جامعة بغداد

كلية العلوم

قسم التقنيات الاحيائية

فسلجة حيوان/العملي

المرحلة الثانية

الفصل الثاني

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First Lab.

Haematology

Is concerned with the study of blood and its components, and includes the study of etiology, diagnosis, treatment.

Blood

is a bodily fluid in animals and human that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from these cells.

Blood is composed of

1.Formed elements include: (erythrocytes, leukocytes, platelets)
2-Plasma which composed of: 92% water, 8% blood plasma components

Plasma components are:

1. Proteins such as (Albumin, lipoproteins, immunoglobulins (antibodies), clotting factors Serum Albumin.

2. Glucose and other nutrients.

3. Electrolytes (mainly sodium and chloride).

4. Mineral ions, hormones

5. Removed waste products, such as carbon dioxide, urea and lactic acid.

Blood collection

1- Capillary puncture method ; we can take the blood from:

a- Thumbs.

b- Ear lobe.

c- Foot of the children.

2- vein puncture method

We can take the blood from the median cubital vein (if we need a large amount of blood)

Preparation of serum :

Put the collected blood in a clean test tube (without anticoagulant) , left it for 5-10 minutes , and then put the test tube in the centrifuge for 10 minutes / 3000 .

The clear is represent the serum .

Preparation of plasma:

Put a blood in test tube **containing anticoagulant**. After a while we see a yellow fluid that called a plasma

Anticoagulants:

They are substances that prevents coagulation; that is, it stops blood from clotting.

Types of anticoagulants:

1- EDTA tri- potassium (Ethylene Diamine Tetra Acetic acid) : It work as a chelating agent to get rid of the calcium ions

2- Heparin :it is a biological substance. It works by activating antithrombin III, which blocks thrombin from clotting blood .

4- Sodium citrate: It gets rid of the calcium ions , but not as strongly as EDTA , it use in blood transfusion .

5- Oxalate : it has a mechanism similar to that of citrate.

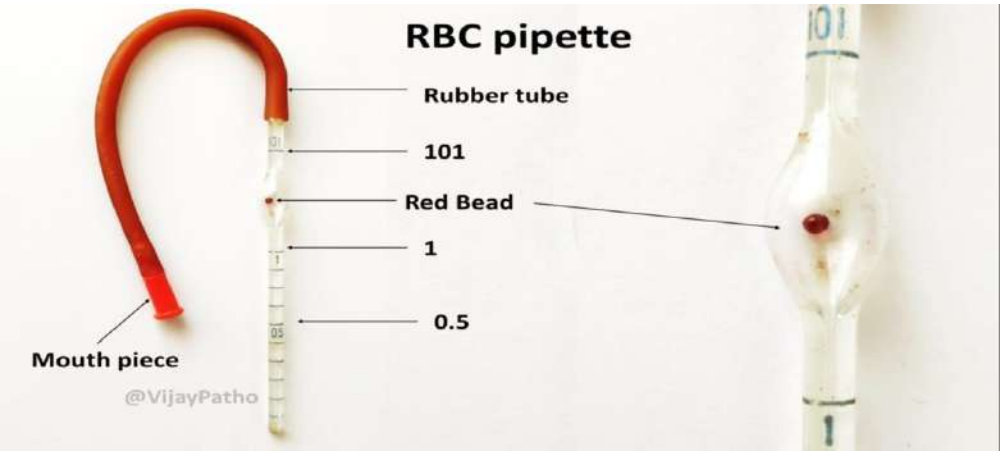
Red Blood Cell Count (RBC)

1. Manual counts = **Hemocytometer (Neubauer) count method**

Materials : Hemocytometer chamber, Cover slip, Light microscope , RBC pipette, and RBC diluting fluid (Hayes's solution or physiological saline (0.85% NaCl)

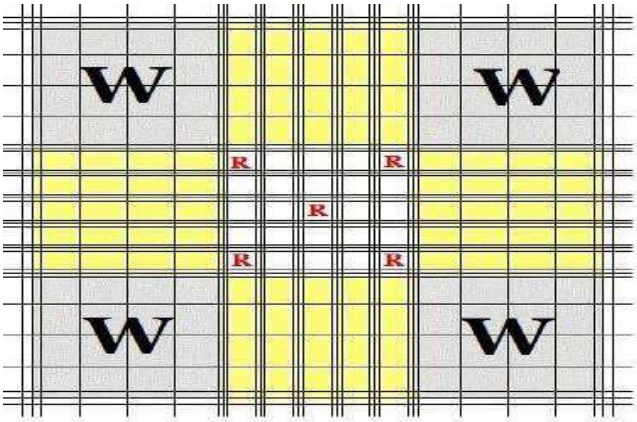
Hayme's solution consists of
Na Cl = 1 G (Isotonic solution).
Na_2SO_4 = 5 g. (prevent rouleux formation).
$HgCl_2$ = 0.5 G acts as antiseptic.
$D.H_2O$ = 200 mL

Hemocytometer chamber



Procedure to Count RBC

1. Fill the red bulb pipette up to 0.5 marks.
2. Draw the solution to mark 101 of the RBC pipette.
3. Mix the blood thoroughly in the pipette.
4. Discard the first few drops (4 to 5) and then fill the Neubauer chamber.
5. The distribution of the cells should be uniform over the ruled area.
6. Allow for 2 minutes to settle the cells.



Total RBCs count = No. of cells counted x Multiply factor

Multiply factor = Depth factor x Dilution factor x No. of counted squares

= 10 x 200 x 5= 10000

Normal range:

For men**, 4.7 to 6.1** million RBCs per microliter of blood

For women, **4.2 to 5.4** million RBCs per microliter of blood

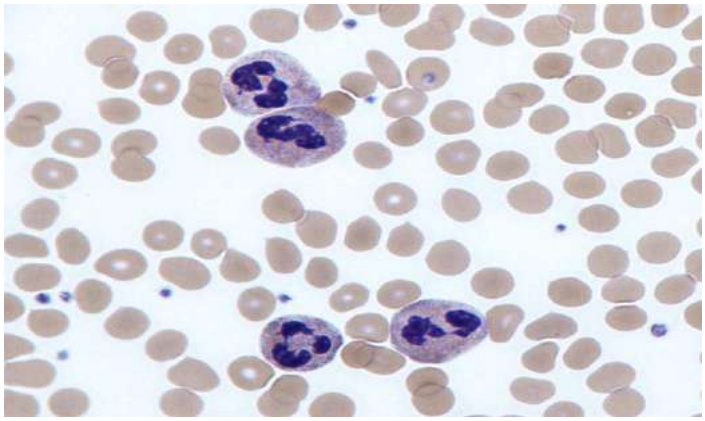
Second Lab. White blood cells

- White blood cells (WBCs) are a part of the body's immune defense system. WBCs count is important in the diagnosis of disease especially when accompanied by a differential white cells count.
  WBCs have size larger than RBC, they contain nuclei, they are completely colorless.

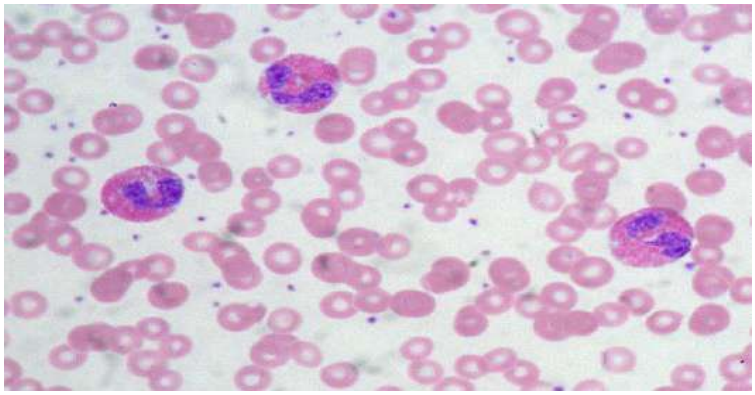
Classification of WBCs:

- The Leukocytes WBCs are divide according to the presence of granules in the cytoplasm into two types
- Granulocytes, which have specific granules.
- Agranulocytes, which lack specific granules.
- Granulocytes are divided according to the size and shape of their nucleus and their interaction with the stains to three types : Neutrophils, Eosinophils, and Basophils .
- Agranulocytes are divided into
- Lymphocytes and Monocytes.

- **Neutrophils:**
- The most common type of WBC (65% of total blood). Its nucleus is lobed (2-5 segment). Its function is the primary defense against bacterial infection.
- Neutrophils increase (**neutrophilia**): occurs in acute infection (eg. Bacterial infection, rheumatoid fever)
- Neutrohpil decrease (**neutropenia**)**:** occurs in some viral infection, hepatitis.



- **Eosinophils**:
  Five % of total blood, contain large granules that stain reddish-orange (eosinophilic), Its nucleus is lobed into two segment.
  Functions include: phagocytosis of antigen-antibody complexes and defense against parasitic infection.
- It increases (**eosinophilia**) occurs in parasitical infection and asthma
- it Decrease (**eosinopenia**) in severe stress.

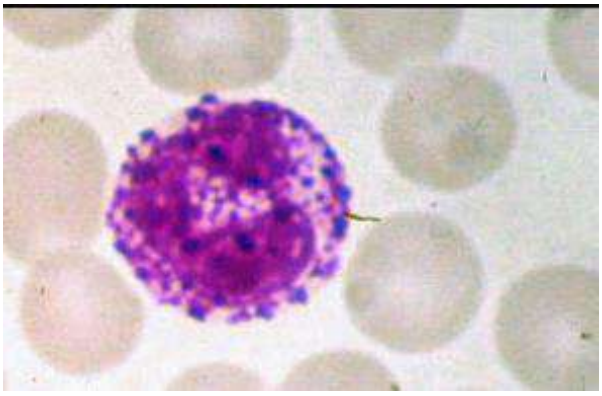


- **Basophiles**:

(1 %) of total blood, contain large dark blue or purple (basophilic) granules. It's nucleus is lobed into two segment, It has a light blue color.

It's increase (**Basophilia)** occurs in anaphylaxis and hemolytic anemia.

It's decrease (**Basopenia**) occurs in hyperthyroidism.

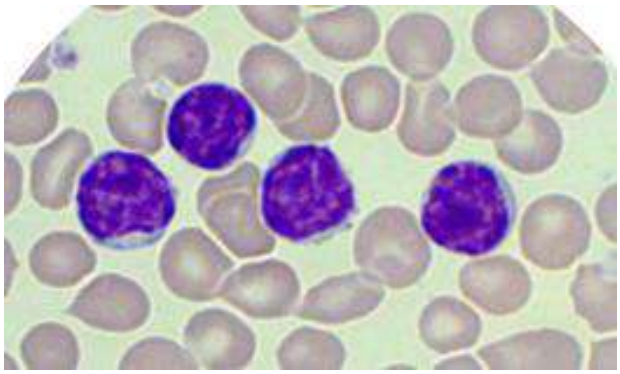


Lymphocytes:

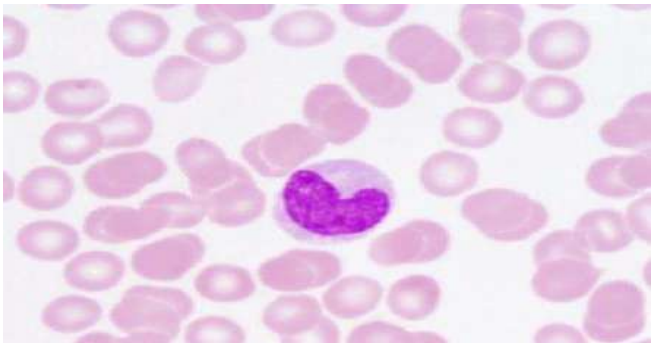
• 25 – 35 % of total blood, are the second most common type of leukocytes.

They are the smallest WBCs; it's nucleus takes almost size of the cell.

• Lymphocyte number is higher in children and also increases with viral infections and tumor.



- **Monocytes**:
- (3 – 9 %) of total blood cells, the largest WBCs, the nucleus is bean shaped, or kidney shape . they enter tissue to become tissue macrophages (also called histiocytes).
- It increases in malaria, typhoid fever and endocarditis.



WBCs count:

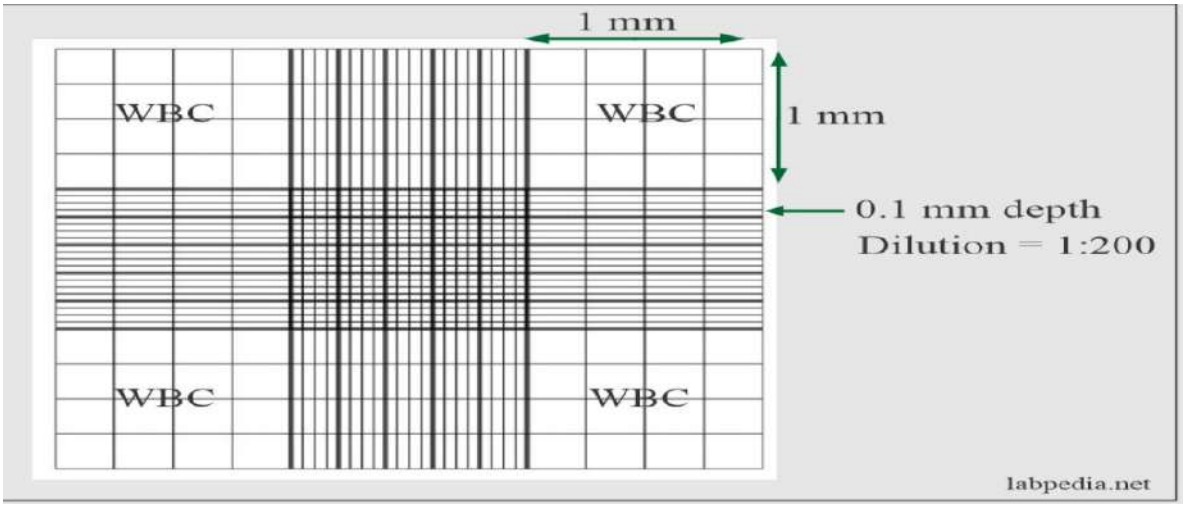
Fill the white bulb pipette up to 0.5 marks.

- Draw the Türk's solution (diluting solution) to mark 11 of the WBCs pipette.
- Mix the blood thoroughly in the pipette.
- Discard the first few drops (4 to 5) and then fill the Neubauer chamber.
- The distribution of the cells should be uniform over the ruled area.
- Allow for 2 minutes to settle the cells.

Türk's solution

- Distilled water 98 ml.
- 2 ml of glacial acetic acid for hemolysis of and .
- 1 ml of crystal violet (gentian violet) to stain the nuclei of WBCs.

Haemocytometer



Total WBCs count = No. of cells counted x 10,000 = WBCs million/cmm

- Normal range:

For men, 4500- 11000 million WBCs per microliter of blood

For women, 4000 - 11000 million WBCs per microliter of blood

Normal increase of WBC number:

- during pregnancy, after birth, after exercise, in newborn.

Pathogenic increase of WBC number:

- Bacterial infection, parasitical infection, viral infection, Allergy, Tumors

Decrease in WBC number :

Some viral infection, Disorder in bone marrow, Typhoid and paratyphoid .

Drugs that may lower your WBC count include:

- Antibiotics, Anticonvulsants, Anti thyroid drug, Chemotherapy drugs.

Drugs that may increase WBC counts include:

Corticosteroids, Epinephrine, Granulocyte colony stimulating factor, Heparin

Third Lab. **Packed Cell Volume (PCV)/ Hemoglobin (Hb)**

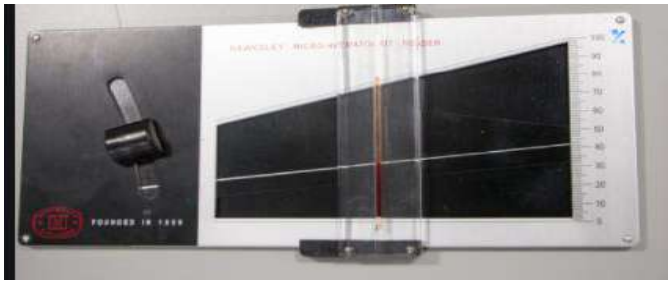
- PCV: Is the proportion of blood volume that is occupied by red blood cells. The value is expressed as a percentage or fraction of cells in blood (in 100 ml).
- For example, a PCV of 40% means that there are 40 millilitres of cells in 100 millilitres of blood.

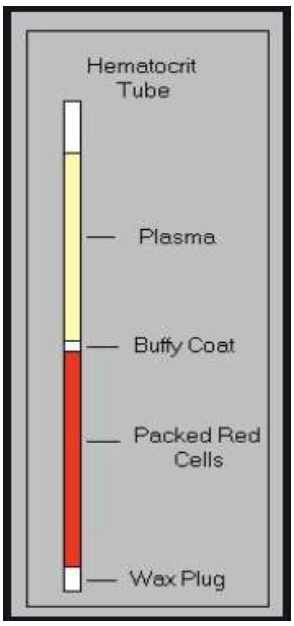
PCV normal values: 37 - 48% for men

38 – 45 % for women

Procedure:

- Capillary tubes are filled by capillary forces. (A minimum of two capillaries is required to ensure balance in the centrifuge). It is important that the tubes are sealed thoroughly.
- The packed erythrocytes are followed by first a small turbid layer – the buffy coat layer – and then a clear column of plasma. Hematocrit is estimated by calculating the ratio of the column of packed erythrocytes to the total length of the sample in the capillary tube, measured with a graphic reading device.
- The measurement should be performed within 10 minutes to avoid merging of the layers.





Raised PCV is seen in:

- Erythrocytosis.
- Extreme physical exercise or excitement.
- High Altitude.
- Dehydration leading to Hemoconcentration e.g. diarrhea, burns, and vomiting.
- Severe chronic pulmonary obstructive disease (COPD).

- **Decreased PCV is seen In:**

1. Anemia
2. Hemorrhage.
3. Renal diseases.
4. Pregnancy.
5. Autoimmune diseases.
6. Malignancies like lymphoma, leukemia, multiple myeloma, and Hodgkin's diseases.

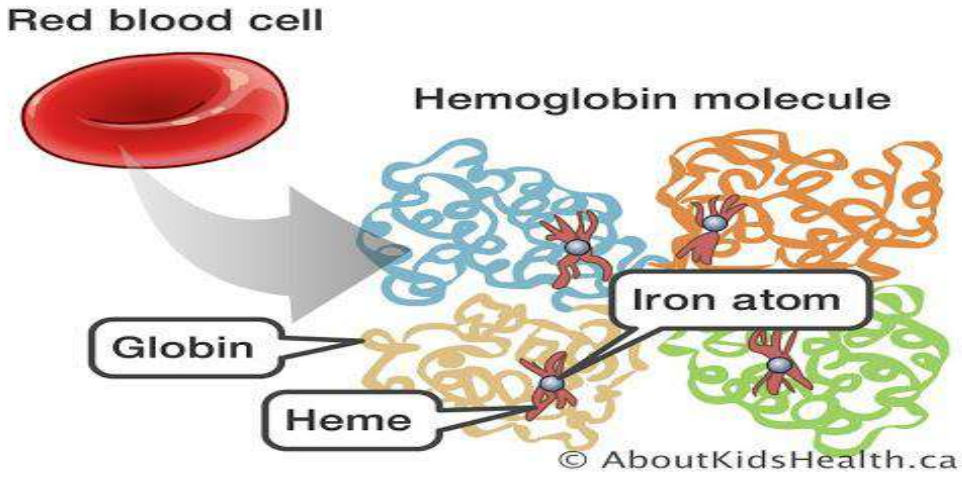
Hematocrit (Hct) is a macroscopic observation where the percentage volume of the packed RBCs is measured. Hct is usually three times the Hb concentration in grams/dl, While in electronic counters, it is calculated by MCV.

Hb = Hct/3

One g/dL Hb unit = 3 Hct unit
For example, if the Hct = 39 %
Then Hb = 39%3= 13

Hemoglobin

A protein in red blood cells that transports oxygen and CO_2 throughout the body. Each molecule of hemoglobin contains 4 units, each unite contain heme and globin



Hemoglobin test may be performed as part of a routine health exam or during a time of illness, but it's often done as part of a complete blood count (CBC). The hemoglobin test is primarily used to detect various types of anemia or erthrocytosis

** Anemia is a common condition that occurs when the amount of healthy red blood cells a person's blood is too low.

Some signs and symptoms of anemia include:

Weakness or fatigue, Lack of energy, Fainting, Paleness, Shortness of breath

Some signs and symptoms of erthrocytosis include:

Disturbed vision, Dizziness, Headache, Flushing, Enlarged spleen

There were two classic method to measure Hb :

Sahli's method .

Drabkin method (by spectrophotometer in length 540 nanometer)

Sahli 's method:

Sahli device is an empty graduated tube with two standard tubes.

2. Put an amount of blood into the Capillary pipette . (0.02 ml)

3. Put drops of HCL solution (7-8 drops) and mix the blood with the acid glass rod.

4. Put drops of distilled water until we get the standard color in the two tubes are compared by placing a tube of blood between the two standard tubes.

The normal ranges for hemoglobin :

Adult males: 14-17 gm/dl
Adult women: 12-16 gm/dl

Increase of hemoglobin levels in :

- Dehydration,
- Excess production of red blood cells in the bone marrow,
- Severe lung disease.
- Several other conditions.

Normal increase and decrease in Hb level occur in these cases:

1. During pregnancy
2. Hemoglobin levels peak around 8 a.m. and are lowest around 8 p.m. each day.
3. Heavy smokers have higher hemoglobin levels than nonsmokers.
4. Living in high altitudes increases hemoglobin values due to an increase in the number of red blood cells.
5. Hemoglobin levels are slightly lower in older men and women and in children.
6. A recent blood transfusion can affect a person's hemoglobin level.

Fourth Lab.

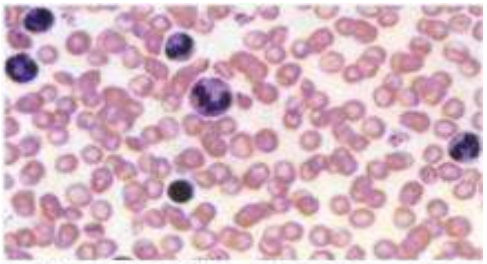
Physical features of the RBCs and ESR

- Mean corpuscular volume (MCV):
- The average **volume** (or size) of the individual erythrocyte.

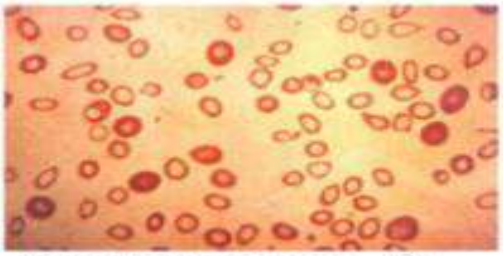
$$MCV = \frac{PCV * 10}{RBC\ (per\ mm3)}$$ •

expressed in femtoliters (fl) or cubic micrometers

The normal value is typically 80-100 fL



Normocytic Normochromic



Microcytic Hypochromic – IDA

Mean corpuscular hemoglobin (MCH):

$$MCH = \frac{Hb\ (gldl)}{RBC\ (millions\ per\ mm3)} \times 10$$

- The value is expressed in picograms (pg/cell)
- Normal value in adult : 27-35 Pg/

High MCH

Are commonly a sign of macrocytic anemia (when the blood cells are too big, which can be a result of not having enough vitamin B12 or folic acid in the body).

Causes: liver diseases, overactive thyroid gland, drinking alcohol, taking too many medications containing estrogen

Low MCH

Are commonly a sign of microcytic anemia occurs when the blood cells are too small and cannot take in as much hemoglobin as they should.

Causes: Low amounts of iron, celiac disease can prevent the body from properly absorbing iron, gastric surgery , lacking key vitamins like B vitamins such as folate and B12

Mean corpuscular hemoglobin concentration (MCHC) :

- MCHC indicates the amount of hemoglobin per unit volume. In contrast to MCH, MCHC correlates the hemoglobin content with the volume of the cell. It is expressed as g/dl of red blood cells or as a percentage value.

MCHC = $(Hb \times 10) / PCV$

The value is expressed in g/dL

The normal values are 32 to 36 g/dL

Causes of a High MCHC: Autoimmune hemolytic anemia, Liver disease, Sickle cell disease, drugs like corticosteroids, splenectomy.
Causes of low MCHC: Iron deficiency (with or without anemia) , Thalassemias, Anemia

Red cell distribution width (RDW):

Is a number that reflects the variation in sizes of the red blood cells

- Normal red blood cells maintain a standard size of 6 to 8 micrometers (µm)
- If RBCs are very small, or very large, RDW will be elevated.
- Nutrient deficiency and anemia are the most causes for high RDW

The Erythrocyte Sedimentation Rate (ESR)

It is the rate at which red blood cells precipitate in a period of 1 hour.

- It is a common hematology test which is a non-specific measure of inflammation.
- The use of the ESR as a screening test in asymptomatic persons is limited by its low sensitivity and specificity. When there is a moderate suspicion of disease, the ESR may have some value as a "sickness index."
- ESR values tend to rise with age and to be generally higher in women. Values are normally increased in black populations.
- The ESR test can be used to help your doctor diagnose conditions that cause inflammation:
  temporal arteritis, autoimmune diseases, cancers, infections

- Normal values:

	Normal ESR test results	Abnormal ESR test results
Females under 50	between 0 and 20 mm/hr.	greater than 20
Males under 50	between 0 and 15 mm/hr.	greater than 15
Females over 50	between 0 and 30 mm/hr.	greater than 30
Males over 50	between 0 and 20 mm/hr.	greater than 20
Children	between 0 and 10 mm/hr.	greater than 10

Westergren Method

- Measure exactly 0.4 ml of the 3.8% Trisodium citrate solution, with the help of a pipette or a syringe into a clean and dry test tube.
- Draw 2ml of venous blood and immediately place 1.6 ml into the Trisodium citrate solution.
- Mix the blood and Trisodium citrate solution well.
- Fill a clean and dry Westergren ESR Tube with the mixture up to the 0 mark
- Close the top of the tube firmly while you place the tube into the tube holder, otherwise the mixture could escape the tube.
- Immediately set your timer for 1 hour or write down the time on a sheet of paper.
- Exactly after 1 hour read how far the red cell layer has fallen. Give the result in mm per hour.

Lab 5 **Blood Groups and Blood Transfusion**

Blood groups

A classification of blood based on the presence or absence of inherited antigenic substances on the surface of red blood cells (RBCs).

- The blood group divided into four categories being A, B, AB, O. there are two distinct chemical molecules called antibodies in plasma and antigens on the surface of the red blood cells. If these categories were not matched properly it could cause clumping or agglutination in the arteries and veins of the recipient and can result in loss of life.

Blood group A:

when you have A antigens on the surface of your red blood cells and B antibodies in your blood plasma.

Blood group B:

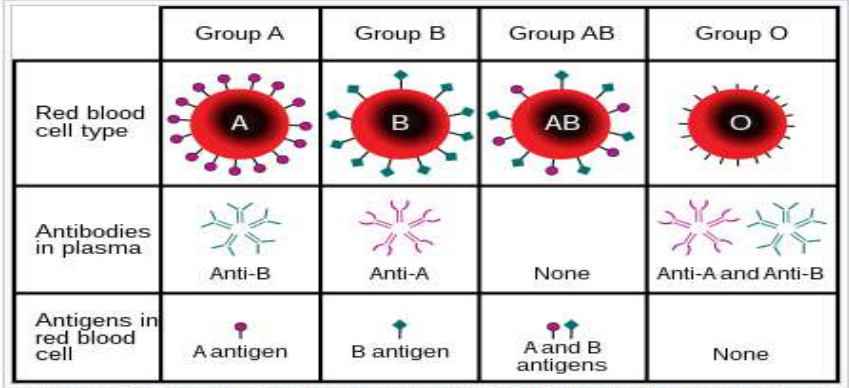
when you have B antigens on the surface of your red blood cells and A antibodies in your blood plasma.

Blood group AB:

when you have both A and B antigens on the surface of your red blood cells and no A or B antibodies at all in your blood plasma.

Blood group O:

when you have neither A or B antigens on the surface of your red blood cells but you have both A and B antibodies in your blood plasma.



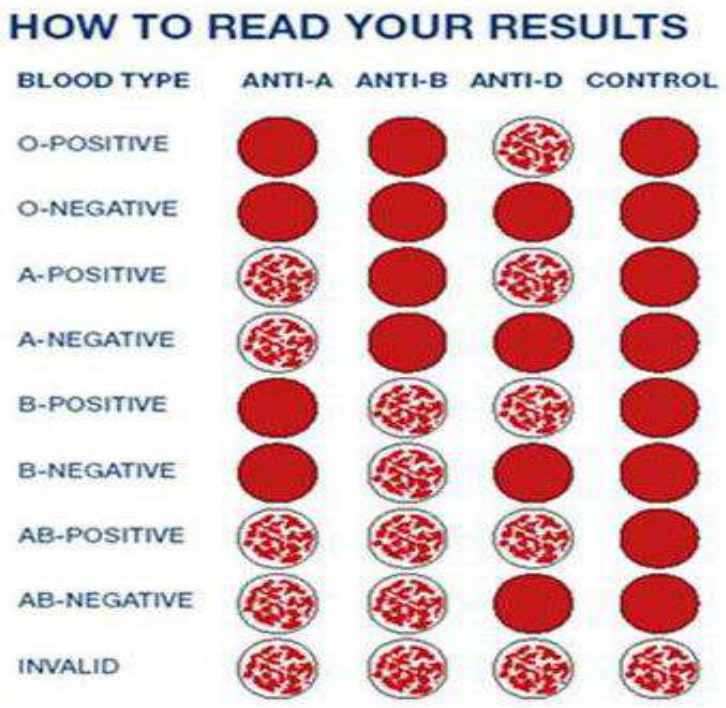
Blood type (or blood group) is determined, in part, by the ABO blood group antigens present on red blood cells.

Rhesus (Rh) factor

- Is an inherited protein found on the surface of red blood cells. If your blood has the protein, you're Rh positive. If your blood lacks the protein, you're Rh negative.
- During pregnancy, problems can occur if mother's Rh negative and the baby is Rh positive. Usually, mother's blood doesn't mix with baby's blood during pregnancy. However, a small amount the baby's blood could come in contact with the mother blood during delivery. Here, mother's body might produce proteins called Rh antibodies after exposure to the baby's red blood cells. The problem will be with the next pregnancy. If the next baby is Rh positive, these Rh antibodies can cross the placenta and damage the baby's red blood cells.

Procedure:

In order to determine your blood type, a lab technician will mix your blood sample with antibodies that attack types A and B blood to see how it reacts. If your blood cells clump together when mixed with antibodies against type A blood, for example, you have type A blood. Your blood sample will then be mixed with an anti-Rh serum. If your blood cells clump together in response to the anti-Rh serum, it means that you have Rh-positive blood.



Blood transfusion:

- Is a routine medical procedure in which donated blood (or it's components) is provided to patient through a narrow tube placed within a vein in your arm.
- Blood has several components, including:
- **Red cells** carry oxygen and help remove waste.
- **White cells** help your body fight infections
- **Plasma** is the liquid part of your blood
- **Platelets** help your blood clot properly
- Blood banks test donated blood to reduce the risk of transfusion-related infections such as HIV or hepatitis B or C.

Complication of transfusion:

- **Mild complications** of transfusion: allergic reactions, which might cause hives and itching, and fever.
- **Serious reactions of blood transfusion:**
- Acute immune hemolytic reaction**:** the immune system attacks the not matched transfused red blood cells
- Delayed hemolytic reaction**:** Similar to an acute immune hemolytic reaction but it can take four weeks to notice a decrease in red blood cell levels.
- Graft-versus-host disease**:** In this condition, transfused white blood cells attack your bone marrow. Usually occurs when patient being treated for leukemia or lymphoma.

Hives

Graft-versus-host disease

Sixth Lab. **Clotting time, Bleeding time**

Coagulation is a complex process by which blood form a clots. It is an important part of hemostasis (the cessation of blood loss from a damaged vessel). Wherein a damaged a blood vessel wall is covered by a platelets and fibrin – containing clot to stop bleeding and begin repair of the damaged vessel. Disorders of coagulation can lead to an increased risk of bleeding (hemorrhage) or clotting (thrombosis).

There are two pathways to coagulate the blood:

1- **Intrinsic pathway**: it happens as a response when there is abnormal in the wall of blood vessel & no damage in blood vessel.

2- **Extrinsic pathway**: its happen when there is a damage in blood vessel.

Process of coagulation:

1- Rupture of platelets ---------- release of thromboplastin

Ca

2- Prothrombin ---------- thrombin

thromboplastin

3- Fibrinogen ---------- fibrin

Thrombin

Clotting time: The time required for blood to form a clot.

There are various methods for determining this, the most common being capillary tube method.

There are two methods to do this test:

1- Full the blue capillary with the blood. and after 30 second broke the end of capillary, and then will see a thread of fibrin. The time from full the capillary with blood to formation of thread of fibrin is call the clotting time.

2- Full the blue capillary with blood and then up and down the capillary, and we will see the clotting formed inside the tube. The time from full the capillary with blood to formation of clots is called the clotting time.

The normal time to formation of clot is 4 – 10 minutes .

- **Bleeding time:** The time required for blood to stop flowing from a tiny wound. Normal bleeding time is from 2 to 9 minutes.

Two techniques are used:

1- Duke's method (Earlobe) (Obsolete)

2- Ivy's method: Forearm (Today use).

Duke's method: The patient is pricked with a special needle or lancet, preferably on the earlobe or fingertip, after having been swabbed with alcohol. The patient then wipes the blood every 30 seconds with a filter paper.

- **Bleeding time is prolonging in:**
  - 1- Defect of blood vessel wall,
  - 2-Decrease in platelet count and in its function.
  - 3-Decrease in coagulating factors.
  - 4- In some diseases like hemophilia, uremia,
  - 5- Ingestion of aspirin.