PRACTICAL PHYSIOLOGY

Leukocytes count (white blood cells; WBCs)

Edition by
Dr.Firas Alaasam
M.Sc Pharmacology & Toxicology

FIRST LABORATORY

Leukocytes count (white blood cells; WBCs)

White blood cells (WBCs), also called leukocytes, are an important part of the immune system. These cells help fight infections by attacking bacteria, viruses, and germs that invade the body. White blood cells originate in the bone marrow, but circulate throughout the bloodstream. There are five major types of white blood cells:

1-neutrophils

2-lymphocytes

3-eosinophils

4-monocytes

5- basophils

Leukocytes count (white blood cells; WBCs)

* A WBC count is a test that measures the number of white blood cells in your body. This test is often included with a complete blood count (CBC). Your blood contains a percentage of each type of white blood cell. Sometimes, however, your white blood

cell count can fall or rise out of the healthy range

* WBC count is reported in cell per cubic millimeter of blood.

* WBCs; all are larger than RBCs and have nuclei (one or several lobes)

* Normal value : 4,000-10,000 cells/mm3.

.

Leukocytes Disorders :

Leukopenia: is the medical term used to describe a low WBC count. Conditions or illnesses that can trigger a low number include:

1- HIV
2- autoimmune disorders
3- bone marrow disorders/damage
4- lymphoma
5- severe infections
6-liver and spleen diseases
7- lupus
8-radiation therapy

Leukocytes Disorders :

Leukocytosis : is the medical term used to describe a high WBC count. Conditions or illnesses that can trigger a high number include:

1-anemia

2-tumors in the bone marrow

3- leukemia

4-inflammatory conditions, such as arthritis and bowel disease

5- stress

6-exercise

7- tissue damage

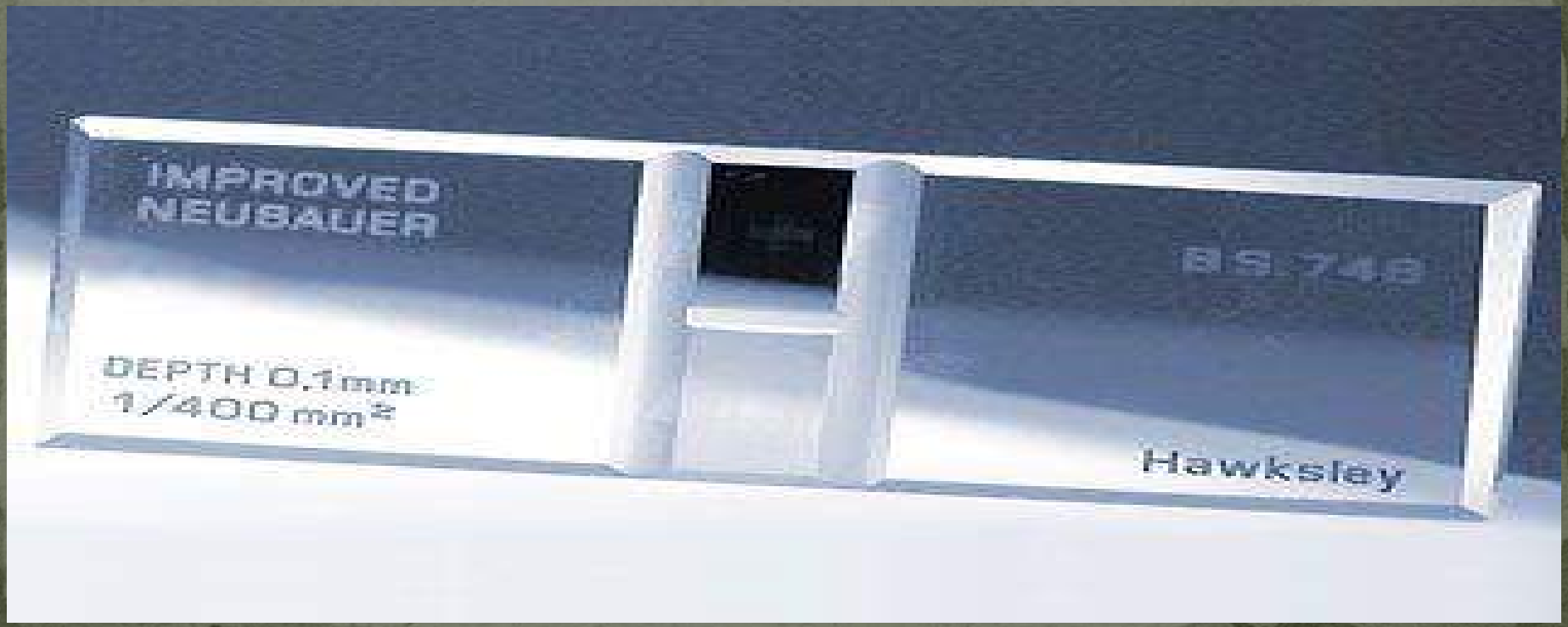
8- pregnancy

9-allergies

10-asthma

Hemocytometer :

The hemocytometer counting chamber (Neubauer) is used for cell counting. The surface of the chamber contains two ruled areas separated by an H-shaped moat.



- The four corner secondary squares are subdivided into 16 smaller squares to aid in cell counting; they are used counting.

- The center square are used when counting. The center secondary square differ from four corner a squares in that it is divided into 25 squares, each of the 25 squares is subdivided into 16 smaller squares.

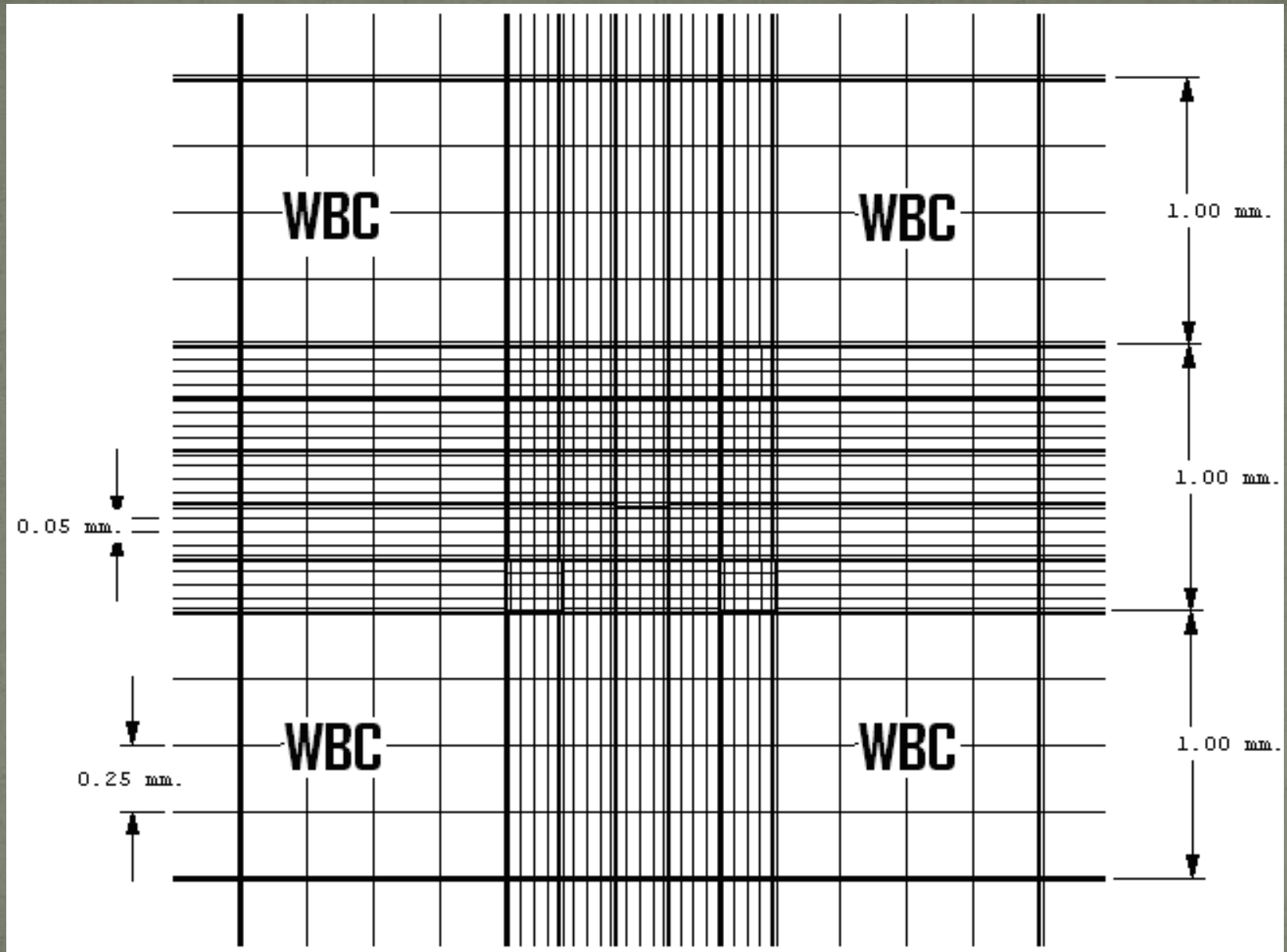


Figure 3.

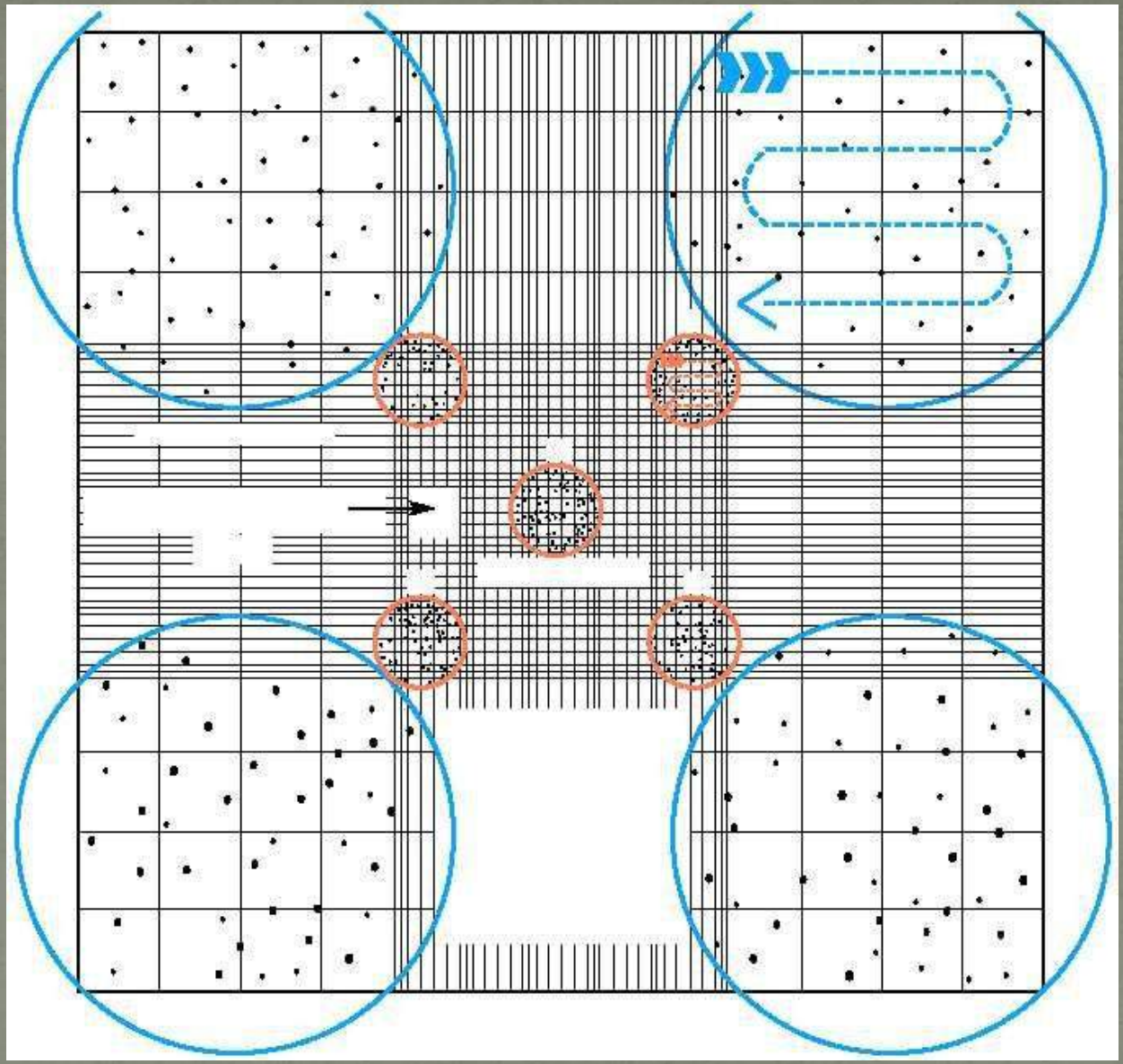
Blood Diluent for Leukocytes

- Acetic A cid (20%).....20mL.
- Methylene blue.
- Distilled water.....1L.

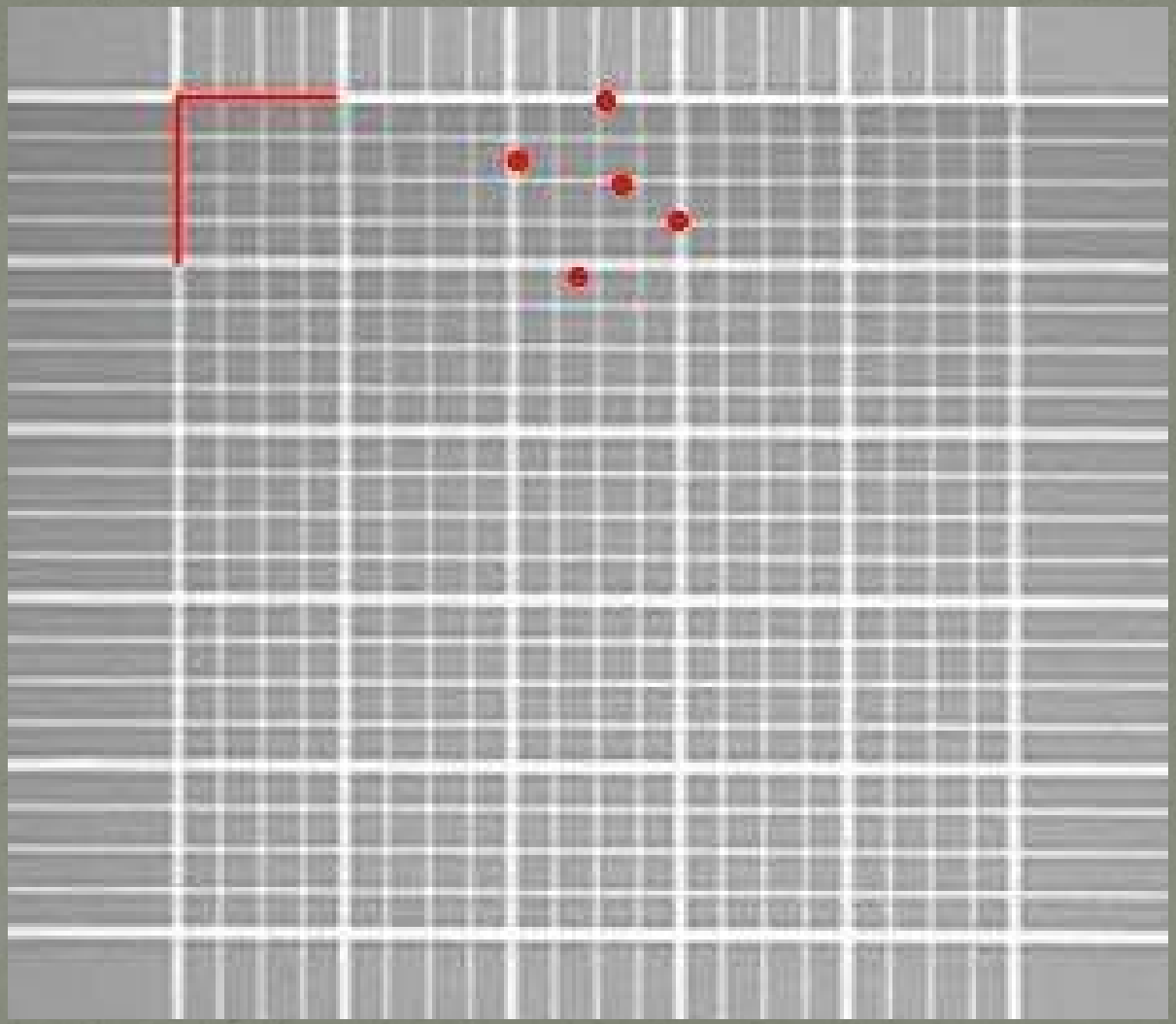
- For WBC count, This diluting fluid contains acid solution that lyses the RBC and a stain the nuclei of WBCs and allows for easy identification and counting.

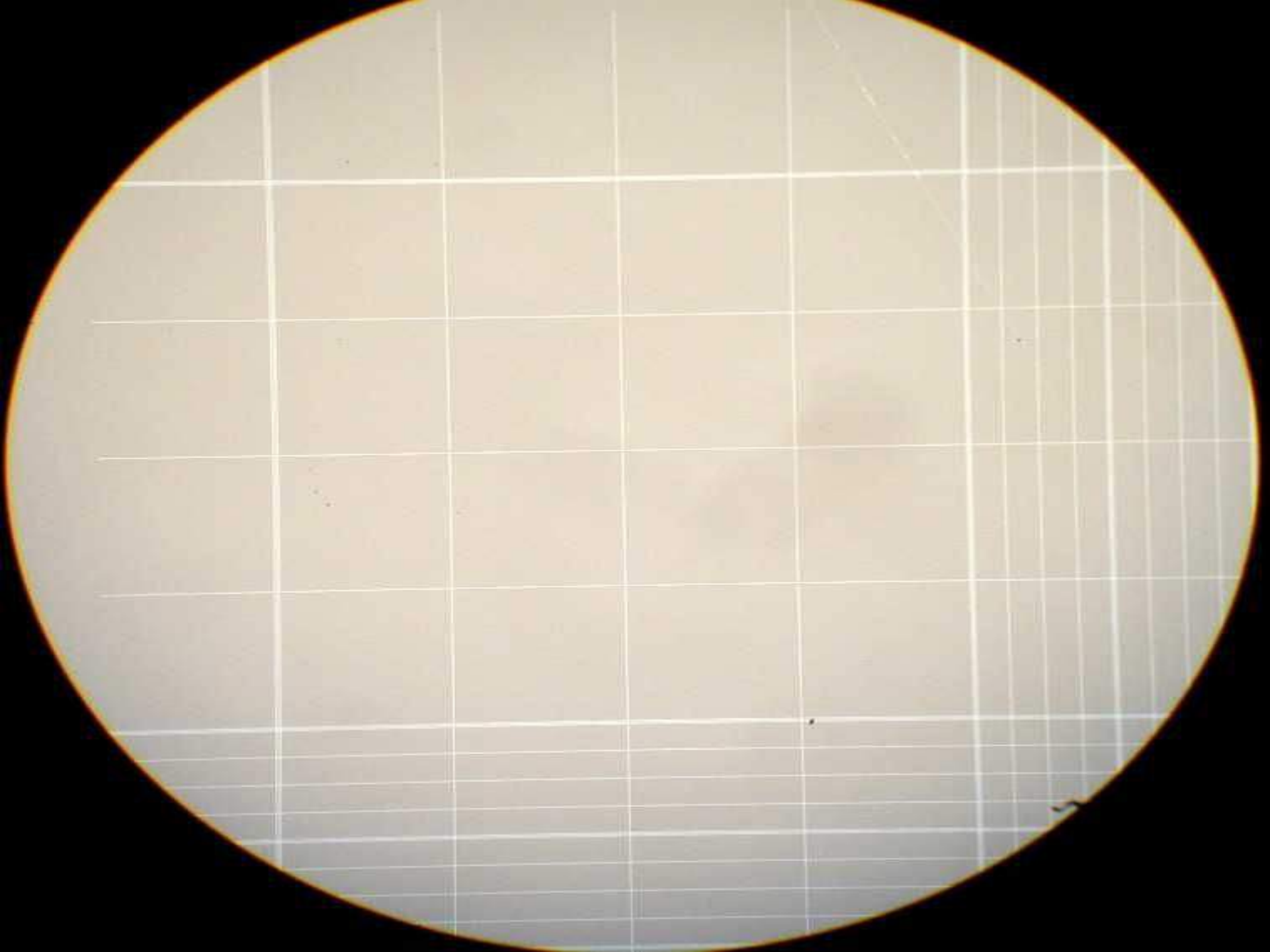
❖ Procedure for the count :

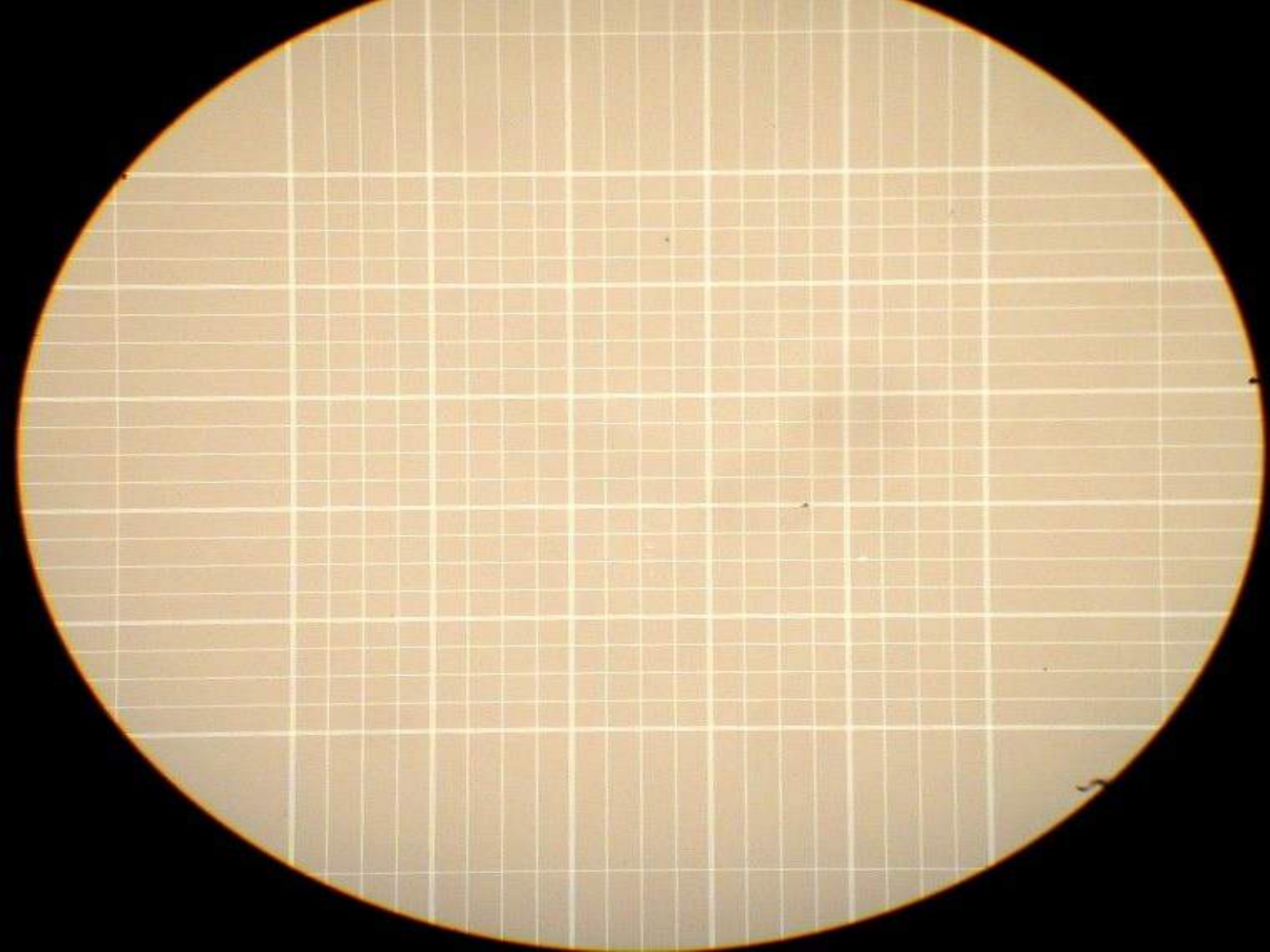
1. Cells are scanned under a 10X objective to determine the distribution.
2. Use the 40X objective to count WBC in each of the four of the corner secondary square on both sides of the chamber.
3. Count cells starting in the upper left corner square, continue counting to the right hand square, drop down to next row continue counting from the right square to the left square.
4. Count all cells that touch any of the upper and left lines, Do not count any cell that touches a lower or right line.



Procedure for the count







Methods for estimation of Leukocytes count as follows :

1) Hemocytometer.
2) Automated method.

Apparatus and Reagent :

1. Hemocytometer cosist of (pipette+ chamber slide).
2. Cover slide.
3. Cotton.
4. (Whole blood +EDTA).
5. Gauze
6. Petri dish.
7. Wood-stick.
8. Distal water.
9. Microscope.

Procedure

1. Draw blood up to (0.5) mark by RBC pipette.
- Must be clean and dry.
- In case EDTA tube must be mix before draw.
- In case draw blood direct patient let first drop.
2. Wipe tip by cotton.
3. Draw the diluting fluid up to (11) mark (dilution 1:20).
- Wipe tip by cotton.
- In (2,4) Because cotton will absorb extra blood or diluting fluid and insure from draw quantity and same time clean the tip pipette to prevent contamination.
- In (1,3) Avoid from hose (mouthpiece) not kink.

5. Pipette should be gently rotated to obtain good mixing.

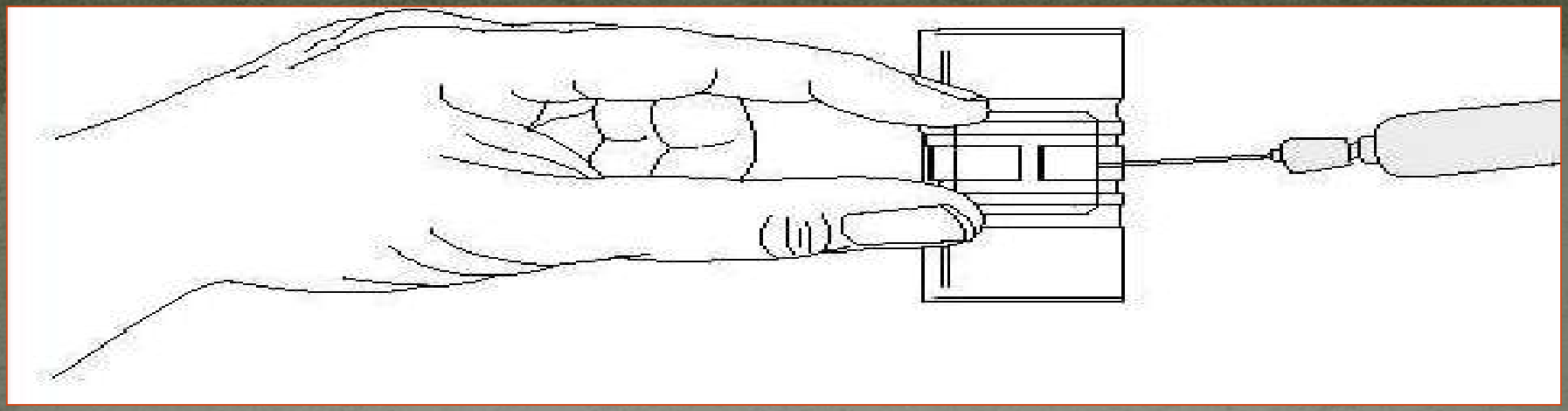
- In (5) by shake must be with the pipette end sealed with your finger.

6. Prepared of Hemocytometer .

- Must be clean and dry both (Hemocytometer and cover slide).place cover slide with some drop of water in four angle to settled above Hemocytometer .

7. Load Hemocytometer with sample.

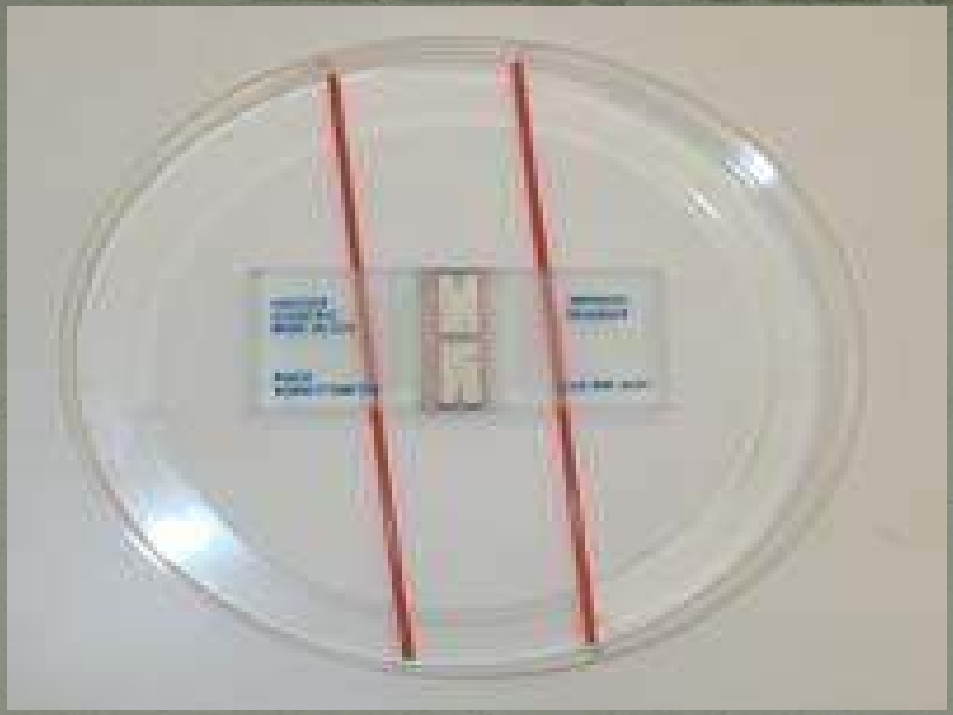
- It must be clean and dry.
- Mix the contents of pipette for 3 minute.
- Expel about (6 drop) or 1/2 pipette stem.
- To expel pipette stem content that did not mix between blood and fluid.
- By holding pipette at an angle (45 degree) and touching the space between cover slide and Hemocytometer than add drops of mixture is allowed to run under cover slide by capillary action.
- avoid air bubbled.
- it should flow in to fill. (Do not over fill).



8. Let the preparation sit for a minute.

- for cells to settle to are count easy.
- Through period mute be washing RBC pipette and mouthpiece with soap and water, finish with distilled water rinse, to prevent from clotting blood and error during next tests.

9. Examine under microscope.
10. In case let sample without examine place in Petri dish contain on little of moist gauze to prevent dry sample.



11. Wash the hemocytometer, with soap and water, finish with distilled water rinse.

2. Automated method :

- The impedance method (coulter-method) counts and sizes cells by detecting and measuring changes in electrical impedance when a practice in a conductive liquid passes through a small aperture. Each cell passing through the aperture- there is a constant DC current flowing between the external and internal electrodes - causes some change in the impedance of the conductive blood cell suspension. these changes are recorded as increases in the voltage between the electrode. the number of pulses is proportional to the number of particles.
- the intensity of each pulse is proportional to the volume of that particle. the volume distribution of the cells are displayed on diagrams :WBC, RBC, and PLT histograms.

PRACTICAL PHYSIOLOGY

ERYTHROCYTE SEDIMENTATION RATE (ESR); SEDIMENTATION RATE (SED RATE).

EDITION BY
DR.FIRAS ALAASAM
M.SC PHARMACOLOGY & TOXICOLOGY

Lab 4

E.S.R OVERVIEW

- Erythrocyte Sedimentation Rate (ESR) ;Sedimentation Rate (Sed Rate).

- (ESR) is a nonspecific measurement used to detect and monitor an inflammatory response to tissue injury (an acute phase) in which there is a change in the plasma concentration of several proteins (termed acute phase proteins).
- ESR : is distance that erythrocytes fall per unit of time in specific column.

- Reported is millimeter/ hour (mm/h; hr)

Principle of E.S.R

- Anticoagulant is added to the blood and allowed to stand in specific tube in vertical form.
- Red corpuscles slowly sediment to the bottom of the tube leaving clear plasma as the supernatant.
- The rate of sedimentation under standard conditions and specific period is know as ESR.

Factors affecting the ESR

A- Effect of plasma protein:

Increased in the concentration of fibrinogen and Immunoglobulin's due to tissue injury will increase rouleaux formation and hence the rate of sedimentation. plasma albumin retards sedimentation of RBCs .

B- The RBC size and number:

- The size and number of RBCs that show alterations in their bioconcavity, like spherocyte and sickle cells, usually do not exhibit increase rate, unless there is severe anemia.
- Increase red cell mass will retard the sedimentation rate e.g. polycythemia.

Factors affecting the ESR

C- *Technical factors*

- Perpendicularity of sedimentation tube, slight deviations from the absolute. Vertical can increase the result.

- Temperature (RT 18-25 C) higher temperature cause false high results due to reduction in plasma viscosity

- Vibration can reduce the ESR.

METHODS OF ESTIMATION FOR E.S.R

Wintrobe method

- Wintrobe tube :
- Length (110 mm).
- Diameter (3.0 mm).
- Graduated from zero (top) to 100 (bottom).
- For children.

Westergren method

- Westergren tube :
- Length (300 mm).
- Diameter (2.5 mm).
- Graduated from zero (top) to 200 (bottom).
- All.

Normal Values by Westergren's Method

1. Men: 0-15 mm/h.
2. Women: 0-20 mm/h.
3. Children: 0-10 mm/h.

- Over age 50 years: (0-30 mm/h).
- Increase by (5-10 mm/h) in persons older than 50 years of age.

SOME STATUS WHICH INCREASE E.S.R:

- Microcytes (small size RBC, B12 deficiency).
- Rheumatoid arthritis.
- Tuberculosis.
- Anemia.
- Autoimmune & inflammation disease.
- Pregnancy.
- Increased level of plasma protein.

- Technical factors: high room temperature.

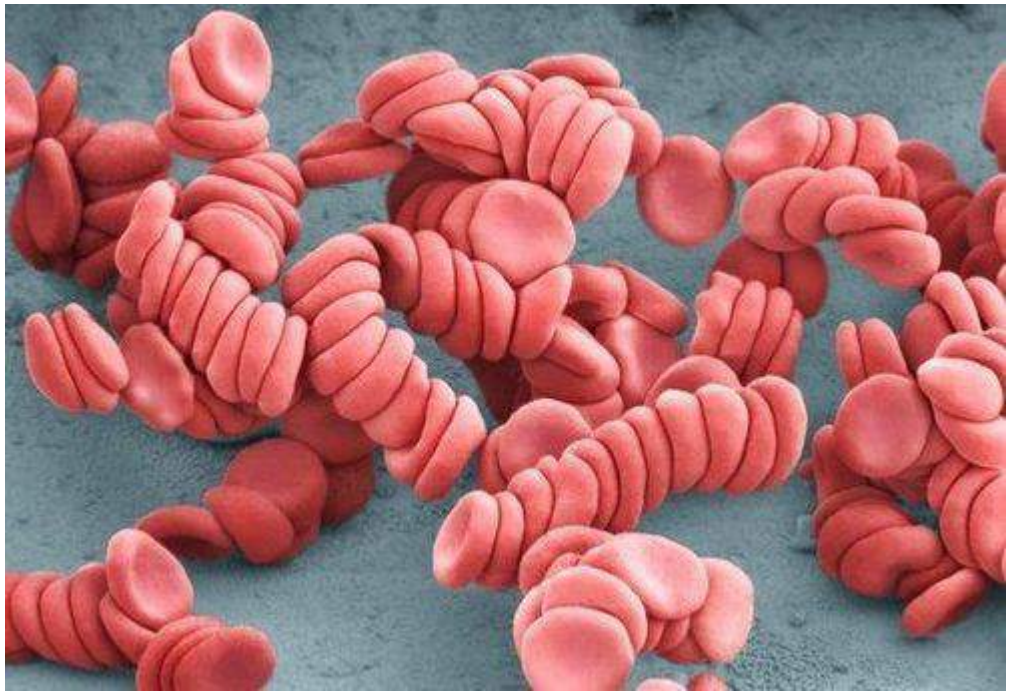
SOME STATUS WHICH DECREASE E.S.R:

- Macrocytes (large size RBC, iron deficiency).
- Sickle cells anemia.
- Polycythemia.

- Technical factors: low room temperature, clotted blood Sample, excess anticoagulant, bubbles in tube, and time.

ROULEAUX

- The stacking up of RBC, caused by extra or abnormal protein in blood that decrease the normal distance RBC between each other.

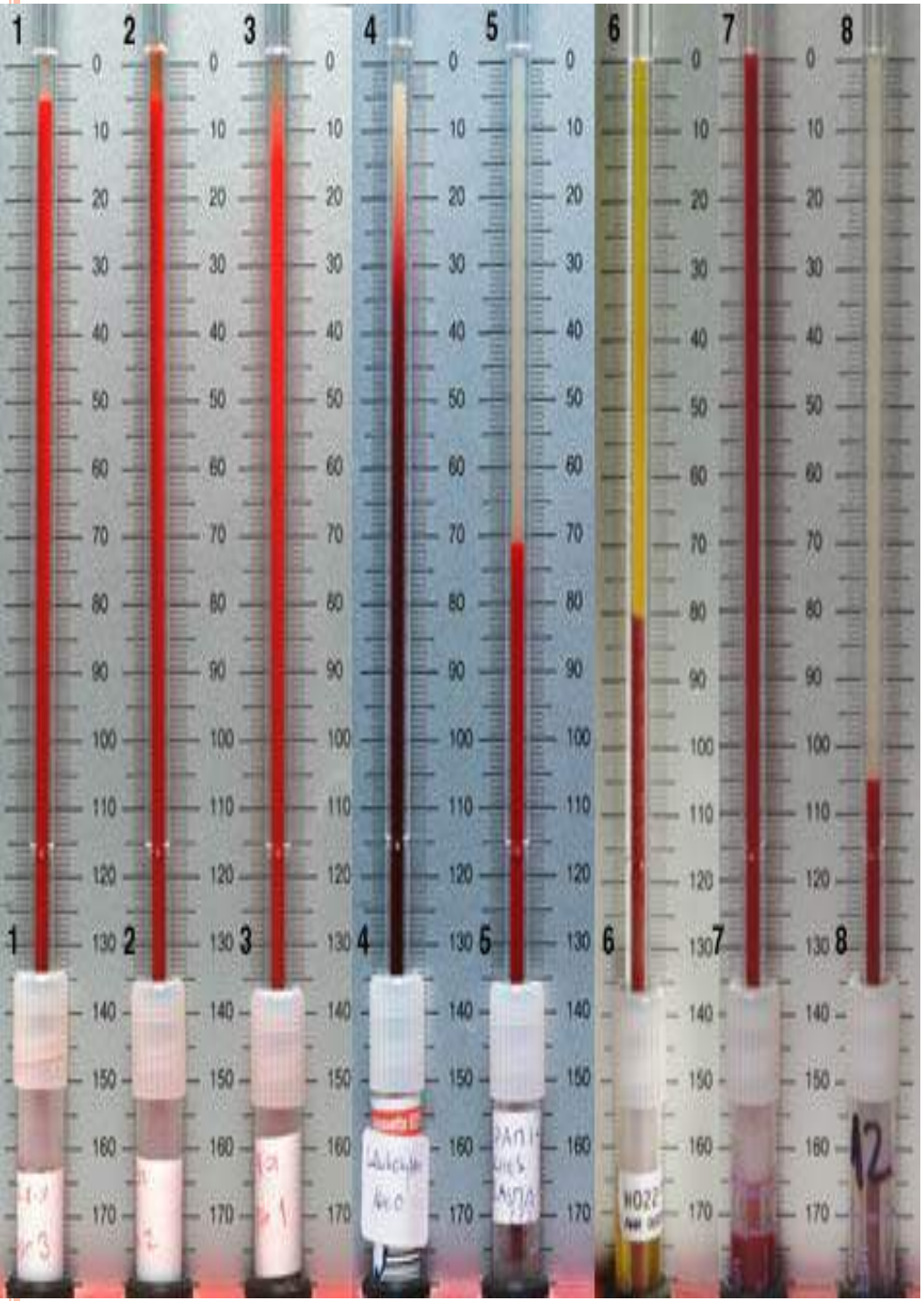


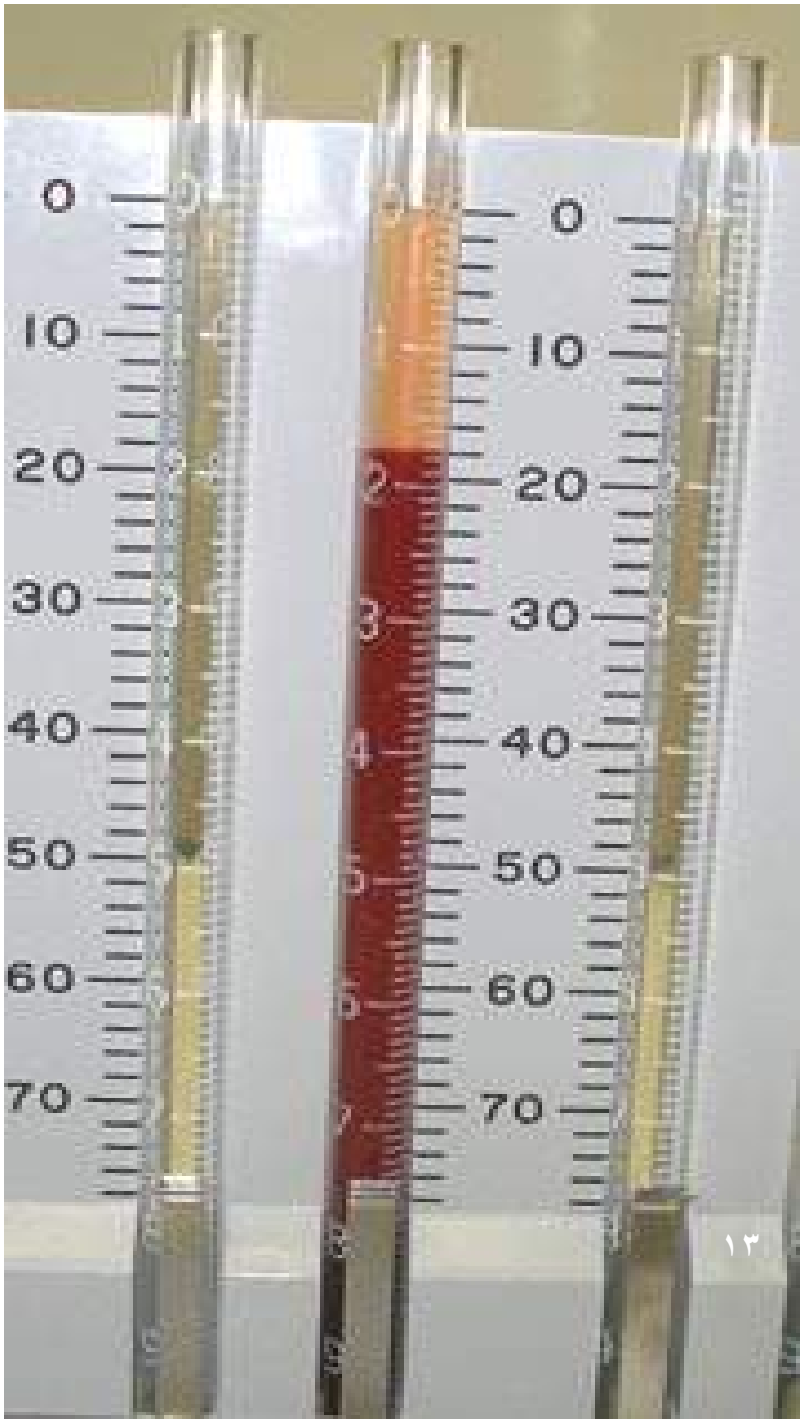
Apparatus and reagent for E.S.R

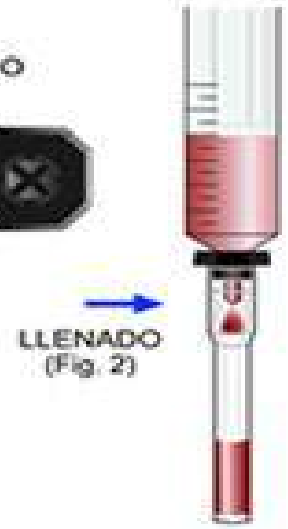
1. Blood samples.
2. Anticoagulant (dilute fluid).
- **Tri-sodium citrate (23 g dissolve 1 L D.W).**
3. Wintrobe or wastergren tube.
4. ESR rack.
5. Pipette.
6. Cotton.

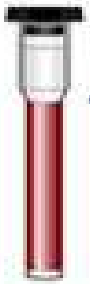
PROCEDURE OF E.S.R

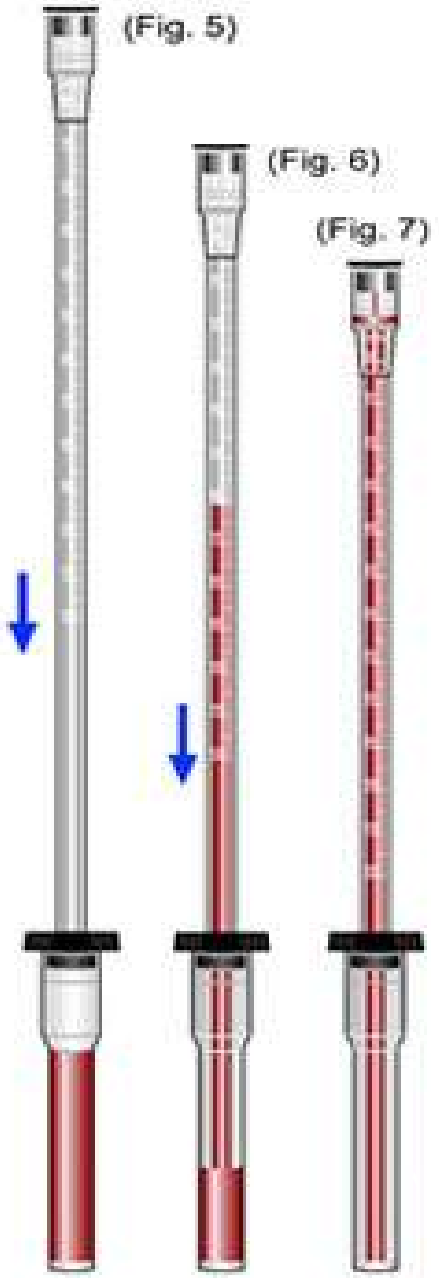
- Patient must fasting at least 4 hours before testing.
- The blood sample must be mixed with anticoagulant agent in this test.
- Put 0.4 ml sodium citrate + 1.6 ml blood . OR put 0.2 ml sodium citrate + 0.8 ml blood . (1:4)
- Mix gently with out shaking then put in the graded tube and leave it stand vertically on the stand for 1 hour.
- Read the amount of plasma that appeared without moving it then leave it to the second hour and read another time.



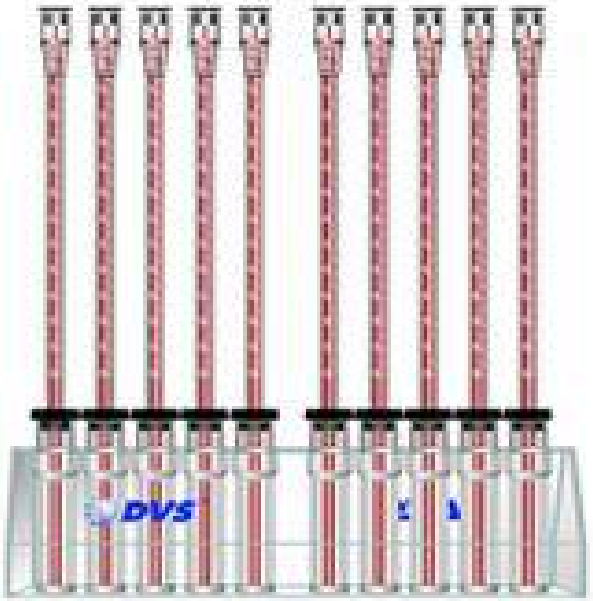








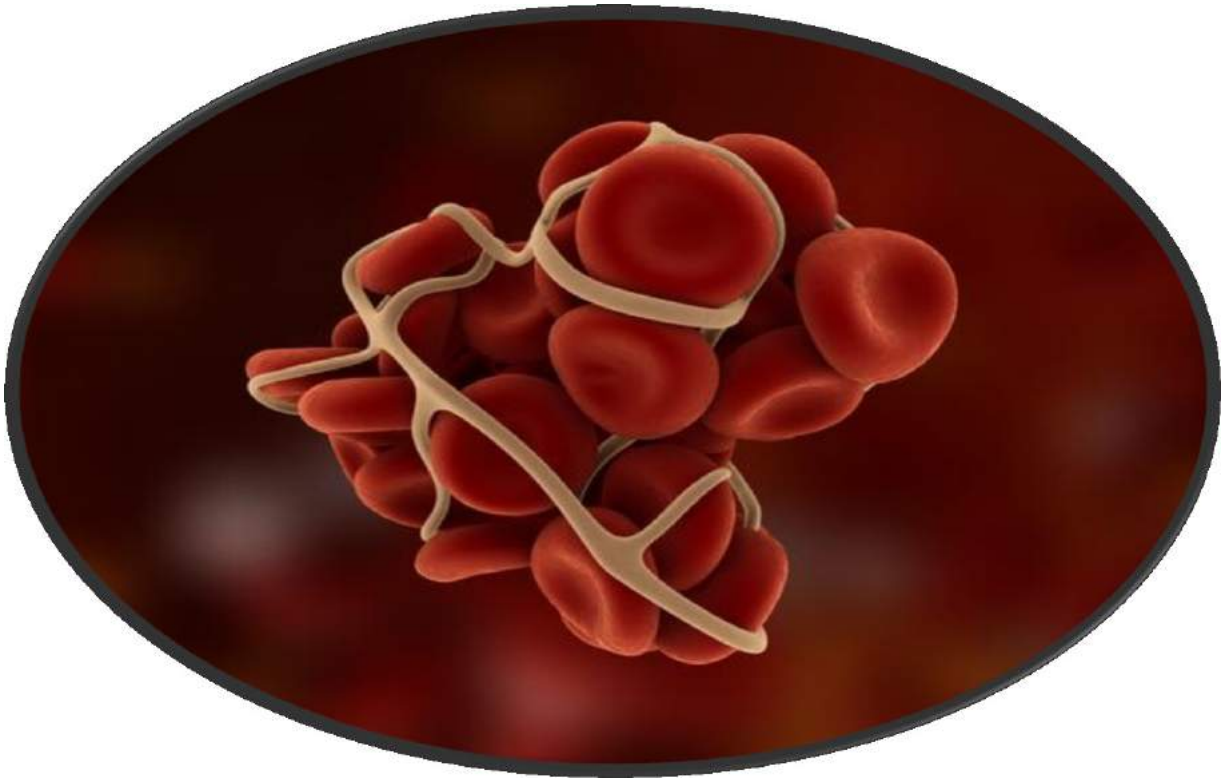
SOPORTE (fig. 8)



Thank You

PRACTICAL PHYSIOLOGY

BLEEDING AND CLOTTING TIME



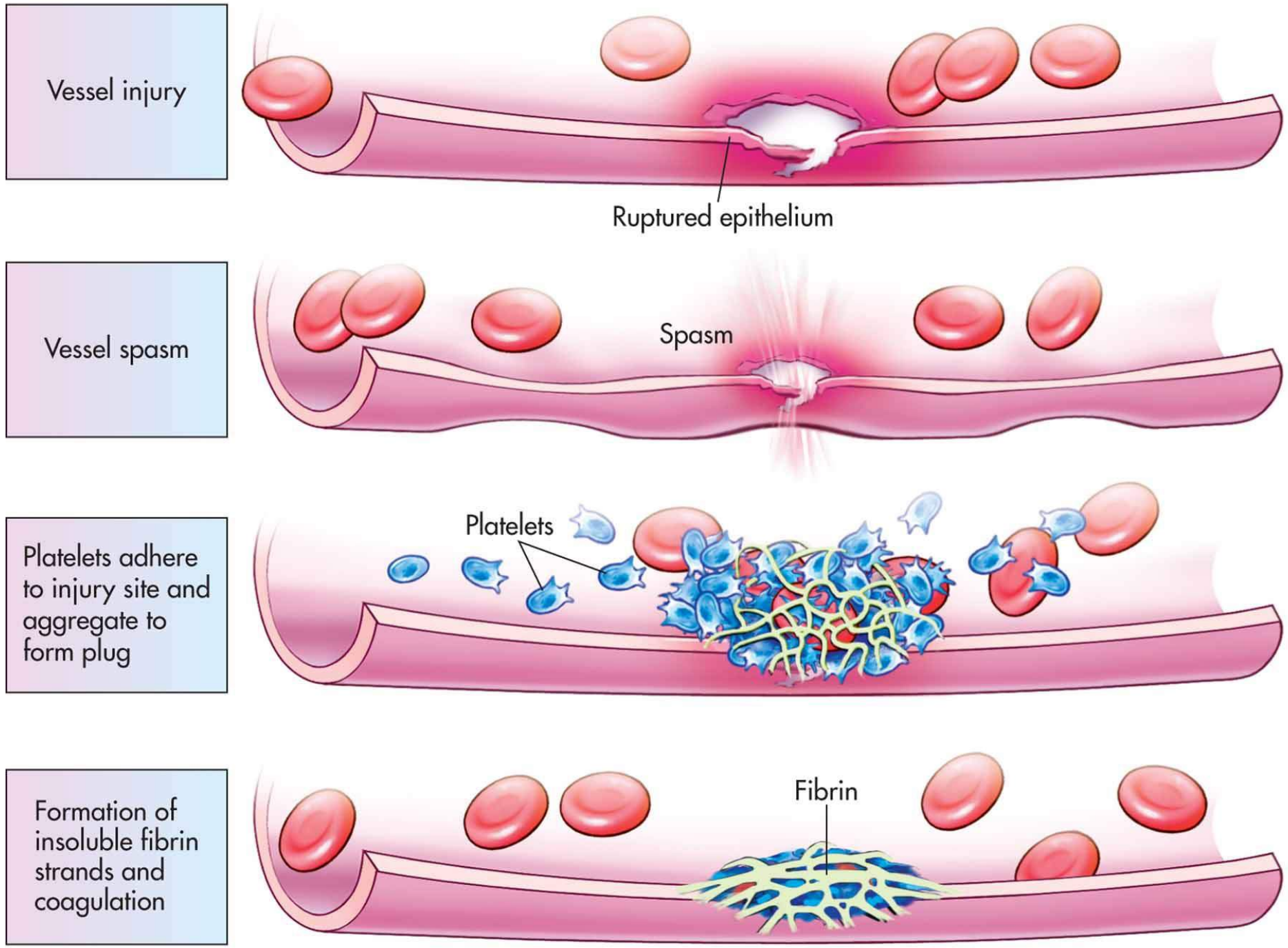
Seventh LABORATORY

Bleeding and clotting time

- **Bleeding :** means loss of blood from damaged or injured small vessels.
- **Bleeding time :** is the time taken from the onset of wound until bleeding cease.
- **Hemostasis :** is the process or mechanism of prevent of blood loss from the injured vessel.
- **This process has three main events :**

1. Contraction of blood vessel.
2. Aggregation of platelets.
3. Formation of clot.

- This test is detecting abnormality or deficiency in function of platelet.


Vessel injury
Ruptured epithelium
Vessel spasm
Spasm
Platelets adhere to injury site and aggregate to form plug
Platelets
Formation of insoluble fibrin strands and coagulation
Fibrin

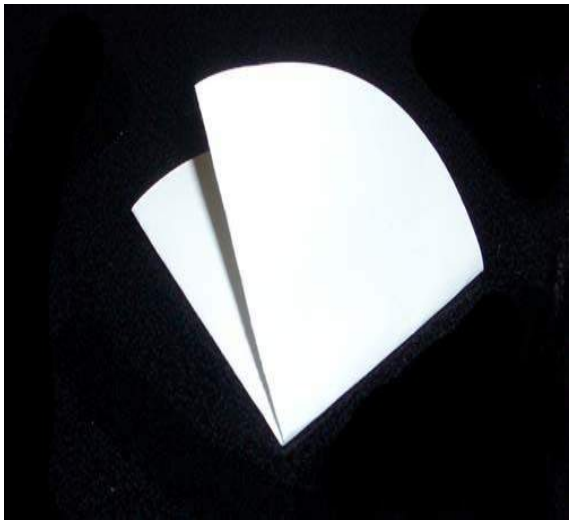
Type test

- Defect in the blood vessels by **Hesses test**
- Defect in the number of platelet by **Count test**
- Defect in the blood vessels or platelet function by **Bleeding time (Duke, Ivey method)**
- Defect in the clotting factors by **Clotting time (Capillary tube, Lee and White method)**

BLEEDING TIME

Apparatus and Reagent

- Alcohol.
- Cotton.
- Sterile disposable lancet.
- Stopwatch.
- Filter paper.



Procedure : Bleeding Time

- Clean the lobe of the ear or tip of finger with alcohol and let dry.
- Puncture of ear lobe or finger tip using lancet.
- For ear lobe, glass slide is place behind the ear lobe.
- While for finger tip, filter paper is using.
- Start the stopwatch at the moment of the puncture.
- Blot the blood with filter paper every 30 second move the filter paper after each drop of blood touches into clean area.
- When filter paper no longer shows signs of blood.
- Stop the stopwatch and record the time.
- Normal value is (1-5 minutes).

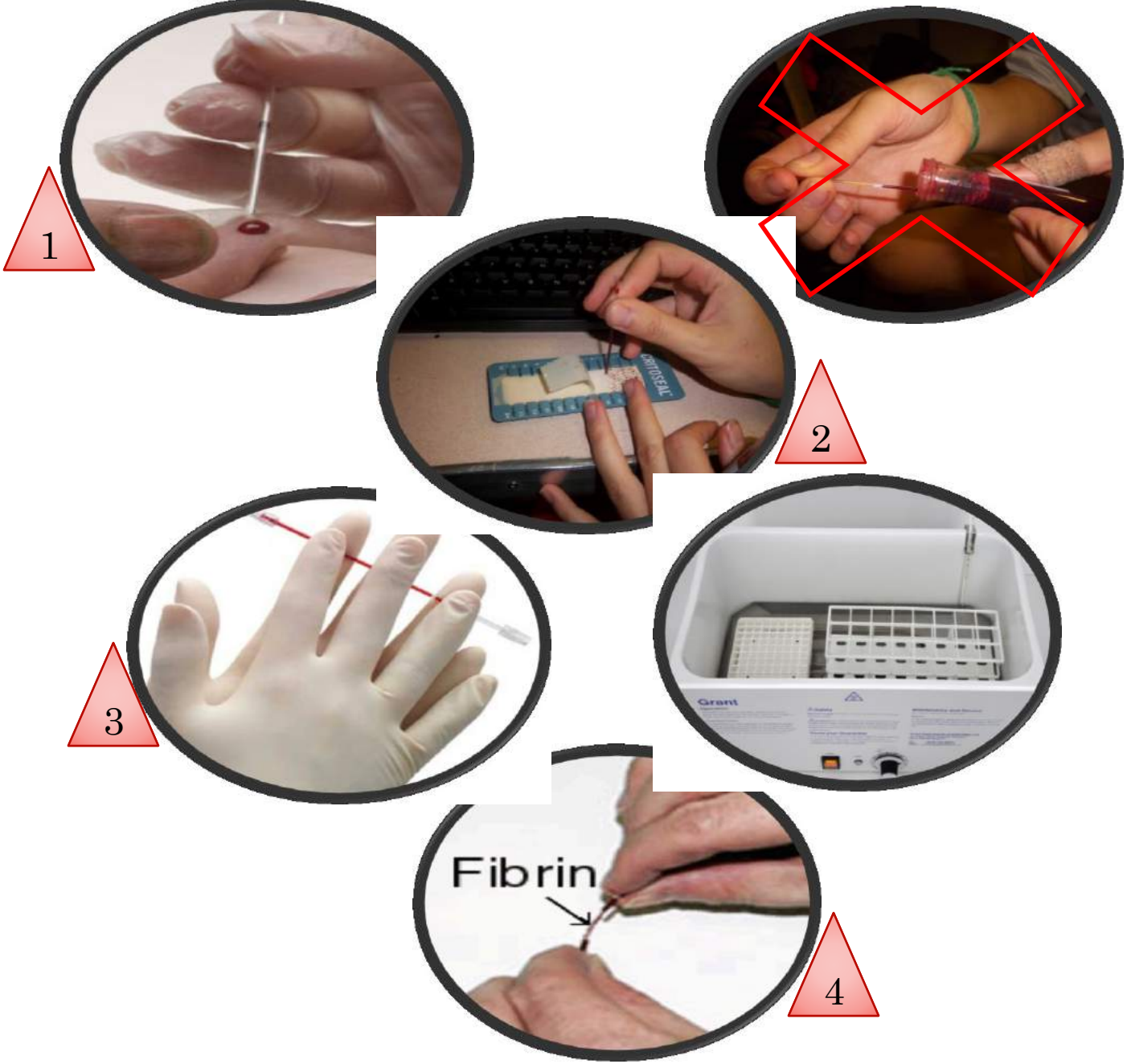
Clotting time

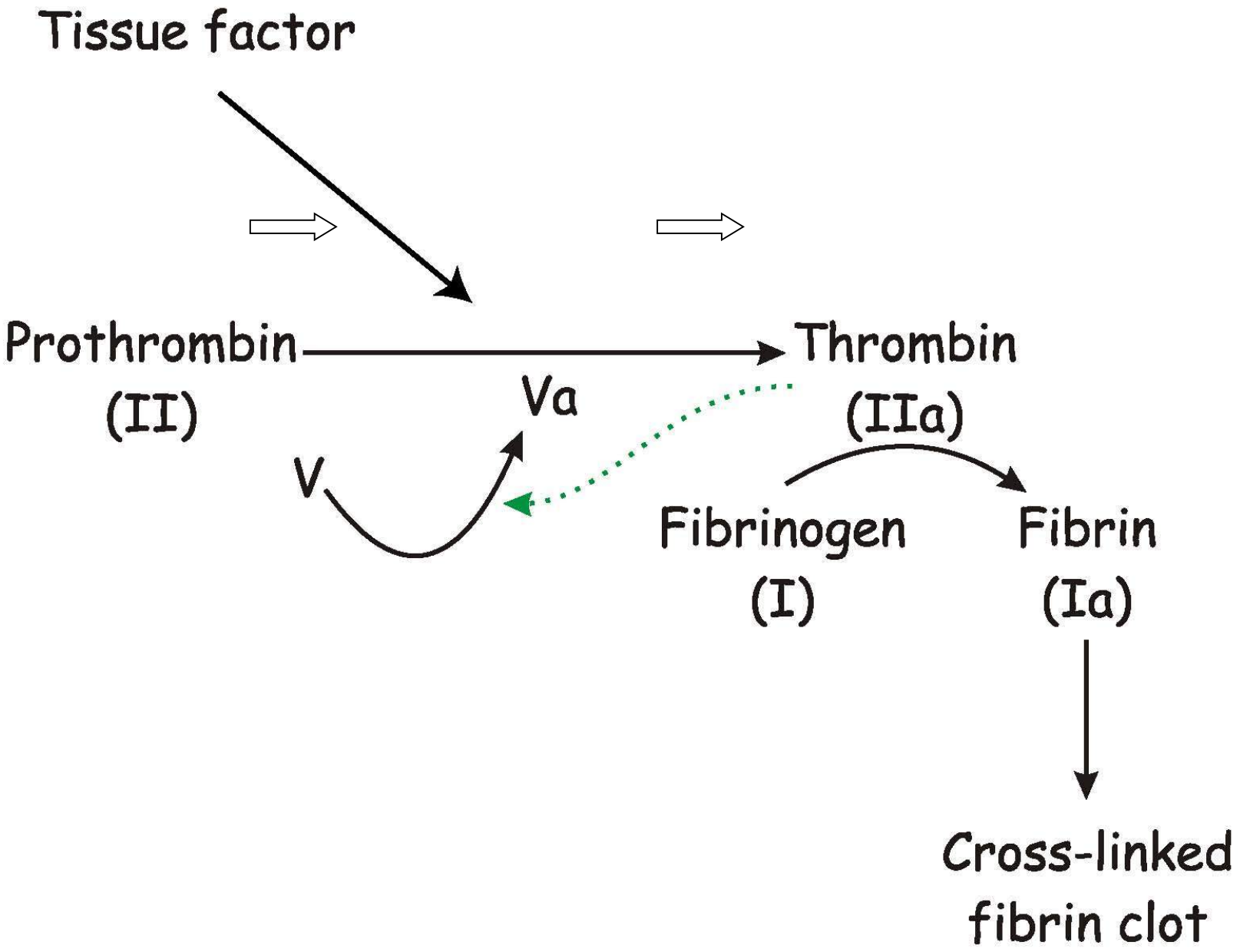
Apparatus and Reagent

- Alcohol.
- Cotto.
- Sterile disposable lancet.
- Stopwatch.
- Capillary tube (without heparin, have blue ring).
- Water bath 37 c°.
- Plasticine clay.

Procedure : Clotting time

1. Clean tip of finger with alcohol and let dry.
2. Puncture of finger tip using lancet.
3. Squeeze the finger to obtain a large drop of blood and fill the capillary tube with blood.
4. The capillary tube are sealed plasticine clay and immersed in water bath at 37 c°.
5. After one minute start breaking small pieces of the capillary tube every 30 second slowly and gently ;until a fibrin thread is seen between the two broken end.
6. Normal value is (5-10 minutes).


1
2
3
4
Fibrin


Tissue factor
Prothrombin
(II)
Thrombin
(IIa)
Va
V
Fibrinogen
(I)
Fibrin
(Ia)
Cross-linked
fibrin clot

PRACTICAL PHYSIOLOGY

Bleeding and clotting time

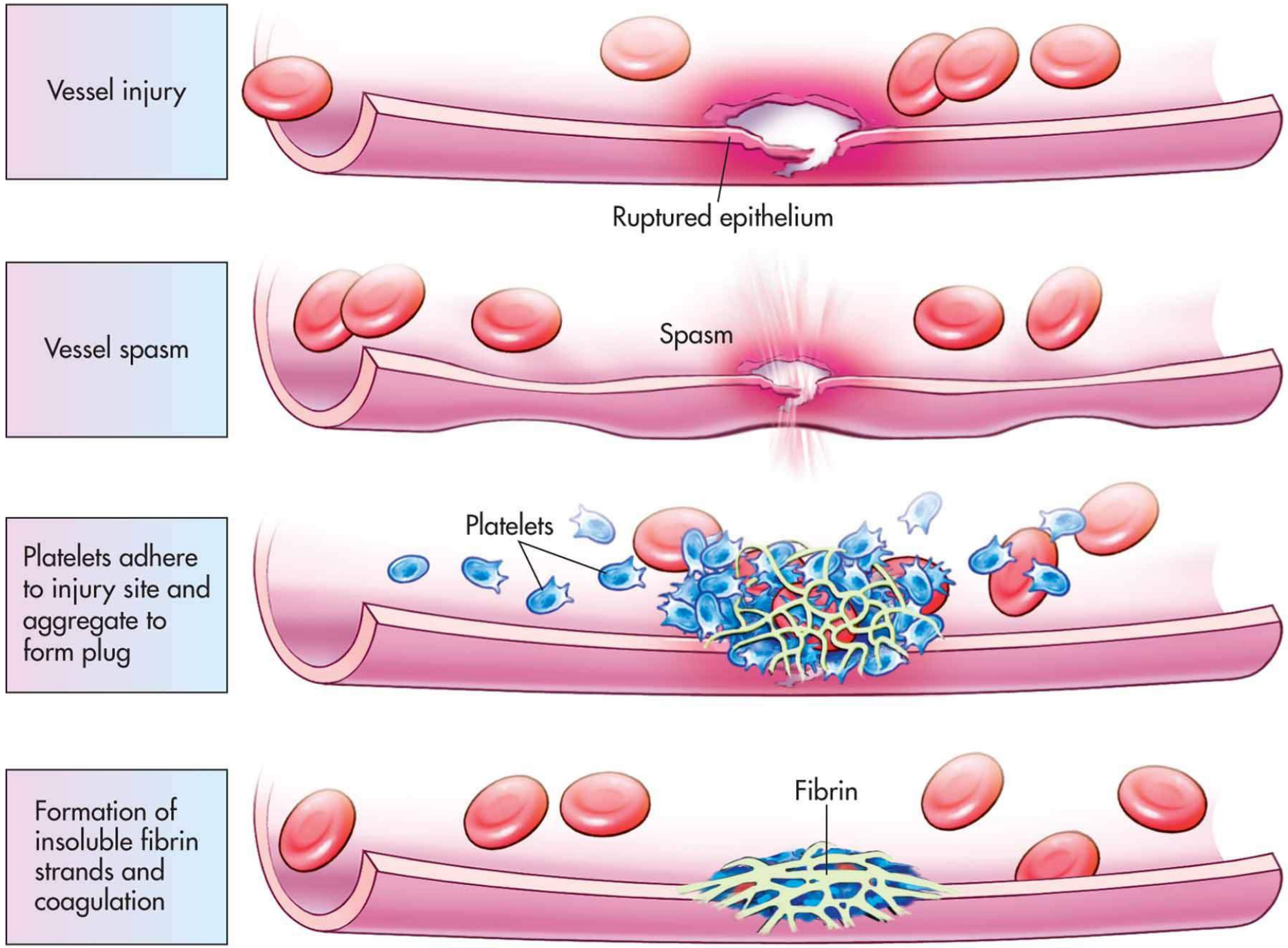
Edition by
Dr.Firas Alaasam
M.Sc Pharmacology & Toxicology

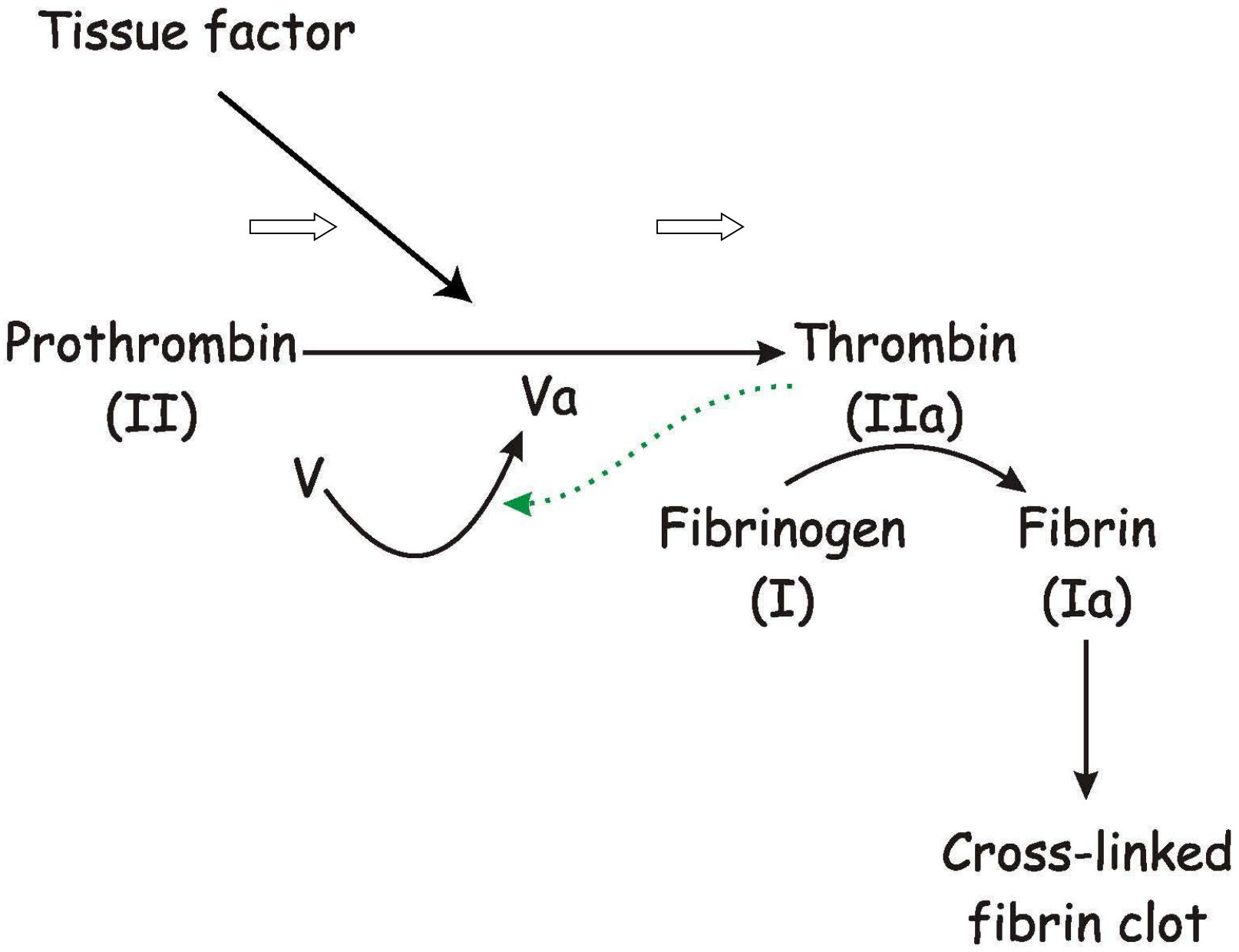
Bleeding and clotting time

- ***Bleeding*** **:** means loss of blood from damaged or injured small vessels.
- ***Bleeding time*** **:** is the time taken from the onset of wound until bleeding cease.
- **Clotting time** is the time required for a sample of blood to coagulate under standard conditions
- ***Hemostasis*** **:** is the process or mechanism of prevent of blood loss from the injured vessel.
- ***This process has three main events*** **:**

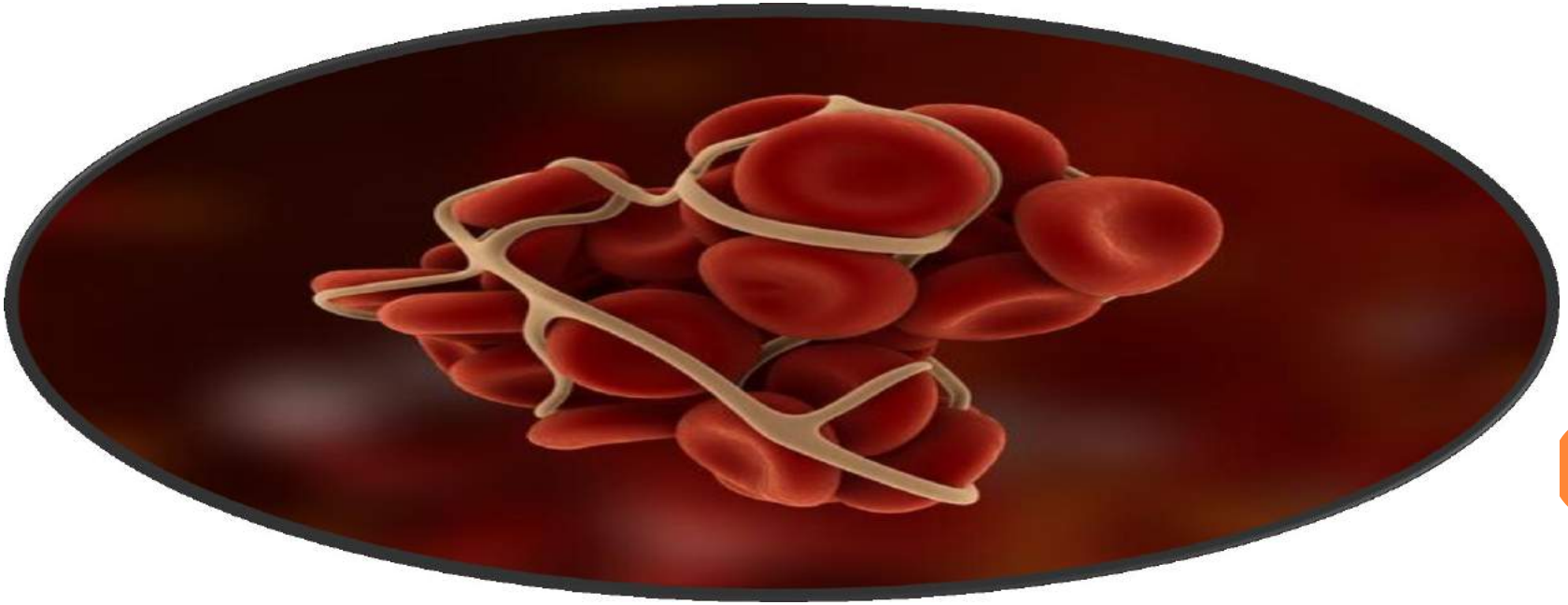
1. Contraction of blood vessel.
2. Aggregation of platelets.
3. Formation of clot.

- This test is detecting abnormality or deficiency in function of platelet.


Vessel injury
Ruptured epithelium
Vessel spasm
Spasm
Platelets adhere to injury site and aggregate to form plug
Platelets
Formation of insoluble fibrin strands and coagulation
Fibrin


Tissue factor
Prothrombin
(II)
Thrombin
(IIa)
Va
V
Fibrinogen
(I)
Fibrin
(Ia)
Cross-linked
fibrin clot

. The bleeding time can be abnormal when the platelet count is low or the platelets are dysfunctional. Causes of abnormal bleeding time can be hereditary or acquired



Hereditary causes of abnormal bleeding time are as follows:

1- Willebrand disease

2-Connective-tissue diseases

hereditary hemorrhagic telangiectasia

Acquired causes of abnormal bleeding time are as follows:

-Medications (aspirin, nonsteroidal anti-inflammatory drugs [NSAIDs], antibiotics [penicillin, cephalosporins], anticoagulants [eg, heparin, streptokinase], tricyclic antidepressants, antipsychotics, theophylline)

-Vitamin C deficiencya

-Alcohol intoxication

-Uremia

-Liver failure

-Leukemias

- Bleeding normally stops within 1-9 minutes but may be longer in children (1-13 minutes) and tends to take slightly longer in females than in males

- General interpretations of bleeding time are as follows:

-1-9 minutes: Normal

-9-15 minutes: Platelet dysfunction

-More than 15 minutes: Critical; test must be discontinued and pressure should be applied

- The patient should not take aspirin, NSAIDs, or alcohol for 7 days prior to the test, since they will prolong the bleeding time and lead to false-positive results

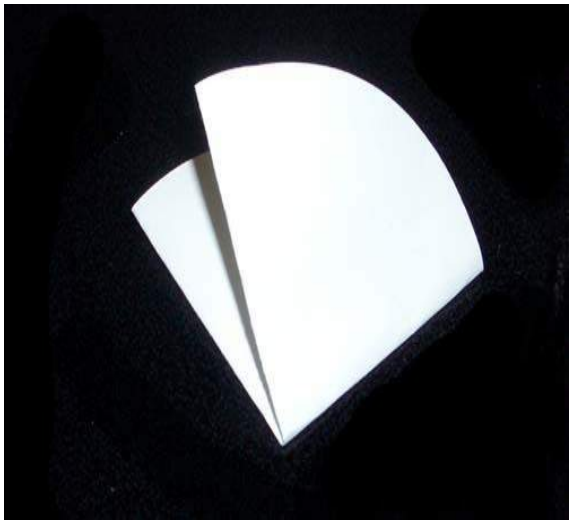
Type test

- Defect in the blood vessels by **Hesses test**
- Defect in the number of platelet by **Count test**
- Defect in the blood vessels or platelet function by **Bleeding time (Duke, Ivey method)**
- Defect in the clotting factors by **Clotting time (Capillary tube, Lee and White method)**

BLEEDING TIME

Apparatus and Reagent

- Alcohol.
- Cotton.
- Sterile disposable lancet.
- Stopwatch.
- Filter paper.



Procedure : Bleeding Time

- Clean the lobe of the ear or tip of finger with alcohol and let dry.

- Puncture of ear lobe or finger tip using lancet.
- For ear lobe, glass slide is place behind the ear lobe.
- While for finger tip, filter paper is using.
- Start the stopwatch at the moment of the puncture.
- Blot the blood with filter paper every 30 second move the filter paper after each drop of blood touches into clean area.
- When filter paper no longer shows signs of blood.
- Stop the stopwatch and record the time.
- Normal value is (1-9 minutes).

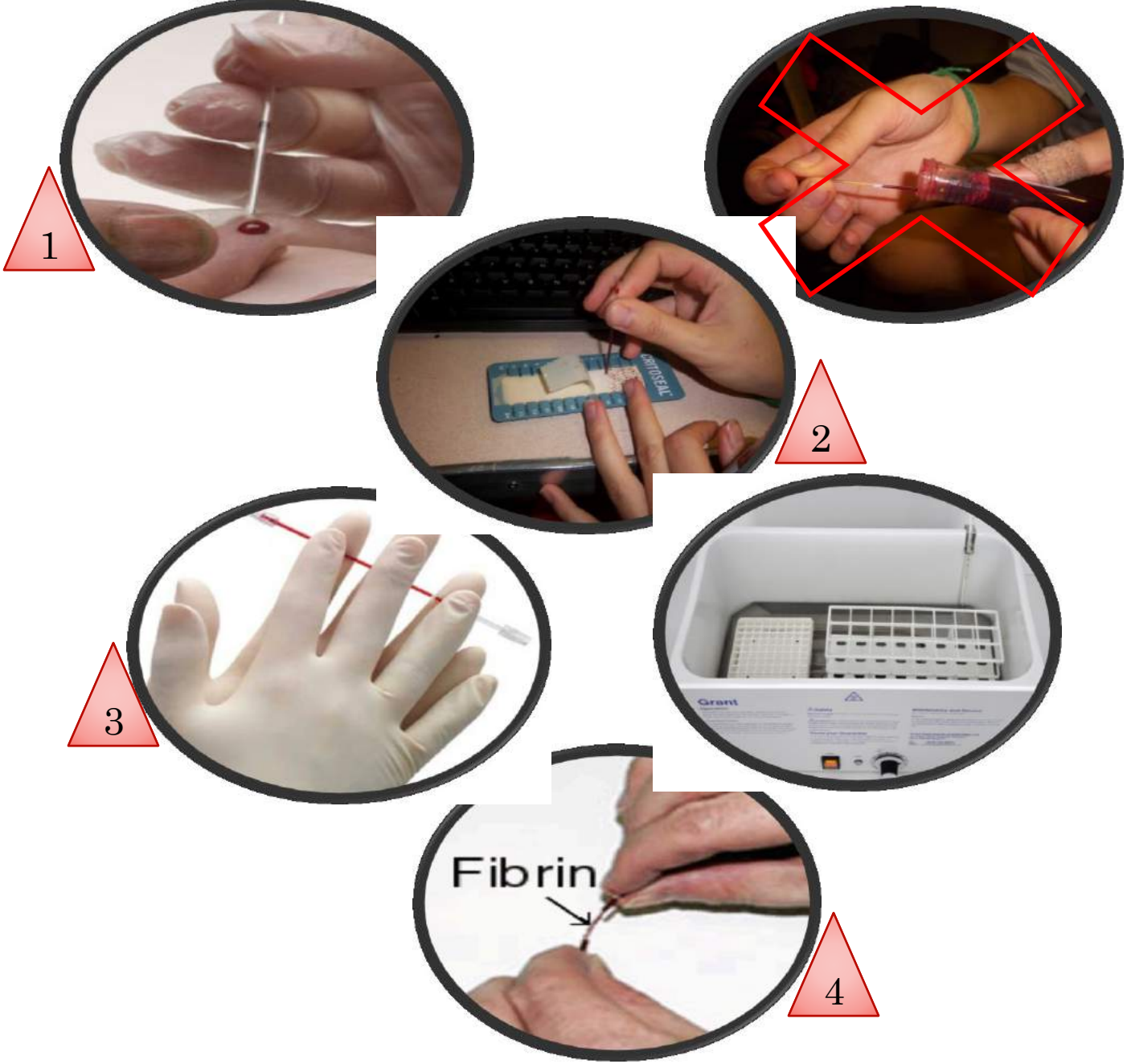
Clotting time

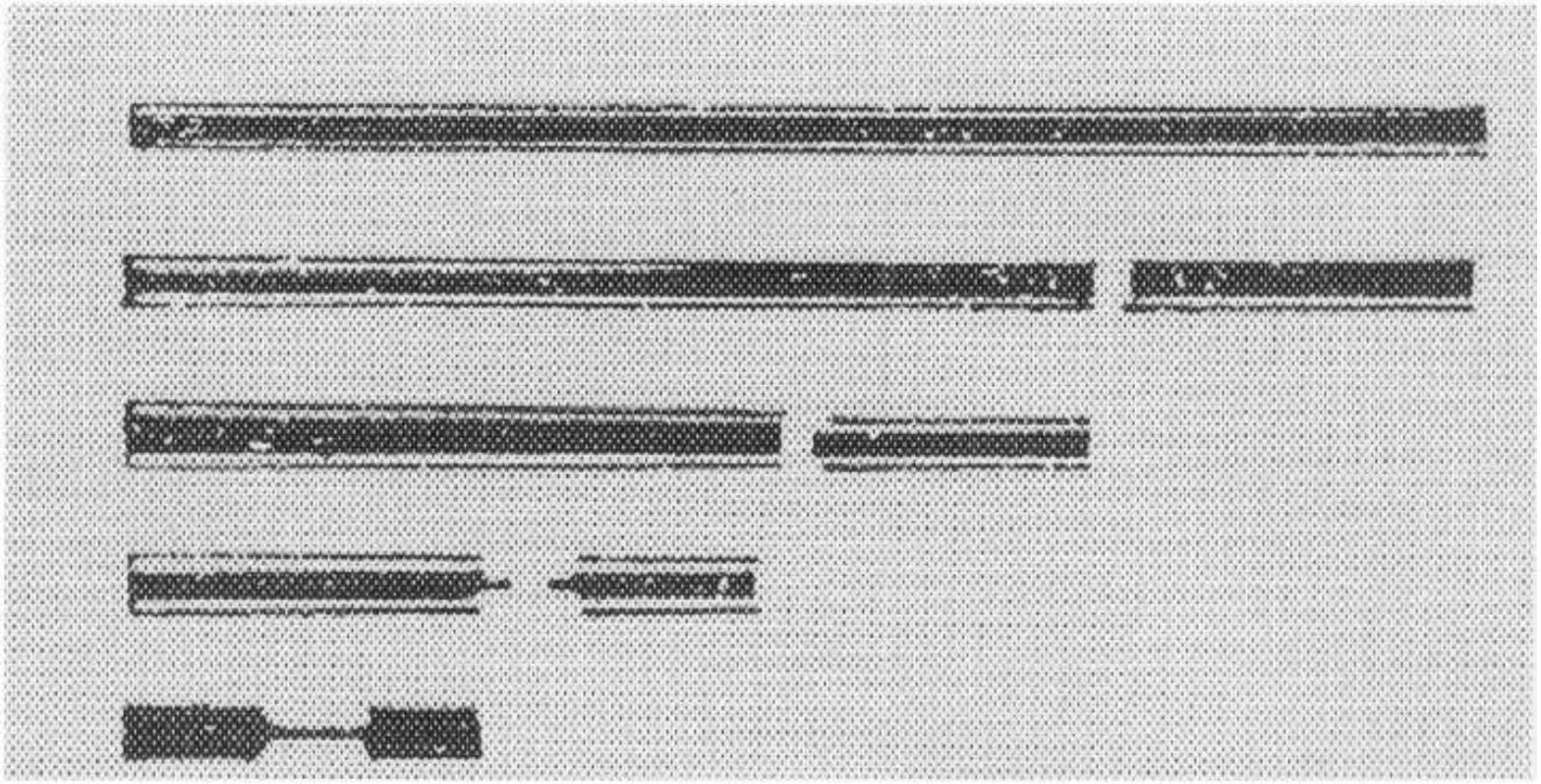
Apparatus and Reagent

- Alcohol.
- Cotto.
- Sterile disposable lancet.
- Stopwatch.
- Capillary tube (without heparin, have blue ring).
- Water bath 37 c°.
- Plasticine clay.

Procedure : Clotting time

1. Clean tip of finger with alcohol and let dry.
2. Puncture of finger tip using lancet.
3. Squeeze the finger to obtain a large drop of blood and fill the capillary tube with blood.
4. The capillary tube are sealed plasticine clay and immersed in water bath at 37 c°.
5. After one minute start breaking small pieces of the capillary tube every 30 second slowly and gently ;until a fibrin thread is seen between the two broken end.
6. Normal value is (5-10 minutes).


1
2
3
Grant
Fibrin
4



PRACTICAL PHYSIOLOGY

BLOOD GROUP

Edition by
Dr.Firas Alaasam
M.Sc Pharmacology & Toxicology

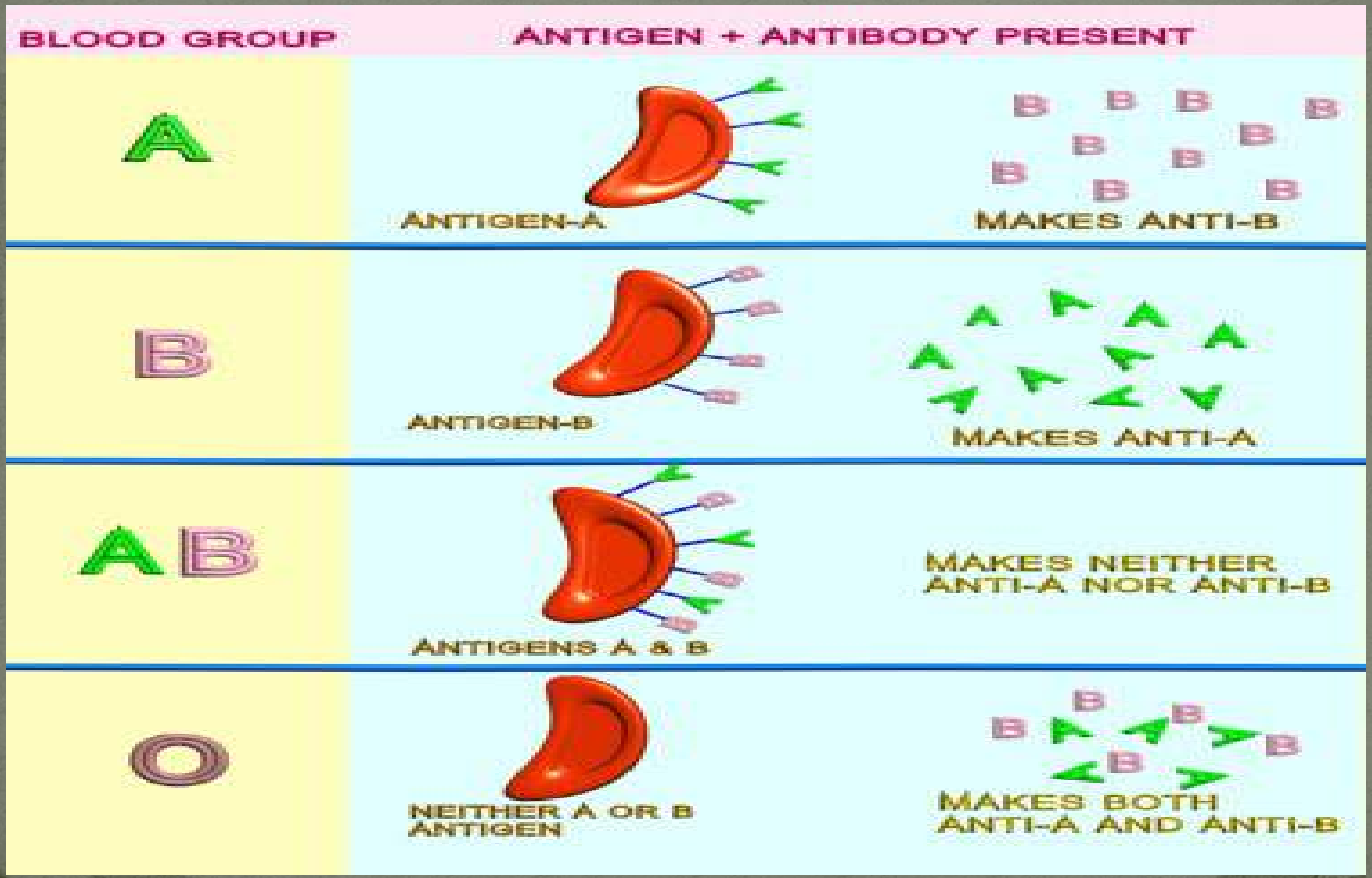
❖ *blood group*

Blood type (also called a blood group) is a classification of blood based on the presence or absence of inherited antigenic substances on the surface of red blood cells (RBCs). These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Some of these antigens are also present on the surface of other types of cells of various tissues. Several of these red blood cell surface antigens can stem from one allele (or very closely linked genes) and collectively form a blood group system.

❖TYPE OF BLOOD GROUP

- **Type A:** blood has RBCs with surface antigen A only AND your plasma contains anti-B antibodies. reported is A
- **Type B:** blood has RBCs with surface antigen B only AND your plasma contains anti-A antibodies. reported is B
- **Type AB:** blood has RBCs with both A and B surface antigens AND your plasma has neither anti-A nor anti-B antibodies. reported is AB
- **Type O** : blood has RBCs lacking both A and B surface antigens AND your plasma contains both anti-A and anti-B antibodies. reported is O

❖TYPE OF BLOOD GROUP



❖TYPE OF BLOOD GROUP

Blood group	Antigen(s) present on the red blood cells	Antibodies present in the serum
A	A antigen	Anti-B
B	B antigen	Anti-A
AB	A antigen and B antigen	None
O	None	Anti-A and Anti-B

RH SYSTEM

-Depends on the presence AND absence of another antigen on the surface of RBC called Rhesus(RH).

-**The term Rh positive (Rh+)** indicates the presence of the Rh surface antigen.

-The absence of this antigen is indicated as **Rh negative**

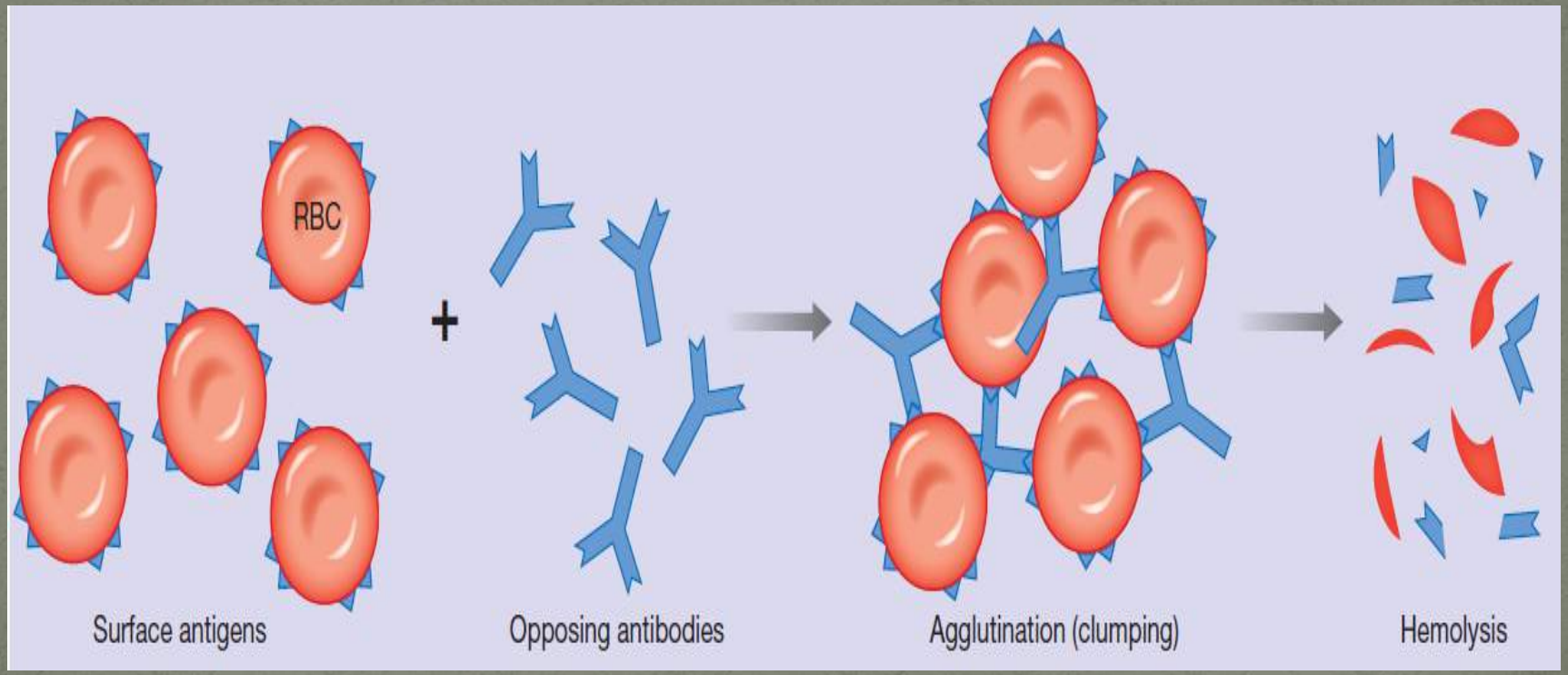
(Rh–).

- the term Rh is usually omitted, and a positive or negative sign is use

- General form blood group reported is (A+,B+,AB+,O+,A-,B-,AB-,O-).

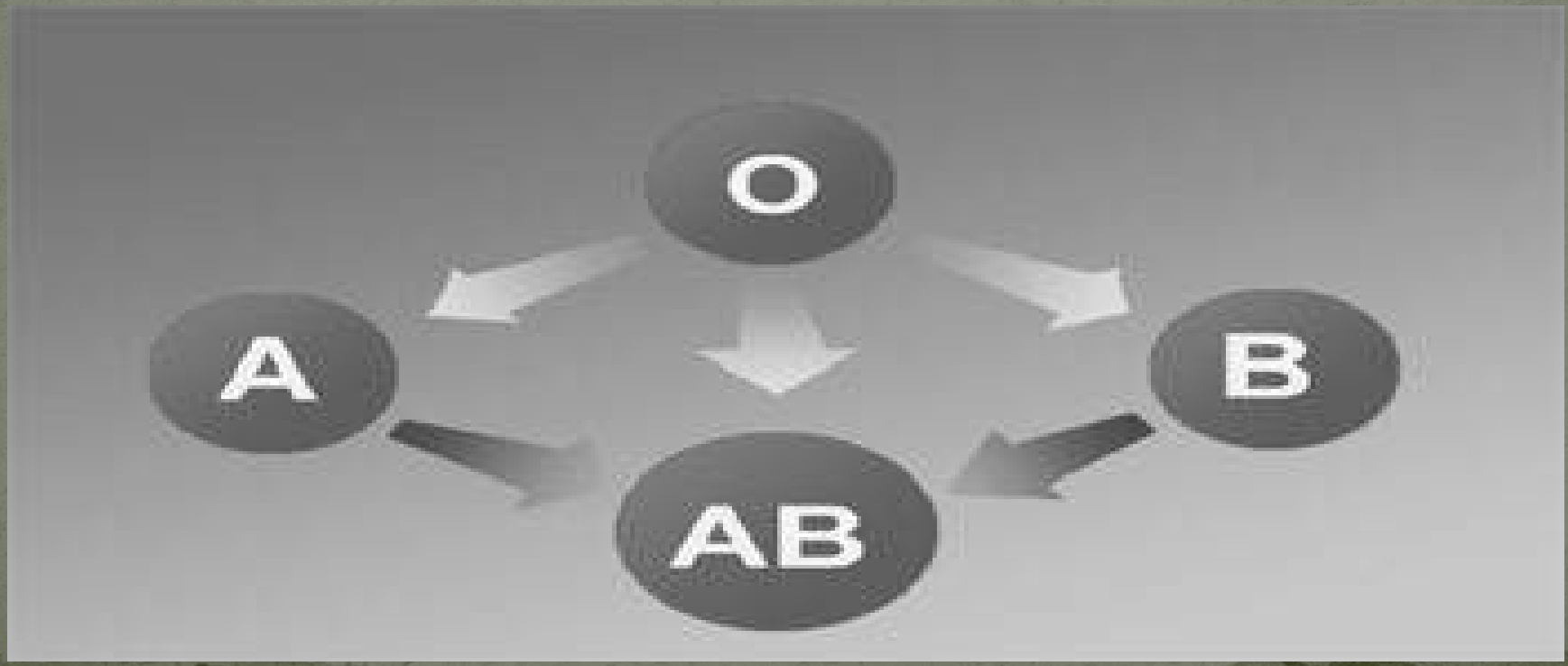
Transfusions

- Normal status : (compatibility type).
- Abnormal status. (incompatibility type).



- Many pregnant women carry a fetus with a blood type which is different from their own, and the mother can form antibodies against fetal RBCs. Sometimes these maternal antibodies are IgG, a small immunoglobulin, which can cross the placenta and cause hemolysis of fetal RBCs, which in turn can lead to hemolytic disease of the newborn called erythroblastosis fetalis, an illness of low fetal blood counts that ranges from mild to severe. Sometimes this is lethal for the fetus; in these cases it is called hydrops fetalis..

- **Type O** is the universal donor, since it does not have (antigens) on the surface of the RBC's.
- **Type AB** is the universal recipient, since it does not have (antibody) in its plasma.



APPARATUS AND REAGENTS

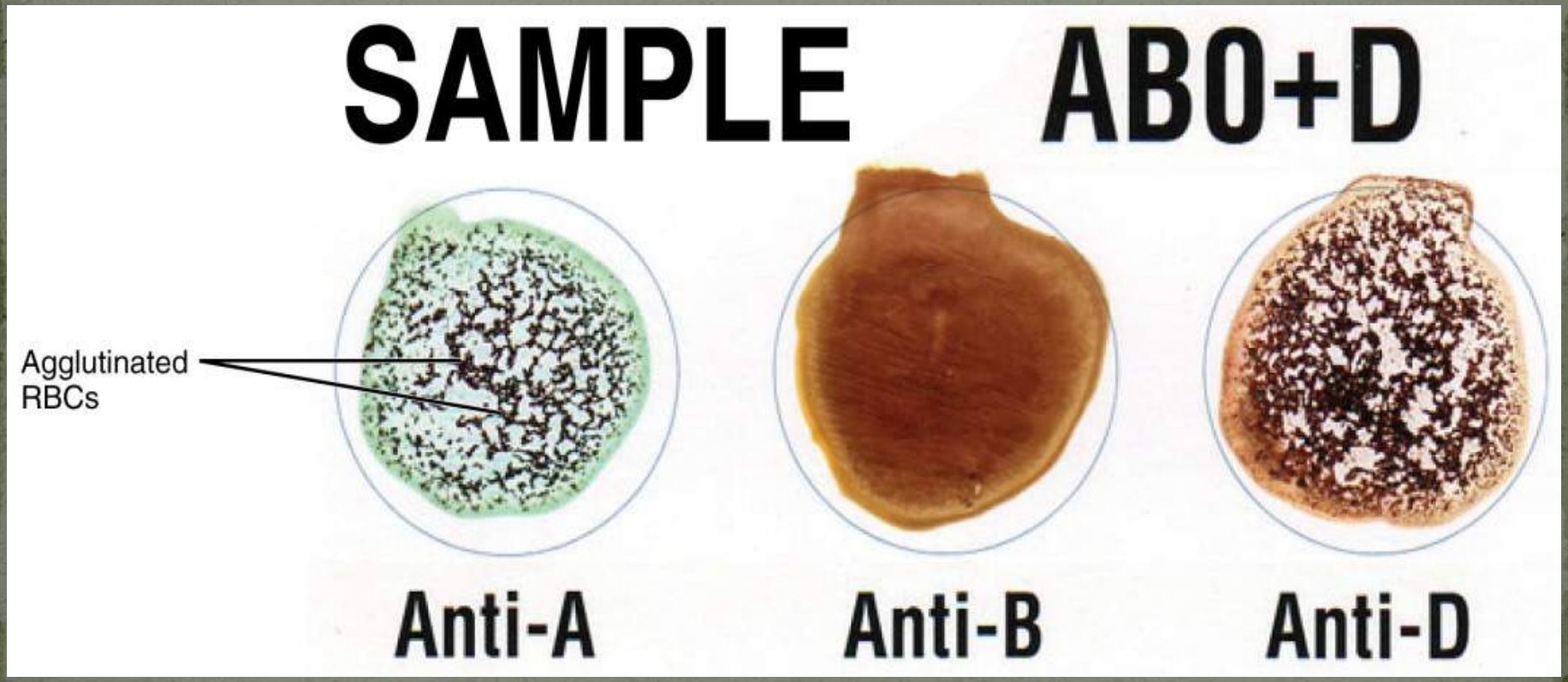
- Anti-ABO,D kit.
- Glass slide.
- Microscope.
- Sticks.
- Blood sample.


LABOESCUELA

PROCEDURE

A	B	D(RH)

A+BLOOD	B+BLOOD	RH+BLOOD



PRACTICAL PHYSIOLOGY

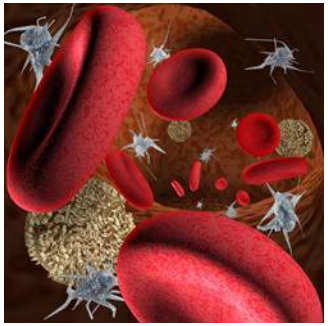
PREPARE BLOOD SMEAR

By

Dr. Firas Alaasam

M.Sc. Pharmacology and Toxicology

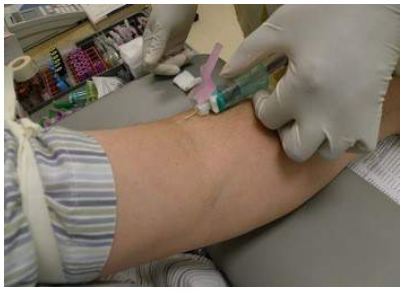
Samples for Hematology



- Capillary blood and venous blood can be used for hematology tests.

- Capillary blood is beneficial if a small amount of blood is needed.

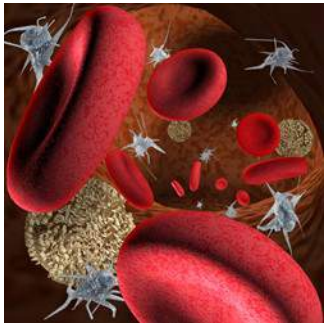
- Venous blood via venipuncture is beneficial if a larger amount of blood is needed for testing.



- Most hematology tests are run using blood collected with the anticoagulant, ethylenediaminetetraacetic acid, or EDTA for short.

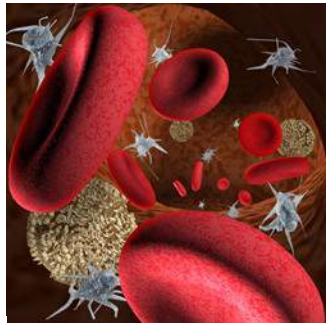
- EDTA is the anticoagulant found in lavender top tubes.

Samples for Hematology: CBC

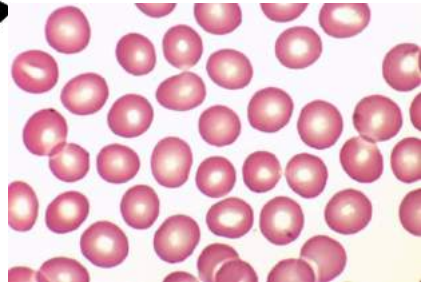


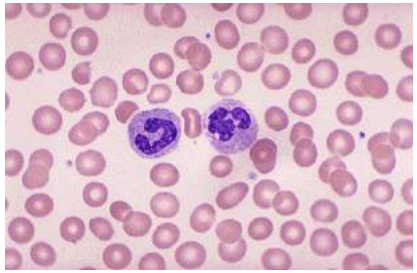
- A complete blood count or CBC is one of the most commonly ordered test in the hematology department.
- When running a CBC there are several separate tests run.
- Tests included in a CBC are….
  - — Red Blood Cell Count (RBCs)
  - — White Blood Cell Count (WBCs)
  - — Differential count (Diff)
  - — Platelet Count (PLTs)
  - — Blood Cell Morphology

Blood Smears

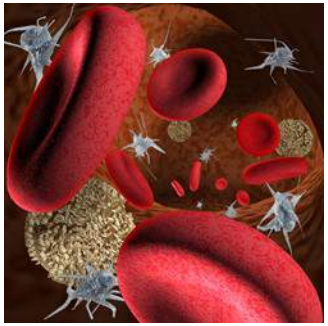


- A CBC also includes an examination of a blood smear if there are issues with the CBC.
- A smear of blood is made and then stained and examined by the laboratory personnel.
- Stained slides can be read manually (by a person) and sometimes by a machine.
- Ultimately a person must review the slides before results are reported.
- Blood smears are checked for types of WBCs, shapes of RBCs, and the presence of platelets.
- Morphology → the structure and shape of cells
- The morphology off all cells are reviewed.

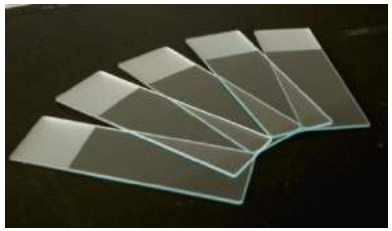




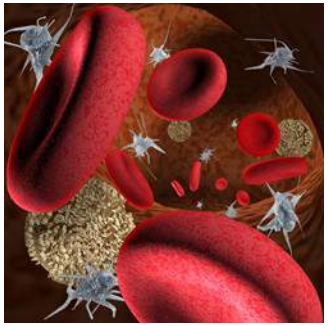
Preparing a Blood Smear



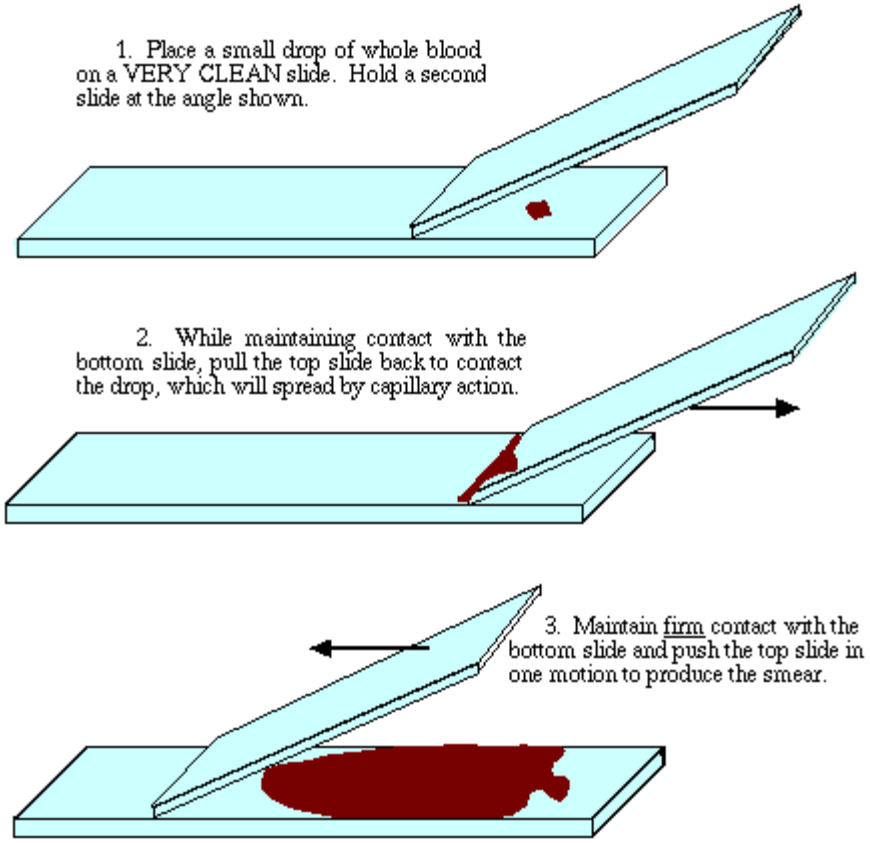
- Equipment needed to perform a blood smear include…..
  - — Clean slides (pre washed or cleaned)
  - — Capillary supplies or Venipuncture supplies
  - — Fresh tube of blood



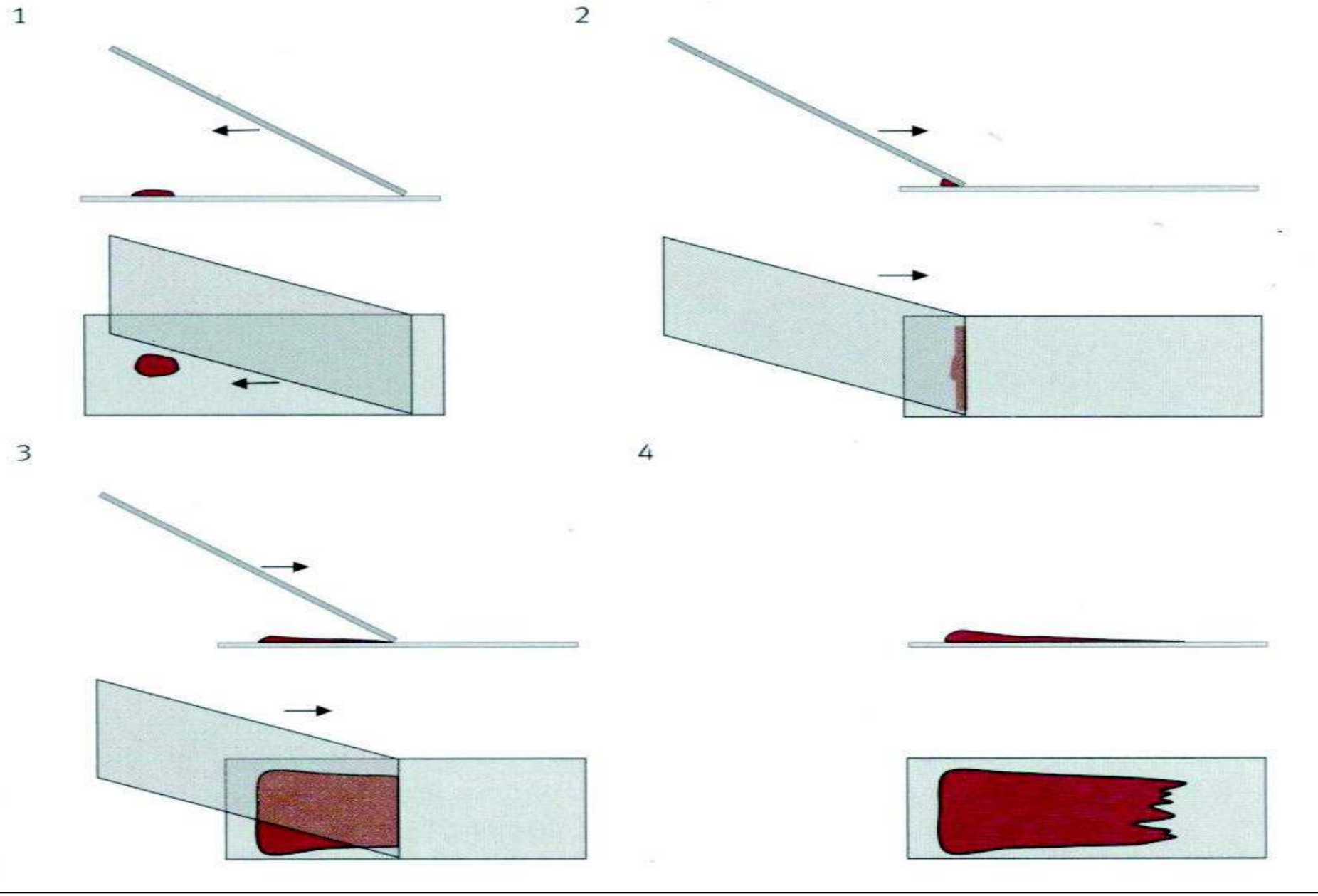
Preparing a Blood Smear



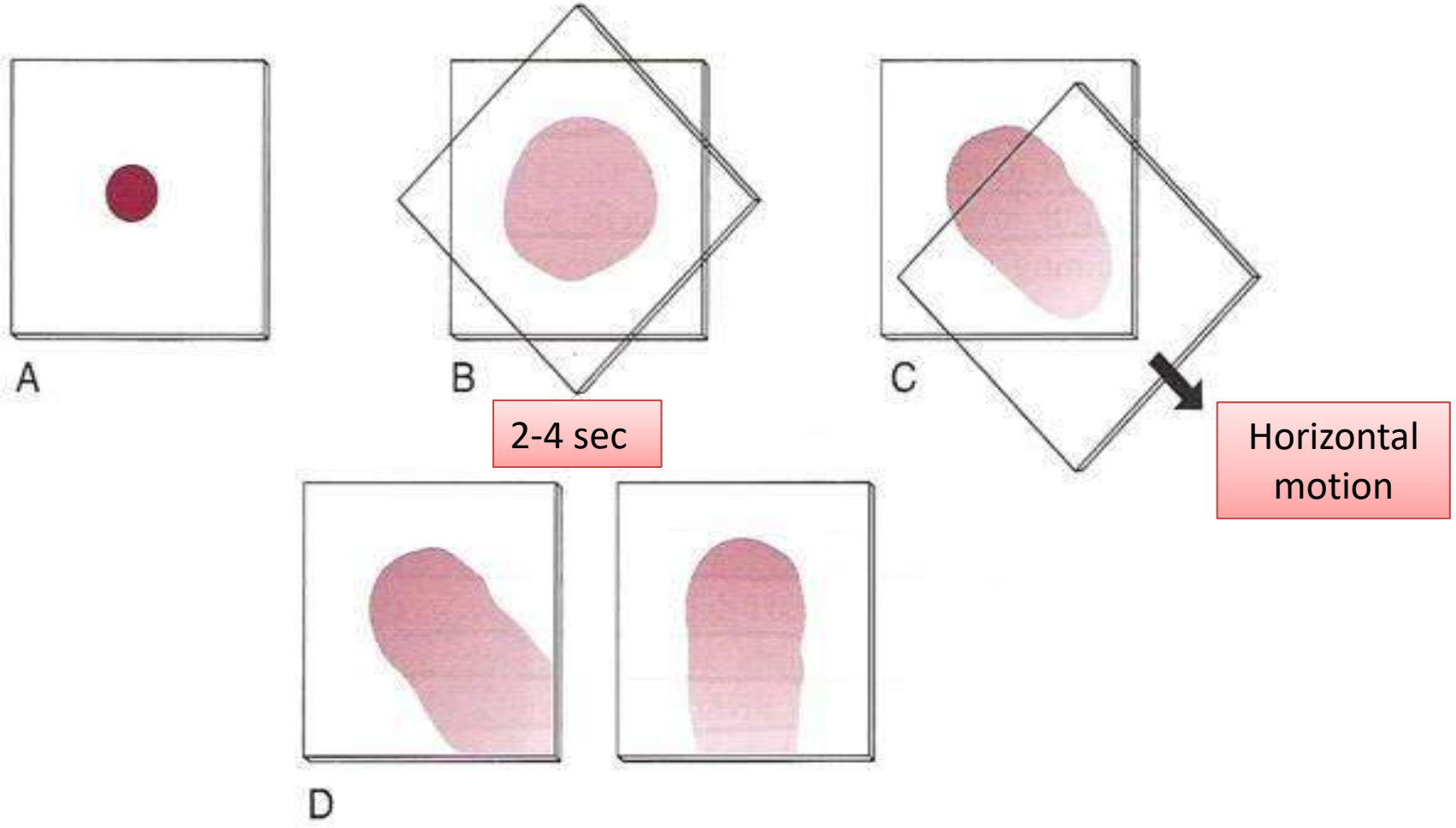
- Making a blood smear
  - — Place a small drop of blood ½ inch from the frosted end of a slide.
  - — The end of a second slide is used to spread the blood drop out on the first slide.
  - — Pull the end of the slide through the blood drop toward you and then push in the opposite direction forcing the blood in your direction.



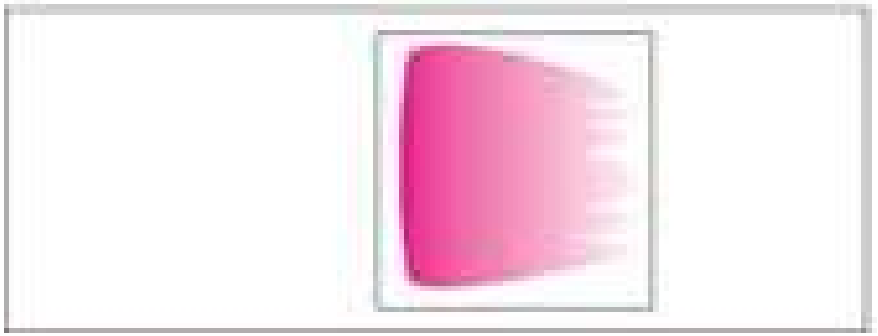
Prepare blood smear (SLIDE-TO- SLIDE)

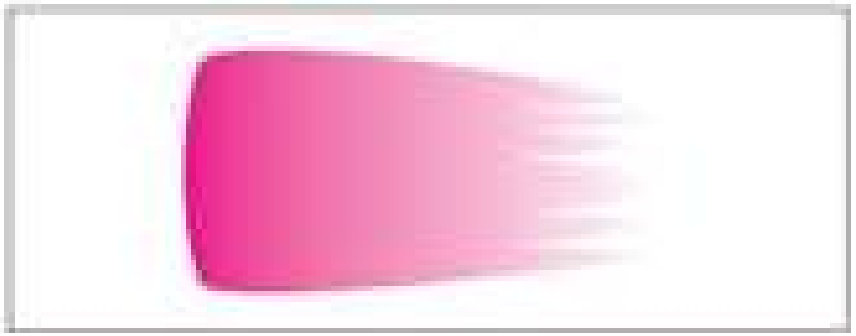


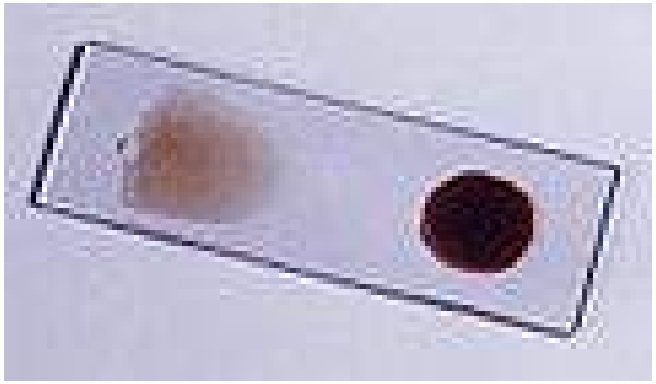
Prepare blood smear (COVERSLIP-TO-COVERSLIP)



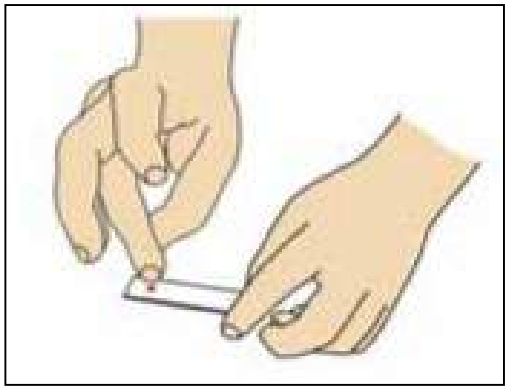
Prepare blood smear (COVERSLIP-TO-SLIDE)

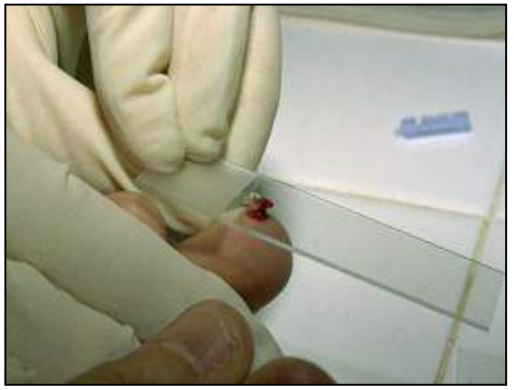






Taking sample





staining

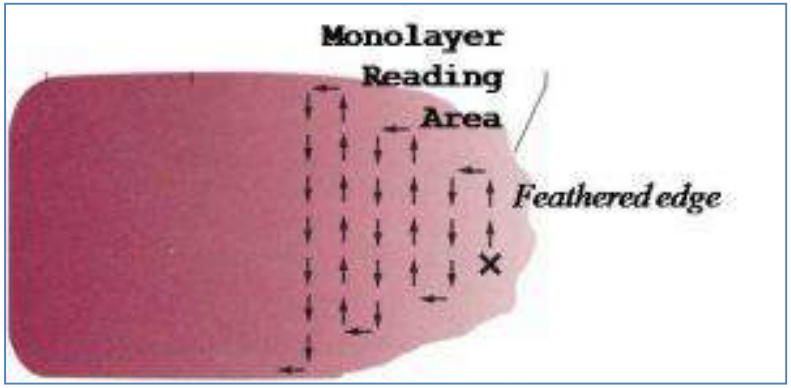
Wright stain

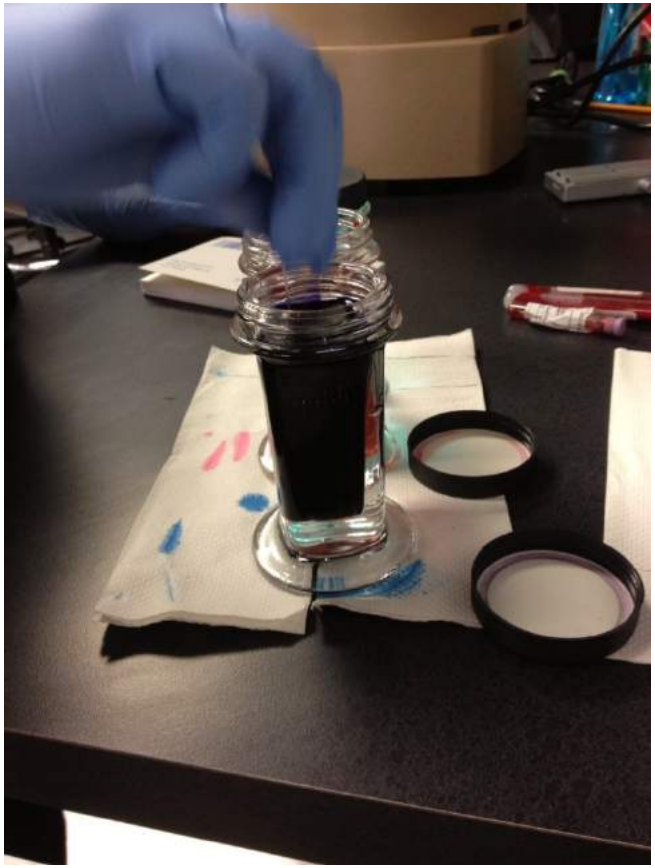
- 1) Flood the slide with stain (1-2min)not dry.
  2) Dilute with equal amount water(2-3min).
  3) Wash and dry.

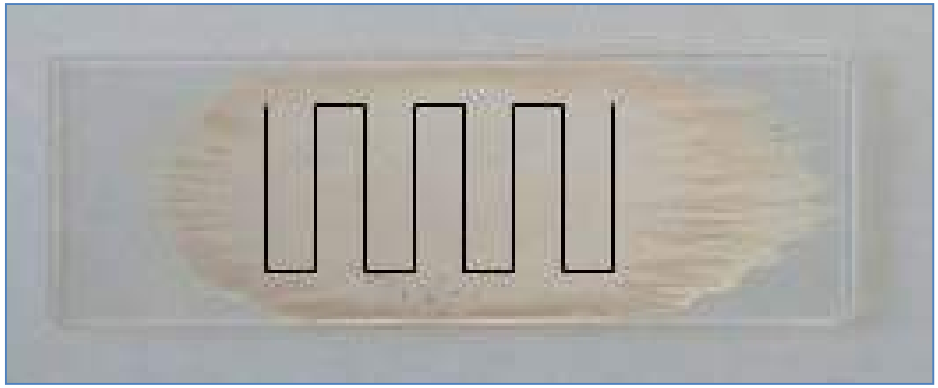
- **Leishman stain**
  - Like wright stain but double dilution of water.
    1) Add (8drops) wait (2min).
    2) Dilute (16 drops) wait(7-10min).
- **Giemsa stain**
- 1)Fixation (2min)
- 2) Add stain diluted 1:9 with buffer (8-10min).

Fixation

- Before staining, the blood films need to be fixed with acetone free methyl alcohol (1/2-1min).
- To prevent hemolysis when contact with water in subsequently.
- Alcohol denature the protein and hardens the cell contents.
- Wright and Leishman staining on pre-fixation(because dilute with alcohol)
- But Giemsa stain need to pre-fixation (because dilute by adding 1 ml stain to 9 ml water.


Monolayer
Reading
Area
Feathered edge





PRACTICAL PHYSIOLOGY

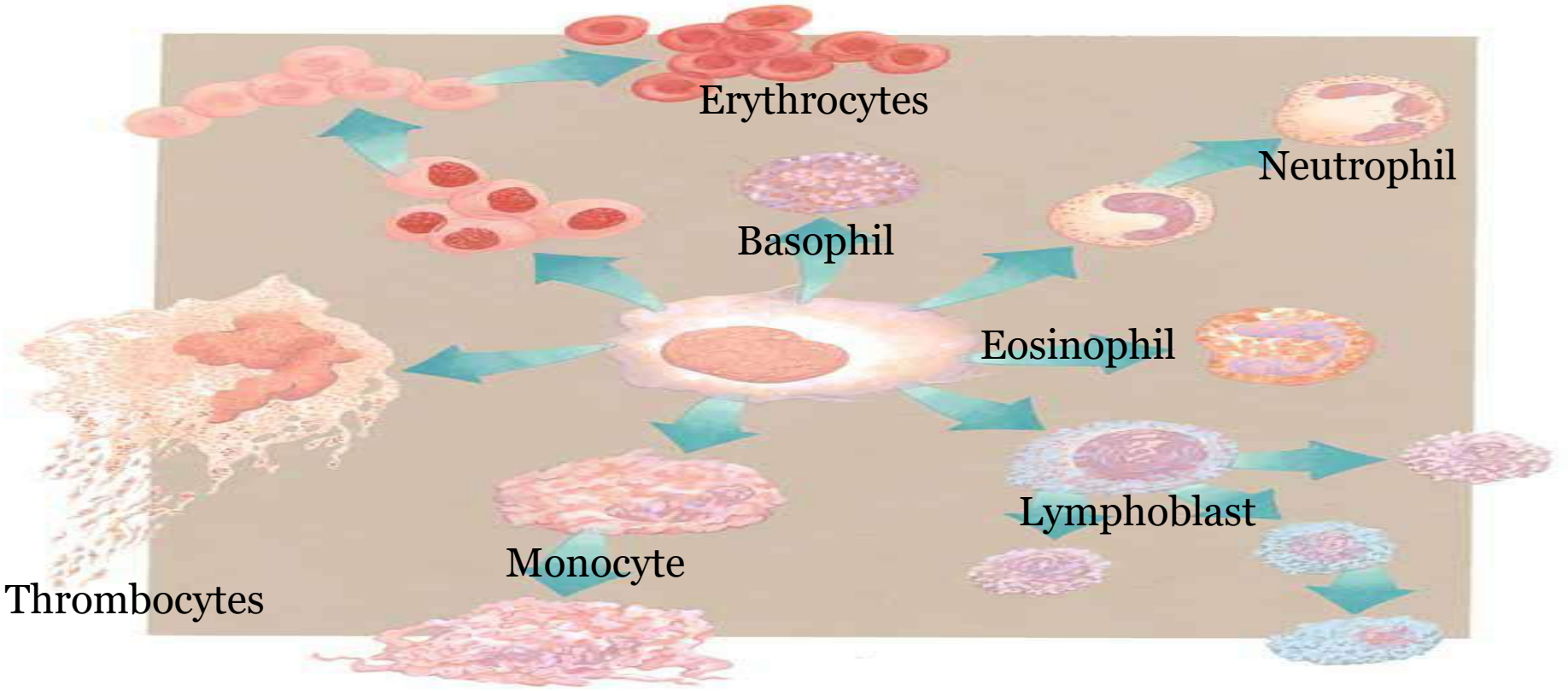
(DLC)

By

Dr. Firas Alaasam

M.Sc. Pharmacology and Toxicology

Differential White Blood Cell Count (Diff; Differential Leukocyte Count); (DLC):



DLC

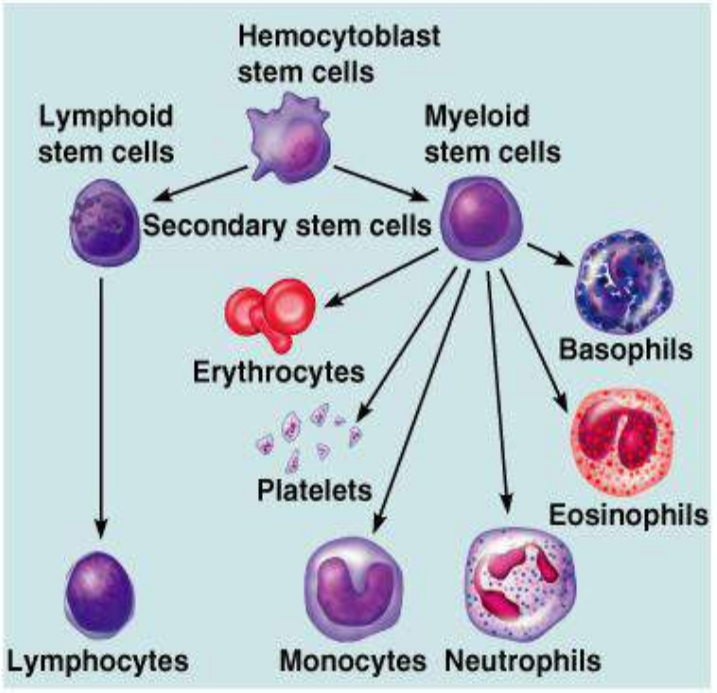
A white blood cell (WBC) count measures the number of white blood cells in your blood. A WBC differential determines the percentage of each type of white blood cell present in your blood. A differential can also detect immature white blood cells or any abnormalities, both of which are signs of a potential problem.

DLC

White blood cells are an important part of your body's immune system: They are responsible for protecting your body against infections and invading organisms. You have five types of white blood cells: neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Each of these is affected in a different way depending on the condition or disease that is affecting your WBC counts.

Types of Leukocytes

- **Types of Leukocytes**
  - **Granulocytes** Granules in their cytoplasm can be stained
  - Include neutrophils,
  - eosinophils, and
  - basophils



Types of Leukocytes

Eosinophil's .....2-4%.

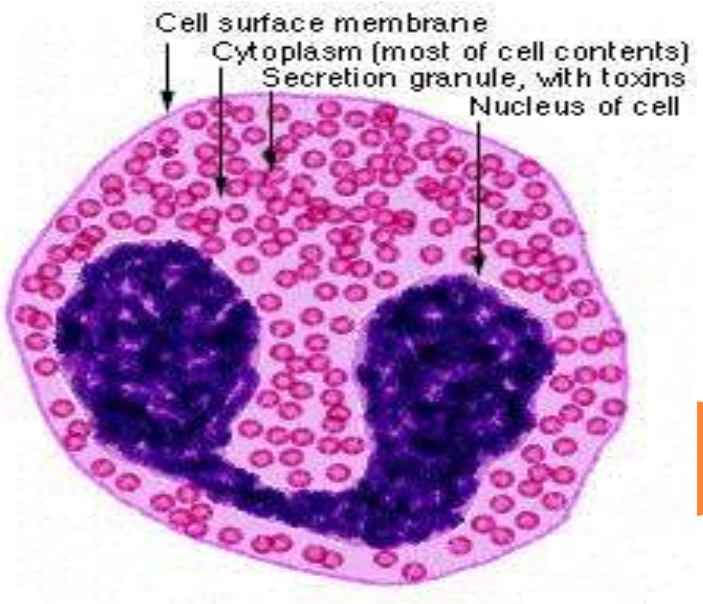
Function: Phagocytosis of antigen-antibody complexes; allergens

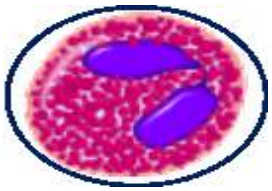
Release enzymes to weaken or destroy parasites such as worms.

Appearance: Nucleus has

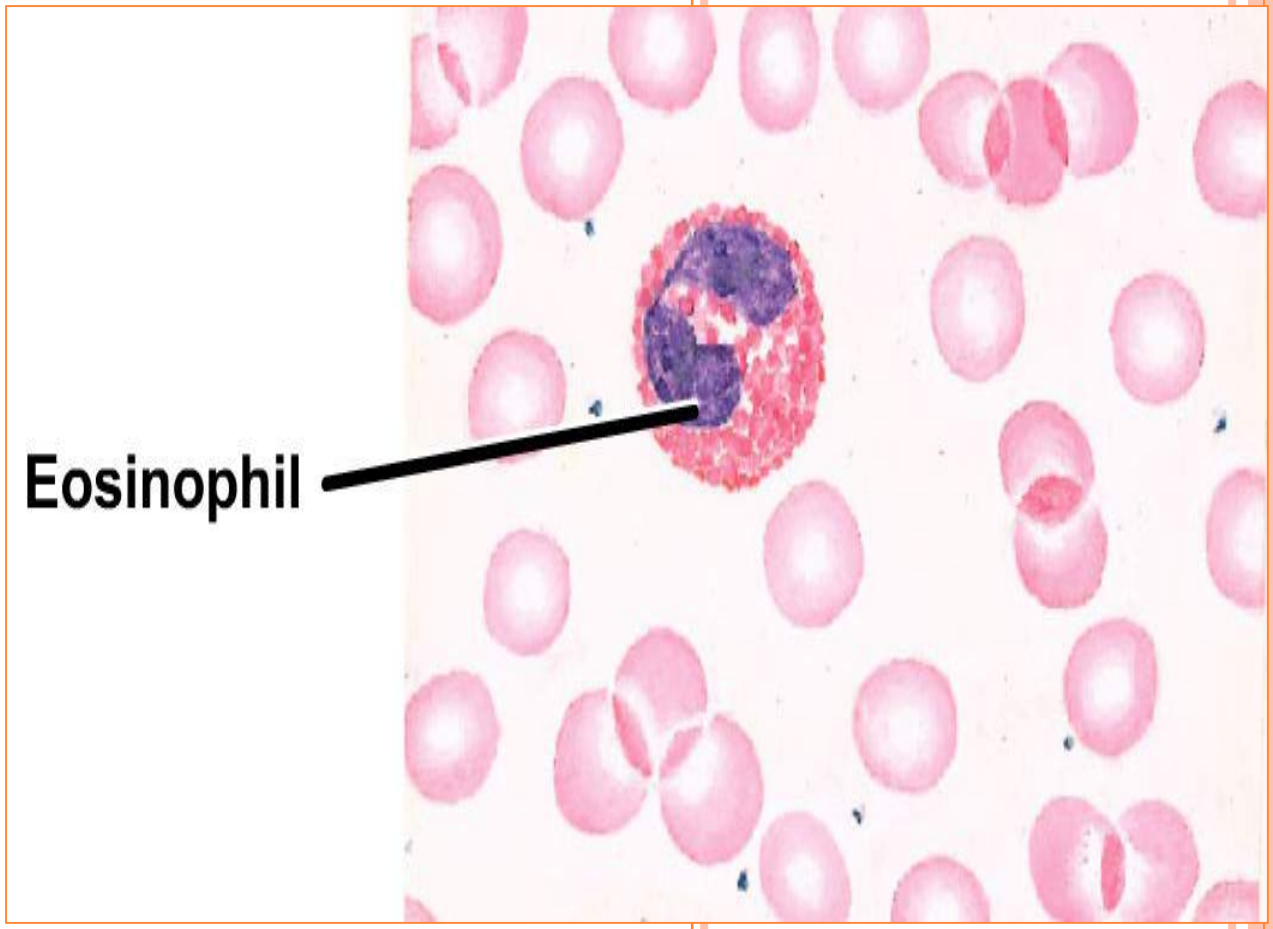
2 large

lobes





1. Identified in blood smear by Their cytoplasm, which is filled with distinct, large, eosinophilic (bright-pink) **granules**.
2. The Nucleus in Eosinophils typically is **bilobed**, but a small, third lobe may be present.
3. **Form about 2-4%.**



EOSINOPHILS (ACIDOPHILIC LEUKOCYTE):

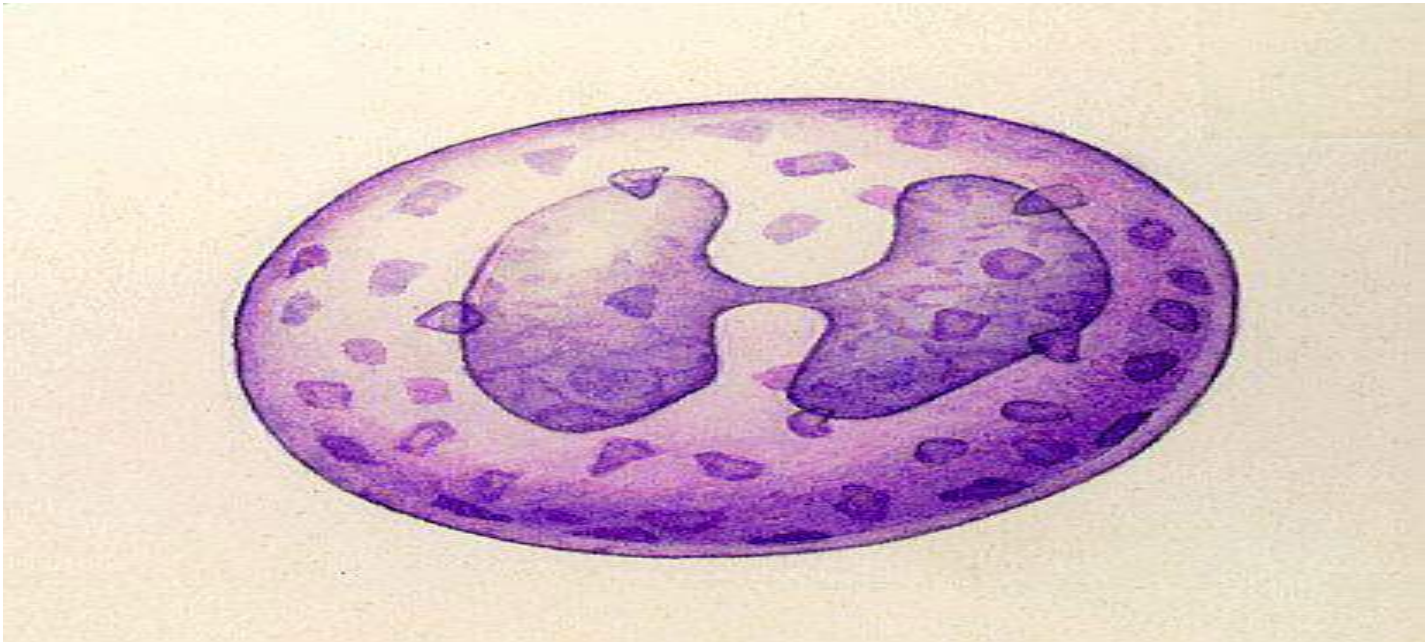
Types of Leukocytes

Basophil less 1%.

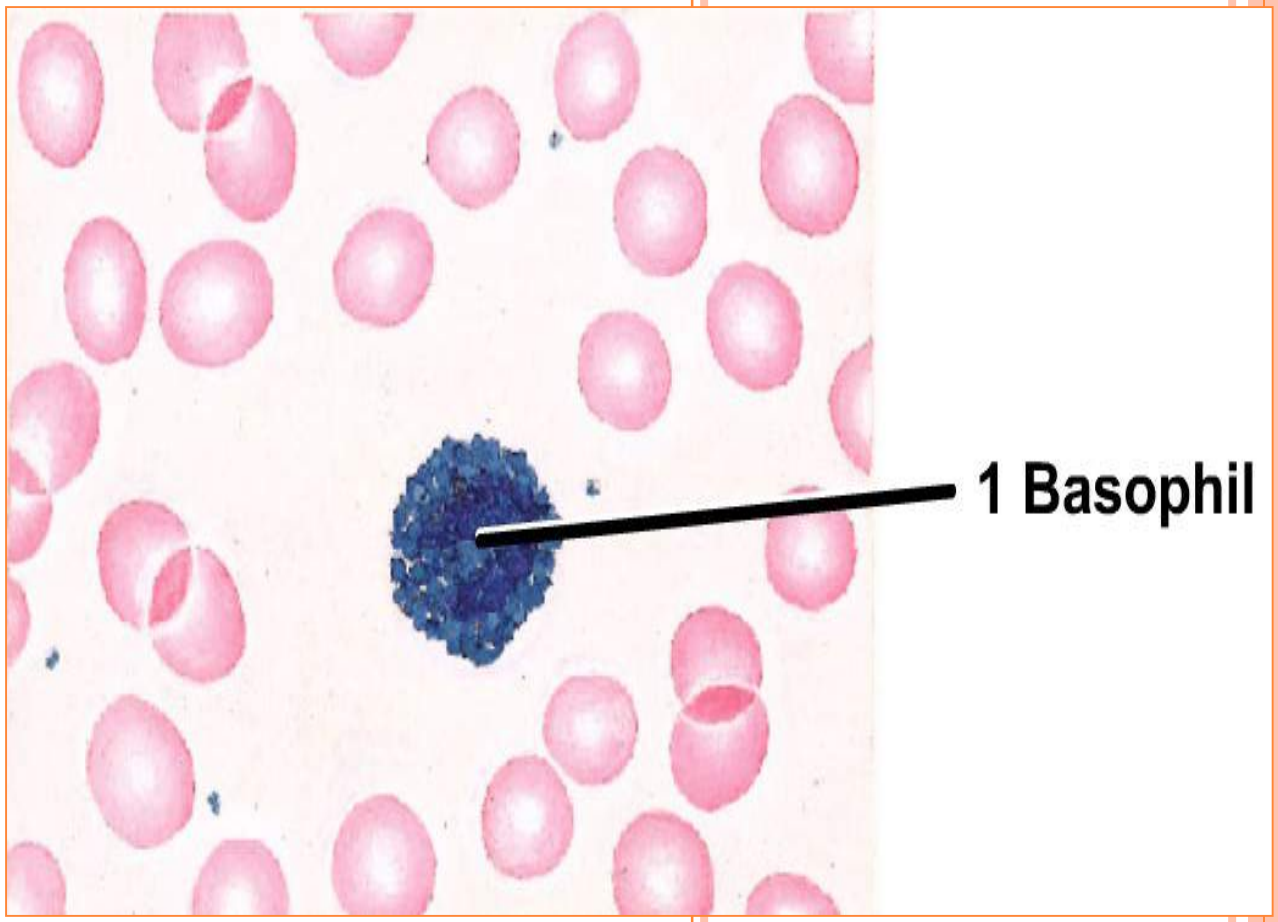
type of white blood cell (leukocyte) that is characterized histologically by its ability to be stained by basic dyes and functionally by its role in mediating hypersensitivity reactions of the immune system.

Types of Leukocytes

Function: Secretes Histamine,(a vasodilator)
Secretes heparin (an anticoagulant)



1. The nucleus is not lobulated and stain pale basophilic, but it usually obscured by the density of the granules
2. Form about less 1%.



BASOPHILS LEUKOCYTE :

Types of Leukocytes

Neutrophils 60-70%

Appearance

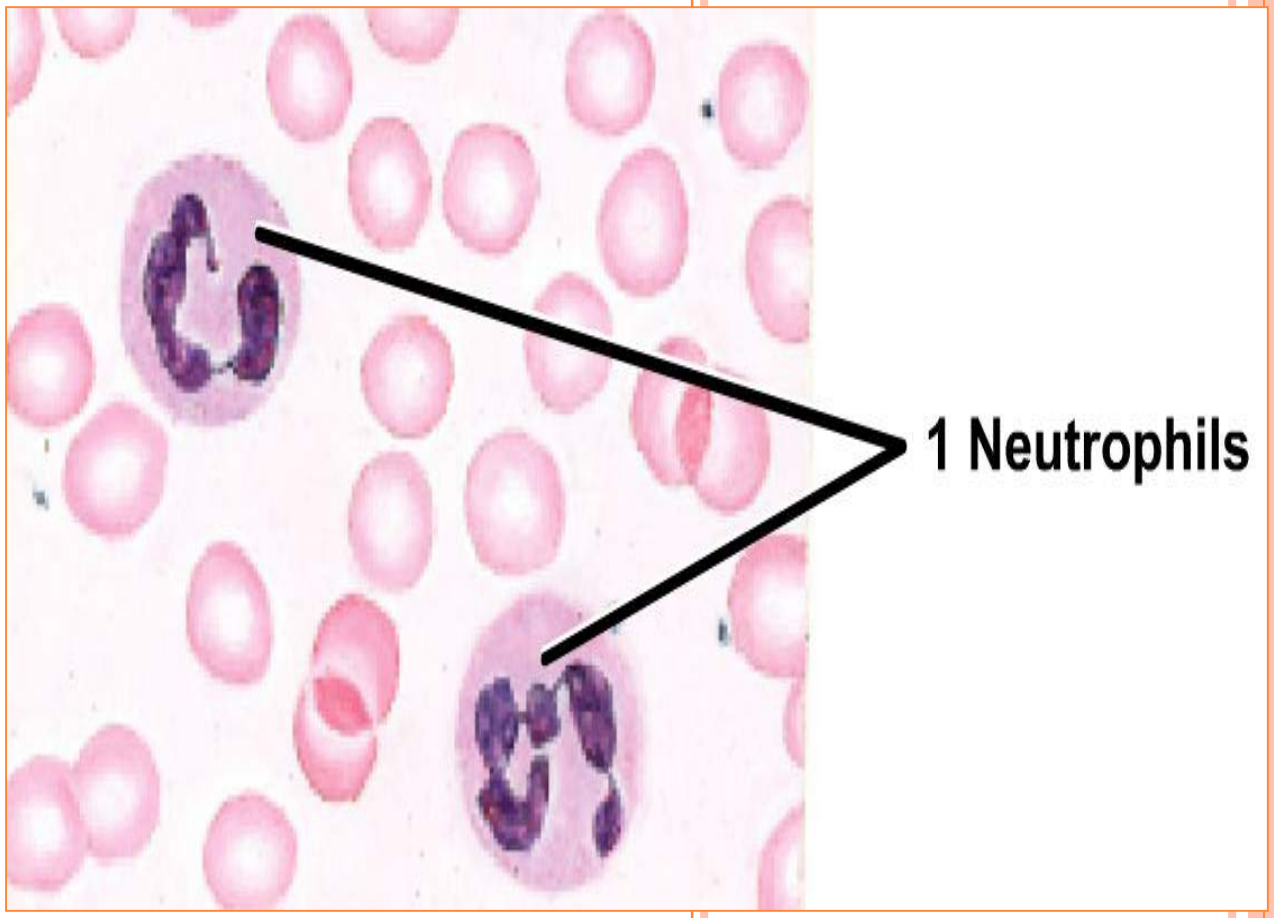
Nucleus usually with 3-5 lobes in S-C shaped array

Functions:

Phagocytosis of bacteria

Release of antimicrobial chemicals

1. Nucleus consist of **several lobes** that are connected by narrow chromatin strand are Polymorphonuclear.
2. Cytoplasm contain **Fine light blue granules** that are difficult to see with a light microscopic as a result cytoplasm appear clear.
3. Most abundant leucocytes.
4. **Form about 60-70%.**



NEUTROPHILS LEUKOCYTE :

Types of Leukocytes

- ***A granulocytes***
  - **Lymphocytes** Nucleus fills most of the cell
  - Play an important role in the immune response
  - **Monocytes** Largest of the white blood cells
  - Function as macrophages
  - Important in fighting chronic infection

Types of Leukocytes

Lymphocytes

These are cells involved in the…..

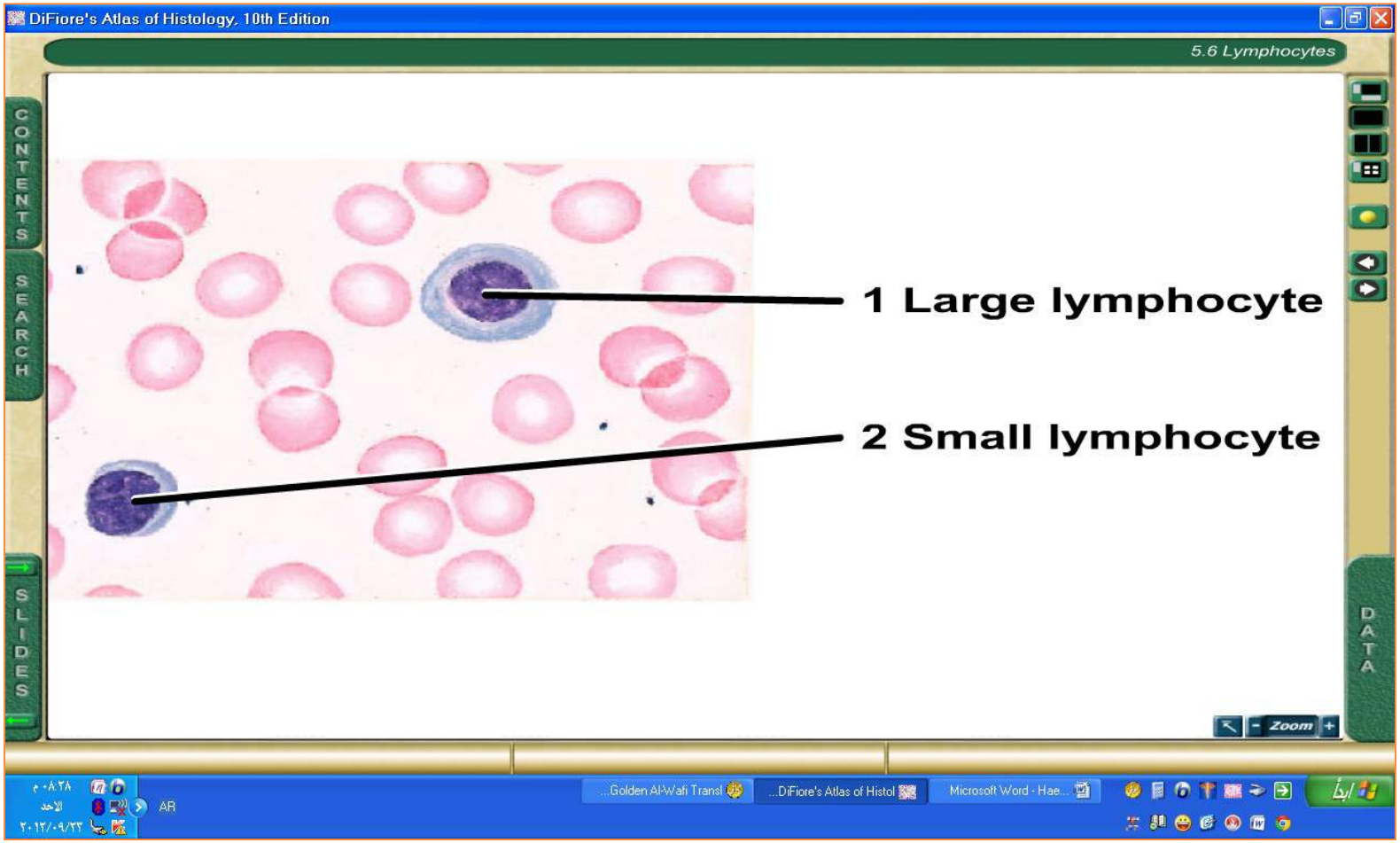
IMMUNE RESPONSE

There are**:** ***B-Lymphocytes*** (antibody production)

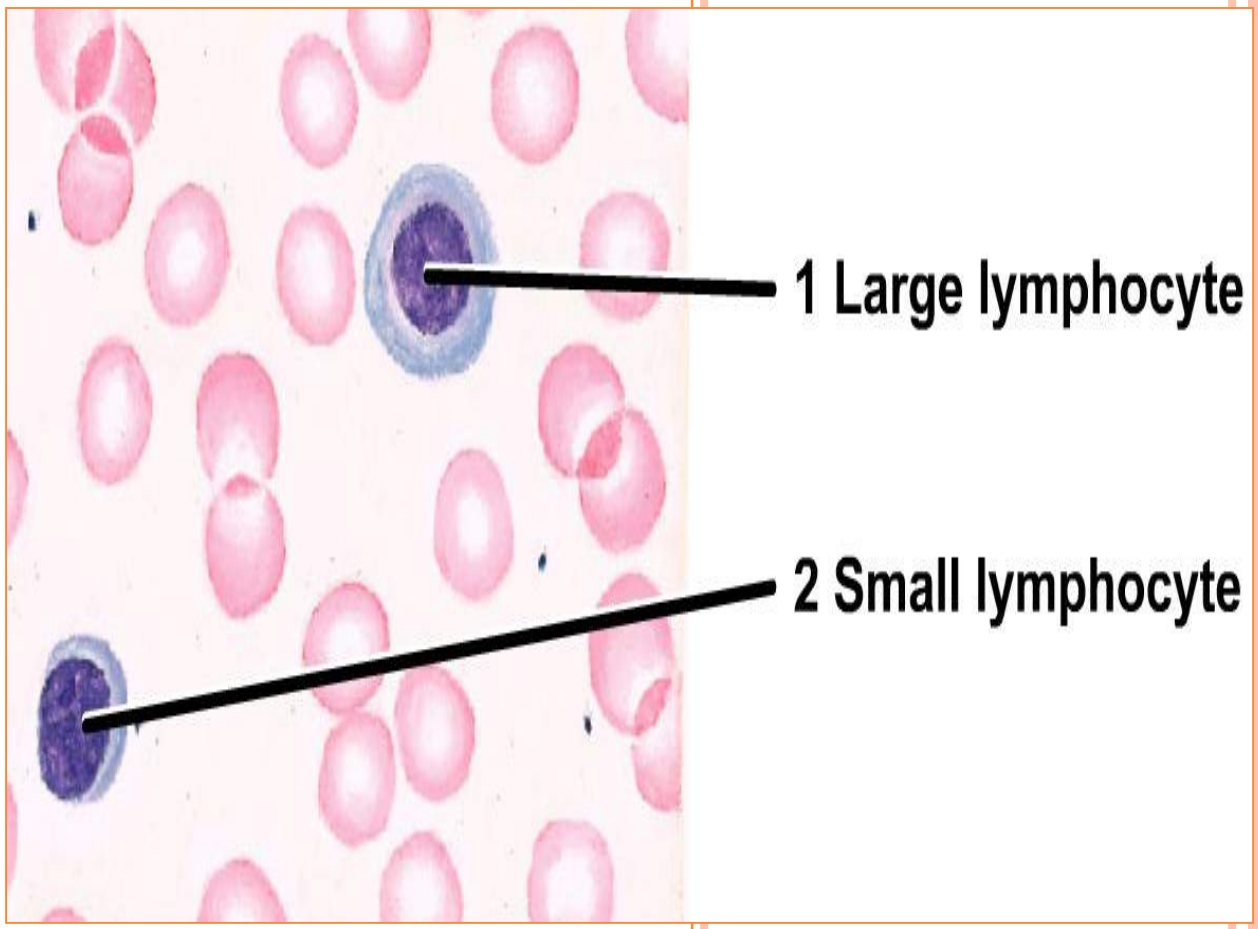
and

T-Lymphocytes: play important role of phagocytosis

Types of Leukocytes

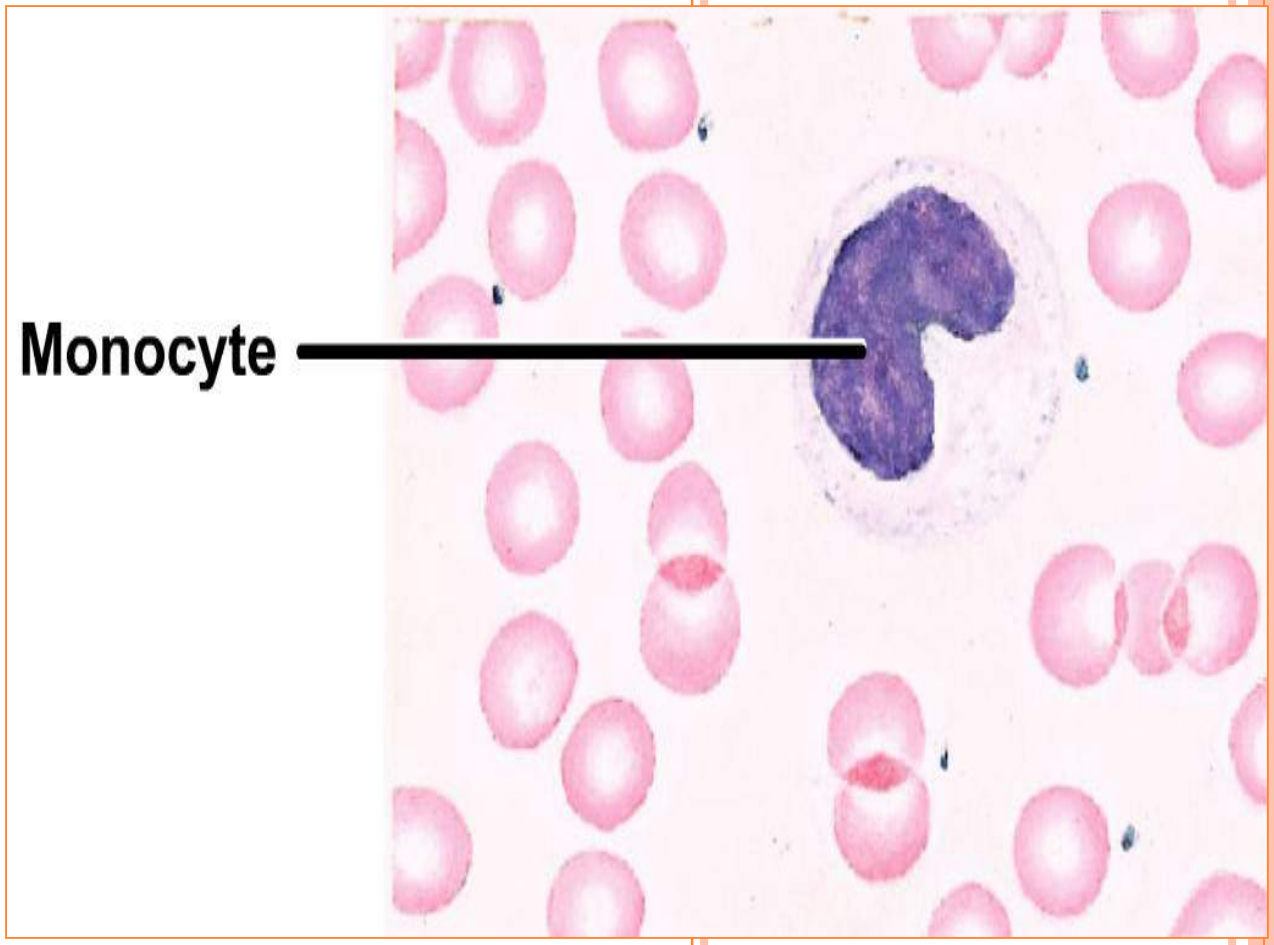


1. Have few or no cytoplasm granules.
2. Round-shaped nuclei.
3. Vary in size from cell that are smaller than red blood corpuscles to that are almost twice as large.
4. In small lymphocytes the densely stained nucleus occupies most of the cytoplasm, which appears as a thin, basophilic rim around the nucleus.
5. In large lymphocytes cytoplasm is more abundant, and the lager and paler nucleus.
6. Form about 20-30%.



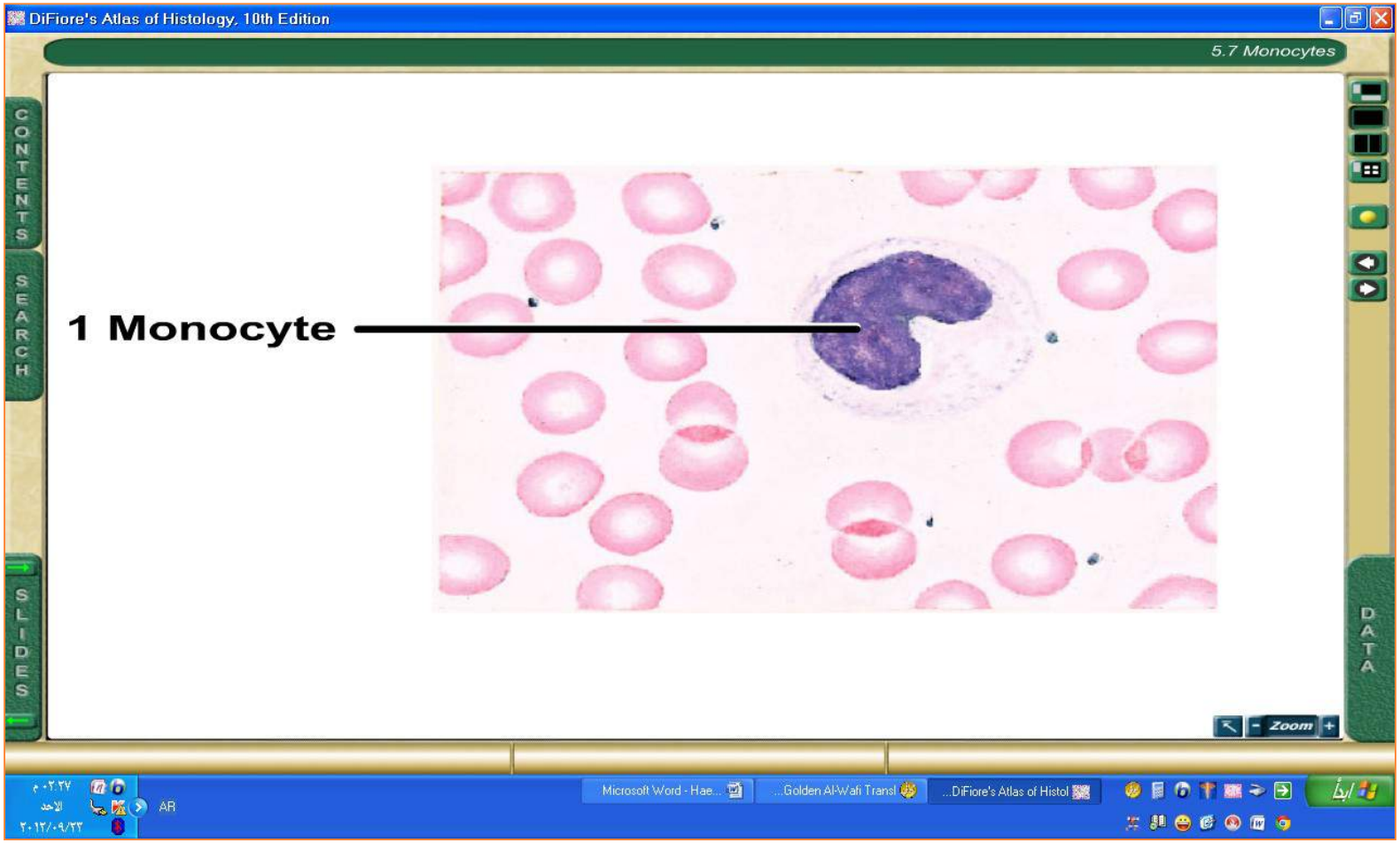
LYMPHOCYTES LEUKOCYTE :

1. Largest A granular leucocytes.
2. Nucleus varies from round to indented horseshoe-shaped or kidney-shaped and it Stained lighter than the lymphocytes nucleus.
3. Abundant cytoplasm is lighter basophilic.
4. Form about 3-8%.



MONOCYTES LEUKOCYTE :

Types of Leukocytes



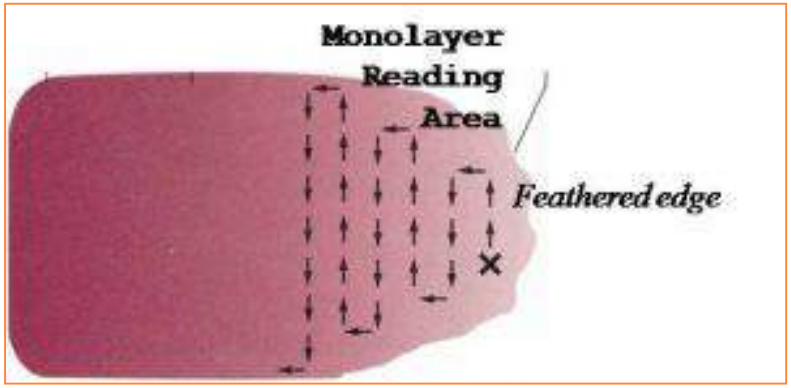
Differential leucocytes count

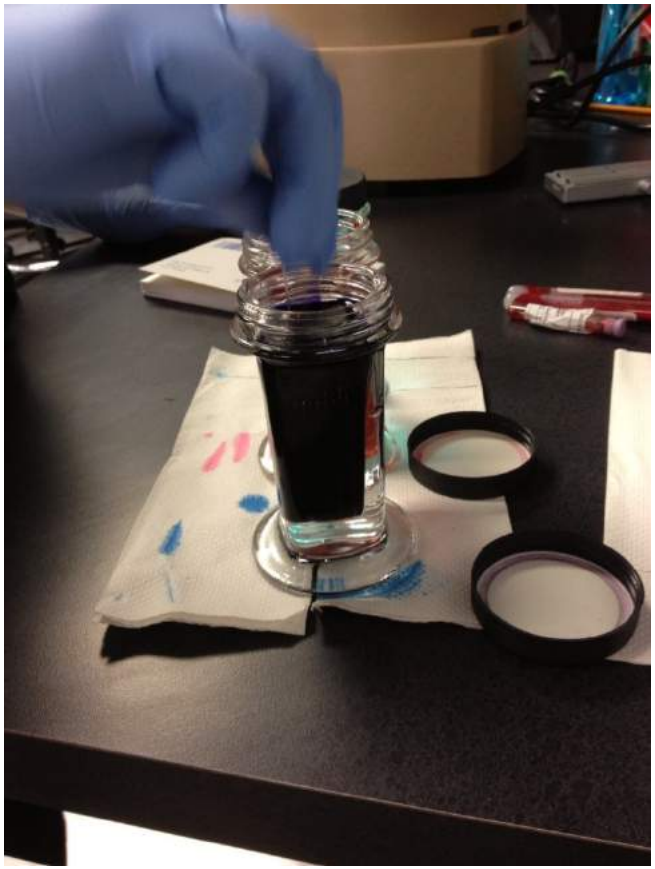
- Prepare blood smear
  - Slide to slide
  - Coverslip to slide
  - Coverslip to coverslip
  - Automated
- Fixation and staining
  - Wright stain
  - Leishman stain
  - Giemsa stain
- Test
  - Low power (4X,10X)
  - High power (40X)
  - Oil immersion (100X)

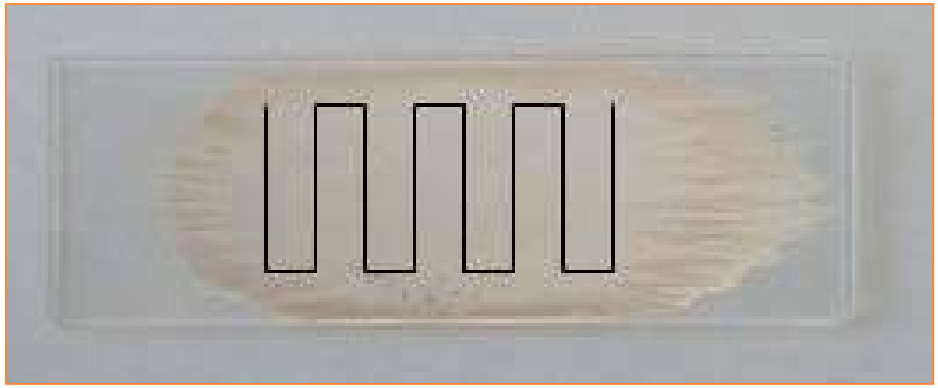
Count

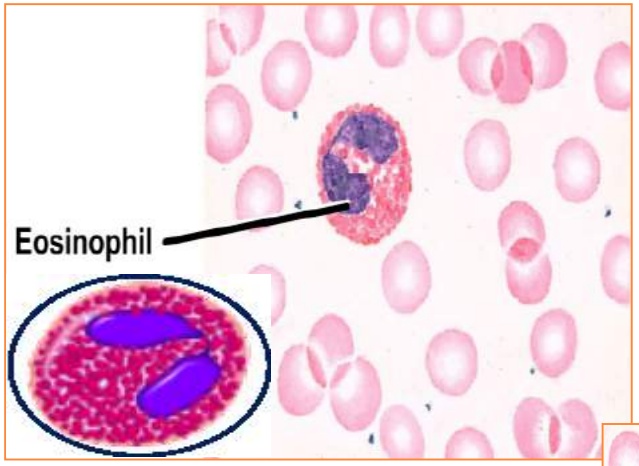
10x	40x	100x
Scan Overall, good distributed, no clumps.	Determine the WBC estimate.	Perform differential white cell count

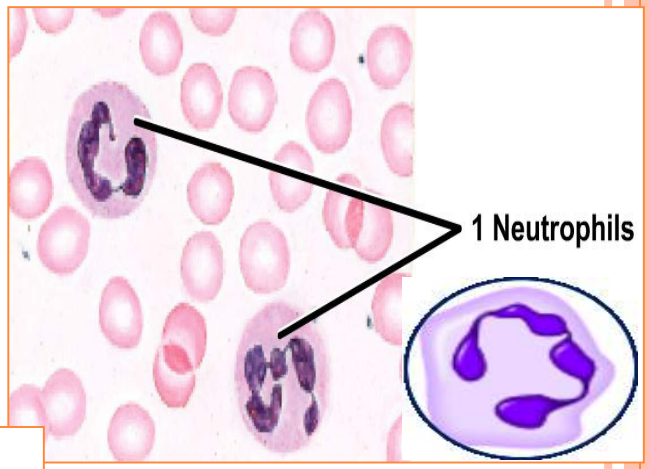
- A total of 100 cells should be counted and record in table under the following : neutrophil, eosinophil, basophil, monocyte, and lymphocyte.

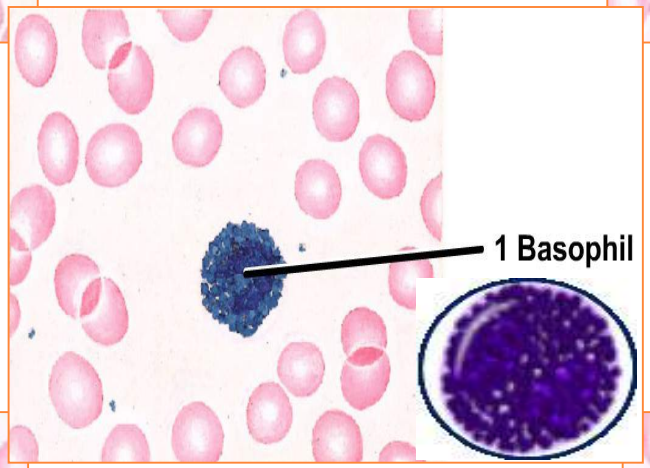

Monolayer
Reading
Area
Feathered edge

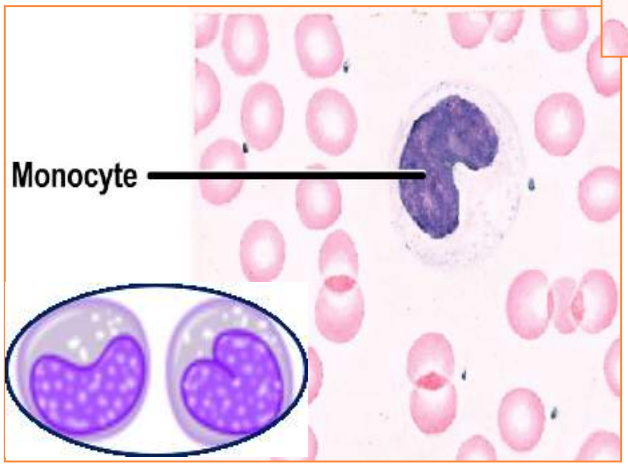


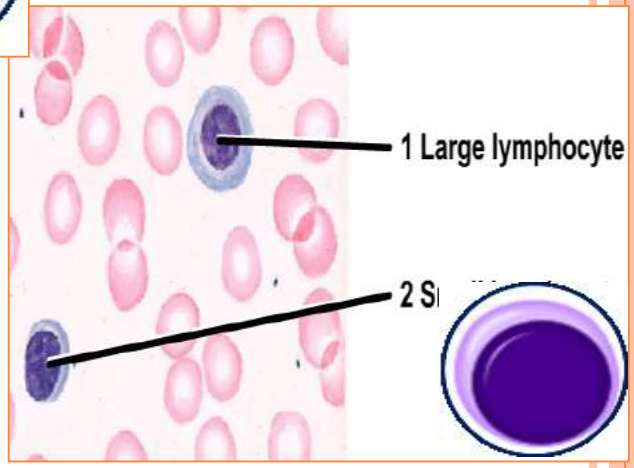



Eosinophil


1 Neutrophils


1 Basophil


Monocyte


1 Large lymphocyte
2 S

PRACTICAL PHYSIOLOGY

Some terms and technical in physiology laboratory

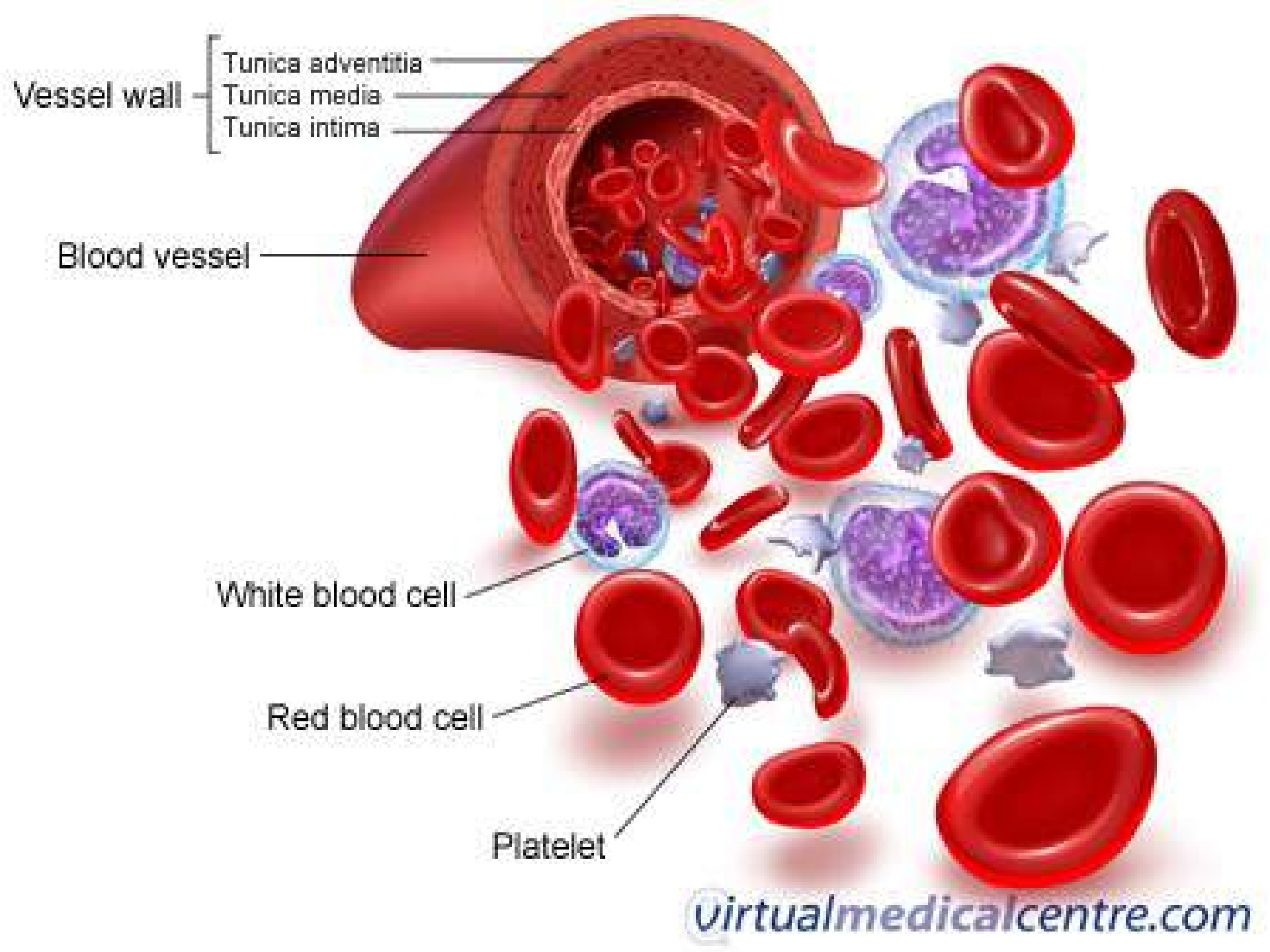
Edition by
Dr.Firas Alaasam
M.Sc Pharmacology & Toxicology

First Year

FIRST LABORATORY

The blood

Blood: The familiar red fluid in the body that contains white and red blood cells, platelets, proteins, and other elements. The blood is transported throughout the body by the circulatory system. Blood functions in two directions: arterial and venous. Arterial blood is the means by which oxygen and nutrients are transported to tissues while venous blood is the means by which carbon dioxide and metabolic by-products are transported to the lungs and kidneys, respectively, for removal from the body


Vessel wall
Tunica adventitia
Tunica media
Tunica intima
Blood vessel
White blood cell
Red blood cell
Platelet
virtualmedicalcentre.com

❖Collection of the blood :

- **Collect the blood from several ways:**

1. Venous blood.
2. Capillary blood.
3. Artery blood.

Blood collection and anticoagulants :

1- Capillary puncture methods.

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- Vein puncture method.

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.

❖Anticoagulant :

- Is chemical material to salt some metal such as sodium, potassium and lithium. That act on prevent clot blood and preserved normally.

❖Form blood in analyzing :

1. Whole blood : require to anticoagulant tube and shaking, but no require to centrifuge.

2. Plasma : require to anticoagulant tube, shaking and centrifuge.

3. Serum : no require to anticoagulant tube and shaking, but require to period clot and centrifuge.

- **serum:** it has no anticoagulant, it contains all the contents except clotting factor .
- Serum uses :blood chemistry, serology, immunology
- **Plasma:** it has anticoagulant .the plasma has all clotting factor .
- plasma uses; blood chemistry and coagulation study

❖There are several of anticoagulant :

1. **Disodium or Dipotassium Ethylenediamine Tetra acetic acid (EDTA):**

- Is powerful anticoagulant that prefer utilize in hematology tests, that act by cheating effect to calcium and prevent do to clotting formation.

2. **Heparin :**

- Consider of essential constitutive blood. but with blood film gives faint blue coloration to background when stain.

3. **Acid Citrate Dextrose solution (ACD) :**

- May be use in blood transfusion and enzyme studies because preservative on red blood corpuscles.

3. **Tri-sodium citrate :**

- Prefer using in coagulating studied and ESR test.


MediPlus
SODIUM CITRATE
MediPlus
SODIUM CITRATE

❖The following steps should be taken in examining blood samples :

1. Select proper anticoagulant in case test that required (plasma or whole blood) depending of test.
2. Prevent clotting samples in case test that required (plasma or whole blood) by proper mixing.
3. Sure of dry and clean any apparatus before using.
4. Label specimen.
5. If sample in tube must mix before drawing.
6. Must wipe off any blood drops outside the tip of pipette.
7. Prepare blood smear and run test.

❖Type of blood tube :

There are several of tube having cap color refer to present or absent and type anticoagulant :

1. **Blood tube absent anticoagulant :**

A. Ordinary glass tube.

B. Ordinary plastic tube.

C. Red tube

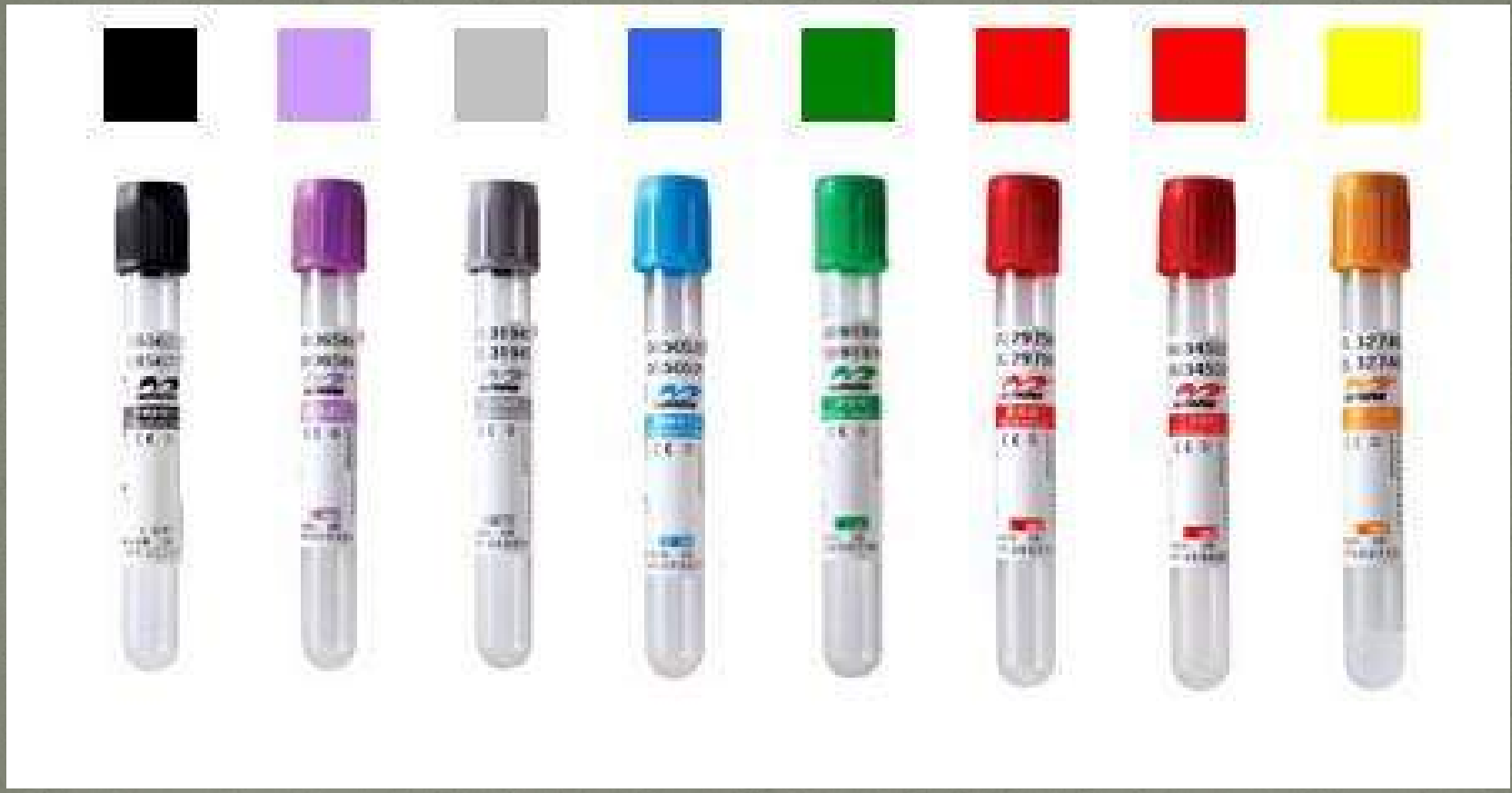
D. Yellow tube

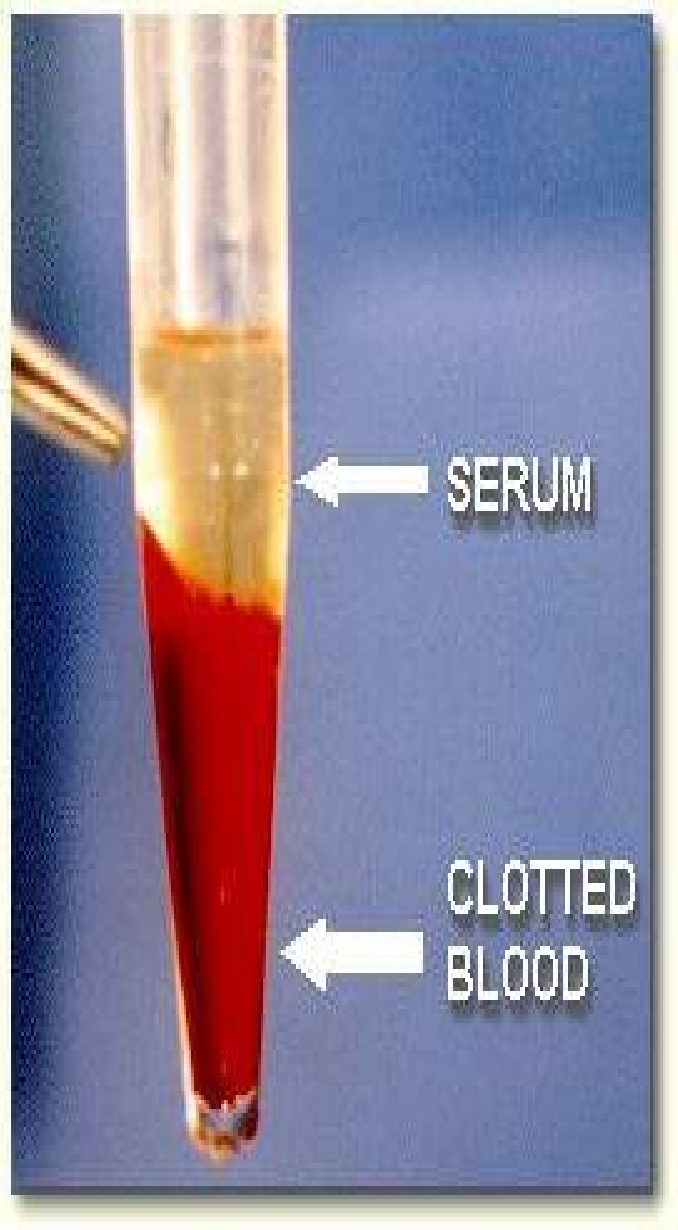
- Using such as this tubes to obtain on serum than no required to shaking but let up to blood clotting than centrifuge will separate serum in upper than transfer to clean tube to keep or test, such as serology ,hormonology and biochemical tests.

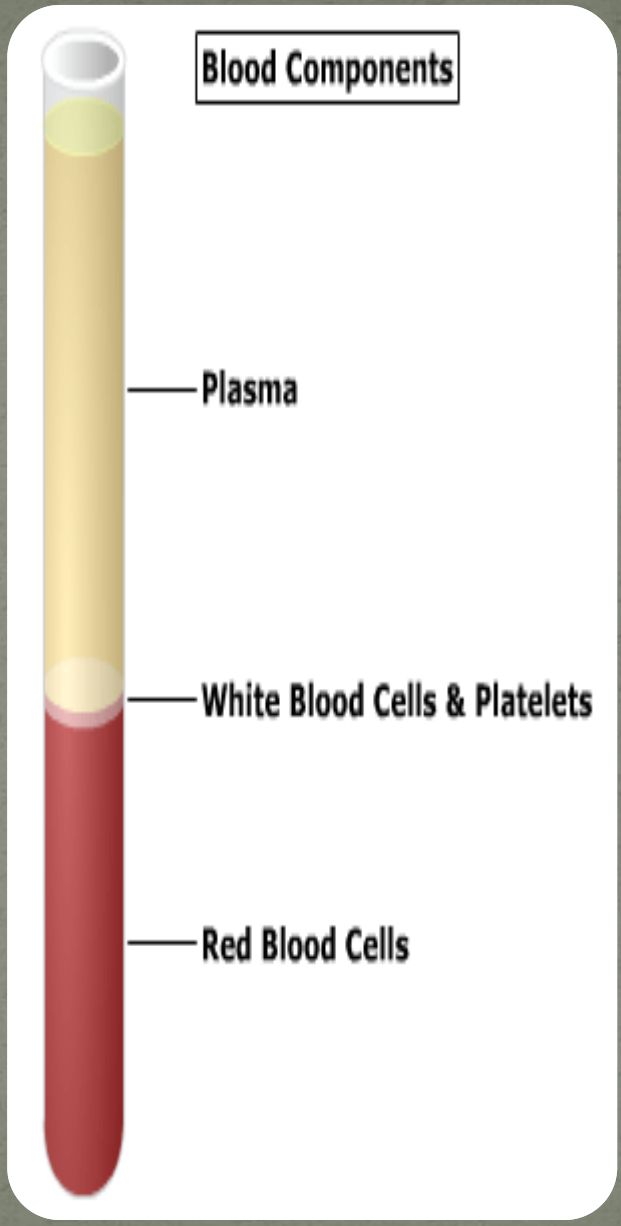
2. **Blood tube present anticoagulant :**

- Using such as this tubes to obtain on plasma or whole blood which required to shaking than centrifuge in case plasma will separate plasma in upper such as hematology tests.

A. Lavender tube : contain on (EDTA) use in hematology tests.
B. Green tube :contain sodium or lithium (Heparin) use in PH, gas blood, electrolytes and drug concentration and G-6-PDH.
C. Blue tube : contain (sodium citrate) use in coagulant tests.
D. Gray tube : contain (sodium fluoride) use in glucose test due to sodium fluoride prevent change concentration glucose in blood.




SERUM
CLOTTED BLOOD


Blood Components
Plasma
White Blood Cells & Platelets
Red Blood Cells

Thank You

PRACTICAL PHYSIOLOGY

Estimation of Hemoglobin concentration ; Hb

EDITION BY

DR.FIRAS ALAASAM

&

M.SC PHARMACOLOGY & TOXICOLOGY

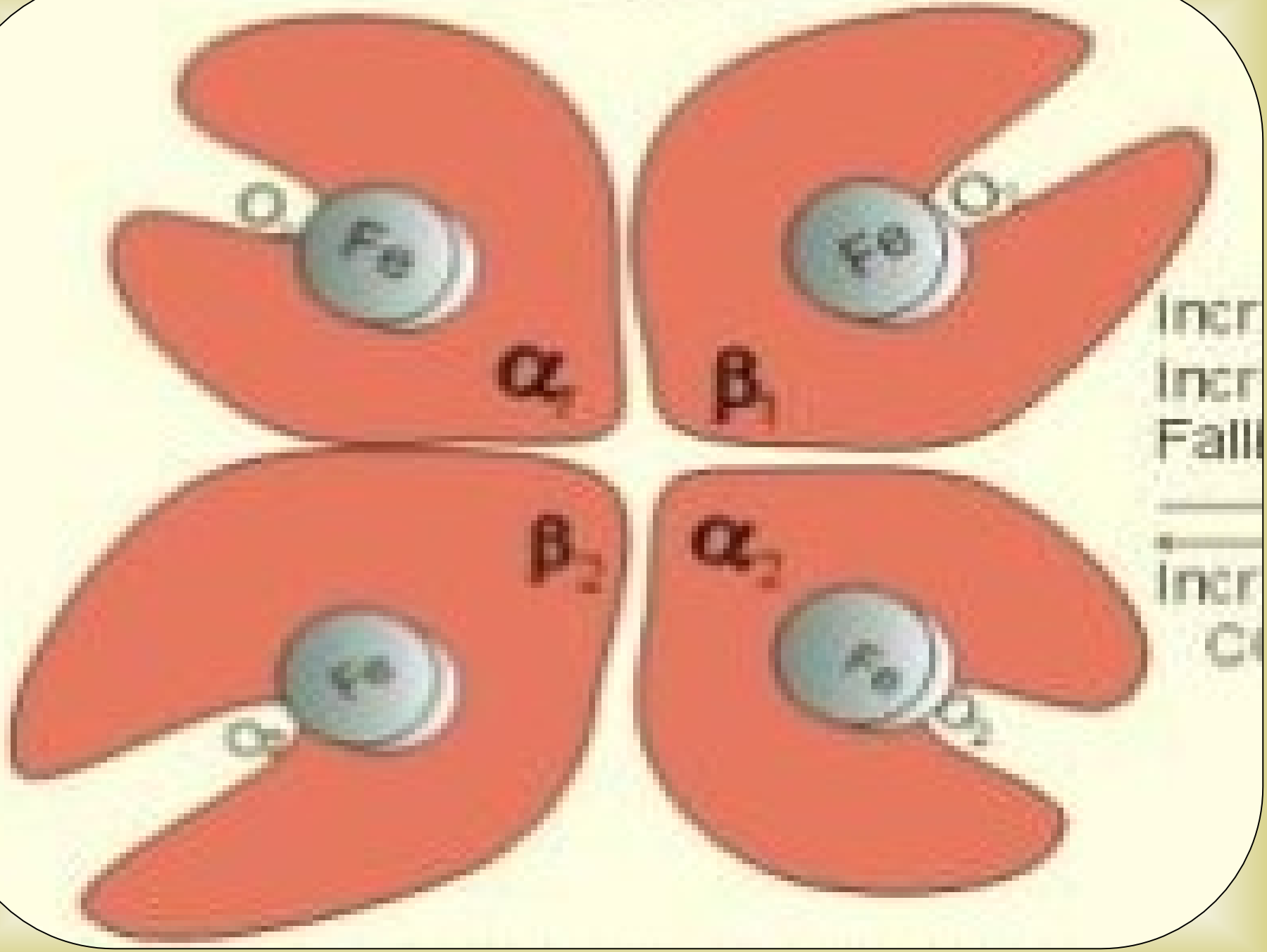
Hemoglobin overview :

Hemoglobin is the protein molecule in red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from the tissues back to the lungs.

The Estimation of Hemoglobin concentration ; Hb)

Hemoglobin overview :

A. Haemoglobin concentration is reported in grams per deciliter of blood.
B. transports O2 and CO2 between lungs and various tissues
C. Haemoglobin is a chromoprotein consist of the colorless globin molecule attached to four red color haem molecules.
D. The globin molecule consist of two alpha and two beta polypeptide chains.


O
Fe
α1
β1
β2
α2
Incr
Incr
Fall
Incr

Types of Hb:

Hb A or HbA1: is the normal Hb in adults represents about 97% of total Hb. it is composed of 2 α and 2 β chains.

HbA2: first appears 12 weeks after birth- a minor component of normal adult HB

HbF(fetal Hb): . normally synthesized only during fetal development

HBA1C : has glucose residues attached to β-globin chains – increased amounts in (diabetes mellitus DM)

Normal value

1. *Women*: 12.0-16.0 g/dL or 120-160 g/L.
2. *Men*: 14.0-17.4 g/dL or 140-174 g/L.
3. *Children*:

i. 0-2 weeks: 14.5-24.5 g/dL or 145-245 g/L.
ii. 2-8 weeks: 12.5-20.5 g/dL or 125-205 g/L.
iii. 2-6 months: 10.7-17.3 g/dL or 107-173 g/L.
iv. 6 months-1 year: 9.9-14.5 g/dL or 99-145 g/L.
v. 1-6 years: 9.5-14.1 g/dL or 95-141 g/L.
vi. 6-16 years: 10.3-14.9 g/dL or 103-149 g/L.
vii. 16-18 years: 11.1-15.7 g/dL or 111-157 g/L.

Hemoglobin Interpretation

A. Increased values :

- **Physiological** : 1) High attitude. 2) Young age.
- **Pathological** : 1) Dehydration.

B. Decreased values :

- **Physiological** : 1) Fluid therapy.
- **Pathological** : 1) Anemia. 2) Hemorrhage. 3) Blood parasites. 3) Malignant tumors.

Methods for estimation of hemoglobin as follows

1) *Sahli's* **method.**
2) *Drabkin's* **method.**
3) *Automated* **method.**

Hemoglobin estimation by *Sahli's* method (Acid hematin method)

1. Principle

- Hemoglobin + (0.1 N) HCl $\xrightarrow[\text{Globin}]{}$ Acid hematin (brown colour)
- The brown color of compound is matched against a brown glass standard in a comparator.

Apparatus and Reagent

1. Sahli-type Haemoglobinometer consisting :

A. Comparator (box contain on two standard tube).

B. Hb tube (Sahli tube).

- Hb tube marked both in grams and percentage scale.

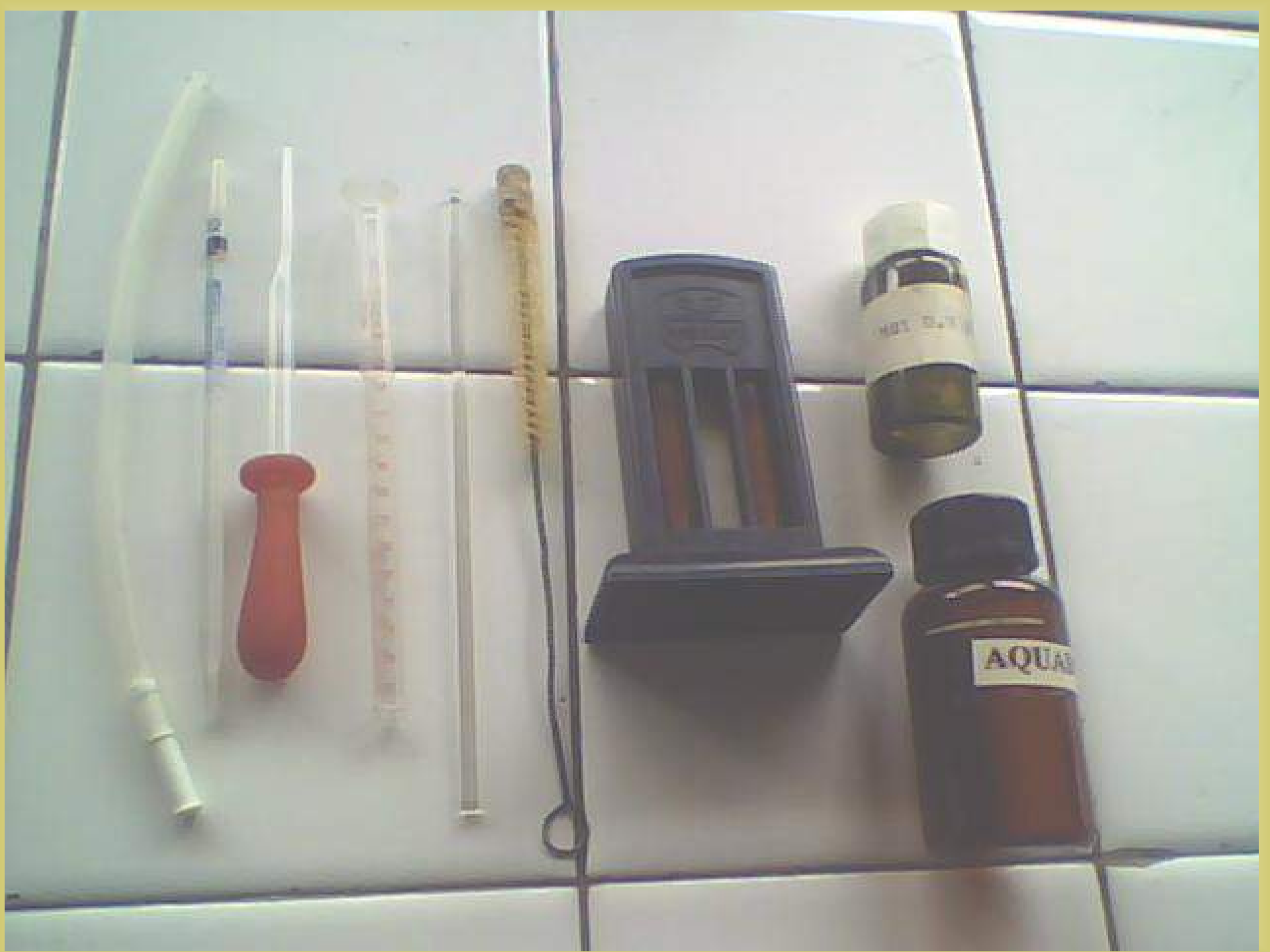
C. Hb pipette (Sahli pipette), (Marked at 20 microliter).

D. Clean brush.

E. Glass Dropper with rubber teat.

F. Glass-rod (Use to mix the solution in tube).

2. (Whole blood +EDTA) or Lancet.
3. Cotton.
4. Distal water.
5. Hcl solution (0.1 N).


AQUA

Sahli Pipette

Procedure

1) Fill the Hb tube to the (20 mark) on percentage scale with Hcl by means of a dropper.
2) Fill the Hb pipette exactly to (20microliter).

- by gentle controlled sucking.
- The pipette is hold horizontally while taking the blood.
- If a slight excess is drawn in, it may be removed by touching the point of pipette with the Cotton.

Procedure

- if a great excess has been draw in, inaccuracy will result, in this case the pipette must be clean ,dried and refilled.
- wipe off with gauze the blood on the outside of the pipette.

3. Empty Pipette contain into tube.

- By keeping the point of the pipette to bottom of the tube and gently blowing off the blood without causing bubbles.

Procedure

- Rinse pipette at least three times by drawing in and discharging the blood acid mixture.
- Hb is converted into acid haematin by Hcl.

4) Mix the solution in the tube by glass rod.

5) Leave the tube for 10 min.

- In this interval at least 95 percent of the color of acid haematin is developing.

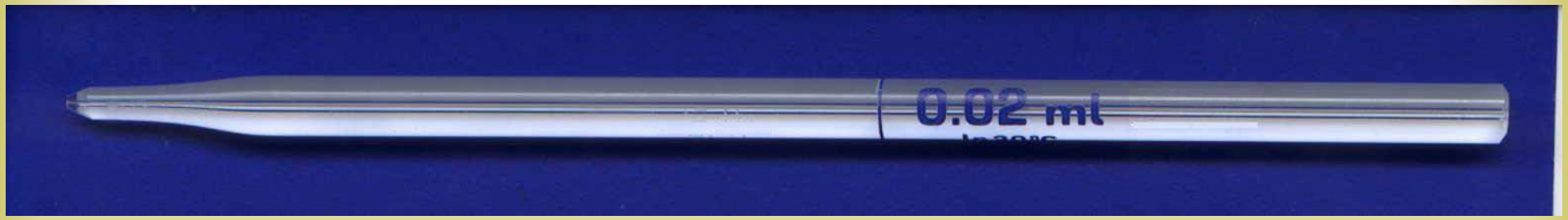
Procedure

6) Now dilute the solution by adding distilled water, drop by drop, stirring and mixture all the time with glass rod until the color matches with standard.

- The bottom of the meniscus is read and on mg/dl scale.


H.B.
METER
(SAHLI'S)
SQUARE
TUBE TYPE
(UNBREAKABLE)


2
10
22
140
(gm%)
(%)


0.02 ml


MARIENFELD
LABORATORY GLASSWARE
Haemometer
Haemometer acc. to Sahli, complete set consisting of
polystyrene support with 2 colored rods and opal glass
plate, comparator tube, haemoglobin pipette 20 µl with
Made in Germany
32 430 00
MARIENFELD
LABORATORY GLASSWARE

Hemoglobin estimation by *Drabkin's* method (Cyannmethaemoglobin method; Photometric method).

Principle

- Hemoglobin + potassium Ferric cyanide ⟶ Methemoglobin +potassium Cyanide ⟶ Cyanmethemoglobin

Apparatus and Reagent

1. Colorimeter at 540 nm.
2. Pipette 0.02 ml.
3. Test tubes
4. Blank solution.
5. Standard solution.
6. Specimens.
7. Drabkin's solution.

: component of Drabkin's solution :

- Potassium cyanide (50mg)
- Potassium ferric cyanide (200mg)

Dissolve in 1 liter of distal water.


163
Hb. g/l
TECHNOMEDICA
MiniHEM 540
PORTABLE PHOTOMETRICAL HEMOGLOBINOMETER

Procedure

Calibration :

In to three clear and dry test tubes adds solution as describes bellows :

1) First tube add 5.0 mL of Hb diluting reagent which serves as a blank .
2) Second tube add 5 mL of Hb diluting reagent and 0.02 mLof Hb standard.
3) Third tube add 5 mL of Hb diluting reagent and 0.02 mL of blood.
4) Leave tubes to 10 min and mix well in room temperature to convert hemoglobin to cyanmethemoglobin.

Procedure

5. Set the wavelength of photoelectric colorimeter at 450 nm.
6. Pour the blank (tube1) into the cuvette. Set the optical density to zero.
7. Pour standard (tube2) into the cuvette and record the optical density (O.D)
8. Pour specimen (tube3) into the cuvette and record the optical density.
9. Calculating may be used

$$\mathbf{Hb=\frac{O.D\ of\ specimen}{O.D\ of\ standard}*\frac{conc.\ of\ standard(60mg/dl)*diluting\ factor(250)}{1000}*10}$$

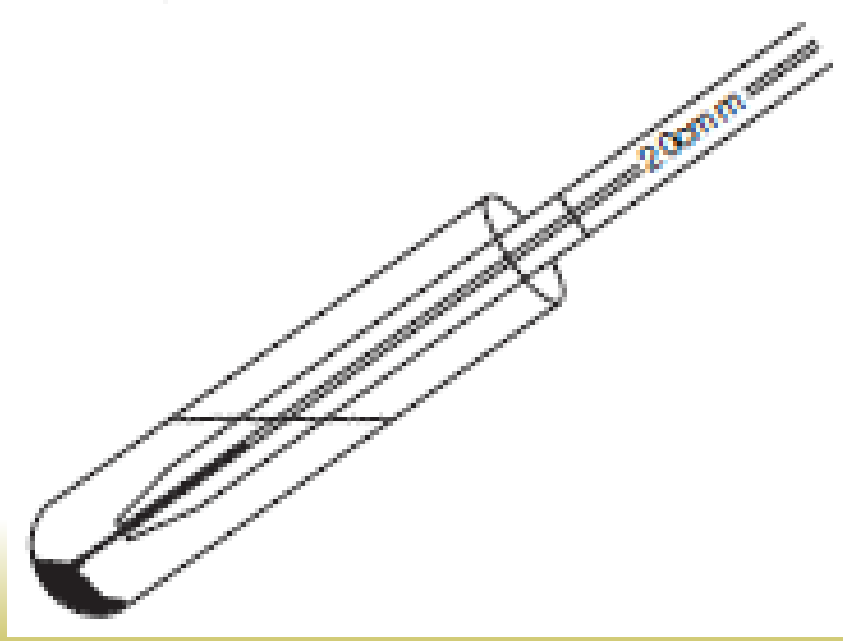
Hemoglobin estimation by *Automated* method

- **Principle**
- Adding lyse in the blood, the red blood cell will be broken and release hemoglobin. hemoglobin and lyse form a new mixture, which can absorb the wavelength 540 nm. measure the absorbency. through the comparison of the absorbency between the pure diluent and the sample- the concentration of the sample hemoglobin is calculated.

HemoCue Hb 301


20cm
20cm


20cm


20cm

HINOTEK AE-11D
Photoelectric Colorimeter

PRACTICAL PHYSIOLOGY

ERYTHROCYTES COUNT (RED BLOOD CORPUSCLES COUNT; RBC)

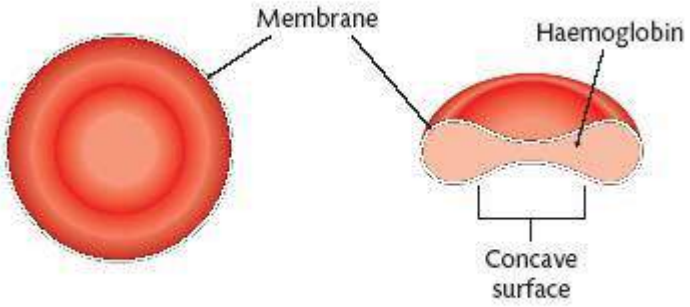
Edition by
Dr.Firas Alaasam
M.Sc Pharmacology & Toxicology

ERYTHROCYTES STRUCTURE:

- Biconcave disk shape; ideal for gas exchange.
- Mature cells are do not have nucleus (anucleate) and contains a hemoglobin.
- Red blood corpuscles count reported : cells per cubic millimeter (cells/mm^3)

i. Normal male count: 5.1 - 5.8 million.

ii. Normal female count: 4.3 - 5.2 million



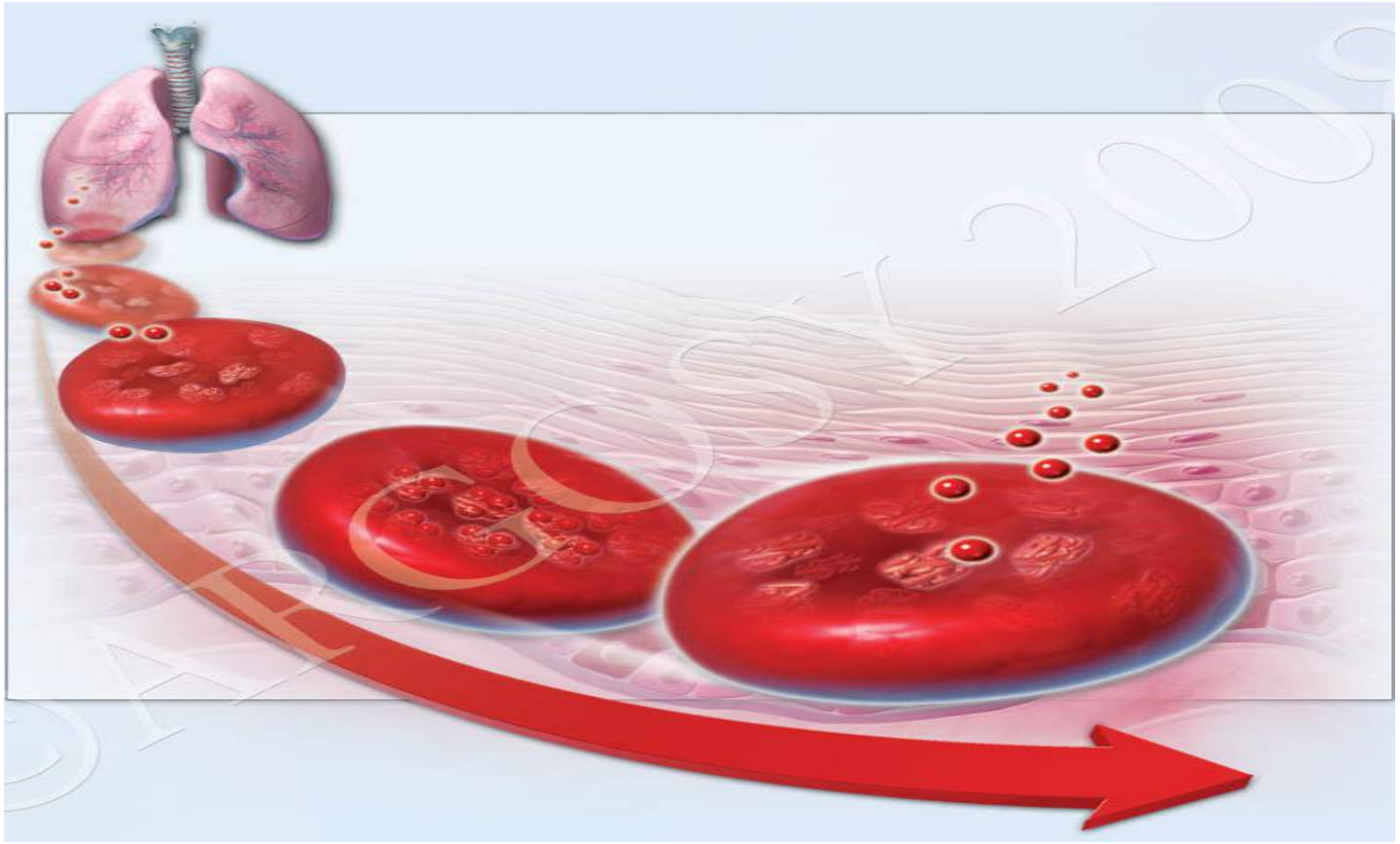
ERYTHROCYTES

- An RBC count is a blood test that measures how many red blood cells (RBCs) you have.

- RBCs contain Hemoglbine which carries oxygen. How much oxygen your body tissues get depends on how many RBCs you have and how well they work.

ERYTHROCYTE FUNCTION :

(Oxygen & Carbon Dioxide Transport)



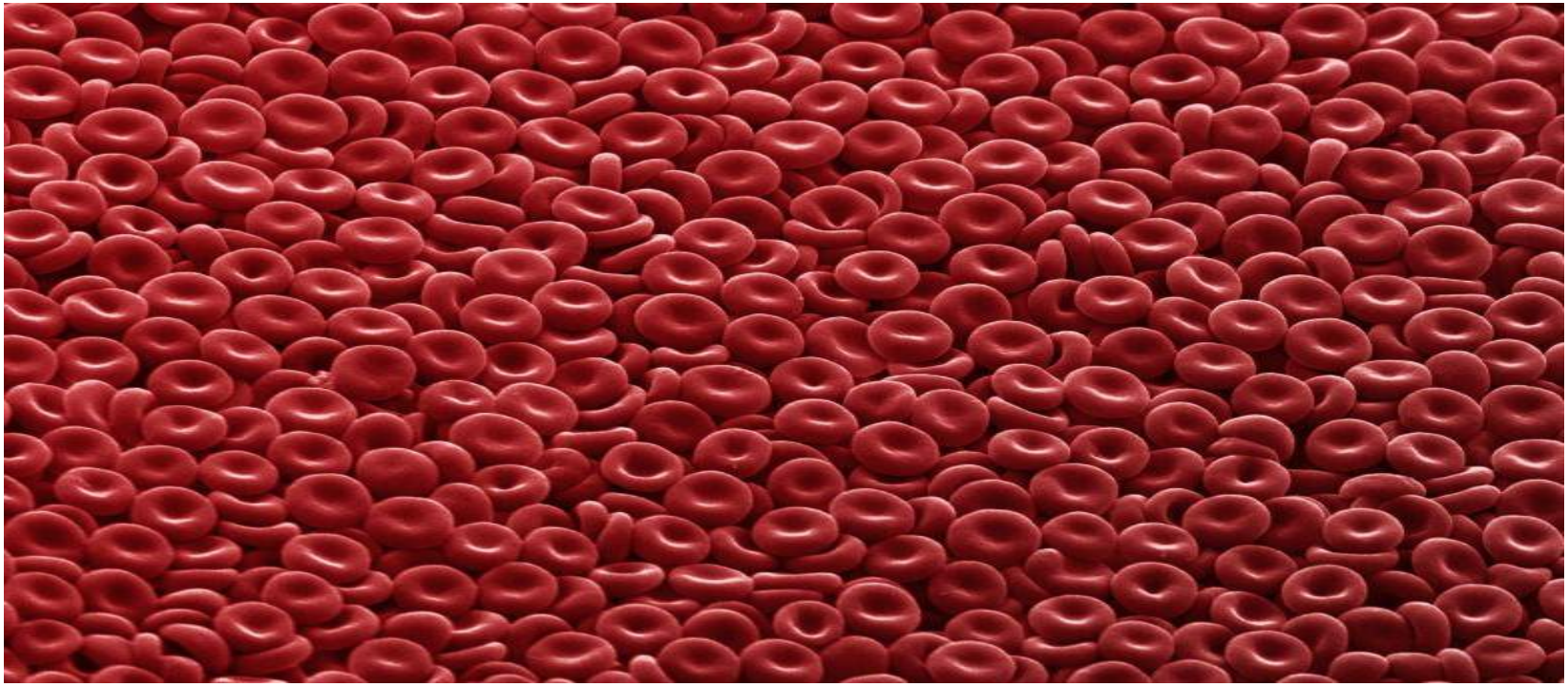
ERYTHROCYTE DISORDERS

Anemia, , is usually defined as a decrease in the . amount of red blood cells (RBCs) or hemoglobin in the blood It can also be defined as a lowered ability of the blood to carry oxygen

- Anemia
- Bleeding
- Bone marrow failure (for example, from radiation, toxins, or tumor)
- RBC destruction (hemolysis) due to transfusion, blood vessel injury, or other cause
- Malnutrition
- Bone marrow cancer called multiple myeloma
- Nutrition deficiencies of iron, copper, folic acid, vitamin B6, or vitamin B12
- Pregnancy

POLYCYTHEMIA

is usually defined as a increase in the amount of red blood cells (RBCs) or hemoglobin in the blood

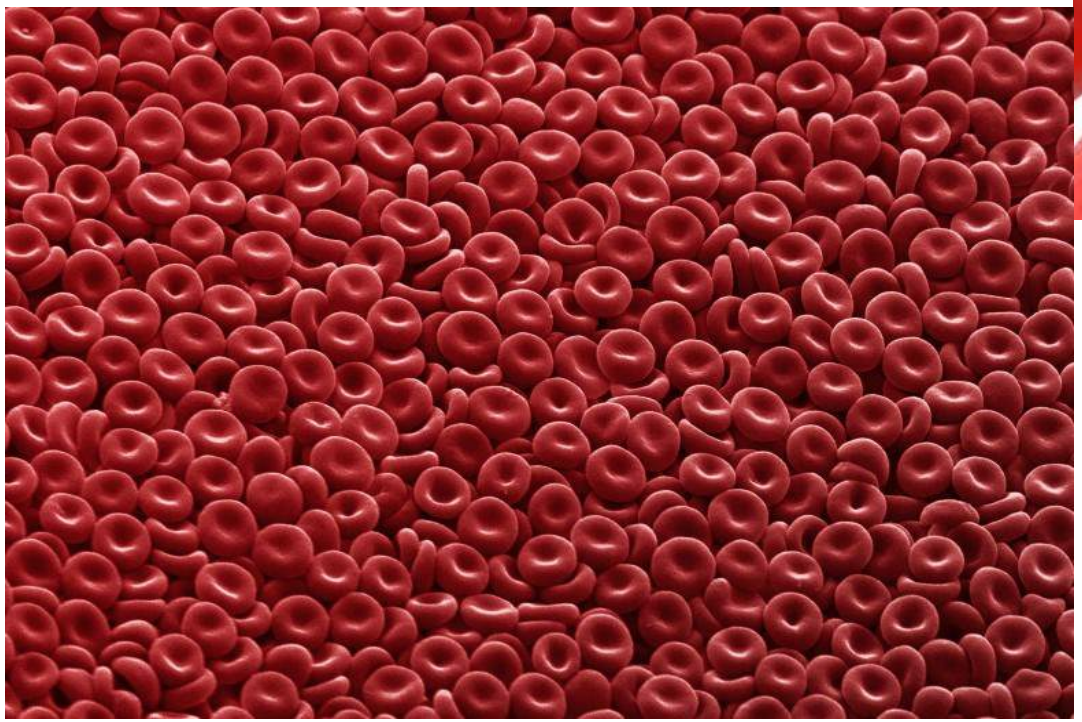


- **Cigarette smoking**
- **Problem with heart's structure and function that is present at birth (congenital heart disease)**
- **Dehydration (such as from severe diarrhea)**
- **Kidney tumor (renal cell carcinoma)**
- **Low blood oxygen level (hypoxia)**
- **Scarring or thickening of the lungs (pulmonary fibrosis)**
- **Bone marrow disease that causes abnormal increase in RBCs (polycythemia vera)**

ERYTHROCYTE Disorders :

⦿**Anemia :** decreased RBC count.

⦿**Polycythemia** : increased RBC count.



DILUTING FLUID

(NORMAL SALINE_0.9% NACL).

Sodium chloride……9 gm.

Distilled water…..1 L.

HAYEMS FLUID :

Mercuric chloride..0.5 gm.

Sodium chloride….1 gm.

Sodium sulphate….5 gm.

Distilled water….to 200 ml.

TRI-SODIUM CITRATE SOLUTION

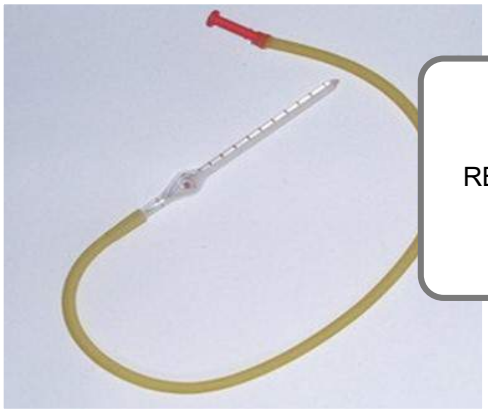
- Trisodium citrate.....3.8 gm.
- Formalin.....1 mL.
- Distilled water……..99 mL.

APPARATUS AND REAGENT

RBC count

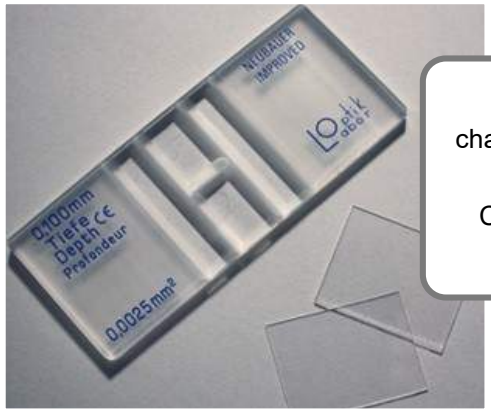
Hemocytometer

RBC count



RBC Pipette

RBC count



chamber slide
AND
Cover slip

1) Cotton.
2) (Whole blood +EDTA) or Lancet.
3) Shaker.
4) Gauze.
5) Petri-dish.
6) Distal water.
7) Wood-stick.
8) Microscope.

PROCEDURE

1. Draw blood up to (0.5) mark by RBC pipette.

- Must be clean and dry.
- In case in EDTA tube must be mix before draw.
- In case draw blood direct patient let first drop.

2. Wipe tip by cotton.
3. Draw the diluting fluid up to (101) mark.
4. Wipe tip by cotton.

- In (2,4) Because cotton will absorb extra blood or diluting fluid and insure from draw quantity and same time clean the tip pipette to prevent contamination.
- In (2,3) Avoid from hose (mouthpiece) not kink.

PROCEDURE

5. Pipette should be gently rotated to obtain good mixing.

⦿In (4) by shake must be with the pipette end sealed with your finger.

6. Prepared of Hemocytometer .

⦿Must be clean and dry both (Hemocytometer and cover slide).

⦿Place over slide with some drop of water in four angle to settled above Hemocytometer.

PROCEDURE

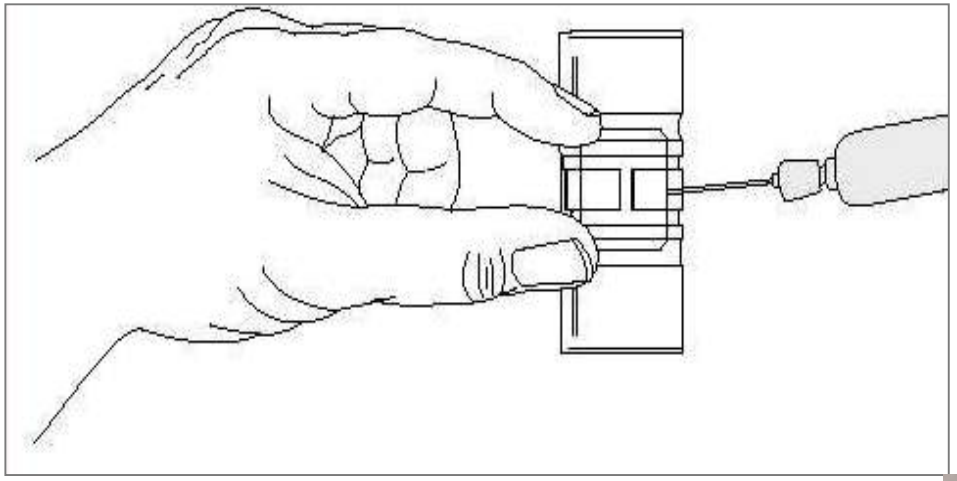
7. Load Hemocytometer with sample.

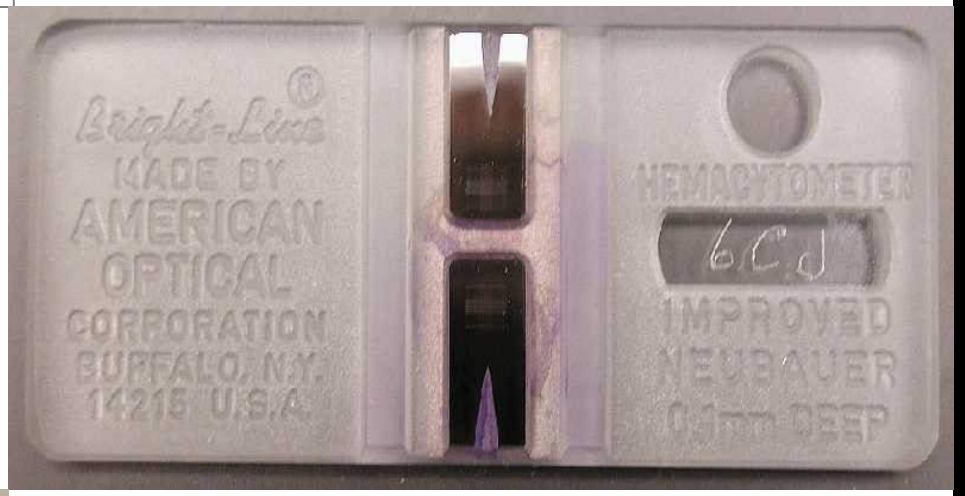
- It must be clean and dry.
- Mix the contents of pipette for 3 minute.
- Expel about (6 drop) or 1/2 pipette stem.
- To expel pipette stem content that did not mix between blood and fluid.
- By holding pipette at an angle (45 degree) and touching the space between cover slide and Hemocytometer than add drops of mixture is allowed to run under cover slide by capillary action.
- Avoid air bubbled.
- It should flow in to fill. (Do not over fill).
- Let the preparation sit for a minute.

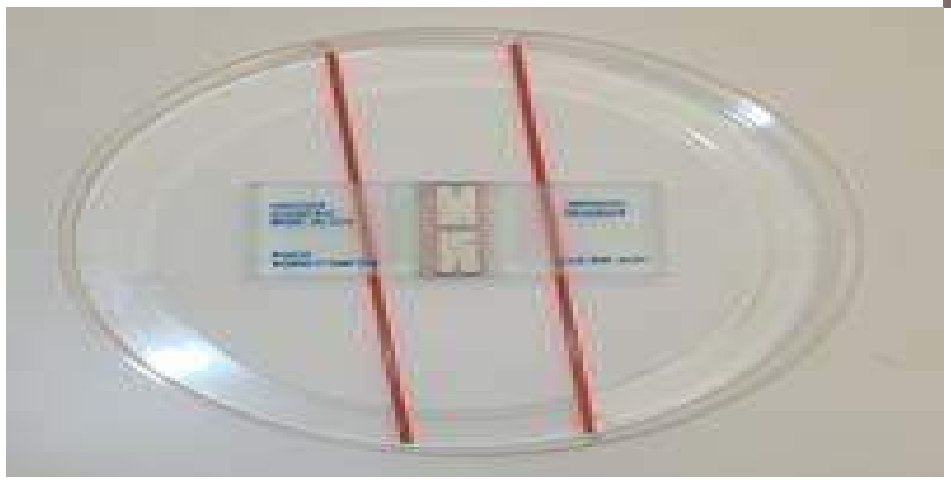
PROCEDURE

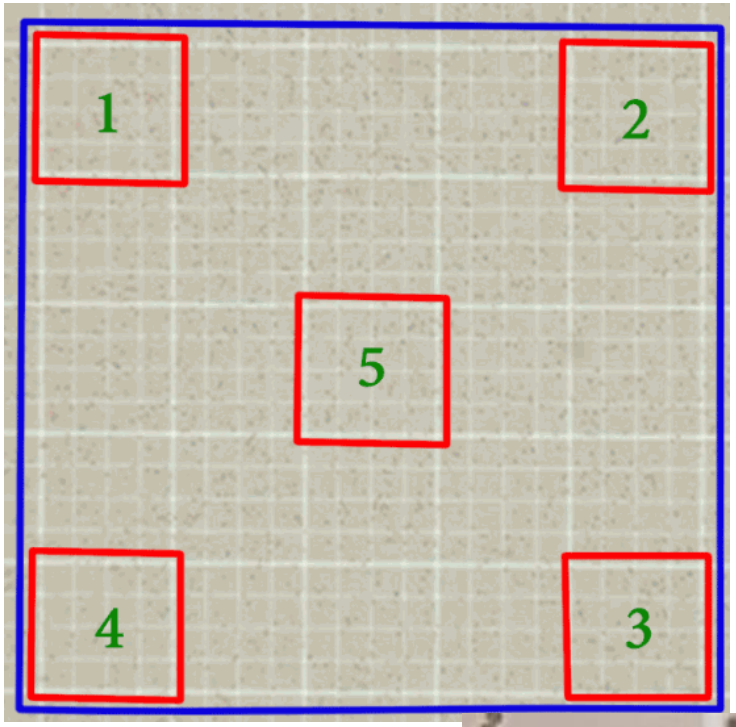
8. For cells to settle are count easy.

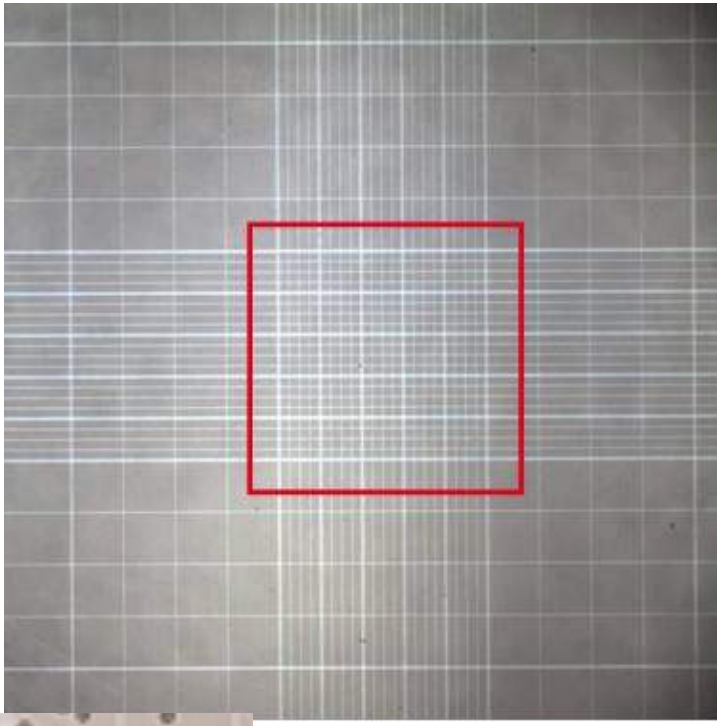
- Through period mute be washing RBC pipette and mouthpiece with soap and water, finish with distilled water rinse, to prevent from clotting blood and error during next tests.
- Examine under microscope.
- In case let sample without examine place in Petri dish contain on little of moist gauze to prevent dry sample.
- Wash the hemocytometer, with soap and water, finish with distilled water rinse.

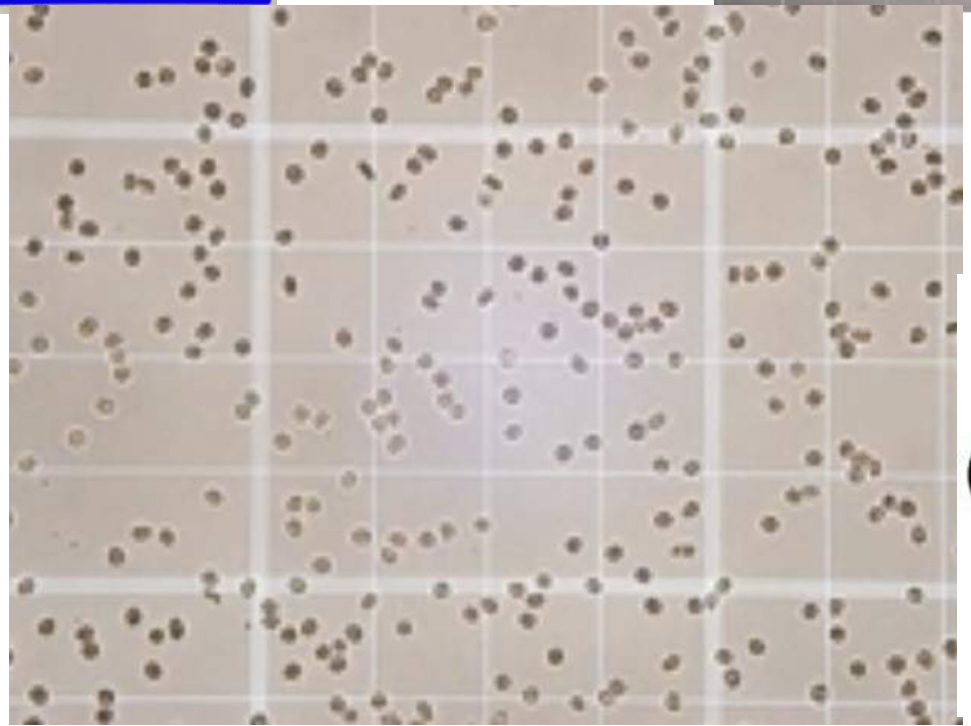



Bright-Line®
MADE BY
AMERICAN
OPTICAL
CORPORATION
BUFFALO, N.Y.
14215 U.S.A.
HEMACYTOMETER
IMPROVED
NEUBAUER




1
2
5
4
3






1 2 3 4

CALCULATION

$$RBC count(cell / mm^3) = N * 10.000$$

PRACTICAL PHYSIOLOGY

Estimation of Packed cell Volume (PCV)

EDITION BY

DR.FIRAS ALAASAM

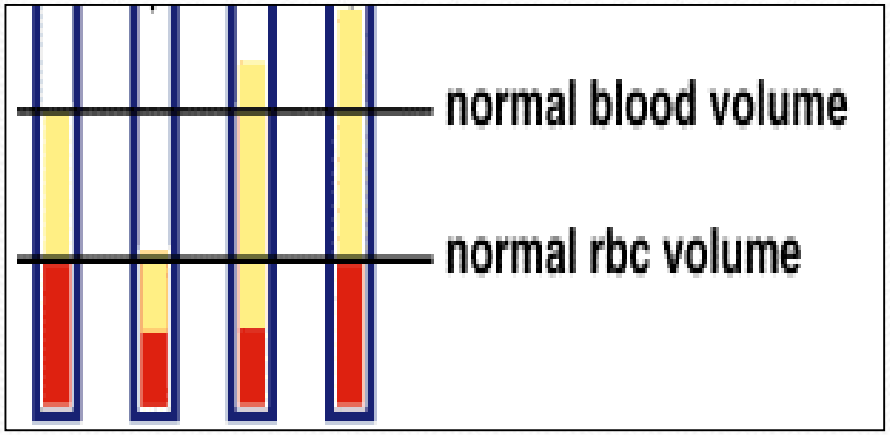
&

M.SC PHARMACOLOGY & TOXICOLOGY

3 lab

Hematocrit (Ht or HCT) or Packed cell Volume (PCV)

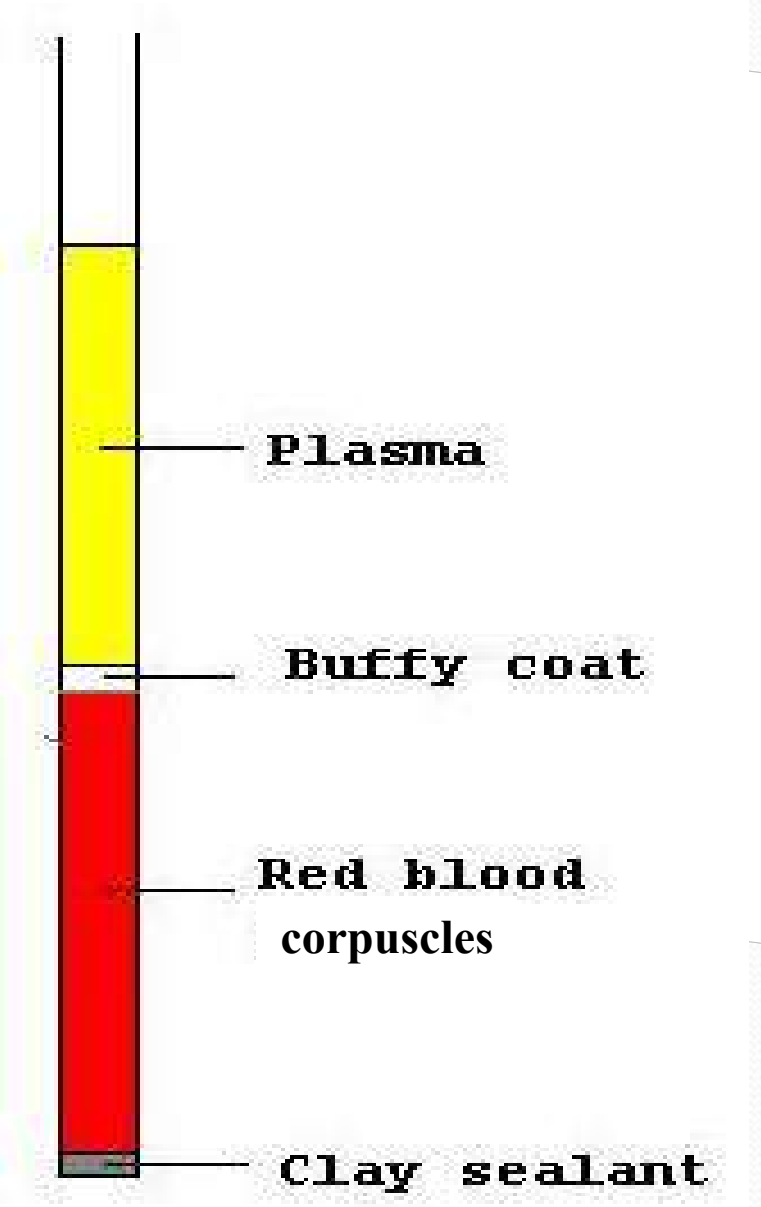
- The percentage by volume of packed red blood cells in a given sample of blood after centrifugation
- PCV, the volume of packed red cells in milliliter s per 100 ml of blood.
- usually reported as the volume percentage (%) of red blood cells in blood.



PCV can be determined by centrifuging heparinized blood in a capillary tube.*

- In a centrifuge blood is separated into three layer including :-

1. **The mass of the erythrocytes** at the bottom which is referred to as packed corpuscles volume (P.C.V).
2. White or gray layer of leukocytes and thrombocytes immediately above the red corpuscles mass that referred to as the **buffy coat**.
3. The blood **plasma**.


Plasma
Buffy coat
Red blood
corpuscles
Clay sealant

Normal Value :

- Women: 36% - 48% or 0.36-0.48.
- Men: 42% - 52% or 0.42-0.52.
- Children:
- 0-2 weeks: 44%-64% or 0.44-0.64.
- 2-8 weeks: 39%-59% or 0.39-0.59.
- 2-6 months: 35%-49% or 0.35-0.49.
- 6 months-1 year: 29%-43% or 0.29-0.43.
- 1-6 years: 30%-40% or 0.30-0.40.
- 6-16 years: 32%-42% or 0.32-0.42.
- 16-18 years: 34%-44% or 0.34-0.44.

Apparatus and reagent :-

- Sample (EDTA tube or lancet).
- Microhematocrit tube (disposable heparinize Capillary tube, red ring).
- Microhematocrit centrifuge.
- Microhematocrit reader.
- Placticine clay.

Fill hematocrit tube to about three-fourth of its length.

EDTA tube

*Use: no heparin capillary tube

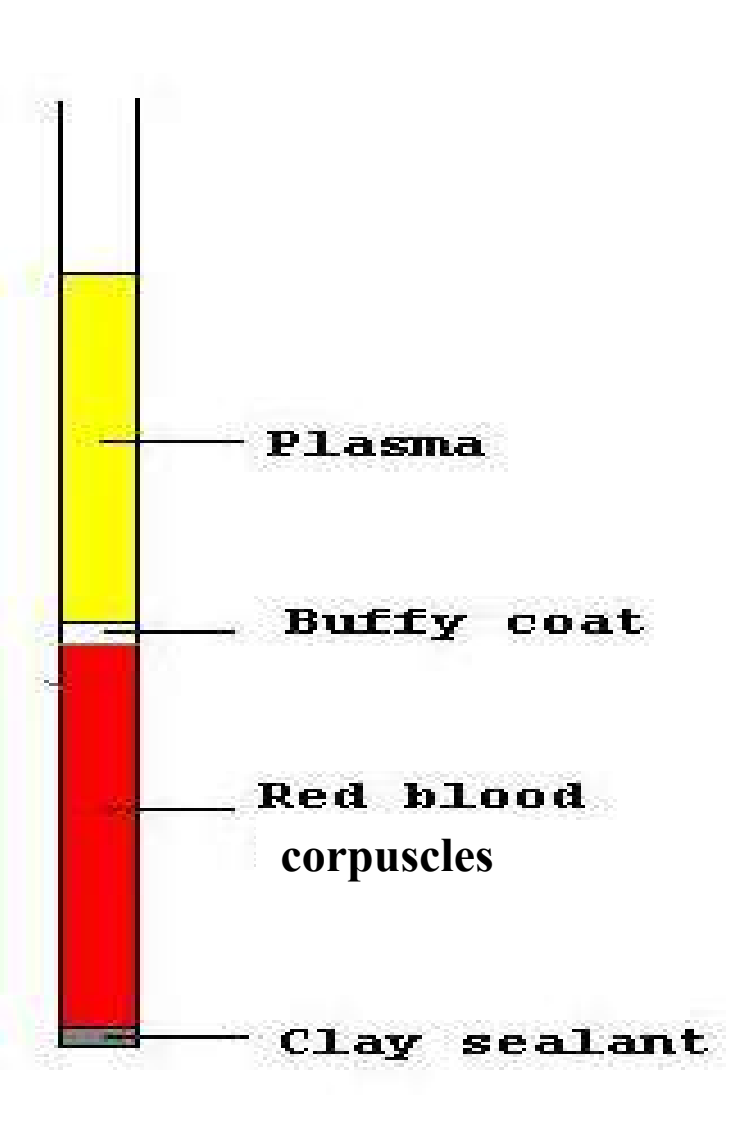
Lancet

Use: heparin capillary tube

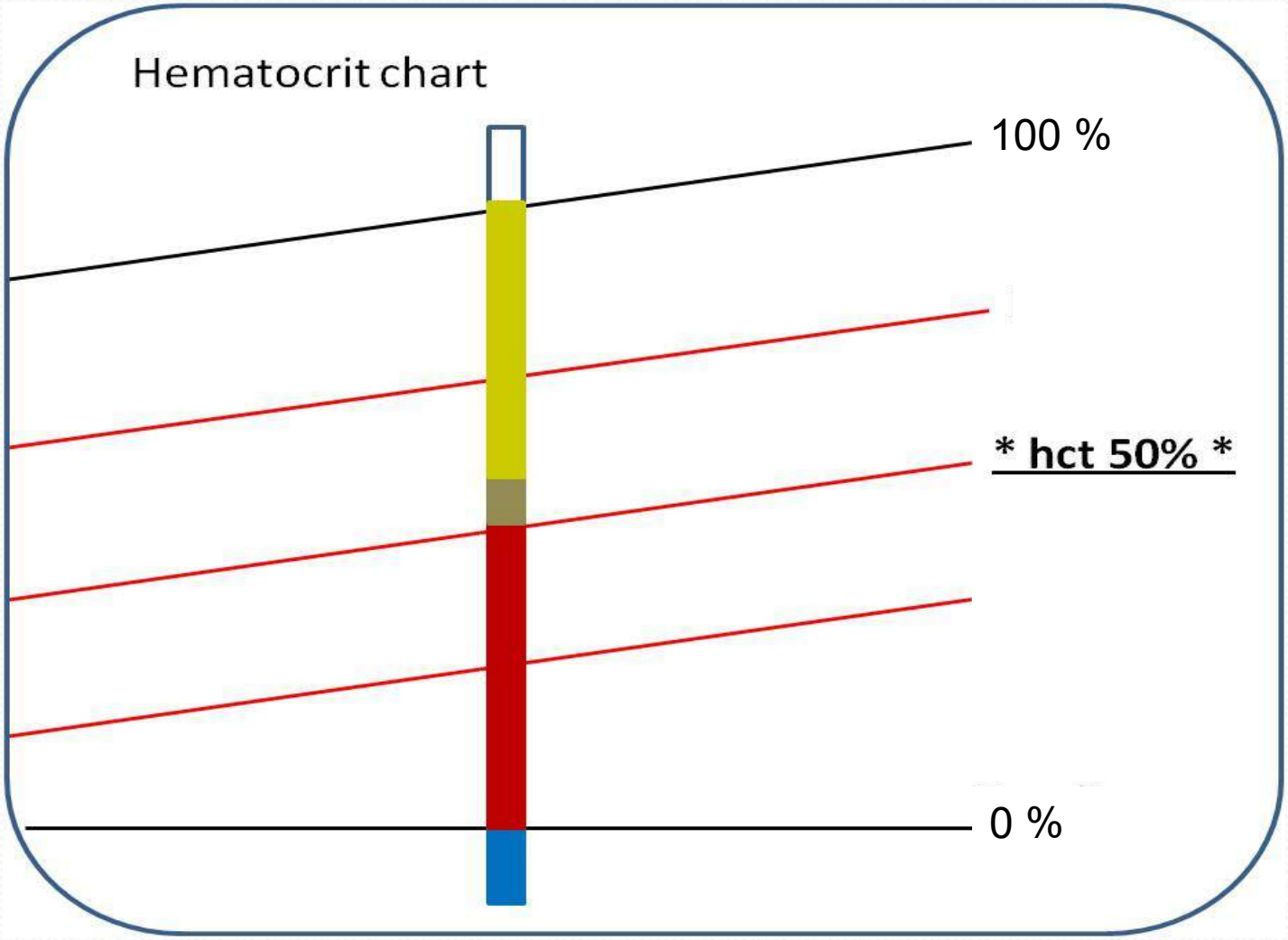
Place finger over the "non-blood" end of the tube and push the opposite end into a clay sealant 3-4 times (clay length about 1 cm)

Place the hematocrit tube in a centrifuge with the clay end toward the periphery. Screw on the safety cover then close the lip and spin. Centrifuge (3 minutes at 15,000; 5 minutes at 10,000).

Allow the Centrifuge to stop on its own; do not hand brake.



Place centrifuged hematocrit tube on a reader with the top of the clay sealant at the 0% mark and the top of the plasma layer at 100%. And seen level of RBC.



Hematocrit Interpretation

1-Increased PCV

-Polycythemia: newborns,high altitude,hypoxia due to lung and heart dieases

- Congestive heart failure,burns(loss of plasm),dehydration,sever exercise,

2- Decreased PCV

-All types of Anamea

-Pregnancy

-Ingestion of large amount of water

Procedure

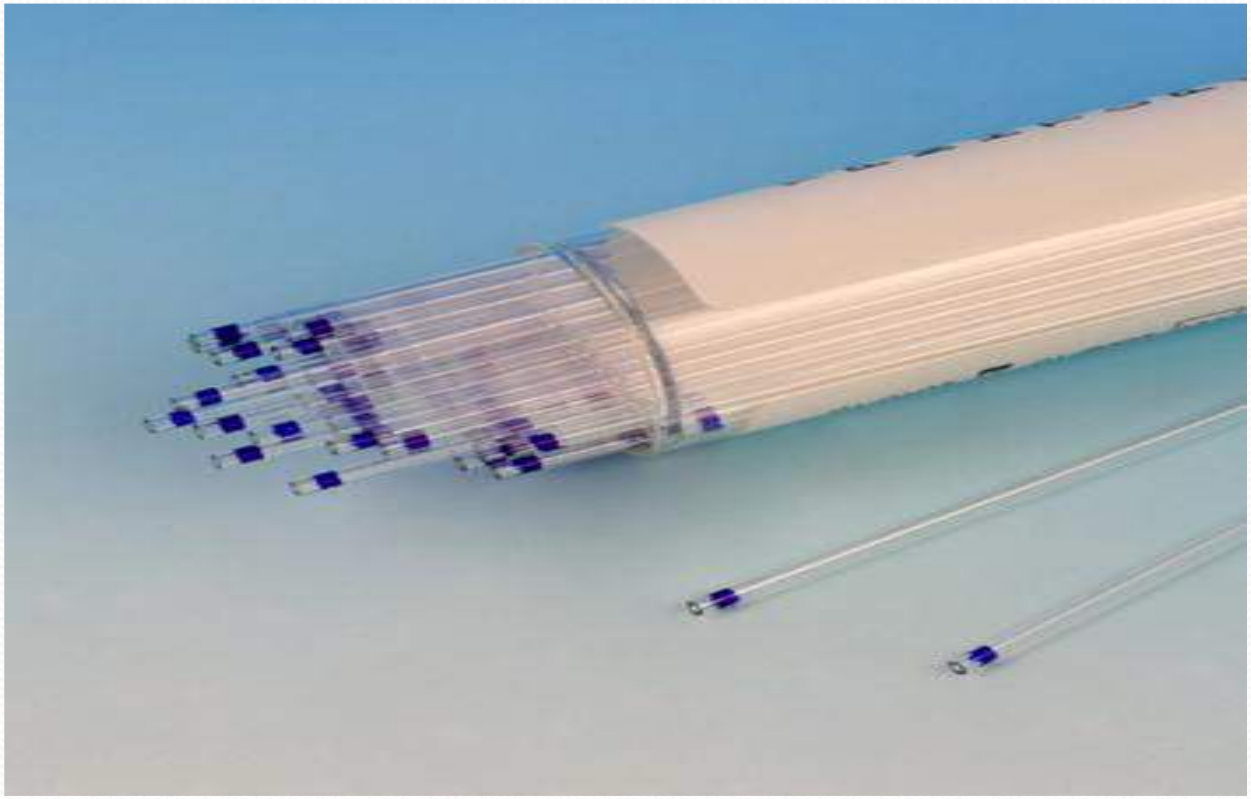
1. Fill two hematocrit tube per patient, Once adequately mixed and full with blood containing anticoagulant. If blood is obtained directly from the patient, use a hematocrit tube containing an anticoagulant. the unmarked end of capillary tube is placed in the blood and permitted to fill rapidly (by hold horizontally) to approximately three-fourth of its length. Then removed from the blood and wiped excess blood outside tube of by cotton.
2. Place finger over the "non-blood" end of the tube and push the opposite end into a clay sealant 3-4 times (clay length about 1 cm).
3. Place the hematocrit tube in a centrifuge with the clay end toward the periphery. Screw on the safety cover then close the lip and spin. Centrifuge for 2 - 5 minutes. (3 minutes at 15,000; 5 minutes at 10,000).

Procedure

4. Allow the Centrifuge to stop on its own; do not hand brake.
5. Place centrifuged hematocrit tube on a reader with the top of the clay sealant at the 0% mark and the top of the plasma layer at 100%.
6. Read the % of RBC which is read at the top of the RBC layer, do not include the buffy coat.
7. Automated by ((Hct %=RBC*MCV*100)).

Procedure

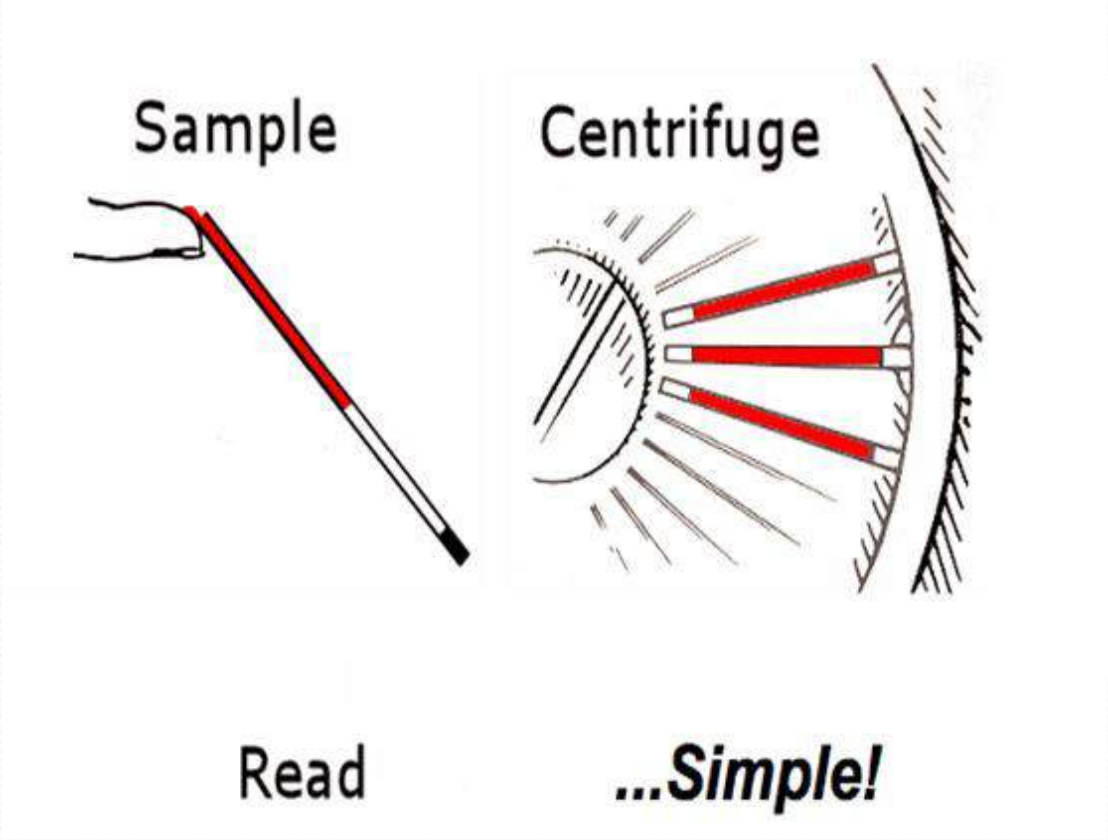
1. Capillary tube is used



2. The capillary is filled with blood to half of the tube , but not filled too much

3. Sealing the capillary with clay (cement)

4. Centrifugation (10000 rpm ad two minute)



5. Reading the Hct