Biochemistry

- Chemistry
- Biochemistry
- Clinical biochemistry
- Atoms , elements , molecules & compounds

Chemistry:

Chemistry: is the science that studies matter and the changes that occur to it, specifically by studying its properties, structure, composition, behavior, interactions and what happens through it.

Chemistry studies atoms and the bonds that occur between them to form molecules, and how these molecules are

subsequently linked to form matter.

- **branches of chemistry:** physical chemistry, organic chemistry, inorganic chemistry, biochemistry , analytical chemistry.

Biochemistry:

Biochemistry: is a branch of natural science specialized in studying the chemical composition of cell parts in various simple and complex organisms.

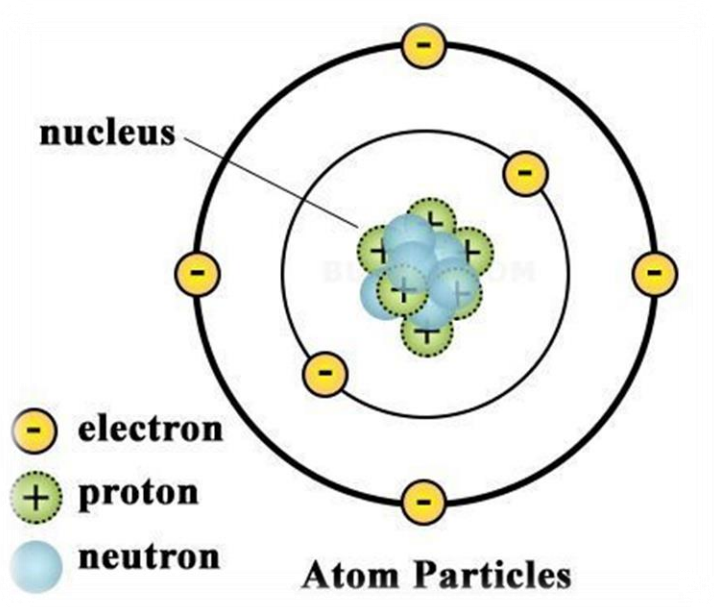
E.x: carbohydrate , proteins , lipids , etc.

Clinical biochemistry: It is the science that deals with various body fluids in order to diagnose diseases.

E.x: laboratory tests such as sugar test , urinary test , Na test , etc.

Atoms , elements , molecules & compounds:

Atoms: atoms are the basic units of matter and It’s the smallest unit of substance.



Each atom consists of three basic components:

1- the protons have appositive electric charge.

2- the Electrons have a negative electric charge.

3- the Neutrons have no electric charge.

* All atoms are electrically neutral, because every atom has an equal number of electrons and protons.

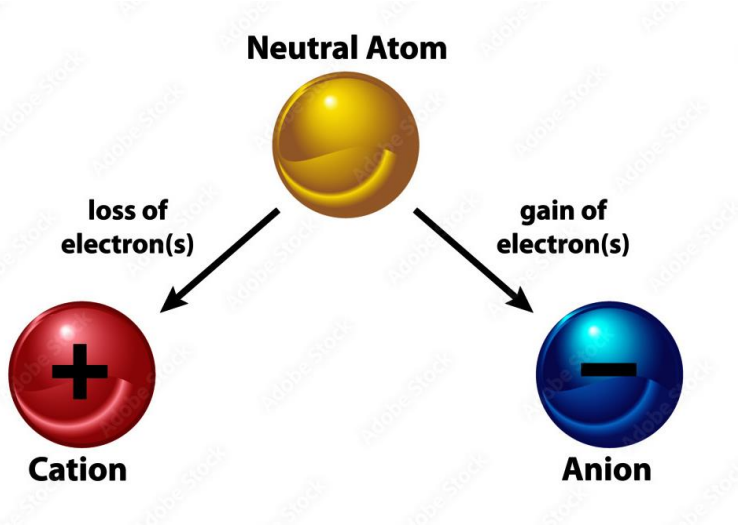
Nucleus: Small dense center of atom and contains almost the mass of the atom and contains protons and neutrons.

* Nearly all of the atom's mass is located in the nucleus.

* The nucleus is tiny compared with the total size of the atom.

Electrons: It is subatomic particle that is almost spherical in shape of an atom and carries a negative electric charge.

- If the number of protons and electrons are equal , the atom electrically neutral.
- If the atom has mor protons than electrons it will has a positive charge.
- If the electrons number mor than protons the atom has a negative charge.

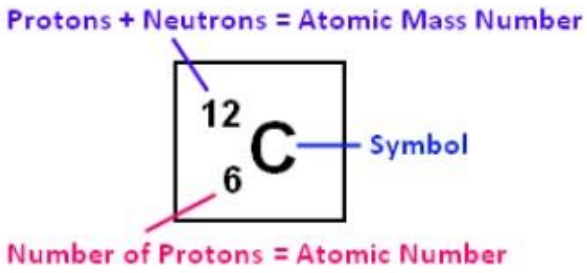


Elements: Composed of one type of atoms . My be define as , a pure substance that cannot be changed into a simple form of matter by any chemical reaction.

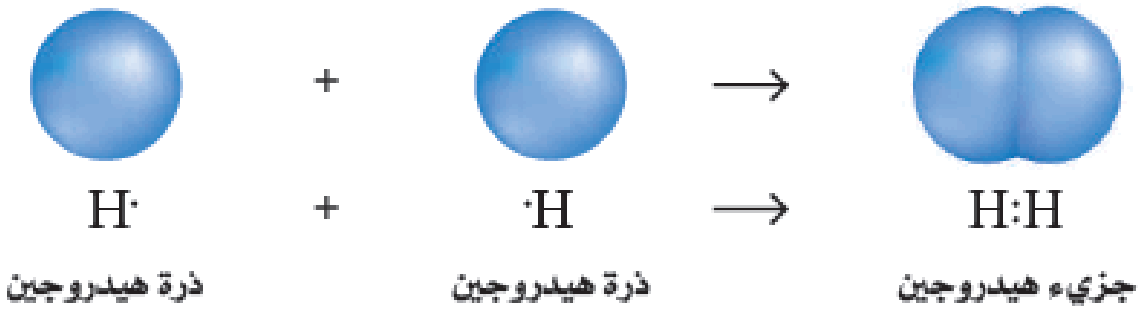
- Each element is identified by two numbers:

- Atomic number : number of protons.

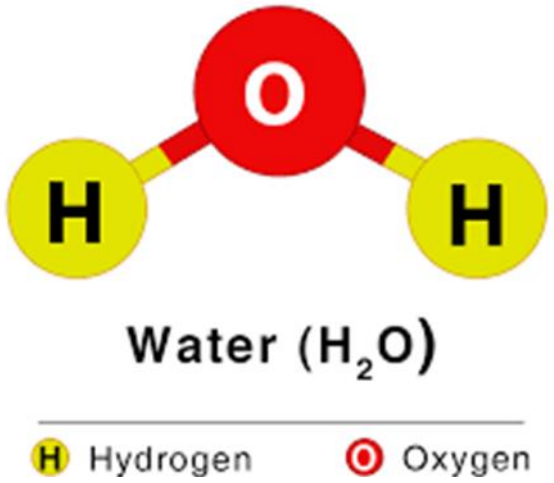
- Atomic weight (mass number): number of protons and neutrons.



Molecule: A molecule is two or more atoms connected by chemical bonds, which form the smallest unit of a substance that retains the composition and properties of that substance. Molecules form the basis of chemistry. Molecules are noted with the element symbol and a subscript with the number of atoms.



Compounds: A chemical compound is a chemical substance composed of many molecules containing atoms from more than one chemical element held together by chemical bonds. A molecule consisting of atoms of only one element is therefore not a compound. A compound can be transformed into a different substance by a chemical reaction, which may involve interactions with other substances. In this process, bonds between atoms may be broken and/or new bonds formed.



BLOOD

Blood: is fluid connective tissues, composed of

- Plasma
- Cells : - Erythrocytes (red blood cells, or RBCs)

  - Leukocytes (white blood cells or WBCs)

  - Platelets (thrombocytes)

Blood has many different functions, including:

- Transport CO2 from tissues to the lungs.
- Forming blood clots to prevent excess blood loss
- Carrying cells and antibodies that fight infection
- Bringing waste products to the kidneys and liver, which filter and clean the blood
- Transporting oxygen and nutrients to the lungs and tissues.
- Regulating body temperature.

Types of Specimens used in Clinical Chemistry :-

Whole blood: The blood that flows through veins & Arteries. This blood sample is obtained in the test tube containing anticoagulant. This sample will contains cells and plasma.

Serum: a clear, pale yellow fluid separated from clotted blood by centrifugation, free of fibrinogen because it was used in clot formation.

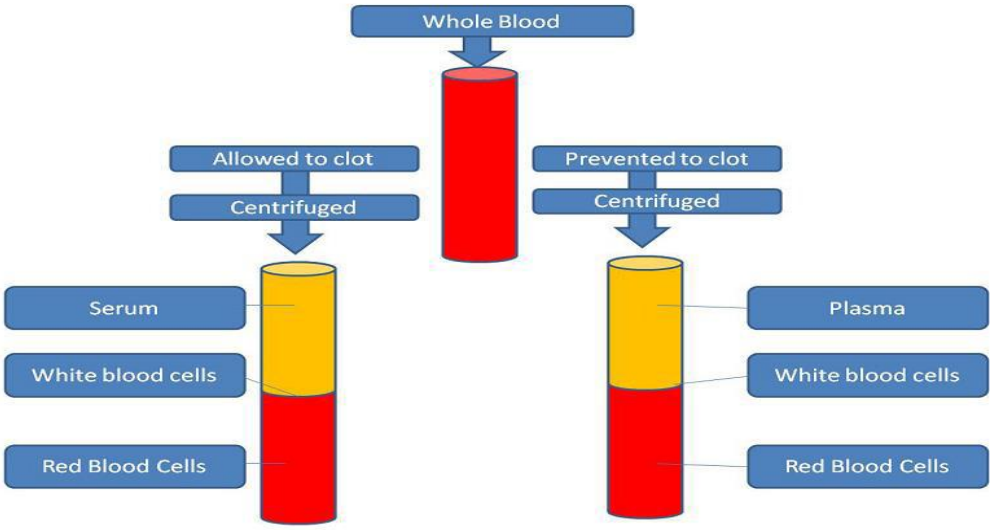
Clotted blood is kept at 37 C for at least 20 minutes and then centrifuged; the upper portion is called serum. There is no fibrinogen.

Plasma: a clear, pale yellow fluid that separates from the cells when blood in an anticoagulant tube is centrifuged

Plasma forms with the help of anticoagulant, which will prevent the clotting. There is presence of fibrinogen in the plasma.

Buffy Coat*:* This is the middle layer between the plasma and RBCs. This will contains white cells and platelets.

Difference between plasma & serum.



The major difference between plasma and serum is that serum does not contain fibrinogen (used in clot formation).
Plasma is produced when we centrifuge blood into an anticoagulant tube. However, Serum is produced when we centrifuge blood into a plain tube.

Clotting factors

The clotting factors are compounds present in tissues of the blood vessels, responsible for the formation of a blood clot.

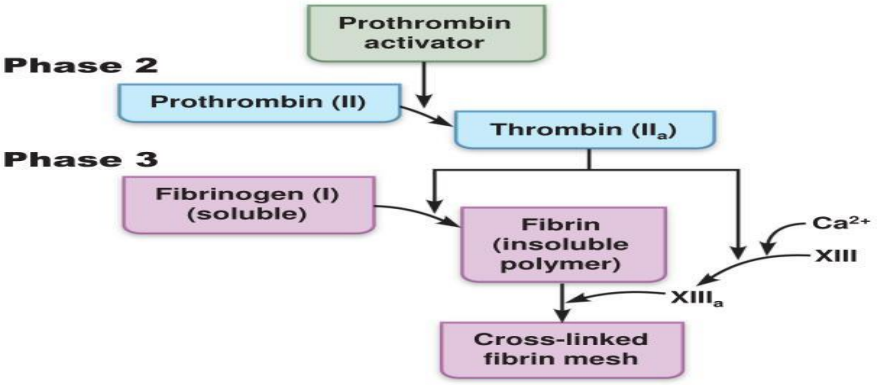
Mechanisms *of* Blood Coagulation:-

The three stages of this process are :-

1- Formation of **Prothrombinase** by interactions between coagulantion factors.

2- **Prothrombin** converted into **Thrombin** by Prothrombinase that is formed in stage 1.

3- **Fibrinogen** (soluble) converted to **Fibrin** (insoluble) by Thrombin.



Anticoagulants: It is the substances that prevent coagulation of blood and it is used to prepare a plasma sample in laboratory

Type of Anticoagulants:-

1- Heparin: is available as lithium heparin (LiHep) and sodium heparin (NaHep). It facilitates the action of anti-thrombin III that is inhibit conversion of fibrinogen to fibrin and Inhibits thrombin formation.

Some samples must be collected on heparin such as
1- Blood gases.
2-plasma potassium measurements to avoid an elevation due to the release of potassium from platelets.

2- **EDTA (Ethylene diamine tetra-acetic acid**): is available as disodium or potassium salts. It is a chelating agent, which binds the calcium; it is used in tests that need whole blood such as CBC, blood group, and cannot be used for coagulation tests or serum electrolytes.

4- **Sodium Citrate**: used as 3.8% solution.
It converts ionized calcium into unionized soluble complex but not as strongly as EDTA.
The most popular tests use citrate is:
- ESR test (
- PT & PTT test

3- Sodium Fluoride (NaF): has a weak anticoagulant action through the formation of a weakly dissociated Ca complex. It is often combined with potassium oxalate anticoagulant (NaF/KO).
Prevents glycolysis, by inhibiting enzyme, enolase.
Inhibit urease so urea determination cannot be done

Types of blood tubes

- **Red Tube:**

- Dry tube with no anticoagulant (does not need shaking)
- In order to get the Serum.
- Used in blood chemistry analyzes, serums, hormones, and some Other analyses.

- **Yellow tube:**

- Does not contain any anticoagulant (does not need shaking)
- It contains a gel material, which helps separate the serum from the blood cells.

- **Lavender tube:**

-Contains EDTA (EDTA K2 or EDTA K3), needs shaking.
- It is used in CBC, blood group analysis, and others.

- **Blue Tube:**

-Contains Sodium Citrate for plasma, needs shaking.
-It is used to measure clotting factors, the most important of which is PT.

- **Black tube:**

-Contains Buffer Sodium Citrate, needs shaking.
- It is used in ESR. Analysis.

Reasons for specimen rejection

1. Unlabeled specimen
2. Improper blood collection tube
3. Inadequate sample volume
4. Clots present in an anticoagulated specimen
5. Specimen is hemolyzed
6. Specimen is lipemic
7. Improperly timed specimens
8. Contaminated specimen
9. Specimen was contaminated with intravenous fluid

BIOCHEMISTRY

Biochemistry : is the study of chemical processes in living tissue (change in material & energy).

Cells

The structure of all living tissues &are the site of most of the biochemical processes which occur in living organisms.

Types of Cells:

1. Prokaryotic cells : are cells without a nucleus, the cell's DNA suspended in the cytoplasm. Most prokaryotes are single-celled organisms such as bacteria.

2. Eukaryotic cells: are cells that contain a nucleus and other organelles . Eukaryotic cells are usually larger than prokaryotic cells, and they are found mainly in multicellular organisms.

Cell Organelles

1- Nucleus—controls cell growth and reproduction.
2- Mitochondria—provide energy for the cell.
3- Endoplasmic Reticulum—synthesizes carbohydrates and lipids.
4- Golgi Apparatus—manufactures, stores and ships certain cellular products.
5- Lysosomes—digest cellular macromolecules.
6- Peroxisomes—detoxify alcohol, form bile acid, and use oxygen to break down fats.
7- Ribosomes—responsible for protein production via translation.

Cell membranes

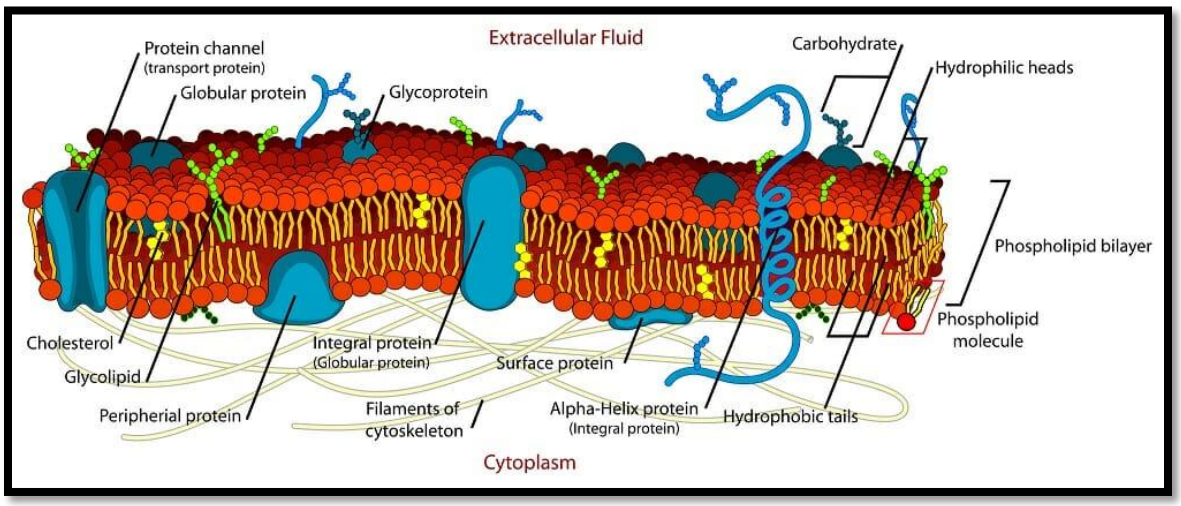
Chemical composition of the cell membrane

(i) lipids (28 – 79%) The main lipid components of the membranes are phospholipids, cholesterol and glycolipids.
(ii) proteins (20 – 70%). depending on the type of the cell. They can be classified into two types. Integral proteins and peripheral membrane proteins.

(iii) oligosaccharides (only 1 – 5%)
(iv) water (20%).

Function of the cell membrane:

1- Gives the cell its structure .
2- Regulates the materials that enter and leave the cell.
3- Plays a role in cell signaling and communication.
4- The cell membrane performs phagocytosis process.



Cell membrane diagram

TRANSPORT MECHANISMS

The permeability of substances across cell membrane is dependent on their solubility in lipids and not on their molecular size.

Transport mechanisms are classified into

1. Passive transport(passive diffusion) : the substances move from higher concentrations to lower concentrations without the help of any protein. The transport continues until the concentration of the substance becomes same on both the sides of the membrane. O2, CO2 and urea.

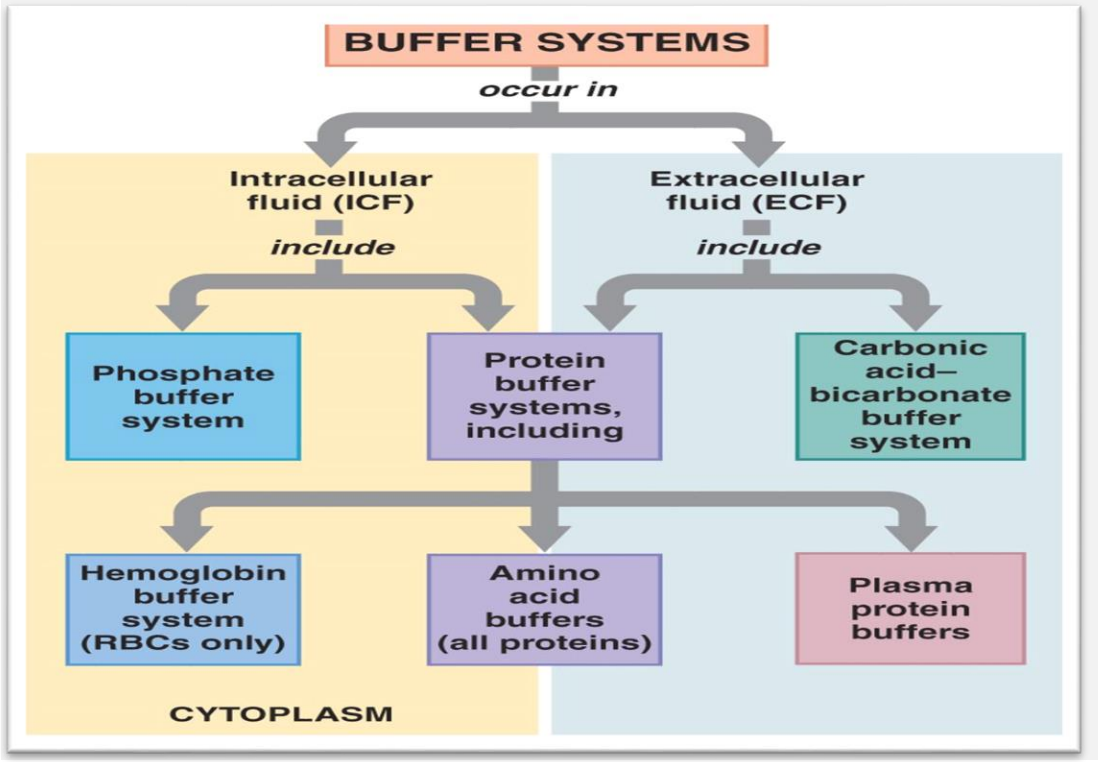
2. Facilitated diffusion. **:**transport substances from high concentrations to low concentrations. Need a carrier carrier protein named as **transporters** or **channels**. No energy is needed.

3. Active transport : transport substances from low concentrations to high concentrations,(drive molecules against the gradient). Needs a carrier protein named as **pumps**, using energy

Cellular Buffers

A solution which resists the change in pH from the addition of acid or base.

Physiologic range of the pH is **7,36-7,44.**



Buffer Systems in Body Fluids

The three major buffer systems of our body:

1- **Carbonic acid bicarbonate buffer**:

- Most important in ECF
- Most body cells constantly generate carbon dioxide
- Most CO_2 is converted to $H2CO_3$, which dissociates into H+ and HCO_3.

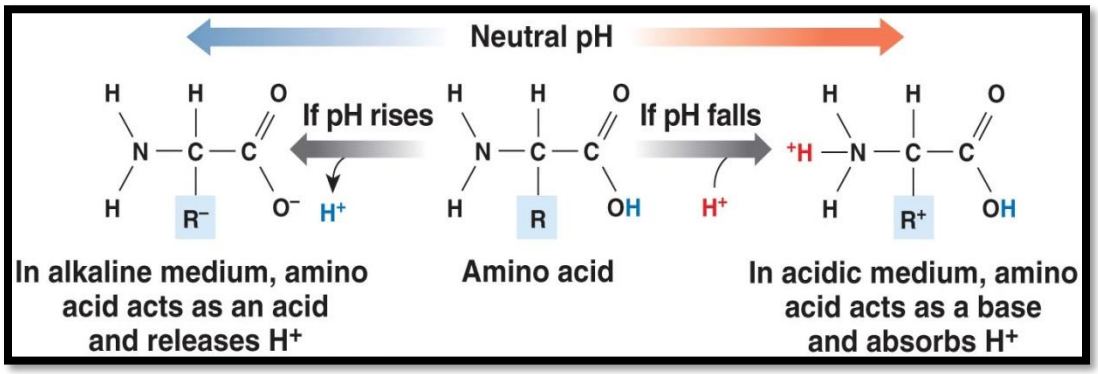
2- **Phosphate buffer system**

- Buffers pH of ICF and urine
- Consists of anion $H_2PO_4^-$ (a weak acid)

- Works like the carbonic acid–bicarbonate buffer system.

3- **Protein buffer system**

- Protein and amino acid regulate pH in ECF and ICF
- Hb regulate pH in ICF
- Hb prevent major changes in pH when plasma PCO2 is rising or falling.



The Role of Amino Acids in Protein Buffer Systems

Homeostasis mechanisms

For maintenance of Acid–Base Balance coordinates actions of buffer systems with

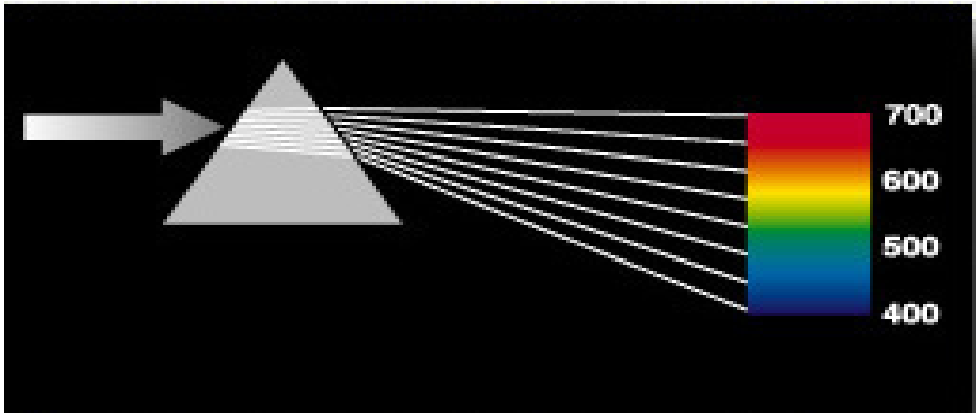
- Respiratory mechanisms
- Metabolic mechanisms

Respiratory acidosis and alkalosis are due to a problem with the lungs(are caused by an imbalance in the production of acids or bases and their excretion by the kidneys.).
-Metabolic acidosis and alkalosis are due to a problem with the kidneys(are caused by changes in carbon dioxide exhalation due to lung or breathing disorders.).

Colorimetric analysis

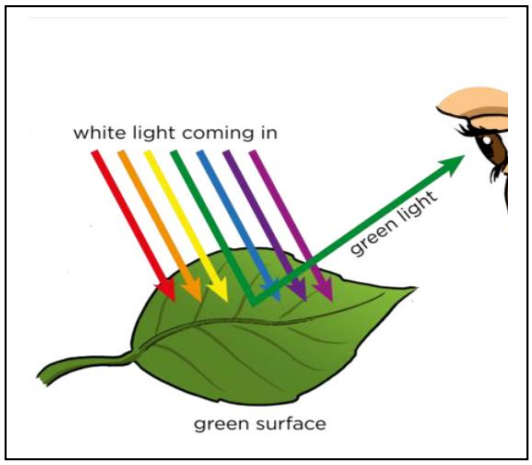
Colorimetric analysis is a method to measure color at a specific wavelength. Reagent is used to react with a specific component in the serum and a colored complex is formed which is directly proportional to the concentration of the compound in the serum.

<u>Visible light</u> is the narrow range of electromagnetic waves with the wavelength of **400-700 nm**.



Polychromatic light is the light containing all wavelengths, including ultraviolet, visible light, and infrared (it is called white light).

monochromatic light : each color of light e.g., red, blue, etc.



Beer-Lambert Law(a.k.a. Beer's law) - the linear relationship between absorbance and concentration of an absorbing species

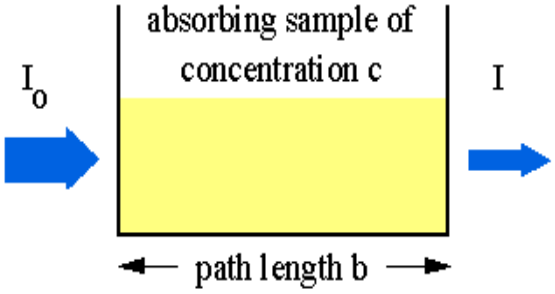
A = abc

A : absorbance of colored solution , **a :** proportional constant

b : thickness of solution , **c :** molar concentration

Main use of Beer's Law is to **determine the concentration**

of various solutions.



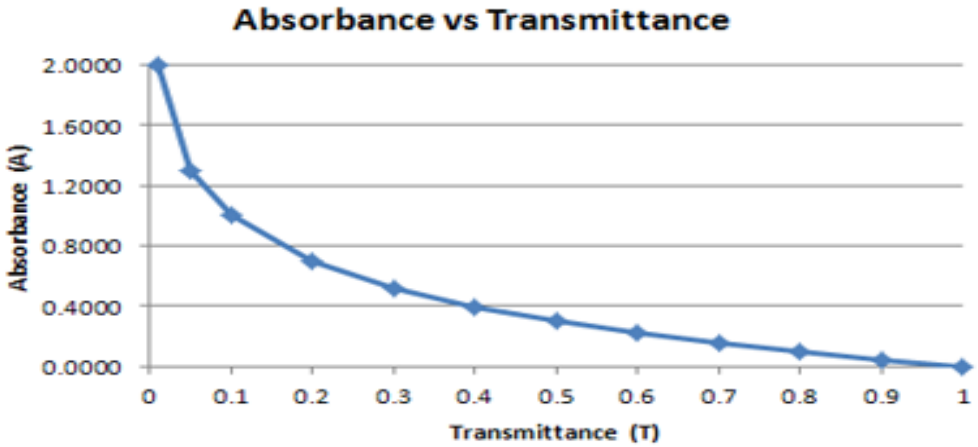
Absorbance (A): Also called optical density (OD), it is the measure of the amount of light absorbed by a sample.

Transmittance (%T): The ratio of the intensity of the transmitted light (**I) to the intensity if the** incident light (**I_o).**

$$\%T = I/I_o \times 100$$

What is the relation between absorbance & transmittance?

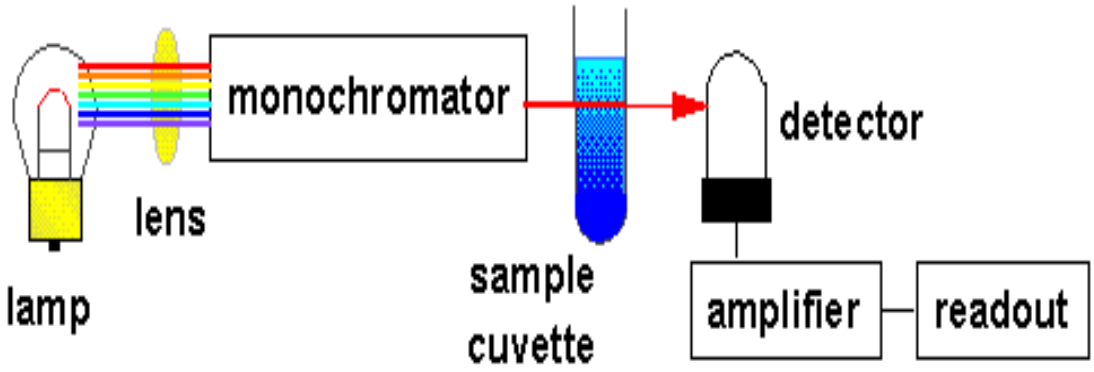
A = 2 – log %T



Spectrophotometer

Spectrophotometry is an instrument that measures the amount of light absorbed, or the intensity of color at a given wavelength.

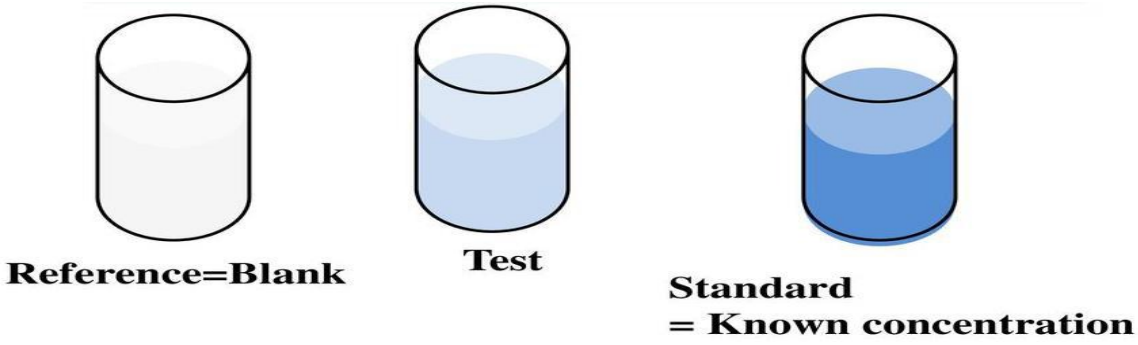
Simple Spectrophotometer Schematic



Component of Spectrophotometer

- **The lamp** emits all colors of light (i.e., white light).
- **The monochromator** selects **one wavelength** and that wavelength is sent through the sample.
- **Cuvette:** contains for sample.

- **<u>The detector</u>** detects the wavelength of light that has passed through the sample.
- **<u>The amplifier</u>** increases the signal so that it is easier to read against the background noise.
- Galvanometer: to record the electrical current.
- **How colorimetric measurement is done?**
- In colorimetric assays three tubes should be prepared as follow:
- 1-Reagent blank: which contain the reagents only (without sample or standard) and used to set instrument at zero absorbance.
- 2-Standard solution: or the calibrator is usually identical to the test solution, except that it contains a known amount of the substance being determined.
- 3-Test solution: contains the unknown concentration of the substance with the reagents used in the test.



Calculations

Test concentration = $\frac{A\ of\ test - A\ of\ blank}{A\ of\ standard - A\ of\ \ blank}$ **x standard concentration**

$C_t = C_s\ A_t/A_s$

Serum electrolytes

Electrolytes : - are substances when dissolved in solution separate into ions.

-Cat ion : positively charged electrolyte such as Na^+ , K^+, Ca^{+2} , Mg^{+2}

-Anion : negatively charged electrolyte (ions of inorganic acid Cl , HCO_3 , PO_4 , SO_4 , I and proteins and some organic acid).

Electrolytes are well distributed in the body fluids in order to maintain the osmotic equilibrium and water balance.

Law of electrical neutrality: Fluid in any body compartment will contain equal number of positively charged and negatively charged ions.

Extracellular fluid (plasma)				*Intracellular fluid (muscle)*			
Cations		Anions		Cations		Anions	
Na^+	142	Cl^-	103	K^+	150	HPO_4^{2-}	140
K^+	5	HCO_3^-	27	Na^+	10	HCO_3^-	10
Ca^{2+}	5	HPO_4^{2-}	2	Mg^{2+}	40	Cl^-	2
Mg^{2+}	3	SO_4^{2-}	1	Ca^{2+}	2	SO_4^{2-}	5
		Proteins	16			Proteins	40
		Organic acids	6			Organic acids	5
	155		155		202		202

Serum sodium

In humans, sodium is an essential mineral. The body obtains sodium through food and drink and loses it primarily in sweat and urine.

Sodium homeostasis

Sodium concentration outside of the cell (***extracellular fluid***) is greater the inside (***intracellular fluid***). This equilibrium is mainly maintained through the transmembrane enzyme Na+/K+ ATPase (***sodium-potassium pump***) which transports $2K^+$ inside and $3Na^+$ outside at the cost of 1 ATP molecule.

Normal serum sodium levels = 135 and 145 mmol/L.

- **Function** : 1- Maintains water distribution in cell.
  2- Maintains proper osmotic pressure in cell.

3- Transmission of nerve impulse.
4- Neuromuscular and myocardial impulse transmission.

Disorder of Sodium:

Hypernatremia: sodium concentration in the serum is higher than normal.

Causes : Cushing disease, Severe dehydration, Diabetic insipidus

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Hyponatremia: sodium conc. in the serum is lower than normal.

Causes : liver disease, kidney disease, congestive heart failure, Excessive sweating, Severe vomiting or diarrhea.

Serum potassium

- Main intracellular cation, & regulated by kidney.
- Potassium concentration outside of the cell (***extracellular fluid***) is lessthan the inside (***intracellular fluid***). This equilibrium is mainly maintained through sodium-potassium pump
- Potassium is slightly higher in serum than in plasma, because some K+ is released from platelets during coagulation of blood
- Normal concentration of K = 3.5- 5.3 meq/l.
- Function : 1- Maintains extracellular cell osmolarity.
  2- Maintains cell electro- neutrality.
  2- Assists in conduction nerve of impulse.
  3- Directly affects cardiac muscle contraction.
  4- Play major role in acid- base balance .

Disorder of Potassium

Hyperkalemia : Concentration of the electrolyte potassium (K+) in the blood is elevated.

Causes : - Impaired potassium excretion
- Excessive release from cells

Hypokalemia : Concentration of potassium (K+) in the blood is low.

Causes : - Inadequate potassium intake.
- Gastrointestinal loss
- Urinary loss.

Serum calcium

- Calcium is the most abundant element in extracellular fluid in the body.
- Regulated by action of ; parathyroid hormone , Vitamin D, Calcitonin.
- Ca ions are concerned in the regulations of the heart action. The effect of Calcium is to promote systole which is balanced by K ions.

Function of ionized calcium

1- Growth and formation of bones.

2- It plays role in muscle contraction and in the electrical conduction system of the heart.

3- It plays role in neurotransmitter release from neurons.

4- Aids in blood coagulation.

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Hypercalcaemia: is an elevated calcium level in the blood.

Total calcium of more than 10.6 mg/dl is hypercalcaemia, with levels over 15.12 mg/dl generally fatal.

Causes : Hyperparathyroidism, Hypervitaminosis, Paget's disease , Malignancy

Hypocalcaemia: low serum calcium levels in the blood.

Total calcium of less than 8.0 mg/dl is hypocalcaemia, with levels below 6 mg/dl generally fatal.

Causes : Hypoparathyroidism, Tetany, Rickets, Nephritis , Pregnancy.

Serum phosphorus

Phosphorus is found as

- free phosphate ion is called inorganic phosphate (consists of a mixture of HPO_4^{-2} and $H_2PO_4^-$ ions)
- phosphate esters.

Bone contains about 85% of the body's phosphate. The rest is located primarily inside cells and less than 1% in extracellular fluids.

Phosphates are most commonly found in the form of:

Phosphates are most commonly found in the form of:

▪ Adenosine phosphates (AMP, ADP, and ATP)

▪ Phosphate backbone in DNA and RNA

▪ Phospholipid of cell membranes .
▪ Calcium-phosphate-hydroxyapatite in bone and teeth.

Pathophysiology

Hypophosphatemia

Causes : Decreased intestinal absorption, Hyperparathyroidism.

Hyperphosphatemia

Causes: An increased intake of phosphate, Hypoparathyroidism

Serum magnesium

Magnesium is the second most abundant element inside human cells and the fourth most abundant positively charged ion in the human body.

The average human body contains about 25 grams of magnesium 53% is located in bone, 19% in non-muscular tissue, and 1% in extracellular fluid

Biological role

1- magnesium's function as an enzyme cofactor.
2- Energy production
3- Nucleic acid protection
4- Bone development

Pathophysiology

Hypomagnesemia: is a low level of magnesium in the blood (It is defined as a level less than 0.7 mmol/l).
Causes : Reduced intake, Gastrointestinal disorders, Renal disorder.

Hypermagnesemia: is a high level of magnesium in the blood (It is defined as a level greater than 1.1 mmol/l).
Causes : Decreased excretion, Endocrine disorders

IRON

Iron is one of the most essential trace elements in humans, being the central ion in haem, the nonprotein component of haemoglobin, myoglobin and the cytochromes. Iron deficiency causes a failure in haem synthesis and

since haemoglobin is required for delivery of oxygen to the tissues, this leads to anaemia and tissue hypoxia. However, free iron is highly toxic to cells and must be bound to protein at all times.

Iron Present in Body:

A. Essential (or functional) iron: *ex: Haemoglobin, Myoglobin- and* s*Cytochromes*
B. Storage iron.: Ferritin and Haemosiderin
C. Transferrin: iron in the plasma is associated with the iron binding glycoprotein, each molecule of which binds two Fe2+ ions.

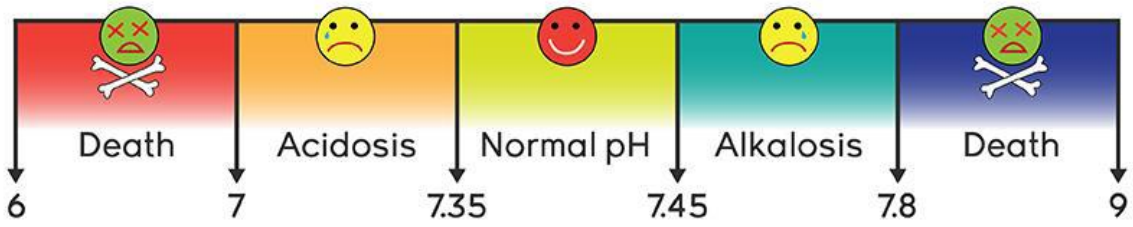
Normal adult concentrations are 10–40 µmol/L.

- Serum iron concentrations differ with age and sex.
- Decreased in Anemia &pregnancy.
- Increased in Hemolytic anemia.

Acid base homeostasis

Blood pH

The pH of any fluid is the measure of the hydrogen ion (H+) concentration.
A pH of 7 is neutral, the lower the pH, the more acidic the blood.



Blood is normally slightly basic, with a normal pH range of 7.35 to 7.45
Value of pH higher than 7.45 in arteries is denoted as alkalemia, pH lower than 7.35 is acidemia.
Value of pH in arterial blood higher than 7.8 or lower than 6.8 are ncompatible with life.

Acid-Base Disorders

Acid-Base Disturbances describe any condition which results in changes of the extracellular fluid pH from the normal range of pH.
A**cidemia** describes the state of low blood pH (**below 7.35**),
Acidosis describes the processes causing increased acidity in the blood and other body tissues (i.e., an increased H^+). It is further sub divided into
1. Metabolic acidosis
2. Respiratory acidosis

1. Metabolic acidosis : More acids are produced by metabolism. This leads to decreased bicarbonate concentration.
It occurs in uncontrolled diabetes mellitus and starvation. Loss of bicarbonate due to diarrhoea and vomiting also cause metabolic acidosis. Increased elimination of bicarbonate by kidneys also leads to metabolic acidosis.

2. Respiratory acidosis :
Plasma partial carbon dioxide pressure is more due to abnormal lung function.
Decreased respiration or hypoventilation occurs due to depression of respiratory centre.
Sedatives like morphine and barbiturates depress respiratory centre.
Hypoventilation also occurs due to obstruction to air passage. In emphysema and asthma.

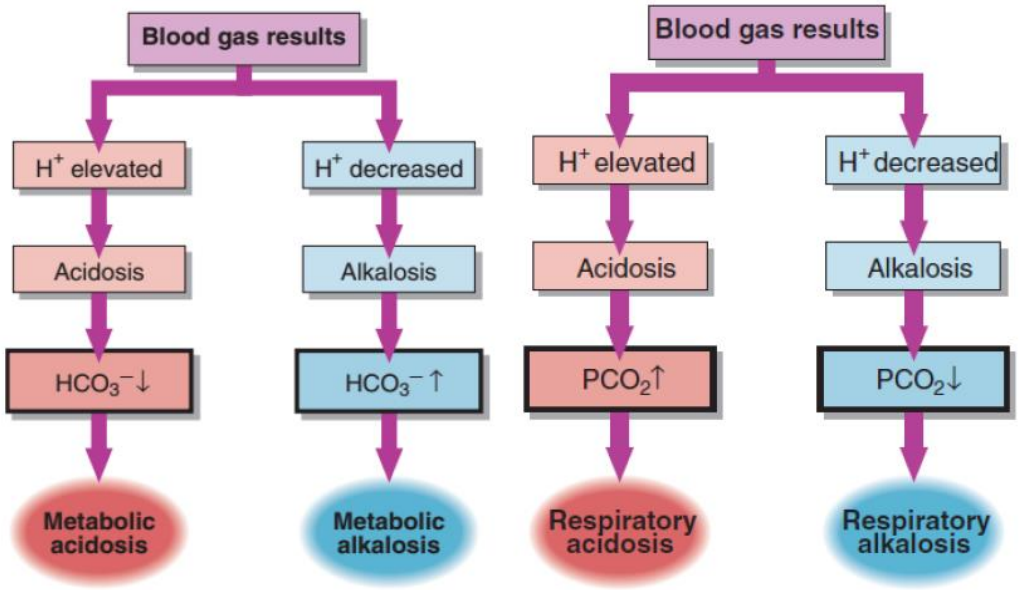
Alkalemia when the serum pH is higher than7.45.
Alkalosis is the result of a process that reduce hydrogen ion concentration of arterial blood plasma .

Type of Alkalosis

1. Metabolic alkalosis
2. Respiratory alkalosis.

1. Metabolic alkalosis : Bicarbonate concentration is more in blood. It occurs due to loss of HCl. More HCl is lost in prolonged vomiting. Ingestion of alkali cause metabolic alkalosis.

2. Respiratory Alkalosis : Partial pressure of carbon dioxide is less. It occurs due to hyperventilation. When respiratory centre is stimulated hyperventilation occurs.
Hyper ventilation occurs at high altitudes, head injury, drug poisoning, fever and anxiety.



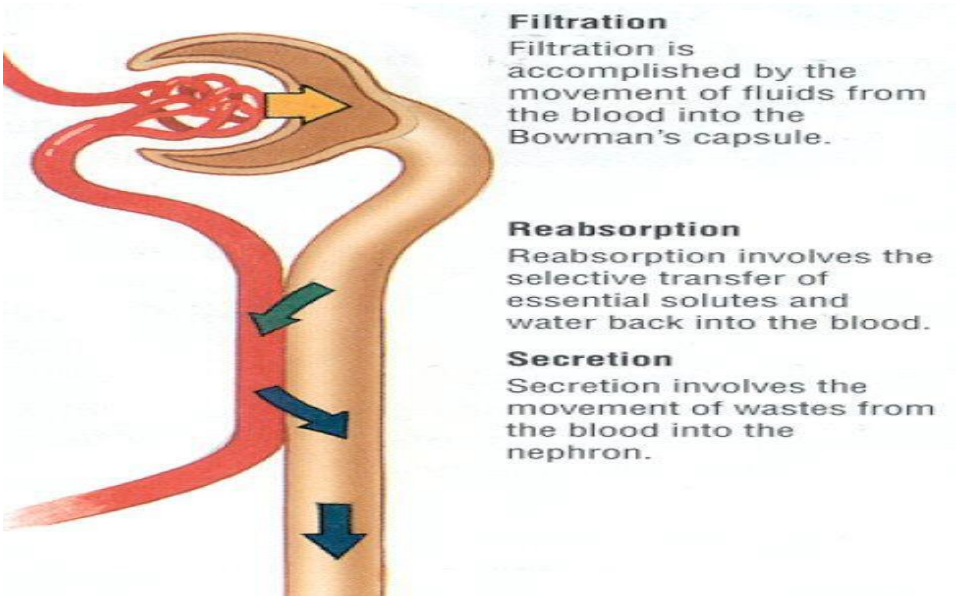
Urine

Urine: complex aqueous solution for inorganic and organic substances resulting from the metabolism processes in the body.

Waste materials in urine:-

Organic substances: Urea, Uric acid, Creatinine.

Inorganic substances: Ammonia, Sulphate salt, Chloride.



URINE – Formation –Excretion

Glomerular filtration: The first step in urine formation is filtration of blood plasma at the nephrons, by passive process that results in the formation of ultrafiltrate of blood.

Tubular reabsorption: In the proximal tubule, 60% to 80% of the ultra-filtrate is reabsorbed by passive or active process.

Tubular secretion: is the transfer of materials from peritubular capillaries to renal tubular (mainly Proximal Convoluted Tubule(PCT) and Distal Convoluted Tubule(DCT).

- Tubular secretion is caused mainly by active transport.
- Usually only a few substances are secreted. These substances are present in great excess, or are natural poisons

Renal threshold: Certain substances appear in the urine when their plasma levels are above certain set-point, or "threshold levels ", such as glucose and amino acids.

Physical properties:

1. Volume:

- Normally 1.5 liter/day.
- Polyuria: an increase in urine amount (at least 2.5 L/day in adults) (In diabetic mellitus).
-Oliguria: decrease in urine amount. In adults, less than 500 ml/day. (In dehydration, Nephritis).
-Anuria: the complete absence of urine, in adults as less than 100 ml/day. (In kidney failure).

2. Color:

- Normal color: is yellow or amber yellow.
- Abnormal colors: Colorless (polyuria).
- Orange (due to antibiotic intake or eating certain foods)
- Brownish or greenish (Bilirubin >2mg/dl in blood).
- Reddish (RBC‟s due to stone or blood menstruation).
- Cloudy with offensive odor (due to pus, crystals or epithelial cells).

3. Clarity: clear to cloudy due to leukocytes, bacteria, epithelial or precipitation of phosphates.

4. Specific gravity (sp.gr): may range between 1.001 and 1.025.

Chemical properties:

1. **pH:** 4.5 - 8 (average pH around 6) .

2. Protein or albumin

- Normally: Nil
- Abnormally: trace, +, ++, +++. According to the read of protein strip.

3. Glucose : Trace, + (160-195), ++ (>200), +++ (250-300).According to the read of glucose strip.

4. Acetone:

Mainly: acetone increases by increasing glucose (>200).

Microscopic exam of sediment:

- Red blood cells (RBCs): negative or rare .
- White blood cells (WBCs): negative or rare .
- Casts: negative (occasional hyaline casts).
- Crystals: negative
- Epithelial cells: few