Almaaqal University
College of Health and Medical Techniques
Department of Medical Laboratory Techniques

Introduction to

Clinical biochemistry laboratory

& Safety measures

Clinical biochemistry . stage 2 . practical

Assist. Lecturer: Abbas Al Eidany

Lab. 1: Introduction to clinical biochemistry laboratory & safety measures.

1.1. Introduction to clinical biochemistry laboratory:

Clinical biochemistry lab main aim is to analyze and measure the value and concentration of one or more substances in biological specimen of the patient and compare it with the standard references of healthy individuals.

1.1.1. Main clinical biochemistry tests:

- **Lipid profile :**

(cholesterol, TG, HDL, LDL)

- **Blood sugar test:**

(Random, fasting, GTT, HbA1c)

- **LFT (Liver functions test):**

(AST, ALT, ALP, GGT, TP, Alb, globuline, bilirubin)

- **KFT (Kidney functions test):**

(urea, creatinie, creatnine clearance, uric acid, Na^+,K^+)

- **Cardiac profile:**

(AST, LDH, CK, K^+)

- **Bone profile :**

(ALP, minerals: Mg^{2+}, Ca^{2+}, phosphate)

- **Electrolytes :**

(Na^+, K^+, Cl^-, Mg^{2+}, phosphorus)

- **Hormones:**

(prolactin, FSH, LH, ADH, TSH, ACTH, STH)

The test procedure flow cycle could be divided into three phases:
1- Pre-analytical : Test ordering, specimen collection, transport and collection.
2- Analytical: Testing.
3- Post-analytical: Result transmission, interpretation, follow-up, retesting.

1.2. Types of biological specimens:

Blood is approximately 55% fluid and 45% blood cells. Tests can be performed on serum or plasma derived from the fluid portion, or on whole blood. The type of specimen tested depends on the test, how urgently results are needed, and the equipment used.

■ **Serum** is normally a clear, pale yellow fluid (non-fasting serum can be cloudy due to lipids) separated from clotted blood by centrifugation. Many chemistry tests are performed on serum.

■ **Plasma** is normally a clear to slightly hazy, pale yellow fluid that separates from the cells when blood in an *anticoagulant* tube is centrifuged. Plasma contains fibrinogen; serum does not because it was used in clot formation. Many chemistry tests can be performed on either serum or plasma. Stat and other tests requiring a fast turnaround time (TAT) are often collected in tubes containing *heparin* anticoagulant because they can be centrifuged immediately to obtain plasma.

■ **Whole blood** contains both cells and plasma, like blood in the body. As with plasma, it must be collected in an anticoagulant tube to keep it from clotting. Whole blood is used for most hematology tests.

■ **Urine** analysis should be obtained from first morning or single random specimens. obtaining an exact reading of the quantity of urine collected over the collection period, usually 24 hours, is always problematic. First morning urine collection poses a similar problem, since it has to be delivered for sediment analysis within one hour of collection. There is often a delay in delivering the collected urine to the laboratory, which leads to false negative or false positive results (increase in the bacteria count, increase in the pH value due to the urease of bacteria and cell element degradation). Urine specimen is needed to measure renal functions and some molecules excretion and urinary system diseases.

■ **CSF (Cerebro-spinal fluid)** is a watery fluid, continuously produced and absorbed, which flows in the ventricles (cavities) within the brain and around the surface of the brain and spinal cord. In some tests we need to determine the value of glucose, protein, albumin, lactate and immunoglobulins.

1.3. Blood collection:

Specimen:
The laboratory staff is permitted to collect blood from two sources only: capillary (peripheral) and venous blood. Arterial blood may be used in certain testing situations, but such blood must be collected only by physicians or by staff with documented training in the collection of arterial blood specimens.

Reagents and materials:
-70% isopropyl alcohol (pre-packaged alcohol wipes)
-Gauze sponges or cotton balls
-Assorted needles, sterile, single use: 18-23 gauge, 3/4" to 1/2” length
-Multi-draw needles for use with evacuated collection tubes
-Or Hypodermic for use with a syringe
-Or Butterfly tubing for use with a syringe and/ or evacuated tubes
-Or Lancets, sterile, single use for collection of capillary blood
-Multi-draw needle holder
-Disposable gloves
-Tourniquets
-Sharps disposal container for contaminated needles, lancets, syringes, test tubes
-Soap, water, paper towels or antimicrobial foams or gels for hand cleansing

Procedure:
It is imperative that the patient, whether sitting or reclining, be made as comfortable as possible. Psychological comfort may also be required, so take time to explain the procedure to the patient.
Always wear gloves, throughout the entire procedure, when obtaining blood specimens.

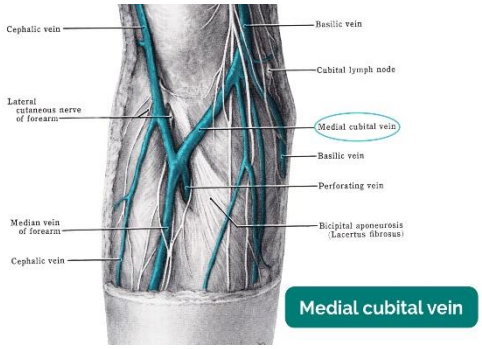
Venous collection:
The *median cubital and cephalic veins* are preferred for blood sampling, but other arm and hand veins may be used. The cephalic vein is located on the lateral (radial) side of the arm, and the basilic vein is located on the medial (ulnar) side.
Securely fasten a tourniquet around the patient’s arm, just above the elbow. Instruct the patient to keep his arm as straight as possible and to make a fist (if possible). Gently palpate the antecubital area in search of a suitable vein. It may be necessary to examine the other arm, wrists, or hands.
When a vein has been located, thoroughly cleanse the site with disinfectant. Allow to dry.

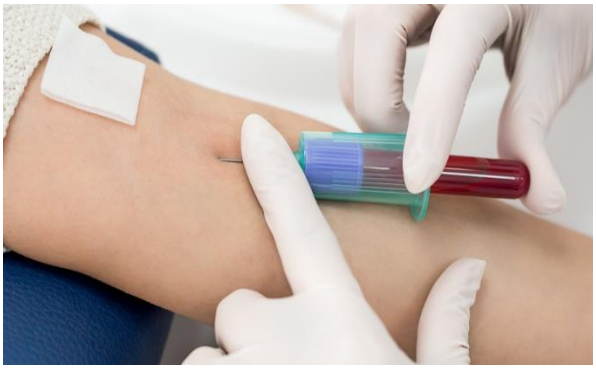
Assemble the vacuum collection set of syringe and needle. Remove the needle cap or sheath.
Grasp the patient's arm (or hand) with your free hand to hold the skin and underlying tissue taut.

Insert the needle into the vein by using a quick, deliberate motion. Begin to withdraw the blood by (a.) inserting the vacuum tube(s) onto the sheathed end of the multi-draw needle, or by pulling back on the syringe plunger. If blood does not return, it may be necessary to make small adjustments to the position of the needle to penetrate the vein.

When sufficient blood has been withdrawn, release the tourniquet. Place the clean gauze sponge or cotton ball over the puncture site. Simultaneously withdraw the needle while using the sponge to apply pressure to the site.
Instruct the patient to apply pressure to the puncture site with the other hand. If the patient is unable, you should continue to apply pressure to the site until bleeding has stopped.
Failure to apply adequate pressure can result in a hematoma, or bruise, to the draw site.
Tourniquets are discarded immediately after use.

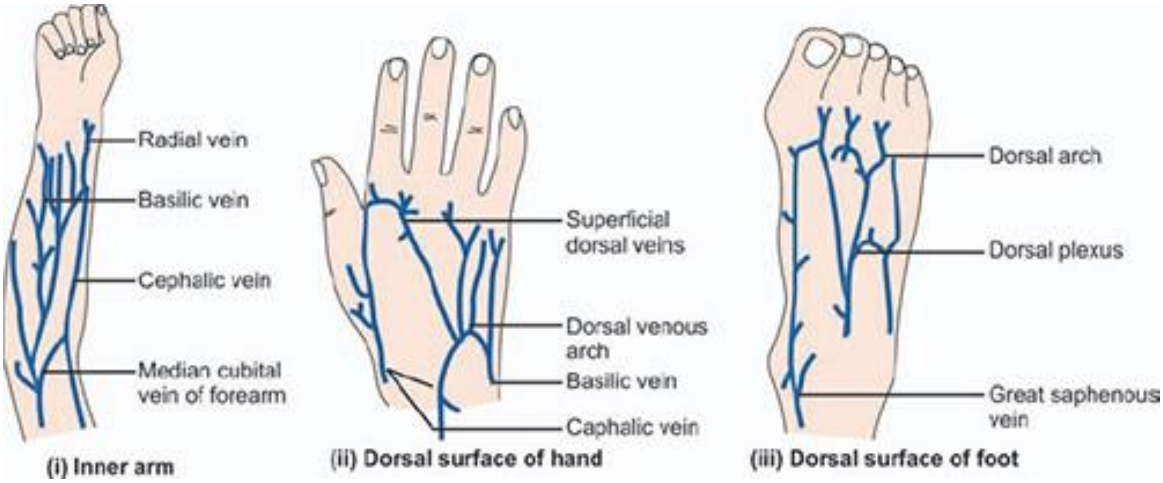
After blood has been dispensed into the tubes dispose of the needle apparatus into appropriate Sharps biohazard waste containers.
All tubes except plain "red top" tubes must be mixed immediately by gently inverting 8 to 10 times. ALL tubes must be properly labeled immediately AFTER filling them.





Take care that you have matched the specimens to the correct patient and label them:
-Patient's first and last names
-Patient's secondary identifier (Patient ID or medical record number)
-Date and time of collection
-Initials of phlebotomist
When the puncture site has stopped bleeding, apply an adhesive bandage, gauze or other suitable material.
Clean any blood spills with disinfectant. Remove and discard gloves. Thoroughly wash your hands with soap and water, or use an appropriate antimicrobial foam or gel for hand cleansing.

Anticoagulants:
Most anticoagulants prevent blood clotting by removing ionic calcium from the blood with the formation of a unionized calcium salt. Heparin prevents coagulation by removing one of the clotting factors - thrombin. The anticoagulant effect of Heparin lasts approximately 24 hours.
Most chemical analyses are performed on serum. To obtain serum, the blood is allowed to clot in the absence of anticoagulant.
Serum or plasma may be stored at room temperature, under refrigeration, or in the freezer, depending on the determination to be run. With few exceptions, the lower the temperature, the greater the stability of the constituents.



1.4. Clinical biochemistry laboratory most needed equipment:

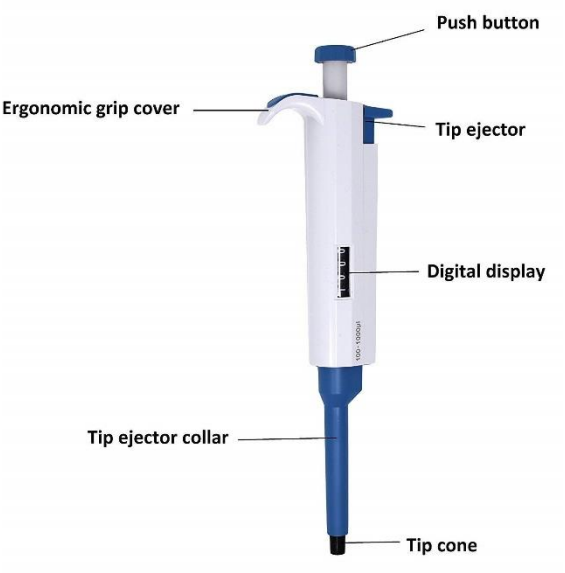
1.4.1. Blood collecting tubes: blood collecting tubes can be used according to the tests needed from the sample.

Tube	Color	Name	Additive	Test used for
	Blood Culture Bottle	Culture Bottle	Sodium Polyanethol sulfonate (anticoagulant) and Growth media for microorganisms	Two bottles are typically collected, in one blood draw; one for aerobic organisms and one for anaerobic organisms.
	Light Blue	Sodium Citrate	3.2% Sodium citrate (anticoagulant)	Coagulation tests
	Red	Red or plain	No additive, No Anticoagulant	Immunology, Serological examination
	Gold	Serum Separating Tube	Serum Separating Gel and clot activator	All Biochemistry test
	Light Green	Heparin Tube	Sodium heparin or Lithium heparin (anticoagulant)	prevent clotting, Chromosome testing, HLA typing, ammonia, lactate
	Purple/Lavender	EDTA	Ethylene diamine tetra acetic acid (EDTA) (Anticoagulant)	Hematological examination like complete Hemogram
	Pink		Ethylene diamine tetra acetic acid (EDTA) (Anticoagulant) Used only for Whole Blood sample being send to transfusion lab	Blood typing and cross-matching, direct Coombs test for autoimmune haemolytic anemia, HIV viral load, Group and save (G & S) These tubes are preferred for blood bank tests.
	Grey	Sodium fluoride	Sodium fluoride (glycolysis inhibitor) Potassium oxalate (anticoagulant)	Glucose, lactate testing
	Yellow	Acid-citrate-dextrose (anticoagulant)		Tissue typing, DNA studies, HIV cultures

1.4.2. Micropipette:

Micropipettes are utilized in the laboratory to transfer small quantities of liquid, usually down to 0.1 uL. They are most commonly used in chemistry, biology, forensic, pharmaceutical, and drug discovery labs, among others. Common micropipette sizes used in labs include:

Common Micropipette Sizes	Volume Range
P2	0.2-2 uL
P10	1-10 uL
P20	2-20 uL
P100	20-100 uL
P200	20-200 uL
P1000	100-1000 uL



1.4.3. Spectrophotometer:

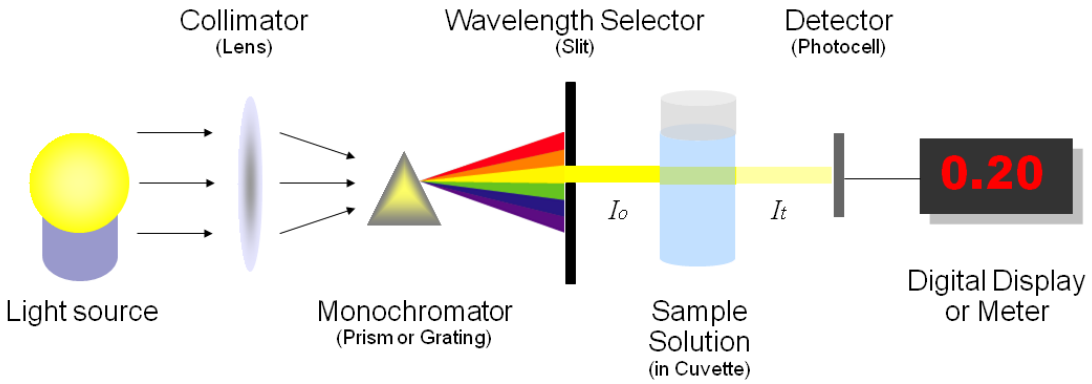
Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance. Spectrophotometry is one of the most useful methods of quantitative analysis in various fields such as chemistry, physics, biochemistry, material and chemical engineering and clinical applications.

- **UV-visible spectrophotometer**: uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum.
- **IR spectrophotometer**: uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum.

Devices and mechanism:

Figure below illustrates the basic structure of spectrophotometers. It consists of a light source, a collimator, a monochromator, a wavelength selector, a cuvette for sample solution, a photoelectric detector, and a digital display or a meter. Detailed mechanism is described below.

A spectrophotometer, in general, consists of two devices; a spectrometer and a photometer. A spectrometer is a device that produces, typically disperses and measures light. A photometer indicates the photoelectric detector that measures the intensity of light.



1.5. Biochemistry laboratory safety: -

Students, Teachers, Researchers and Technicians who work in laboratories are exposed to the various hazards chemicals. In laboratories, involves a greater variety of possible hazards chemicals and some of these hazards chemicals needs to take precautions. In the all laboratory widely used 'laboratory Safety Rules' procedures are listed below:

A) Personal protective equipment (PPE): -

Personal protective equipment is used in the laboratory to protect ourselves when working with chemical hazards.

Examples: laboratory coats, footwear, gloves, safety goggles and mask.

1) Laboratory Coats: - The primary purpose of coats is used in the laboratory to protect against splashes and spills. In the laboratory coats should be nonflammable and easily removable and buttoned when in use. The rubber coated aprons can be worn to protect against chemical splashes and may be worn over a laboratory coat for additional protection. We should not be wear laboratory coats, gloves, or any other personal protective clothing outside of laboratory areas.

2) Footwear: - Shoes must be worn in the laboratory at all times, regardless of the performance experimental works and use leather shoes which is completely protects the toes, heel and top of foot provide the best general protection. The shoes must be water proof materials used. The shoes must have a nonslip sole firmly attached to the foot and sandals, sneakers and open-toed shoes. do not provide adequate protection when handling heavy objects that might fall onto the feet.

3) Gloves: - Gloves are required for routine laboratory practicals to protect the hands when the handling chemical, physical, or biological hazards that can enters in to the body through the skin and it is important to wear the proper protective gloves that is made up of polyvinyl or other nonlatex gloves are an acceptable alternative for people with latex allergies. Nitrile gloves, offer a wider range of compatibility with organic solvents than do latex gloves.

4) Eyewear: - Laboratory Safety goggles is the best protection against hazards chemical splashes, mists, vapors and dusts. Eye wear is required to be worn at any time projectile objects are being used in the laboratory works. Contact lenses is strongly recommended that they are not be worn in the laboratory experimental works. We should wear ultraviolet absorbing protective safety glasses while working with ultraviolet light.

5) Mask: - The face masks is a partial cover for the face used for protection. It provides protective covering for nose and mouth. Face masks generally used in biomedical research laboratories are called dust masks.

B) First Aid Box In Laboratory :-

1) Skin burns should be washed under running water or ice water and petroleum jelly or burn ointment should be applied and then covered with sterile gauze. Any blister formed must not be punctured.
2) Chemicals injury to the eyes must be treated by through washing with water.
3) In accidental swallowing of chemicals, the mouth must be thoroughly rinsed with water.
4) Contamination with infected material in wounds caused by broken glassware, must be thoroughly rinsed with water and washed with soap solution before applying antiseptic solution.

C) Eye Wash Station :-

Eye wash stations it must be required mirror and a set of bottles containing saline solution that can be used to flood the injured eye with water. The eye wash station is intended to allow us to flood the eye with a continuous stream of water.

Laboratory notes :

- Mouth pipetting is never be allowed, using only rubber teat (Bulb) or used automated micropipette.
- Hair must be tied back.

- Do not wear jewelry and loose / baggy clothing.
- Never touch, taste or smell any chemical unless instructed to do so.
- Don't mix the chemicals unless instructed to do so.
- Keep the lids on chemical containers when there is not in use.
- Pencils, pen or any other materials should never be placed in your mouth.
- Don't eat food / drink water in the laboratory. Never use glassware as food / water containers.
- Wash the hands after every laboratory works / practicals and handles glasswear sharp tools and heated containers carefully.
- Do not engage in laboratory practical jokes.
- Keep the nonessential books and clothing far away from your work area.
- Reporting to all accidents including the minor incidents to your instructor immediately.
- Returns the reagent bottles to their respective shelf immediately after use so as to avoid accidental breaking of bottles on the working bench.
- Throw solids in the waste bins if it is necessary to pour strong acid or alkali into the sink, run water freely to wash it away.

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Estimation of blood glucose

(Serum glucose level test)

Clinical biochemistry . stage 2 . practical

Assist. Lecturer: Abbas Al Eidany

Lab. 2: Estimation of blood glucose (GOD-PAP Method).

2.1. Introduction:

The cells in the human body use the sugar called glucose as their major source of energy. Glucose molecules are broken down within cells in order to produce adenosine triphosphate (ATP) molecules, energy-rich molecules that power numerous cellular processes. Glucose molecules are delivered to cells by the circulating blood and therefore, to ensure a constant supply of glucose to cells, it is essential that blood glucose levels be maintained at relatively constant levels.

If blood glucose falls below 60 mg/dl a condition called ***hypoglycaemia*** develops. If this is not quickly reversed, the person may faint. If the body and brain continue to be deprived of adequate glucose levels, then convulsions and hypoglycaemic coma follow, which can be fatal.

An abnormally high concentration of blood glucose, known as ***hyperglycaemia***, is also a problem. Since high concentrations of any soluble metabolite lower the water potential of the blood plasma, water is drawn out of the cells and tissue fluid by osmosis, back into the blood. As the volume of blood increases, water is excreted by the kidney in an attempt to maintain the correct concentration of blood. As a result the body tends to become dehydrated, and the circulatory system is deprived of fluid. Ultimately, the correct blood pressure cannot be maintained.

Diabetes mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces.

Type 1 diabetes (previously known as insulin-dependent, juvenile or childhood-onset) is characterized by deficiency in insulin production and requires daily administration of insulin. The cause of type 1 diabetes is not known and it is not preventable with current knowledge.

Type 2 diabetes is the most common form of diabetes and is characterized by disorders of insulin resistance and insulin secretion, either of which may be the predominant feature.

2.2. Clinical presentation:

Clinical indications of DM are stand principally on glucose excretion by urine along with osmotic diuresis. The patient will have polyuria (higher amounts and recurrence of urine) as well as polydipsia (extreme drinking) and a weight loss as a result of energy supplying molecules loss (ketone bodies and glucose) in the urine. In type 1 Symptoms could establish suddenly, counter to type 2 diabetes, while in type 2 The disease could be diagnosed several years after its onset, once complications have appeared and arisen already.

2.3. Test principle:

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD). The hydrogen peroxide (H_2O_2) formed reacts, under catalysis of peroxidase , with phenol and 4-aminophenazone to form a red-violet quinoneimine.

$$\textbf{β-D-Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-aminophenazone (PAP)} \xrightarrow{\text{POD}} \textbf{Quinoneimine} + 4\ H_2O$$

2.4. Reagents composition:

Reagent 1a (buffer)	Phosphate buffer Phenol
Reagent 1b	Glucose oxidase Peroxidase 4-aminophenazone
Reagent 2 (standard)	Standard glucose (5.44 mmol/l, 98 mg/dl).

2.5. Preparation of working reagent:

1) Dissolve the entire powder of vial (R1b) with buffer of vial (R1a).

Procedure:

Pipette into well Identified test tubes	**Blank**	**standard**	**Sample**
Working reagent	1 ml	1 ml	1 ml
Standard	—	10 µl	—
Sample	—	—	10 µl

2) Mix and incubate for 10 minutes at 37 ◦C or (15-25) minutes at room temperature (20-25 ◦C).

3) Read the absorbance at 500 nm (490–550) against blank reagent. The color is stable for 30 minutes.

2.6. Calculations:

$$\text{Blood glucose conc.} = \frac{\text{Abs (sample)}}{\text{Abs (standard)}} \text{ X Standard conc.}$$

2.7. Normal values:

Fasting Blood Sugar (FBS)	(70 – 110 mg/dl)	(3.8 – 6.1 mmol/L)
Random Blood Sugar (RBS)	(120 – 180 mg/dl)	(6.6 – 10.0 mmol/L)
Postprandial Blood Sugar (PBS)	(Up to 140 mg/dl)	(Up to 7.8 mmol/L)

- Fasting means while not having anything to eat or drink (except water) for at least 6-8 hours before the test.
- Random Blood Sugar determines the amount of glucose at random time.
- Postprandial Blood Sugar determines the amount of glucose in the plasma two hours after a meal.

Convertion units: mg/dl ÷ 18 = mmol/L

Linearity:

This method is linear up to 500 mg/dl (27.8 mmol/L). If the glucose concentration is greater than 500 mg/dl repeat the determination using a sample diluted 1 to 2 with saline solution and multiply the result by 2.

Interfering factors:

1. Unless plasma is separated from the blood cells within about an hour of collection the whole blood sample must be mixed with an inhibitor of glycolysis, such as fluoride. This helps prevent an in vitro fall in the plasma glucose concentration as glycolysis continuous, which may result in *pseudohypoglycemia*.
2. Many forms of stress (e.g. trauma, burns, myocardial infarction (MI), infection) can cause increased serum glucose level.
3. Most intravenous (IV) fluids contain dextrose, which is quickly converted to glucose. Most patient receiving IV fluids will have increased glucose levels.
4. Many drugs affect glucose levels.

2.8. Clinical significance:

Hyperglycemia occurs when the patient has:

- Diabetes mellitus
- Hyperthyroidism
- Adrenal diseases
- Encephalitis
- Pituitary gland tumor

Hypoglycemia occurs when the patient has:

- Diabetes mellitus type 1 intake excessive insulin
- Insulinoma
- Exercised more than usual
- Starvation
- Alcohol drinking
- Liver diseases
- Addison's disease
- Hypothyroidism.

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Department of Medical Laboratory Techniques

Estimation of

serum total protein & albumin

Clinical biochemistry . stage 2 . practical

Assist. Lecturer: Abbas Al Eidany

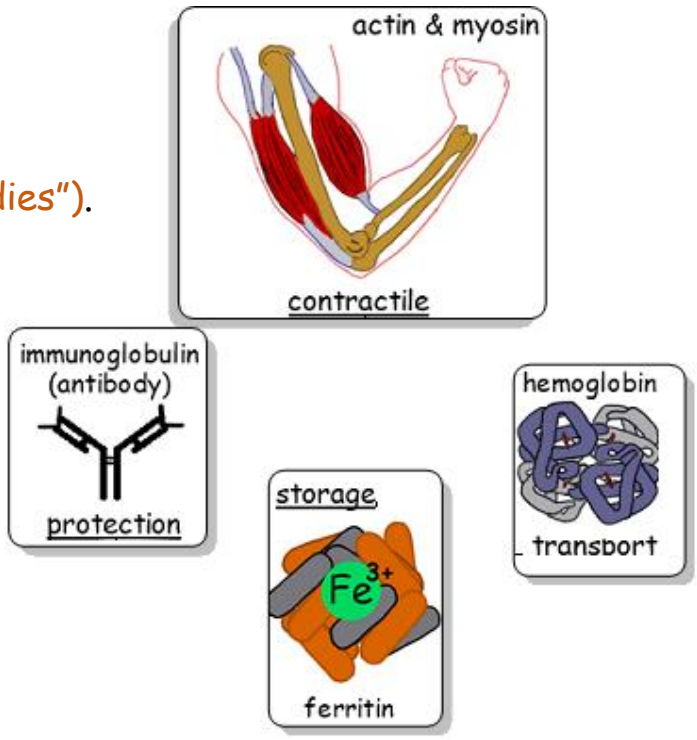
Lab. 3: Estimation of serum total protein & albumin.

Proteins

Proteins are macromolecules composed of amino acids linked together through peptide bonds.

Biological importance of proteins:

1- Catalysis (Enzymes) are proteins.
2- Structure (muscle protein).
3- Movement (myosin & actin).
4- Defense (Immunoglobulins "antibodies").
5- Regulation (Enzymes & Hormones).
6- Transport (globin).
7- Storage. (Mb & Ferritin).



Classification of proteins:

1- According to shape:

Characteristics	Fibrous	Globular
Shape	Long, narrow fiber	Rounded (spherical)
Water solubility	Insoluble	Soluble
Stability	More stable	Less stable
Examples	Collagen	Albumin, globulin

2-according to their chemical structure:

Proteins can be classified as:

(a) Simple proteins.

- On hydrolysis they yield only the amino acids ,examples are: albumins.

(b) Conjugated proteins.

- These are simple proteins combined with some non-protein material in the body.

(c) Derived proteins.

-These are proteins derived from simple or conjugated proteins.

Estimation of serum total protein:

- Total serum proteins are a combination of prealbumin, albumin, and globulins.

Biuret method:

- The biuret method is a colorimetric technique specific for protein.
- The peptide bonds of proteins react with Cu^{2+} in alkaline solution to form a colored complex which absorbance, proportional to the concentration of total protein in the specimen.

The Biuret reagent contains sodium potassium tartrate to complex cupric ions and maintains their solubility in alkaline solution.

Manual procedure:

1- Add 1000 µL of reagent R1 in 2 tubes.
2- Add 20 µL of each standard R2 and serum in different tubes, mix well and wait 10 min. at room temperature and check absorbance at 550 nm.

Calculations:

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Normal values:

Total Protein	(g/dL)
Newborn	4.6-7.0
Adult	6.0--8.3

Clinical significance:

Hyperproteinemia (Hemoconcentration)	Hypoproteinemia (Hemodilution)
Decrease ⬇ in plasma water volume	Increase ⬆ in plasma water volume
Noted in dehydratation	Water intoxication
Severe vomiting & diarrhea	During massive intravenous infusions
Diabetic acidosis	Low levels of albumin

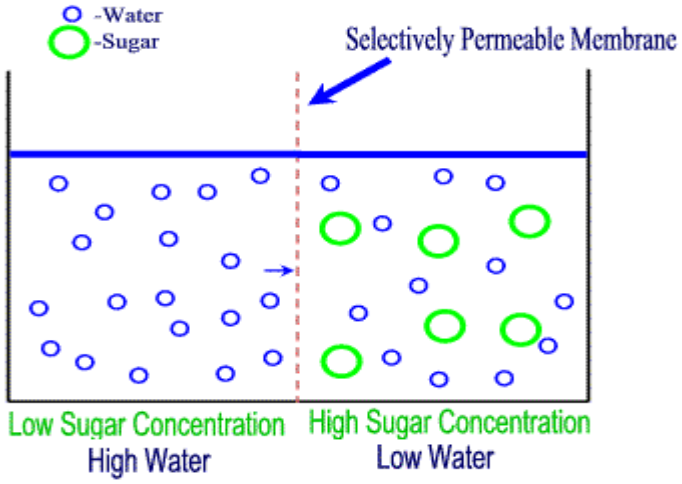
Albumin

Notes about albumin:

1. Is the most abundant protein in the blood, constituting 2/3 of total proteins.
2. Plasma proteins are separated into three major groups:
   a) Fibrinogen (4%).
   b) Globulins (38%).
   c) Albumin (58%).

Albumin functions:

- One of the most important function is to maintain the osmotic pressure of the intravascular fluid.



- Albumin acts as a carrier protein for bilirubin, calcium, progesterone, other drugs, hormones, and enzymes.
- Albumin has an important role in the endogenous metabolism of calcium, fatty acids, bilirubin, drugs, and hormones.

Estimation of serum Albumin (BCG):

Precautions for estimation of serum Albumin:

1. A fasting sample is preferred.
2. Specimens with lipemia or hemolysis should be avoided.
3. Avoid prolonged tourniquet. This may increase albumin and proteins.
4. Take into account physical exercise and fever where there is increased filtration.
5. Blood samples after the I/V therapy may give low value.

This test is needed:

1. In liver diseases.
2. Kidney diseases.
3. In patients with a severe burn.

4. In a patient suspected of malnutrition.
5. Patients with cancers.
6. As a part of other tests.

Manual procedure:

1- Add 1000 µL of reagent R1 in 2 tubes.
2- Add 5 µL of each standard R2 and serum in different tubes, mix well and check absorbance at 630 nm within 3 min. at room temperature.

Calculations:

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Normal values of Albumin:

Albumin	g/dL
Normal value:	3.2-4.8

Clinical significance:

Hypoalbuminemia	**Hyperalbuminemia**
Nephrotic syndrome	Dehydration
Burns	High protein diet
Blood loss	False value due to prolonged tourniquet
Malignancies	
Inflammatory process	
Liver diseases	
Decreased protein intake	
Ascites	

AL-Maaqal University
College of Medical and Health Techniques
Division of Laboratory Techniques

Qualitative Analysis of Proteins

Lab (4)

Prepared by Dr : **Ibrahim Samy Kamel**

Assistant lecturer of biochemistry- Almaaqal university

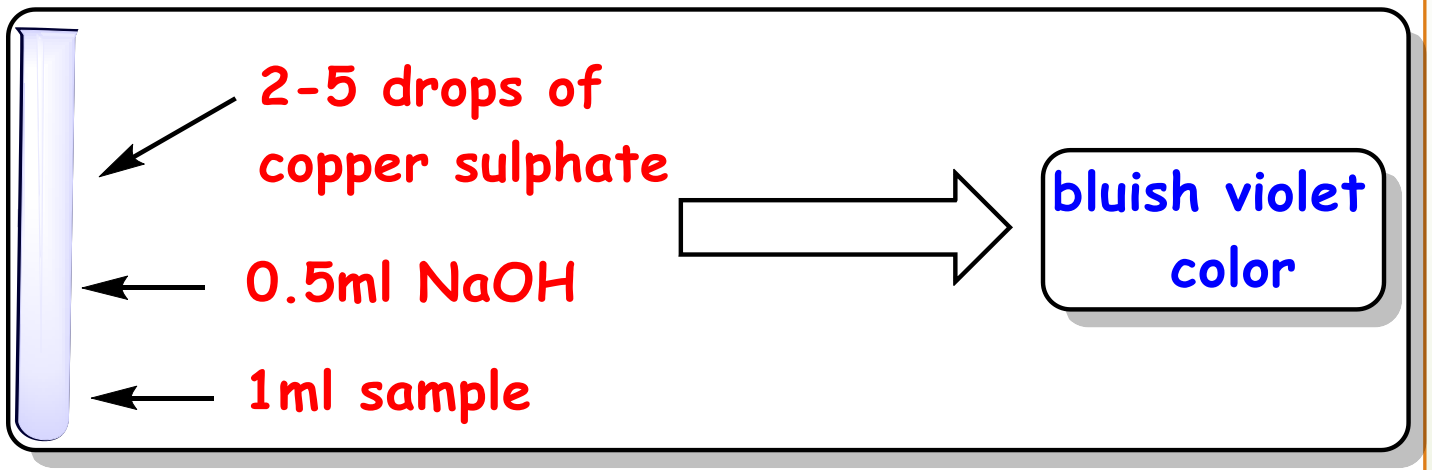
Experiment No.1 Biuret Test

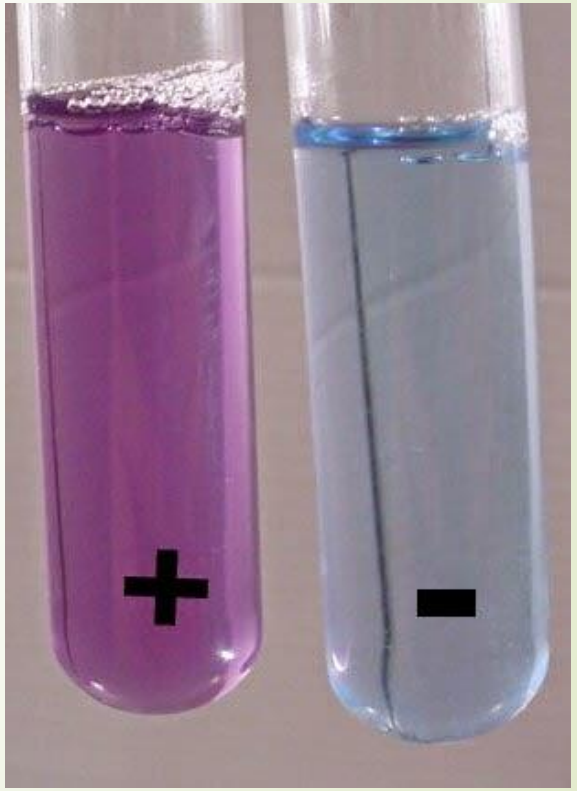
Detection of the Presence of peptide bonds in a given sample

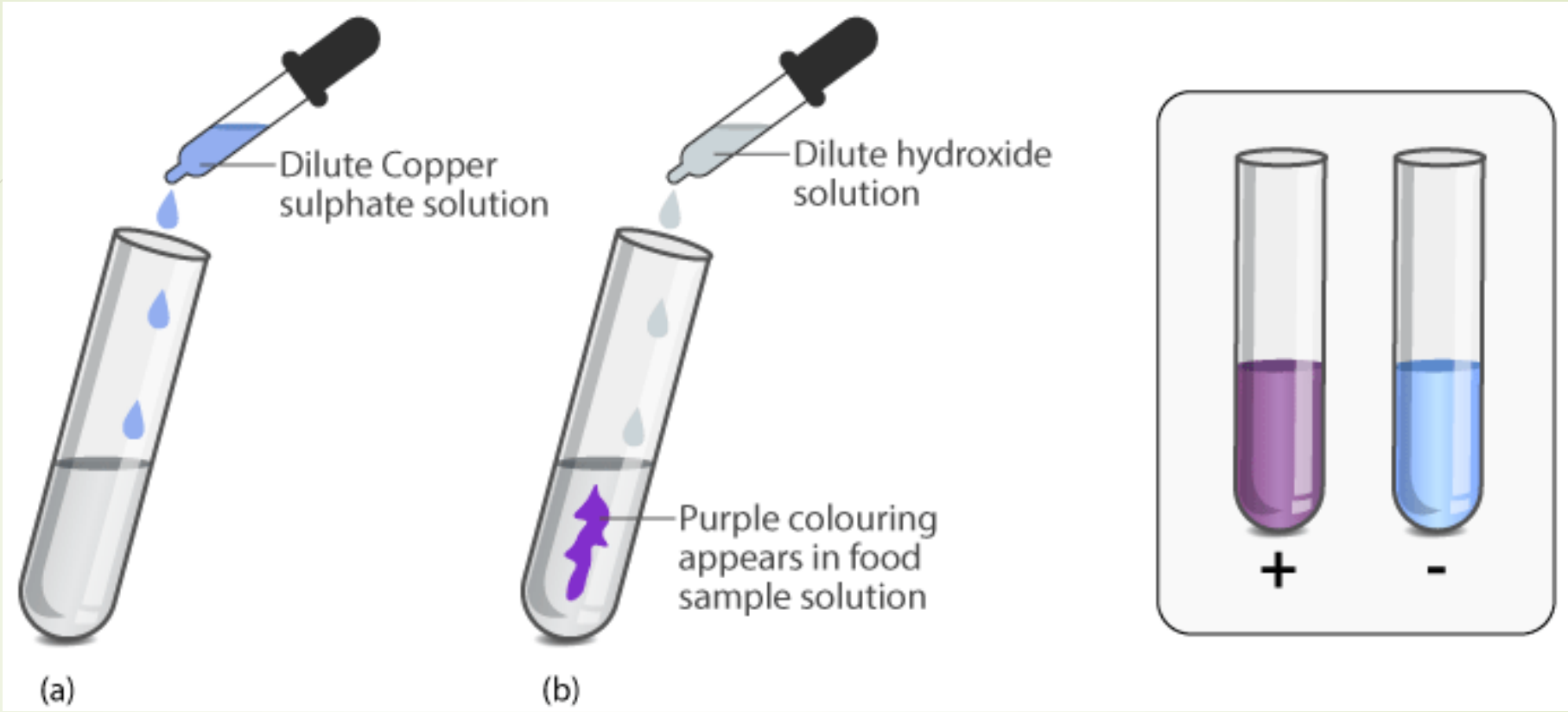
Principle:

- Used for the identification of proteins
- The compounds containing at least two peptide linkage undergoes this test.
- An alkaline solution of protein is treated with a drop of aqueous copper sulfate when a bluish violet colour is obtained.

Procedure






Dilute Copper sulphate solution
Dilute hydroxide solution
Purple colouring appears in food sample solution
(a)
(b)
+
-

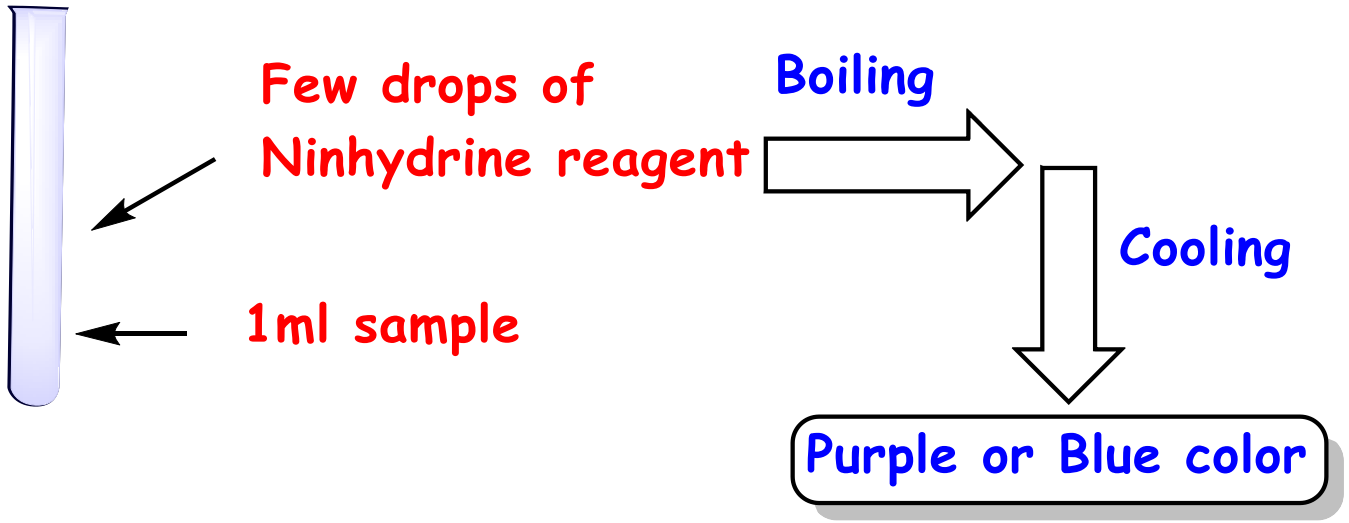
Experiment No.2 Ninhydrin Test

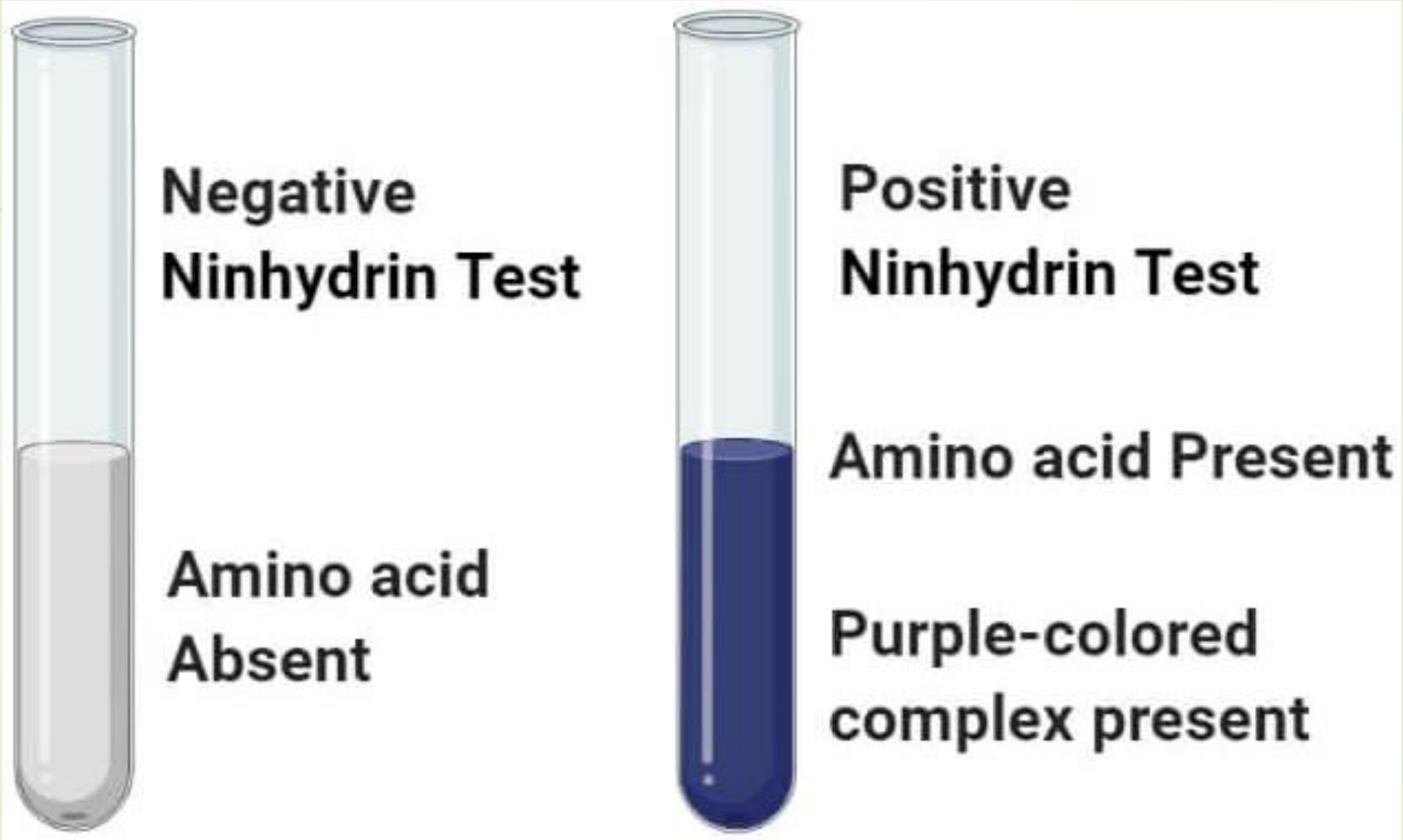
Detection of the Presence of amino group in a given sample

Principle:

- Two molecules of ninhydrin react with a free amino group to produce a deep purple or blue color.

Procedure




Negative
Ninhydrin Test
Amino acid
Absent
Positive
Ninhydrin Test
Amino acid Present
Purple-colored
complex present

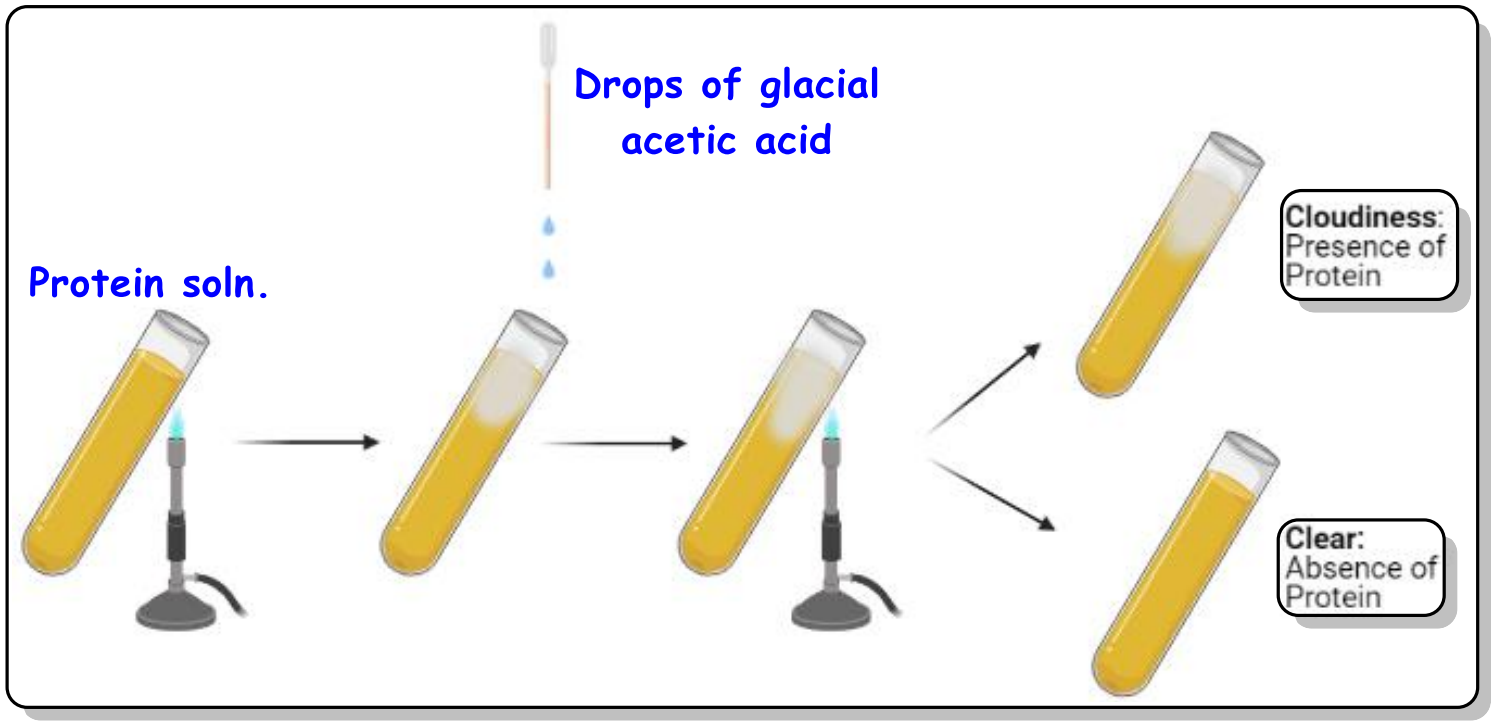
Experiment No.3 Coagulation Test

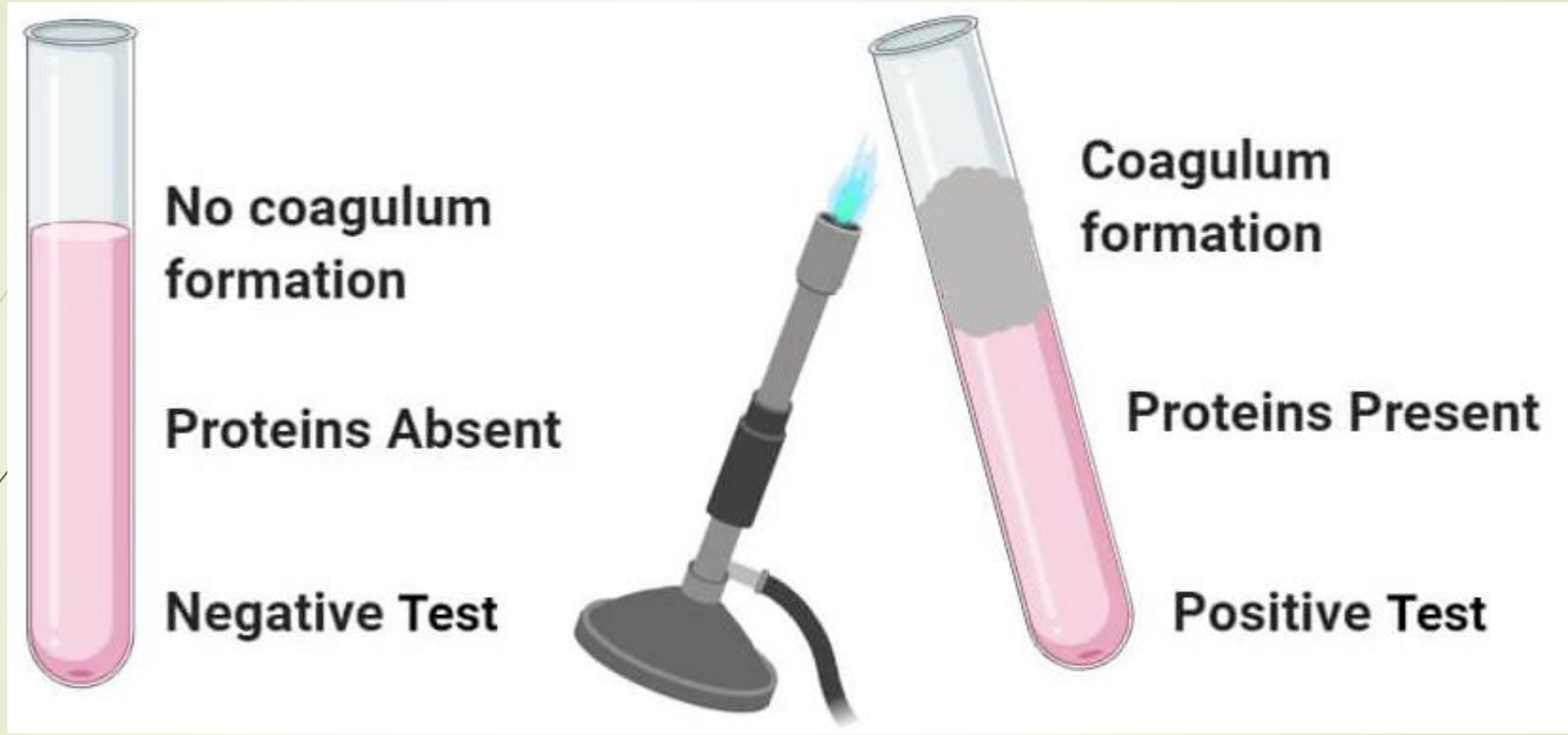
Detection of albumin

Principle:

- Albumin and globulin are heat coagulable proteins

Procedure




No coagulum formation
Proteins Absent
Negative Test
Coagulum formation
Proteins Present
Positive Test

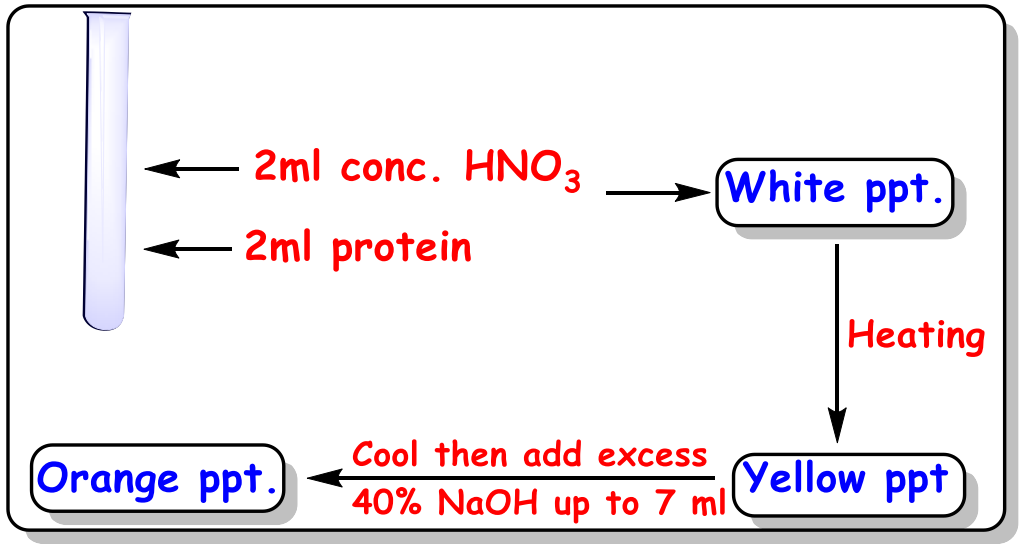
Experiment No.4 Xanthoproteic Test

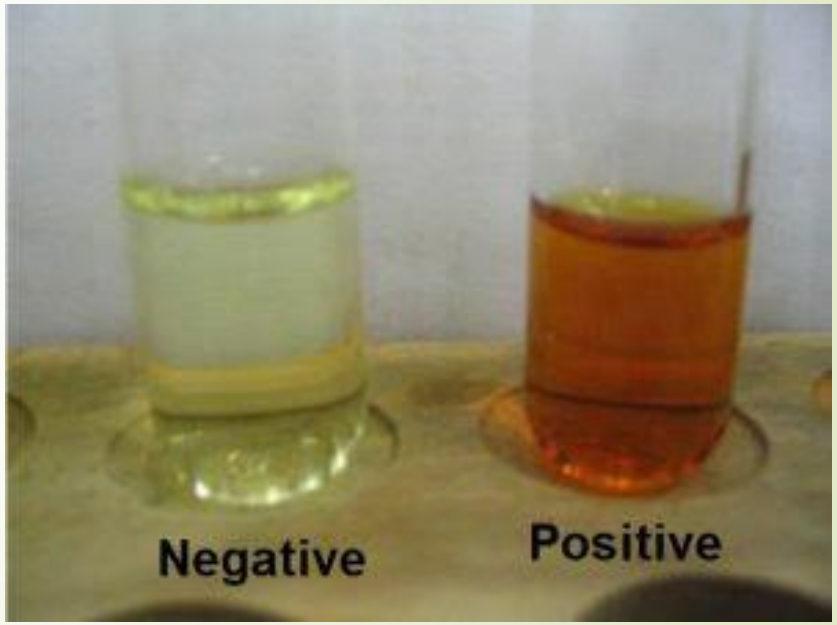
Detection of the Presence of aromatic amino acids in a given sample

Principle:

- Aromatic amino acids such as phenyl alanine, tyrosine and tryptophane give positive result to this test.
- Proteins that contain these amino acids also give positive result to this test.
- In presence of Conc. HNO_3, **yellow** color occurs
- This yellow color changes to **orange** in alkaline medium

Procedure:

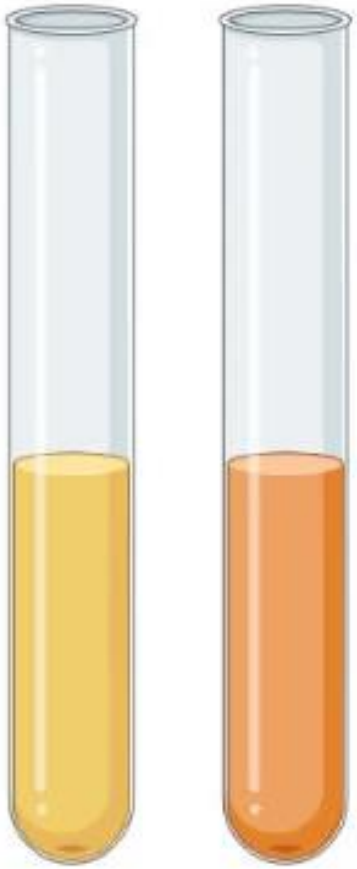




Xanthoproteic Test Negative

Absence of aromatic amino acids (tyrosine and tryptophan)

Absence of the dark yellow or orange color



Xanthoproteic Test Positive

Presence of aromatic amino acids (tyrosine and tryptophan)

Presense of the dark yellow or orange color

AL-Maaqal University
College of Medical and Health Techniques
Division of Laboratory Techniques

Qualitative Analysis of carbohydrates

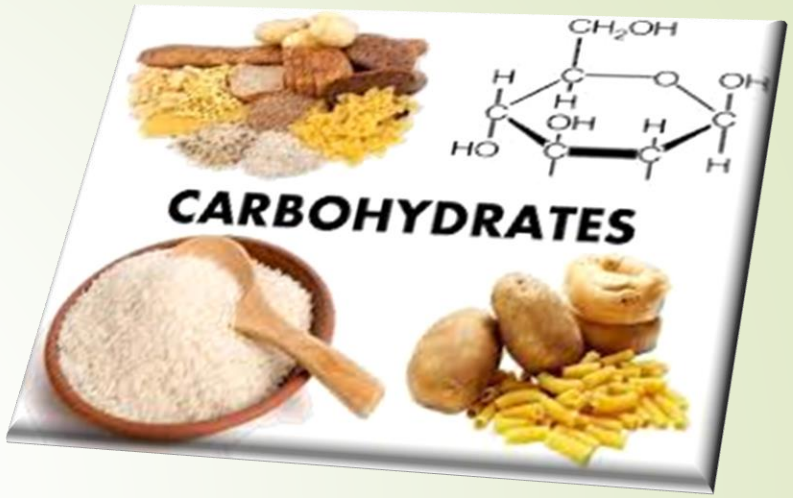
Lab (5)

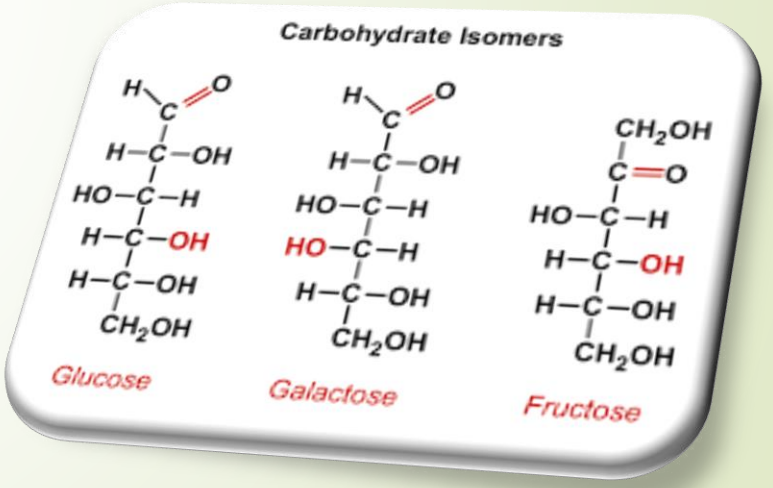
Prepared by Dr : **Ibrahim Samy Kamel**

Assistant lecturer of biochemistry- Almaaqal university

Introduction

- **Carbohydrates** may be defined as polyhydroxy aldehydes or ketones or as substances that yield one of these compounds on hydrolysis.
- Many carbohydrates have the empirical formula $(CH_2O)_n$, *where n* is 3 or larger.
- Basic units of carbohydrates are monosaccharides that cannot be split further by hydrolysis.
- They are named according to the number of carbon atoms in the chain so that triose contains three, tetrose four, pentose five, and hexoses six carbon atoms.





Importance of Carbohydrates:

i. A source of energy for the body e.g. glucose and a store of energy, e.g. glycogen and starch.
ii. Building blocks for polysaccharides, e.g. cellulose in plants and glycogen in the human body.
iii. Components of other molecules e.g. Nucleic acid, glycolipids, glycoproteins, coenzymes.
iv. They play key roles in the immune system, development, fertilization, and blood clotting.

Classification of carbohydrates:

Carbohydrates are classified into groups according to the number of individual simple sugar units

1) **Monosaccharides:** Carbohydrates that contain only **one** sugar unit are called monosaccharides. These are the simplest form of sugar and are usually colourless , water soluble and crystalline solids, e.g. glucose, fructose , galactose etc.

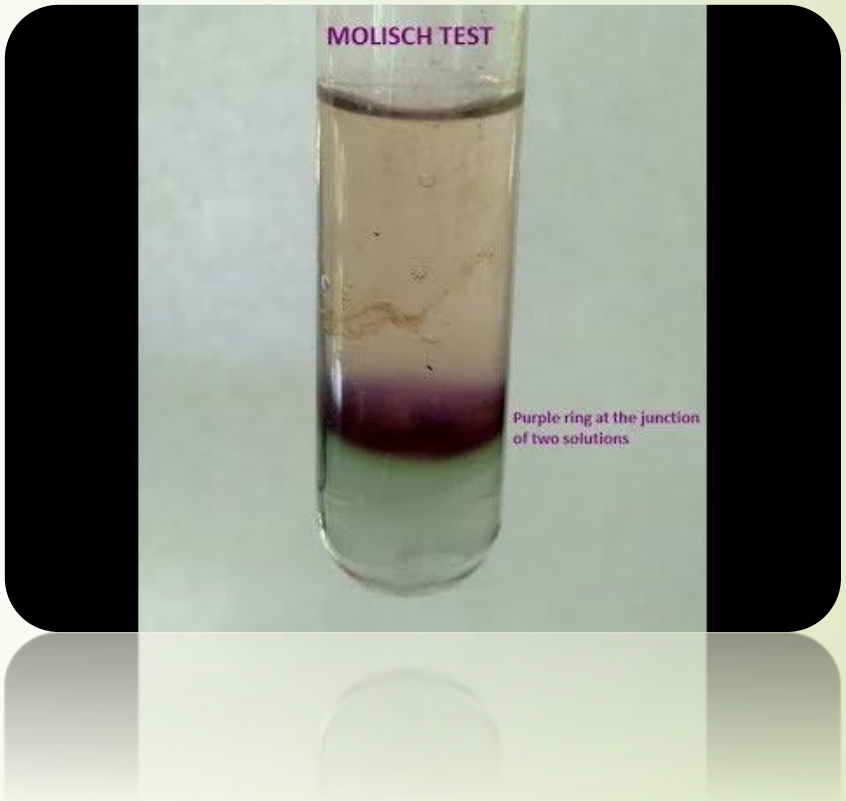
2) **Dissacharides:** Carbohydrates that contain **two** sugar units are called disaccharides. It is formed when two monosaccharides undergo condensation reaction and are water soluble, e.g. sucrose, lactose etc.

3) **Polysaccharides:** Carbohydrates that contain **more than two** sugar units are called disaccharides. It is formed when more than two monosaccharide units bound together by glycosidic bonds, e.g. starch, glycogen , cellulose etc.

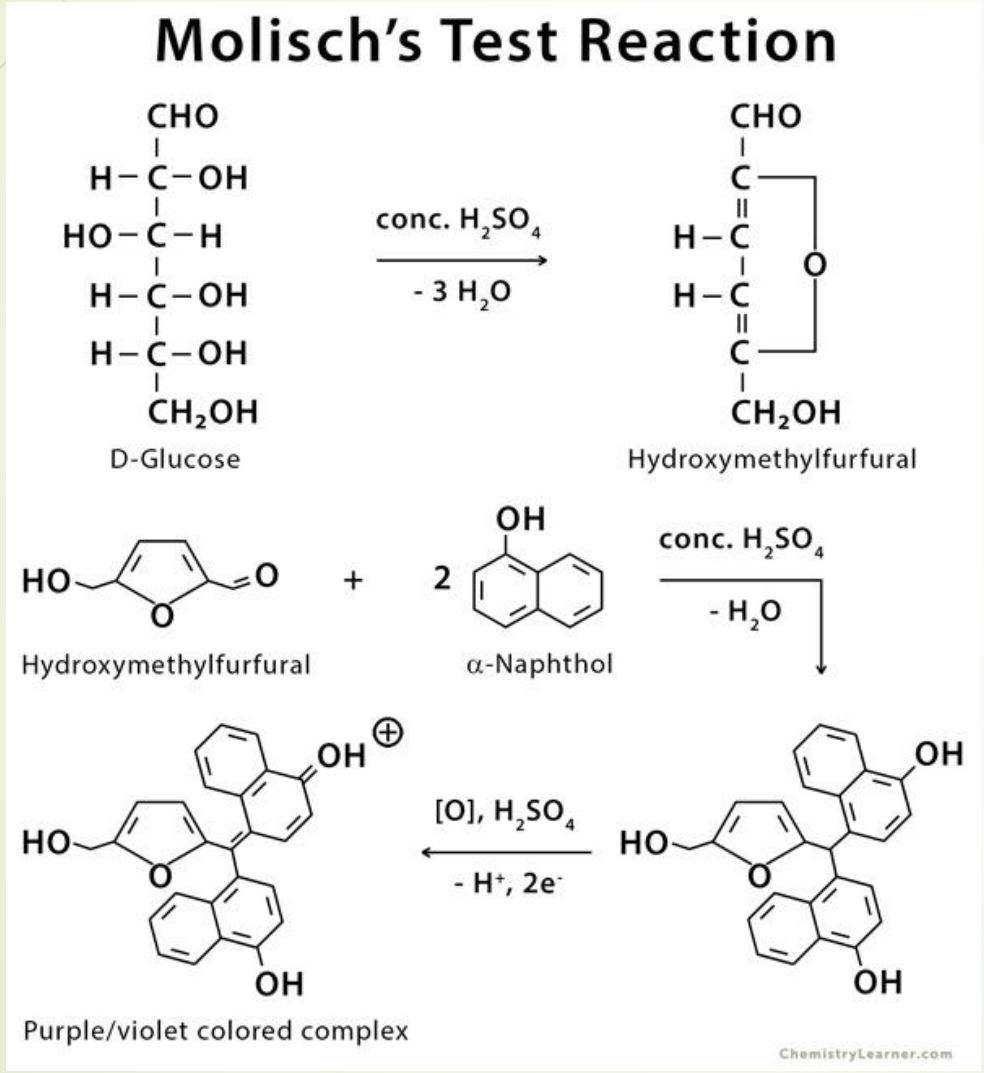
Experiment No.01

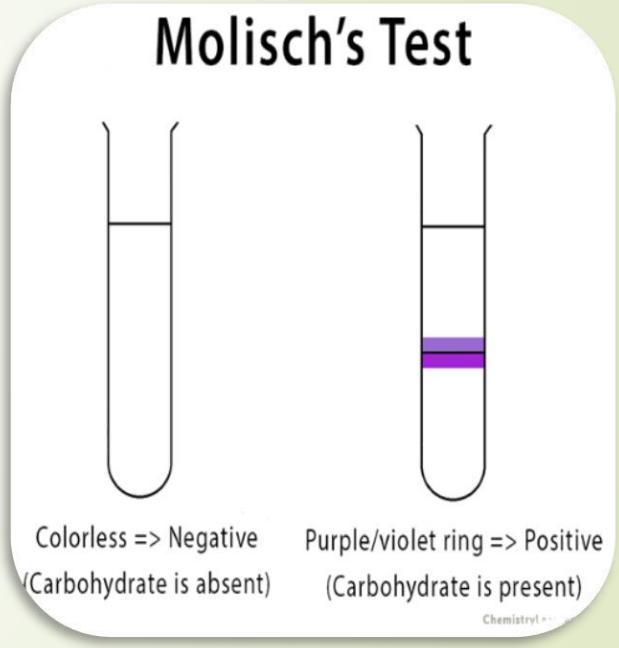
Detection of the Presence of Carbohydrate in the Given Samples by Molisch's Test

- **Principle:**
- Molisch's test is a sensitive chemical test for the presence of carbohydrates,
- Most carbohydrates should give a positive reaction
- Nucleic acids and glycoproteins also give a positive reaction.



Mechanism of reaction





Procedures

- **REAGENTS:**

1) **Molisch's Reagent: 5% α-napthol in 95% alcohol**
2) **Concentrated H_2SO_4**
3) **1% solution different carbohydrates.**

Steps

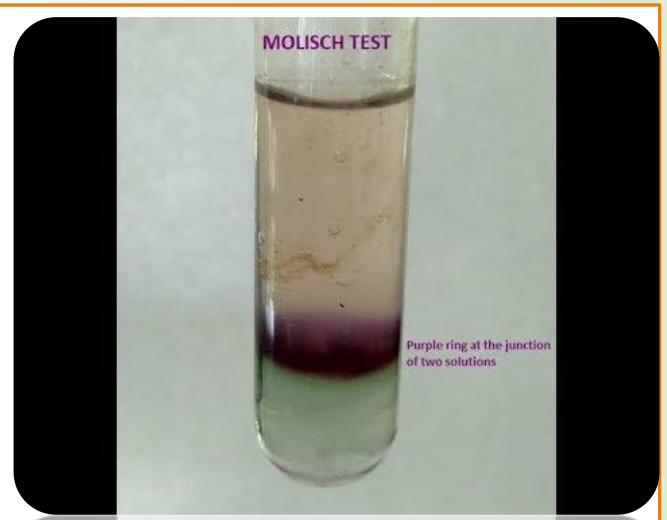
1. Take 2 ml of sugar solutions in a test tube.
2. Add 2-3 drops of Molisch reagent(α-napthol), and mix well.
3. Gently pour 2 ml of concentrated H_2SO_4 on the test tube's wall.
   (The acid should not be mixed with the solution).

Procedures

2 ml conc. H_2SO_4

2-3 drops of a-napthol

2ml sugar solution



Violet ring

Molisch's test