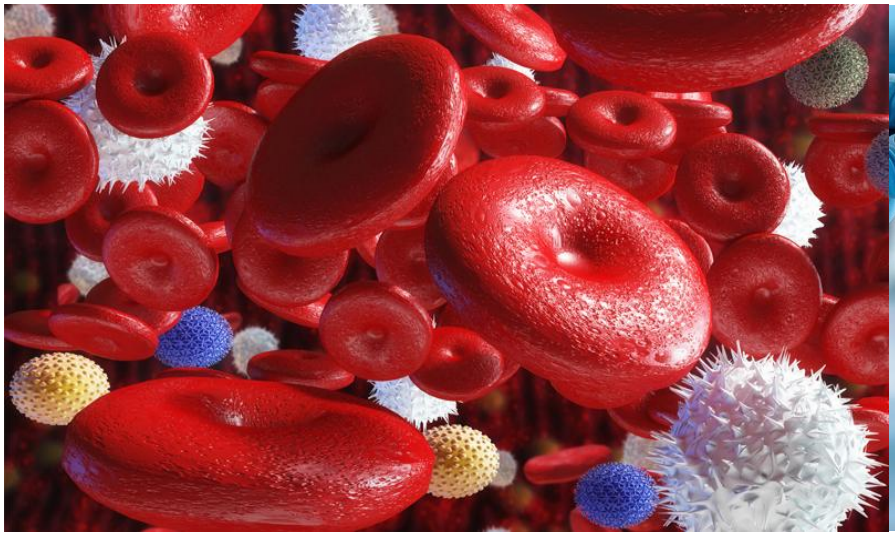
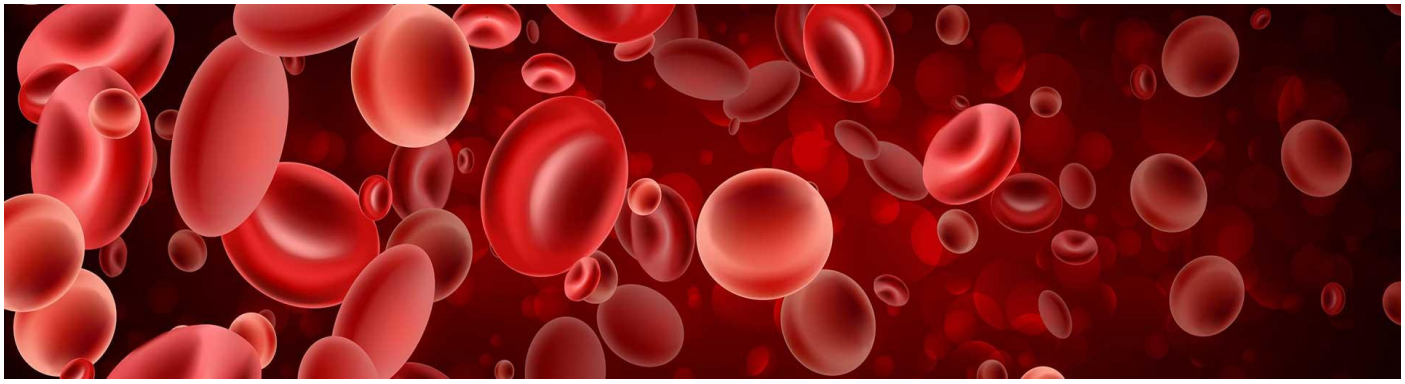
Practical Animal physiology

3rd Level




TOPICS	
Lab 1	Blood physiology
Lab 2	Complete Blood Count (CBC), Hb, PCV
Lab 3	Erythrocyte Sedimentation Rate (E.S.R.)
Lab 4	Blood Cell Count, RBCs count
Lab 5	Total white blood cell count
Lab 6	Differential White Blood Cells Count
Lab 7	Blood Group System
Lab 8	Laboratory Diagnosis of Clotting Disorders

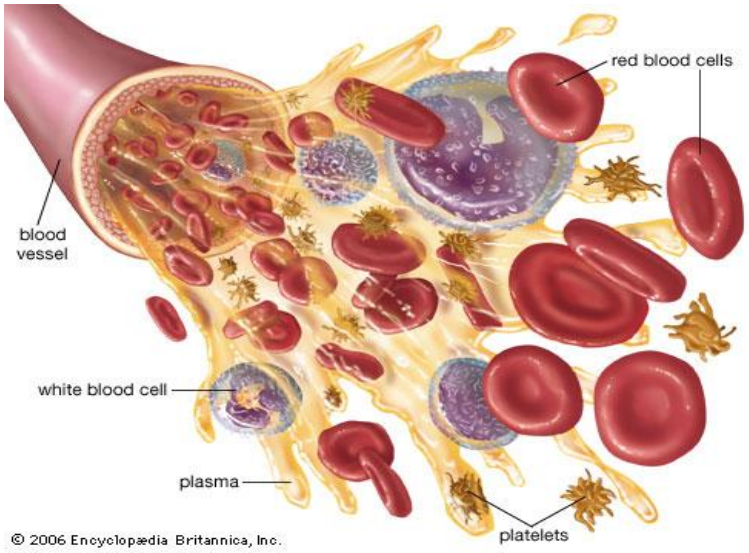
First Exam	
Second Exam	

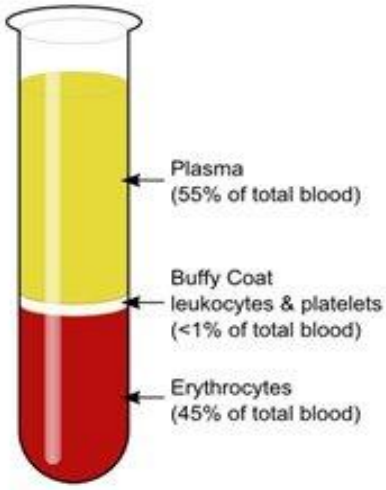


By: Assis. Lec. Ammar B. Al-Asadi

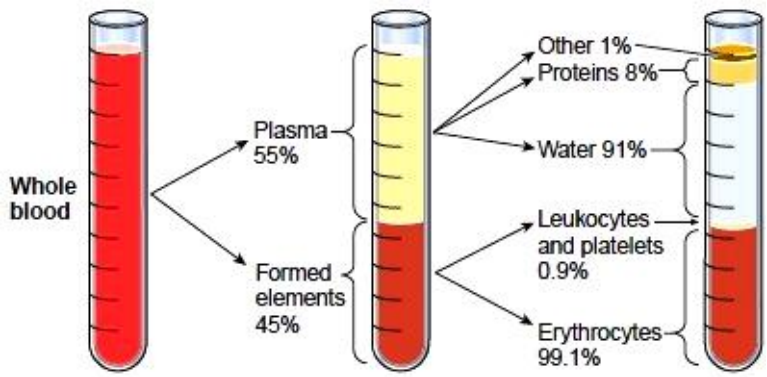
Blood physiology

Composition of blood: blood has been defined as a highly specialized connective tissue composed of two phases; a liquid phase and a Solid phase . The liquid phase is known as plasma and the solid phase is made up of cellular elements which include: **red blood cells**, **white blood cells** and **platelets**.





The average human body contain about 4 – 6 liter of blood. The cells or cellular elements occupy about **45%** of the volume of the blood is settle. The most obvious component of plasma is water about 91% of the plasma. The water has many functions as vehicle for the transport of blood cells and many materials and also important in the temperature regulation. Electrolytes and other substances constitute for about 2% plasma. 7% of the plasma is made up of proteins which perform number of different function e.g. (transport, coagulation, inflammation, antibodies production and regulation of the osmotic pressure and PH.



Blood collection and anticoagulants:

Blood sampling used for the laboratory tests can be either:-

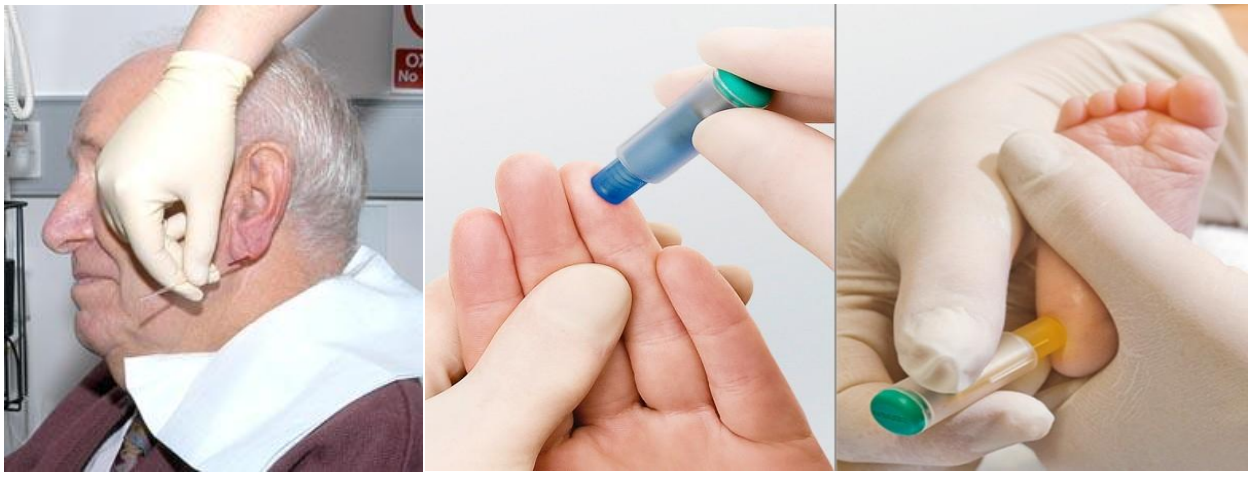
1- Capillary blood. In this method blood can be taken by pricking:-

A- The lobe of the ear

B- The side surface of the finger

C- The infants from the planter surface of the heel or The great toe.

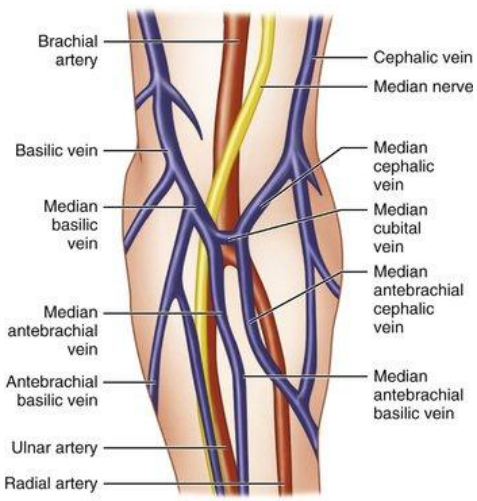
This method is carried when the test needs little amount of blood.



2- Venous blood.

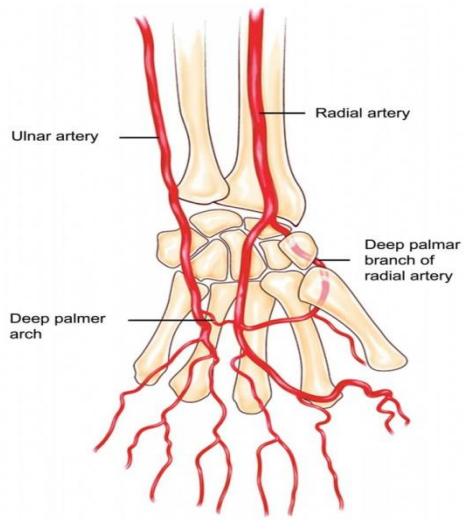
It is preferred for most hematological examination. In this method blood can be collected from many sites especially the **antecubital veins** of the forearm

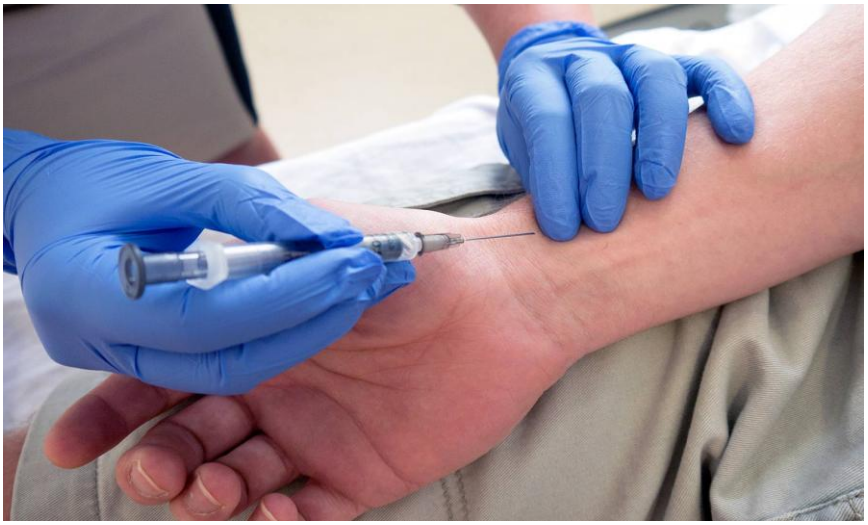
This method is carried when the test needs a lot of amount of blood.



3- Arterial blood.

This is done by a special type of syringe the artery usually chosen is the **radial artery**. The test is especially valuable for blood gas analysis.

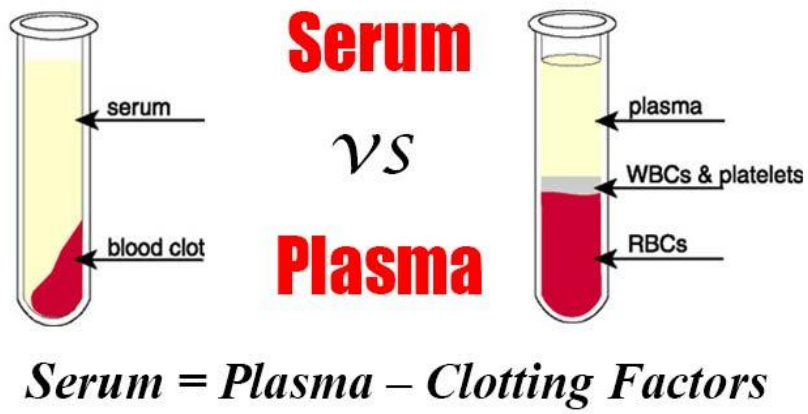




Serum and Plasma

If we want **SERUM**, after collection of the blood, its put in a dry and clean test tube for 5 – 15 min under room temperature to allow coagulation, after this put the test tube in centrifuge for 10 min. at 3000 r.p.m. the supernatant represent the serum, this withdraw to another tube to performing the tests.

If we want **PLASMA**, after collection of blood its put in an-anticoagulant tube (to avoid coagulation) mix gently by inverting the tube not shaking, leave the sample for period of time, notice the yellow – colored liquid above, this is the plasma. Also can use the centrifuge to obtain all the plasma, which withdraw to another tube to perform the tests.



Anticoagulants

Anticoagulant are chemical reagents that ceased the series of the reactions that lead to the blood clotting, anticoagulant differing in the following properties :-

1- Mood of action 2- Preparation 3- Utilization cautions

The commonly used anticoagulants are the following:-

1- EDTA (Ethylene Diamine Tetra Acetic acid)

This anticoagulant removes the free calcium ions from the blood by chelating.

2- Sodium citrate

This anticoagulant removes the calcium ions by loosely binding them to form calcium citrate complex.

3- Oxalate

This anticoagulant removes the free calcium ion from the blood through formation of calcium oxalate as insoluble precipitates. Sodium oxalate is now considered as the anticoagulant for coagulant tests.

4- Heparin

Heparin is acts as antithrombin and is the only naturally occurring anticoagulant used in the laboratory some anticoagulants are drugs that reduce the action of the blood clotting factors. Heparin, because its acts fast, is the first anticoagulant administered after thrombosis event. Heparin is given intravenously and requires close supervision, so it is only given while the patient is in the hospital, and seldom for more than five days.



Complete Blood Count (CBC)

We can define the CBC as many tests which determine the different component of blood (cells or Hb). A major portion of the CBC is the measure of the numbers of white blood cells, red blood cells and platelets in the blood. The CBC can provide important information on the types of blood cells, their condition, and numbers; this can help in diagnosis many condition and diseases, such as anemia, leukemia and inflammation.

The CBC includes many tests :-

1- Hemoglobin (Hb) determination.

2- Hematocrite of Packed Cell Volume (P.C.V.)

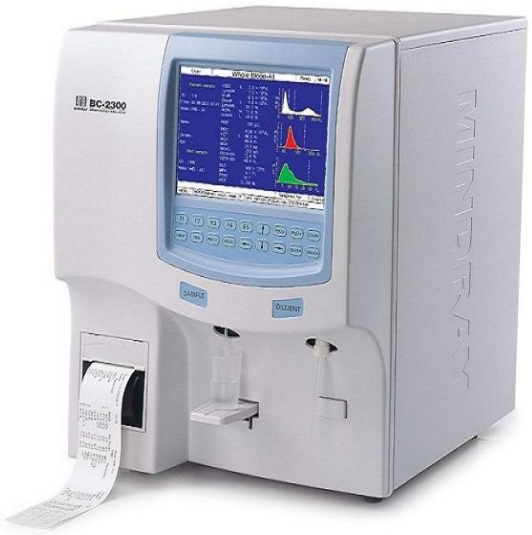
3- Erythrocyte Sedimentation Rate (E. S. R.)

4- Red Blood Cells Count (R.B.C count)

5- White Blood Cells Count (W.B.C count)

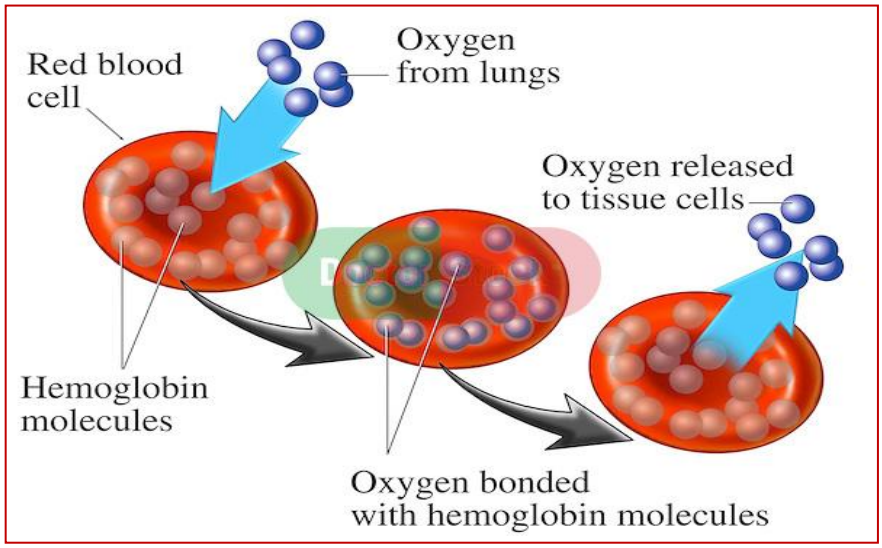
6- Differential W.B.C count

7- Platelets count

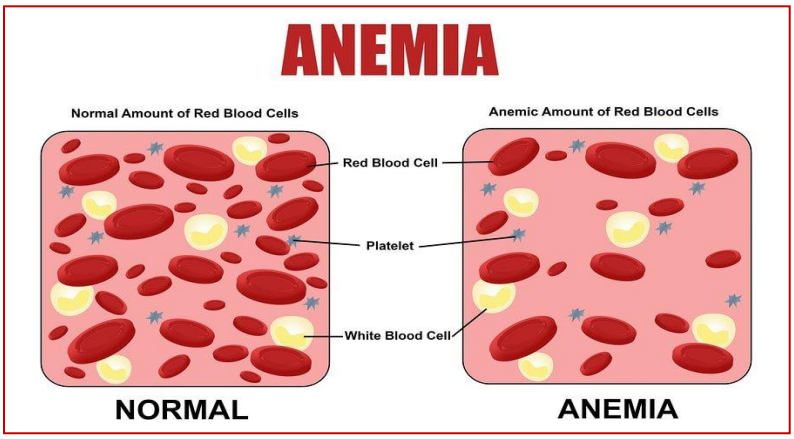


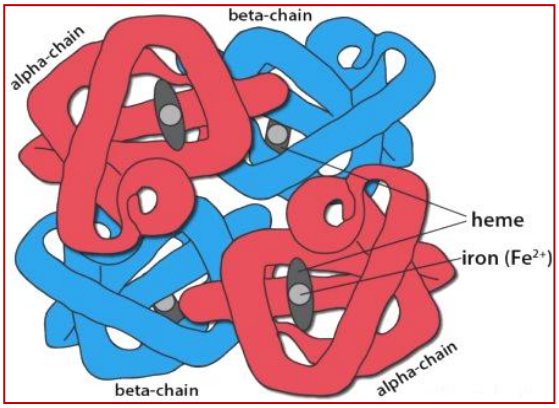
Hemoglobin (Hb) Determination

Hb is a respiratory pigment of red blood cells that carries O2 and CO2. A molecule is a tetramer made up of four monomers. The monomer consists of a heme (Pigment with iron) and Globin unit (protein). The globin units are made up of 4 polypeptide chains (2 α chain & 2 β chain) there are many normal types of Hb such as fetal Hb (HbF) which is gradually replaced by adult Hb (HbA).



Hb values are affected by age, sex, pregnancy, disease, and altitude. During pregnancy increase in body fluids lead to the red cells become less concentrated, causing the red cell count to fall. Since the Hb inside the red cells, also falls. Disease may also affect the values of Hb for example; anemia drops Hb values. Above normal Hb values may occur. In dehydration or change in altitude, at higher altitude there is low O2 pressure in the air resulting in an increase in red cells and Hb values.





There are several methods for Hb estimation, two of them:-

1- Sahli s method (acid hematin method)

2- Drabkin s method

We are going to deal with the first one.

Sahli s method (acid hematin method)

The method is based upon conversion of Hb to acid Hematin compound (brown color) by using acid.

<u>Reagents & Equipments:-</u>

1- Hb pipette.

2- Stirrer (glass rod)

3- Standard Hb comparative

4- Graduated tube (Hb tube) 140%

Specimen:-

The specimen to be used is capillary blood or EDTA anticoagulant blood.

Procedure:-

1- fill Hb tube with (0.1 N) HCL till 20 marks

2- Withdraw the blood sample by Hb pipette to 0.02 ml. mark (20µl).

3- Add the blood sample to the Hb tube quickly and mix well by the stirrer.

4- Used the acid to dilute the mixture of Hb until a match is seen with brown glass Hb standard.

5- Read the lower level of the fluid on % report Hb in g\ 100 ml. or (g \ dl.) of blood unit.

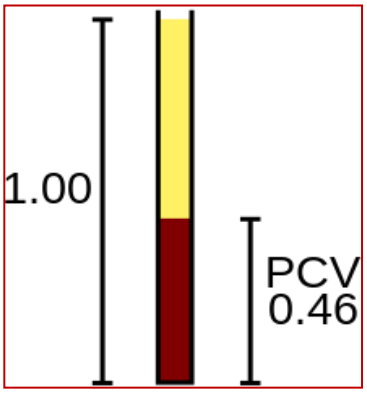
Normal ranges:-

Male adult: 14.0 – 18.0 g / dl.

Female adult: 11.5 – 16.5 g / dl.

Determination of Hematocrite (Packed Cell Volume; P.C.V.)

P.C.V. may be defined as the percentage of the packed red cell volume to the total amount of blood. There are two methods used in determining the hematocrite value are :-



1- Macrohematocrite method (wintrobe method)

2- Microhematocrite method

The method of choice is the second, because of its advantage; requires less blood and less time.

Microhematocrite Method :-

Anticoagulant whole blood is centrifuged, and total volume of the red cells mass is expressed as a percentage of the total blood volume or decimal fraction. Ex. (46% or 0.46).

Reagents & Equipments:-

1- Heparinized capillary tubes

2- Microhematocrite centrifuge speed 10,000 rpm.

3- Hematocrite reader (%)

4- Artificial clay

Specimen:-

The specimen is heparinized capillary tube or venous blood which adds to EDTA tube (whole blood)

Procedure:-

1- If capillary blood sample is used then we must use the heparinized capillary tube (prevent clot). But if anticoagulant blood is used (venous blood add to anticoagulant) the capillary tube used should not coated with anticoagulant (non – heparinized capillary tube) Because the excess anticoagulant may cause cell shrinkage & produce false low values. Ensure that is not air bubbles. Leave at least 15 mm of capillary tube is empty.

2- Sealed the tube by artificial clay.

3- Put the tubes in centrifuge with the sealed ends toward the outside of the holder for 5 min. at 10,000 r.p.m.

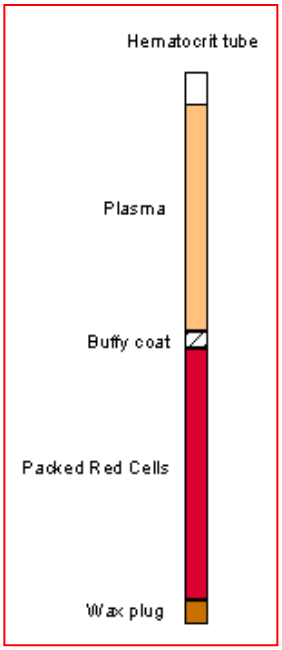
4- Read with reader & report the result as percentage or decimal fraction.

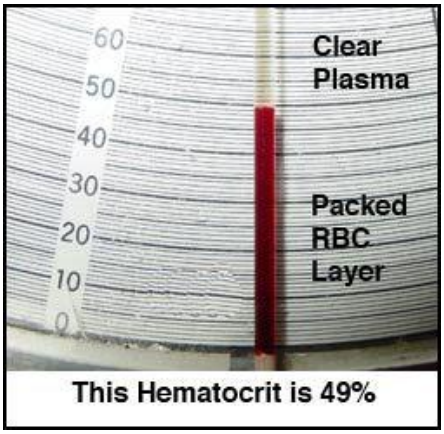
Δ Now look at the tube the blood is separated into 3 layers :-

1- Column of R.B.Cs at the bottom.

2- Narrow middle zone of buffy coat consists of W.B.Cs & Platelets.

3- Fluid of plasma at the top.





Normal range:-

Male adult: 36 – 52 %

Female adult: 33 – 47 %

When P.C.V. is Above normal range this indicate many medical condition such as **Polycythemia** (increase R.B.Cs numbers) and (dehydration) occur in sever diarrhea, vomiting or drinking too little of water and use diuretics, because the loose of the fluids lead to decease the volume of plasma compared with R.B.Cs.

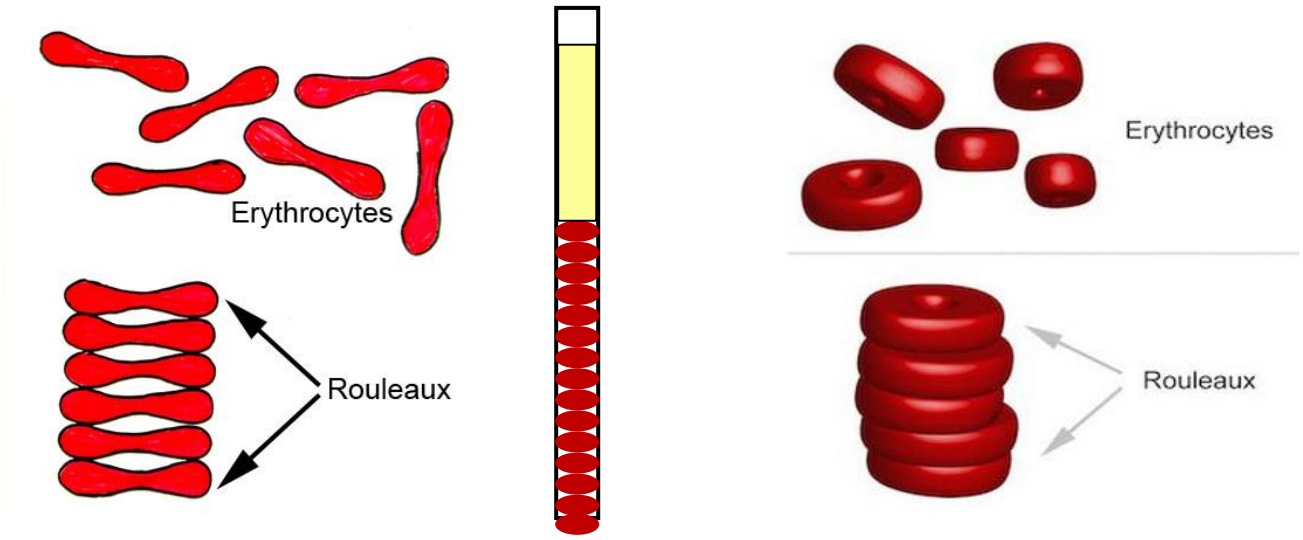
Below normal range occur in **anemia** and leukemia because the disorder in bone marrow function that leads to low numbers of R.B.Cs or in severe bleeding and in pregnancy.

Erythrocyte Sedimentation Rate (E.S.R.)

Is the rate of sink the R.B.Cs in graduated tube fixed vertically for a given time and expressed as millimeters per hour (mm./hr)

If anti-coagulated blood is allowed to stand for some time, the R.B.Cs gradually sink and the plasma upward. The rate of this action is constant in healthy human and is known as ESR.

in certain conditions this rate is increased because the R.B.Cs align face to face in columns like a stack or coins this called a rouleaux and this proportional to ESR.



ESR is affected by the following factors:

1- The number, size and density of R.B.Cs.

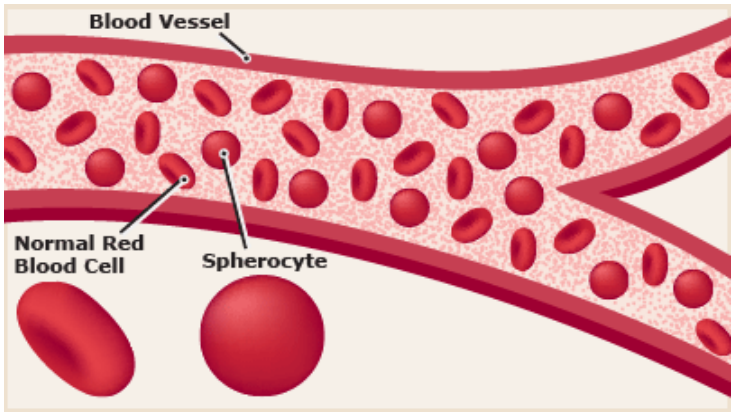
2- The composition of plasma especially the protein

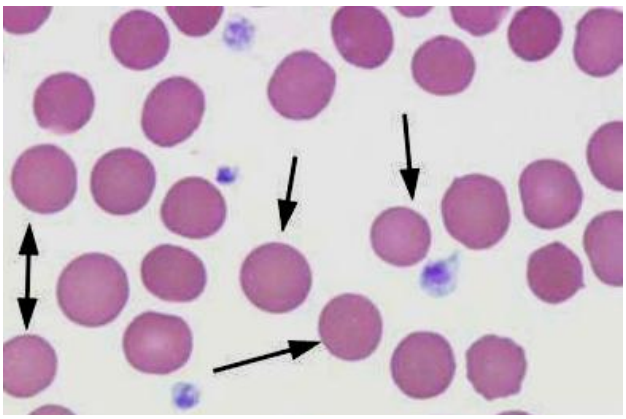
3- Plasma viscosity

4- Room temperature

We try to discuss each of them :-

* When cells are suspended in normal plasma, rouleaux formation is minimal and the sedimentation of cells is low, the changes occur in the R.B.Cs numbers like anemia leads to increase rouleaux formation and accelerate the ESR, but there is some changes not correlate with this state like some special conditions of anemia, ex: Spherocytic anemia these, usually do not exhibit increased sedimentation, so normal & even reduced ESR. Why ?





* The principle cause of rouleaux formation is the composition of plasma, especially the protein (Albumin & Globulins).

* Plasma viscosity is affected by the concentration of large proteins (fibrinogen & some immunoglobins)

Lower levels of plasma viscosity are seen in neonates because of low levels of proteins particularly fibrinogen, while there is a slight increase in viscosity is not affected by anemia unlike ESR.

*Temperature is proportional to the rate of sedimentation, the typical tem. To the ESR test in lab. Is 20 – 25 ^{o}C (room temp.)

There are two methods to measure the ESR :-

1- Westergrene method

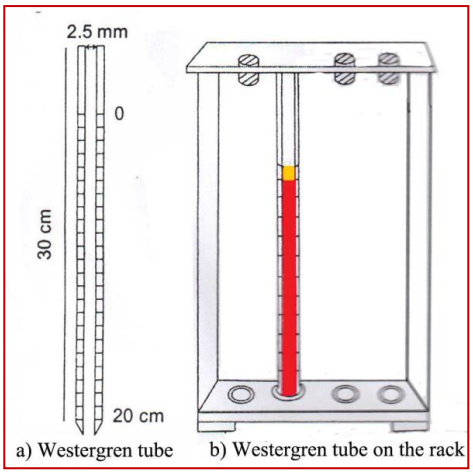
2- Wintrobe method

The method of choice is the **first**.

Westergrene method

This test measures the precipitation rate of R.B.Cs in diluting plasma.

Reagents & Equipments:



1- Anticoagulant: - Trisodium citrate solution.

2- Westergrene s pipette graduated form 0 (top) to 200 (bottom).

3- Westergrene s rack.

Specimen

Venous blood should be treated with sodium citrate only as an anticoagulant to dilute the plasma in a ratio of 4 volume solution.

Procedure:-

1- 1.6 ml. of venous blood is dispensed into 0.4 ml. of the sodium citrate.

2- Mixed gently & fill the westergrene s tube to the 0 mark.

3- put the tube in the rack under room temp. & record the time.

4- Exactly after 1 hour read the upper level of R.B.Cs in (mm/h).

Normal ranges:-

Male adult: 0 – 15 mm. / hr.

Female adult: 0 – 20 mm. / hr.

The highest ESR levels are usually seen in a cancer of W.B.Cs and rheumatoid disease, infections, anemia and kidney disease .

Finally the ESR test is considered an indication of presence of inflammation but not the type of inflammation.

Blood Cell Count

Counting of blood cells are done either by an electronic cell counter or manually with the special glass slide known **(Hemocytometer)**. Since many different formed elements are in the blood in high concentration, blood must be diluted before counting. The diluting fluid is selected for its ability to :-

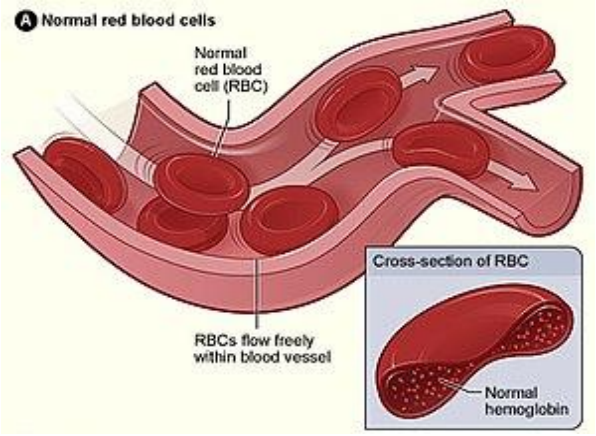
1- Dilute the blood
2- lyses cell types not wanted
3- stain cell types want to count

Blood cell counts are usually reported in number of cell per cubic millimeter (no. of cells /mm^3) or per micro liter (no. of cells / µl.) (1 µl. = 1 mm^3).

Red Blood Corpuscles Count

Normal erythrocytes may be described as biconcave discs ; they are thicker at the edges than in the middle. During development R.B.Cs loss their nuclei and most of their organelles thus they are unable to divide . Each erythrocyte contains large quantities of Hb that allow O2 & CO2 to be carried in R.B.Cs.

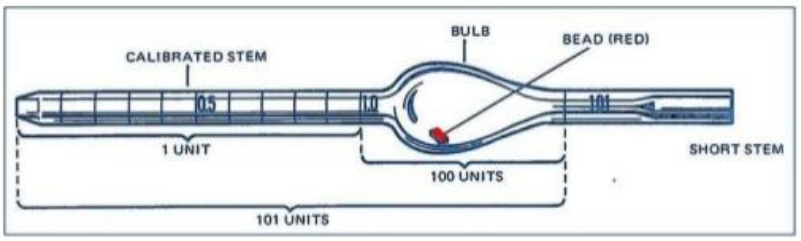
The biconcave shape increases the surface of area thus greatly facilitating to exchange the blood gas.

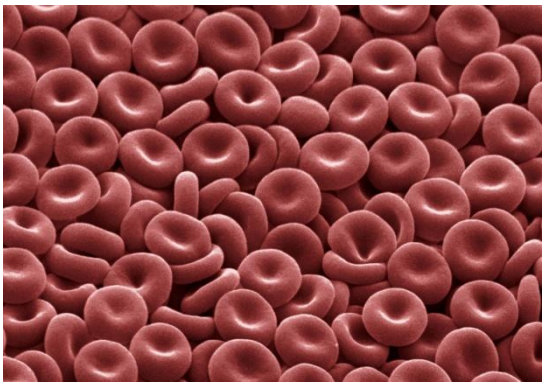


The lack of nucleus gives the R.B.C great flexibility & elasticity and folded when move through very narrow blood capillaries . Also the round edges reduce the friction the cells in the capillaries. Erythrocytes live for about 120 days in males and 110 days in females.

Principle:

Whole blood is diluted with an isotonic diluents, the number of cells in affixed volume is counted and the final count is expressed as the number of cells in one cubic millimeter (No. of cells /1 mm^3) of undiluted blood.





Reagent & Equipments:

1- Anticoagulated blood EDTA is the anticoagulant of choice.

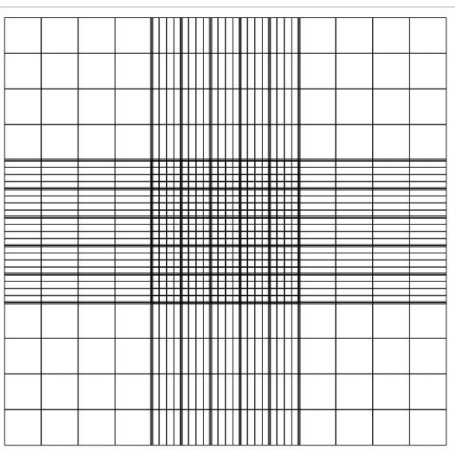
2- The pipette used is a thoma pipette. The blood will be diluted 200 times (0.5 to 100), it does not measure blood in µl. But provides a dilution ratio.

3- R.B.Cs diluting fluid :- the dilution fluid is known as Hayme s Solution that should be isotonic to keep the shape of the R.B.Cs & prevent R.B.Cs from clumping.

4- Hemocytometer & cover slip.

The special design slide called improved Neaubaur the central square is divided into 25 small squares by triple lines; each of 25 small squares is divided into 16 tiny squares by single lines. We use 5 of these 16 counts R.B.Cs

5- Microscope with both a low power (10X) and a high power (40X) objective.



Procedure:

1- Insert the stem of the R.B.C thoma pipette into the blood sample and withdraw the blood into the stem to slightly above the 0.5 mark.

2- Wipe the outside of the pipette stem and adjust the level of the blood in the stem to the mark 0.5 exactly.

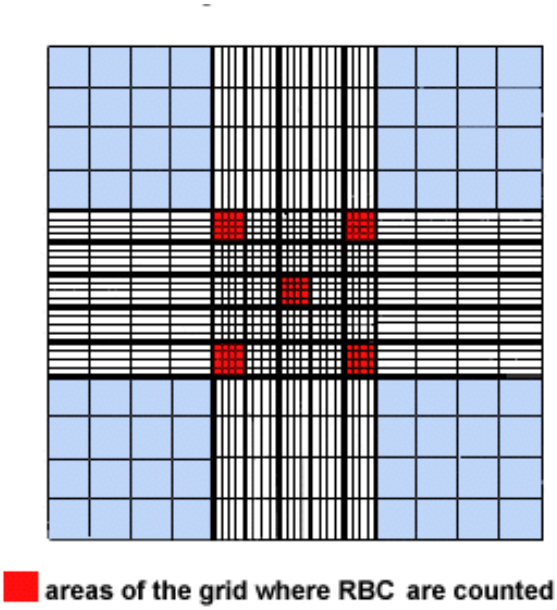
3- Using constant suction. Draw the diluent into the pipette until the bulb is filled and the fluid reaches the 101 mark. Mix the blood with the diluent.

4- Discard the first 5 drops to remove the cell-free diluent present in the pipette stem. Charge the chamber by touching the tip of the pipette gently to the edge of the cover slip. Blood will be draw into the counting chamber by capillary action.

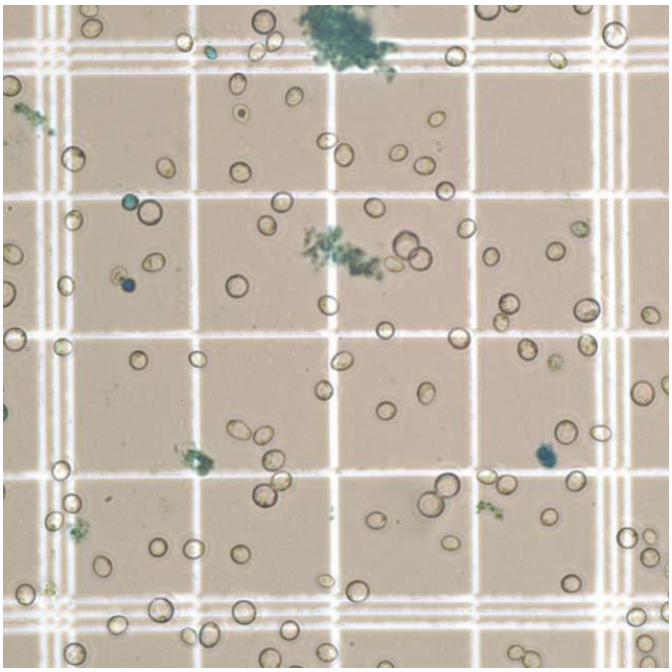
5- Place the Hemocytometer on the microscope stage.

6- First find the lined area with the low power (10X) objective and focus properly switen to (40X) and find one of the small corner squares in the corner of the triple lines. Notice that each of these smaller squares is divided into 16 tiny squares. Count all the R.B.Cs in each of these 16 squares.

7- The total number of R.B.Cs in each of the squares should be relatively uniform and calculate the numbers.



areas of the grid where RBC are counted



Calculation:

Calculating the R.B.Cs includes this formula:-

$$\text{No. of RBCs (cells/mm}^3\text{)} = \frac{N}{80} \text{ X 400 X 200 X 10}$$

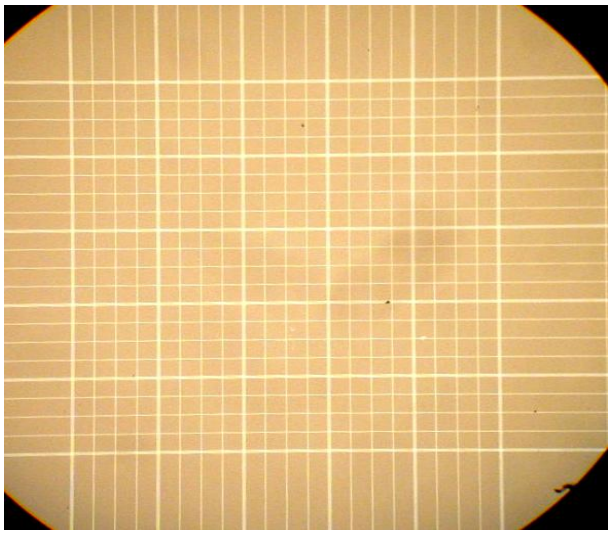
N = cells counted

80 = 16 X 5

400 = 16 X 25

200 = Dilution factor

10 = Volume factor



Normal range:

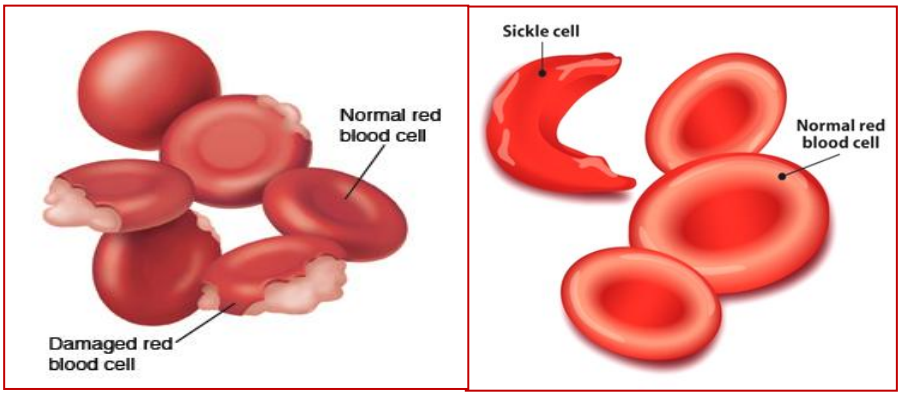
Male adult: 4.000000 – 6.000000 cell/mm^3

Female adult: 3.000000 – 5.000000 cell/mm^3

R.B.Cs count is useful in the diagnosis of some disease including anemia & polycythemia there is a direct relationship between R.B.Cs count & Hb, PCV, because Hb is the main component of R.B.C & the PCV rate increases & decreases in the same conditions.

The high R.B.Cs count called Polycythemia or lack of O2 because of living at high altitude, Smoking or in case of dehydration.

The low level of R.B.Cs count is called anemia that caused by a large loss of blood or poor production in the R.B.Cs. Because absence of nutrient also the R.B.Cs number is decreased in the case of large destruction of R.B.Cs in hemolytic anemia or sickle cell anemia.



Total white blood cell count

W.B.Cs (leucocytes) are special cells that are whitish in color because they lack Hb. Large than R.B.Cs & have a nucleus. Their function to protect the body against invading microorganisms and remove dead cells & debris from the tissues by phagocytosis

Leucocytes can be classified according to their appearance in stains into 2 types:

1- Granulocytes. These contain large cytoplasmic granules.

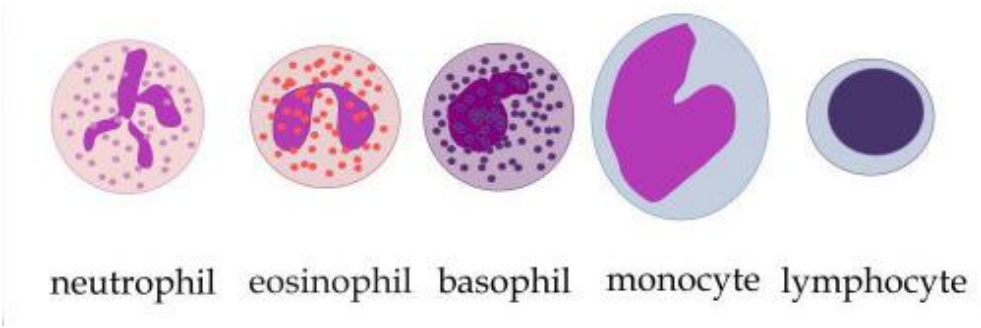
2- A granulocytes. These contain very small granules cannot seen with the light microscope.

The first one is classified into 3 types according the reactivity with the stain :-

1- Neutrophiles 2- Eosinophiles 3- Basophiles

The second is classified into 2 types:-

1- Monocytes 2- Lymphocytes



<u>Principle:</u>

Whole blood is diluted with a fluid to:-

1- Hemolyse the R.B.Cs.

2- Stain the nuclei of W.B.Cs to see easier under microscope.

3- Keep the W.B.Cs in normal state (isotonic solution)

The number of W.B.Cs is counted in a fixed volume of fluids and the final result is expressed as the number of cells in cubic millimeter of undiluted blood. (No. of cells / 1 mm^3 of blood)

Reagent & Equipment:

1- Anticoagulated blood.

The anticoagulant of choice is EDTA because it prevents blood coagulation and preserves the morphology of W.B.Cs.

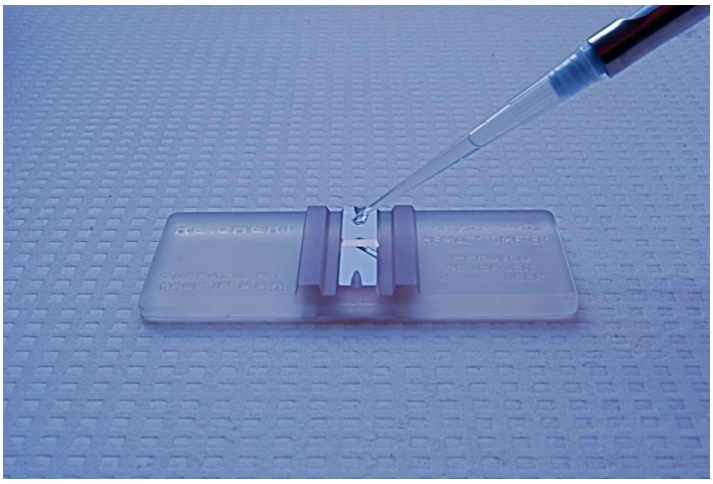
2- W.B.C pipette is known as thoma pipette, allows a 1:20 dilution of blood, it does not measure blood in µl. But provides a dilution ratio

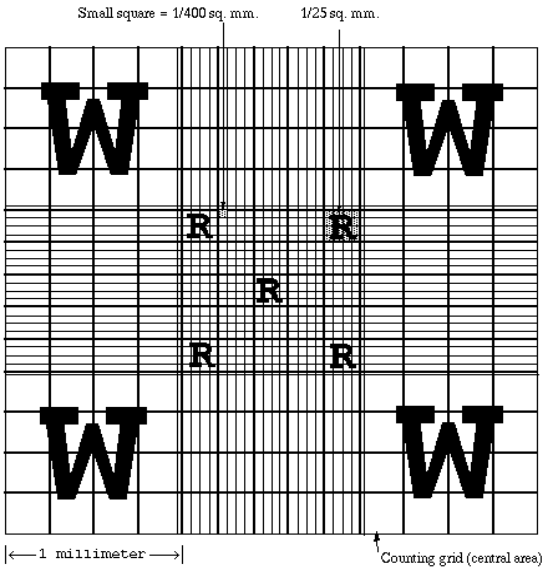
Recently this pipette is not used, because not accurate, so the Hb (sahli's) pipette is better.

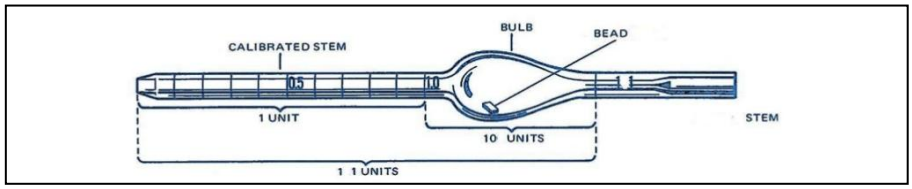
3- W.B.C diluting fluid Known as Turk's fluid.

4- Hemocytometer & cover slips. It's a special designed counting chamber. The most commonly used is the improved neubauer. We used the 4 corner squares each one is divided into 16 small squares by single lines.

5- Microscope the W.B.Cs are counted under low power (10X) objective lens.







Procedure:

1- Pour 0.4 ml. of diluting fluid into a tube.

2- Gently invert the fresh blood sample to ensure mixing.

3- Insert the stem of pipette into blood and withdraw the blood into the stem to slightly above 0.02 marks.

4- Remove the pipette from blood and wipe the outside of its stem, adjust the blood level to the 0.02 mark exactly. Be careful it's easy to remove too match blood and then you will have to begin again.

5- Pour the blood to the diluting fluid tube and shake gently for 15-30 sec.

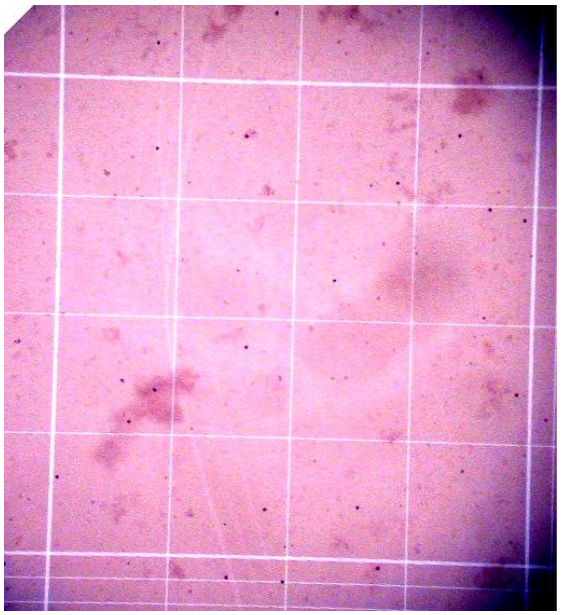
6- Now the blood is ready to be placed in the chamber, blood will be drawn into chamber by capillary action, make sure that no bubbles.

7- Put the chamber on the stage of the microscope.

Counting of W.B.Cs

Find the lined square with the 10x objective. The W.B.Cs are count in each of the 4 corner square. (The design of the slide is shown in the figure).

Calculating the W.B.Cs



The total number of W.B.Cs in the 4 corner square should be uniform.

The final calculation includes this formula:-

$$\text{WBC (cell/mm)} = \frac{N}{4} \times D \times 10$$

N= cells counted in 4 corner square

D= Dilution factor (=20)

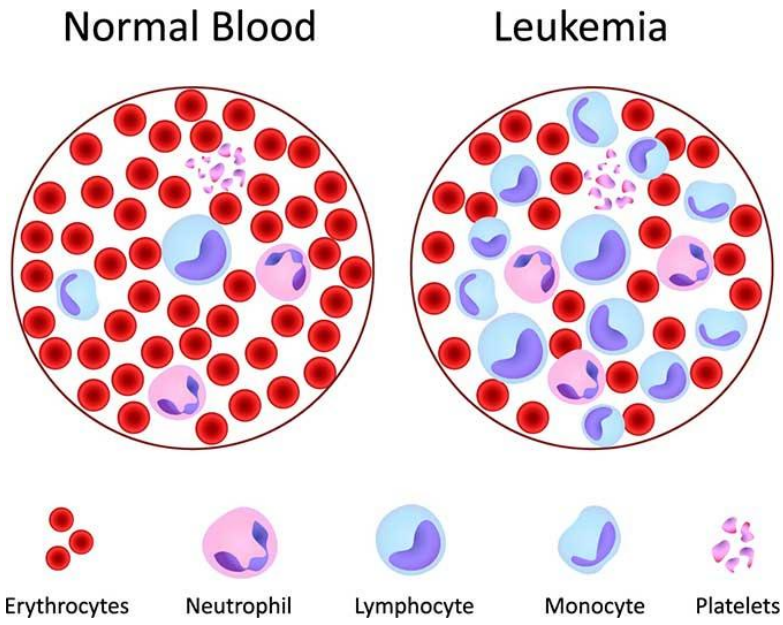
10= Volume factor

Reference range:

Male adult : 5.0 – 10.0 x 1000 cells /mm^3

Female adult : 4.5 – 9.0 x 1000 cells / mm^3

High W.B.Cs count is Known "Leucocytosis" caused by either physiological or pathological conditions, like in the neonates, pregnancy and sever exercise or in fever, injury, surgery, and in the medical conditions such as heart attack, kidney failure and cancer. Very high level of W.B.Cs can sometimes indicate Leukemia.



Low W.B.Cs count is known "Leucopenia", because the body not produces enough W.B.Cs after chemotherapy or radiation therapy, bone marrow failure (a plastic anemia), viral infections such as AIDS.

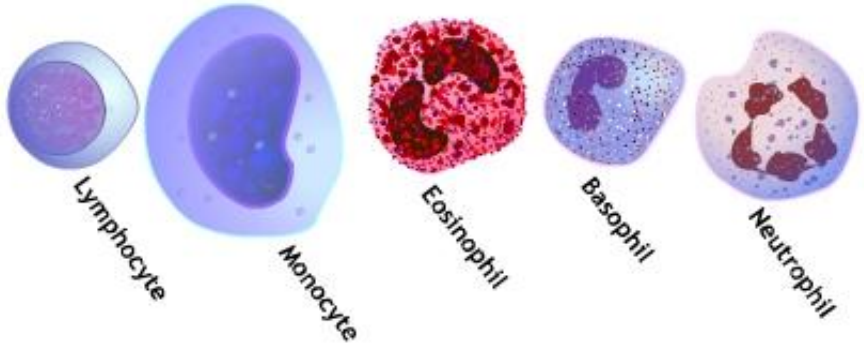
Differential White Blood Cells Count

The differential W.B.Cs count is a part of the C.B.C tests and is the most important, includes the preparation, staining, and examination of a thin film of blood smear on a glass slide.

The total W.B.Cs count is not necessarily indicative of the severity of a disease, since some done, to exam the presence the different type of W.B.Cs

There are 5 types of W.B.Cs are normally found in the blood:-

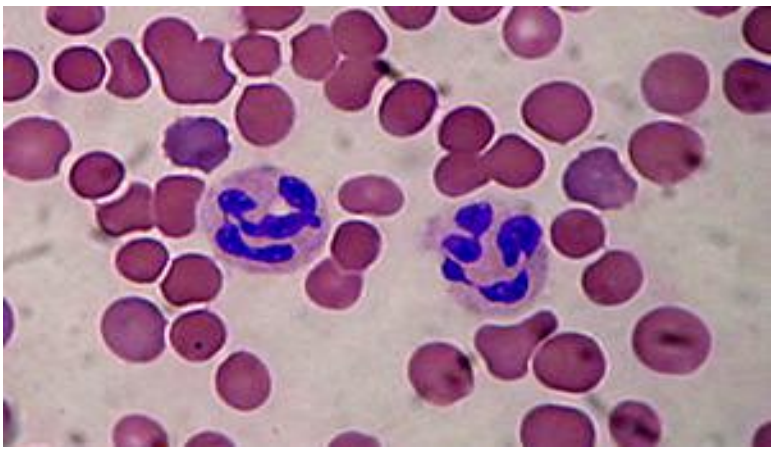
1- Neutrophiles
2- Eosinophiles
3- Basophiles
4- Lymphocytes
5- Monocytes

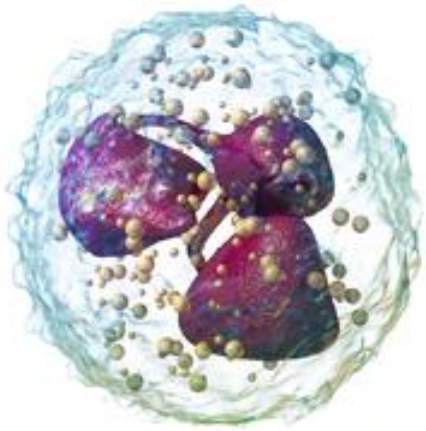


1- Neutrophils

Has very tiny light staining granules (difficult to see) the nucleus is multi-lobed. These cells have a phagocytosis work against toxins and viruses. In the normal blood smear this type is the most numerous. Normally neutrophils account = 50 – 70% of all W.B.Cs.

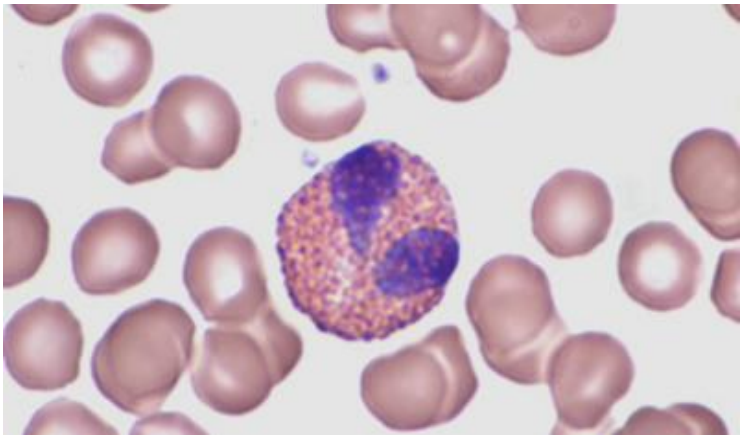
Above normal range, this due to an acute infection such as rheumatic fever burns. While if the count is less normal, it may be due to virus's infection ex: influenza, hepatitis, Rubella or lack of vit.B12 (folic acid).

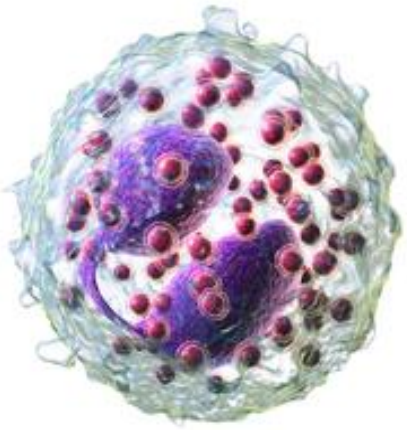




2- Eosinophiles

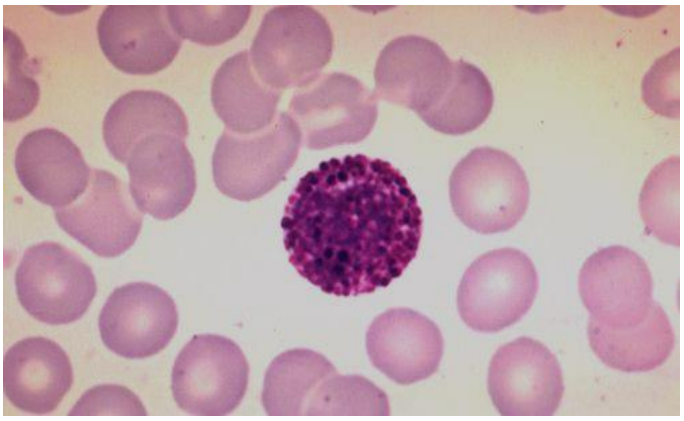
Have large acidophilic granules contain digestive enzymes that are effective against parasitic worms. Appear pink or red in stained preparation with 2 lobes of nucleus. These cells account for less than 5% of the W.B.Cs increases may be due to parasitic diseases and asthma.

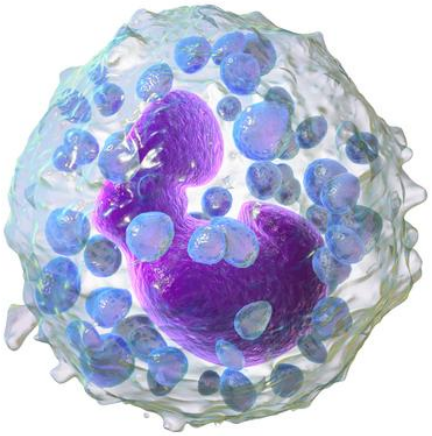




3- Basophiles

The basophilic granules in this cell are large. Stain deep blue to purple. These granules contain histamines (cause vasodilation) and heparin (anticoagulant). We rarely see these cells because they represent less than 1% all W.B.Cs high no. caused by hemolytic anemia and some types of leukemia.

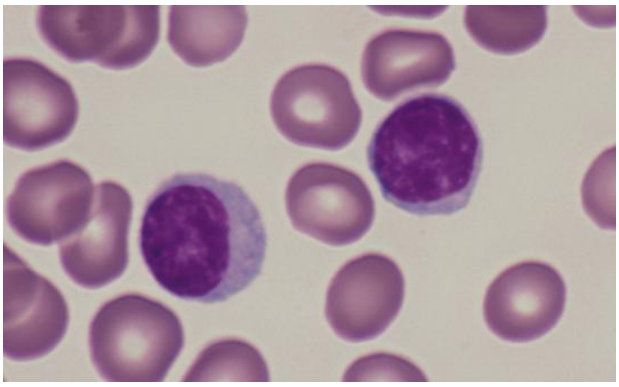


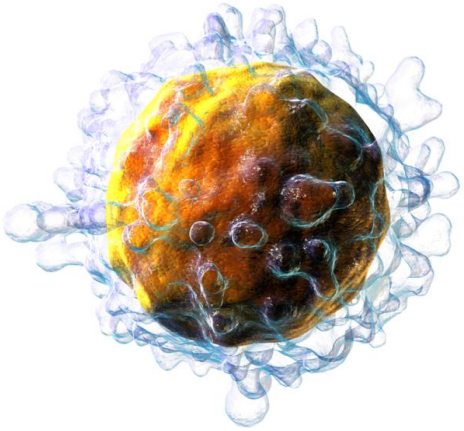


4- Lymphocytes

It's a granular cell with very clear cytoplasm which stains pale blue. Its nucleus is very large and dark purple. Its size is much smaller than the three previous cells. Normal account 25 - 35% of all the W.B.Cs

When the no. is increased, due to chronic infection, especially in AIDS the patients have increased no. of T- cell, an indicative of the AIDS.

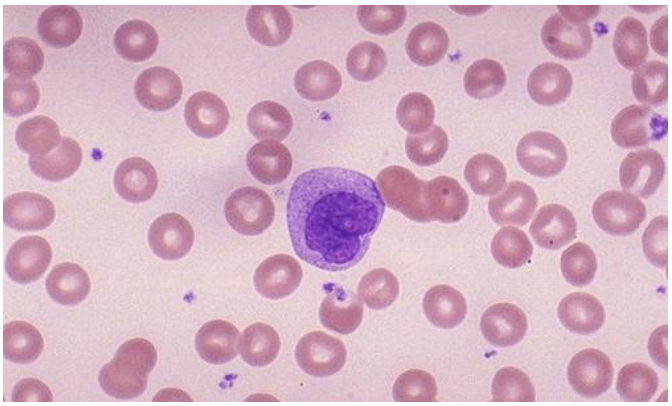


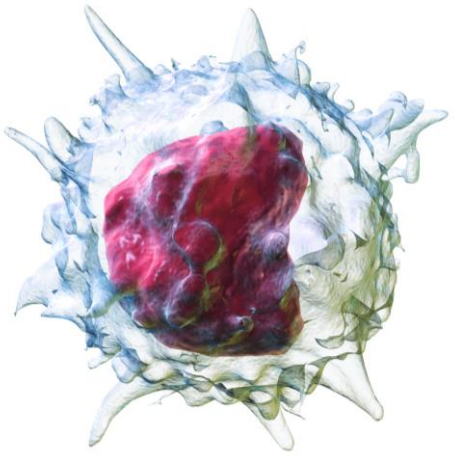


5- Monocytes

This cell is the largest of the W.B.Cs and is agranular, the nucleus has kidney shaped the cytoplasm is light blue. These cells leave the blood steam to the infected tissues and called macrophages.

These cells account for 3 – 9 % of all W.B.Cs in cases of fungus and bacterial infections the no. is increased like in the tuberculosis (TB).





<u>Reagents & Equipments:-</u>

1- Glass microscope slides, clean & dry.

2- Leishman s stain

3- D.W

4- Microscope

5- Oil immersion

Differential count procedure:-

This procedure is done in 3 steps

1- Making the blood smear.

2- Staining the cells.

3- Counting the cells.

1- Making the blood smear:-

To prepare a blood smear for a differential count Follow the steps below:-

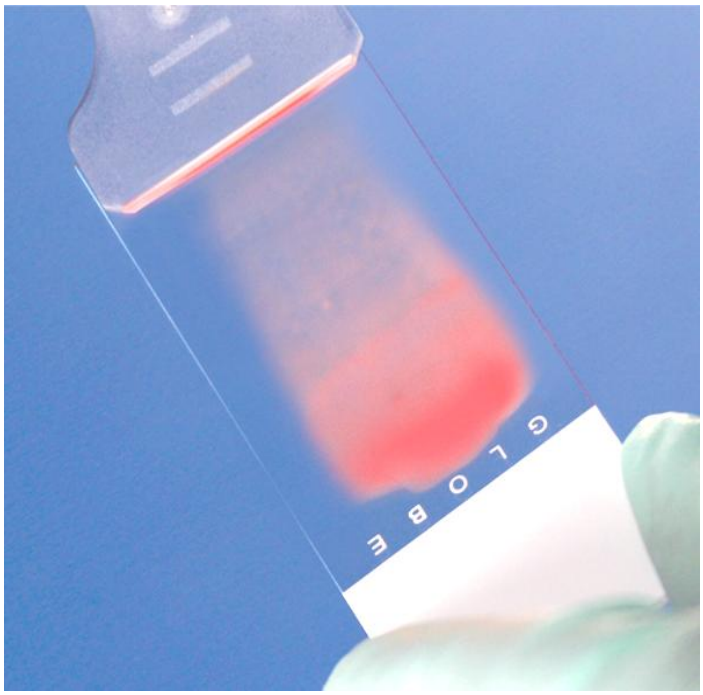
1- Collect anticoagulant blood by using Heparinized – capillary tube, from capillary blood.

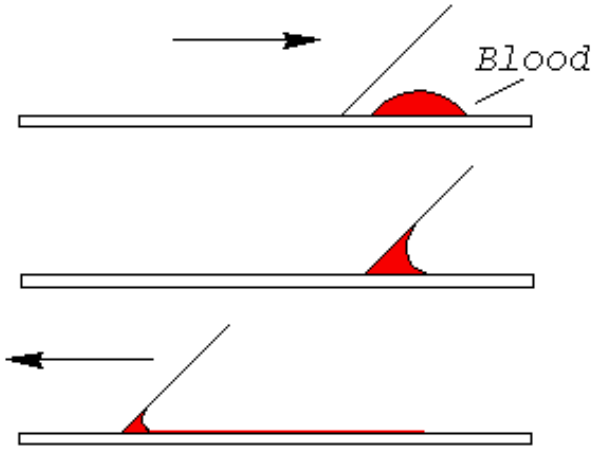
2- Deposit a drop of blood from capillary tube to the slide on a flat surface.

3- Hold a second slide between your thumb and forefinger. Back the second slide down until it touches the drop of blood

4- Push the second slide along the surface of the other slide in smooth & uniform manner. When you are making the smear, prevent blood from reaching the edges of the slides.

5- Once the blood smear in made, let it dry in the room temp.





2- Staining the cells:-

1- Prepare two containers filling one with stain solution and the other with D.W.

2- Immerse the slide in the stain for 15 – 30 sec.

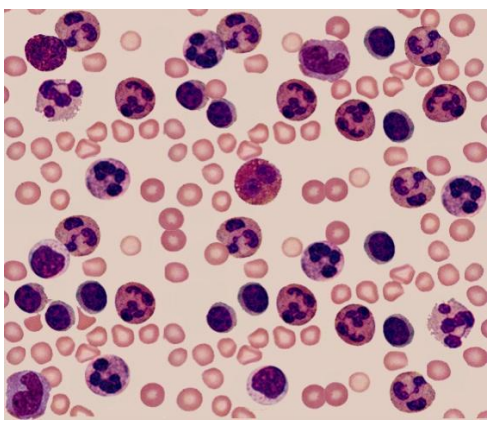
3- Remove the slide & immerse it in the D.W for 5 – 15 sec.

4- Wipe the back of the slide & leave it dry.

5- When the slide is dry, proceed to step 3.

3- Counting the cells:-

1- Place the slide under microscope under the oil immersion objective (100X).
2- Place a large drop of oil on thin area of the smear.
3- Rotate very slowly the coarse adjustment until you see some cells.
4- Count 100 W.B.Cs record cell type and number of cells.
5- Total each type of W.B.C if you count 20 lymphocytes among 100 cells, the diff. count for lymphocytes is 20%.



Blood Group System

Before the discovery of the blood groups system, the attempts to transfuse blood were unsuccessful, because they resulted in clumping of blood cells, rupture and clotting in the blood vessels called transfusion reactions leading to death. Until 1905 the scientist Karl Land Steiner demonstrated that transfusion reactions are caused by interactions between Antigens (also called agglutinogens) and Antibodies (also called agglutinins).

The membranes of the R.B.Cs have glycoprotein molecules called Antigens (Ags) Specific for the individuals, and in the plasma there are proteins called Antibodies (Abs). So in human the Ags on the surface of the R.B.Cs found in **over** than 600 types organized into 22 system one of them called Blood Group System includes only two types ABO & Rh blood group system.

1- ABO blood group system

ABO blood group system is based on the presence or absence of the A & B Ags on the R.B.Cs. (See the table 1).

Blood Group	Ag on RBCs	Ab in serum	Genotypes
A	A	Anti-B	AA or Ai
B	B	Anti-A	BB or Bi
AB	A and B	-	AB
O	-	Anti-A & Anti-B	ii

Table-1 - show the ABO system in human

We can understand that in the same group there is Abs in serum differing from Ags which is carried on R.B.Cs., in order to prevent the spontaneous agglutination and hemolyse the R.B.Cs or cause clots in the blood vessels.

In some rare cases there are incompatibility between a pregnant mother and her baby in the type of ABO blood group, but this is not problem for the child, because; First: the fetus has few ABO Ags and not well developed to activate the immune system of the mother than adult. Second: most of the Abs of the mother is belong to the type IgM is very large to cross the placenta to the baby R.B.Cs.

2- Rh blood group system

Another important blood group is the Rh factor. It's discovered in 1940 by Land Steiner in Rhesus monkeys Rh factor is a protein on R.B.Cs membranes called Antigen-D, this Ag is found in 85% of people (Rh+) and 15 % does not inherit this Ag (Rh-).

The incompatibility of Rh is very common and cause of the **HDN** disease in the new born. This happen when Rh- female marries Rh+ male, there is 50 % chance of producing Rh+ fetus (if the father has genotype Rh+Rh-) and 100 % chance of producing Rh+ fetus (if the father has genotype Rh+Rh+).

During pregnancy the mother and fetal circulations are separate, but at the time of birth, hard contractions in the uterus cause destroy the blood vessels causing mixing of fetal and mother blood for hours, so number of fetus R.B.Cs that carry the Ag-D will enter the mother circulation and stimulate her immune system to produce Abs called Ab-D will cross the placenta to the fetal blood causing clotting and hemolyse of fetus R.B.Cs which will destroyed within 1-2 week.

this case is deadly to the fetus, especially if the level of the Abs was high and the dangerous increase in the continuous pregnancy, but if the level is low, the fetus my live with sever anemia, therefore this disorder is called **hemolytic disease of new born (HDN)**. Also the term erythroblastosis fetalis is also used to describe the HDN, because the blood smear from these babies show the presence of many immature R.B.Cs (erythroblasts) because the R.B.Cs were destroyed before mature.

In mild cases the treatment is the transfusion of blood or in some times used the exchange transfusion without Abs. but the best treatment is the preventive treatment by give to the Rh- mother immediately after birth her first child (Rh+) a **RhoGAM** within 72 h. after birth.

RhoGAM is Rh-positive gamma-globulin (Rh+Abs) this Ab will attach and destroy the fetus R.B.Cs that enters the mother blood before her immune system is activated, so she can become pregnant with another Rh+ child without any dangerous.

Blood group test:

Principle:

The principle is based on the Antigen- Antibody reaction and show agglutination.

Reagents & Equipments:

1-Blood group plate or microscope slides.

2-Anti-A, Anti-B, anti-D solution

3-Stirrer.

Specimen:

The specimen is capillary or EDTA- anticoagulated blood.

Procedure:

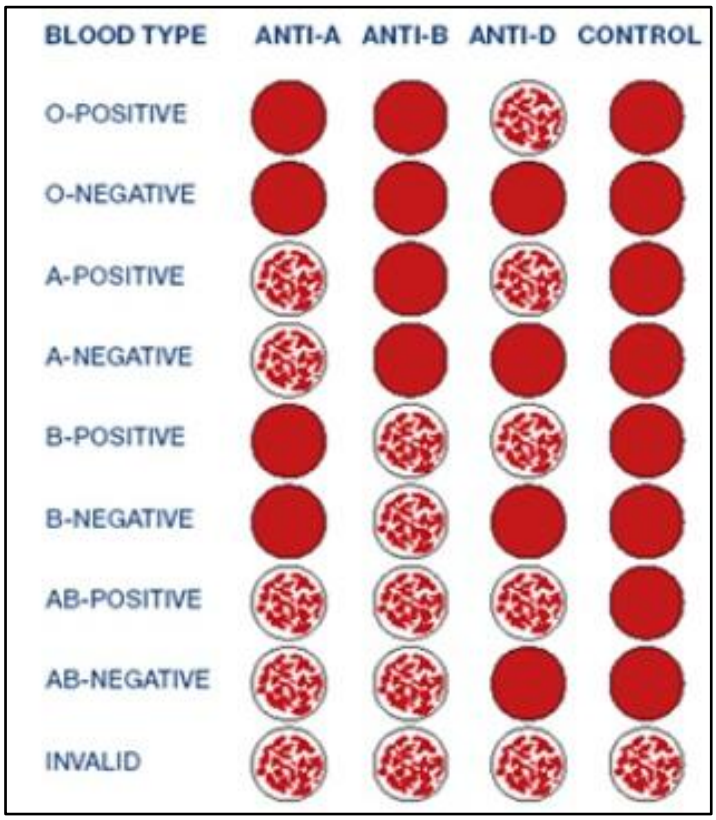
Agglutinalion tests are usually carried out in tubes or on slides for ABO & Rh grouping. Here we will perform slide test.

1- Put 3 drops of the blood on a clean-dry glass slide (these drops represent the Ag).
2- * Add drop of anti-A (represent the Abs) to the first drop.

* Add drop of anti-B (represent the Abs) to the second drop.
* Add drop of anti-D (represent the Abs) to the third drop.

3- Move the slide in circular motion.
4- Slide must read within 5 min. to avoid the evaporation.

- If agglutination occurs in the first drop, it mean that the blood group is A.
- If agglutination occurs in the second drop, it means that the blood group is B.
- If agglutination occurs in the both drops, it means that the blood group is AB.
- If there is no agglutination in both drops, it means that the blood group is O.
- If agglutination occur in the third drop, it means that the blood Rh+. If no it is Rh-

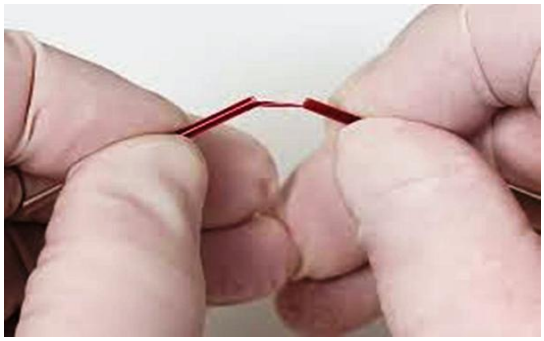


Laboratory Diagnosis of Clotting Disorders

Clotting Time Test (C.T)

The C.T determines the ability of capillary blood to clot within a time, after a puncture of the capillaries

Equipments:



1-Un - Heparinized capillary tube (Blue color).

2-Lancet

3-Watch

Specimen:

The specimen must be used is the capillary blood.

Procedure:

1-Sterilize the surface of finger.

2-Prick by lancet and record the time.

3- Collect the blood with un-heparinized capillary tube.

4- Move the capillary tube between your fingers in sloping, until the blood moving is stopped.

5-Break of a small piece of capillary tubes every 30 sec. until you see the strand of fibrin.

6- Stop the watch, record the total time. This represents the C.T expressed in min.

Normal value:

C.T = 2 – 11min.

Bleeding Disorders

The bleeding disorders associated with abnormalities in the quality and quantity of the coagulation.

These abnormalities are due to structure of blood vessels or impaired platelets function, like a reduction in their number in the blood stream, or disorders of plasma proteins involved in coagulation.

Abnormal synthesis of coagulation factors will result in a bleeding disorder known as **Hemophilia**.

Some dietary reasons can contribute in the disorder of bleeding such as vit.k deficiency, which is essential for synthesis and activation of some coagulation factors. Also in some conditions that the person requires transfusion of large amount of stored blood tend to infect with defects of bleeding because when blood is stored some clotting factors undergo chemical changes and become inactive, also the platelets tend to disappear from the stored blood after 4 - 5 days, and fall in their numbers cue to the dilution of the patient s blood and from increased bleeding.

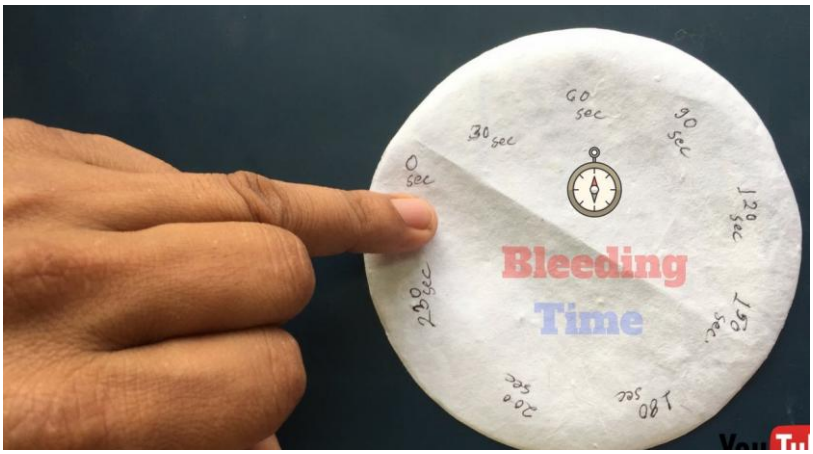
Laboratory Diagnosis of Bleeding Disorders

Bleeding Time Test (B.T)

There are many different tests were developed over the years, including the Duke test we are deal with it:-

Principle:

The Duke test measures the time required for the stop of bleeding after a puncture through the skin.



Equipments:

1-Lancet

2-Filter paper

3- Watch

Specimen:

Capillary blood

Procedure:

1-Sterilize the edge of the ear lobe.

2-Prick the skin by lancet and record the time.

3- Take a drop of blood by the filter paper every 30 sec.

4- Notice the blood, until it clot and stop the watch.

5- Record the total time that represent the B.T expressed by min.

Normal Value:

B.T = 2-8 min