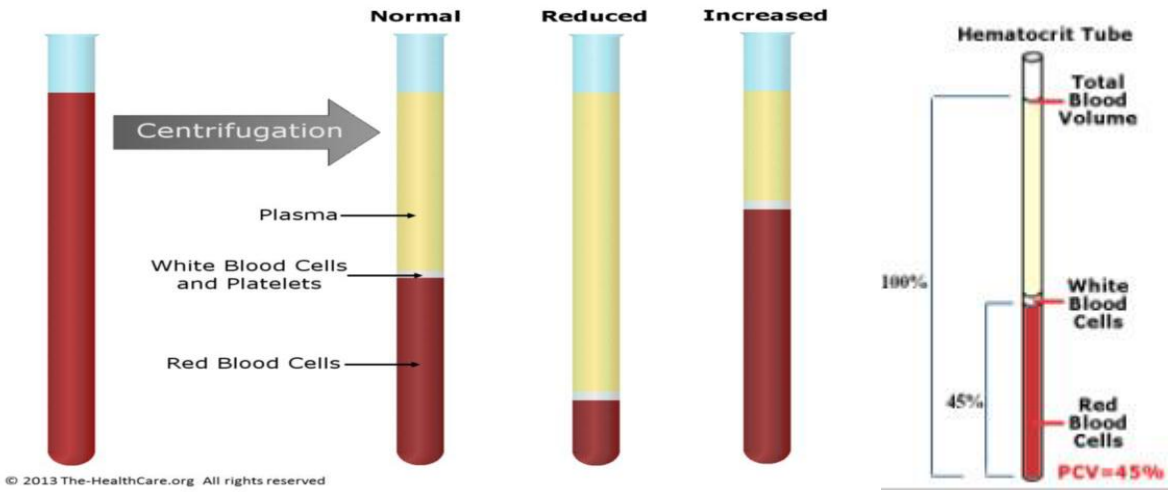
2. Low RBC production from the bone marrow **(**Toxins, cancer, lowEPO)

3. IDA, aplastic anemia. Hemolytic anemia etc

PCV-High : A high hematocrit value may **truly** reflect an increase in the fraction of RBCs

1. polycythemia vera
2. secondary polycythemia (smoking, kidney cancer, high attitude living

3-reactive polycythemia (vomiting and diarrhea, **Burn)**



Diagnostic uses of the buffy coat

1. The buffy coat consist less than 1% of the total volume of the blood
2. The buffy coat is used to

- Extract DNA from the blood of mammals (since mammalian red blood cells are anucleate and do not contain DNA).
- Quantitative buffy coat (QBC) is a laboratory test to detect infection with malaria or other blood parasites.

<u>How is the PCV in the following individuals ?</u>

1. Fluid preservation - the Hct may be decreased explain that? the RBC mass normal
2. Patient with aplastic anemia ? Decrease low RBC mass
3. Patient with relative polycythemia? increase --. Low RBC mass
4. Patient with PRV? Increase increase RBC mass