2. Hematocrit (HCT) or packed cell volume (PCV)

The hematocrit measures the volume of red blood cells compared to the total blood volume (red blood cells and plasma).



Methods of hematocrit

1- Microhematocrit method

2- Macrohematcrit method (wintrobe method)

Microhematocrit method

Material used

· Capillary tubes, plain or heparinized   
· Modeling clay sealant   
· Microhematocrit centrifuge   
· Microhematocrit reader

Procedure of Microhematocrit method

1. Fill the capillary tube two-thirds to three-quarters full with well-mixed, venous blood or fingertip blood (For fingertip blood use heparinized tubes).

2. Seal one end of the tube with clay.   
3. Place the filled tube in the microhematocrit centrifuge, with the plugged end away from the center of the centrifuge.

4. Centrifuge at a preset speed of 10,000 to 12,000 rpm for 5 minutes. If the hematocrit exceeds 50 percent, centrifuge for an additional 3 minutes.

5. Place the tube in the microhematocrit reader. Read the hematocrit by following the manufacturer's instructions on the microhematocrit reading device.

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Advantages

• Rapid.

• Simple.

• Several specimens can be measured at one time.

• Accurate compared with the visual comparator methods.

• Useful for validating the accuracy of other methods.

Reference values

* + - Newborn 42-60%
    - Infant/child 30-43%
    - Adult male 42-52%
    - Adult female 36-46%

3. Red blood cell counts

The total red blood cell (erythrocyte) count is the number of red cells in one cubic millimeter of blood.

Methods:-

1. Manual methods
2. Electronic method

Manual method (thoma)

Apparatus and Reagent

1. Neubauer chamber with coverslip (Hemocytometer Chamber).
2. Red cell pipette.
3. Microscope.
4. Diluting fluid.



***Neubauer Counting Chambers***

Diluting fluids

1. Hayems solution
2. Mercuric chloride 0.5 gm
3. Sodium chloride 1 gm
4. Sodium sulphate 5 gm
5. Distilled water to 200 ml
6. Normal saline (0.9 % Nacl)
7. Sodium chloride 9 gm
8. Distilled water 100 ml

Procedure

1. Draw blood to the 0.5 mark in the RBC pipette, without letting any bubbles into the pipette by holding the pipette almost horizontally. The pipette must be clean and dry.
2. Draw the diluting fluid up to the mark 101 (dilution 1 to 200), while filling the bulb the pipette should be gently rotated to obtain good mixing.
3. The coverslip is placed over the neubauers chamber so as to cover both the ruled platforms evently.
4. Now load the chamber. This is done in three steps:
5. Mix the contents of pipette for 3 minutes.
6. Expel 6 drops from the pipette to remove the fluid in the stem which has not been mixed with blood.
7. By holding the pipette at an angle of 45 degree and touching the space between the coverslip and the chamber by the point of the pipette, an appropriate drop of the mixture is allowed to run under the coverslip by capillary action.
8. Allow two minutes for setting of the cells and then count.
9. The count is done as follows:

In the erythrocyte count, the central double ruled square is used. Red cells lying in 80 very small squares have to be counted. These 80 small squares comprise 5 medium sized squares, each of which is bound by a triple line. It is recommended that the five medium sized squares chosen for counting cells should consist of four corners and one central, this is to secure a never distribution of cells, In counting, cells which touch the left hand lines or the upper lines of the square are taken to be within that square and those which touch the lower or right hand lines are omitted as outside the square.

Calculation

The number of RBCs in 1 mm3 = number of cell counted × 10000

