Hematology: is a branch of medicine concerning the study of blood, the blood forming organs, and blood diseases.

Blood: is a complex fluid consisting of different blood cells suspended in yellowish liquid called plasma. The average person has about 5 liters of blood.

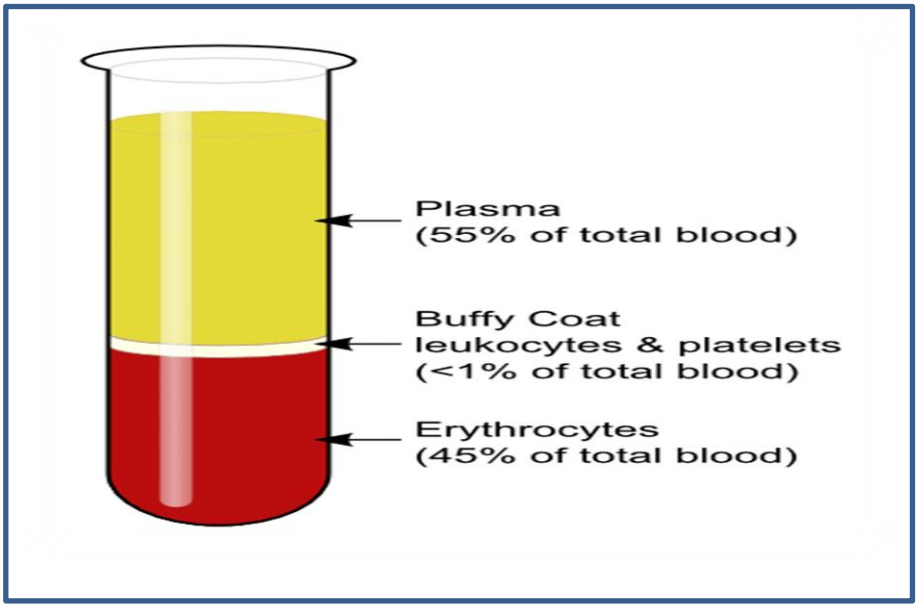
❖ Blood composition:

Blood is composed of two parts: a cellular part (blood cells) and a non cellular part called plasma.

▪ Blood Cells

There are three types of blood cells: Red blood cells, white blood cells (leukocytes) and platelets.

- **Plasma**: A liquid part of blood which makes up about half of the content of whole blood. Can obtain if blood is collected into a tube containing an anticoagulant, so the plasma contain clotting factor.
- **Serum**: a liquid part of blood that collected after the blood was clotting so it not contains fibrin and other clotting factors. (Similar to plasma components excepted not contain clotting factor).



Blood collection:

There are several ways to obtain blood as below:

A-Veinpuncture

The venipuncture technique consists of a series of steps that include:

1- Prepare the patient: Patients should either sit or lie comfortably

2- Select supplies equipment:

- Srynge
- Gloves
- Alcohol
- Cotton ball
- Tourniquet
- Collection tube and tube holder

3- Wash hands and apply gloves.

4- Apply the tourniquet.

5- Select the venipuncture site.

6- Cleanse the site :the skin should be cleaned with 70% ethanol and allowed to dry

7- Syringe is held securely in the dominant hand with the thumb on top and the other fingers below(Before entering the vein, examine the point of the needle for any defects).

8- Before removing the needle, remove the tourniquet.

9- Remove the needle, and apply pressure.

10- Dispose of the needle with the safety device.

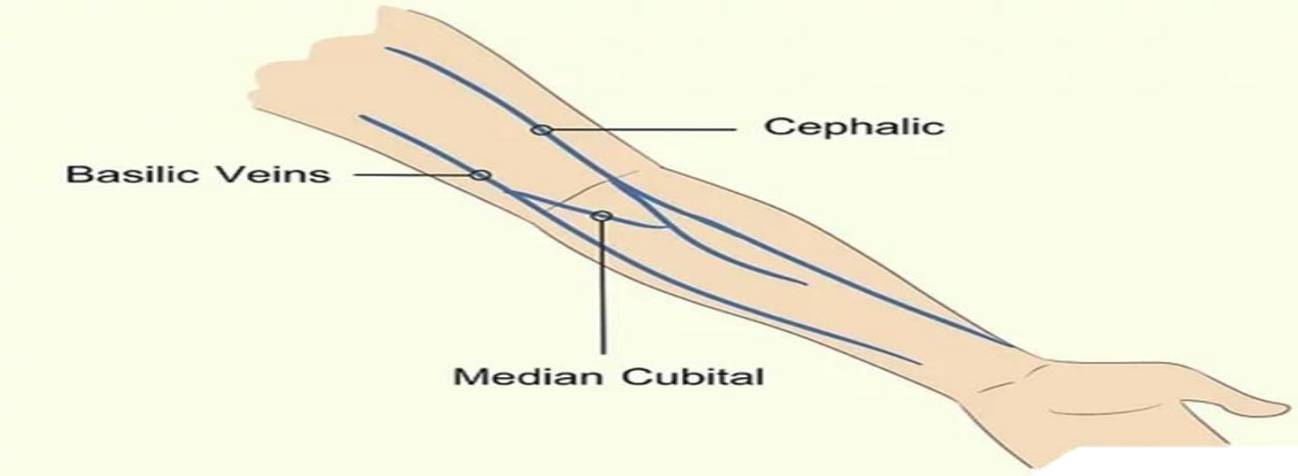
- In some laboratory test(calcium and potassium) the tourniquet shouldn't use.

➢ The position of Veinpuncture:

1- **The median cubital:** is the vein of choice because it is (large, well anchored, and does not tend to move when the needle is inserted).

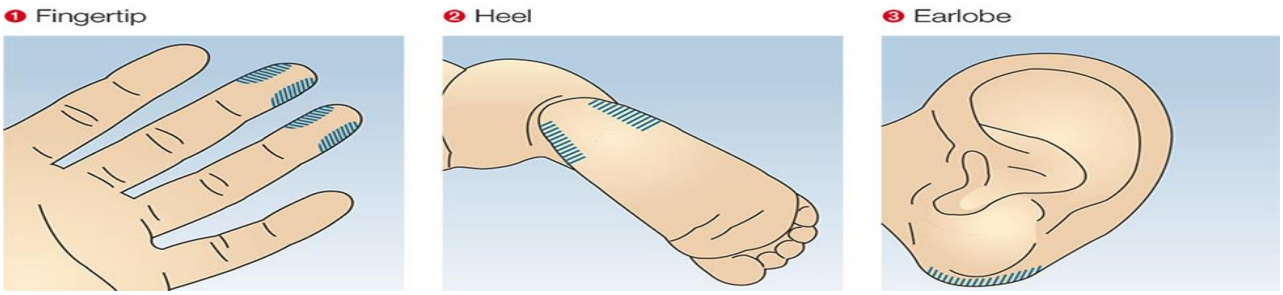
2- **The cephalic vein**: located on the thumb side of the arm, is usually more difficult to locate, except possibly in larger patients, and has more tendency to move. Because this vein is closer to the surface

3- **The Basilic vein**: should be used as the last choice because the median nerve and brachial artery are in close to it, increasing the risk of injury.



B- Capillary blood: It is often necessary to obtain blood by skin puncture in babies and infants and in adults with poor veins.

Blood may be obtained by prick with a sterile lancet on the surface of a heel (babies less than 3 months of age and infants), or a finger, thumb or ear lobe (older children and adults).



➢ Blood samples hemolysis

Hemolysis is defined as the rupture of erythrocytes resulting in the release of its intracellular components in plasma or serum.

Practices to prevent hemolysis

1. Choose the right gauge needle.

2. Alcohol used for cleansing the venipuncture site should be allowed to dry completely before drawing

the blood.

3. One should collect the blood specimen in the correct blood collection tube (serum separator tube (SST) or red top tubes without anticoagulants).

4. After performing venipuncture and removing the needle, transfer the blood gently down the side of the collection tube.

5. Invert the tube gently as recommended by the tube manufacturer.

6. Later, the tube should be placed upright for 15-30 minutes at room temperature until complete clot

formation.

7. Once clotted, store the samples at an upright position at 4°C (refrigerated) until ready to be shipped to

Lab2

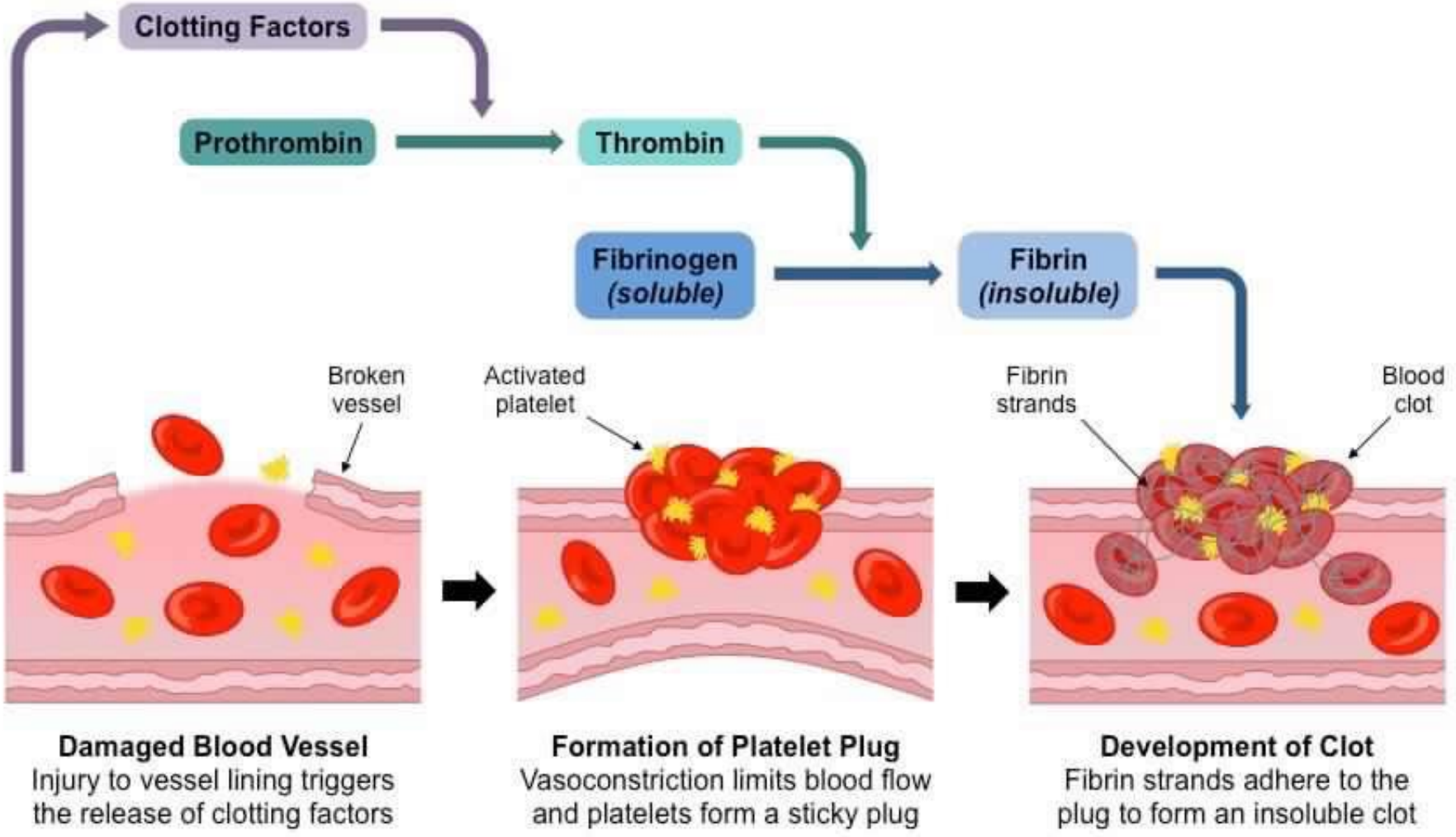
Blood coagulation & Anticoagulants

- **coagulation:** refers to the process of forming a clot to stop bleeding.

The clotting action

- When bleeding occurs, platelets become activated and form a network by attaching to the blood vessel wall at the site of bleeding, and also by attracting other clotting factors in the blood to promote the formation of fibrin to stop ongoing bleeding rapidly.

Blood coagulation



- **Anticoagulants:** are agents that prevent the formation of blood clots both in vitro and in vivo by affecting blood coagulation factors. Anticoagulation occurs by binding calcium ions (EDTA, citrate) or by inhibiting thrombin activity (heparinates, hirudin).
- **Anticoagulants** (sometimes known as “blood thinners”) are drugs that are given to prevent blood from clotting or prevent existing clots from getting larger. They can keep harmful clots from forming in heart, veins or arteries. Examples are Heparin, warfarin, dabigatran, apixaban and rivaroxaban.

- Blood samples must be collected into tubes or bottles containing different anticoagulants to prevent coagulation (clotting).

The anticoagulants commonly used

1- **Ethylene-di-amine-tetra-acetic Acid (EDTA)** EDTA acts by its chelating effect on the calcium molecules in the blood. It removes calcium ions, which are essential for coagulation.

-Use

- EDTA is the anticoagulant of choice for blood counts and blood films. It is also ideal for platelet counts as it prevents platelets from clumping. This anticoagulant, however, is not suitable for coagulation studies because it destroys clotting factors V and VIII.

2- **Tri sodium Citrate**

Trisodium citrate removes free calcium ions by loosely binding to them forming a calcium citrate complex.

-Use

- This anticoagulant is used for coagulation studies and the estimation of the erythrocyte sedimentation rate (ESR). For coagulation tests, nine volumes of blood are added to one volume of the sodium citrate solution. For the ESR, four volumes of blood are added to one volume of the sodium citrate solution.

3- Heparin is a natural substance which is synthesized by the liver. It is prevents clotting by inactivating thrombin, thus preventing conversion of fibrinogen to fibrin. It is the best anticoagulant when absolute minimal hemolysis is required (e.g., osmotic fragility test and hematocrit determination).

Tube cap color	Additive
	3.2% Sodium citrate
	Serum tube with clot activator or gel
	Sodium or lithium heparin
	Potassium EDTA
	Sodium fluoride, and sodium or potassium oxalate

Red Blood Cell Morphology

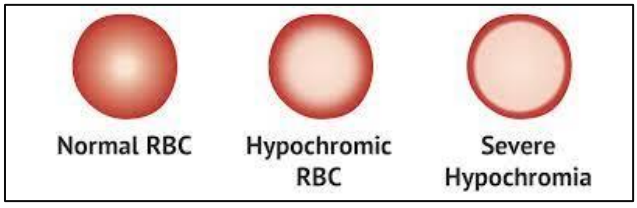
The primary function of the RBC, or erythrocytes, is to carry oxygen from the lung to body tissues and to transfer carbon dioxide from the tissues to the lungs.

- Normal mature RBC are biconcave, round discs that are about 6 – 8 micrometer in diameter.

- The term used to indicate red blood cells of normal size and shape is normocytic. The term used to indicate a normal color or central pallor(i.e.,normal hemoglobin content) is normochromic.

Hypochromic: RBC that demonstrates a central pale area that becomes larger and paler as the hemoglobin content diminishes, Found in:

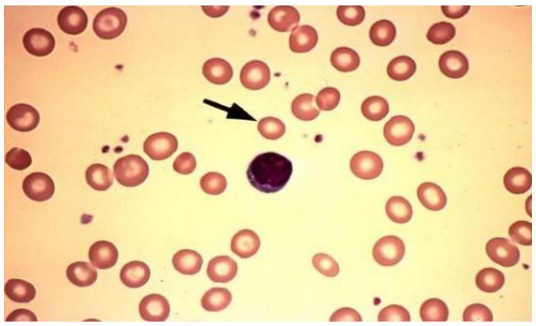
- Iron Deficiency
- Sideroblastic Anemia
- Thalassaemia



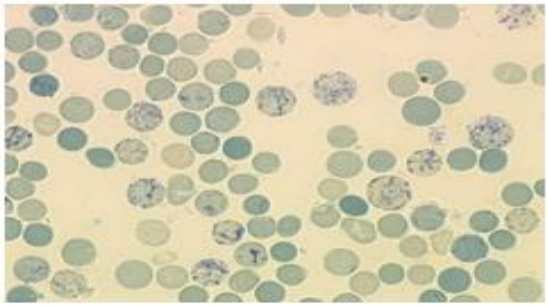
Polychromasia: Terms used to indicate the increased presence of non-nucleated immature erythrocytes (Polychromatophilic erythrocytes) that contain residual RNA which gives a blue-gray stain to the red cells.

- These cells, are slightly larger than mature erythrocytes. After exposure to a supravital stain, the cytoplasm organelles of these cells clump into an easily recognized blue-staining reticulum and the cells is called reticulocytes

Microcytosis: abnormally small erythrocytes (less than 6 µm in diameter). Found with Hypochromic



Microcytosis



polychromasia

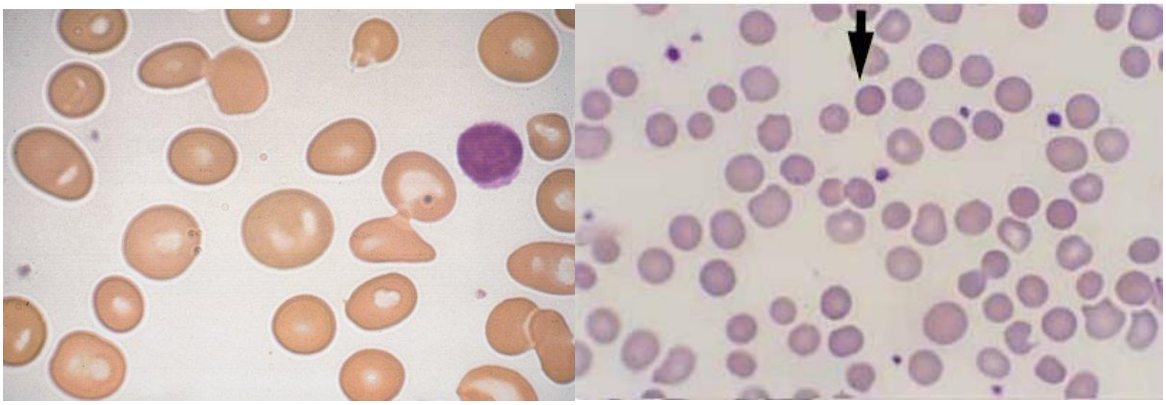
Macrocytosis: abnormally large RBC (larger than 9µm). May be round or oval in shape, the diagnostic significance being different. Found in:

- Folate and B12 deficiencies (oval)
- Megaloblastic Anemia
- High reticulocytes count

Spherocytes: are nearly spherical erythrocytes which usually have a diameter smaller than normal. They lack the central pale area due to their spherical shape.

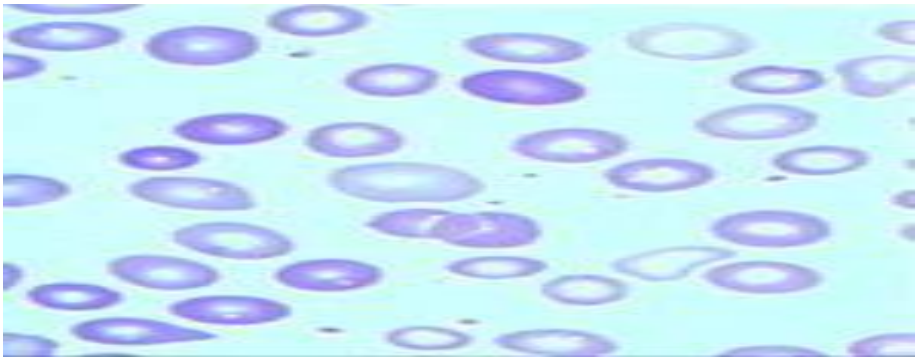
Found in:

- Hemolytic anemia
- Hereditary sphercocytosis

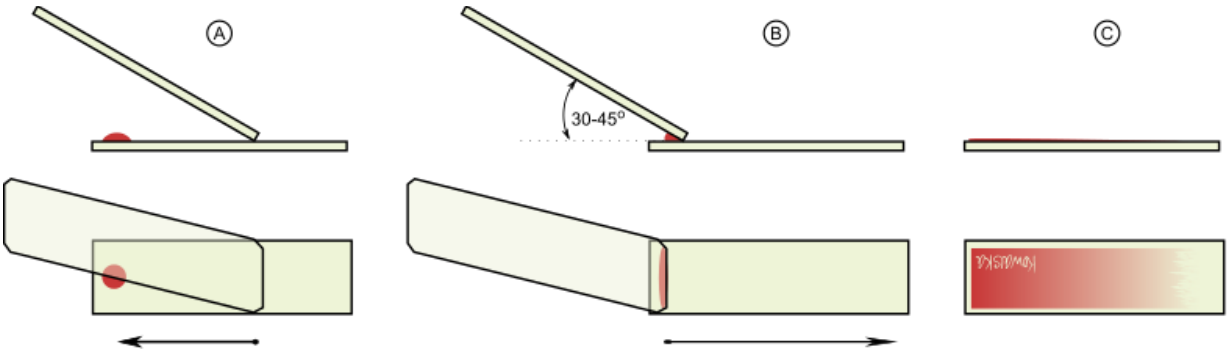


Macrocytosis **spherocytes**

Anisocytosis: increase in the variability of red cell size. Variation in erythrocyte size is now measured by the red cell distribution width (RDW).



Size variation	Hemoglobin distribution	Shape variation	
Normal	Hypochromia	Target cell	Acanthocyte
Microcyte	1+	Spherocyte	Helmet cell (fragmented cell)
Macrocyte	2+	Ovalocyte	Schistocyte (fragmented cell)
Oval macrocyte	3+	Stomatocyte	Tear drop
Hypochromic macrocyte	4+	Sickle cell	Burr cell
	Polychromasia (Reticulocyte)		



Making blood Smear

Staining blood smear with Leshman's stain

1-Fully cover the smears with Leishman’s Stain solution for 2 minutes.

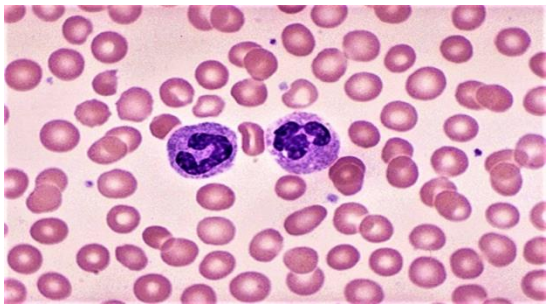
2- Add twice the amount of distilled water and mix by swirling.

3-Incubate for at least 10 min.

4-Rinse thoroughly with distilled water.

5-Dry the slides using blotting paper and air-dry.

6-Examine slide under microscope by oil lens



Hematology

Lab 3

Erythrocyte Sedimentation Rate (ESR)

ESR: Is the distance (in millimeter) that erytrocyte fall after one hour in a vertical column of anticoagulated blood under the influence of gravity.

- The erythrocyte sedimentation rate (ESR) is a relatively simple,inexpensive, non-specific test that has been used for many years to help detect inflammation associated with conditions such as infections, cancers, and autoimmune diseases.
- ESR is said to be a non-specific test because an elevated result often indicates the presence of inflammation but does not tell the health practitioner exactly where the inflammation is in the body or what is causing it.

Erythrocyte Sedimentation Rate (ESR)

Factors affecting ESR

1.Size of RBCs: In vitamin B12 deficiency RBCs are large in size and the sedimentation rate increased, while in iron deficiency RBCs are small in size and the sedimentation rate decreased.

2.Number of RBCs: In anemia ESR increase ,while in polycythemia ESR decreased .

3.Plasma proteins concentration: increased level of fibrinogen , gamma globulins

Lead to **rouleaux** formation**.**

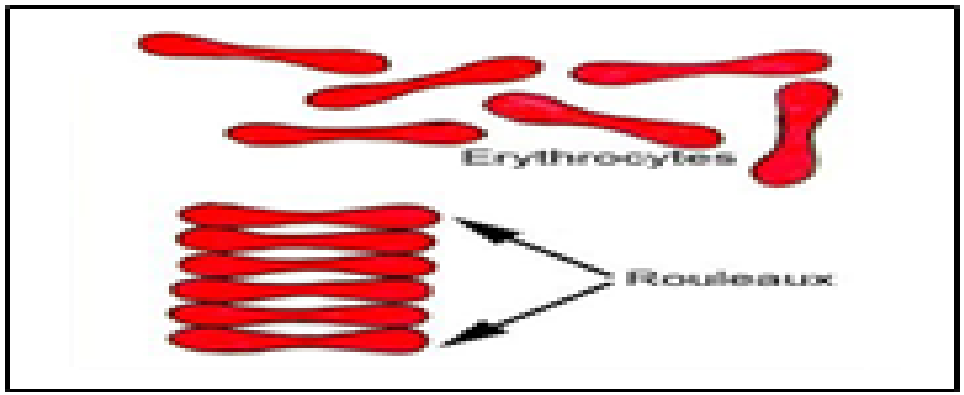
4.Hemoglobin content.

5.Blood viscosity.

Factors affecting ESR

Rouleaux:

The stacking up of red blood cells caused by extra of abnormal protein in the blood that decrease the normal distance red cells maintain between each other.



ESR increased in

1.Rheumatoid arthritis and Tuberculosis.

2.Multiple myeloma

3.Anemia.

4.Kidney disease.

5.Autoimmune and inflammatory disease.

6.Pregnancy.

ESR decreased in

1.Abnormally shaped RBC (sickle cells, spherocytosis).
2.Polycythemia.
3.Congestive heart failure.

Normal value:

Newborn: 0-2 mm\hr

Adults age lower than 50 y

Men: 0-15 mm\hr

Women: 0-20 mm\hr

Adults age older than 50 y

Men: 0 to 20 **(**mm/hr**)**
Women: 0 to 30 **(**mm/hr**)**

Procedure

1.Place 0.5 ml of Ttrisodium Citrate in test tube.

2.Add 2 ml of blood sample to the test tube.

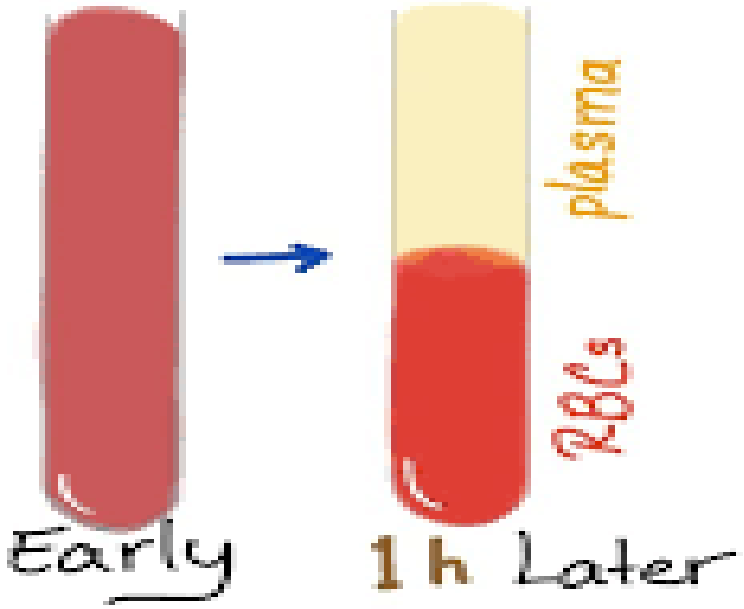
3.Mix the contents of test tube gently.

4.Fill the westergren tube to 0 mark.

5.Set up the westergren tube right in a stand with a spring clip on top and rubber at bottom.

6.Read the highest of the clear plasma above the upper limit of the column of sediment red cells in mm\hr.

ESR



lab 6

WBC count

White Blood Cell (WBC) count

WBC: They are a heterogeneous group of nucleated cells that are responsible for the body's defenses and are transported by the blood to the various tissues where they exert their physiologic role, e.g. phagocytosis.

- Their production is in the bone marrow and lymphoid tissues (lymph nodes, lymph nodules and spleen).
- White blood cells (WBCs) are an important part of the immune system. These cells help fight infections by attacking bacteria, viruses, and germs that invade the body.

TYPES OF WHITE BLOOD CELLS

Granulocytes

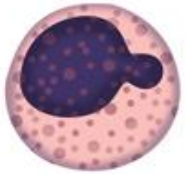
1. Neutrophil

Helps in phagocytosis

2. Eosinophil

Fights against parasitic infection

3. Basophil



Produces inflammatory and allergic reactions

Agranulocytes

4. Lymphocyte

Produces specific immune responses

B Lymphocyte

T Lymphocyte

Natural Killer Cell

5. Monocyte

Fights off bacteria, viruses and fungi

Leukopenia is the medical term used to describe a low WBC count. Conditions or illnesses that can trigger a low number include: HIV, bone marrow disorders/damage, severe infections, liver and spleen diseases, lupus, and radiation therapy.

Leukocytosis is the medical term used to describe a high WBC count. Conditions or illnesses that can trigger a high number include: anemia, tumors in the bone marrow, leukemia, inflammatory conditions, such as arthritis and bowel disease, tissue damage, pregnancy, allergies.

- An average normal range for white blood cells is between 4,500 and 11,000/ mm3 of blood. Abnormal test results are classified by numbers that are higher or lower than this range.

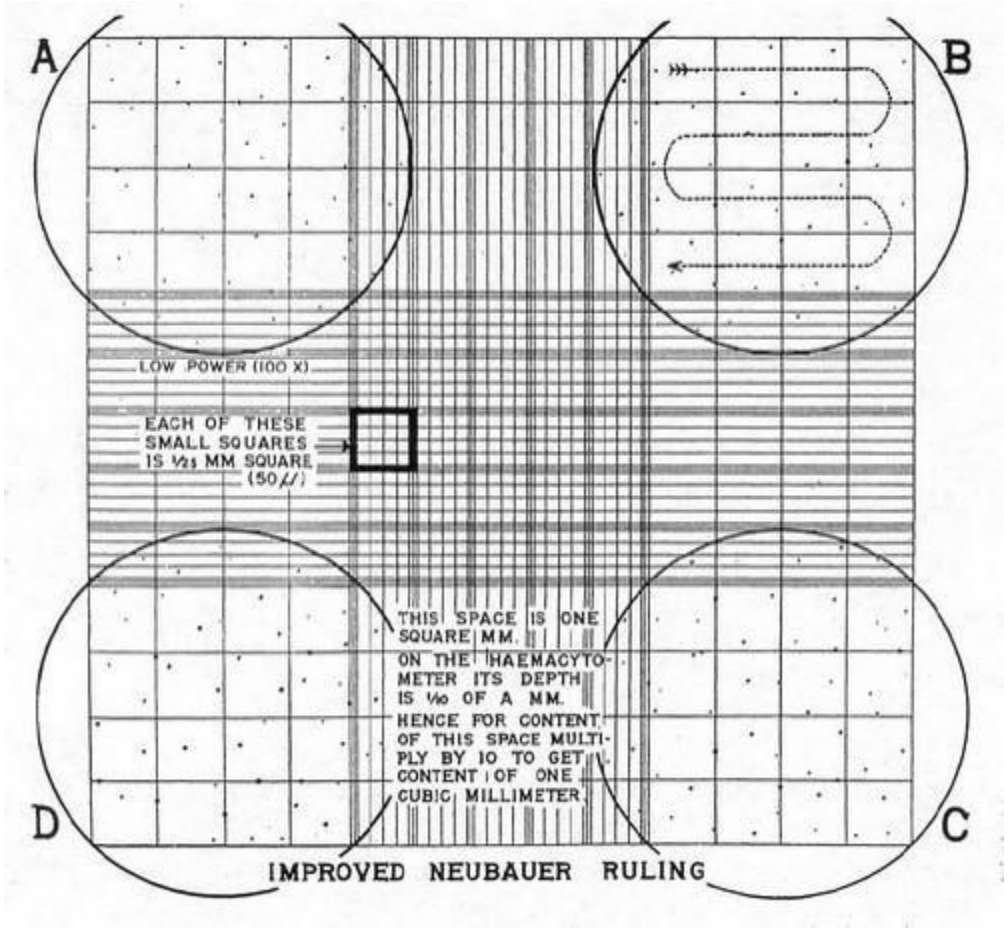
- **Procedure:**

1- Withdrawn 20 µl of blood.
2-Immediately draw diluting fluid(Turk's solution) 0.4 ml in clean tube
3- Mix the contents for 2-3 minutes to ensure even distribution of cells.

4- Prepare the chamber slide and the cover glass
5- Allow the mixture to flow under the cover glass until the chamber is completely charged.
6- Count the white cells in the four squares in corner areas.

Calculation:

WBC/mm3 = No. of cell in 4 square x 50



IMPROVED NEUBAUER RULING

In ability of blood to supply tissues with adequate oxygen for proper metabolic function, caused by decreased number of red cells, Decreased level of hemoglobin or decreased of hematocrit (packed cell volume) below the normal physiological limits.

Classification of anemia

A. Clinical Classification:

Have a variety of ways depending on parameters used.

1. According on severity: as a mild (Hb = 10-12 g/dl) moderate (Hb = 7-10 g/dl) and severe (Hb < 7 g/dl).

2. According to etiology (causes): two general forms of anemia:.

a) Absolute anemia:(decrease in blood cell mass or Hb content) due to

bone marrow defect.

b) Relative anemia: which include;

1. nutritional deficiencies (folate , iron and B12).

2. hemolytic disorders.

3. blood loss.

4. bone marrow replacement by malignancy.

5. infections and chronic disorders.

6. toxicity.

7. hemopoetic stem cells damage (aplastic anemia)

B. morphological classification:

Based on microscopic examination of blood smear and red cells indices and color of it.

1. Normocytic Normochromic anemia; the concentration of hemoglobin in normal physiological value and the size of cells in normal range but

2. microcytic hypochromic anemia: the concentration of hemoglobin

bellow the normal physiological value and the size of cells smaller than normal range.

3. microcytic normochromic anemia: the concentration of hemoglobin in

normal physiological value and the size of cells smaller than normal

range.

4. macrocytic normochromic anemia: the concentration of hemoglobin in normal physiological value and the size of cells larger than normal range

---

- **Hemolytic anemia** is the abnormal destruction (breakdown) of red blood cells either in the blood vessels (intravascular hemolysis) or (extravascular hemolysis).

Classification of hemolytic anemia

1. Genetic (hereditary) hemolytic anemias can classify according to:

- Defect in RBC membrane: e.g hereditary spherocytosis , elliptocytosis , and hereditary stomatocytosis
- Defect in RBC metabolism (enzyme defects): e.g Glucose - 6- phosphate dehydrogenase deficiency (G6PD) and Pyruvate kinase deficiency anemia
- Abnormal hemoglobin: e.g sickle cell anemia and thalassemia.

2. Acquired hemolytic anemia

A- Immune-mediated acquired hemolytic anemia , and these include:

- Autoimmune acquired hemolytic anemia e.g warm and cold antibody types
- Alloimmune hemolytic anemia e.g hemolytic disease of the newborn (HDN) , Rh disease , blood transfusion reactions.
- Drug induced immune mediated hemolytic anemia e.g Penicillin

B- Non-immune mediated hemolytic anemias

- Infections: e.g malaria.
- Drugs: some drugs and other ingested substances lead to hemolysis by direct action on RBCs e.g ribavirin.
- Toxins: e.g snake venom and plant poisons such as aesculin.
- Trauma: Mechanical such as heart valves , and extensive vascular surgery

Laboratory findings

1. CBC

A low level of Hb or HCT is a sign of anemia , MCV results may be a sign as to the cause of anemia.

2- Features of increased red cell production :

a) Reticulocyte count

The test shows whether BM is making RBCs at the correct rate. In hemolytic anemia usually have high reticulocyte count because the BM is working hard to replace the destroyed RBCs.

b) BM erythroid hyperplasia

3- Features of increased red cell breakdown :

- Serum bilirubin (unconjugated) is increase.
- Urine urobilinogen increased.
- Fecal stercobilinogen increased.
- Serum haptoglobin absent because the haptoglobins become saturated with Hb and the complex is removed by RE cells.

4- Hb electrophoresis:

This test looks at the different types of Hb in blood .It can help to diagnose the type of anemia.

5- Hemosiderinuria(increase) is a result to the presence Hb in the glomerular filtrate. It is a valuable sign of intravascular hemolysis.

Hematology

Lab 8

Hematocrit (packed cell volume) & Hemoglobin test

- Packed cell volume (PCV): is the volume percentage (%) of red blood cells in blood.

The purpose: This test often ordered when a person has signs and symptoms of a condition affecting RBCs, such as anemia and polycythemia.

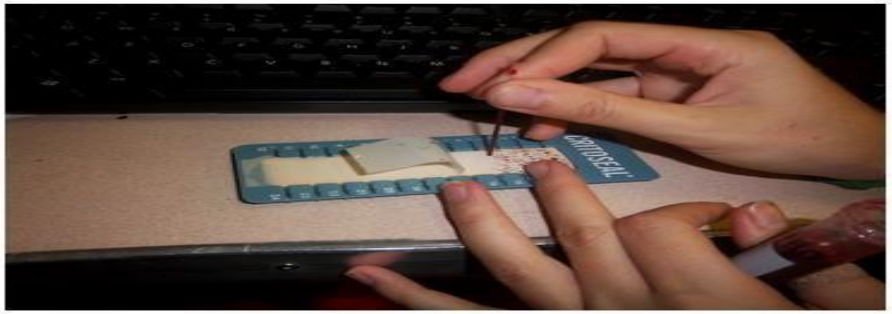
- The normal PCV is:

➢ Newborn	53-65%
➢ Child	30-43%
➢ Adult male	42-52%
➢ Adult female	37-45%

❑Procedure:

1.Puncture the skin of the finger and collect blood from the capillary directly into heparinized micro haematocrit tube; fill 3/4 of the tube.

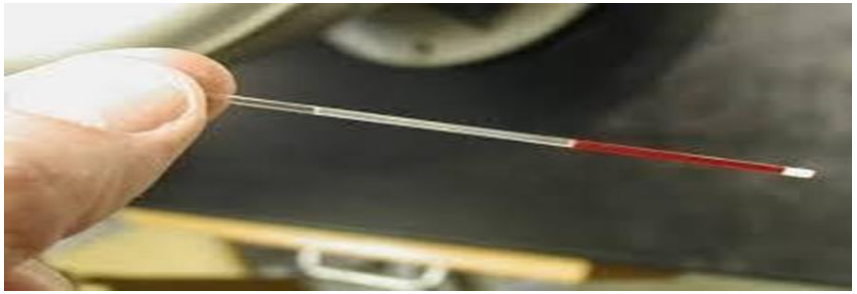
2.Closed one end of the tube with clay. Avoid trapping air between the blood and plug.



procedure:

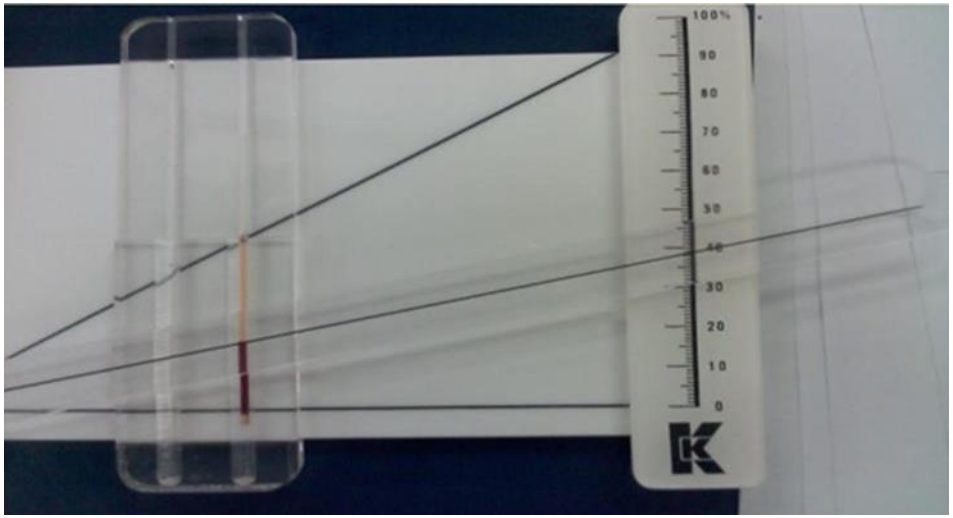
3.Place the tube into a calibrated micro haematocrit centrifuge, Close the cover. Set the timer (most instruments require 3 to 5 minutes centrifugation time).Centrifuge tube(usually at 10,000 RPM).

4.The tube should be removed and read within a minute or two after the centrifuge has stopped to avoid re-dispersion of cells.



Procedure:

5.Use a lined card, wheel or other device to determine the hematocrit value. They all work by the same principle, measuring the height of the total blood column and the height of the red cell layer.



Hematocrit (packed cell volume) & Hemoglobin test

- **Hemoglobin test depend on PCV:**

$$Hb = (PCV/3) - 1$$

Hematocrit (packed cell volume) & Hemoglobin test

When the PCV or Hb tests are used?

Depend on signs and symptoms of patient:

Symptoms such as:

- fatigue, or unexplained weight loss
- Monitoring of anemia and its cause
- Before and after major surgery
- During pregnancy
- Presence of chronic kidney disease or many other chronic medical problems.

Bleeding time: is a screening test performed by measuring how long it takes for bleeding to stop after injury.

Why the Test is performed?

This test helps diagnose bleeding problems.

Bleeding time Procedure:

- **Duke Method**

With the Duke method, the patient is pricked with a special needle or lancet, on the earlobe or fingertip(Do not squeeze the finger) after having been swabbed with alcohol. The patient then wipes the blood every 30 seconds with a filter paper. The test ceases when bleeding ceases.

Normal value:

The normal time is about 2-6 minutes.

- ## What Abnormal Results Mean:

Longer-than-normal bleeding time may be due to:

- Blood vessel defect
- Platelet aggregation defect
- Thrombocytopenia (low platelet count)

Clotting time (CT) It is the whole blood clotting time which investigate the efficiency of blood coagulation.

- **Why Get Tested?**

1- In certain liver diseases that can affect the making of blood clotting factors.

2- Before surgery.

3- If take anticoagulant medication (to check that you are taking the correct dose)

The methods of the test

1-Lee-white method

- **procedure**

1- Blood sample is collected from the vein (about 0.5 ml)

2- The blood is placed into a glass tube

3- the tube is warmed to 37 degree

4- Tilt the tube every 30 seconds interval until blood clots

- **sources of error**

-Low blood volume will give short time

-Low incubation temperature will shorten clotting

- The normal time is about 5-15 minutes.

2-Capillary tube method: (Wright's method)

1- Under sterile precautions make a sufficiently deep prick in the finger tip. Note the time when bleeding starts (start the stop watch).

2- Touch the blood drop at the finger tip using one end of the capillary tube kept tilted downwards. The tube gets easily filled by capillary action.

3- After about two minutes start snapping off small lengths of the tube, at intervals of 15 seconds, each time noting whether the fibrin thread is formed between the snapped ends. Note the time (stop the stop watch) when the fibrin thread is first seen.

4- Clotting time is the interval between the moment when bleeding

starts and the moment when the fibrin thread is first seen.

Capillary tube method:

Normal value is 3 to 10 minute

Iron deficiency is the most common nutritional deficiency worldwide, Also known as microcytic hypochromic anemia due to lack iron in the body which is necessary for hemoglobin production.

Causes of iron deficiency anemia

1. **Inadequate Iron Intake (Nutritional deficiency):** in which insufficient amount of iron is consumed to meet the normal and daily demand (e.g poor diet and imbalanced vegetarian diet)

2. **Inability to Absorb Iron** due to chronic diarrhea associated with celiac disese , Crohn disease , resection of small bowel and gastrectomy.

3. Internal Bleeding: e.g acute and chronic hemorrhages (peptic ulcers , carcinoma of stomach , colon or rectum , hematuria , bleeding hemorrhoids and worm infestation by the hook worm Ancylostoma duodenale.

4. Increased consumption of iron: e.g pregnancy , growth years of children.

Laboratory finding in IDA

- The Hb and PCV are decreased , and the red cell indices (MCV , MCH & MCHC) are also decreased , but the RDW is increased.
- Blood film examination will show hypochromic and microcytic red cells with anisocytosis.
- Reticulocyte count is normal or decreased , but increased when the patient takes iron as treatment.
- Serum iron is low while TIBC (Total iron binding capacity) is increased, because TIBC measures transferring.

- Total iron-binding capacity (TIBC) or sometimes transferrin iron binding capacity is a medical laboratory test that measures the blood's capacity to bind iron with transferrin.
- Serum ferritin is decreased, because it measures iron stores.
- Ferritin, a major iron storage protein, is essential to iron homeostasis and is involved in a wide range of physiologic and pathologic processes.

Hemoglobinopathies

Hemoglobinopathies: are genetic defects that result in abnormal structure of one of the globin chains of the hemoglobin molecule.

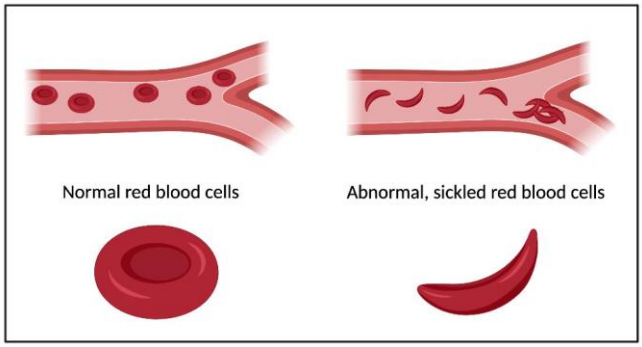
Sickle Cell Anemia

The term sickle cell disease (SCD) describes a group of inherited red blood cell disorders. People with SCD have abnormal hemoglobin, called hemoglobin S or sickle hemoglobin, in their red blood cells.

People who have SCD inherit two abnormal hemoglobin genes, one from each parent. In all forms of SCD, at least one of the two abnormal genes causes a person's body to make hemoglobin S. When a person has two hemoglobin S genes, Hemoglobin SS, the disease is called sickle cell anemia. This is the most common and often most severe kind of SCD.

Pathogenesis:

- Cells in tissues need a steady supply of oxygen to work well. Normally, hemoglobin in red blood cells takes up oxygen in the lungs and carries it to all the tissues of the body.
- Red blood cells that contain normal hemoglobin are disc shaped. This shape allows the cells to be flexible so that they can move through large and small blood vessels to deliver oxygen.
- Sickle-shaped cells are not flexible and can stick to vessel walls, causing a blockage that slows or stops the flow of blood. When this happens, oxygen can't reach nearby tissues.



Screening Tests for Sickle Cell Disease

- These tests depend on the decreased solubility of Hb S at low oxygen tensions. All sickle tests, whether positive or negative, must be confirmed by electrophoresis.
- Hemoglobin S solubility test and sodium metabisulfite test. Both tests are used to screen for hemoglobin S by adding certain chemicals to a patient's blood sample that reduce the amount of oxygen present. The reduced amount of oxygen will cause the abnormal sickle-shaped cells to form. It should not be performed on infants until they are at least 6 months old because of the presence of hemoglobin F as the predominant hemoglobin at birth.

Sickling Test

Principle:

The sickling phenomenon may be demonstrated in a thin wet film of blood sealed with petroleum jelly/paraffin wax mixture or nail varnish. If Hb S is present the red cells lose their smooth round shape and become sickled. Changes should be apparent after 30 min- 1 hour at 37◦ C.

Reagent

The reagent is 2% sodium metabisulphite. It should be freshly prepared.

Procedure

1. On a clean slide, add 1 drop of anticoagulated blood.

2. Add 2 drops of the freshly prepared reagent to the drop of blood.

3. Mix and place a cover slip on top of the slide.

4. Place the slide inside a wet petri-dish.

5. Incubate for 30 min at 37◦ C.

6. Examine under the microscope (x40)

Results

+ve: A positive result is indicated by the presence of sickled cells.

-ve: a negative result means there are no sickle cells