Practical Physiology

3rd year of Zoology

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3rd year of biology and geology
(English program)

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Red blood cells count method

The red blood cells (RBCs) count method comes under hemocytometry, which quantitatively measures the number of RBCs in a blood sample. Hemocytometer slide is a manual method to count RBCs.

Principle of red cell count

It is not possible to directly count the RBCs in a blood sample. Thus, it is necessary to dilute the blood sample or blood specimen using one of the RBC diluting fluids (Nacl 0.9% is isotonic solution for RBCs cells).

What is RBC?

RBCs stand for red blood cells. It is also called erythrocytes, which appears red-coloured due to the coloured pigment (**haem**) and exists as a biconcave disc. RBCs possess a diameter of 7.5 to 8.7 μm and a thickness of 1.7 to 2.2 μm. It lacks a nucleus and has a life span of 120 days.

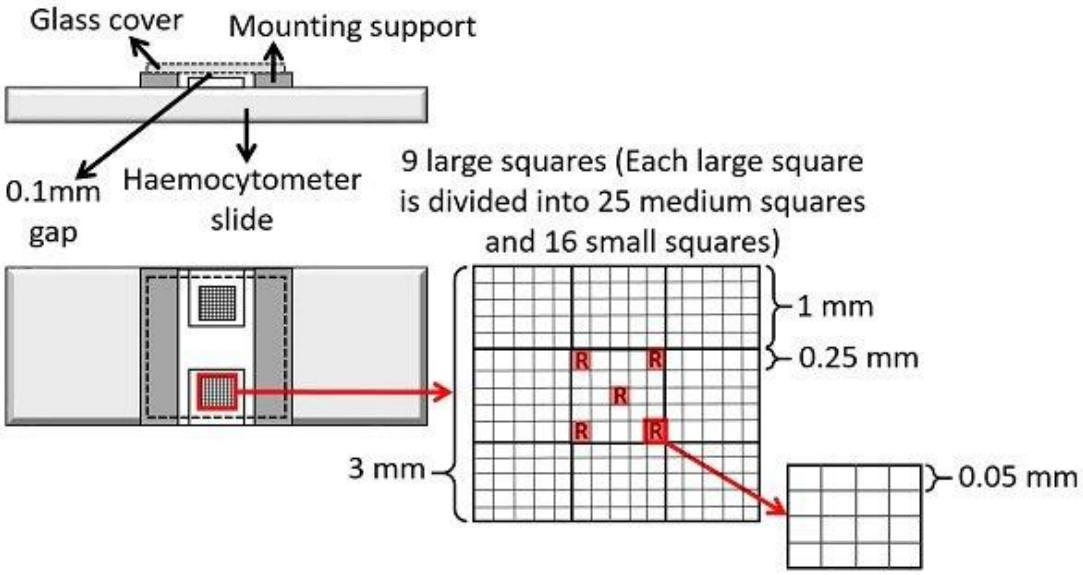
Requirements of Total RBC Count :

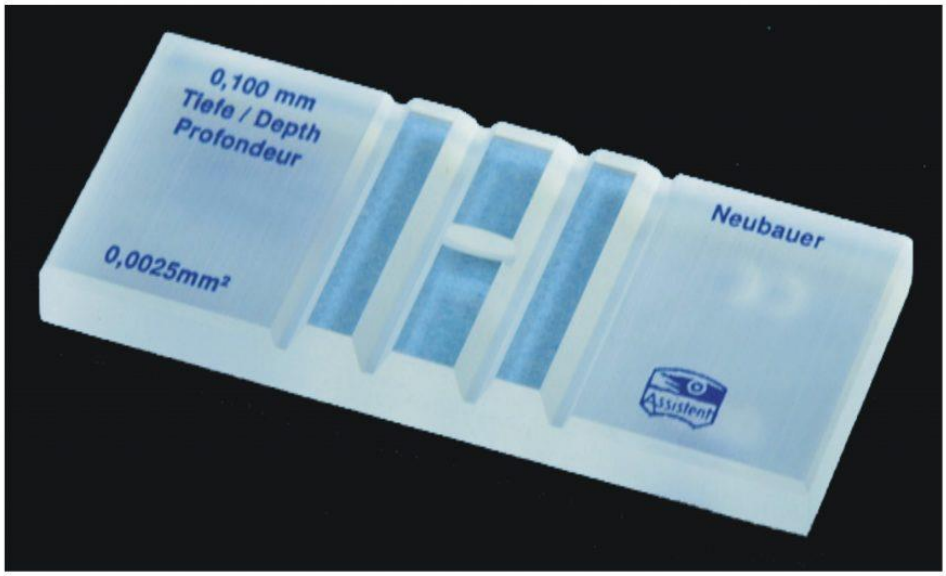
Hemocytometer refers to the micro-slide through which the number of erythrocytes or RBCs can be enumerated.

Hemocytometer:

It is a specialized thick glass slide used to count the eukaryotic cell suspension. Hemocytometer has a size of 30 X 70 X 4 mm. Its central portion is ruled, where the cell counting is performed. The counting grid has a size of 3 mm X 3 mm. One can estimate the number of red blood cells using a hemocytometer after diluting the blood sample with RBC diluent.

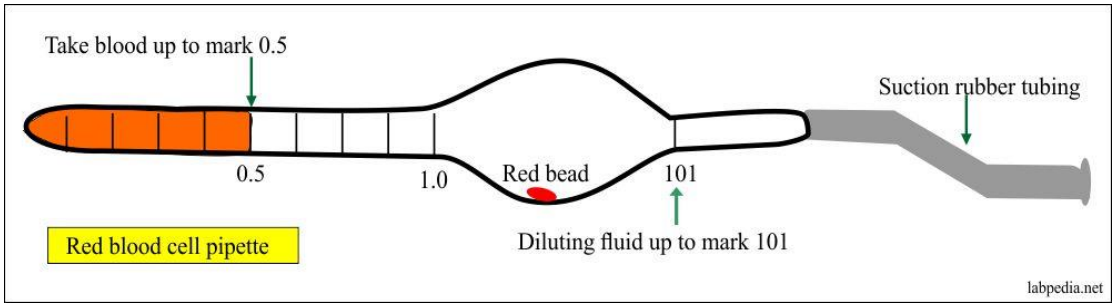
Neubauer's Chamber or Haemocytometer





Procedure:

1. RBCs counting solution is Nacl 0.9% isotonic saline.
2. Make a dilution of 1:200 with a diluting solution. Fill the red bulb pipette up to 0.5 marks.
3. Draw the solution to mark 101 of the RBC pipette.



4-Mix the blood thoroughly in the pipette.

1.Discard the first few drops (4 to 5) and then fill Hemocytometer.

.2Make sure that the chamber is free of air bubbles.

3.The distribution of the cells should be uniform over the ruled area.

5- Allow for 2 minutes to settle the cells.

6- Now count RBCs in the Hemocytometer.

1Use 10 or 40 X to count the RBCs.

For RBCs, use the center square, which has 25 smaller squares.

7- Count the corner 4 squares and one central square.

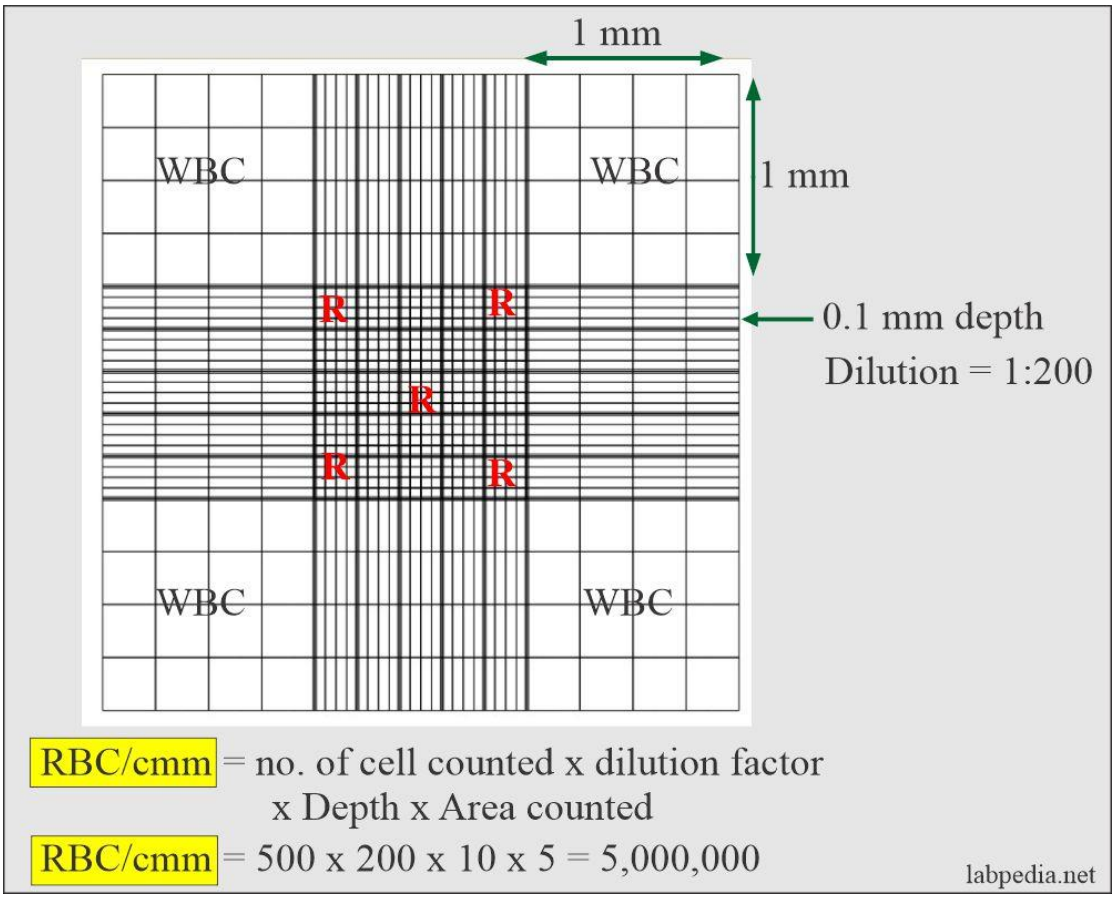
8-Count only the RBCs which fall on the left and top border of these squares.

9- Repeat the count twice and divide by 2 to get the average.

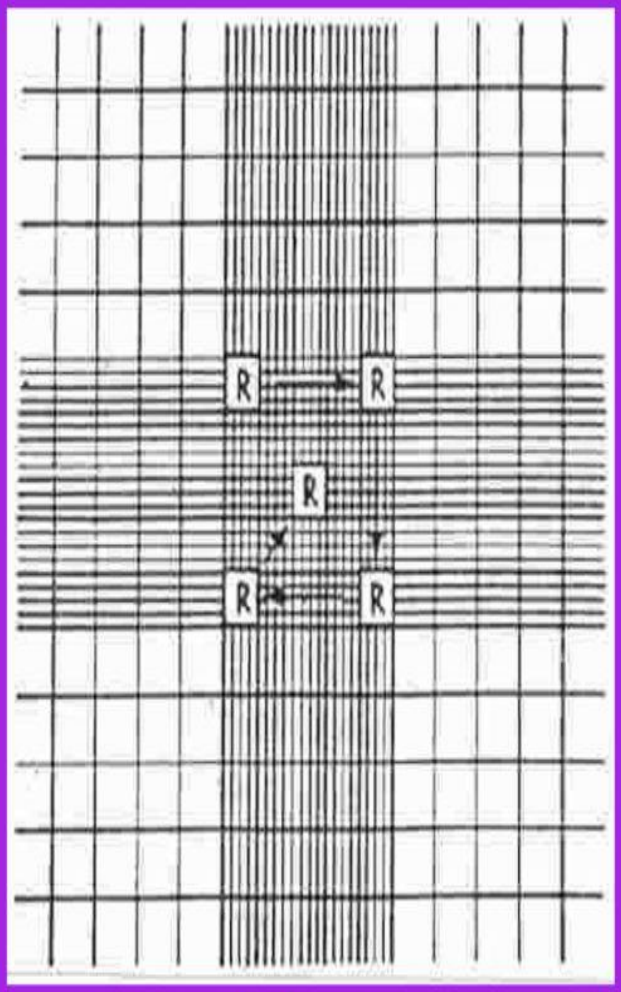
The formula for RBCs count is:

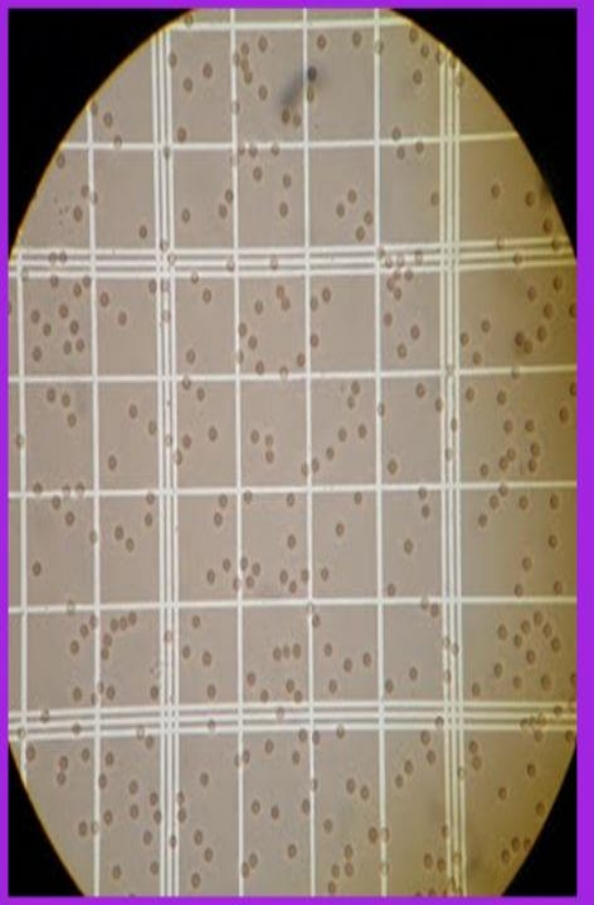
Multiply factor = 10 x 200 / 0.2 = 10,000

Multiply RBCs count with 10,000 = RBCs million/cmm.



Method determination of red blood cell (RBC) count





http://mbbsstudystuff.com/

White blood cells count method

The white blood cells also named leukocyte (WBCs) count method comes under hemocytometry, which quantitatively measures the number of WBCs in a blood sample. Hemocytometer slide is a manual method to count WBCs.

It is not possible to directly count the WBCs in a blood sample. Thus, it is necessary to dilute the blood sample or blood specimen using one of the WBC diluting fluids (acetic acid 0.2%).

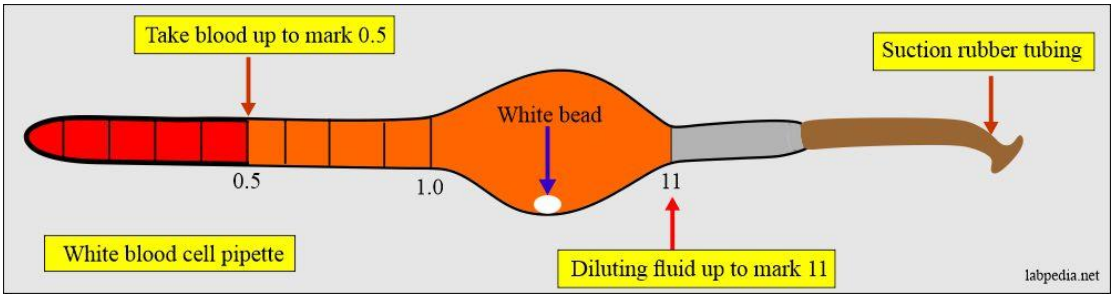
Principle of white cell count

Blood is diluted with a solution (acetic acid) that causes the lysis of RBCs. Acetic acid has no effect on the WBCs. Gentian violet is added to differentiate the WBCs.

1. Blood is diluted with a fluid that causes the RBCs' hemolysis, but WBCs remain intact, and then these are counted in the Neubauer chamber.
2. Gentian violet lightly stains the leucocytes and allowing those to be counted.

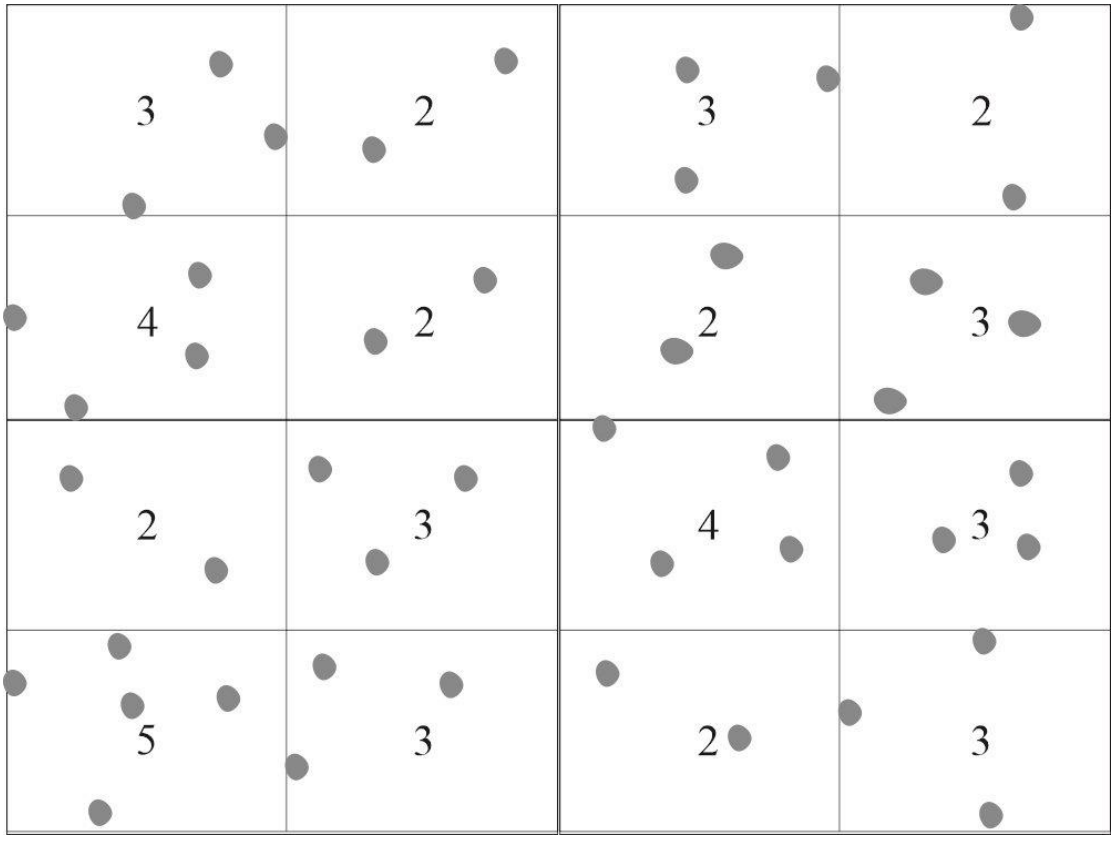
This pipette (also called Thoma pipette) long stem is divided into two parts:

1. The long stem is marked with 0.5 and 1.0
2. While the short arm after the bulb is marked 11.
3. Its central portion is a bulb or a globular shape with one white bead in it.
4. Rubber tubing is attached to suck the blood.
5. Ultimately the dilution of the blood to the TLC fluid is 1:20.



Procedure:

1. Take the WBC pipette, which has a white bead inside.
2. **WBC pipette method:**
3. Fill the blood into the 0.5 marks and then add the diluting solution.
    1. Fill the pipette with the diluting solution to point 11.
    2. Remove the rubber tubing.
    3. Seal both ends or hold in between two fingers.
    4. OR can put this pipette on the mechanical device to shake it.
    5. Shake for 1 minute or preferably for 2 minutes.
    6. After thorough mixing, discard the first few drops and then gently fill the chamber until the platform is filled.
    7. The capillary action will draw the fluid.
    8. Allow the chamber on the microscope stage for 2 to 3 minutes till the cells are settled.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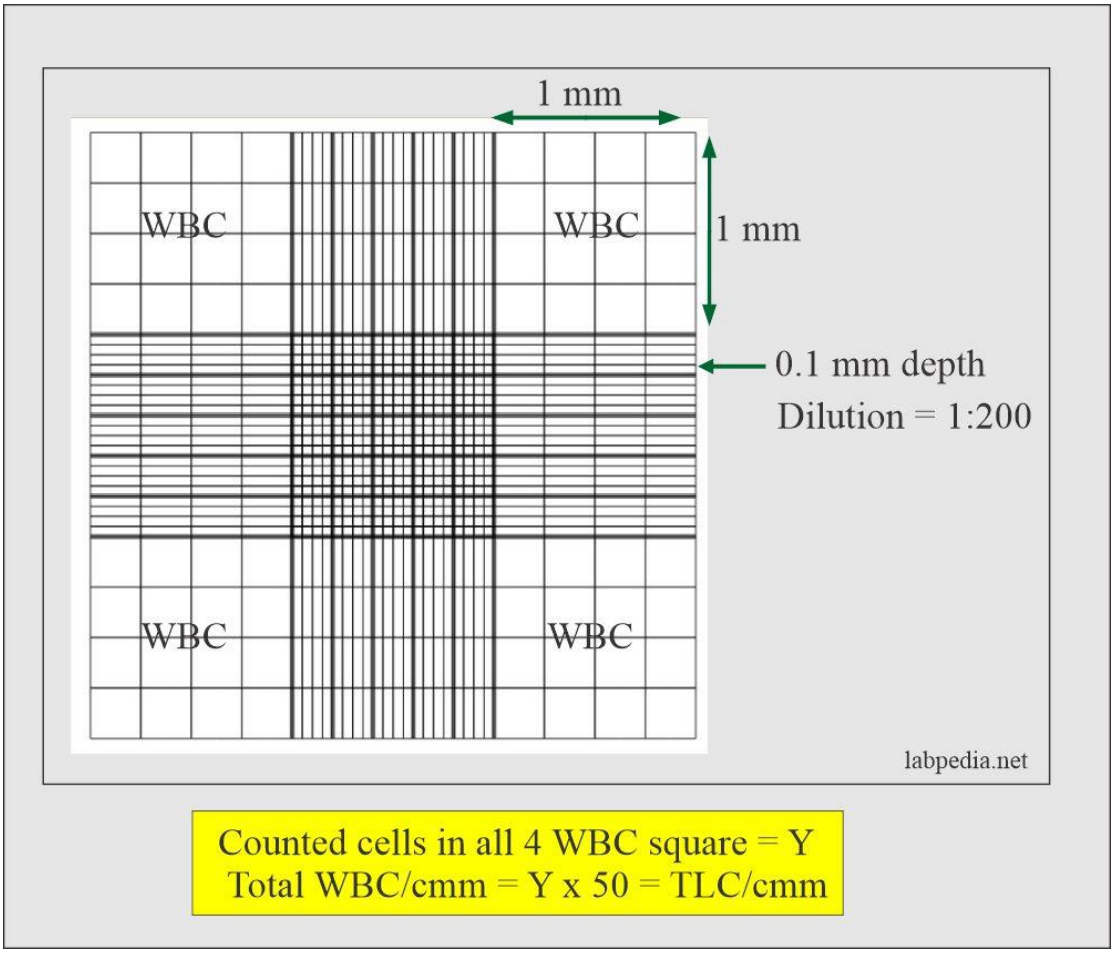
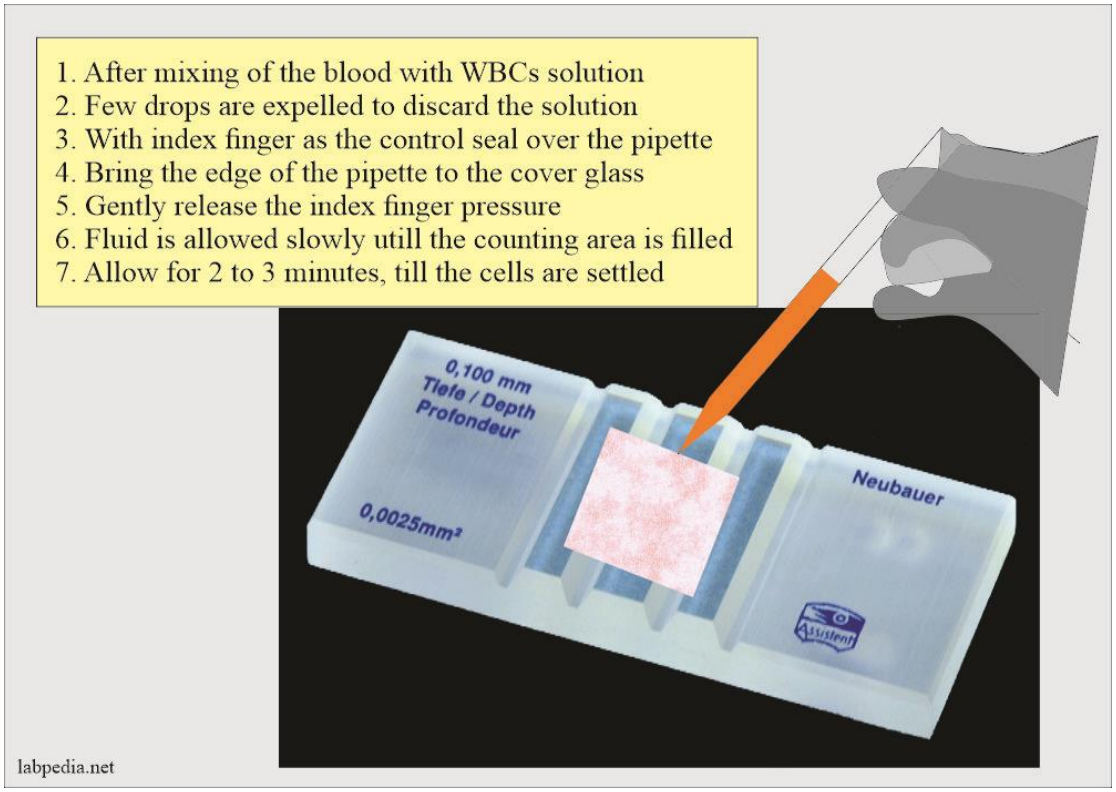

1 mm
1 mm
WBC
WBC
WBC
WBC
0.1 mm depth
Dilution = 1:200
labpedia.net
Counted cells in all 4 WBC square = Y
Total WBC/cmm = Y x 50 = TLC/cmm

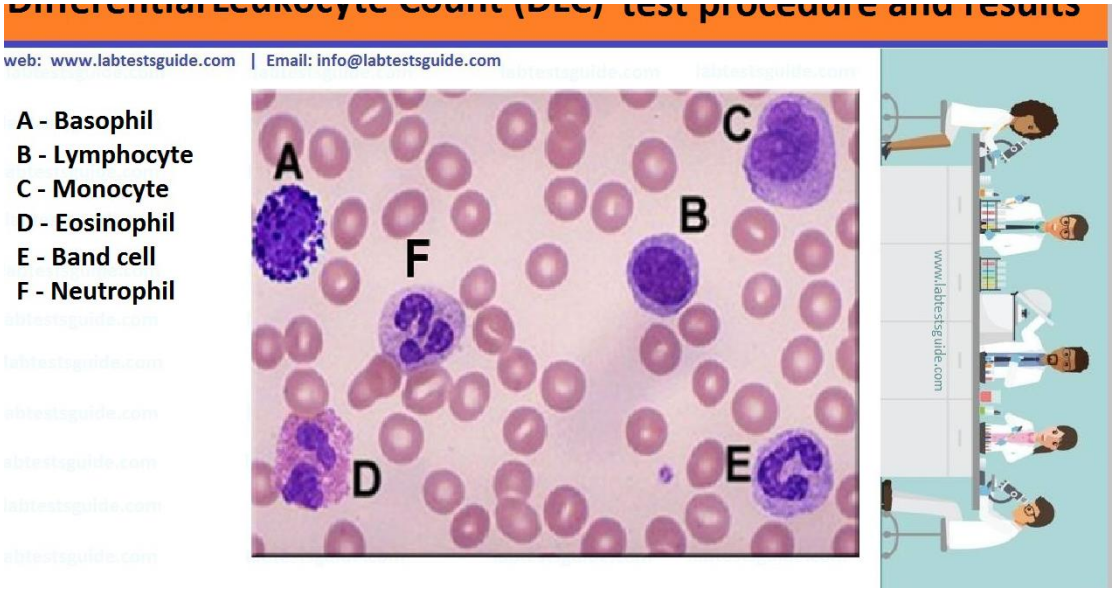


Calculations:

1. Count the cells in the hemocytometry. These are counted in the four large corner squares labeled as WBC and if the number is Y.
    1. One large area is 1 x 1 mm, and the depth is 0.1 mm.
    2. Total area counted in 4 large squares = 4 x 1 x o.1 = 0.4 µL (4/10).
    3. Y x 10/4 is the total WBC in the cell in 1 µL.
    4. Now dilution is 1:20.
    5. Number of WBC in 1µL = Y x 10 x 20/4 = Y x 50 = Total WBC count.
    6. **Total WBC = counted cells (Y) x 50 = WBC COUNT/cmm.**

Differential Leukocyte Count (DLC) Test

A differential blood count gives the relative percentage of each type of white blood cell and also helps to reveal abnormal white blood cell populations (eg,blasts, immature granulocytes, and circulating lymphoma cells in the peripheral blood).



have five types of white blood cells:

Neutrophils

Lymphocytes

Monocytes

Eosinophils

Basophils

Test Procedure

Leukocytes are classified into various groups depending on their size,

features of the nucleus and features of the cytoplasm. The WBCs exist in two forms viz. granulocytes and agranulocytes. Granulocytes are further

classified as eosinophil, basophil, neutrophil, while agranulocytes shows

lymphocytes and monocytes.

Sample Required:

1. The best sample is blood in EDTA.

2. Also, prepare fresh peripheral blood smear.

3. This is inexpensive, easy to perform and rapidly done as a screening test.

Test Requirements:

Well mixed whole or anticoagulent Blood

Glass Slides

Distilled Water

Stain (any one)

Leishman's stain

Wright Stain

Giemsa Stain

Field Stain

Dicrorizer (Any one)

Microscope

Preparing the Slide:

Collect drops of blood on the end side of a glass slide.

Spread the blood drop with another glass slide by placing it at an angle of 45 degree and move sidewise.

Hold the spreader firmly and move it on the previous slide to the other end ina straight line with same force and pressure.

Allow the glass slide to dry after formation of the smear.

Fix the smear with air dry or any other fixative/

Staining the Slide.

Observation the slide and counting of cells

Keep the prepared slide is under low power of microscope and choose a good quality slide.

Then identify different types of WBC under medium power.

Draw a table with 10 boxes both on horizontal and vertical axis on a observation notebook or use hand counter to count the cells.

Fix the slide on the plateform and choose a area towards the corner.

Count the different types of WBC found on the table in an abbreviated.

Move downwards and in chain like manner till 100 cells are observed.

After counting 100 cells prepare the report.

Hemoglobin determination

Hemoglobin (Hb or Hgb) is a red color pigment present in red blood cells (RBCs) comprises Fe2+ and Globin protein. It is Hemoglobin in RBCs that carries the oxygen from the lungs to the tissues and CO2 from body tissues to the lungs for excretion.

Hemoglobin (Hb or Hgb) is responsible for the appearance of Red color RBCs and blood. Hemoglobin is a chromoprotein consisting of Globin molecule attached to 4 red colored Heme molecules. Hemoglobin synthesis requires the coordinated production of Heme and Globin. Heme is a prosthetic group that medicates reversible binding of oxygen by hemoglobin. Globin is the protein that surrounds and protects the Heme molecule.

Sahli's Method Acid Hematin Method :

PRINCIPLE OF SAHLI's METHOD / ACID HEMATIN METHOD

The principle of Sahli's Method or Acid hematin method is quite easy that when the blood is added to 0.1% Hydrochloric acid (HCl), the hemoglobin present in RBCs is converted to acid hematin which is a dark brown colored compound. The color of the formed acid hematin complex corresponds to the Hemoglobin concentration in the blood and is matched with the standard which is a reference brown glass given in the Sahli's apparatus by diluting with 0.1% hydrochloric acid or distilled water until the color of acid hematin complex match with the color of the standard.

REAGENTS REQUIRED FOR SAHLI's METHOD / ACID HEMATIN METHOD

- 0.1% hydrochloric acid (It is prepared by diluting concentrated hydrochloric acid in distilled water and volume is made up 100 ml).
- Distilled water

APPARATUS & EQUIPMENTS REQUIRED FOR SAHLI's METHOD / ACID HEMATIN METHOD

- Sahli's Apparatus
    - Hemoglobin pipette (0.02 ml or 20 µl capacity)
    - Sahli's graduated Hemoglobin tube
    - Thin glass rod Stirrer for Hemoglobin Tube
    - Sahli's Comparator box with brown glass standard

PROCEDURE OF SAHLI's METHOD / ACID HEMATIN METHOD

0.1% Hydrochloric acid is taken in Hemoglobin tube (has two graduations – one side gm/dl, and other side shows the Hb %). ⇒

Dispense the blood into 0.1% hydrochloric acid taken in the hemoglobin tube, rinse the pipette with the same solution and mix properly with the help of stirrer.

⇒ Place the tube at room temperature for 5 minutes for complete conversion of hemoglobin into acid hematin.

⇒ After the reaction completes, place the Hb tube in the column in Sahli's Comparator box and start diluting the dark brown coloured compound (Acid Hematin) formed in the Hb tube using the N/10 HCl or distilled water by adding drop by drop of it into the solution and mix with the help of stirrer after each addition.

⇒ This process is done until the endpoint comes matching the color of standard with the color of the test.

⇒ Once the color is matched with the standard brown glass, lift the stirrer up and note down the reading in Sahli's Hb tube by taking the lower meniscus in consideration.

PRECAUTIONS TO BE TAKEN WHILE PERFORMING ESTIMATION OF HEMOGLOBIN BY SAHLI's METHOD / ACID HEMATIN METHOD:

⇒ Sahli's apparatus especially the Hemoglobin pipette and Sahli's Hemoglobin tube should be clean and dry before use.

⇒ Suck the blood exactly up to the mark of 20 µl (0.02 ml) and air bubbles should not be present in the pipette with blood.

⇒ Mix well the acid and blood and wait for at least 5 minutes after adding the blood in acid.

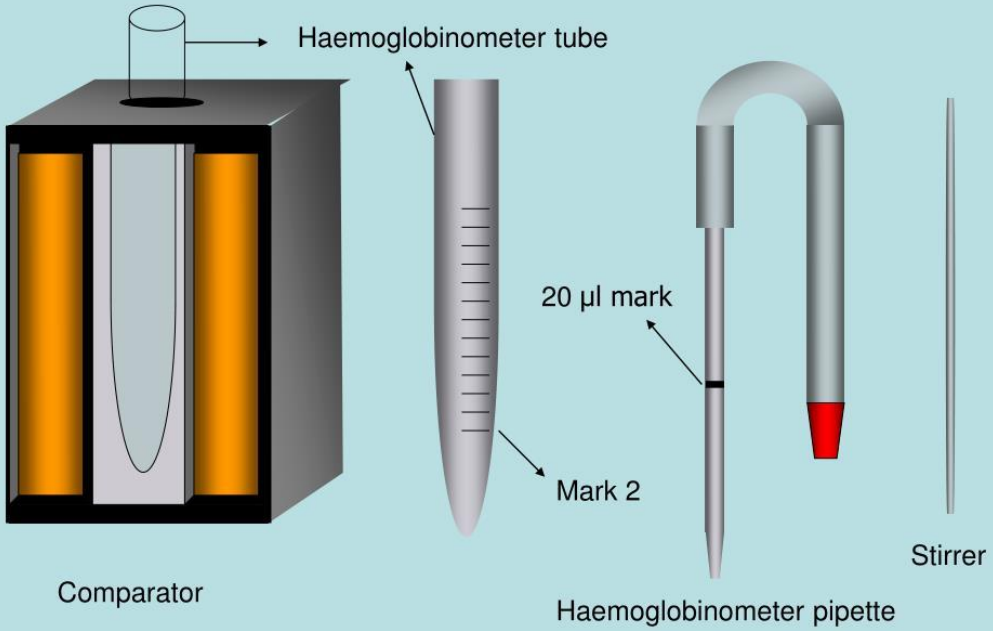
Add distilled water drop by drop and mix well after each dilution. Avoid over dilution of the content.

⇒ The matching of color should be done against the natural source of light or electrical tube light (white light) to avoid any visual errors.

⇒ Blood sample and 0.1% HCl acid should be taken in an accurate and precise amount in the Hb tube.

⇒ The Hb pipette should be wiped off properly in order to avoid the excess addition of blood in the Acid.

SAHLI'S HAEMOGLOBINOMETER



Determination of Hematocrit (Hct) (Packed Cell Volume; PCV)"

Hematocrit:Hematocrit is defined The percentage by volume of packed red blood cells in a given sample of blood after centrifugation.The hematocrit may also be referred to as Packed Cell Volume (PCV) or erythrocyte volume fraction (EVF).

Purpose for doing the Hct

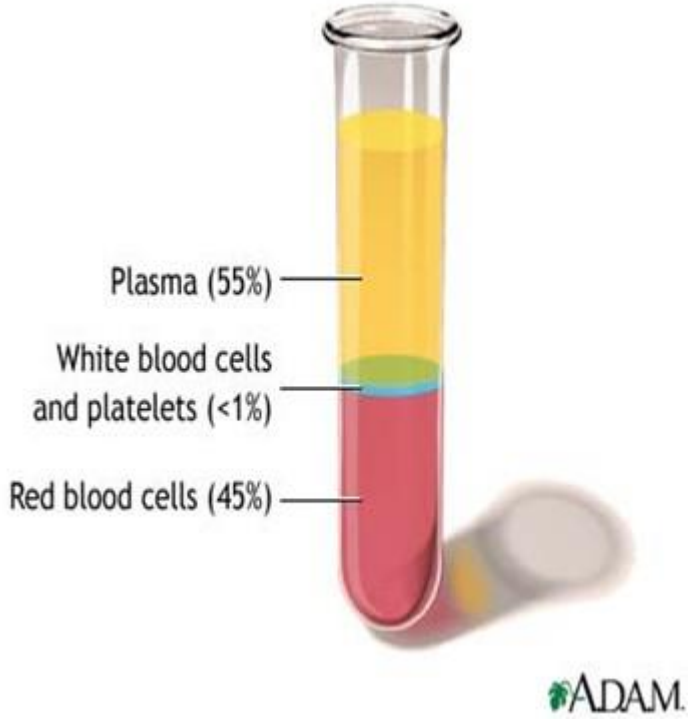
Blood is made up of red and white blood cells, platelets, and plasma.A decrease in the number or size of red cells also decreases the amount of space they occupy, resulting in a lower hematocrit.An increase in the number or size of red cells increases the amount of space they occupy, resulting in a higher hematocrit.

Procedure:

Take blood by capillary tube by capillary.

Close the hole that took blood , close it with wax,clay and soap.

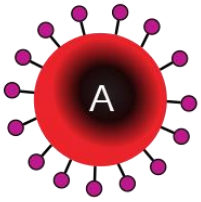
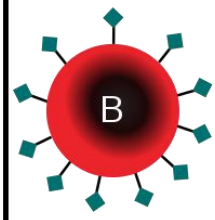
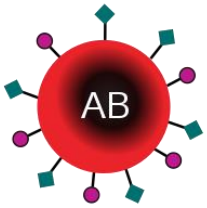
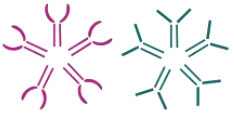
the tubes in a centrifuge (5 minutes at rpm),.7. Using a special reading device(since the capilary tube is not graduated).



ABO blood group system

The **ABO blood group system** is used to denote the presence of one, both, or neither of the A and B antigens on erythrocytes. In human blood transfusions it is the most important of the 38 different blood type (or group) classification systems currently recognized. A mismatch (very rare in modern medicine) in this, or any other serotype, can cause a potentially fatal adverse reaction after a transfusion, or an unwanted immune response to an organ transplant. The associated anti-A and anti-B antibodies are usually IgM antibodies, produced in the first years of life by sensitization to environmental substances such as food, bacteria, and viruses.

The ABO blood types were discovered by Karl Landsteiner in 1901; he received the Nobel Prize in Physiology or Medicine in 1930 for this discovery.[4] ABO blood types are also present in other primates such as apes and Old World monkeys.

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in red blood cell	A antigen	B antigen	 A and B antigens	None

Coagulation Time

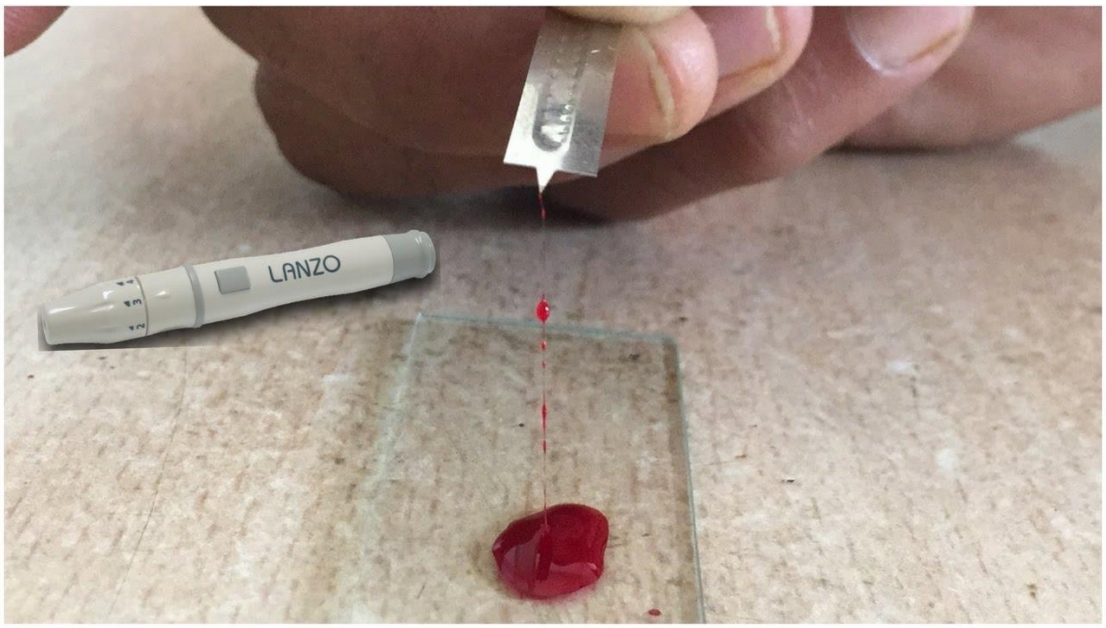
Coagulation Time (Clotting Time) CT.

CT: the of whole blood is length of time required for a measured amount of blood to clot under certain specified conditions (the time required for blood to form aclot).

Slide methode

Procedure:

Place 1 ml from blood on slide then each 30 seconds check blood even formation threads .

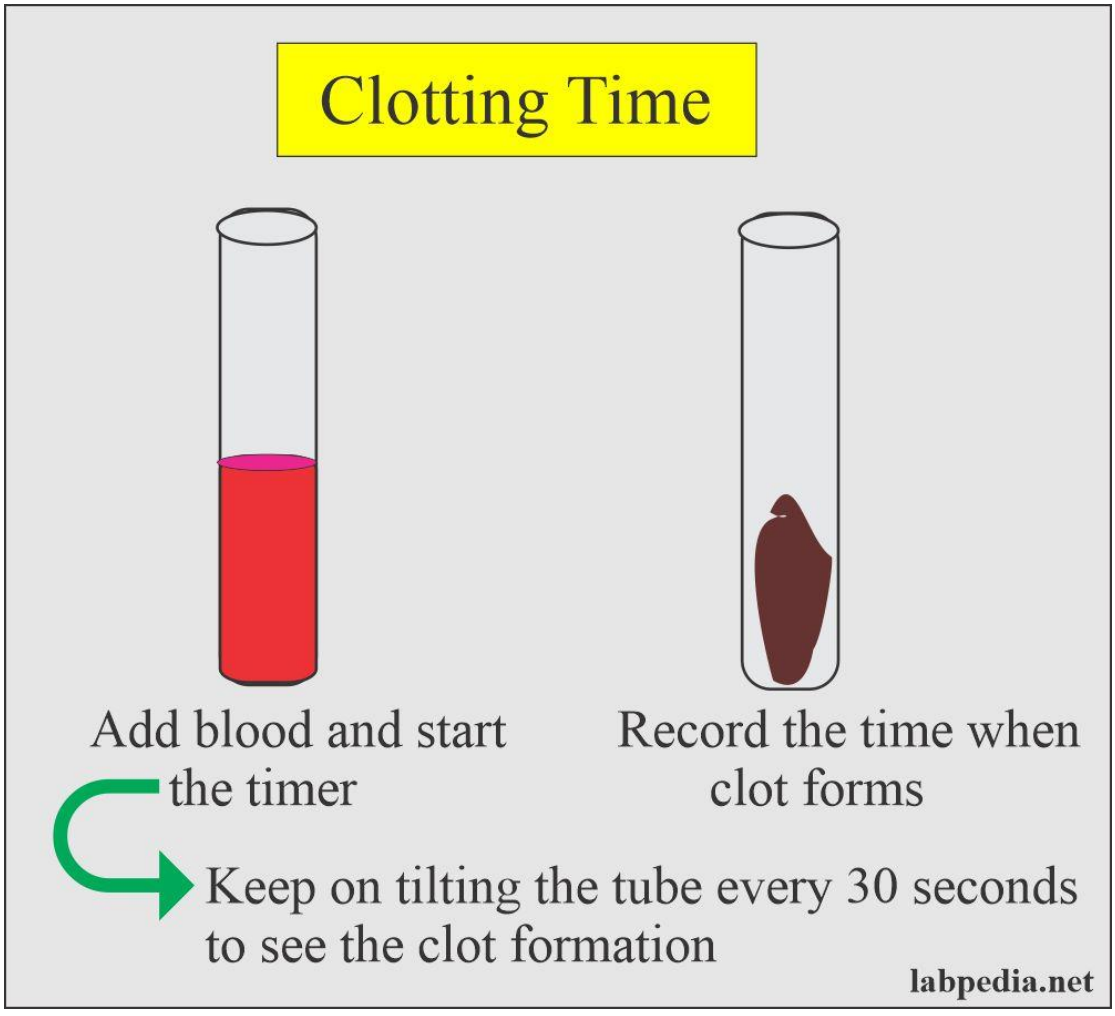

LANZO

test tube method:

1. Perform this test at 37 ° C.
2. For the tube method, take 4 ml of blood and start the time.

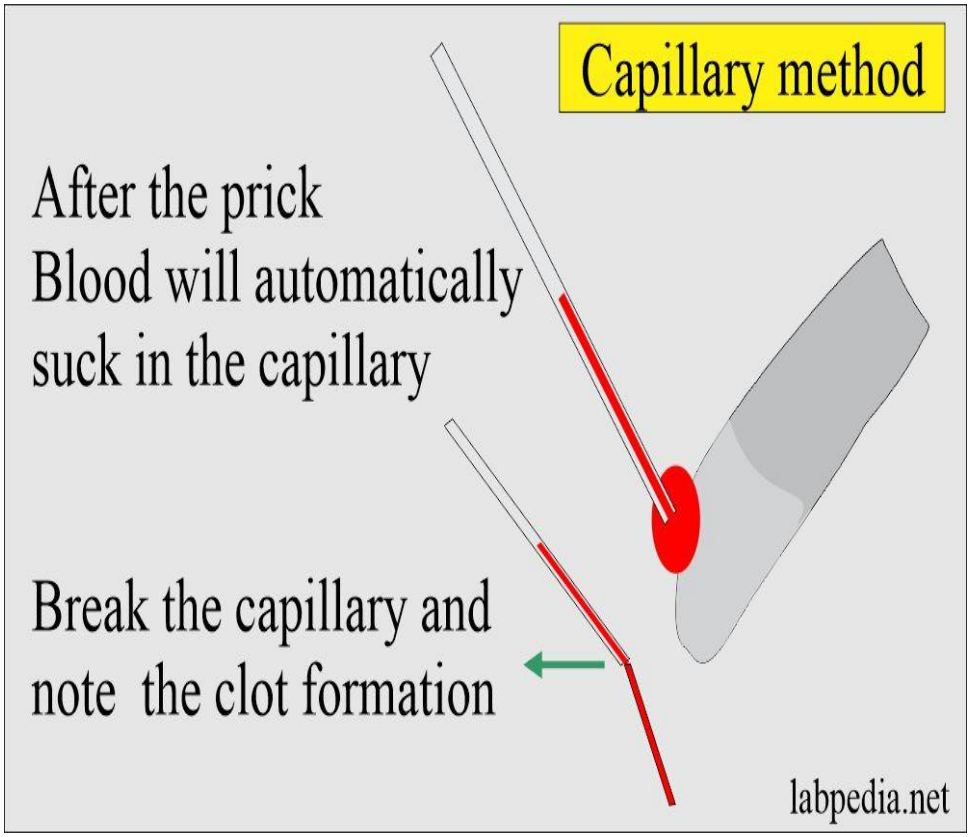
3.Note the time when there is the first appearance of the clot formation.

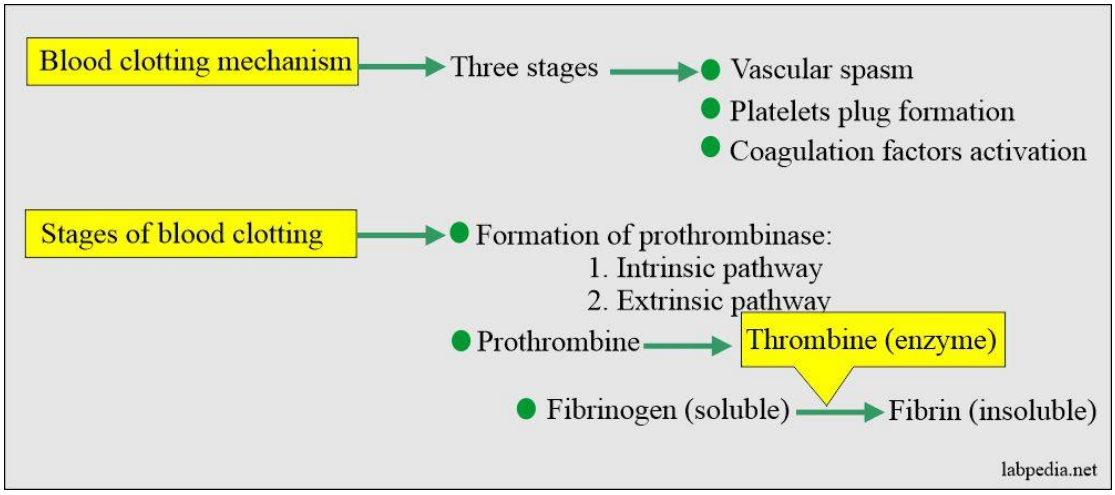
4.This test can be done in multiple tubes to be more accurate.



Capillary method.

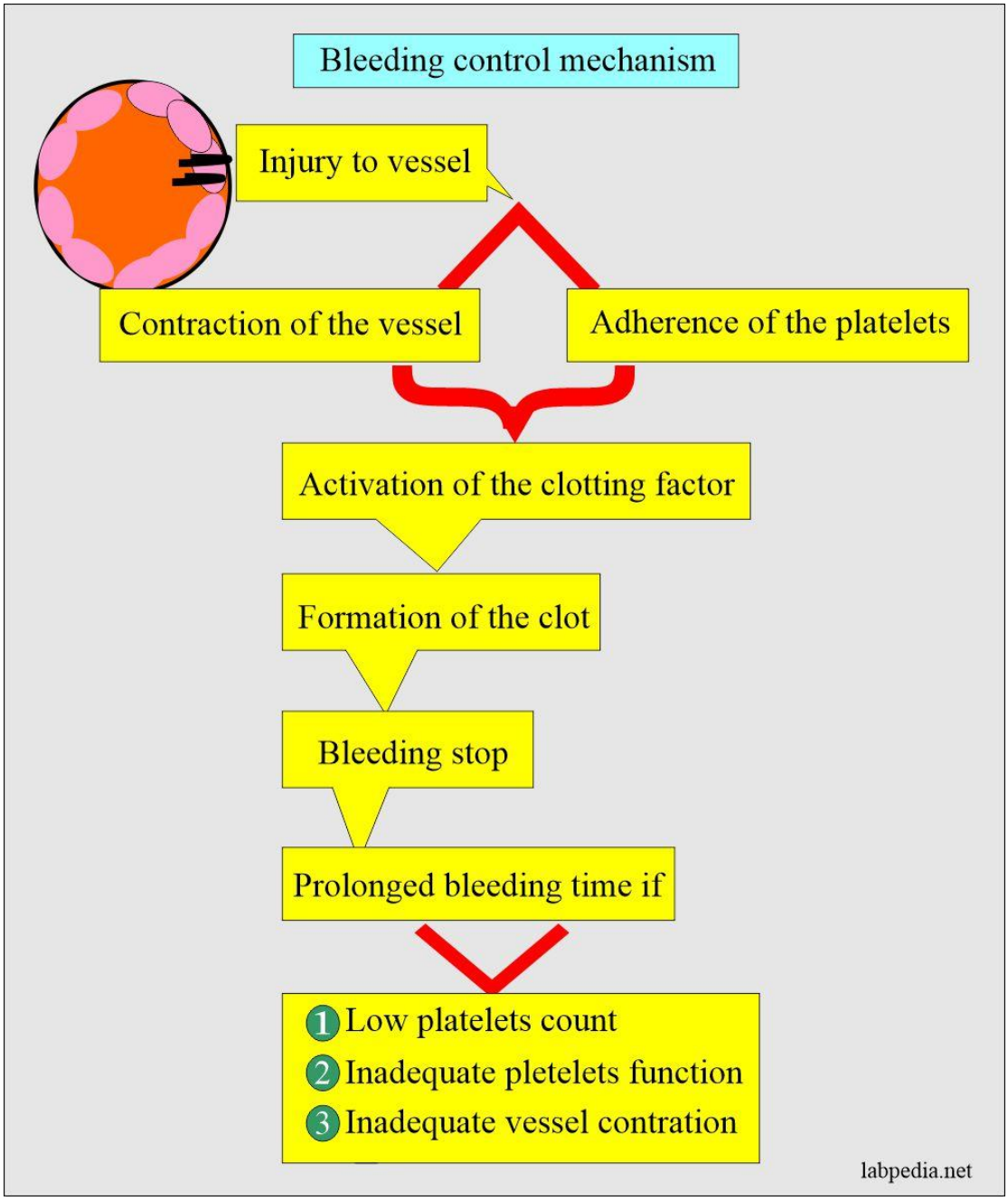
1. Prick the finger with the lancet.
2. Hold the capillary over the blood, and the capillary will fill automatically.
3. Now, after regular intervals, break the capillary.
4. When a clot starts forming, that is the endpoint and clotting time.




Blood clotting mechanism
Three stages
Vascular spasm
Platelets plug formation
Coagulation factors activation
Stages of blood clotting
Formation of prothrombinase:
1. Intrinsic pathway
2. Extrinsic pathway
Prothrombine
Thrombine (enzyme)
Fibrinogen (soluble)
Fibrin (insoluble)
labpedia.net

Bleeding Time (BT)

Bleeding time is a medical test done on someone to assess their platelet function , count and integrity of the blood vessels.



procedure

1-Warm up the finger for skin puncture.

2- Make an incision with a sterile disposable lancet to depth 3 mm.

3-As soon as blood is visible start the stop watch.

4-Wipe off the first drop of blood.

5-When blood stop from finger we take a time from stop watch.

Blood pressure

Arterial blood pressure is most commonly measured via a sphygmomanometer, which historically used the height of a column of mercury to reflect the circulating pressure. Blood pressure values are generally reported in millimetres of mercury (mmHg), though aneroid and electronic devices do not contain mercury.

For each heartbeat, blood pressure varies between systolic and diastolic pressures. Systolic pressure is peak pressure in the arteries, which occurs near the end of the cardiac cycle when the ventricles are contracting. Diastolic pressure is minimum pressure in the arteries, which occurs near the beginning of the cardiac cycle when the ventricles are filled with blood. An example of normal measured values for a resting, healthy adult human is 120 mmHg systolic and 80 mmHg diastolic (written as 120/80 mmHg, and spoken as "one-twenty over eighty").

Lung Volumes

Lung volumes are also known as respiratory volumes. It refers to the volume of gas in the lungs at a given time during the respiratory cycle. Lung capacities are derived from a summation of different lung volumes. The average total lung capacity of an adult human male is about 6 litres of air. Lung volumes measurement is an integral part of pulmonary function test. These volumes tend to vary, depending on the depth of respiration, ethnicity, gender, age, body composition[1] and in certain respiratory diseases. A number of the lung volumes can be measured by Spirometry- Tidal volume, Inspiratory reserve volume, and Expiratory reserve volume. However, measurement of Residual volume, Functional residual capacity, and Total lung capacity is through body plethysmography, nitrogen washout and helium dilution technique.

Lung Volumes :

- **Tidal Volume(TV)**

It is the amount of air that can be inhaled or exhaled during one respiratory cycle. This depicts the functions of the respiratory centres, respiratory muscles and the mechanics of the lung and chest wall.

The normal adult value is 10% of vital capacity (VC), approximately 300-500ml (6-8 ml/kg); but can increase up to 50% of VC on exercise .

- **Inspiratory Reserve Volume(IRV)**

It is the amount of air that can be forcibly inhaled after a normal tidal volume.IRV is usually kept in reserve, but is used during deep breathing. The normal adult value is 1900-3300ml.

- **Expiratory Reserve Volume(ERV)**

It is the volume of air that can be exhaled forcibly after exhalation of normal tidal volume. The normal adult value is 700-1200ml. ERV is reduced with obesity, ascites or after upper abdominal surgery

- **Residual Volume(RV)**

It is the volume of air remaining in the lungs after maximal exhalation. Normal adult value is averaged at 1200ml(20-25 ml/kg) .It is indirectly measured from summation of FRC and ERV and cannot be measured by spirometry.

n obstructive lung diseases with features of incomplete emptying of the lungs and air trapping, RV may be significantly high. The RV can also be expressed as a percentage of total lung capacity and values in excess of 140% significantly increase the risks of barotrauma, pneumothorax, infection and reduced venous return due to high intra thoracic pressures as noticed in patients with high RV who require surgery and mechanical ventilation thus needs high peri-operative inflation pressures.

Lung capacities

- **Inspiratory capacity(IC)**

It is the maximum volume of air that can be inhaled following a resting state. It is calculated from the sum of inspiratory reserve volume and tidal volume. IC = IRV+TV

- **Total Lung Capacity(TLC)**

It is the maximum volume of air the lungs can accommodate or sum of all volume compartments or volume of air in lungs after maximum inspiration. The normal value is about 6,000mL(4-6 L). TLC is calculated by summation of the four primary lung volumes (TV, IRV, ERV, RV).

TLC may be increased in patients with obstructive defects such as emphysema and decreased in patients with restrictive abnormalities including chest wall abnormalities and kyphoscoliosis.

- **Vital Capacity(VC)**

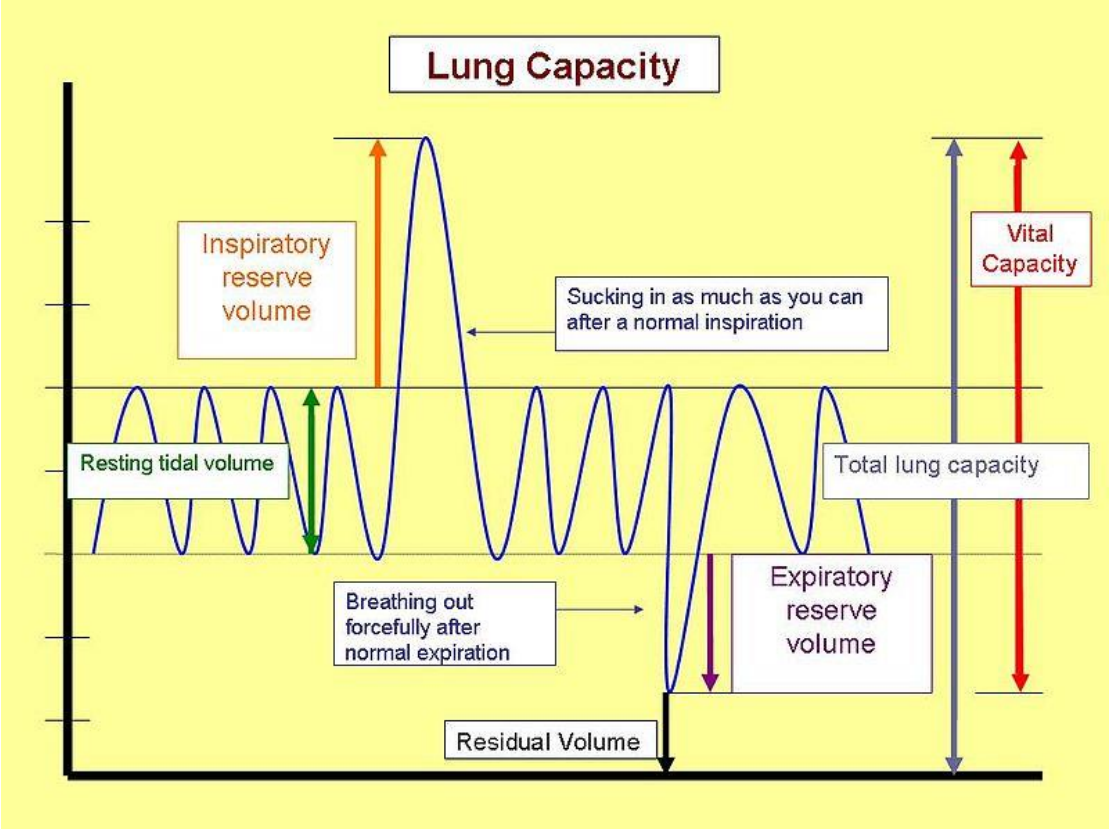
It is the total amount of air exhaled after maximal inhalation. The value is about 4800mL and it varies according to age and body size. It is calculated by summing tidal volume, inspiratory reserve volume, and expiratory reserve volume. VC = TV+IRV+ERV.

VC indicates ability to breathe deeply and cough, reflecting inspiratory and expiratory muscle strength.VC should be 3 times greater than TV for effective cough. VC is sometimes reduced in obstructive disorders and always in restrictive disorders

- **Function Residual Capacity(FRC)**

It is the amount of air remaining in the lungs at the end of a normal exhalation. It is calculated by adding together residual and expiratory reserve volumes. The normal value is about 1800 – 2200 mL. FRC = RV+ERV.

FRC does not rely on effort and highlights the resting position when inner and outer elastic recoils are balanced. FRC is reduced in restrictive disorders. The ratio of FRC to TLC is an index of hyperinflation. In COPD, FRC is upto 80% of TLC.



Links

https://www.youtube.com/watch?v=uSq0-0W5vOk

https://www.youtube.com/watch?v=0f9p9JX4qJk

https://www.youtube.com/watch?v=Pxmt8FdDqN4

https://www.youtube.com/watch?v=A2Jr-9ylzzA

https://www.youtube.com/watch?v=SoWXhpZC1NA

https://www.youtube.com/watch?v=amJAJeWk0NI

https://www.youtube.com/watch?v=b2dZn1HVT-k

https://www.youtube.com/watch?v=odQ6ggM67Ck