جامعة شط العرب الاهلية
كلية العلوم –قسم التحليلات المرضية

Principles of Pathological analysis

Lach 1 – Introduction to pathological analysis

(The Blood)

Prof. Dr. Mohammed A Fayyadh

Important References

1-Mosby's Manual of diagnostic and laboratory test 5th edition (2010) by Kathleen D.Pagana .

2-Clinical biochemistry and metabolic Medicine , 8th by Martin A.Crook

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Terms should we Know

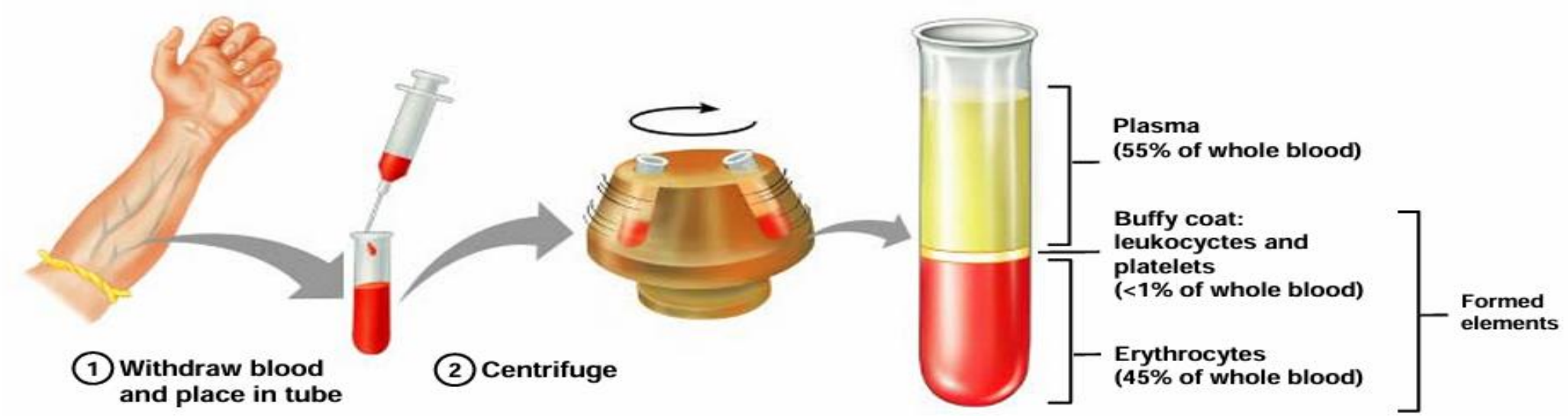
1- Blood **2- liver** **3-kidney(renal)**
4-Cardiac (Heart)

5-gastrointestinal tract **6-Plasma** **7-lungs**

8- White blood cells (WBC) **9-Red Blood Cells (RBC)**

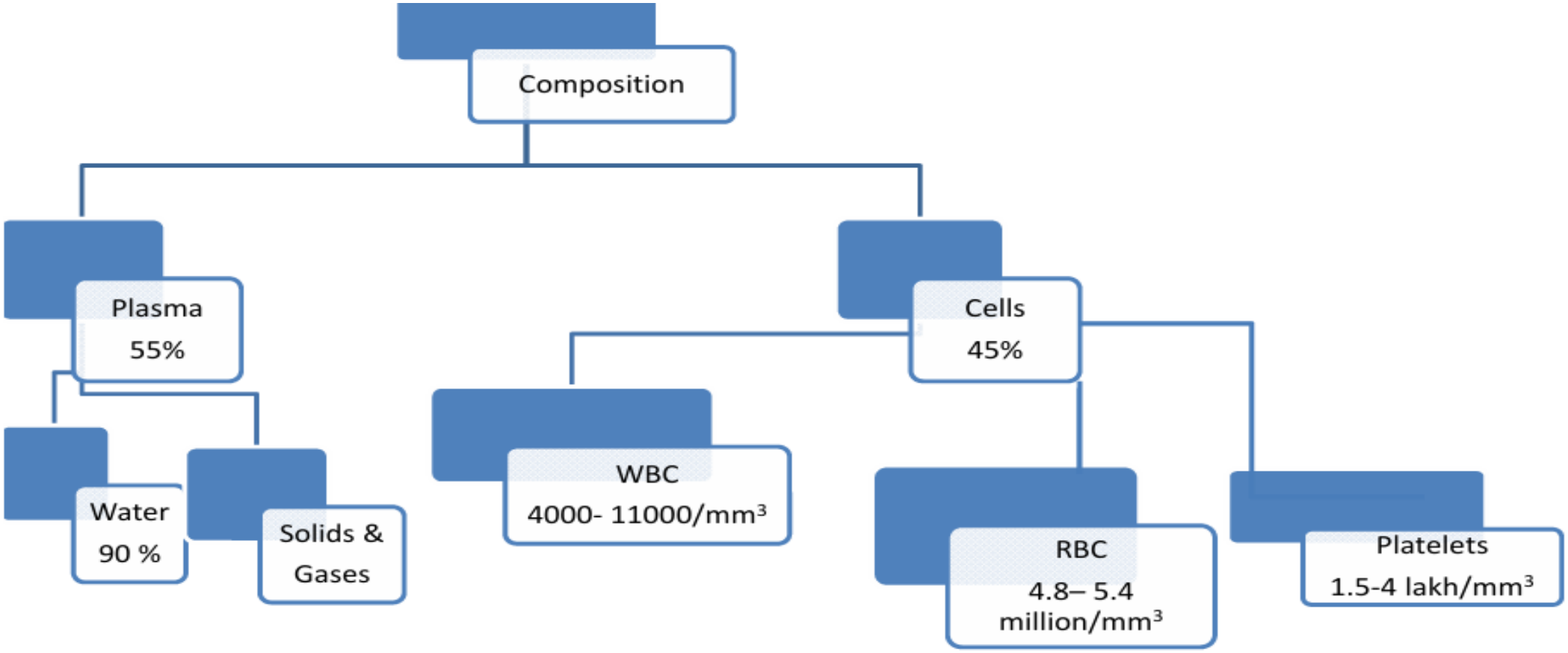
10-Haemocytometer **11-Platelets**

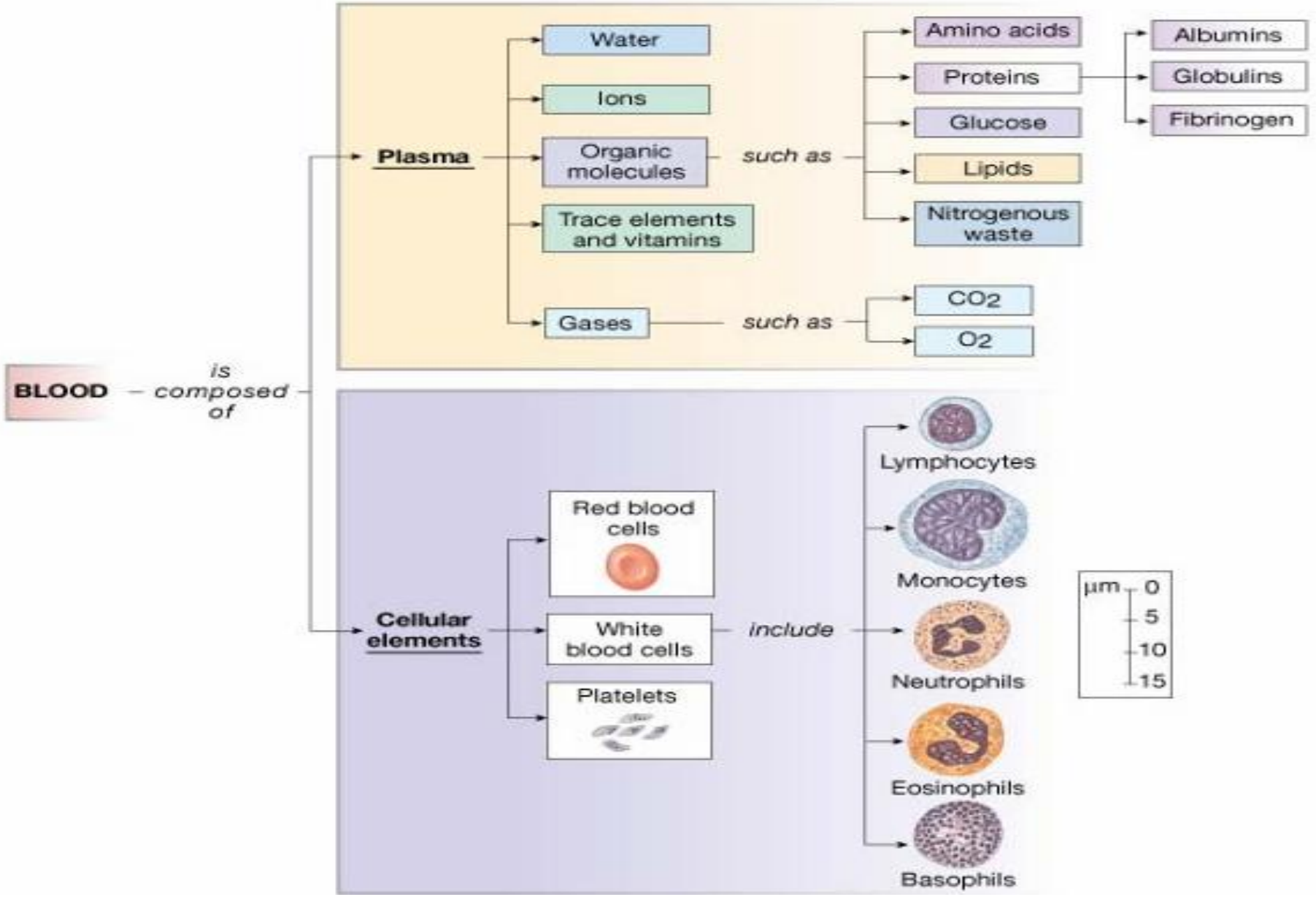
Components of Whole Blood



- Hematocrit
  - Males: 47% ± 5%
  - Females: 42% ± 5%

Components of blood




BLOOD
is composed of
Plasma
Water
Ions
Organic molecules
such as
Amino acids
Proteins
Albumins
Globulins
Fibrinogen
Glucose
Lipids
Nitrogenous waste
Trace elements and vitamins
Gases
such as
CO2
O2
Cellular elements
Red blood cells
White blood cells
Platelets
include
Lymphocytes
Monocytes
Neutrophils
Eosinophils
Basophils
µm
0
5
10
15

Functions of blood

A)Distributive

 # -Carries O2 (from lungs) and nutrients (from GIT and body stores) to all cells

 # -Carries wastes from all cells to elimination sites (lungs for CO2, liver for bilirubin and kidneys for nitrogenous wastes)

#- Carries hormones (chemical signals) from endocrine organs to target tissues.

B) Regulatory functions

- Body T° by absorbing and distributing heat
- pH by virtue of its many buffers
- Maintains adequate fluid volume in the body

C) Protective functions

- Prevents blood loss by initiating clotting mechanisms in response to blood vessel damage
- Prevents infection via WBCs and plasma immune proteins

Types of Blood cells

1. Red Blood Cells (Erythrocytes)- (RBC)

Function: The primary role of red blood cells is to carry oxygen from the lungs to the rest of the body and return carbon dioxide back to the lungs for exhalation.

Structure: They are biconcave in shape (which increases surface area) and lack a nucleus, allowing for more room to carry hemoglobin.

Life Span: Typically around 120 days.

Production: Red blood cells are produced in the bone marrow and are released into the bloodstream.

2. White Blood Cells (Leukocytes)

White blood cells are involved in the immune response and fight infections and foreign substances. There are several different types of white blood cells, each with a specific role:

1-Neutrophils: The most common type of white blood cell. They are the first line of defense against bacterial infections.

2-Lymphocytes: There are two main types:

a)B lymphocytes (B cells): Produce antibodies that fight infection.

b)T lymphocytes (T cells): Help to destroy infected or cancerous cells and regulate the immune system.

3-Monocytes: These cells become macrophages and help engulf and digest pathogens and debris.

4-Eosinophils: Primarily involved in defending the body against parasitic infections and in allergic reactions.

5-Basophils: Release histamine and other chemicals involved in allergic reactions.

3. Platelets (Thrombocytes)

Function: Platelets are involved in blood clotting (hemostasis) by clumping together and forming a plug at the site of blood vessel injury, preventing excessive bleeding.

Structure: They are small, irregularly shaped fragments of larger cells called megakaryocytes.

Life Span: About 7 to 10 days.

Production: Platelets are produced in the bone marrow.

Abnormal Erythrocytes

Abnormal erythrocytes (red blood cells) can occur due to various conditions and diseases, affecting their size, shape, or function. Here are some common types of **abnormal erythrocytes** and the conditions associated with them:

1. **Anisocytosis:-**The presence of red blood cells that vary significantly in size.

Associated Conditions:-

- **Iron deficiency anemia**
- **Thalassemia**
- **Megaloblastic anemia** (due to vitamin B12 or folate deficiency)

2. **Poikilocytosis:-**The presence of abnormally shaped red blood cells

Associated Conditions:

a-Sickle cell anemia b-Hereditary elliptocytosis c-Thalassemia

d-Severe anemia

There are different types of Poikilocytosis

a-Sickle Cells :Red blood cells that are shaped like or sickle, instead of being round and flexible.

b-Target Cells (Codocytes) :Red blood cells that appear to have a target-like shape with a central area of color surrounded by a clear ring.

Associated Conditions: Thalassemia -Liver disease -Iron deficiency anemia

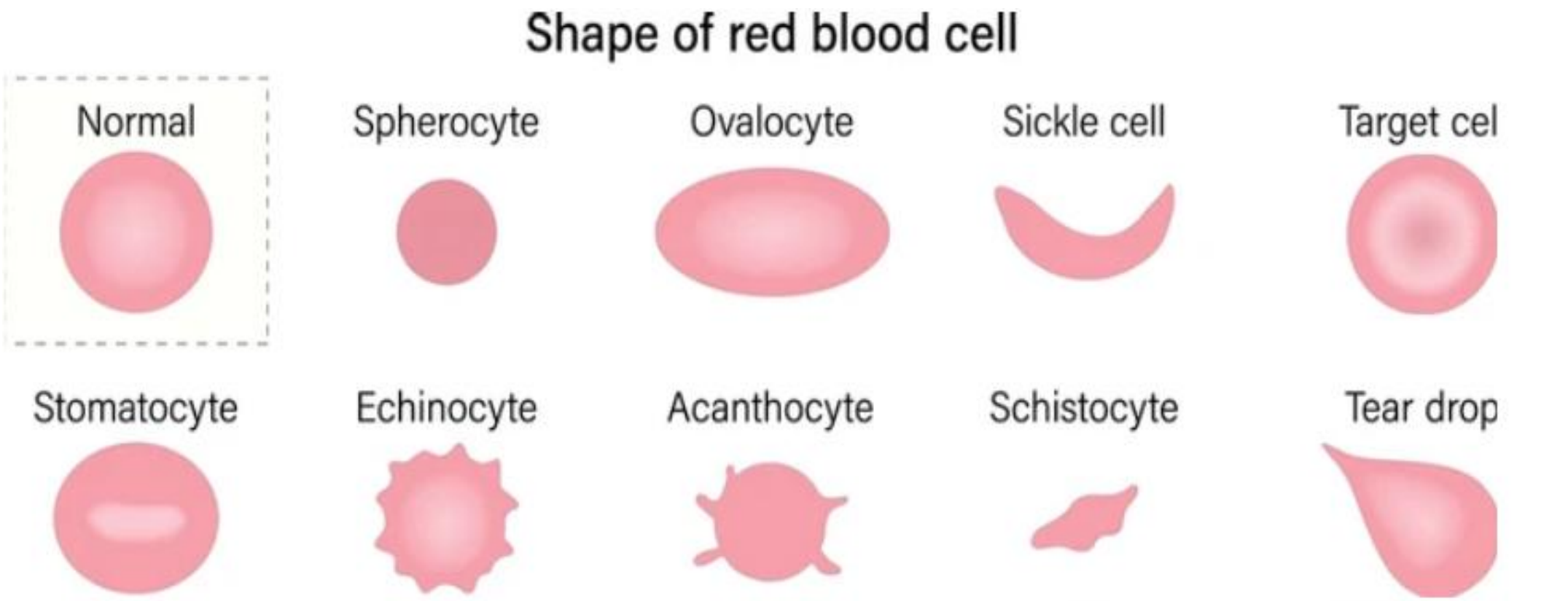
C-Schistocytes (Fragments):-Fragmented pieces of red blood cells, often jagged or irregular in shape.

Associated Conditions:-Hemolytic anemia (due to mechanical damage, such as from artificial heart valves)

D-Microcytes:-Smaller than normal red blood cells

Associated Conditions:-Iron deficiency anemia - Thalassemia

Abnormal Erythrocytes



Most important blood tests

*Blood tests can be used to help a doctor identify a variety of health conditions, including infections, anemia, high cholesterol, vitamin deficiencies, organ failure, HIV, cancer, diabetes, and more.

*Blood tests can help the doctor determine how different organs in your body are working. Examples of organs whose malfunctions can be visible in a blood test include your heart, thyroid, liver, or kidneys. The most important is Complete Blood Count (CBC) which include :-WBC Count : Platelets Count and RBC Count .

WBC Count

Material Needed :

Haemocytometer - Cover slip – WBC dilution fluid (e.g Turk s solution ; glacial acetic acid + gentian violet)- Micropipette – Anticoagulated blood (EDTA).

Procedure:

1-dilution of blood sample .(Mix 20 µL=0.02 mL of blood with 380 µL=0.38 mL of WBC dilution fluid .

This result in a dilution ratio (factor) of 1:20

2- Charging the Haemocytometer .

-place a clean cover slip on the Haemocytometer chamber .

-load a small drop of the diluted blood into the chamber using micropipette .

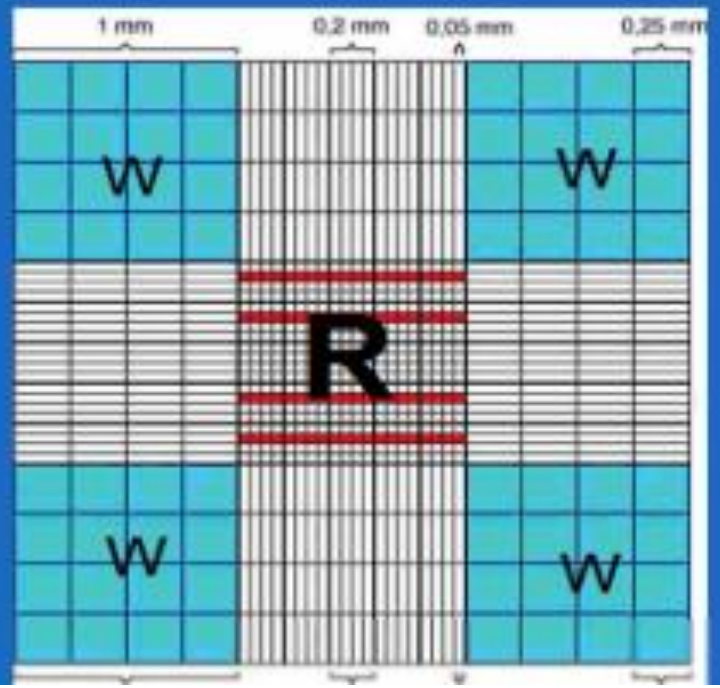
-Allow the WBC to settle for 1-2 minute

3-Microscope Examination .

-Focus the 4 large corner squares (each contain 16 smaller squares using 10X objective ,count all WBC in these 4 large squares.

Use the Following Formula to Count WBC NO

Haemocytometer - Improved neubauer's chamber



Formula for WBC Count

$$\textbf{WBC count} = \frac{\text{Nuber of WBC counted xDilution Factor}}{\text{Volume of counted area (cubic mm)}}$$

If 80 WBC are counted in the large 4 square :-

Dilution factor =20

Volume of counted area= 1mm X 1mm X 0.1= o.4mm^3

WBC = 80 X 20 /0.4 = 4000 cells /µL

The normal **White Blood Cell (WBC) count** typically ranges as follows:

Adults: **4,000 to 11,000 WBCs per microliter (µL)** of blood.

Children: WBC count can vary more, typically ranging from **5,000 to 15,000 WBCs per µL**.

Factors Affecting WBC Count:

Age: Children often have a higher WBC count compared to adults. **Gender**: There is no significant difference between males and females. **Health Conditions**: Infections, inflammation, and certain medications can raise or lower WBC counts. **Pregnancy**: WBC count can be slightly elevated during pregnancy.

Abnormal WBC Counts:

- **Leukocytosis**: A WBC count above **11,000/µL**, typically indicates an infection, inflammation, or other conditions such as leukemia.
- **Leukopenia**: A WBC count below **4,000/µL**, which may indicate conditions like bone marrow disorders, autoimmune diseases, or the effects of certain medications.

1-What is the primary function of blood in the human body?

A) To regulate body temperature
B) To transport oxygen, nutrients, and waste products
C) To control muscle movement
D) To protect the body from viruses

2-Which type of blood cell is primarily responsible for oxygen transport in the human body?

A) White blood cells
B) Red blood cells
C) Platelets
D) Plasma cells

3-An elevated white blood cell (WBC) count is most likely associated with which condition?

A) Anemia
B) Infection
C) Dehydration
D) High blood pressure

4- A condition known as leukocytosis refers to:

A) A low white blood cell count
B) An elevated white blood cell count
C) A normal white blood cell count
D) An absence of white blood cells

5-**An elevated white blood cell (WBC) count is most likely associated with which condition?**

A) Anemia
B) Infection
C) Dehydration
D) High blood pressure

Fill in the blank with suitable term.

Fill in the blank questions with answer about blood functions ?

1-Blood helps to transport oxygen from the lungs to the rest of the body and carbon dioxide from the body back to the lungs for exhalation. This function is carried out by _____.
Answer: Red blood cells

2-One of the key functions of blood is to transport nutrients and hormones to various parts of the body. This function is primarily performed by _____.
Answer: Plasma

3-The blood helps in regulating body temperature by distributing heat throughout the body. This is primarily done by _____.
Answer: Plasma

4-Blood clotting by clumping of platelets is called----------

Happy Ramadan

جامعة شط العرب الاهلية
قسم التحليلات المرضية –كلية العلوم

Pathological analysis

Lach 2 – Platelets and RBC (Role and Count)

Prof. Dr. Mohammed A Fayyadh

Purposes of Platelets Count Experiment

A-Platelets count is part of a complete blood count (CBC).Total Platelets count tells you whether or not you are suffering from Thrombocytosis= Thrombocythemia :-an increase in the no. of platelets or Thrombocytopenia = a decrease in the no. of platelets.

B- To learn how to do the manual method in the lab to get the number of platelets.

C- To get an idea about the possible causes of abnormal platelet count.

Platelets Physiology

Platelets: are not cells (but still considered as a part of the blood cellular elements); rather, they are very small, irregularly shaped, non nucleated fragments from large megakaryocytes.

*The mature platelets are non-nucleated cells with a minute granules present in the cytoplasm and there is no pigment present in platelets.

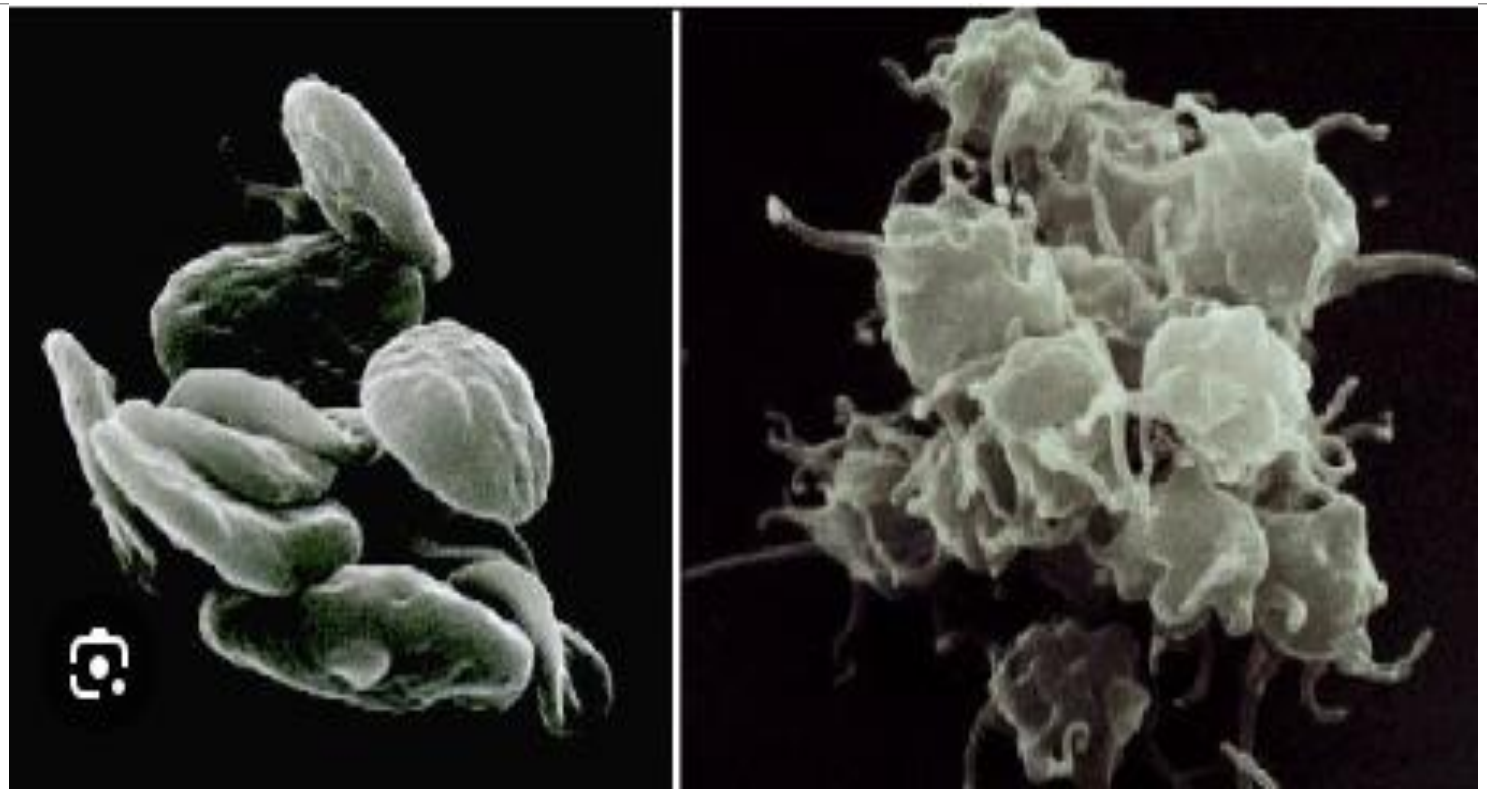
Shape: Shape is spherical or rod and they become oval or disc shaped when inactivated .

Size: On an average, the size of the platelets is 2– 4 µm.

Life Span: The average lifespan of platelets is 3-10 days. Platelets are eliminated from the circulation mainly by the tissue macrophage system in the spleen.

Normal Range: About 150,000– 450,000 cells are present per cubic millimeter (cmm) of blood.

Rest platelets ----- activated



Functions(Role) of Platelets:

1-Hemostasis (Stopping Bleeding): The primary function of platelets is to help stop bleeding when blood vessels are injured. They do this through a series of steps:

- **Adhesion:** When a blood vessel is damaged, platelets are attracted to the site of injury. They adhere to **collagen** in the vessel walls.
- **Activation:** Once adhered, platelets become activated and release various chemicals (such as ADP, thromboxane A2), which recruit more platelets to the site of injury.
- **Aggregation:** Platelets stick together (aggregate), forming a temporary "platelet plug" at the injury site, helping to block the flow of blood.

2-Clot Formation: Platelets also help initiate the **coagulation cascade**, a series of chemical reactions that lead to the formation of a **fibrin clot**. Fibrin, a protein, forms a mesh that strengthens the platelet plug and solidifies the clot.

Functions(Role) of Platelets

3-Wound Healing: Platelets contain growth factors that are released during the clotting process. These factors help with tissue repair and regeneration, assisting in the healing of wounds and injured tissues.

4-Preventing Excessive Bleeding: Platelets play a crucial role in maintaining a balance. While they are essential for forming clots to stop bleeding, their activity is tightly regulated to prevent inappropriate clotting, which could lead to **thrombosis** (formation of blood clots in the wrong place, such as in veins or arteries).

The platelet count

The platelet count in a blood sample is typically measured through a **complete blood count (CBC)** test, The most common methods are:-

1. Automated Hematology Analyzer:

This is the most common way for platelets count .the analyzer uses a laser or electrical impedance to count and measure the size of blood cells, including platelets. the machine gives a count of platelets per microliter (µL) of blood.

Picture-1- Automatical Hematology Analyzer (Model KX – 21) made in Japan 2006.

2-Manual Counting .

a)A blood sample is diluted with a specific solution (e.g., ammonium oxalate).

b)The diluted sample is loaded into a haemocytometer.

c)Platelets are counted under a microscope in specific grid areas of the haemocytometer.

d)The total platelet count is then calculated based on the number of platelets seen in the grid area and the dilution factor.

Formula for Manual Platelet Calculation

$$\text{Platelet Count} = \frac{\text{Total Count of Platelets}}{\text{Volume Counted}} \times \text{Dilution Factor}$$

Example

If you diluted your blood sample 1:100, the dilution factor is **100**.

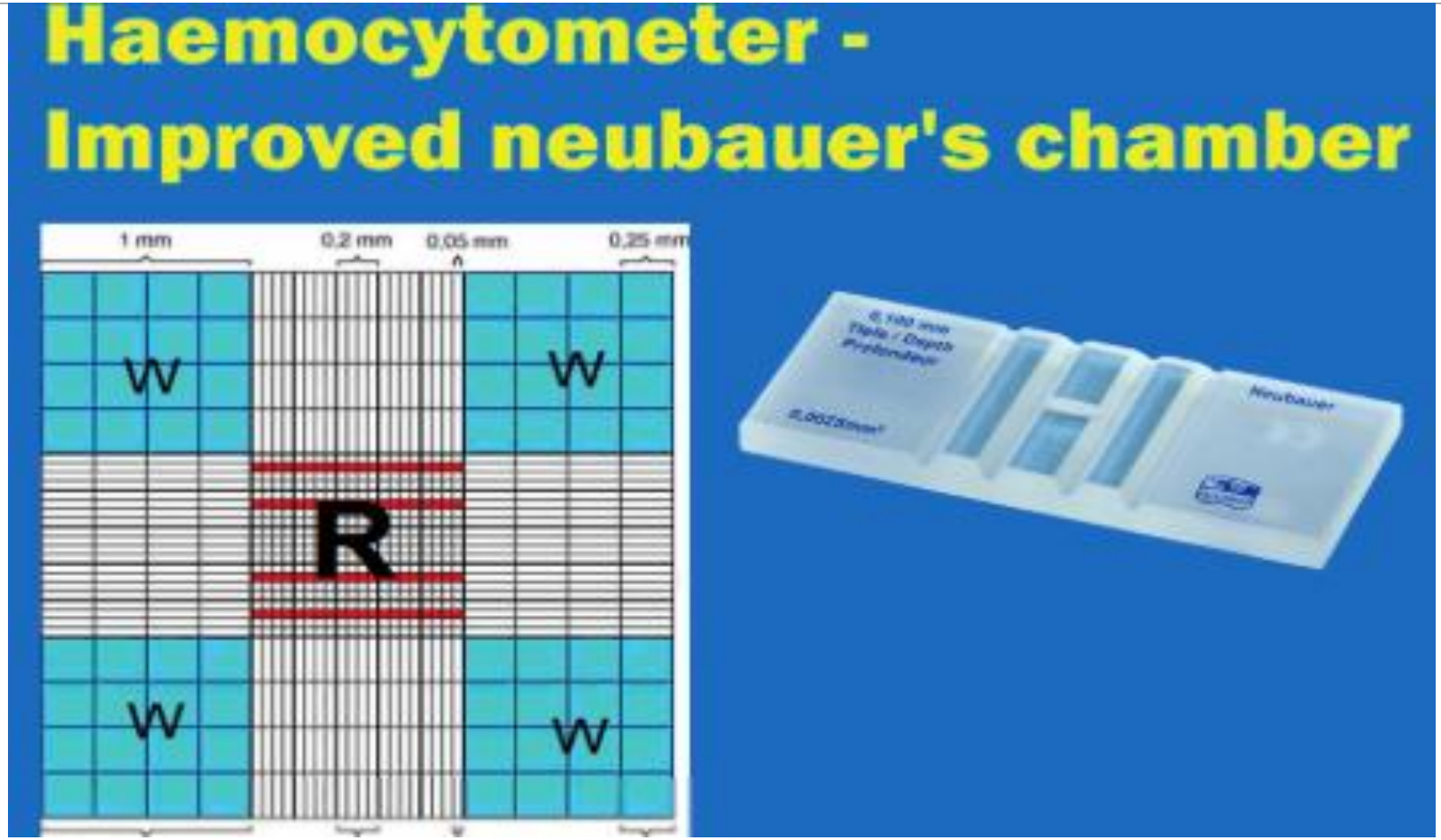
The total No of Platelets counted =200

The volume for the four corner squares is typically **0.1 µL** (0.1 microliter).

$$\text{Platelet Count (per µL)} = \frac{200\,\text{platelets}}{0.1\,\mu\text{L}} \times 100$$

$$\text{Platelet Count (per µL)} = 2000 \times 100 = 200,000\,\text{platelets}/\mu\text{L}$$

The Haemocytometer (Neubauer chamber)



Red Blood Cells

Red blood cells, or **erythrocytes**, are the most abundant type of cell in the blood, playing a key role in transporting oxygen and carbon dioxide throughout the body. Their primary function is to deliver oxygen from the lungs to tissues and organs, and return carbon dioxide from the body tissues back to the lungs to be exhaled.

Structure of Red Blood Cells

Shape: RBCs have a **biconcave disc shape** (a donut-like shape with a flattened center), which increases their surface area and allows for more efficient gas exchange. This shape also helps them move smoothly through the blood vessels.

Size: RBCs are about 6-8 micrometers in diameter.

No Nucleus: Mature red blood cells do not have a nucleus, which makes room for hemoglobin (the protein that carries oxygen and carbon dioxide).

Hemoglobin Content: Hemoglobin is the iron-containing protein inside RBCs that binds to oxygen in the lungs and releases it in tissues that need it.

Role of Red Blood Cells

1-Oxygen Transport:

- RBCs are primarily responsible for **carrying oxygen** from the lungs to the rest of the body. Hemoglobin in the RBCs binds to oxygen in the lungs.

2-Carbon Dioxide Removal:

- After RBCs deliver oxygen to the tissues, they pick up **carbon dioxide** (a waste product of metabolism) from the tissues. Hemoglobin helps to transport the carbon dioxide back to the lungs, where it is exhaled.

3-pH Regulation:

- RBCs also help in **regulating blood pH** by carrying hydrogen ions and bicarbonate ions, which are involved in maintaining the acid-base balance in the body.

Red Blood Cell Count

The **normal RBC count** varies by age, sex.

Typical values are:

- **Men:** 4.7 to 6.1 million cells per microliter (µL) of blood.
- **Women:** 4.2 to 5.4 million cells per microliter (µL) of blood.
- **Children:** 4.1 to 5.5 million cells per microliter (µL) of blood.

Abnormal RBC Counts

Low RBC Count (Anemia):

- When the number of RBCs is low, it can lead to a condition called **anemia**, which reduces the blood's ability to carry oxygen, leading to symptoms such as fatigue, weakness, dizziness, and shortness of breath.
- Causes of anemia include **iron deficiency**, **vitamin B12 deficiency**, **chronic disease**, **blood loss**, and **bone marrow problems**.

High RBC Count (Polycythemia):

A higher-than-normal RBC count is known as **polycythemia**. This condition can make the blood thicker (more viscous), increasing the risk of blood clots, heart attacks, and strokes.

- Causes of polycythemia include **dehydration**, **chronic lung disease**, or conditions like **polycythemia vera**, a type of blood cancer.

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Abnormal erythrocytes (red blood cells) can occur due to various conditions and diseases, affecting their size, shape, or function. Here are some common types of **abnormal erythrocytes** and the conditions associated with them:

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Associated Conditions:-

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- **Megaloblastic anemia** (due to vitamin B12 or folate deficiency)

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Associated Conditions:

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d-Severe anemia

There are different types of Poikilocytosis

a-Sickle Cells :Red blood cells that are shaped like or sickle, instead of being round and flexible.

b-Target Cells (Codocytes) :Red blood cells that appear to have a target-like shape with a central area of color surrounded by a clear ring.

Associated Conditions: Thalassemia -Liver disease -Iron deficiency anemia

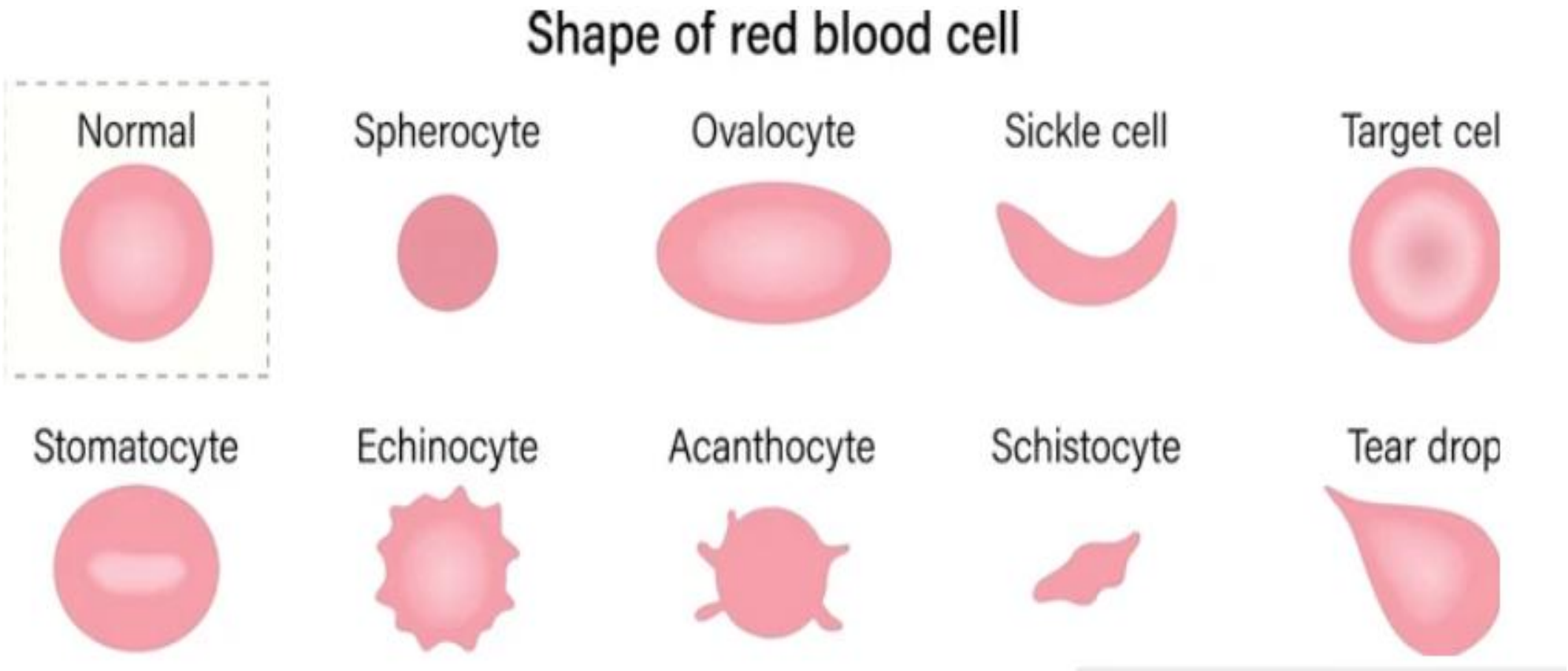
C-Schistocytes (Fragments):-Fragmented pieces of red blood cells, often jagged or irregular in shape.

Associated Conditions:-Hemolytic anemia (due to mechanical damage, such as from artificial heart valves).

D-Microcytes:-Smaller than normal red blood cells

Associated Conditions:-Iron deficiency anemia - Thalassemia

Abnormal Erythrocytes



Manual RBC Count (Using a Haemocytometer)

1-Blood Dilution: A small sample of blood is diluted with a specific solution (like saline or a diluent that helps reduce cell clumping and increases accuracy).

2-Loading the Haemocytometer:

- The diluted blood is placed into the haemocytometer

3-Counting the RBCs:

- Using a microscope, RBCs are counted in the defined grid areas. Multiple grid squares may be counted for accuracy.

4-Calculation: The RBC count is calculated using the following formula:

Red Blood Cells (RBCs)

$$\text{RBC count (cells/}\mu\text{L)} = \frac{\text{Number of cells counted} \times \text{dilution factor} \times 10^4}{\text{Number of squares counted}}$$

MCQ

1. What is the primary function of platelets in the human body?

a) Carry oxygen to tissues
b) Help in blood clotting
c) Transport carbon dioxide
d) Fight infections

2-Which of the following is true about red blood cells (RBCs)?

a) RBCs have a nucleus
b) RBCs are biconcave in shape
c) RBCs are produced in the liver
d) RBCs contain DNA

3-What is the primary component of red blood cells (RBCs) responsible for oxygen transport?

a) Hemoglobin
b) Platelets
c) Plasma
d) Fibrinogen

4-The process of blood clot formation involving platelets is known as:

a) Hemostasis
b) Hemolysis
c) Erythropoiesis
d) Leukopoiesis

5-What is the role of red blood cells (RBCs) in the circulatory system?

a) To fight infections
b) To transport gases such as oxygen and carbon dioxide
c) To aid in blood clotting
d) To produce antibodies

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جامعة شط العرب الاهلية
كلية العلوم –قسم التحليلات المرضية

Pathological analysis

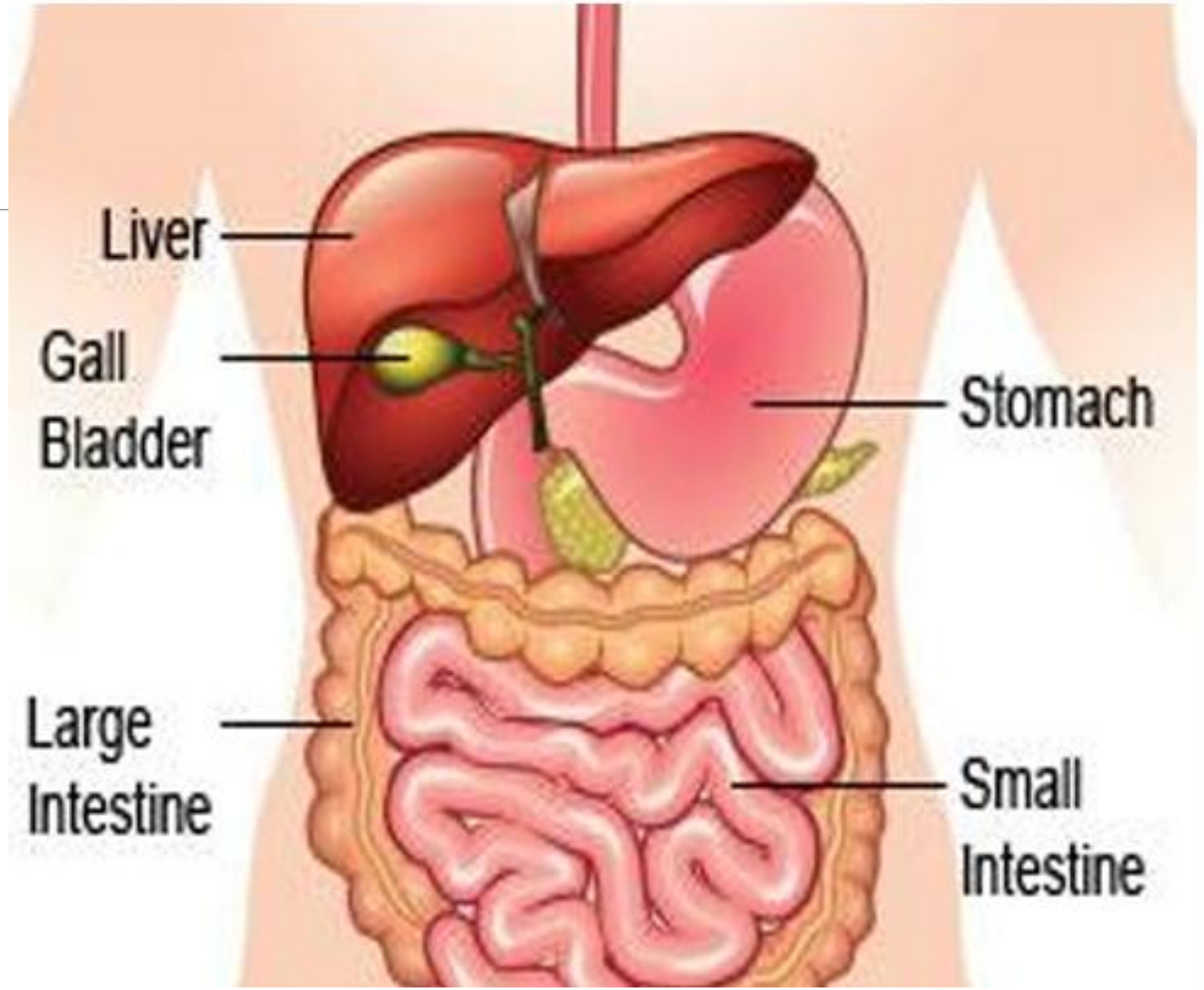
Lach 3 : liver Functions

Prof. Dr. Mohammed A Fayyadh

Liver function test(LFT)

Definition of Liver :

The liver is an organ only found in vertebrates which detoxifies various metabolites, synthesizes proteins and produces biochemical necessary for digestion and growth. In humans, it is located in the right upper quadrant of the abdomen, below the diaphragm. Its other roles in metabolism include the regulation of glycogen storage, decomposition of red blood cells, and the production of hormones. The liver's highly specialized tissue, consisting of mostly hepatocytes, regulates a wide variety of biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions. The total number of liver functions vary, but textbooks generally cite it being around 500 functions .


Liver
Gall Bladder
Stomach
Large Intestine
Small Intestine

Liver Function test

Liver function tests (also called a liver panel) use a sample of blood to measure several substances made by the liver.

The most common liver function tests measure:

Albumin, a protein made in the liver. (synthetic function)

Total protein. This test measures the total amount of protein in the blood, which includes albumin and globulins. **ALP** (alkaline phosphatase), **ALT** (alanine transaminase), **AST** (aspartate aminotransferase), and **GGT** (gamma-glutamyl transferase). These are enzymes that are mainly made in the liver. Enzymes are proteins that speed up certain chemical reactions in the body. (**Cholestasis &Cell damage)**

Bilirubin, a waste product your body makes when it breaks down old red blood cells. the liver removes most of the bilirubin from the body.(**excretory function**)

Prothrombin time (PT), how long it takes your blood to clot. Prothrombin is a protein involved in blood clotting. It's made in your liver.(synthetic function)

Some of these tests can show how well your liver is working and others can show whether your liver may be damaged by liver disease . But liver function tests alone usually can't diagnose specific diseases. So other tests to find the exact cause usually needed .

Objectives of the liver function test .

Liver function tests are most often used to help:

*Find out if liver disease or damage could be causing certain symptoms

*Learn how serious liver disease is after it has been diagnosed

*Monitor(مراقبة)liver diseases over time and/or find out how well treatment is working

*Check(تقيم)for side effects of certain medicines that can affect the liver

The most important symptoms of liver diseases.

-Nausea (غثيان)and vomiting

-Lack of appetite

-Fatigue(تعب)

-Weakness

-Jaundice,(يرقان)a condition that causes your skin and eyes to turn yellow

-Swelling and/or pain in your abdomen .

-Swelling in your ankles and legs

-Dark-colored urine .and/or light-colored stool .

-Frequent itching

Glutamate Oxaloacetate Transferase (GOT) measurement .

Glutamate Oxaloacetate Transaminase (GOT) (Aspartate Aminotransferase – AST)is essential for **amino acid metabolism** and plays an important role in the **liver's function, energy production, and nitrogen balance**. Its levels in the blood can also be used as a diagnostic tool for liver and heart-related health issues.

Steps measurement of GOT

A-Preparation

1-The patient may be asked to Fast for 8-12 hours before the test (depending on the doctor s instruction).

2-Certain medications (like statins or antibiotics)might be paused ,as they can affect enzyme level .

B –Blood sample collection

1-Clean the skin with an antiseptic wipe.

2-Warps a tourniquet around the arm to make veins more visible .

3-Insert the Needle into a vein and collects the sample into sterile tube.

C-sample handling

1- the blood sample is labeled and sent to a laboratory for analysis .

2-In the lab the serum (the liquid portion of blood) is separated by Centrifugation.

D-Enzyme Analysis (Biochemical testing)

1-The test typically uses a colorimetric assay or Spectrophotometry to measure **AST/GOT activity .**

2-The enzyme ability to catalyze the transfer of an amino group is measured by the production of specific by products (like Oxaloacetate) which produce color change .

3-The intensity of color is directly proportional to the enzyme activity --- measured as unit per liter (U/L)

E-Results and interpretation.

1-Normal Range :- (10-40 U/L)

2-Elevated level can indicate :-

*Liver damage (Hepatitis(التهاب الكبد), Cirrhosis (تليف الكبد) ,Fatty liver disease)

*Heart damage * Muscle injury * Alcohol or drug toxicity

Spectrophotometer

Review questions

1. Which of the following liver enzymes is primarily used as a marker for liver damage or dysfunction?

a) Lactate dehydrogenase (LDH)
b) Alkaline phosphatase (ALP)
c) Glutamate Oxaloacetate Transaminase (GOT)
d) Creatine kinase (CK)

2- **Which of the following is a common cause of elevated Aspartate Aminotransferase levels?**

a) Alcoholic liver disease
b) lipid rich food
c) Acute myocardial infarction
d) Renal failure

3- **A prolonged prothrombin time (PT) is indicative of:**

a) Acute liver failure
b) Hyperthyroidism
c) Kidney dysfunction
d) Diabetes mellitus

Fill in the blank

1-The GOT activity can be measured by----------

2-Elevated level of GOT can indicate ----------such as----------and----------

3-Glutamate Oxaloacetate Transaminase also known as----------

4---------- is a condition that causes your skin and eyes to turn yellow.

5- ----------is a waste product results from break down old red blood cells.

GOT -Catalyzes the transfer of an amino group from **aspartate** to **α-ketoglutarate**, forming **glutamate** and **oxaloacetate**.

Alkaline phosphatase (ALP) is an enzyme found in various tissues throughout the body,. It plays a role in the breakdown of proteins by catalyzing the removal of phosphate groups from molecules in an alkaline (basic) environment.

Alanine transaminase (ALT),, ALT catalyzes the conversion of **alanine** and **α-ketoglutarate** into **pyruvate** and **glutamate**. This process is part of the body's mechanism for amino acid breakdown and energy production.

Gamma-glutamyl transferase (GGT) is an enzyme involved in the transfer of the **gamma-glutamyl group** It plays a significant role in **amino acid metabolism**, especially in the **transport and breakdown of glutathione**, which is an important antioxidant in the body.

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كلية العلوم –قسم التحليلات المرضية

Pathological analysis

Lach 4 – Liver Enzyme (GPT,ALP)

Prof. Dr. Mohammed A Fayyadh

Liver enzymes

Liver enzymes are proteins that play a critical role in the chemical reactions within the liver, helping with processes such as **metabolism, detoxification, and the breakdown of nutrients**. The primary liver enzymes include:

1-Aspartate Aminotransferase (AST) – Also called GOT **(glutamate oxaloacetate transaminase),** AST is involved in the conversion of amino acids and is found in several organs, including the liver, heart, and muscles. Elevated AST levels can indicate liver disease, but it can also reflect damage to other organs.

2-**Alanine Aminotransferase (ALT)** – Also known as SGPT (serum glutamate pyruvate transaminase), ALT helps convert amino acids into energy and is primarily found in the liver. **High levels of ALT in the blood often indicate liver damage**.

3-**Alkaline Phosphatase (ALP)** – This enzyme is important in the breakdown of proteins and is found in various tissues, especially in the liver, bones, and bile ducts. **Elevated ALP levels can indicate liver problems, bile duct obstructions, or bone conditions**.

4-**Gamma-glutamyl transferase (GGT)** – GGT helps in the metabolism of glutathione and is involved in the transfer of amino acids. **It is a sensitive marker for liver disease, particularly in cases of alcohol-induced liver damage**.

5-Lactate Dehydrogenase (LDH) – Though not exclusive to the liver, LDH is present in many tissues, including the liver. **Elevated LDH levels can be a sign of liver damage, but they may also reflect damage to other organs**.

Alkaline Phosphatase (ALP) Analysis

ALP catalyzes the hydrolysis of phosphate esters at an alkaline pH, producing an alcohol and free phosphate. Increased ALP levels are often associated with bone disorders, liver diseases, or biliary tract obstructions.

Materials:

#-Serum or plasma sample #-ALP assay reagent (usually includes a substrate like p-nitrophenyl phosphate, buffer, and activators) #-Photometer or spectrophotometer # -Test tubes or cuvettes

Procedure:

1-Sample Collection:

- Collect venous blood from the patient and prepare serum or plasma by allowing the sample to clot and centrifuging it.

2-Reagent Preparation:

- Prepare the ALP assay reagent, typically containing p-nitro phenyl phosphate (a substrate), an alkaline buffer (such as diethanolamine buffer), and magnesium ions for activation.

3-Reaction Setup:

- Pipette a specific volume of serum/plasma (usually 50 µL) into a cuvette and Add the ALP reagent (usually 1 mL) to the sample.

4-Incubation:

- Incubate the sample-reagent mixture at 37°C for a set time (typically 30 minutes).

5-Measurement:

- Measure the absorbance of the reaction mixture at 405 nm, which corresponds to the yellow color produced when p-nitrophenyl phosphate is hydrolyzed to p-nitrophenol.

Example Calculation for ALP Activity

1-**Assume** the laboratory has a standard reaction rate for the ALP enzyme, which is 1.0 absorbance units per minute (A/min) at a particular wavelength.

2-**Sample Measurement**: When the patient's sample is measured, it shows an absorbance change of 0.35 A/min.

3-**Calculate Enzyme Activity**: You can calculate the enzyme activity using the

$$\text{ALP Activity (U/L)} = \frac{\text{Sample Absorbance Change per min}}{\text{Standard Absorbance Change per min}} \times \text{Standard ALP Activity (U/L)}$$

If the standard ALP activity is **150 U/L** and the sample shows an absorbance change of **0.35 A/min**, the calculation would be:

$$\text{ALP Activity} = \frac{0.35}{1.0} \times 150 = 52.5\,\text{U/L}$$

The **normal range** for **Alkaline Phosphatase (ALP)** can vary depending on factors such as age, sex, and the laboratory's methods, but generally:

Adults: **44 to 147** IU/L (International Units per liter)

Children (especially during growth phases): The normal range for children can be from **150 to 600 IU/L**, depending on their age and developmental stage.

Health conditions: Elevated ALP can indicate liver disease, bile duct obstruction, bone disorders (like Paget's disease), or certain cancers. Low ALP levels may be associated with conditions like malnutrition or hypothyroidism.

Glutamate Pyruvate Transaminase(GPT) Analysis =ALT

The **GPT (Glutamate Pyruvate Transaminase)**, also known as **ALT (Alanine Aminotransferase)** test, is a blood test used to measure the level of ALT, an enzyme found primarily in the liver which catalyzes the conversion of alanine and alpha-ketoglutarate to pyruvate and glutamate . It helps assess liver function and detect potential liver damage.

Preparation:-

Fasting: Typically, fasting for 8–12 hours before the test is recommended, though it might not always be necessary.

Medications: Inform the doctor about any medications or supplements you're taking, as some might affect the results.

Alcohol: Avoid alcohol for at least 24 hours before the test, as it can interfere with the enzyme levels.

Materials:

*Serum or plasma sample

*ALT assay reagent (often involving substrates like pyruvate and coenzymes like NADH)

*Photometer or spectrophotometer

*Test tubes or cuvettes

Procedure

Sample Collection:

- Collect venous blood from the patient using appropriate collection tubes (e.g., serum separator tubes).
- Allow the sample to clot, then centrifuge to obtain serum or plasma.

Reagent Preparation:

- Prepare the ALT assay reagent (e.g., alpha-ketoglutarate or pyruvate), coenzymes (NADH), and buffers.

Reaction Setup:

- Pipette a specific volume of the serum/plasma sample into a cuvette or test tube (usually 50 µL).
- Add the reagent to the sample, usually in a 1:1 ratio.

Incubation:

- Incubate the mixture at 37°C (body temperature) for a set period (typically 30 minutes).

Measurement and Calculation

- Measure the absorbance of the reaction mixture at a wavelength of 340 nm (which corresponds to NADH absorption) using a spectrophotometer. The decrease in NADH absorbance is proportional to the ALT activity. And calculate ALT activity by comparing the rate of absorbance change (ΔA) to a standard curve or using the following formula:

- $$ALT\ activity\ (u/L) = \frac{Sam\ Abso\ changes\ \ per\ \min u/L}{Standared\ Abs\ \ change\ Per\ min} XStandard\ \ ALT\ Activity(u/L$$

Result Interpretation:-

Normal Range: The normal ALT range typically varies from 7 to 56 units per liter (U/L) of blood. However, the reference range may vary slightly based on the laboratory and method used.

Elevated ALT Levels: Elevated ALT levels may indicate liver damage or disease, such as hepatitis, fatty liver disease, cirrhosis, or liver injury from alcohol or medication.

cuvettes

MCQ

1- What does an elevated level of ALP (Alkaline Phosphatase) indicate?

A) Liver dysfunction
B) Bone disease
C) Kidney failure
D) Both A and B

2- Which organ is primarily associated with elevated levels of GPT (ALT)?

A) Heart
B) Brain
C) Liver
D) Kidneys

3- What is the normal range for GPT (ALT) in the blood?

A) 5-40 U/L
B) 10-50 U/L
C) 30-100 U/L
D) 50-120 U/L

4- Which of the following is *NOT* a common cause of elevated ALP (Alkaline Phosphatase) levels?

A) Liver diseases
B) Bone diseases (like Paget’s disease)
C) Gallbladder disorders
D) Hyperthyroidism

Fill in the blank

1- The ALP assay reagent, typically containing ----------as substrate, an alkaline buffer such as ----------, and ---------- ions for activation.

2-ALT assay reagent often involving substrates like ---------- and coenzymes like ----------

3- Increased ---------- levels are often associated with bone disorders, ---------- diseases, or biliary tract obstructions.

I wish you the Best

جامعة شط العرب الاهلية

كلية العلوم –قسم التحليلات المرضية

PATHOLOGICAL ANALYSIS

LACH -5 -DIABETES MELLITUS (DISEASE)

PROF . DR . MOHAMMED A FAYYADH

Diabetes Disease

#-It is a chronic disease due to disorder of carbohydrate metabolism, due to insulin deficiency results in hyperglycemia (increased blood glucose level) & glucosurea (presence of glucose in urine).

#-Associated with several changes in metabolism; such as metabolism of proteins & fats.

Clinical Biochemical Findings in Diabetes

*Glucosuria.

*Large volume of urine &

increase urination frequency (Polyuria)

*Polyphagia
(eats more frequently).

*Always thirsty

*Several metabolic changes

Metabolic changes in diabetes

Include increase in:

- Fat catabolisim leads to increase in FFAs(Free fatty acids) in blood & liver.
- Acetyl.coA leads to increase formation of cholesterol & risk of atherosclerosis.
- ketone bodies generation in blood and urine leads to acidosis.
- catabolism of tissue protein due to energy requirement (because glucose can't uptake by cells) lead to weight loss and increase in level of amino acids in blood & more formation of urea by deamination of amino acid.

Types of diabetes

-Type I diabetes mellitus (TIDM)

-Type 2 diabetes mellitus (TIIDM)

- Gestational diabetes mellitus (GDM)

- Other "due to drugs or chemicals"

Types of diabetes:

1) ***Type 1 diabetes(T1D)/ Juvenile diabetes/ Insulin dependent diabetes:*** T1D affects both adults and children at any age and occurs when the person's pancreas stop producing insulin. Affected individuals depend on daily injections of insulin to maintain normal blood glucose levels.

2) ***Type 2 diabetes/ Non-insulin dependent diabetes mellitus (T2D or NIDDM):*** This is the most common form of diabetes that most often occurs in adulthood. In T2D, fat, muscle and liver cells do not respond correctly to insulin. This is called insulin resistance. As a result, blood sugar cannot enter these cells to be used for energy production . Insulin resistance is a gradual process that develops slowly over time.

3) Gestational diabetes: This refers to diabetes that is first diagnosed during pregnancy. As many as eight out of 100 pregnant women develop gestational diabetes. Weight gain and changing hormones that occur during pregnancy can impair insulin function, resulting in high blood sugar. This form of diabetes usually disappears after pregnancy, however, women who have had gestational diabetes have a 40-60% chance of developing T2D within 5 to 10 years.

Diabetic profile:

- Is group of tests that are used to diagnose diabetes or its complications , it includes:

1. C-peptide.
2. Blood glucose (4 types: FBS, PPBS, RBS, OGTT)
3. HbA1C
4. Insulin
5. ICA (islet cell antibody) for type I
6. Ketones
7. Microalbuminurea.

Types of blood glucose tests:

(1) Fasting blood sugar (FBS):

measures blood glucose after fasting for at least 8-12 hrs

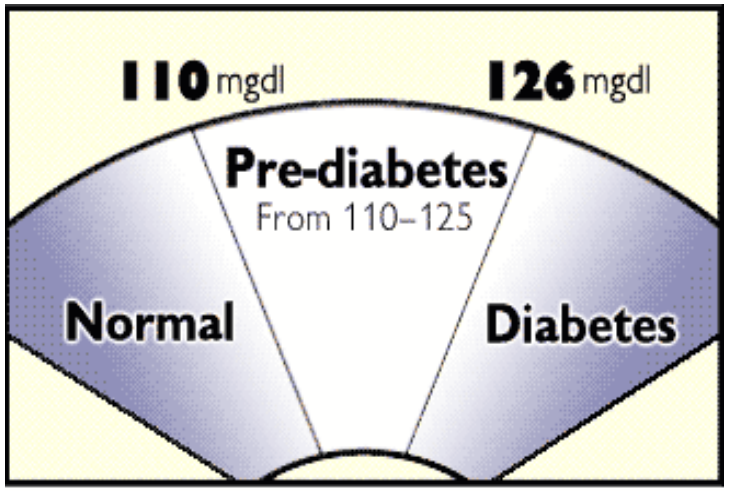
It often is the first test done to check for diabetes.

patient with mild or borderline diabetes may present with normal FBG values.

If diabetes is suspected, GTT can confirm the diagnosis.

Normal levels:

60-110mg/dl



Fasting Plasma Glucose Test

(2) Post-Prandial Blood Sugar (2-hour PPBS):

After the patient fasts for 12 hours, a meal is given which contains starch and sugar (approx. 100 gm).

Then after 2 hours blood is collected to measure glucose level.

home blood sugar test is the most common way to check 2-hour postprandial blood sugar levels.

For those who don't have diabetes:

less than 140 mg/dL.

For those who have diabetes:

less than 180 mg/dL

(3) Random blood sugar (RBS)

*measures blood glucose randomly at any time throughout the day without patient fasting.

*it is useful because glucose levels in healthy people don’t vary widely throughout the day.

*blood glucose levels that vary widely may indicate a problem.

(4) Oral glucose tolerance test (OGTT)

*Glucose Tolerance is defined as the capacity of the body to tolerate an extra load of glucose or it measures the body's ability to use glucose.

*it is series of blood glucose measurements taken after drink glucose liquid

*It is considered as definitive diagnostic test for DM.(Diabetes Mellitus)

*It is ordered to:

#- confirm the diagnosis, in pre-diabetic
-diagnose gestational diabetes (most commonly)

Recommended if 100-126 mg/dl or (5.5 mmol/L-7.0 mmol/L).

Procedure for (OGTT)

-Arrive FBS: After an overnight fasting of 12-16 hrs

-Drink: 75-100g of glucose dissolved in 250-300ml of water and given orally.

-After drink: blood samples and urine are collected every 30min for 3hrs (1 hr, 1.5 hr , 2hr, 2.5hr, 3hr)

-A curve between time and blood glucose concentration, is plotted.

Interpretation:

Normal Response :

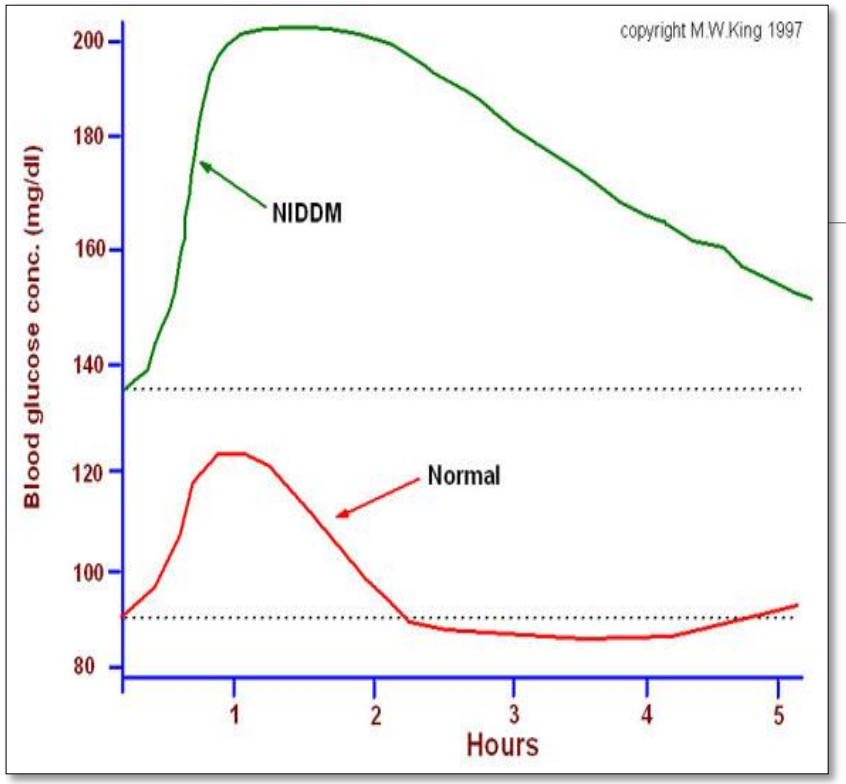
FBS is normal. After 1 hr it will rise, returns to normal fasting level within 2 hours.

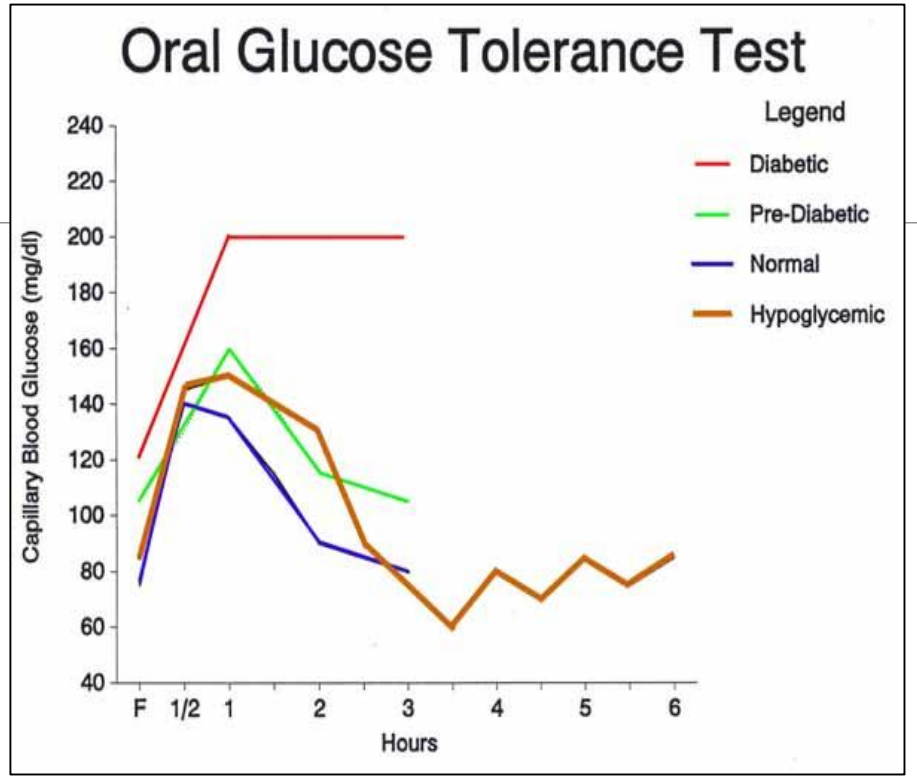
Diabetic curve :

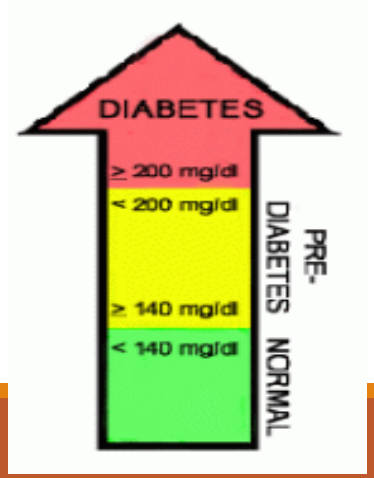
FBS: 140mg/dl or 7.8 mmol/L. After 2 hr: 200mg/dl (11 mmol/L) or more. Glucosuria is usually seen

Impaired GTT:

- with 2hrs glucose level between 140mg/dl - 200mg/dl
- it is not abnormal but must be followed up for DM.


copyright M.W.King 1997
Blood glucose conc. (mg/dl)
200
180
160
140
120
100
80
NIDDM
Normal
1
2
3
4
5
Hours


Oral Glucose Tolerance Test
Legend
Diabetic
Pre-Diabetic
Normal
Hypoglycemic
Capillary Blood Glucose (mg/dl)
240
220
200
180
160
140
120
100
80
60
40
F
1/2
1
2
3
4
5
6
Hours


DIABETES
≥ 200 mg/dl
< 200 mg/dl
≥ 140 mg/dl
< 140 mg/dl
PRE-DIABETES
NORMAL

3- HbA1c:(Glycosylated hemoglobin)

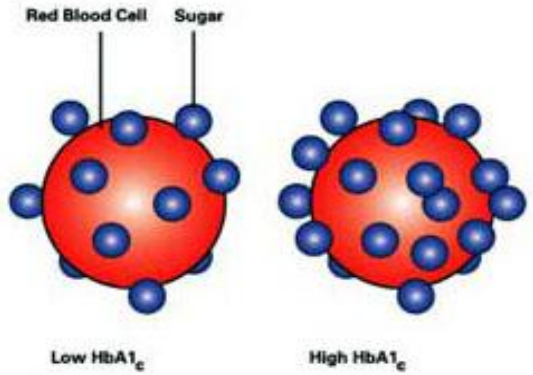
* HbA1C: is glucose bound to hemoglobin

* Measures blood glucose conc. over a longer period of time

* it indicates how well diabetes has been controlled in the 2-3 months before the test.

*The A1C level is directly related to complications from diabetes (lower the A1C level lower risk for complications)

* Type of sample: whole blood in EDTA tube



Normal Values:

Glycohemoglobin A1c:4.5%-5.7%

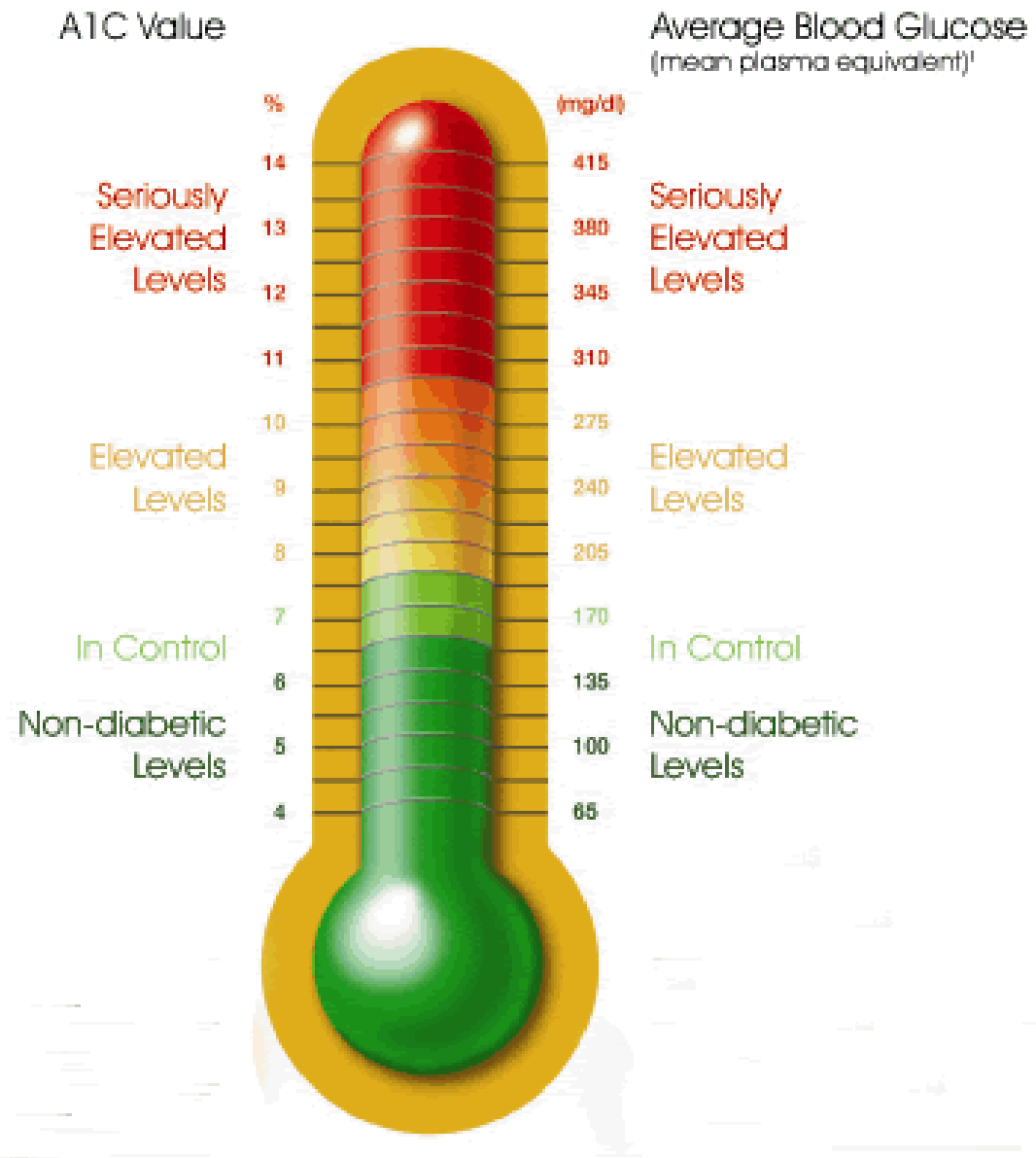
Total glycohemoglobin:5.3%-7.5%

Expected range of Hb A1c:

Sugar: 90-150 5 to 7%

Sugar: 150-180 7 to 8 %

Sugar: 180-360 9 to 14 %



Kit components

****Glucose Oxidase Reagent :**

mixture of:

glucose oxidase + peroxidase+ aminoantipyrine+ buffer

****Glucose standard Reagent :**

conc. 100mg/dl or 5.55 mmol/L

Calculations:

$$Glucose.conc = \frac{Abs.sample}{Abs.stander} \; x \; conc.stander$$

Hypoglycemia :

When blood glucose falls below 60 mg/dl.

Causes:

Most commonly seen in overdose of insulin in treatment of DM.(Diabetes mellitus)

1. **Hypothroidism. الدرقية**
2. **Insulin secreting tumours of pancrease – (rare)**
3. **Hypoadrenalism (Addison's disease)الكظرية**
4. **Hypopitruitism. النخامية**
5. **Severe exercise.**
6. **Starvation.**

MCQ

1-What is the main cause of Type 1 diabetes mellitus?

a) Insulin resistance

b) Insulin deficiency .

c) Obesity

d) Poor diet

2-**Which of the following tests is used to diagnose diabetes mellitus?**

a) Hemoglobin A1c

b) Complete blood count (CBC)

c) Electrocardiogram (ECG)

d) Chest X-ray

3-Which organ produces insulin in the body?

a) Liver

b) Heart

c) Pancreas

d) Kidney

4-What is the recommended target blood sugar level for most people with diabetes?

a) 70-100 mg/dL before meals

b) 120-160 mg/dL before meals

c) 200-250 mg/dL before meals

d) 50-70 mg/dL after meals

I wish you the Best

جامعة شط العرب الاهلية
قسم التحليلات المرضية –كلية العلوم

Principles of Pathological analysis

Lach 6 – Blood Urea –Blood Creatinine

Prof. Dr. Mohammed A Fayyadh

Introduction

The kidneys are two bean shaped organs lying retroperitoneally (خلف الصفاق)on each side of the vertebral column slightly above the level of umbilicus. (السرة)

•The range in length & weight, respectively, from approximately 6cm & 24gms in a full term infant to more than/equal to 12cm & 150gms in an adult .

Kidney Functions

Filtration of Blood: The kidneys filter waste products and excess substances, including fluids, from the bloodstream. These wastes are then excreted as urine.

Regulation of Fluid and Electrolyte Balance: The kidneys help maintain the balance of water, salts, and minerals (such as sodium, potassium, and calcium) in the body. This is essential for proper cell function, nerve signaling, and muscle contractions.

Regulation of Blood Pressure: The kidneys play a critical role in regulating blood pressure by controlling the volume of blood (through fluid regulation) and by producing the enzyme Renin, which helps adjust blood pressure.

Kidney Functions

Acid-Base Balance: The kidneys help maintain the body's pH level (acid-base balance) by excreting hydrogen ions and reabsorbing bicarbonate from urine, helping keep the blood's pH within a healthy range**.(7.35-7.45)**

Detoxification: The kidneys filter out toxins and waste products like urea, creatinine, and excess drugs or medications from the bloodstream, helping detoxify the body.

Hormone Production: The kidneys produce and release hormones, such as erythropoietin, which stimulates red blood cell production, and E renin, which helps regulate blood pressure. They also convert **vitamin D** into its active form, which is essential for calcium absorption.

Blood Urea

Blood urea :is the amount of urea present in the blood. Urea is a waste product that forms when the body breaks down proteins. The liver produces urea as a byproduct of metabolizing nitrogen from protein, and it is then transported through the bloodstream to the kidneys, where it is filtered and excreted in urine.

The **blood urea nitrogen (BUN)** level is a commonly measured marker of kidney function. Elevated levels of blood urea can indicate kidney dysfunction or dehydration, as the kidneys are responsible for filtering urea from the blood. When the kidneys aren't functioning properly, urea accumulates in the bloodstream. The amount of excreted urea varies directly with dietary protein intake, increased excretion in fever, diabetes, and increased adrenal gland (الغدة الكظرية)activity.

Blood urea & serum creatinine

Estimation of blood urea & serum creatinine are useful.

- **Serum creatinine is a better indicator than urea.**
- **But a markedly increased blood urea is conclusive evidence of severe impaired glomerular function. In chronic renal disease, the blood urea level correlates better with symptoms of uremia than does the serum creatinine.**

Blood Urea Estimation

The biochemical estimation of **Blood Urea Nitrogen (BUN)** involves measuring the concentration of urea in blood, which is an important marker of kidney function. The process generally involves converting urea into a measurable product through a series of enzymatic or chemical reactions. Here's an overview of the most commonly used methods:

1. **The Urease Method :** This method is based on the principle that the ammonia released reacts with a reagent to produce a color change, which is directly proportional to the amount of urea present in the sample.

Urea in the blood reacts with the enzyme urease, which catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} \text{Ammonia } (NH_3) + CO_2$$

The ammonia produced is then measured either directly or indirectly.

Procedure:

Sample Preparation: Blood plasma or serum is used for this test.

Urease Reaction: Urea in the sample is broken down into ammonia using urease.

Color Development: The ammonia produced reacts with an acid or a colorimetric reagent (often phenol and hypochlorite), resulting in a color change.

Measurement: The intensity of the color change is proportional to the concentration of urea in the sample. The absorbance is measured spectrophotometrically at a specific wavelength (e.g., 580 nm).

2. **Glutamate Dehydrogenase (GLDH) Method.**

This method involves the coupling of the urea hydrolysis reaction with a series of enzymatic reactions to form a measurable compound.

-Urea is first hydrolyzed by urease to ammonia and carbon dioxide.

#-The ammonia then reacts with α-ketoglutarate in the presence of glutamate dehydrogenase (GLDH) to form glutamate and NADH.

#-The NADH formed can be measured spectrophotometrically at 340 nm, and its concentration is proportional to the urea concentration.

3. **Diacetyl Monoxime Method.**

This method involves the reaction of urea with Diacetyl monoxime and Thiosemicarbazide in an acidic medium.

Urea reacts with diacetyl monoxime in the presence of thiosemicarbazide, producing a pink-colored complex. The intensity of the color is proportional to the concentration of urea in the blood .The color intensity is measured spectrophotometrically.

Reference Range :-

11-40 mg /dL

#- A normal level of blood urea is often mistakenly regarded to indicate normal kidney function.

#-In a steady state the blood urea may not rise beyond the upper range of normal(40mg/dl) even when 75% of the renal function is lost.

#-There may be transient rise in blood urea level due to :-

#- high protein intake **#-**severe infections **#-** tissue breakdown **#-** trauma (صدمة)

#-use of large doses of corticosteroids or Tetracycline)

#- gastrointestinal bleeding & inhibition of anabolism.

Additional Tests for Kidney Function:

To get a complete picture of kidney function, blood urea levels are often assessed alongside other tests, such as:

Creatinine: A waste product from muscle metabolism, which is also filtered by the kidneys. Elevated creatinine can also indicate impaired kidney function.

Glomerular Filtration Rate (GFR): A calculation based on blood creatinine levels, age, gender, and other factors to estimate kidney function.

Creatinine Test

Creatinine is a waste product that is produced continuously as a result of muscle metabolism. It is generated from the breakdown of **creatine**, a molecule that plays a key role in energy production in muscles. Once creatinine is formed, it enters the bloodstream and is then filtered out by the kidneys, ultimately being excreted in urine.

Creatinine levels in the blood are commonly used as an indicator of **kidney function**. Normally, the kidneys filter creatinine out of the blood efficiently, so a rise in blood creatinine levels can indicate that the kidneys are not functioning well or are impaired.

How to Estimate Creatinine:

Blood Sample Collection: usually from a vein in the arm.

Laboratory Analysis: The sample is sent to a laboratory, where the creatinine concentration in the blood is measured.

Results: The laboratory provides a creatinine value (usually in mg/dL), which is:-

Men: 0.6 to 1.2 mg/dL

Women: 0.5 to 1.1 mg/dL

Children: 0.2 to 0.7 mg/dL (depending on age and size)

What High Creatinine Levels Can Indicate:

Kidney disease: Chronic kidney disease (CKD), acute kidney injury (AKI), or kidney infections can reduce kidney function, leading to elevated creatinine levels in the blood.

Dehydration: When the body is dehydrated, the kidneys have less fluid to filter out waste, leading to a higher concentration of creatinine in the blood.

Muscle damage: Conditions that cause muscle breakdown, like rhabdomyolysis) انحلال العظلات المخططة(, can lead to increased creatinine production.

Obstruction: Any blockage in the urinary tract can lead to a buildup of creatinine in the blood.

Measurement of Creatinine

To measure the concentration of creatinine in blood, the most common biochemical procedure involves the **Jaffe reaction**, which is based on the reaction between creatinine and alkaline picrate. Here's a general outline of the biochemical procedure:

1) **Sample Collection:**Blood samples are collected, and the plasma or serum is separated for analysis.

2) **Reagent Preparation:**

*The Jaffe reaction requires an alkaline solution of **picric acid** (a yellow compound) as the reagent.

*The reagent is prepared by dissolving picric acid in an alkaline solution (usually sodium hydroxide NaOH or sodium carbonate, Na2CO3).

3) **Reaction:**

*A portion of the serum or plasma is mixed with an alkaline picrate solution.

*Under these conditions, creatinine in the blood reacts with the alkaline picrate to form a red-orange complex .

*The color intensity of the red-orange complex is measured using a spectrophotometer at a wavelength of 520 nm.

*The absorbance is directly related to the concentration of creatinine in the sample.

4) Calculation:

*A calibration curve (منحنى قياسي ،معياري)is prepared by measuring the absorbance of known concentrations of creatinine.

*The creatinine concentration in the sample is then determined by comparing its absorbance to the calibration curve.

Creatinine clearance

Creatinine clearance is a measure of kidney function that estimates how well the kidneys are clearing creatinine from the bloodstream. It's used to assess kidney health, particularly the glomerular filtration rate (GFR).

The most common method for estimating creatinine clearance is the **Cockcroft-Gault equation**, which uses the patient's serum creatinine, age, weight, and gender.

For men:

$$C_{\mathrm{Cr}} = \frac{(140 - \mathrm{age}) \times \mathrm{weight\ (kg)}}{72 \times \mathrm{serum\ creatinine\ (mg/dL)}}$$

For women:

$$C_{\mathrm{Cr}} = \frac{(140 - \mathrm{age}) \times \mathrm{weight\ (kg)}}{72 \times \mathrm{serum\ creatinine\ (mg/dL)}} \times 0.85$$

Where:

- C_{Cr} = creatinine clearance (mL/min)
- Age is in years
- Weight is in kilograms (kg)
- Serum creatinine is in mg/dL
- The factor of 0.85 is used to account for lower muscle mass in women.

Uric acid

Uric acid is a waste product formed when the body breaks down **purines**, which are substances found in certain foods and drinks, as well as naturally occurring in the body. Purines are also found in many cells throughout the body, and when they break down, uric acid is produced.

Once uric acid is formed, it is carried in the bloodstream to the kidneys, where it is excreted in urine. If the body produces too much uric acid or the kidneys do not excrete enough, it can lead to elevated levels of uric acid in the blood (a condition known as **hyperuricemia**).

What High Uric Acid Levels Can Indicate:

Gout(النقرس): A condition where uric acid builds up in the joints, causing inflammation, pain, and swelling.

Kidney stones: Uric acid crystals can form in the kidneys and cause kidney stones, which can be painful and may block the urinary tract.

Chronic kidney disease: Poor kidney function can lead to reduced clearance(تصفية) of uric acid from the bloodstream, causing its accumulation.

Metabolic syndrome: High uric acid levels may be associated with conditions like high blood pressure, obesity, and insulin resistance.

(Not)Diet: Diets rich in purines (from foods like red meat, shellfish, and alcohol) can increase uric acid levels.

Normal Uric Acid Levels:

Men: 3.4 to 7.0 mg/dL

Women: 2.4 to 6.0 mg/dL

Children: 2.0 to 5.5 mg/dL (can vary with age)

Uric Acid Clearance:

Uric acid can be estimated using the following formula:

$$\text{Uric Acid Clearance}(mL/min) = \frac{\text{Urine Uric Acid}(mg/dL) \times \text{Urine Volume}(mL)}{\text{Serum Uric Acid}(mg/dL) \times 1440}$$

- **Urine Uric Acid**: The concentration of uric acid in the urine.
- **Urine Volume**: The total volume of urine collected over 24 hours (in milliliters).
- **Serum Uric Acid**: The concentration of uric acid in the blood.
- **1440**: This is the number of minutes in 24 hours.

MCQ

1- **How do the kidneys help regulate blood pH?**

A) By releasing oxygen
B) By excreting hydrogen ions and reabsorbing bicarbonate
C) By increasing heart rate
D) By storing excess glucose

2- **What is the primary function of the kidneys?**

A) Producing digestive enzymes
B) Filtering blood and forming urine
C) Regulating body temperature
D) Producing red blood cells

3- **6. What waste product is formed from the breakdown of proteins and excreted by the kidneys?**

A) Glucose
B) Urea
C) Bile
D) Glycogen

4-What is the main purpose of measuring blood urea nitrogen (BUN) and creatinine levels?

A) To assess liver function
B) To diagnose diabetes
C) To evaluate kidney function
D) To monitor heart disease

5- **What is creatinine?**

A) A carbohydrate stored in muscles
B) A waste product from muscle metabolism
C) A hormone that regulates blood pressure
D) A vitamin stored in fat

Fill in the blank

1- The kidneys play a critical role in regulating ----------by controlling the volume of blood and by producing the enzyme ----------

2- The kidneys produce and release hormones, such as ---------- which stimulates red blood cell production, and ----------

3- The most commonly used methods for urea estimation are :-

a----------b----------c----------

4-In urease method the level of urea in the blood is measured by a spectrophotometer at a wavelength of----------

I wash you the Best

جامعة شط العرب الاهلية –كلية العلوم
قسم التحليلات المرضية

Pathological analysis

Lach -8- lipid profile

Dr .prof .Dr. Mohammed A.Fayyadh

Lipids profile

Lipid profile test: is a test that measures the amount of certain fat molecules called lipids in your blood..

A lipid profile usually includes the levels of:

* Total cholesterol

*High-density lipoprotein (HDL) cholesterol

*Triglycerides

*Very low-density lipoprotein (VLDL)

*low-density lipoprotein (LDL)

*Cholesterol :HDL ratio

- it ordered to determine the risk of heart disease .

Lipids profile

The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease, certain forms of pancreatitis, and other diseases.

What is **Cholesterol?**

*** it Is steroid**

***Cholesterol is a type of fat, found in your blood. It is produced by your body and also comes from the foods you eat (animal products). Cholesterol is needed by your body to maintain the health of your cells. Too much cholesterol leads to coronary artery disease. Your blood cholesterol level is related to the foods you eat or to genetic conditions (passed down from other generations of family members).**

Roles of Cholesterol

***Has important function in body:**

***important part in membrane of cells, organs and tissues in the body**

***is used to make hormones,**

***forms acids that are needed to absorb nutrients from food.**

Therefore, cholesterol deficiency is not good.

***Source: 70% synthesized in body, 30% from food (animal source as meat, eggs and dairy products)**

Cholesterol levels:

*** High level associated with heart disease**

*** Good level: below 200 mg/dl (low risk of heart disease).**

*** Border line: 240mg/dl (if higher at high risk)**

Notes:

* Measuring blood cholesterol level not need fasting?

Cholesterol level is not affected by single meal but affected by long term pattern of eating

* This test may be measured any time of the day without fasting. However, if the test is drawn as part of a total lipid profile, it requires a 12-hour fast (no food or drink, except water). For the most accurate results, wait at least two months after a heart attack, surgery, infection, injury or pregnancy to check cholesterol levels.

* Cholesterol level is elevated during pregnancy (till 6 weeks after delivery)

* Some drugs are known to increase cholesterol levels as anabolic steroids, beta blockers, epinephrine, oral contraceptives and vitamin D.

Blood lipoprotein

*They are lipids carrier particles .

***Composed of:**

cholesterol, cholesterol ester, TG, phospholipids and protein

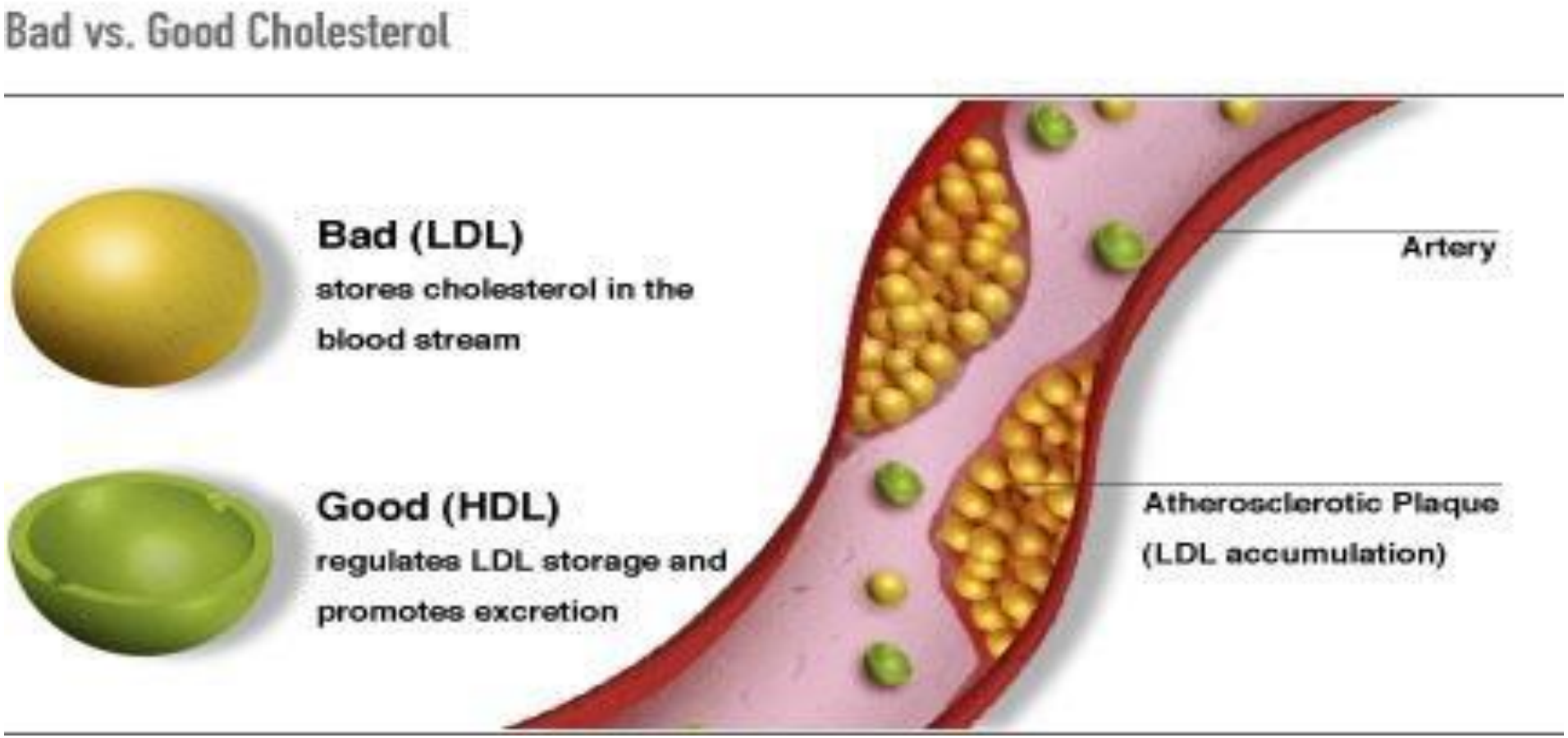
*Four major types: VLDL, LDL, HDL and chylomicron

*They differ in the contents of each composition.

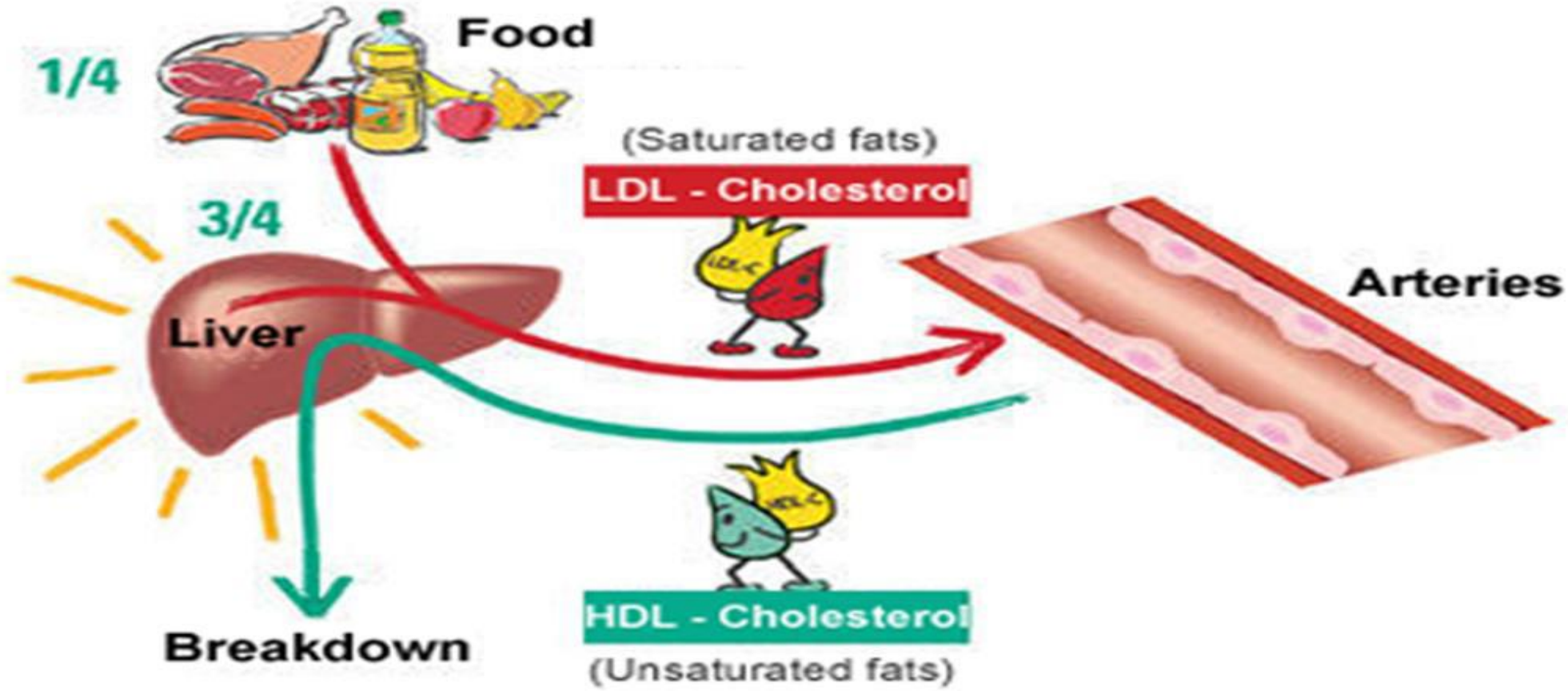
*Function: transport lipids in blood to organs (lipids are hydrophobic and can't transport in blood without carrier)

*Then these lipids are either: stored in adipose tissue or oxidized to give energy.

Blood lipoprotein



Blood lipoprotein



LDL (low density lipoprotein)

 # -LDL: bad cholesterol " carry cholesterol from liver to blood then to organs

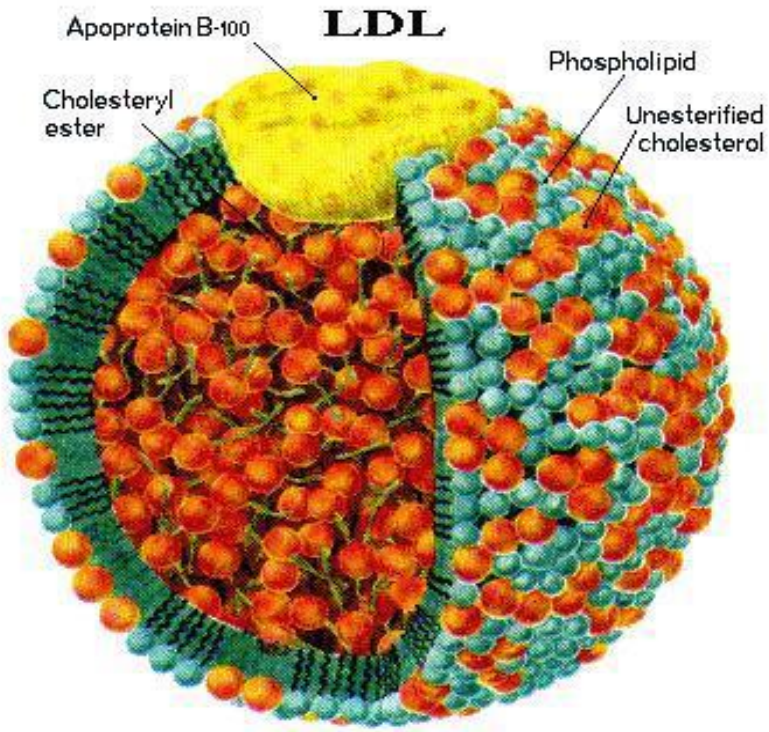
#-It has less protein content and contains more cholesterol.

#-LDL cholesterol is easy to stick to the walls of blood vessels.

#-High LDL in blood associated with atherosclerosis, heart disease and myocardial infraction

#- Reducing LDL levels is a major treatment target for cholesterol-lowering medications.

#-Because high LDL in blood will deposited in blood artery and trigger clot formation



LDL-Cholesterol levels

Blood should be collected after a 12-hour fast .For the most accurate results, wait at least 2 months after a heart attack, surgery, infection, injury or pregnancy to check LDL levels.

Goal values:

#- Good level: below 100 mg/dl (low risk of heart disease).

#-Border line: 100- 120mg/dl

#-High risk : < 120mg/dl

Measuring LDL-C level:

#-LDL level calculated either : directly or by equation

#- LDL= Total cholesterol -(HDL+TG/5)

HDL (high density lipoprotein)

#-HDL: good cholesterol, carry cholesterol from organs and blood to liver to get rid of it .

#-It removes excess cholesterol from tissues (it cleans blood).

#-High levels linked to a reduced risk of heart and blood vessel disease. The higher your HDL level, the better.

HDL levels:

Goal value:

#-Greater than 40 mg/dL

#- A good level of HDL is 60 mg/dl or more.

Preparation:

#-This test may be measured any time of the day without fasting. However, if the test is drawn as part of a total lipid profile, it requires a 12-hour fast .For the most accurate results, wait at least two months after a heart attack, surgery, infection, injury or pregnancy to check HDL levels.

Triglycerides TG

#-Triglyceride is body storage form of fat and energy , Give energy in case of absence of carbohydrates

#-Most TG found in adipose tissue

#-Some triglycerides circulate in the blood to provide fuel for muscles to work.

#-Extra triglycerides are found in the blood after meal.

#-Elevated in obese or diabetic patients.

#-It increases after eating sugars or drinking alcohol.

#- Associated with heart and blood vessel disease.

TG levels

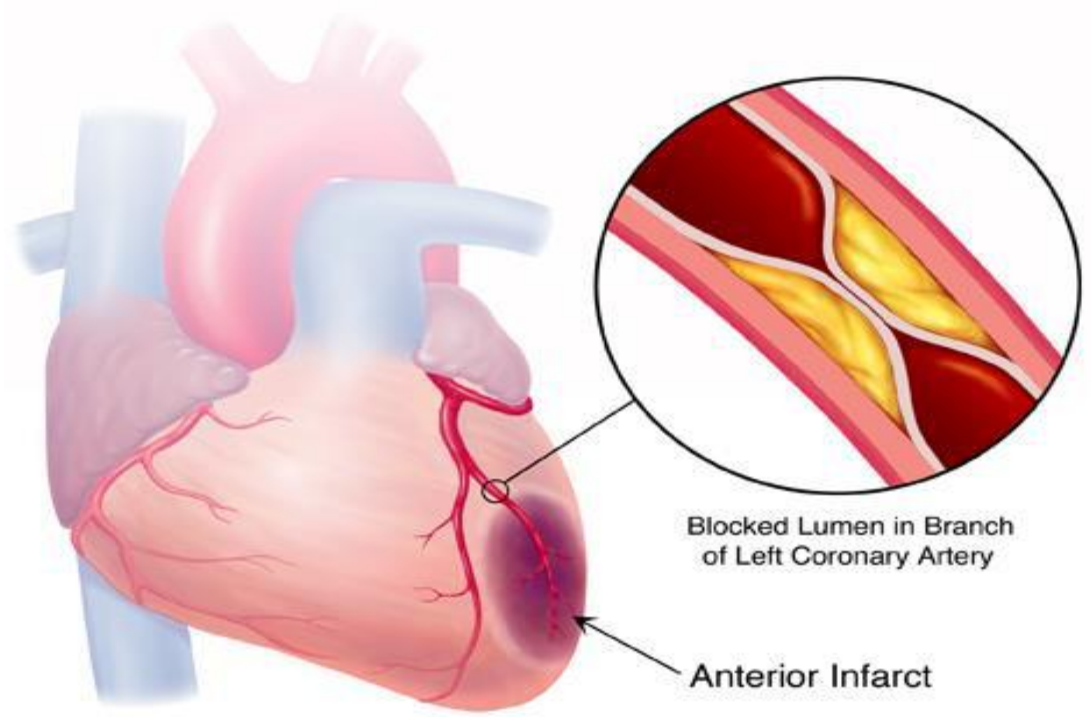
#-TG test needs 12 hrs fasting because its level is effected by meal (fatty meal, high carbohydrates meal)

#-Level should be: Less than 150 mg/dl

#-High TG leads to fatty liver

HYPERLIPIDEMIA

#- the condition of abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. It consider a heterogeneous group of disorders.



TYPES OF HYPERLIPIDEMIA

#-Primary hyperlipidemias are probably genetically based, but the genetic defects are known for only a minority of patients

#-Secondary hyperlipidemia may result from diseases such as diabetes, thyroid disease, renal disorders, liver disorders, and Cushing's syndrome, as well as obesity, alcohol consumption, estrogen administration, and other drug-associated changes in lipid metabolism .

Calculations

Total cholesterol = HDL + LDL + VLDL

VLDL = Triglyceride / 5

LDL = Total cholesterol – (HDL + Triglyceride / 5)

MCQ

1-Which of the following lipid profile components is primarily used to assess the risk of cardiovascular disease?

A) Triglycerides
B) Total cholesterol
C) Low-Density Lipoprotein (LDL)
D) High-Density Lipoprotein (HDL)

2-Which of the following components of the lipid profile is commonly referred to as "bad cholesterol"?

A) High-Density Lipoprotein (HDL)
B) Low-Density Lipoprotein (LDL)
C) Triglycerides
D) Total Cholesterol

3-**The lipid profile test measures all of the following EXCEPT:**

A) Total cholesterol
B) Triglycerides
C) High-Density Lipoprotein (HDL)
D) Blood glucose levels

4-**Which of the following is a desirable level for LDL cholesterol?**

A) Less than 100 mg/dL
B) Less than 130 mg/dL
C) Less than 160 mg/dL
D) Less than 200 mg/dL

I wish you the Best

جامعة شط العرب الاهلية
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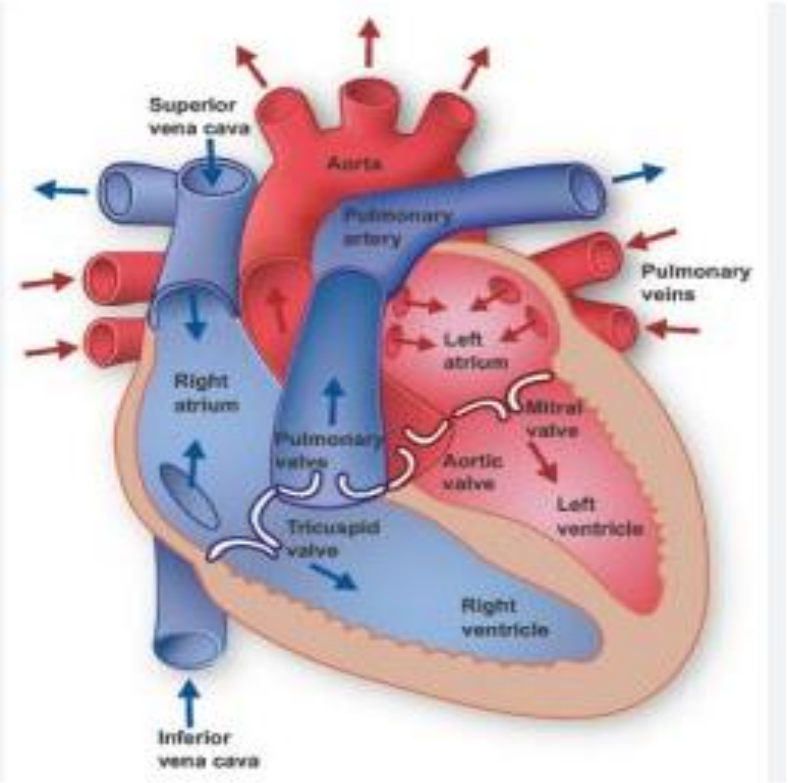
Pathological analysis

Lach 8 – Cardiac Function

Cardiac Function

Blood tests can provide valuable information about cardiac function by measuring various markers that indicate the heart's health and potential underlying conditions. Some common blood tests related to cardiac function include:



1-Troponin: This protein is released when heart muscle cells are damaged. Elevated levels indicate heart injury, often from a heart attack or other forms of cardiac stress.

2-B-type natriuretic peptide (BNP): High levels of BNP can indicate heart failure. BNP is released by the heart in response to increased pressure and volume, often seen in heart failure.

3-Creatine kinase-MB (CK-MB): CK-MB is an enzyme found in the heart muscle. Elevated levels can suggest heart muscle damage, such as from a heart attack.

4-C-reactive protein (CRP): This is a marker of inflammation, and elevated CRP levels may indicate an increased risk of cardiovascular disease.

Lipid panel: This test measures levels of cholesterol and triglycerides, helping assess the risk for atherosclerosis (narrowing of the arteries) and other cardiovascular diseases.

High-sensitivity C-reactive protein (hs-CRP): This is a more sensitive measure of inflammation and can help identify individuals at risk for cardiovascular events, even when traditional risk factors like cholesterol levels appear normal.

Homocysteine: Elevated homocysteine levels are associated with an increased risk of cardiovascular disease, as high homocysteine can damage blood vessel walls.

Copeptin: A relatively newer marker, copeptin can aid in diagnosing heart failure and other cardiac conditions.

Troponin test

A **Troponin test** is a blood test used to measure the levels of troponin proteins (Troponin I and Troponin T) in the blood. Troponins are proteins found in heart muscle cells and play a key role in muscle contraction. When heart muscle cells are damaged, such as during a heart attack (myocardial infarction), these proteins are released into the bloodstream.

The test is commonly used to:

Diagnose a heart attack: Elevated troponin levels indicate that heart muscle damage has occurred, which is often associated with a heart attack.

Assess the severity of heart injury: Higher troponin levels can reflect more significant damage to the heart muscle.

Evaluate other heart-related conditions: It can also be used to monitor other heart conditions like heart failure, myocarditis, or unstable angina.

Troponin levels typically rise within a few hours of heart injury and can remain elevated for up to a week, making it a valuable diagnostic tool in emergency situations.

How is the Troponin test calculated?

The **Troponin test** is typically measured using a blood sample, and the results are reported as the concentration of troponin in the blood (usually in nanograms per milliliter, ng/mL).

The **calculation** of troponin levels is done using an **immunoassay** method, which detects and quantifies the specific troponin proteins in the blood. There are different types of assays and techniques for measuring troponin, but the most common include:

1-Enzyme-linked immunosorbent assay (ELISA)

2- Chemiluminescent immunoassays (CLIA)

3-Cardiac-specific troponin assays using automated analyzers.

The result is usually given in the following way:

Normal levels: Typically, troponin I levels are <0.04 ng/mL and troponin T levels are <0.01 ng/mL. However, normal ranges may vary depending on the laboratory and test method used.

Elevated levels: Elevated troponin levels, especially those that rise and remain high, suggest heart muscle injury. For example, values above 0.1 ng/mL might be concerning, depending on the clinical context.

How do troponin levels change over time?

Initial rise: Troponin levels usually rise within **3 to 6 hours** after the onset of heart muscle injury (e.g., during a heart attack).

Peak levels: Troponin levels typically peak around **12 to 24 hours** after the event.

Return to baseline: Levels generally return to normal after **7 to 14 days**.

Interpretation of Results:

Negative troponin result: If the troponin levels are normal or low (below the detection threshold), it is unlikely that a heart attack or major heart injury has occurred.

Positive troponin result: Elevated troponin levels, especially if they are rising or persistently high, are indicative of **heart muscle damage**, such as in a heart attack or other cardiac conditions.

Troponin testing in a clinical setting

-Troponin tests are often performed multiple times over a period (e.g., at 3-hour intervals) to track the rise and fall of troponin levels, which helps doctors assess the timing and extent of heart damage.

- It's important to interpret troponin levels in conjunction with clinical symptoms, ECG findings, and other diagnostic tests.

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Lach -2

Phlebotomy Supplies

Prof. Dr. Mohammed A Fayyadh

Phlebotomy Supplies

1. **Phlebotomy Chair:-** These chairs are designed with adjustable arm rests that allow the phlebotomist to easily access the patient's arms.

2. Supplies for Locating a Vein:- There are many kinds of phlebotomy equipment available that help the phlebotomist locate a vein to obtain a blood sample:

a- **Tourniquet** – This rubber-band-like device is used to occlude venous blood flow and help phlebotomists discover the vein. After applying the tourniquet, the phlebotomist will use their fingers to locate a vein. The vein will feel bouncy under the pressure of the finger. The tourniquet is used for almost every patient.

b- Hot Pack – Phlebotomists often use instant warm packs on the patient's arm or heel to encourage venous dilation, which makes the veins more exposed.

c- Vein Finder – This electronic device utilizes light to locate veins. This is helpful when a phlebotomist has difficulty locating a vein.

3- Skin Disinfectant:- There are different disinfectants used to clean the skin before a needle puncture.

Alcohol Swabs : Chloraprep : Iodine

4- Needle :- There are different types of needles used by phlebotomists. The phlebotomist must determine the best needle type and size depending on the type of blood draw required.

a- **Butterfly Needles** :- These are small needles with wings that come connected to flexible tubing. The end of the butterfly needle tubing can be connected to a vacutainer device.

b-BD Eclipse – This is a multi-sample vacuum collection needle. The BD eclipse has a safety device attached and is used for venipuncture using the vacuum tube collection system.

c-Multi-Sample Blood Collection Needle -

This all-in-one device has two needles that are screwed into the holder. One needle is inserted into the vein while the vacuum blood collection tube is then inserted into the holder. The blood collection tube is punctured by the needle on the other end of the tool which allows for sample collection.

d-**Lancet :-**A small device that contains a very small needle. When the lancet is engaged, the needle quickly enters and exists the skin, resulting in a small amount of bleeding from the site. This device is used on heels and fingers, usually on people with small or fragile veins.

5- Blood Collection Device:-These devices attach to the end of the selected needle to allow the phlebotomist to draw blood back from the vein. This device allows blood to transfer from the vein, through the needle, and into the blood collection tube.

- **Vacutainer System** – A plastic tube that attaches to the end of butterfly needles, the BD eclipse, or multi-sample blood collection needles. This plastic tube contains a rubber stopper that allows for accessing the vacutainer blood collection vials without using a needle.
- **Syringe** – Syringes are used to collect blood from patients with small or fragile veins instead of using the vacutainer system. The syringe is attached to the end of the selected needle tubing. After entering the vein with the needle, the phlebotomist will draw back blood with the syringe. *Using a syringe for blood collection requires an extra step: using a blood transfer device.*

6- Transfer Devices:-If blood is collected in a syringe, this device screws onto the syringe and contains a mechanism that transfers the blood from the syringe into the blood container tube. This protects phlebotomists from an accidental needle stick.

7- Blood Collection Tubes

a-Blood Culture Bottle – Blood culture bottles are used to collect blood from patients suspected of septicemia or bacteremia. These bottles are often compatible with the vacutainer phlebotomy equipment.

b-Vacutainer Tubes – Vacutainer tubes are used with vacuum container systems or with a blood transfer device. There are various types of containers that have different additives to allow for certain laboratory tests. A few examples of these tubes may include:

Purple – A blood collection tube containing EDTA which is an anticoagulant. It is used to obtain a complete blood count.

Green **–** A blood collection tube containing heparin which is an anticoagulant. It is used to obtain whole blood samples for analysis.

Gold **–** A blood collection tube containing clot activator and serum separator. It is commonly used for chemistry collections.

Blue **–** A blood collection tube containing sodium citrate. It is used for coagulation studies.

C-Microtainer Tube* - Microtainer® tubes are tiny blood collection tubes that are used to collect blood from a skin puncture obtained by a lancet (usually via finger or heel). These are often used for those patients who are difficult draws. A “difficult draw” includes patients with fragile or difficult veins, such as: infants, small children, or elderly patients.

d- **Microhematocrit Tube** – These small glass blood collection tubes are used for collecting blood from the capillary after using a lancet. They are often used to determine the percentage of red blood cells.

8- Patient Label

After blood collection, a sticker is applied to the blood collection tube to identify the patient it belongs to. The label should include the time, date, and initial of the phlebotomist that collected it.

9- Safety:- Phlebotomist’s handle lots of blood and safety is very important. Certain devices exist to avoid phlebotomist exposure to blood borne pathogens. Phlebotomists should be aware of the safety equipment available when handling this biohazardous material.

1. **Preventing Accidental Needle Sticks** – Devices that minimize the risk of the phlebotomist accidentally being exposed to a dirty needle.
2. **Re-Sheathing Devices** – After the needle is withdrawn from the patient, most blood draw needles have mechanisms that allow the phlebotomist to cover the needle without needing to touch it.
3. **Needle Holders** – This needle-safety device is used along with the vacuum blood collection system to stabilize the needle during the blood draw, so the phlebotomist doesn't have to hold the needle while changing out blood collection tubes.
4. **Sharps Container** – After the needle has been used for a blood draw, the phlebotomist disposes of the needle in a labeled sharps container. This puncture-proof and leak-proof container prevents exposure to blood borne pathogens.
5. **Biohazard Bags** – After the blood is in blood collection tubes and labeled with a patient sticker, the phlebotomist places the blood into a biohazard bag before delivering the blood to the laboratory. These bags clearly label the blood tubes as a possible biohazard material during transport.
6. **Gloves** – The phlebotomist wears gloves throughout the collection process.

MCQ

- **1- What is the purpose of a tourniquet in phlebotomy?**
- a) To clean the skin
  b) To secure the needle
  c) To restrict blood flow and make veins more visible
  d) To prevent contamination of the sample

2-**What is typically used to clean the skin before a venipuncture?**

a) Distilled Water
b) Iodine swab
c) Sterile gauze
d) Cotton ball

- **3- What is the most common anticoagulant used in blood collection tubes for plasma samples?**
- a) Heparin
  b) Sodium citrate
  c) EDTA
  d) Sodium fluoride

1-A _____ is used to tie around the patient’s upper arm to make veins more prominent.

2-Blood collection tubes with a purple top usually contain ---------- and are used for hematology tests.

3-A _____ needle is often used for patients with small or difficult veins.

4-Blood collection tubes with a green top usually contain --------- and are used for hematology tests.

5-Blood collection tubes with a blue top usually contain --------- and It is used for coagulation studies.

6- ----------is a small device that contains a very small needle

I wish you the Best

جامعة شط العرب الاهلية-كلية العلوم

قسم التحليلات المرضية

Pathological analysis

Lach -3 Blood group

Prof .Dr. Mohammed A. Fayyadh

The blood group

Introduction:

- Blood grouping is the classification of blood based on the presence or absence of
- two inherited antigenic substances on the surface of red blood cells (RBCs). The
- ABO and Rh are the major, clinically significant and the most important of all the
- blood group systems. The ABO blood group system was first discovered by Karl
- Landsteiner in 1900. The human ABO blood group system is divided into the
- following four major groups depending on the antigen present on the surface of
- their red blood cells: 1. “A” group 2. “B” group 3. “AB” group
- 4. “O” group

Table 1: ABO Blood Group System

Antigens on the surface of Red Blood Cells	Antibodies in the Serum	ABO Blood Group	Genotype
A	Anti B	A	AA or AO
B	Anti A	B	BB or BO
A and B	Neither Anti A nor Anti B	AB	AB
Neither A nor B	Anti A, Anti B, Anti AB	O	OO

- The Rhesus system (Rh) is the second most important blood group system in humans. The most significant and immunogenic Rhesus antigen is the RhD antigen. The individuals carrying the Rh antigen are considered to have positive blood group whereas those individuals that lack this antigen are considered to have negative blood group.

Principle:

The ABO and Rh blood grouping system is based on agglutination reaction. When red blood cells carrying one or both the antigens are exposed to the corresponding antibodies they interact with each other to form visible agglutination or clumping. Blood group A individuals have A antigens on RBCs and anti-B antibodies in serum. Similarly, blood group B individuals have B antigens on RBCs and anti-A antibodies in serum. Blood group AB individuals have both A and B antigens RBCs and neither anti-A nor anti-B antibodies in serum . Whereas, blood group O individuals have neither A antigens nor B antigens, but possess both anti-A and anti-B antibodies in serum .The Rh antigens are transmembrane proteins in which the loops exposed on the surface of red blood cells interact with the corresponding antibodies.

Materials

Blood group kit.

Reagents: 70% Alcohol/ Spirit, Blood Grouping Kit Blood Grouping Teaching Kit is stable for 6 months from the date of receipt without showing any reduction in performance. Store the Anti A Sera, Anti B Sera and Anti RhD Sera at 2-8 C. Other contents can be stored at room temperature (15-25oC). **Other requirements:** Cotton , lancet.

Blood Grouping Kit

Procedure:

Important Instructions:

Procedure:

1. Dangle the hand down to increase the flow of blood in the fingers.
2. Clean the fingertip to be pierced with spirit or 70% alcohol (usually ring or middle finger).
3. With the help of the sterile lancet, pierce the fingertip and place one drop of blood in each of the cavities.
4. Add one drop of antiserum into each cavity as shown below:
1. Before starting the experiment the entire procedure has to be read carefully.
2. Always wear gloves while performing the experiment.

3. Ensure the slide is clean and dry prior to use.

4. Do not allow the antisera reagent dropper to touch the blood sample.

5. The result of the reaction should be interpreted immediately after mixing.

6. Avoid intermixing of the antisera reagents while performing the experiment as it may give false result.

7. Mix each blood drop and the antiserum using a fresh mixing stick.

7. Observe agglutination in the form of fine red granules within 30 seconds. Anti RhD takes slightly longer time to agglutinate compared to Anti A and Anti B.

Note: Proper care should be taken while disposing the lancet and mixing sticks.

Interpretation:

_ If agglutination is observed when blood is mixed with Anti A reagent, then the individual is said to have blood group “A”.

_ If agglutination is observed when blood is mixed with Anti B reagent, then the individual is said to have blood group “B”.

_ If agglutination is observed when blood is mixed with Anti A and Anti B reagent, then the individual is said to have blood group “AB”.

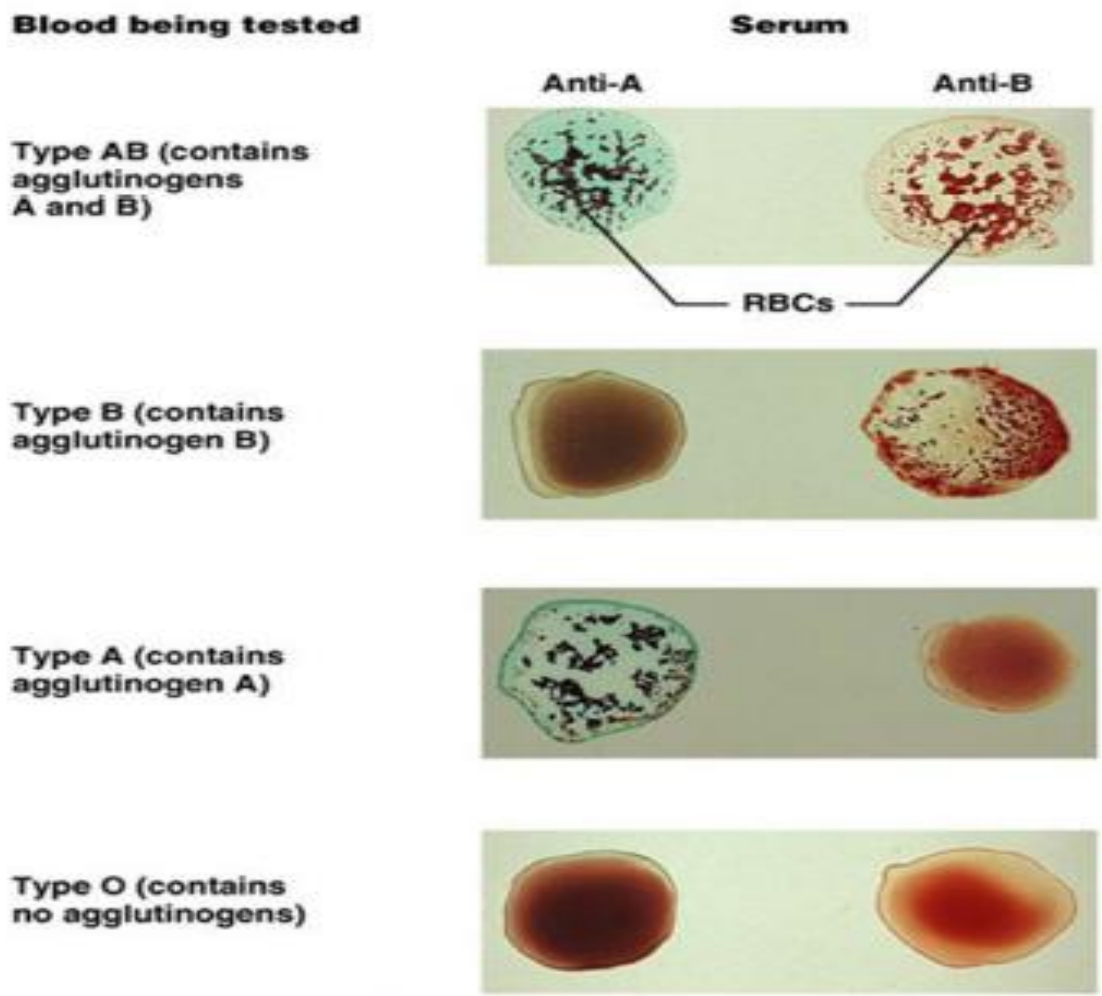
_ If no agglutination is observed when blood is mixed with Anti A and Anti B reagent, then the individual is said to have blood group “O”.

_ If agglutination is observed when blood is mixed with Anti RhD reagent, then the individual is said to have “+ve” Rh factor.

_ If no agglutination is observed when blood is mixed with Anti RhD reagent, then the individual is said to have “-ve” Rh factor.

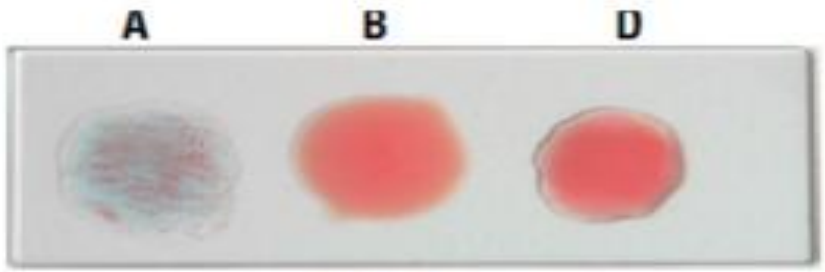
Observation and Result:

Figure: group AB (ngative)

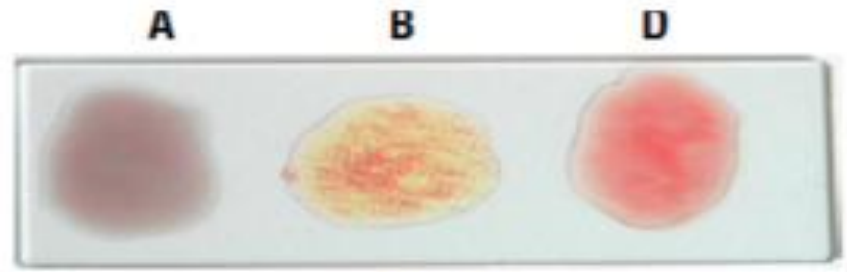


This slide shows how blood typing is done. A specific anti-serum is added to a blood sample. If it agglutinate, it indicates the presence of the antigen which is is how the blood type is determined.

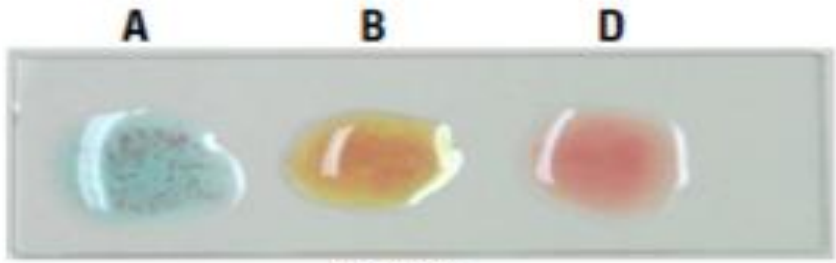
This sample agglutinated after Anit-A serum added But not with Anti-B serum.



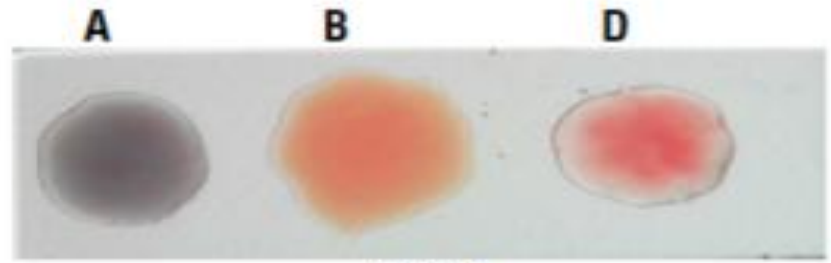
Slide 1



Slide 2



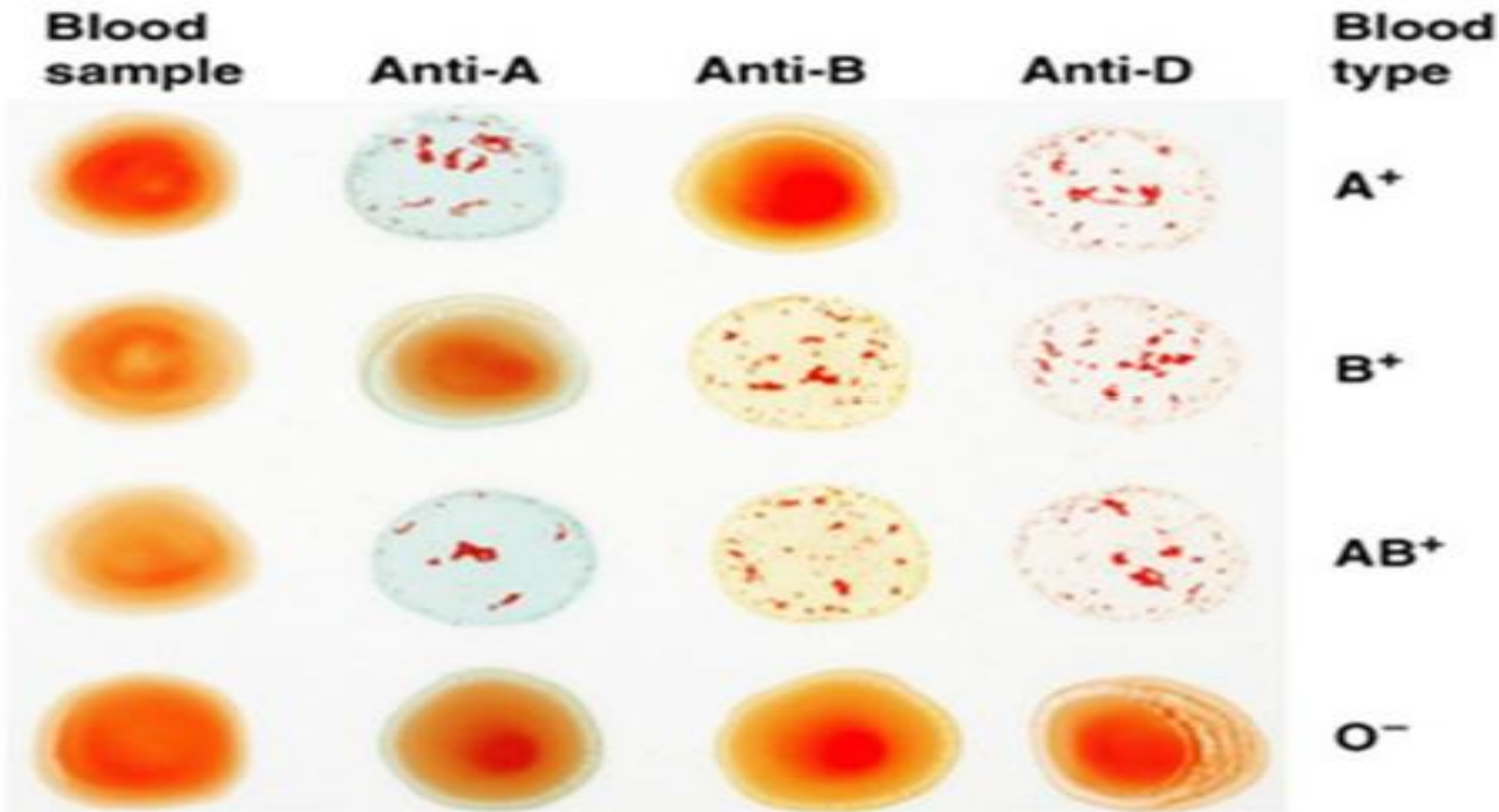
Slide 3



Slide 4

Table 3: Determination of blood group and Rh factor based on agglutination seen

Sr. No.	Anti A	Anti B	Anti RhD	Blood Group
Slide 1	✓	✗	✓	A +ve
Slide 2	✗	✓	✓	B +ve
Slide 3	✓	✓	✓	AB +ve
Slide 4	✗	✗	✓	O +ve


Blood sample
Anti-A
Anti-B
Anti-D
Blood type
A+
B+
AB+
O−

MCQ

1- Which of the following blood groups is known as the universal donor?

A) AB - B) O - C) A - D) B

2-**Which blood type can receive blood from all other ABO blood groups?**

A) A B) O C) AB D) B

3-**Which blood group is considered the universal recipient?**

A) O B) A C) AB D) B

4-**If a person with blood type O receives blood from a person with blood type A, what type of reaction is likely to occur?**

A) No reaction B) Minor allergic reaction C) Agglutination and hemolysis

D) Increased oxygen levels

1-The universal donor blood group is _____, while the universal recipient blood group is _____.

2- **Blood group AB individuals have both A and B ---------- and neither anti-A nor anti-B ---------- in serum.**

3-If no agglutination is observed when blood is mixed with ----------and ----------reagent ,then the individual is said to have blood group 'O'

4-If agglutination is observed when blood is mixed with ----------and ----------reagent ,then the individual is said to have blood group 'AB'

5-A person with blood group B can receive blood from group B and group ___.

6-The presence or absence of the ___ factor determines if blood is positive or negative

Q3) Define the following terms

1- Antigen 2-antibodes 3-Rhs 4-reagent. 5- lancet

I wish you the Best