Hashemite University

Faculty of Allied Health Sciences

Department of Medical Laboratory Sciences

Human Physiology Laboratory Manual

2018

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Grading :

A. Exams:
   1. Midterm exam: 35 marks.
   2. Final exam : 45 marks.

B. Report + Quizzes : 20 marks.

Notes:

- Lab coat is a must.
- Attend your assigned lab sessions.

Session No. 1

The osmotic fragility of Red Blood Cells

Introduction:

The cell membrane acts as a semi-permeable membrane, allowing the passage of water into the cell, but not the movement of salts out of the cell. When RBC`s placed in distilled water they swell, burst and then hemoglobin released. The breakdown of the cell and the liberation of hemoglobin are known as hemolysis. It is due to osmosis. According to the effect of the solutions on the RBCs, we can classify them into **Figure (1):**

1-Hypertonic solution: The solution contains a higher concentration of solutes than are present in the RBCs cells, water leaves the cell faster than enters causing the cell shrink.This shrinkage is called crenation.

2-Hypotonic solution: A solution that has a lower concentration of the solutes than are present in the cell. In such solution, water flows rapidly into the cell causing them to swell and may burst (lyse) as the plasma membrane disintegrates and finally the cell membrane ruptures.

3- Isotonic solution: A solution that contains the concentration of the solutes as the cell. Blood cells immersed in such solution gain and lose water molecules at the same rate establishing an osmotic equilibrium.

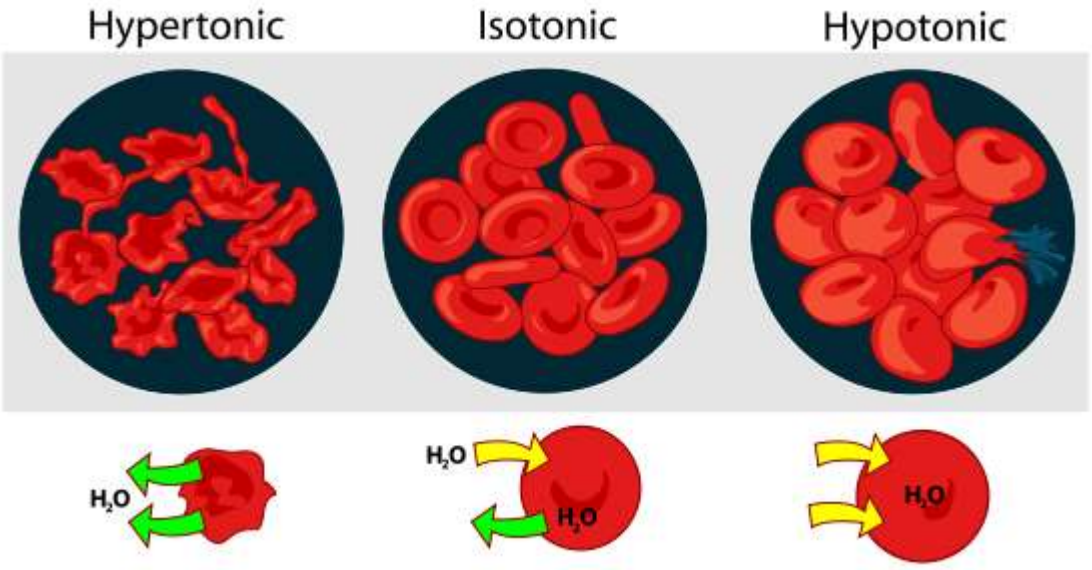


Figure (1) Different concentration of solution that affect RBCs

Material:

1- One test tube rack containing ten test tubes.

2 - Volumetric pipette (2ml)

3- A dropping pipette with a rubber teat.

4- Blood obtained by vein-puncture from a volunteer.

5- Nacl 1%.

6- 0.3 molar of Urea.

8- 0.3 molar of Glucose.

9- Soap.

10- 70% Alcohol.

Exercise 1:

1. Label tubes from one to ten in sequence.

2. Prepare from 1% Nacl solution different solutions of a progressively increasing concentration of Nacl (0.3 up to isotonic saline of 0.9 concentrations) as the **table (No 1) Figure (2).**

3. Dispense one drop of blood to each of the 10 test tubes. Mix well and let stand for 3 minutes.

4. Centrifuge the test tubes at 3000 RPM for 2-3 minutes.

5. Hold the rack of tubes up to the light and compare them. If the solution is red and transparent, hemolysis has occurred. If the solution transparent and there is a precipitate at the bottom, remix it

6. Report the Nacl concentration at which:

A) Complete hemolysis occurred

b) Partial hemolysis occurred

c) No hemolysis occurred.

7. Explain the results.

Table (1)

Solution	**Tube No**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Nacl **1**% (ml)	1.8	1.6	1.4	1.2	1.1	1	0.9	0.8	0.7	0.6
H2O (ml)	0.2	0.4	0.6	0.8	0.9	1	1.1	1.2	1.3	1.4
% Nacl	**0.9**	**0.85**	**0.7**	**0.6**	**0.55**	**0.5**	**0.45**	**0.4**	**0.35**	**0.3**

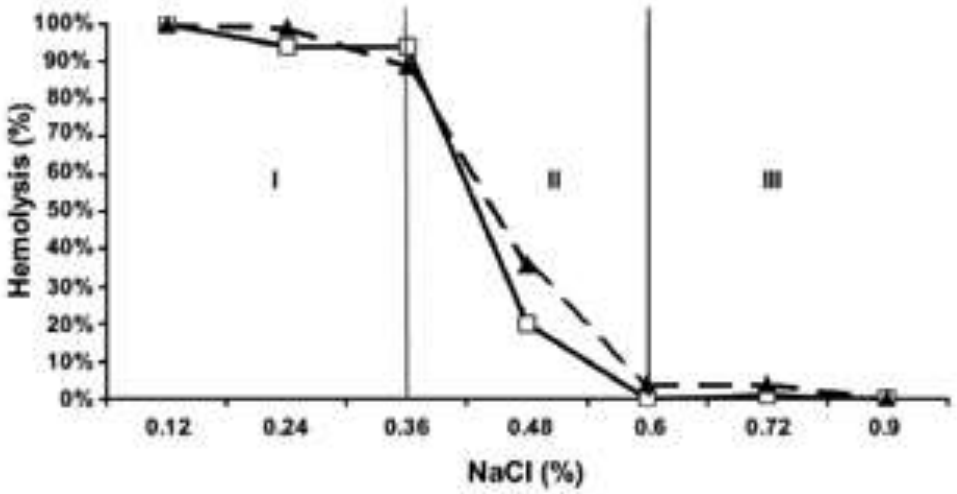


Figure (2)

Exercise 2;

To see the osmotic effect of substances that penetrate or damage the cell membrane:

1. Prepare 10 ml of each of the following substances (see table 2).
2. Dispense a drop of blood to each tube, mix well then let stand for 3 minutes.
3. Centrifuge at 3000 RPM for 1 minutes.
4. Discuss the results of hemolysis and speed of hemolysis related to the type of tested substances and their osmolarities.

Table (2)

Type	Substance	Molarity	Osmolarity	Permeability	Hemolysis
Ionic	Nacl	0.15	0.3	Non - permeable	No hemolysis
Polar Small size	Urea	0.3	0.3	Freely permeable	Complete hemolysis
Polar Large size	glucose	0.3	0.3	Selectively permeable	No hemolysis
Detergent	Nacl + Soap	-	-	Damages cell membrane	Complete hemolysis
Organic solvent	Alcohol	-	-	Dissolve the lipid of Cell membrane	Complete hemolysis

Session No. 2

Composition of blood and the total white blood cells (Leukocytes) count

Introduction:

Blood composed of the following:

1. **Plasma (55%):** contains water, salts, plasma proteins, and some substances that are transported by blood.
2. **Cellular elements (45%):** erythrocytes, leukocytes and platelets.

Blood collection:

There are many different ways to collect blood samples and these ways depend on the purpose of what blood is collected for:

There are two major ways to collect the blood:

1. Capillary puncturing:

The puncture is usually made on the middle finger of the right hand. To increase the circulation, the fleshy portion of the middle finger is massaged. When the finger is punctured, the blood forms a rounded drop so then it can be sucked into a capillary tube. This method usually used if you want to know the type of your blood and doing pcv test (not much blood is needed).

2. Vein puncture:

If few cubic centimeters of blood are required to perform the test, the blood is obtained from a vein. The blood sample can be collected in an anticoagulant tube (tube contains anticoagulant substances such as Sodium citrate, EDTA and Heparin) in this case we can obtain *plasma* or it can be collected in a tube without an anticoagulant substance and in this case we can obtain *serum* (plasma minus fibrinogen).

Introduction:

The leukocytes, or white blood cells, range in the adults of both sexes between 4,000 and 11,000 cells per microliter (or cubic millimeter) of blood. The circulating leukocyte are either granulocytic (neutrophil, basophil, or eosinophils) or agranulocytic (lymphocytes or monocytes). The life span of leukocytes varies according to their function: the range extend from neutrophils, which generally survive a few hours to a few days, to the lymphocytes, which may survive for more than a year.

Abnormal WBC's Count:

1) **Leukocytosis**: An increase in the number of leukocytes above the upper limit of the normal range (11,000 leukocytes per microliter), This increase can be normal protective response to stress such as invading microbes or a malignant proliferation of leukocytes such as in **Leukemia**.

2) **Leukopenia:** A decrease in the number of circulating leukocytes below the lower limit of the normal range (4000 per microliter). It may be caused by certain infections as well as a depression of the bone marrow due to radiation, poisoning, or chemotherapeutic agents**.**

Materials:

1. Hematocytometer (counting chamber)
2. Cover slip
4. WBC diluting fluid (Turk's solution, which consist of 1 ml of glacial acetic acid, 1 ml of gentian violet solution and 98 ml of distilled water): glacial acetic acid destroys RBC's & gentian violet stains WBC's nuclei.
5. Micropipettes (1000µL & 50µL).

Procedure:

1. Mix the blood sample thoroughly.
2. Add 50µL of blood to 950µL of diluent and mix.
3. Clean the hemacytometer with alcohol and let to dry.
4. Place the coverslip over the ruled area.
5. Charge the hemacytometer gently with diluted blood using a capillary tube.
6. Let WBC's to settle for 2-3 minutes.
7. Examine the hemacytometer under microscope as follows:
   a. Under 10× objective lens (to count the cells in 4 W-squares).
   b. In each of W-squares, count cells starting from left to right, then from up to down (zigzag motion).
   c. Count cells that touch any of the upper and left lines only.

Calculation:

1- Find the dilution factor:

$$DCF = \frac{50\mu L + 950\mu L}{50\mu L} = 20$$

DCF= Sample (µL) + Diluent (µL) / Sample (µL)

2- Find the correction volume factor:

$$VCF = \frac{Desired\ volume}{Used\ volume} = \frac{1mm^3}{0.4mm^3} = 2.5$$

Used volume is calculated as the following:

1. The length of the side of each W-square = 1mm
2. The volume of each of these squares =

   $1mm \times 1mm \times 0.1mm = 0.1mm^3$
3. The volume of 4W-squares (used volume for counting) =

   $4 \times 0.1 = 0.4\ mm^3$

$$Total\ WBC's\ Count\ (10^3 / mm^3) = Cells\ counted \times DCF \times VCF$$

So, $Total\ WBC's\ Count\ (10^3 / mm^3) = Cells\ counted \times 50$.

EXAMPLE:

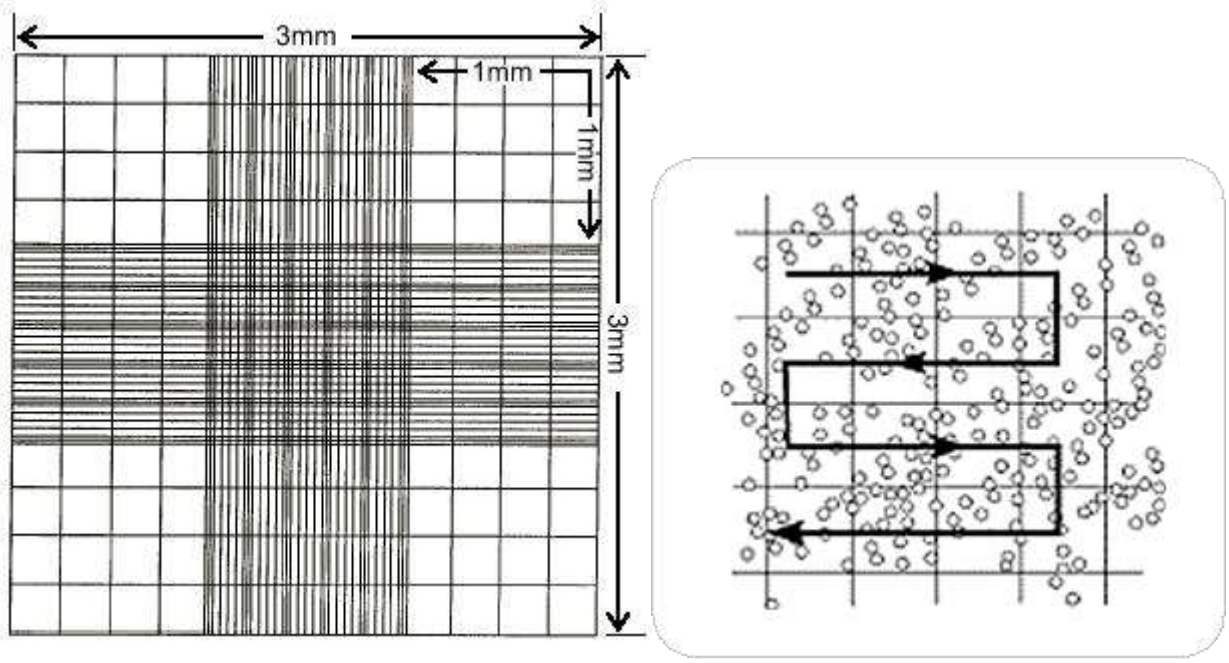
If you count an average of 30 WBC's per sequar your WBC's count is:

30 x4 =120 WBC's.

120 x 50 = 6000 WBC's /mm^3

RESULTS:

……WBC's / mm^3



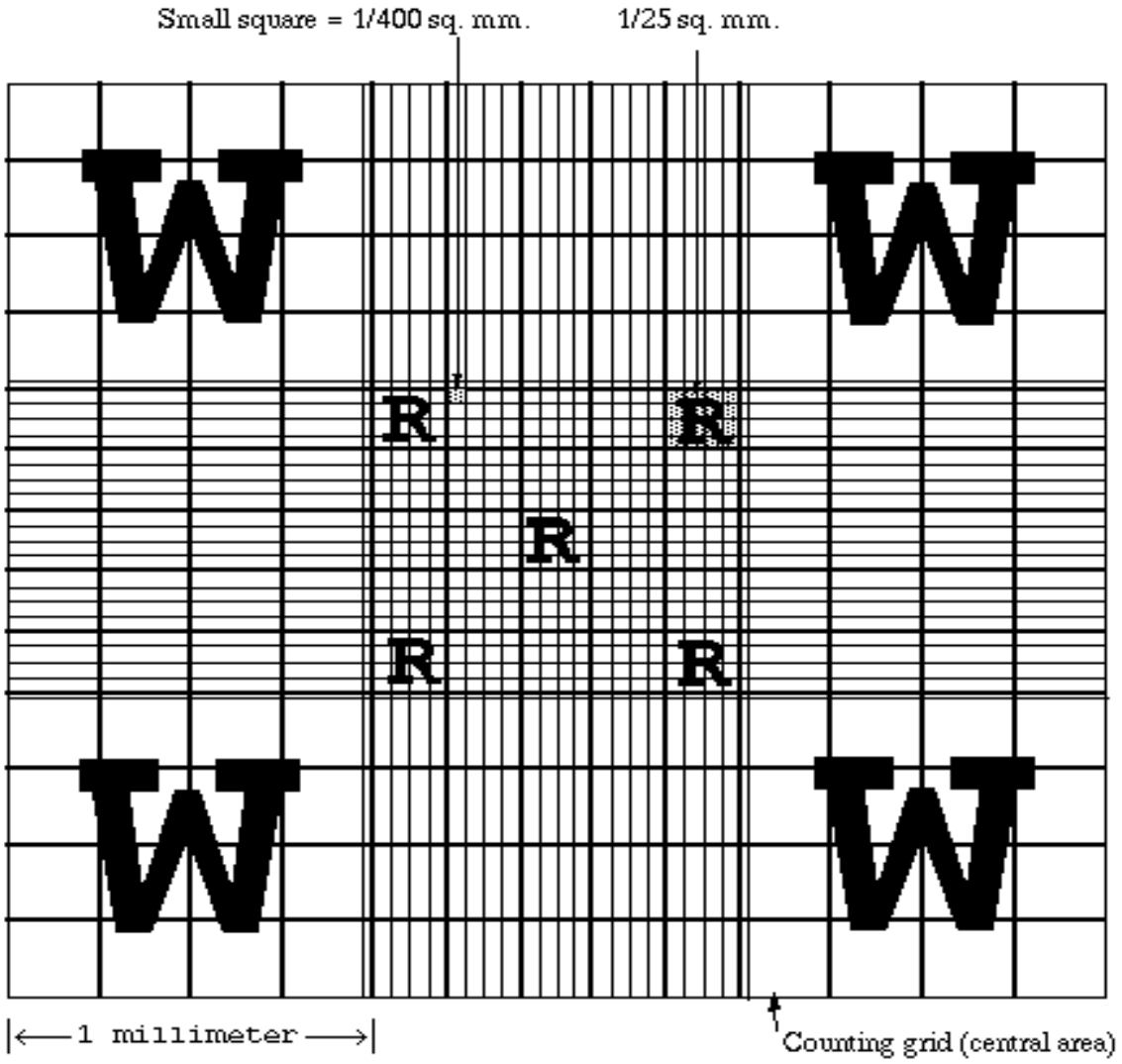


Figure (1): Hematocytometer.

Session No. 3

The total Red blood cells (Erythrocytes) count

Erythrocytes:

- Erythrocytes, or red blood cells, originate in the red bone marrow of the adult, yolk sac, liver, spleen, and red bone marrow of the fetus.
- The mature normal erythrocyte is a biconcave, disc-shaped.
- The diameter approximately 7.5 µm and thickness of 1µm.
- It lacks a nucleus, and its cytoplasm consists of a meshlike framework composed of proteins and lipids within which hemoglobin is bound. Despite the lack of a nucleus, erythrocyte normally lives for 120 days in circulation.
- Their function in oxygen and carbon dioxide transportation.
- The normal values for RBC's vary between males and females due to sex differences. The normal **men** average number of RBC`s per µL (or cubic millimeter) is 5.400,000 (±300,000) and in normal **woman** 4.800, 000 (±300,000).

Abnormal RBC's Count

An excess of RBC's is called **Polycythemia** which occurs in three types:

1. **Relative Polycythemia** (pseudopolycythemia) results from an increase in the concentration of erythrocytes without an increase in the total red blood cell mass, such as may occur in dehydration, severe burn, and shock.
2. **Primary Polycythemia Vera** (true Polycythemia), is an increase in the concentration of erythrocyte, accompanied by an increase in total red blood cell mass due to hyperactivity of bone marrow. In most cases, the cause is unknown, but in others, the Polycythemia may be symptomatic of malignancies in the bone marrow, kidney, or brain. The RBC count may be as high as 11 million/µl.
3. **Secondary Polycythemia** (erythrocytosis) occurs as a physiologic response to chronic arterial hypoxia. The hypoxia may be associated with living in low oxygen-environments (high altitude) or may occur in emphysema, pulmonary fibrosis, carbon monoxide poisoning. Characterized by RBC count as high as 6-8 million RBC/ µl.

A decrease in the number of circulating erythrocytes below normal range constitutes **Erythrocytopenia**: commonly seen in many types of anemia (hemorrhagic anemia, hemolytic anemia, iron deficiency anemia, pernicious anemia and a plastic anemia) due to decrease in RBC count or quantity of hemoglobin or both.

Materials:

1. Hematocytometer (counting chamber)
2. Cover slip
3. RBC diluting fluid (Isotonic solution such as physiological saline or Hayemn's solution)
4. Blood samples (using EDTA tubes).
5. Micropipettes (1000µL & 10µL).

Procedure:

1. Mix the blood sample thoroughly.
2. Add 5µL of blood to 995µL of diluents and mix.
3. Clean the hemacytometer with alcohol and let to dry.
4. Place the coverslip over the ruled area.
5. Charge the hemacytometer gently with diluted blood using a capillary tube.
6. Let RBC's to settle for 2-3 minutes.
7. Examine the hemacytometer under microscope as follows:
   a. Under 10 x objective lens (to ensure an even distribution of cells).
   b. Then, under 40 x objective lens (to count the cells in 5R-squares).
   c. In each of R-squares, count cells starting from left to right, then from up to down (zigzag motion).
   d. Count cells that touch any of the upper and left lines only.

Calculation:

1. Find the dilution correction factor (DCF):

$$\text{Dilution factor} = \frac{Total volume}{Sample volume} = \frac{1000}{5} = 200$$

2. Find the volume correction factor (VCF):

$$VCF = \frac{Desired\ volume}{Used\ volume} = \frac{1 mm^3}{0.02 mm^3} = 50$$

Used volume is calculated as the following:

1. The length of the side of each small R-square =
   1mm/ 5 squares = 0.2mm
2. The volume of each of these squares =
   0.2mm x 0.2mm x 0.1mm = 0.004mm^3

3. The volume of 5 R-squares (used volume for counting) =

5 x 0.004 = 0.02 mm^3

3. $Total\ RBC's\ Count\ (10^6 / mm^3) = Cells\ counted\ (5R) \times DCF \times VCF$

So, $Total\ RBC's\ Count\ (10^6 / mm^3) = Cells\ counted \times 10000$

Example:

If you count 120 RBC`s per square your

RBC count in 5 R-squares is 120 X 5= 600 RBC's.

Total RBC's count = 600 x10000= 6,000,000 RBC's / mm^3

Results:

_____ RBC's/ mm^3.

Session No. 4

Preparation of blood smear and leukocytes differential count

Introduction:

Leukocytes, or white blood cells, are nucleated cells that are formed in the bone marrow from the same stem cells as red blood cells. Basically, white blood cells are protective, pathogen destroying cells that are transported to all parts of the body in the blood or lymph.

They are classified in to two major groups, depending on whether or not they contain conspicuous granules in their cytoplasm. These are **granulocytes** and **agranulocytes**.

The structure and function of each group are discussed in the table (1).

Deviations of the white blood cells from their normal percentages may indicate serious pathological conditions:

Neutrophilia: High neutrophil counts, often a signal of bacterial infection.

Neutropenia: A condition, in which there is a marked decrease in the number of neutrophils,

Eosinophilia: High eosinophil count, may indicate parasitic infection.

Basophilia: Is an increase in the number of basophil, which occurs in allergic reaction.

Lymphocytosis: High lymphocyte count, are present in viral infections.

Monocytosis: an increase in the number of monocytes, which occurs in infectious mononucleosis.

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Materials:

1. Clean glass slides
2. Anticoagulant blood
3. Alcohol
4. Buffer (ph= 6.8)
5 .Romanowsky stains are usually used in staining blood films; they are composed of polychrome **methylene blue** and **eosin**, or we can use Leishman's stain or Wright's stain.
6 .Distilled water.
7 .Immersion oil

Procedure:

1. Label the end of the slide.
2. Carefully place a small drop of the blood in the middle of the slide, being careful not to touch the skin of the finger with the slide.
3. Spread the drop of the blood using other slide (spreader slid) , see figure(1).

The thickness of the film may be regulated by increasing or decreasing the angle between die slides or by varying the rate of spreading. .A spread made quickly gives a thick film, the wider the angle, the thicker die film.

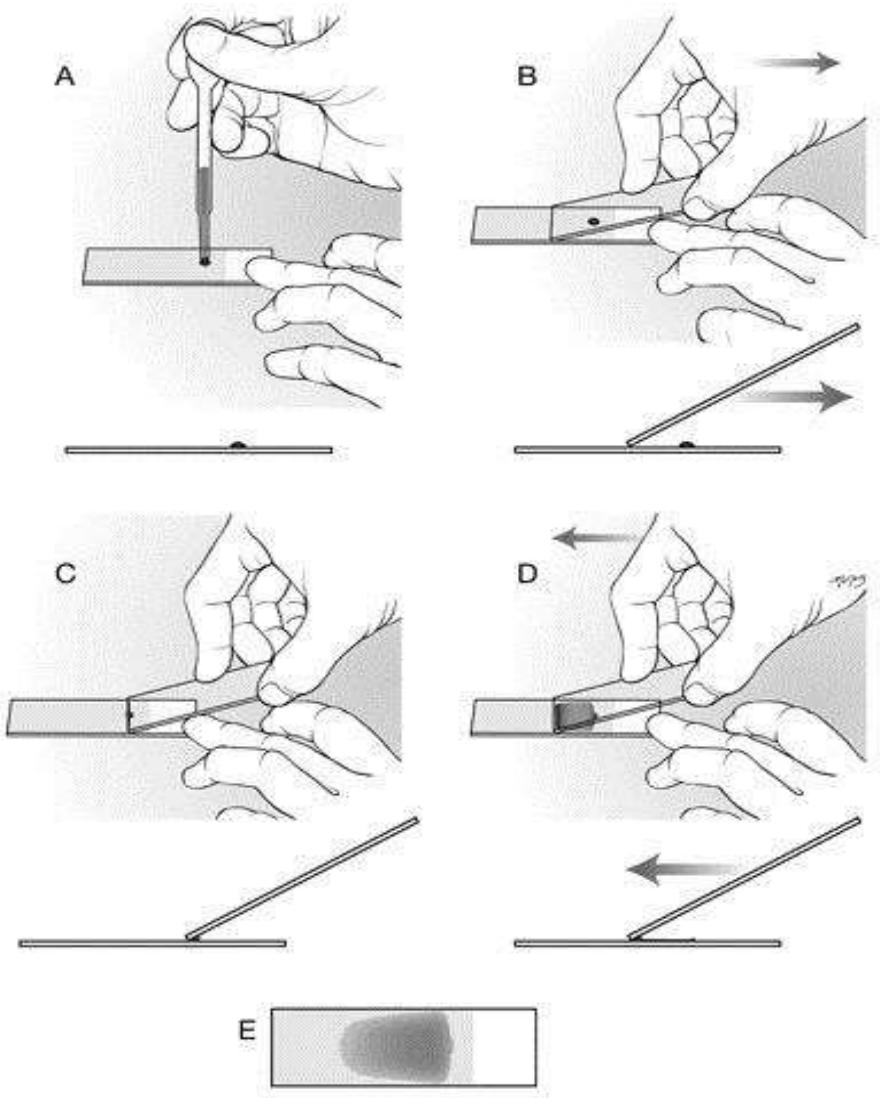


Figure (1): Blood smear preparation steps.

4. The spread film should be allowed to dry by shaking it in air at room temperature
5. Flood the smear with 10 drops of Wright or Leishman stain and wait for 3 minutes.
6. Add 10 drops of phosphate buffer to the stain on the slid and wait for 5 minutes.
7. Wash the slid gently with distilled water and leave it to air dry.
8. Examine the slid under the microscope using the low power first then the oil immersion objective. The dry and stained film is examined without cover slip under oil immersion objective Every white cell seen should be recorded in a table under the following headings; neutrophil, eosinophil, basophil,

monocyte and lymphocyte. Scan the slide following the pathway demonstrated by figure (2). A total of 100 cells should be counted. Find the percentage of each type. From the total and differential WBC's count we can find the absolute number of each type present in each mm^3 of blood. The normal values for the differential leukocyte count:

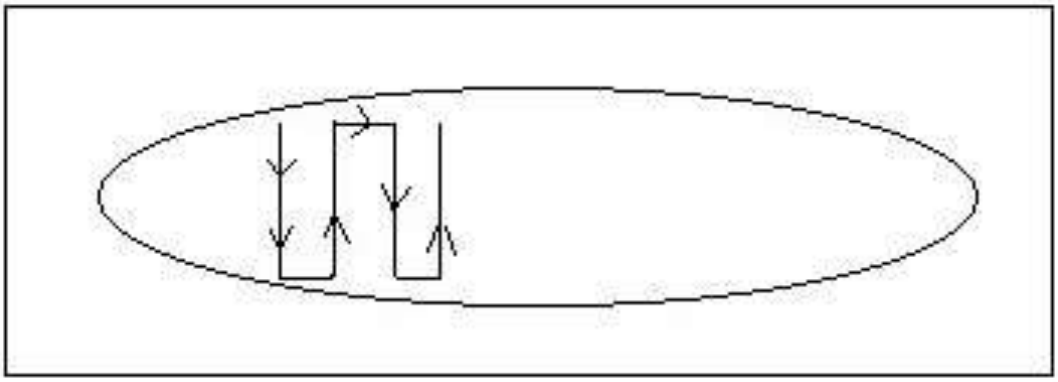


Figure (2): Blood smear examination directions.

Result;

Differential WBC counts (according to table (1)):

_____ **% Granulocytes**

_____ % Neutrophils

_____ % Eosinophils

_____ % Basophils

_____ **% Agranulocytes**

_____ % Lymphocytes

_____ % Monocytes

FORMED ELEMENTS	Function and Description	Source
Red Blood Cells (erythrocytes)  4 million–6 million per mm^3 blood	Transport O_2 and help transport CO_2. 7–8 μm in diameter; bright-red to dark-purple biconcave disks without nuclei.	Red bone marrow
White Blood Cells (leukocytes) 5,000–11,000 per mm^3 blood	Fight infection. Remove dead/dying cells. Destroy cancer cells.	Red bone marrow
Granular leukocytes		
• Neutrophils  40–70%	Phagocytize pathogens. 10–14 μm in diameter; spherical cells with multilobed nuclei; fine, lilac granules in cytoplasm if stained.	
• Eosinophils  1–4%	Phagocytize antigen-antibody complexes and allergens. 10–14 μm in diameter; spherical cells with bilobed nuclei; coarse, deep-red, uniformly sized granules in cytoplasm if stained.	
• Basophils  0–1%	Release histamine and heparin, which promote blood flow to injured tissues. 10–12 μm in diameter; spherical cells with lobed nuclei; large, irregularly shaped, deep-blue granules in cytoplasm if stained.	
Agranular leukocytes		
• Lymphocytes  20–45%	Responsible for specific immunity. 5–17 μm in diameter (average 9–10 μm); spherical cells with large, round nuclei.	
• Monocytes  4–8%	Become macrophages that phagocytize pathogens and cellular debris. 10–24 μm in diameter; large, spherical cells with kidney-shaped, round, or lobed nuclei.	
Platelets  150,000–300,000 per mm^3 blood	Aid hemostasis. 2–4 μm in diameter; disk-shaped cell fragments with no nuclei; purple granules in cytoplasm.	Red bone marrow

Table (1): Types of WBC's cells

Session No. 5

Hemoglobin concentration, Hematocrit (Packed cell volume, PCV), Erythrocyte sedimentation rate (ESR) & Blood grouping

Part One: hemoglobin concentration

Introduction:

Erythrocytes contain a respiratory pigment, hemoglobin, which imparts a characteristic color to the cells. Hemoglobin readily associates and dissociates with oxygen and carbon dioxide and is responsible for the red blood cell's ability to transport these gases**.** Normal adult ranges from (13.8 to 17.2) grams per deciliter (g/dl) for males and (12.1 to 15.1) g/dl for females. At birth, hemoglobin concentration ranges from 14 to 24 g/dl decreases approximately 11.5 g/dl at 1-2 years of age for both sexes. **Hemoglobin can be estimated either by Sahli method hemolyzing blood by strong acid in a solution and then using standard colors to estimate hemoglobin or by dividing packed cell volume over 3 or Hb=PCV/3.**

Results

Hemoglobin content:

_____ g /100 (g/dl) ml blood for male.

_____ g /100 (g/dl) ml blood for female.

Part Two: Hematocrit (packed cell volume, PCV) Determination

Introduction:

PCV is the volume of RBC to whole blood volume or percentage ratio of packed RBC's. Determination of **PCV** depends on centrifuging the whole blood sample , the heaviest particles (RBC's) falling to the bottom of the tube, the white blood cells are heavier than platelets and will therefore sediment on top of the RBC while the platelets being the lightest formed elements in the blood. The fluid portion of the blood (plasma) is the top portion of the centrifuged specimen. Normal PCV values for **male** and **female**, respectively, are 47.0±7 (40-54%) and 42.0±5 (37-47%).

Abnormal PCV Values:

A **high hematocrit** reading indicates either an increase in the number of red blood cells or a reduction in the circulating plasma volume. The high hematocrit (65 or more) seen in cases of cholera is due to the large loss of water in the stool. Abnormal increase in hematocrit, also may result from increase in the number of RBC's which is due to bone marrow cancer, or from living at high altitude, where less oxygen is available.

A **low hematocrit** usually indicates a reduction in the number of circulatory red blood cells as in anemia.

Materials:

1. Capillary tubes: Plain capillary tubes (blue color) if anticoagulant blood is used or anticoagulant capillary tubes (red color) if capillary blood is used.
2. Sealing material (clay).
3. Microhematocrit centrifuge.
4. Microhematocrit reader.

Procedure:

1. Allow the capillary or well - mixed anticoagulated whole blood to enter the capillary tube
   A. If a finger blood is used: Get an anticoagulant capillary tube (red ring). Make a finger puncture. Put one end of the tube in the drop of blood and allow the blood to flow into the tube.
   B. If anticoagulated blood is used, draw blood into a plain capillary tube (blue ring) until it is approximately two – thirds filled with blood.
2. Remove excess blood on the outside of capillary tube with a piece of gauze.
3. Seal one end of the capillary tube with the sealing material by placing the dry end of the tube into the sealer in a vertical position. The plug should be 4 - 6 mm long.
4. Place the capillary tube in a radial groove of the microhematocrit centrifuge with the sealed end away from the center of the centrifuge.
5. Centrifuge for 5 minutes at 11,000 rpm.
6. Remove the tube from the centrifuge and read the PCV value using the microhematocrit reader. It's important that the buffy coat not included in the result.

Results:

Hematocrit:_____ %

Part Three: Erythrocyte Sedimentation Rate (ESR) Determination

Introduction:

The method depends on the fact that RBC’s tend to sediment or precipitate to the bottom of the containing tube. The sedimentation rate depends upon plasma viscosity and the mass of surface area of red blood cells. In inflammation cases ,increase in fibrinogen which leads to increase rouleaux formation,(attachment of fibrinogen to RBC's surfaces) result in increased the mass to surface area ratio of the red cells and this will cause an increase in the sedimentation rate.

The ESR increases in many diseases involving inflammation and tissue destruction, such as **Rheumatoid arthritis**. The sedimentation rate is greater than normal during menses and pregnancy and anemia. ESR decreases in polycythemia. Although the ESR is a non-specific test it is employed clinically to judge the progress of such disease.

The normal ESR values in Westergren's method are:

Male: 0-15 mm/ hr.

Female: 0-20 mm/ hr.

Materials:

1. Westergren pipette.
2. Test tube rack.

EDTA anticoagulated blood.

Procedure:

1. Add blood (one volume) to a test tube contains 2 ml (4 volume) of EDTA **(blood** Anticoagulant)
2. Mix the tube for 1 minute.
3. Fill the Westergren pipette to exactly the zero mark, making certain there are no air bubbles in the blood.
4. Place the tube in the rack.
5. Allow the pipette to stand for exactly 60 minutes.
6. At the end of 60 minutes, measure the number of millimeters of visible clear plasma (which indicates the amount of settling RBC`s); record this figure on the data sheet.

Results:

ESR=---------- mm/hr

Part Four: **ABO & Rh-Blood grouping**

Introduction:

Blood of different individuals can be grouped according to the presence or absence of certain antigens on the surface of the RBC's. There are several blood typing systems based on the various possible antigens, but the factors typically typed for are the antigens of the ABO and Rh blood groups which are commonly involved in the transfusion reactions.

Table (1): ABO and Rh blood group

Blood type	RBC's (antigen)	Plasma (antibody)	Population distribution
A	**A**	**B**	**41%**
B	**B**	**A**	**10%**
AB	**AB**	**Neither A nor B**	**4%**
O	**Neither A nor B**	**A and B**	**45%**
Rh+	**Presence of Rh antigen**	**None**	**85%**
Rh-	**Absence of Rh antigen**	**Anti-Rh**	**15%**

Materials:

1. Clean dry glass slides.
2. Wooden sticks.
3. Blood of unknown grouping obtained by finger puncture.
4. Specific antisera, anti -A , anti -B , anti -Rh (anti -D) .
5. Lancet, alcohol soaked gauze pads.

Procedure:

1. Puncture your thumb by sterile lancet and allow three separated drop fall on the slid
2. To the first blood drop add one drop of anti-A serum, to the second blood drop add one drop of anti-B serum and to the third one add one drop of anti-D serum.
3. Mix the blood and the serum well using the wooden sticks then rotate the slide for 1-2 minutes. Use different stick for each mixing,
4. Notice the presence or absence of agglutination
5. Read the results of the major blood groups (ABO system) as follows:

Status	Observed (+)	Not observed (-)
Presence of clumping with anti-A		
Presence of clumping with anti-B		
Presence of clumping with anti-Rh		

Result:

Blood typing is_____

Session No. 6

Pulse Rate & Arterial blood pressure Measurement

The pulse gives an idea about the condition of the vessel walls and amount of variation of pressure of the contained blood, therefore state of the heart and circulation may be obtained. Your pulse is the rate at which your heart beats. As your heart pumps blood through your body, you can feel a pulsing in some of the blood vessels close to the skin's surface.

Examination of pulse:

Arterial pulse may be examined in the radial, brachial, femoral, posterior tibial and dorsalis pedis. Examination of radial pulse is more commonly practiced, figure (1 a, b).

Feel the pulse (palpate) by placing two or three fingers on the radial artery. Do not use the thumb. Forearm is pronated and the wrist slightly flexed.

Pulse Rate: count beats for not less than 1/2 minute. The rate is accelerated (tachycardia), by emotion, exercise, fever, and atrial fibrillation. The rate is slowed (bradycardia) in heart block. The typical pulse of healthy adult man should be at rate of 72/min.

Figure (1, a)

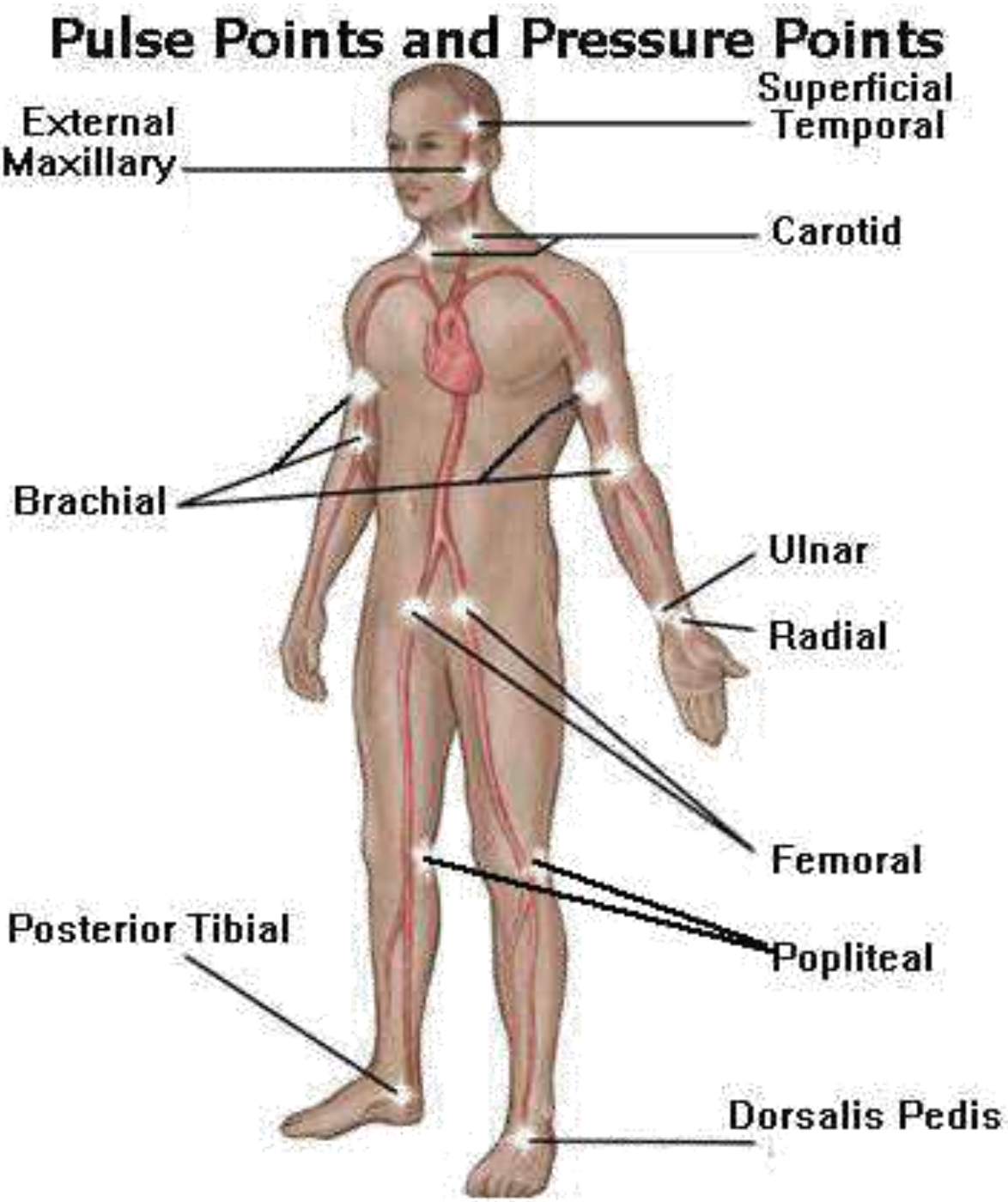


Figure (1, b)

Part Two: Arterial blood pressure

Blood pressure is defined as the pressure exerted against any unit area of the blood vessel walls and is generally measured in arteries. Because the heart contracts and relaxes, the resulting rhythmic flow of blood into the arteries causes the blood pressure to rise and fall during each beat. There are two types of blood pressure (recorded in millimeters Mercury (mm Hg)) Figure (2)

:

1. **The systolic pressure:** is the pressure in the arteries at the peak of ventricular ejection. When your heart beats, it squeezes and pushes blood through your arteries to the rest of your body. This force creates pressure on those blood vessels, and that's your systolic blood pressure. The diastolic pressure: it reflects the pressure during the ventricular relaxation.

2. **The diastolic reading**, or the bottom number, is the pressure in the arteries when the heart rests between beats. This is the time when the heart fills with blood and gets oxygen. For example, if the systolic blood pressure is 120 and the systolic blood pressure is 80, the blood pressure is expressed as 120/ 80 (120 over 80).

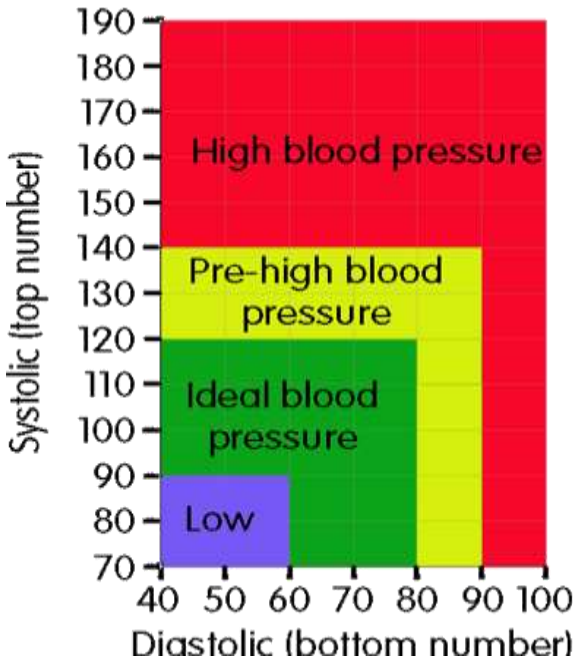


Figure (2)

Abnormal blood pressure values:

1. **Hypertension**: if the systolic pressure > 140 mm Hg or the diastolic pressure > 90 mm Hg.
2. **Hypotension**: if the systolic pressure < 90 mm Hg or the diastolic pressure < 60 mm Hg.

Materials:

1. Stethoscope.
2. Sphygmomanometer (mercury, inflatable cuff, pulb).

Procedure:

The blood pressure is estimated mainly by a sphygmomanometer and stethoscope (auscultatory method).

1. Clean the earpieces of the stethoscope with alcohol swab, and check the cuff for the presence of trapped by compressing it against the laboratory table.

2. The subject should sit in a comfortable position with one arm resting on the laboratory table (approximately at the heart level if possible).
3. Wrap the cuff around the subjects' elevated arm, just above the elbow, with the inflatable area on the medial arm surface (over the **brachial** artery) (figure 3). Secure the cuff by tucking the distal end under the wrapped portion or by bringing the Velcro areas into position.
4. Palpate the brachial pulse and lightly mark its position with a felt pen. Place stethoscope diaphragm over the pulse point. <u>The cuff should not inflate for more than one minute.</u>
5. Inflate the cuff to approximately 160-mm Hg pressure, and slowly release the pressure valve. Watch the pressure gauge as you listen carefully for the **first soft** thudding sounds of the blood spurting through the partially occluded artery. Note this pressure (**systolic** pressure), and continue to release the cuff pressure. You will notice first an increase, then a muffling, of the sound.

Note: Make two blood pressure determinations, and record your result below.

<u>Effect of various factors on blood pressure:</u>

Many factors—age, weight, time of day, exercise, body position, emotional state, and various drugs for example alter blood pressure

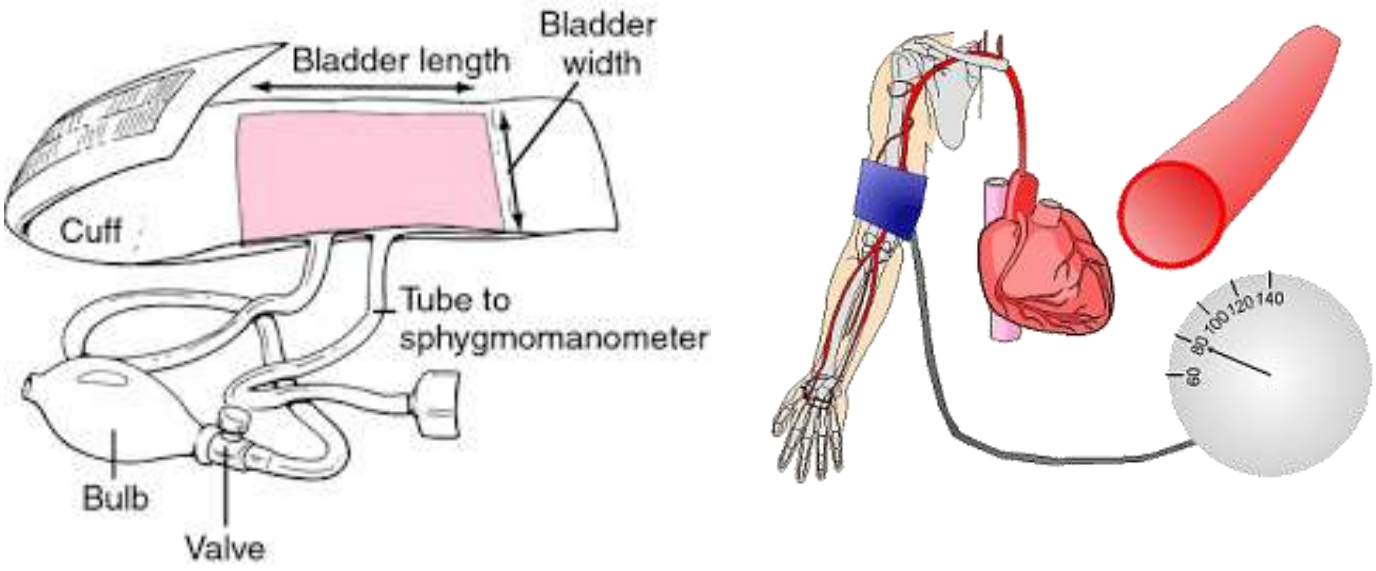
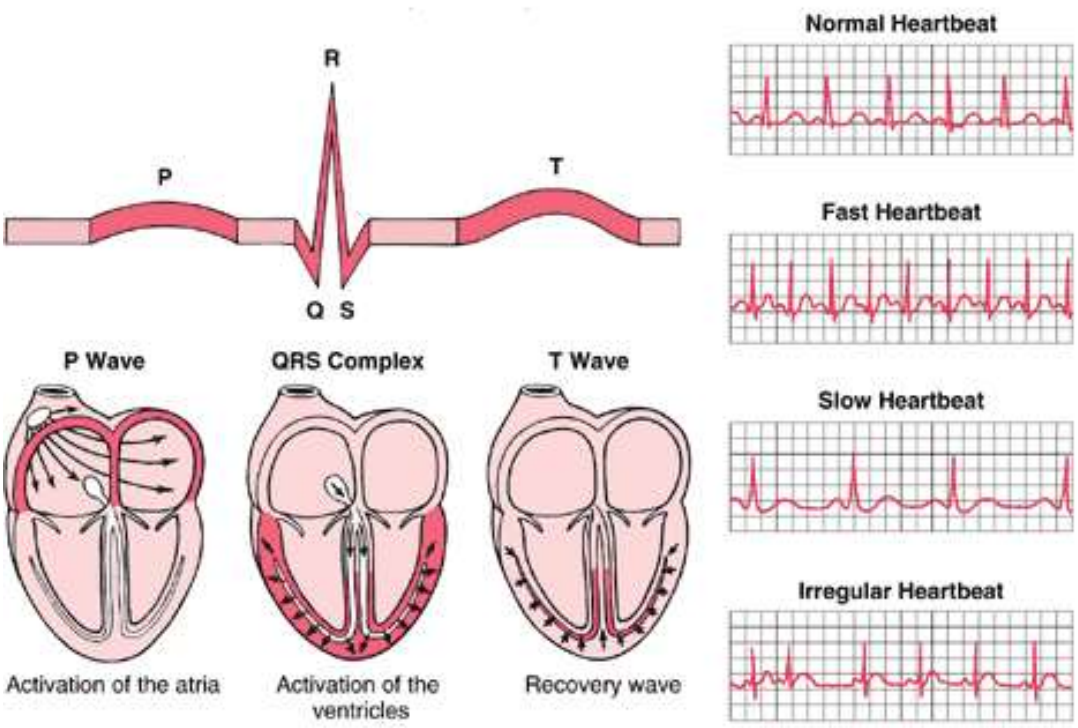


Figure (3): Sphygmomanometer

Session No. 7
Conduction system of the heart and Electrocardiography

Introduction:

Heart contraction results from a series of electrical potentials changes (depolarization waves) that travel through the heart preliminary to each beat. The conduction of impulses through the heart generates electrical currents that eventually spread throughout the body. These impulses can be detected on the body's surface and recorded with an instrument called an **electrocardiogram (ECG) machine**. The graphic recording of the electrical changes (depolarization and repolarization) occurring during the cardiac cycle is called an **Electrocardiograph.**



Figure(1): Normal Electrocardiogram**.**

- The typical ECG consists of a series of three recognizable waves.

1- The **P wave**: Indicates the depolarization of the atria immediately before atrial contraction.

2- The large **QRS complex**: Resulting from ventricular depolarization has a complicated shape (primarily because of the variability in size of the ventricles and the time differences required for these chambers to depolarize). It precedes ventricular contraction. The repolarization of the atria, which occurs during QRS interval, is generally obscured by the large QRS complex.

3-The **Twave**: Results from currents propagated during ventricular repolarization.

***Standards leads are used to record an ECG for diagnostic purposes:**

1- Three bipolar standard limb leads: Lead I, II, III

A- Lead I: Detects potential difference between the left arm and right arm (LA-RA).

B- Lead II; Detects potential difference between the right arm and left leg (RA-LL).

C-Lead III: Detects the potential difference between the left arm and left leg (LA-LL).

3- Six unipolar chest leads: They called V1 – V6 (V: Stand for vector) or C1 – C6 (C: Stands for chest)

1- V 1: At the right margin of the sternum in the fourth intercostals space.

2- V 2: At the left margin of the sternum in the fourth intercostals space.

3- V 3: Midway between position 2 and 4.

4- V4: At the left midclavicular line in the fifth intercostals space.

5- V5: At the left anterior axillary line and at the same level as position 4.

6- V6: At the left midaxillay line and at the same level as position 4.

Note: the right leg is used as ground or earth.

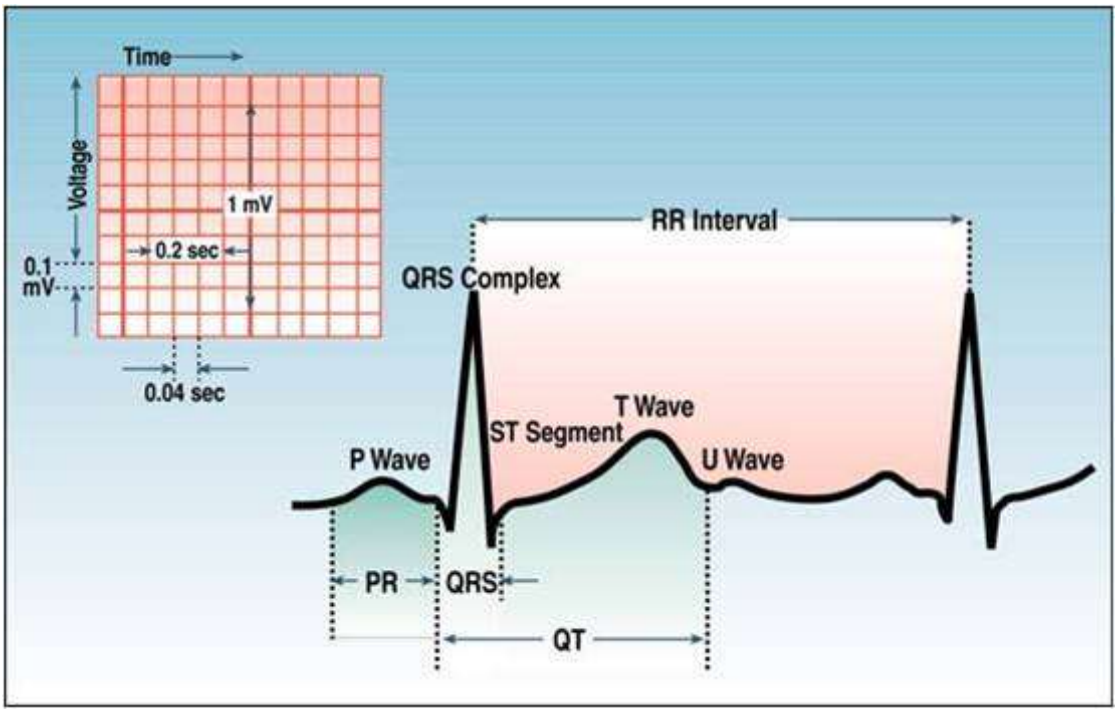
<u>Measuring heart rate from ECG</u>

1. The paper speed is 25 mm/sec, each mm (1 small square) speed = 1/ 25= 0.04 sec.

2. Measure the duration of one cardiac cycle represented by the length of R-R interval (number of small squares between two successive R waves (in mm).

3. Multiply R-R length by 0.04 sec (the time of each cardiac cycle).

4. $\text{Heart rate/ min} = \dfrac{60\,\text{sec}}{(\text{R - R}) \times 0.04\,\text{sec}}$



Abnormal Heart Rate Values:

Tachycardia; A heart rate over 100 beat/min.

Bradycardia : A heart rate below 60 beat/min.

Example

R-R interval length = 18 mm

So cardiac cycle duration = 18 x 0.04 =0.72 sec

Heart Rate = 60/ 0.72 = 83 beat/ min.

Materials:

1- ECG recording apparatus.

2- ECG gel.

3- Alcohol swabs cot.

Procedure:

1- Clean the skin with a cotton moistened with **alcohol** to **remove** *died cells and oil*.

2- Apply the **ECG gel** on the cleaned skin sites (limb, chest) for **good conduction.**

2- Attach the electrodes to the cleaned skin sites. Take care not to let the electrodes come in contact with each other, then connect the electrodes with the machine through leads, see figure (2).

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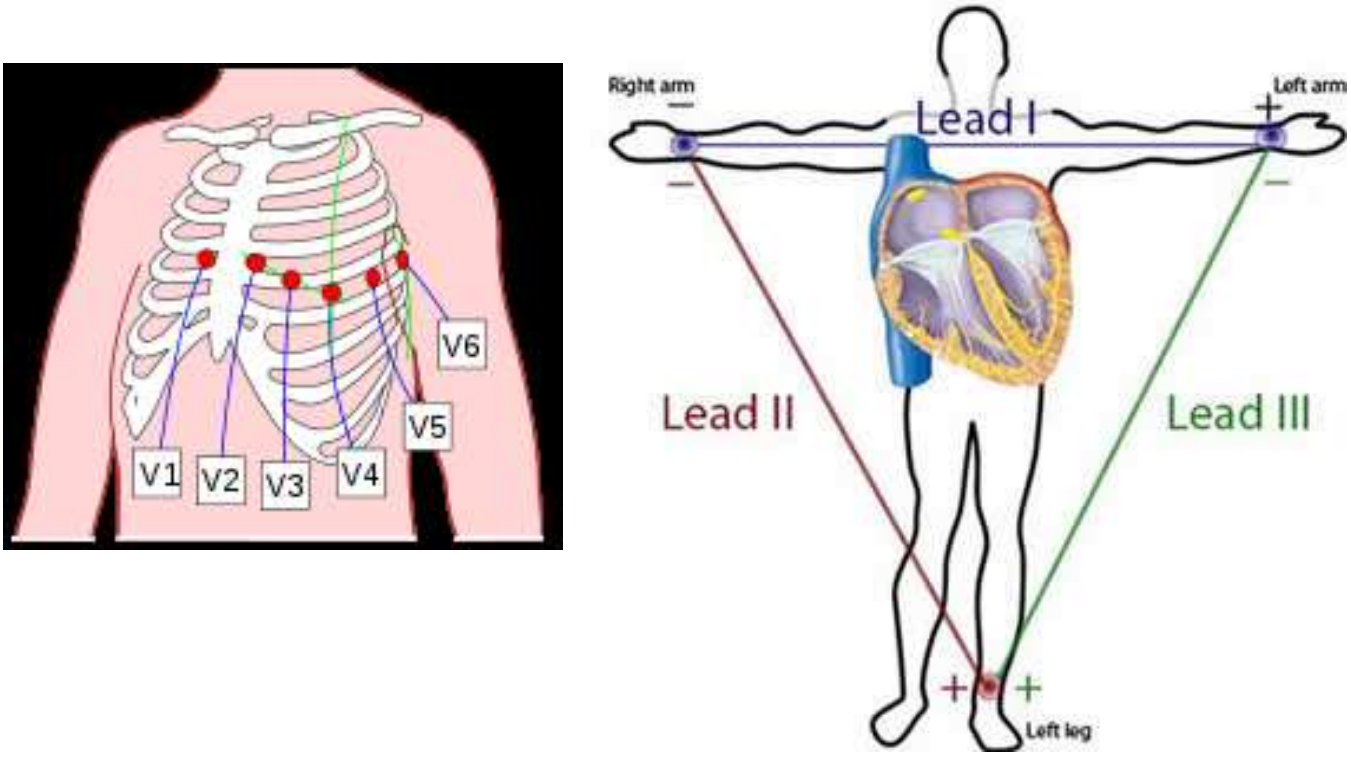
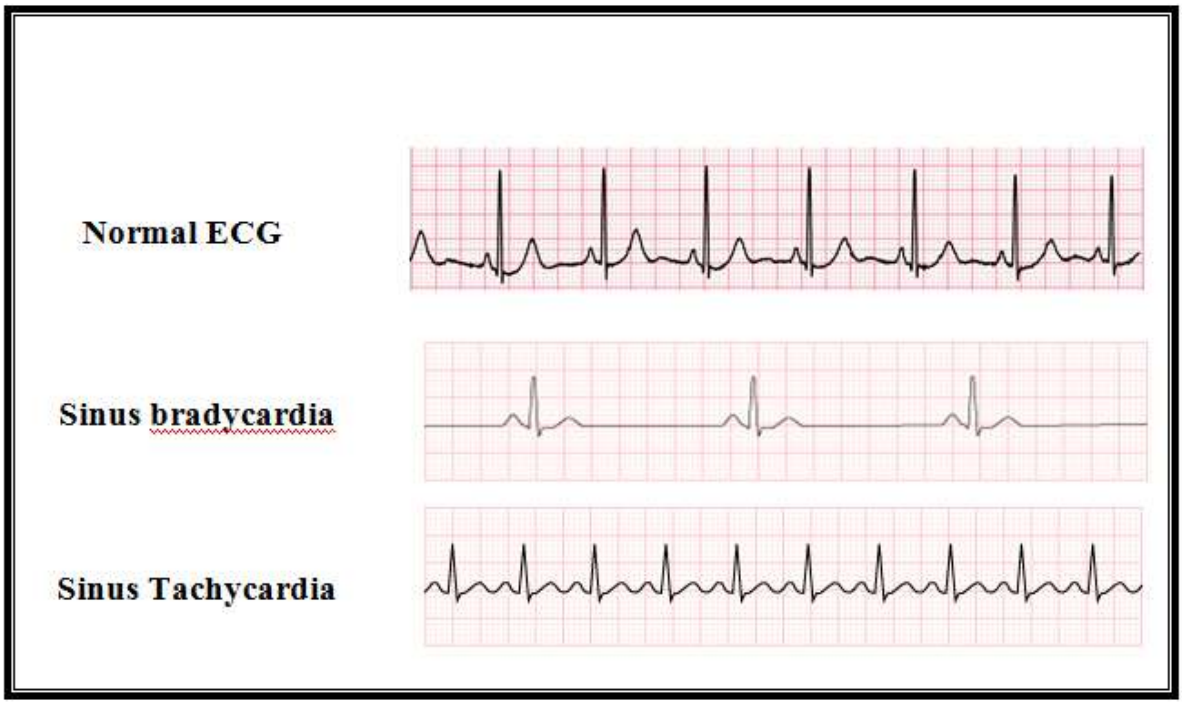


Figure (2): Attaching the electrodes**.**



Figure(3): Electrocardiograms (Lead II) of common cardiac arrhythmias and abnormalities.

Session 8

Pulmonary Function Tests (Lung Volumes and Capacities)

Introduction:

The volume of air a person inhaled (**inspires**) and exhales (**expires**) can be measured with a Spirometer (Spiro=breath, meter = to measure). is an apparatus for measuring the volume of air inspired and expired by the lungs.. The resultant record of volume changes versus time is called a **spirogram.**

Lung volumes

1- **Tidal volume**: It is the volume of air expired or inspired per breaths during normal quite breathing. The tidal volume of 70g adult man is about **500ml** per inspiratory breath; this can be increased dramatically during exercise.
2- **Inspiratory reserve volume**: Volume of air inhaled maximal (deepest) inspiration (started after normal tidal inspiration). This volume is about **3000ml**.
3- **Expiratory reserved volume**: Volume of air expelled during maximal active contraction of expiratory muscles starts at the end of normal tidal expiration. This about **1500ml**.
4- **Residual volume**: Volume of air remaining in lungs after a maximal forced expired, which is about **1000ml**.

Lungs Capacities:

1- **Vital capacity**: It is a useful clinical measurement; it's the maximal volume of air that can be expelled after maximal inspiration. It is about **5000ml**. It is also equal to the sum of tidal volume and inspiratory and expiratory reserved volumes
2- 2**- Functional residual capacity**: Volume of air remaining in lungs at the end of normal tidal expiration. It is about **2500ml** [Sum of residual volume and expiratory reserved volume (1000+1500=2500)].

3- **Total lung capacity**: Volume of air in lungs after a maximal inspiration. It consists of all four lung volumes: Residual volume (1000ml) +Tidal volume (500ml)+Inspiratory residual volume(3000ml)+Expiratory reserved volume(1500ml). It is about **6000ml**.

4- **Inspiratory capacity**: Volume of air inhaled during maximal inspiratory begins after end of normal tidal expiration. It is tidal volume+ inspiratory volume, it is about **3500ml**

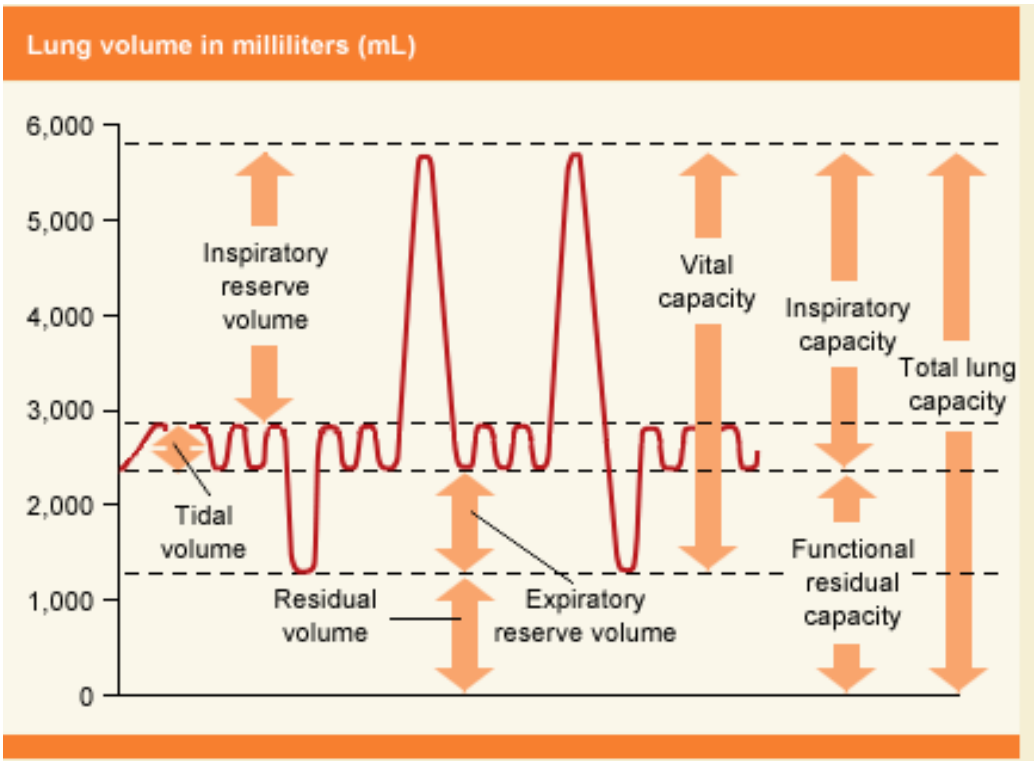


Figure (1): Lung volume and capacities spirogram.

Chronic pulmonary diseases are classified into two physiological categories:

1. **Obstructive pulmonary disorders**, such as emphysema and bronchial asthma. In chronic obstructive pulmonary disease (COPD) such as bronchial asthma, excessive mucus secretion partially blocks airways, increasing airways resistance and thus making breathing more difficult. The asthmatic may take longer to inspire and expire, but pulmonary volumes may be normal or near normal.

2. **Restrictive pulmonary disorders** such as pulmonary fibrosis and other chronic diseases of the lung interstitial. In a restrictive pulmonary disease, the ability to change lung volume is

decreased. As a result, lung capacities and volumes are generally reduced (e.g., decreased vital capacity

Notes:

1- **Body size**: Size of lungs depends on subject height and weight or body surface area. All volumes are larger in larger people.
2- **Age**: Size of lungs depends on age, volumes are smaller in children, and alternation in elasticity and compliance of lung in old age affect lung volumes and capacities
3- **Sex**: All volumes are slightly smaller in females due to difference in body size
4- **Exercise**: This increase lung volumes
5- **Posture**: Vital capacity is larger in sitting than in recumbent position because during sitting the viscera drop down by gravity, helping free decent of the diaphragm. Therefore, lung volume and capacities depend on mechanics of the lung and chest wall (elasticity and airway resistance) and the activity of inspiratory and expiratory muscles.

Materials:

1-Spirometer

2-Clamp

3-Steril mouth piece

4-Alcohol 70%

Procedure:

1. Close the nose by a clamp

2. Let the subject while sitting to breathe through the mouth piece into the spirometer without looking at the record.

3. After few normal respiratory movement, the subject is asked to perform Vital capacity (VC), Forced vital capacity (FVC) and the maximum voluntary ventilation (MVV).

1. To determine the (**VC**): Inspire slowly (fully), then expire slowly (fully).
2. To determine the (**FVC**): Inspire slowly (fully), then expire rapidly (fully).
3. To determine the (**MVV**): Breath rapidly for 12 – 15 seconds.

RESULTS:

_____ **% Vital capacity (VC)**

_____ **% Forced vital capacity (FVC)**

_____ **% maximum voluntary ventilation (MVV)**

Session 9:

Human Reflex Physiology

Introduction

Reflexes are rapid, predictable, involuntary motor responses to stimuli; they are mediated over neural pathways called reflex arcs. There are five essential components of **reflex arc** Figure 1: Receptor, sensory neuron, integration center, motor neuron, and effectors. Reflex **testing** is an important diagnostic tool to assess the condition of the nervous system. Exaggerated, distorted,, or absent reflex may indicate degeneration or pathology or portions of the nervous system, such conditions includes damage to intervertebral discs, tumors, polyneuritis, and apoplexy.

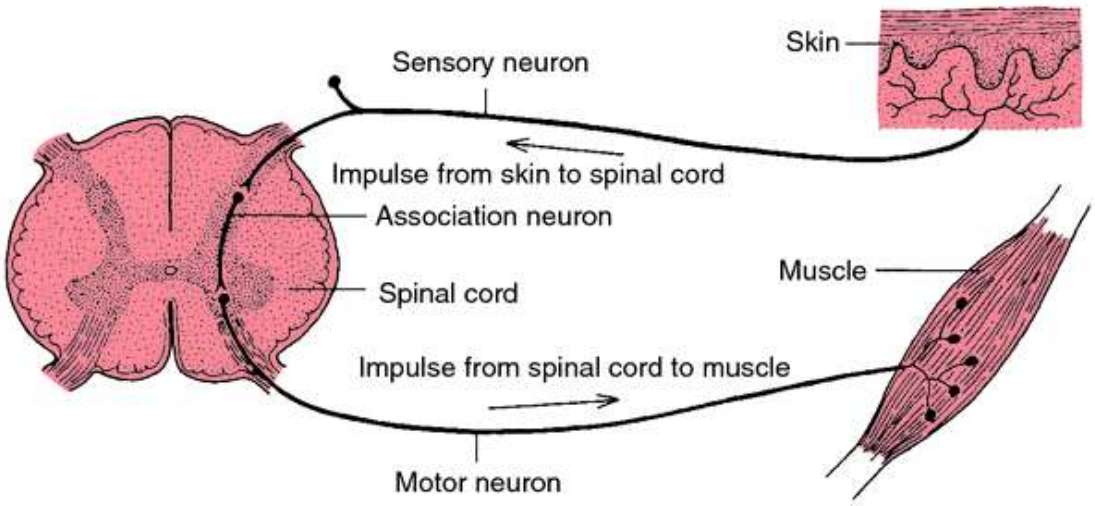


Figure 1

Reflexes can be categorized into one of two large groups:

1. **Autonomic reflexes** are mediated through the autonomic nervous system and are not subject to conscious control. These reflexes result in the activation of smooth muscles, cardiac muscle, and the glands of the body; they include the regulation of such body functions such as digestion, elimination, blood pressure, salivation and sweating.

2. **Somatic (spinal)** include all these reflexes that involve the stimulation of skeletal muscles by the somatic division of the nervous system.. An example of such reflex is the rapid withdrawal of a hand from a hot object. Clinically, **Somatic** reflexes are categorized as being either **deep** or **superficial**:

    1- Deep tendon reflex = Stretch reflexes

These type of reflexes provide information on the integrity of the central nervous system and peripheral nervous system. And they can be used to detect the presence of a neuromuscular disease.

2- superficial reflex = cutaneous reflexes

A reflex elicited by stimulation of the skin.

Materials

Reflex hammer.

Method:

A. Deep reflexes:

Reflexes of the upper limb

A. **Biceps reflexes:** This reflex causes flexion of the arm. It is elicited by holding the subject`s elbow with the thumb pressed over the tendon of the biceps brachii, as shown in figure 2- A. To produce desired response, strike a sharp blow to the first digit of the thumb with the reflex hammer.

B. The brachioradialis reflex

In normal individuals this reflex will cause flexion of the forearm at the elbow joint and halfway between pronation and supination .To demonstrate this reflex, direct the subject to rest the hand on the thigh in the position shown in illustration (figure 2- b). Identify the brachioradialis tendon at the wrist. It inserts at the base of the styloid process of the radius, usually about 1 cm lateral to the radial artery then strike the tendon with the wide end of the reflex hammer

C. **Triceps reflex**: This deep reflex causes extension of the arm in normal individuals. To demonstrate this reflex, flex the arm at the elbow holding the wrist as shown in illustration figure 2-c , with the palm facing the body. Strike triceps brachii tendon above the elbow with pointed end of the reflex hammer.

Reflexes of the lower limb

D. **Patellar reflex:** It is referred to as the **knee reflex, knee jerk, or quadriceps reflex**. the quadriceps muscle cause extension of the leg at knee joint. To perform this test, the subject should be seated on the edge of a table with the leg suspended and somewhat flexed over the edge. Figure 2-D .To elicit the typical response, strike the patellar tendon, which is just below the knee cap.

E. **Achilles reflex:** This reflex is also referred to as the **ankle jerk**. It is characterized by plantar

flexion when the Achilles tendon is struck a sharp blow. To perform this reflex the subject must be relaxed then strike the Achilles tendon . Figure 2-E

B. Superficial reflexes

F. **Plantar flexion:** This reflex is the one in this series that is of superficial nature. Note in illustration G figure2-F, and figure 3, that the normal reaction to stroking the sole of the foot in an adult is plantar flexion. If **dorsiflexion** occurs, starting in the great toe and spreading to the other toes (**Babiniski's sign**), it may be assumed that there is myelin damage to fibers in the pyramidal tracts. Incidentally, dorsiflexion is normal in infants, especially if they are a sleep. Babiniski`s sign disappears in infants once myelinization of nerve fibers is complete (after 6 months of birth). Perform this test on the bottom of the foot of a subject, using a hard object such as key. Follow the pattern shown in the middle illustration.

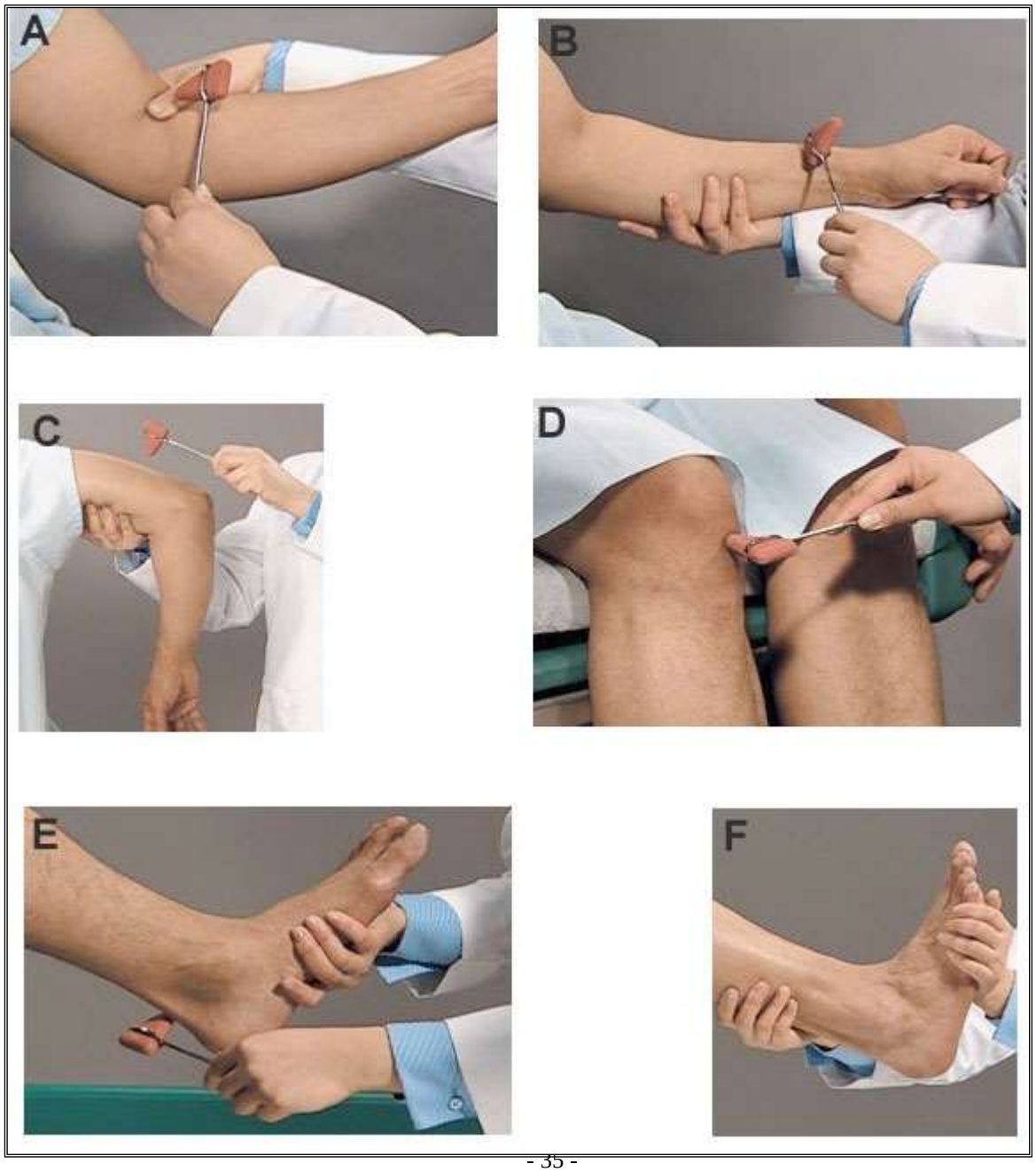


Figure (2): Type of Reflexes

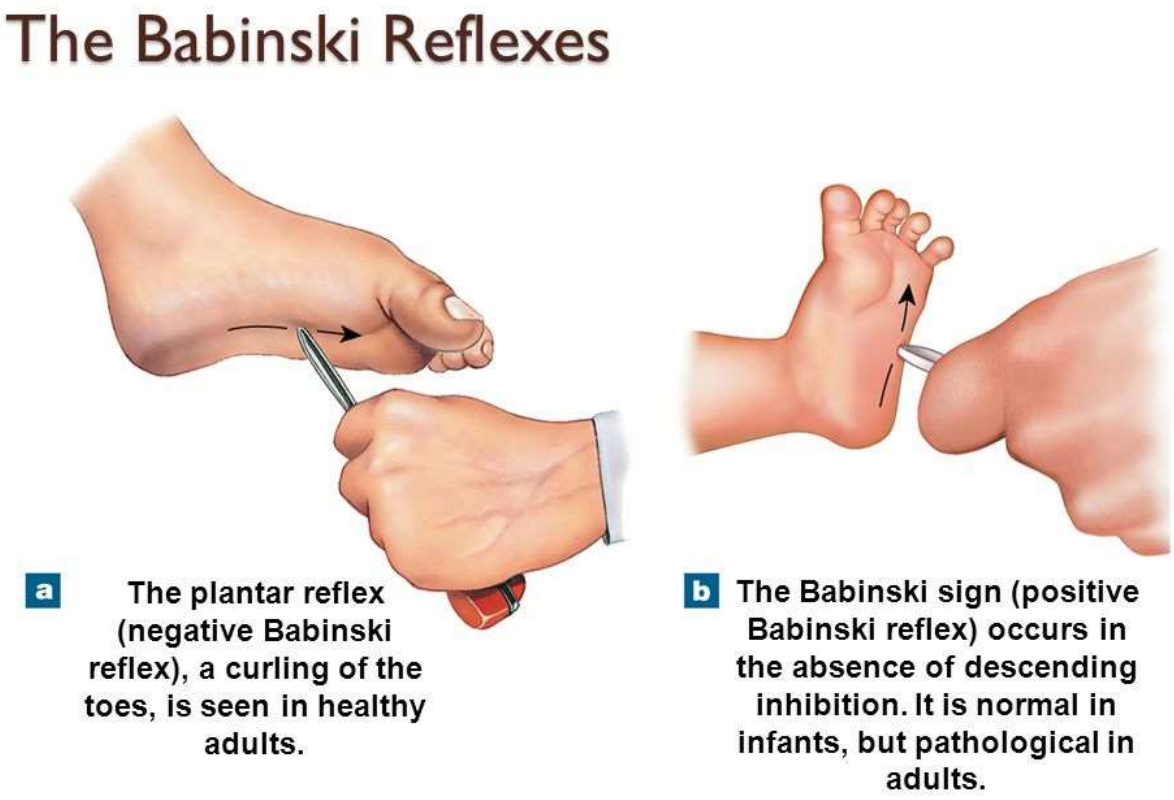


Figure (3): Plantar flexion

Session 10:
Special Senses (Vision & Hearing)

Part One: Vision

Introduction:

The image of an object is formed by refraction of light rays from it by the cornea and the lens of the eye, and then the rays are focused on the rods and cones at the retina. Rods and cones are photoreceptors contain light-sensitive pigments which are altered when exposed to visible wave length of light, leading to changes in ion flow which generate neural signals. This signal is translated in the occipital cortex as an image.

The various tests outlined in this exercise related to the observation of normal conditions, as well as the election of the more common types of abnormalities. By performing these tests you will learn more about the physiology of vision.

Materials:

1. Snellen test letter chart.
2. Green's astigmatic chart.
3. Vision disc.
4. Ishihara color-blindness test booklets.

Procedure:

1. Test for Visual Acuity

Visual acuity, or sharpness of vision, is generally tested with a **Snellen eye chart**, which consist of letters of various sizes pointed on a white card. This test is based on the fact that letters of certain size can be seen clearly by eyes with normal vision at a specific distance. The distance at which the normal, or emmetropic, eye can read each line of letters is printed at the end of that line.

1. Have your partner stand 20 feet from the posted Snellen eye chart, with one eye covered by a card or hand. As your partner reads each consecutive line aloud, check for accuracy. (If this individual wears glasses, the test should be taken twice-first with glasses off and then with glasses on).
2. Record the number of the line with the smallest sized letters read. If it is 20/20, the person's vision for the eye is normal. If it is **20/40** (or any ratio with a value less than one), his or her vision is **less** than the normal acuity. (Such an individual is **myopic**). If the visual acuity is **20/15**, vision is **better** than normal, because this person can stand 20 feet from the chart and read letters that are only discernible by the normal eye at 15 feet.
3. Have your partner test and record your visual acuity. If you wear glasses, the test result without

glasses should be recorded first.

Results:

Visual acuity right eye: _____

Visual acuity left eye:_____

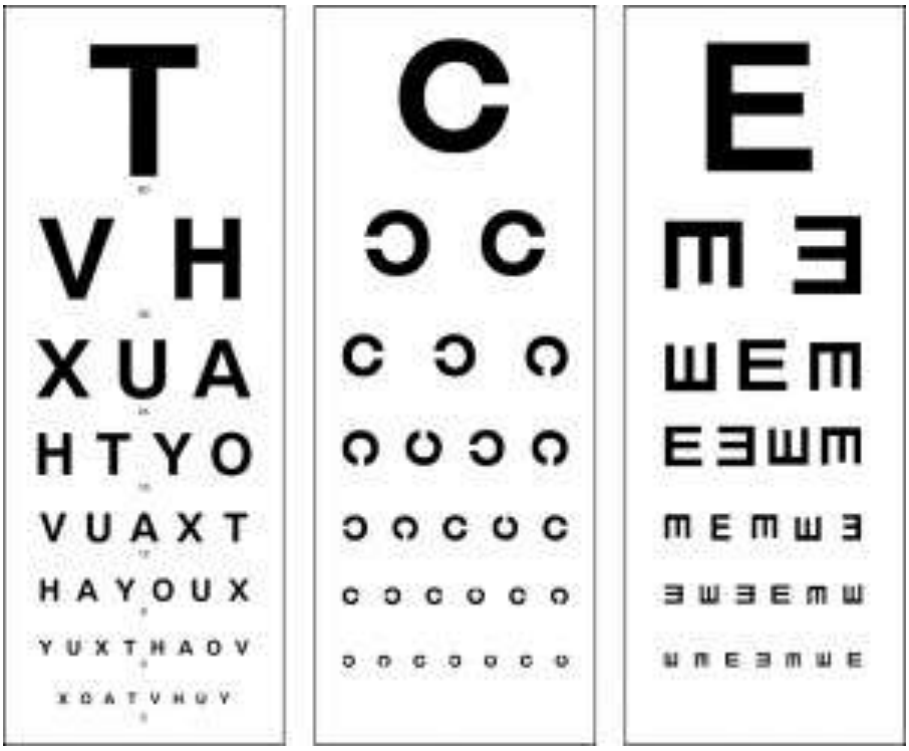


Figure (1): Snellen test letter chart.

2. Blind Spot:

The blind spot of the retina is the optic disc where nerve fibers of the retina converge and exit the eyeball via the optic nerve. There are no cones or rods present in the optic disc; therefore no image can be formed in this area.

1. Hold figure 5 about 18 inches from your eye, and focus your right eye on the X, which should be positioned so that it is directly in line with your right eye. Move the figure slowly toward your face, keeping your right eye focused on the X. When the dot focuses on the blind spot, which lacks photoreceptors, it will disappear.

Have your laboratory partner record in metric units the distance at which this occurs. (The dot will reappear as the figure is moved closer).

Results:

Distance at which the dot disappears:

Right eye:_____

Repeat the test for the left eye, this time closing the right eye and focusing the left eye on the dot.

Record distance at which the X disappears:

Left eye:_____

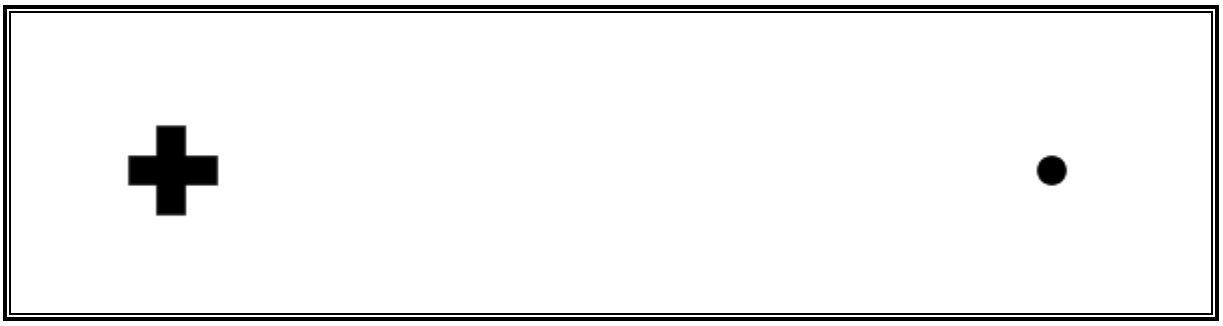


Figure (2): Blind spot test**.**

3. Test for Near point Accommodation:

The elasticity of the lens decreases dramatically with age, resulting in difficulty in focusing for near or close vision. Measuring the near point of accommodation can test lens elasticity. The near point of vision is about 7 cm at age 10, 10 cm at age 20, 14 cm at age 30, 22 cm at age 40, 40 cm at age 50 and 100 cm at age 60.

To determine your near point of accommodation, hold a common straight pin at arm's length in front of one eye. Slowly move the pin toward that eye until the pin images becomes distorted. Have your lab partner measure the distance from your eye to the pin at this point, and record the distance below. Repeat the procedure for the other eye.

Near point for right eye:_____

Near point for left eye:_____

4. Test for color blindness:

Ishihara`s color plates are designed to test for deficiencies in the cones, or color photoreceptor cells. Studies suggest that there are three cone types, each contains a photoreceptor pigment. One type primarily absorbs the red wavelengths of the visible light spectrum, another blue wavelength, and a third the green wavelengths, nervous impulses reaching the brain from these different photoreceptor types are then interpreted (seen) as red, blue, and green, respectively.

The interpretation of the intermediate colors of the visible light spectrum is a result of overlapping input from more than one cone type. Color blindness is a **sex linked hereditary condition** that affects 8% of the male population and 0.5% of females. The most common type is

red-green color blindness, in which either the red or green cones are lacking. If red cones are lacking, a condition called **protanopia** exists. Individuals that have this condition see blue-green and purplish-tinted reds as gray. A lack green cones is designated as **deuteranopia.**

1. View the various color plates in bright light or sunlight while holding them about 30 inches away and at right angles to your line of vision. Report to your laboratory partner what you see in each plate. (Take no more than three seconds for your decision).
2. Your partner is to write down your responses and then check your accuracy with the correct answers given at the front of the color plate book.

Is there any indication that you have some degree of color blindness?

If so what type?

Part Two: Hearing and Equilibrium

Materials:

1. Ear model.
2. Tuning forks (middle C 256 Hz).

Hearing Tests:

1- Weber Test: (**to determine conductive and nerve deafness**).

Strike tuning fork on the heel of your hand or with a mallet and place the handle of the tuning fork medially on your forehead (figure 5).

Is the tone equally loud in both ears, or is it louder in one ear?

If it is equally loud in both ears, you have equal hearing, or equal loss of hearing, in both ears. If nerve deafness is present in one ear, the tone will be heard in the unaffected ear but not in the ear with nerve deafness. If conduction deafness is present, the sound will be heard **more strongly** in the ear in which there is a hearing loss. Conduction deafness can be stimulated by plugging one ear with cotton to interfere with the conduction of sound to the inner ear.

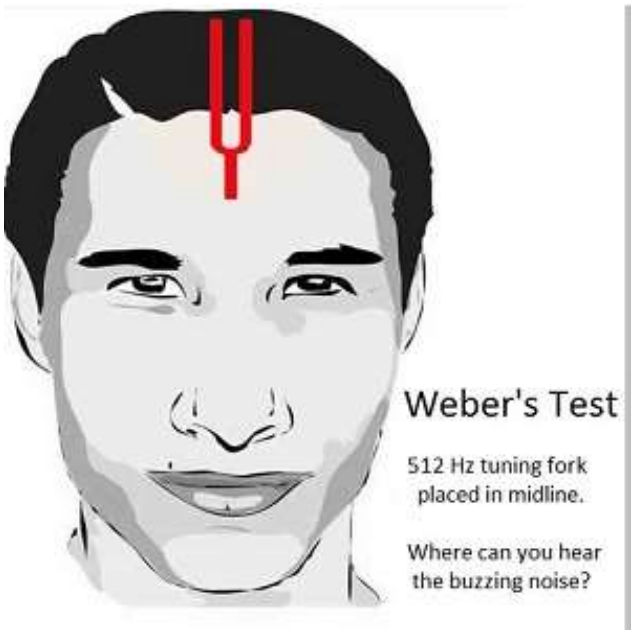


Figure (1): *Weber Test.*

2- Rinne Test: (**for comparing Bone-and Air-conduction hearing**)

Procedure:

1. Strike the tuning fork, and place its handle on your partner's mastoid process (figure 6 (a)). When the sound is no longer audible to your partner, hold the still-vibrating prongs close to his auditory canal (figure 6(b)). If your partner hears the fork again when it is moved to that position (**by air conduction**), hearing is not impaired and the test result is recorder as positive (+). (Record below).
2. Repeat the test, but this time test air-conduction-hearing test.
3. After the tone is no longer heard by air conduction, hold the handle of tuning fork on the bony mastoid process. If the subject hears the tone again by bone conduction after hearing by air conduction is lost, there is some conductive deafness; and the result is recorded as negative.
4. Repeat the sequence for other ear.

Right ear:_____

Left ear:_____

Does the subject hear better by bone or air conduction?

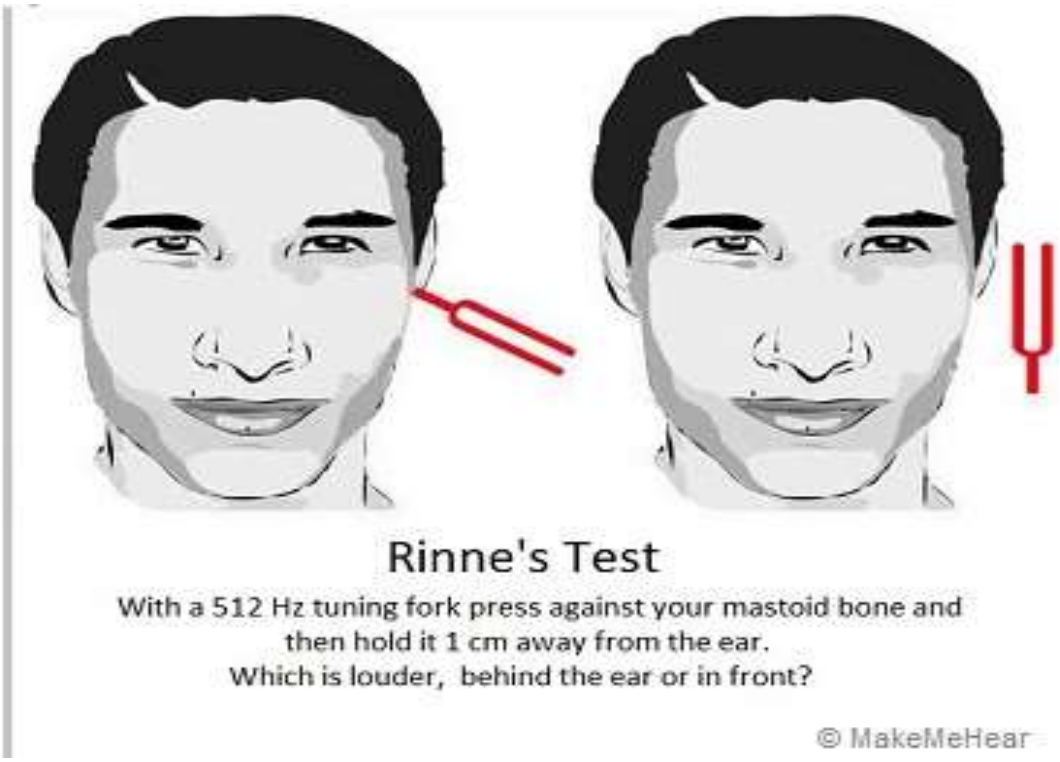


Figure (2): Rinne Test

Table (1): The comparison between the Rinne test and the Weber's test

Case/ Test name	Rinne test	Weber's test
Normal	Hears vibration of air after bone conduction is finished	Hears equally in the two ears
Conduction deafness	Does not hear vibration of air after bone conduction is finished	Hears is louder in defective ear.
Nerve deafness	Hearing is very Diminished (almost lost)	Sound is better in normal ear