Lab: 1

Specimen collection and preservation

Introduction to Clinical Laboratories

When a person is ill, diagnosis begins with physical examination by a doctor. It may not possible to diagnose a disease only on the basis of physical examination. There are various diagnostic tests to confirm a suspected diagnosis. The clinical pathological laboratory tests are extremely useful to find out the causes of disease.

The functional components of the clinical laboratory are:
1) Clinical pathology
2) Hematology
3) Clinical biochemistry
4) Clinical microbiology
5) Serology
6) Blood bank
7) Histology and cytology

Functions: Clinical biochemistry deals with the biochemistry laboratory applications to find the cause of a disease as well as the severity of diseases of many organs such as liver, stomach, heart, kidneys, brain as well as the endocrine disorders and related status of acid-base balance of the body.

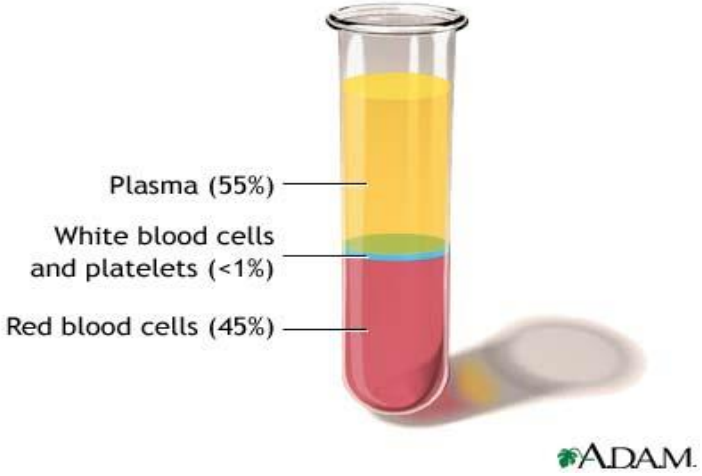
Specimen collection:

There are three types of blood specimens- serum, plasma and whole blood. Each different specimen is collected for various reasons. When blood is removed from the body, typically, it will coagulate or clot within 30 to 60 minutes. Serum can be separated from blood by centrifugation. Centrifugation is a process that spins the blood at high speeds in a machine called a centrifuge. This spinning separates the serum from the blood cells enmeshed in blood clot. Blood serum looks pale-yellow and has a similar composition to plasma. However, serum does not contain fibrinogen. Laboratory tests, like chemistry and immunology test are commonly performed on serum.

Coagulation tests cannot be performed on serum because the coagulation factors are separated out of the serum during the centrifuge process. Phlebotomy or blood collection: The term phlebotomy refers to blood draw from a vein, artery, or the capillary bed for lab analysis or blood transfusion

Blood:

Blood is a liquid tissue. Suspended in the watery plasma are seven types of cells and cell fragments. -Red blood cells (RBCs) -White blood cells (WBCs) -Platelets -Five kinds of Leukocytes (lymphocytes, monocytes, neutrophils, eosinophils, basophils) -After centrifugation of blood, the blood separate into three layers (see the figure)



Blood plasma: -

Plasma is the liquid component of blood. -It is mainly composed of water, blood proteins and inorganic electrolytes. -Roughly 92% water, mixed with organic and inorganic-substances. - The most abundant plasma solute is the plasma protein, of which there are three groups: albumin, globulins, and fibrinogen.

Blood clot:

-When a blood sample is left standing without anticoagulant, it forms a coagulum or blood clot. One of the normal components of plasma is a soluble plasma protein called fibrinogen. -On standing, this protein will be converted to insoluble substance called fibrin; this occurrence is referred to as blood coagulation or clotting. -The clot contains coagulation proteins, platelets, and entrapped red and white blood cells.

Blood serum:

Serum is the same as plasma except that clotting factors (such as fibrin) have been removed. -For many biochemical laboratory tests, plasma and blood serum can be used interchangeably. Serum resembles plasma in composition but lacks the

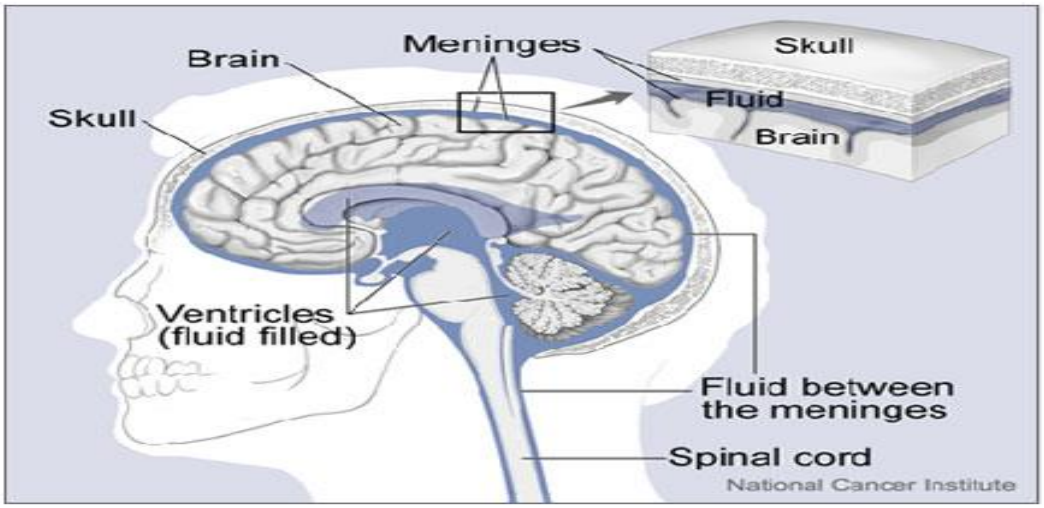
coagulation factors. - It is obtained by letting a blood specimen clot prior to centrifugation.

1- Whole blood specimens are usually required for hematology tests. These types of tests require the blood to remain in the same form as it is in the bloodstream. It is important that the blood specimen does not clot or separate. An anticoagulant must be added and the specimen should be mixed for at least 2 minutes immediately before performing the test. It must be analyzed within limited time (why?)

2- Urine has a long, rich history as a source for measuring health and well-being and remains an important tool for clinical diagnosis. The clinical information obtained from a urine specimen is influenced by the collection method, timing and handling.

3- Saliva testing is a diagnostic technique that involves laboratory analysis of saliva to identify markers of endocrine, immunologic, inflammatory, infectious, and other types of conditions. Saliva is a useful biological fluid for assaying steroid hormones such as cortisol, genetic material like RNA, proteins such as enzymes and antibodies, and a variety of other substances. Saliva testing is used to screen for or diagnose numerous conditions and disease states, including Cushing's disease, anovulation, HIV, cancer, parasites, hypogonadism, and allergies.

4- Cerebrospinal fluid (CSF) is a clear watery liquid that is formed and secreted by the choroid plexus, a special tissue that has many blood vessels and that lines the small cavities or chambers (ventricles) in the brain. About 17 ounces (500 mL) are produced each day. This rate of production means that all of the CSF is replaced every few hours. A CSF analysis is a group of tests that evaluate substances present in CSF in order to diagnose conditions affecting the central nervous system.



Phlebotomy:

The term phlebotomy refers to blood draw from a vein, artery, or the capillary bed for lab analysis or blood transfusion. Usually vein is used to collect blood by vein puncture procedure. In adults: most venipuncture procedure use arm vein.

On arm, one of three arm veins is used: **median cubital** vein "located on the middle", **cephalic** vein or **basilic** vein "located on both sides".

Median cubital vein is the best choice (why?).

However if venipuncture procedure is unsuccessful in median capital; cephalic or basilica is used.

Artery blood is rarely used in special cases as when blood gases, pH, PCO2, PO2 and bicarbonate is requested. It is usually performed by physicians.

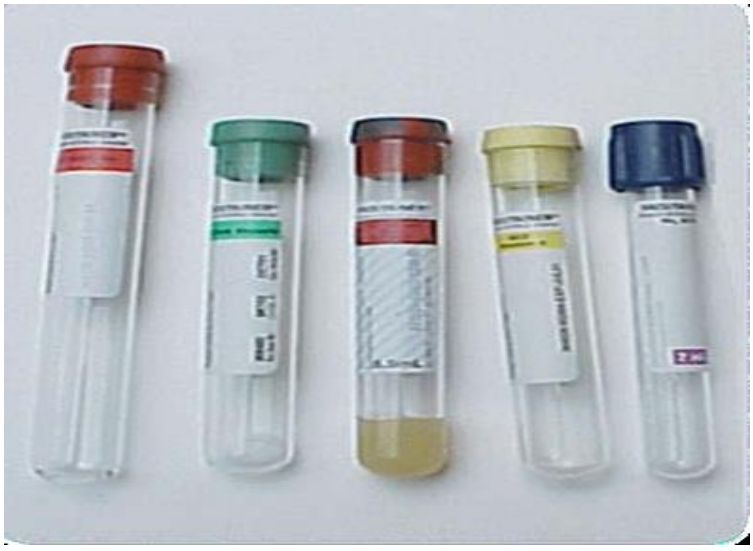
Hemolysis :

- It means liberation of hemoglobin due to rupture of RBCs.
- Due to hemolysis plasma or serum appears pink to red color.
- It causes elevation in: K^+, Ca^{2+}, phosphate, SGOT, SLDH and acid phosphatase.
- Hemolysis is occurred due to sampling, transporting and storage (too hot or too cold).

Changes in the serum color indicate one of the following:

- **Hemolyzed**: serum appears **pink** to red due to rupture of RBCs
- **Icteric**: serum appears **yellow** due to high bilirubin.
- **Lipemic**: serum appears **milky** or turbid due to high lipid.

Blood collection tubes:



The tubes are covered with a color-coded plastic cap. They often include additives that mix with the blood when collected, and the color of the tube's plastic cap indicates which additives that tube contains. The tubes may contain additional substances that preserve the blood for processing in clinical laboratory. Using the wrong tube may therefore make the blood unusable.

Top Color	Additives	Principle	Uses
Lavender	**EDTA**	**-The strongest anti-coagulant** **- Ca^{+2} chelating agent** **- To preserve blood cells components**	**- Hematology** **- Blood bank (ABO)** **- HbA1C (Glycosylated Hb)**
Light Blue	**Sodium Citrate**	**Ca^{+2} chelating agent**	**- PT: Prothrombin Time** **- PTT: Partial Thromboplastin Time** **(in case of unexplained bleeding and liver disease)**
Green	**Sodium Heparin or Lithium Heparin**	**Heparin binds to Thrombin and inhibits the second step in the coagulation cascade** **(Prothrombin → Thrombin)** **Fibrinogen ↛ Fibrin**	**Enzymes** **Hormones** **Electrolytes (Na^+, K^+, Mg^+, Cl^-**

Top Color	Additives	Principle	Uses
Black	**Sodium Citrate**	**Ca^{+2} chelating agent**	**ESR (Erythrocyte Sedimentation Rate) to test how much inflammation in the patient, unexplained fever, Arthritis, Autoimmune Disorder**
Gray	**-Sodium Fluoride** **-Potassium Oxalate**	**Glycolysis inhibitor** **Anti-Coagulant**	**Glucose tests**
Royal Blue	**Heparin** **Na-EDTA**	**Anti-Coagulant** **Tube should not be contaminated with metals**	**Toxicology** **Trace Elements and metals**
Yellow	**ACD (Acid-Citrate Dextrose)**	**Anti-Coagulant**	**DNA Studies** **Paternity Test** **HLA Tissue Typing (Human Leukocyte Antigen)** **The body used this protein to differentiate the self-cells from non-self-cells**

Anticoagulant

An **anticoagulant** is a substance that prevents coagulation (clotting) of blood. A group of pharmaceuticals called anticoagulants can be used in vivo as a medication for thrombotic disorders. Some anticoagulants are used in medical equipment, such as test tubes, blood transfusion bags, and renal dialysis equipment.

Heparin is a biological substance, usually made from pig intestines. It works by activating antithrombin III, which blocks thrombin from clotting blood. Heparin can be used in vivo (by injection), and also in vitro to prevent blood or plasma clotting in or on medical devices.

Anticoagulants outside the body

Laboratory instruments, blood transfusion bags, and medical and surgical equipment will get clogged up and become nonoperational if blood is allowed to clot. In addition, test tubes used for laboratory blood tests will have chemicals added to stop blood

clotting. Apart from heparin, most of these chemicals work by binding calcium ions, preventing the coagulation proteins from using them.

- **EDTA** is denoted by mauve or purple caps on Vacationer (A **vacutainer** blood collection tube is a sterile glass or plastic tube with a closure that is evacuated to create a vacuum inside the tube facilitating the draw of a predetermined volume of liquid. Most commonly used to draw a blood sample directly from the vein, these also are used to collect urine samples) brand test tubes. This chemical strongly and irreversibly binds calcium. It is in a powdered form.
- **Citrate** is usually in blue Vacutainer tube. It is in liquid form in the tube and is used for coagulation tests, as well as in blood transfusion bags. It binds the calcium, but not as strongly as EDTA. Correct proportion of this anticoagulant to blood is crucial because of the dilution. It can be in the form of sodium citrate or ACD.
- **Oxalate** has a mechanism similar to that of citrate. It is the anticoagulant used in fluoride (grey top) tubes.

Spectrophotometry

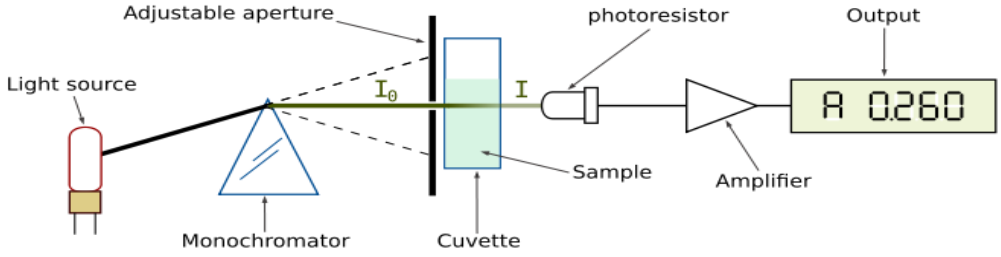
In chemistry, **spectrophotometry** is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared.

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer that can measure intensity as a function of the light source wavelength. Important features of spectrophotometers are spectral bandwidth and linear range absorption or reflectance measurement

In short, the sequence of events in a modern spectrophotometer is as follows:

1. The light source is imaged upon the sample
2. A fraction of the light is transmitted or reflected from the sample
3. The light from the sample is imaged upon the entrance slit of the monochromator

The monochromator separates the wavelengths of light and focuses each of them onto the photodetector sequentially.



Beer-Lambert Law

Introduction

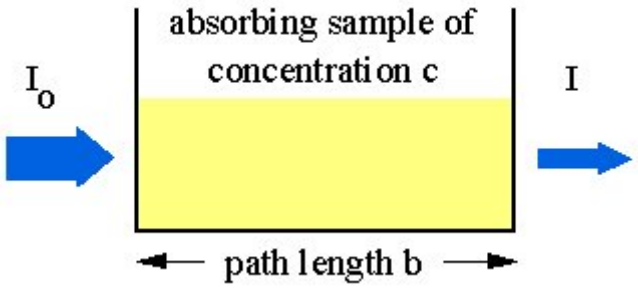
The Beer-Lambert law (or Beer's law) is the linear relationship between absorbance and concentration of an absorbing species. The general Beer-Lambert law is usually written as:
$A = a(\lambda) * b * c$

Where A is the measured absorbance, $a(\lambda)$ is a wavelength-dependent absorptivity coefficient, b is the path length, and c is the analyte concentration. When working in concentration units of molarity, the Beer-Lambert law is written as:
$A = \varepsilon * b * c$
where ε is the wavelength-dependent molar absorptivity coefficient with units of M^{-1} cm^{-1}.

Instrumentation

Experimental measurements are usually made in terms of transmittance (T), which is defined as:
$T = I / I_o$
where I is the light intensity after it passes through the sample and I_o is the initial light intensity. The relation between A and T is:
$A = -\log T = -\log (I / I_o)$.

Absorption of light by a sample



Modern absorption instruments can usually display the data as either transmittance, %-transmittance, or absorbance. An unknown concentration of an analyte can be determined by measuring the amount of light that a sample absorbs and applying Beer's law. If the absorptivity coefficient is not known, the unknown concentration can be determined using a working curve of absorbance versus concentration derived from standards.

LAB:2

Carbohydrate Metabolism

(Diabetes mellitus)

Carbohydrates are metabolized in the body to glucose. Glucose is taken by the muscle to produce energy (insulin required). Glucose is stored in the liver as glycogen and in adipose tissues as fat.

Blood sugar concentration, or glucose level, refers to the amount of glucose present in the blood of a human or animal. Normally, in mammals the blood glucose level is maintained at a reference range between about 3.6 and 5.8 mM (mmol/l). It is tightly regulated as a part of metabolic homeostasis

Diabetes mellitus (DM) is a group of diseases characterized by high levels of blood glucose resulting from defects in insulin production, insulin action, or both.

Regulation of blood glucose level

The homeostatic mechanism which keeps the blood value of glucose in a remarkably narrow range is composed of several interacting systems, of which hormone regulation is the most important. The concentration of blood glucose level is maintained constant by the action of two general opposing factors:

-The rate of glucose entrance to the blood.

- Rate of removal of blood glucose.

There are two types of mutually antagonistic metabolic hormones affecting blood glucose levels:

- Hormones which will increase blood glucose such as glucagon, growth hormone, cortisol and catecholamines.

- Hormone which will decreases blood glucose level (insulin).

Other type is regulation by different tissues and organs such as liver and Extra hepatic tissue , Kidney ,Gastrointestinal tract, Skeletal muscle and adipose tissue.

Types of Diabetes

- *Type 1 Diabetes Mellitus*
- *Type 2 Diabetes Mellitus*
- *Gestational Diabetes*
- *Pre diabetes*

Type 1 Diabetes Mellitus:

Previously was called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes. Type 1 diabetes develops when the body's immune system destroys pancreatic beta cells, the only cells in the body that make the hormone insulin that regulates blood glucose. This form of diabetes usually strikes children and young adults, although disease onset can occur at any age.

Type 2 Diabetes Mellitus:

Previously was called non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. Type 2 diabetes may account for about 90% to 95% of all diagnosed cases of diabetes. It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises, the pancreas gradually loses its ability to produce insulin.

Gestational Diabetes:

A form of glucose intolerance that is diagnosed in some women during pregnancy. After pregnancy, 5% to 10% of women with gestational diabetes are found to have type 2 diabetes. Women who have had gestational diabetes have a 20% to 50% chance of developing diabetes in the next 5-10 years.

Pre Diabetes:

Other specific types of diabetes result from specific genetic conditions (such as maturity-onset diabetes of youth), surgery, drugs, malnutrition, infections, and other illnesses. Such types of diabetes may account for 1% to 5% of all diagnosed cases of diabetes.

What is hypoglycemia?

If blood sugar levels drop too low, a potentially fatal condition called hypoglycemia develops. Symptoms may include lethargy, impaired mental functioning; irritability; shaking, twitching, weakness in arm and leg muscles; pale complexion; sweating; paranoid or aggressive mentality and loss of consciousness. Brain damage is even possible.

Causes:

-Insulinoma

- Non-pancreatic tumors (usually mesodermal)

- Liver disease of various types

- Hypopituitarism

What is hyperglycemia?

Hyperglycemia or high blood sugar is a condition in which an excessive amount of glucose circulates in the blood plasma. This is generally a glucose level higher than (200 mg/dl). Reference ranges for blood tests are 11.1 mmol/l, but symptoms may not start to become noticeable until even higher values such as 250–300 mg/dl.

Signs and symptoms

- Polyphagia - frequent hunger, especially pronounced hunger
- Polydipsia - frequent thirst, especially excessive thirst
- Polyuria - frequent urination
- Blurred vision
- Fatigue (sleepiness)
- Weight loss
- Poor wound healing (cuts, scrapes, etc.)
- Dry mouth

Causes:

- Deficiency of insulin (Diabetes mellitus)

-Increase of anti-insulin hormones:

- Glucocorticoids as in adrenal tumors and Cushing syndrome

- Thyroxin as in hyperthyroidism.
- Pituitary growth hormone as in acromegally

Management of DM

- Diet and Exercise
- Oral hypoglycaemic therapy
- Insulin Therapy

Risk factors of DM:

- Cardiovascular disease

-Kidney damage (nephropathy)

-Eye damage (retinopathy)

-Foot damage

-Nerve damage (neuropathy)

-Alzheimer's disease.

Laboratory Tests:

- **Fasting blood sugar (FBS)**

- **Random blood glucose (RBS)**

- **Two-hr postprandial blood sugar test (2-h PPBS)**

- **Oral glucose tolerance test (OGTT)**

- **Intravenous glucose tolerance test (IVGTT)**

- **Glycosylated hemoglobin (HbA_{1C}).**

- **Urine glucose test**

- Fasting blood sugar (FBS)

This test measures the level of glucose in the blood after fasting for at least 8 hours.

Blood glucose range for this test 70 - 115 mg/dl

- ***Random blood sugar (RBS)***

A blood sample will be taken at a random time. Normal range for this test 110 -160 mg/dl.

- ***Two-hr postprandial blood sugar test (2-h PPBS)***

Measures the glucose level exactly 2 hours after eating a meal.

- ***Glucose tolerance test (GTT)***

For this test, you fast overnight, and the fasting blood sugar level is measured. Then you drink a sugary liquid, and blood sugar levels are tested periodically for the next two hours. This protocol "challenges" the person's body to process the glucose.

- ***Glycated hemoglobin (A1C) test.***

This blood test indicates your average blood sugar level for the past two to three months. The normal range for the hemoglobin A1c test is between 4% and 6%. Because studies have repeatedly shown that out-of-control diabetes results in complications from the disease, the goal for people with diabetes is an hemoglobin A1c less than 7%. The higher the hemoglobin A1c, the higher the risks of developing complications related to diabetes.

- ***Urine glucose test***

The presence of a reducing substance in the urine may be detected by Benedict's test or dipstick test).

Other advanced tests:

Other advanced test used to diagnose diabetes and to measure response to treatment includes **C-peptide** and **insulin level**. A C- peptide is better indicator of B-cell function than peripheral insulin. A C-peptide test can be done when diabetes has just been found and it is not clear whether type 1 diabetes T or type 2 diabetes is present.

A person whose pancreas does not make any insulin (type 1 diabetes) has a low level of insulin and C-peptide. A person with type 2 diabetes has a normal or high level of

C-peptide.

Normal value: Fasting …..0.51 - 2.72 ng/ml

Self-monitoring of blood glucose

Extremely useful for outpatient monitoring specially for patients who need tight control for their glycemic state. A portable battery operated device that measures the color intensity produced from adding a drop of blood to a glucose oxidase paper strip. e.g. One Touch, Accu-Chek, DEX, Prestige and Precision

Lab Practices:

-Collect blood for the test in an appropriate tube.

-Follow the method in the pamphlet.

-Compare the results to the normal value.

Principle of glucose measurement:

Glucose is determined after enzymatic oxidation by the enzyme glucose oxidase, the formed hydrogen peroxide reacts under the catalysis of peroxidase with phenol and 4-aminophenazone to give the red-violet quinoneimine dye as indicator.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{quinoneimine} + 4\,H_2O$$

The intensity of the color formed is proportional to the glucose concentration in the sample.

Procedure:

Wavelength: . . . . 500 nm
Cuvette: . . . . 1 cm light path
Temperature. .. . . 37ºC / 15-25ºC
Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette

Standard	Test	Reagents
	10µl	Serum
10 µl		Standard
1ml	1ml	Enz&dye reagent

Mix, incubate for 10 minutes at 20 - 25 C or for 5 minutes at 37C.
Read the absorbance (A) of the samples and standard, against the Blank. The color is stable for at least 30 minutes.

Calculation:

$$\text{Concentration of serum glucose} = \frac{\text{Absorption of sample}}{\text{Sta}} \times \text{Conc of}$$

Absorption Standard

Absorption of standard (mg /dl or mmol /L)

Concentration of Standard is 100 mg/dl or 5.5 mmol/L.

Remember ....Take control of your life ....Do not let Diabetes control you

Lab: 3 &4

LIPID PROFILE

What is lipid profile?

Lipid profile is a group of tests that are often ordered together to determine risk of coronary heart disease. It includes:

- Total cholesterol
- Triglycerides
- High density lipoprotein cholesterol (HDL-C) — often called good cholesterol
- Low density lipoprotein cholesterol (LDL-C) —often called bad cholesterol
- Very Low density lipoprotein (VLDL)

Estimated LDL = [total cholesterol] − [total HDL] − [estimated VLDL].

Estimated VLDL**=TG/5**

Cholesterol

About 20–25% of total daily cholesterol production occurs in the liver; other sites of high synthesis rates include the intestines, adrenal glands, and reproductive organs. Synthesis within the body starts with one molecule of acetyl CoA and one molecule of acetoacetyl-CoA. Since cholesterol is insoluble in blood, it is transported in the circulatory system within lipoproteins.

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)

Blood cholesterol levels and risk for heart disease:

Interpretation	Level mmol/L	Level mg/dL
Desirable level corresponding to lower risk for heart disease	< 5.0	< 200
Borderline high risk	5.2–6.2	200–240
High risk	> 6.2	> 240

However, as today's testing methods determine LDL ("bad") and HDL ("good") cholesterol separately, this simplistic view has become somewhat outdated. The desirable LDL level is considered to be less than 100 mg/dL.

Clinical significance

Hypercholesterolemia

Hypercholesterolemia is the presence of high levels of cholesterol in the blood. It is not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many forms of disease, most notably cardiovascular disease (CVD). Familial hypercholesterolemia is a rare genetic disorder that can occur in families, where sufferers cannot properly metabolized cholesterol.

abnormally high cholesterol levels (hypercholesterolemia); that is, higher concentrations of LDL and lower concentrations of functional HDL are strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack)(MI), stroke, and peripheral vascular disease.

Hypocholesterolemia:

Abnormally low levels of cholesterol are termed hypocholesterolemia. Research into the causes of this state is relatively limited, and while some studies suggest a link with depression, cancer and cerebral hemorrhage.

Lab practices:

-Collect blood for serum preparation in an appropriate tube.

-Run lipid profile tests.

-Compare the results to the normal values.

Estimation of serum total cholesterol

METHODE: Enzymatic- (Colorimetric End point).

Standard

Cholesterol 200 mg/dl

Reagent Preparation: Reagent provided is ready to use.

Stability of reagents:

To store 2-8°C and protected from light.

PROCEDURE

This reagent can be used on most analysers; Semi automated analysers and manual

Methods

The applications are available on request.

Wavelength : 510 nm

Temperature : 37°C

	BLANK	STANDARD	SAMPLE
Reagent R	1000µL	1000µL	1000µL
Standard	--------	10µL	--------
Sample	--------	-------	10µL

Mix and incubate for 5 min. at 37°C. Read the absorbance of STD. And sample

Against reagent blank.

CALCULATION

A Sample/A Standard × n n = standard concentration

Triglyceride

Triglyceride is a glyceride in which the glycerol is esterified with three fatty acids It is the main constituent of vegetable oil and animal fats.

Triglycerides, as major components of very low density lipoprotein (VLDL) and chylomicrons, play an important role in metabolism as energy sources and transporters of dietary fat.

Reference ranges for blood tests, showing usual ranges for triglycerides (increasing with age)

Interpretation	**Level** mmol/L	**Level** mg/dL
Normal range, low risk	<1.69	<150
Borderline high	1.70-2.25	150-199
High	2.26-5.65	200-499
Very high: high risk	>5.65	>500

Please note that this information is relevant to triglyceride levels as tested after fasting 8 to 12 hours. Triglyceride levels remain temporarily higher for a period of time after eating.

Causes Of *hypertriglyceridemia*

- Obesity
- High carbohydrate diet
- Diabetes mellitus.
- Excess alcohol intake
- Nephrotic syndrome
- Hypothyroidism (underactive thyroid)

Low triglyceride levels may be due to:

- Low fat diet
- Hyperthyroidism
- Malabsorption syndrome
- Malnutrition

Principle of triglyceride measurement;

Enzymatically; lipase hydrolyzes TG to glycerol and fatty acids. Glycerol is then phosphorylated in the following reaction:

glycerokinase

Glycerol + ATP ⟷ glycerophosphate + ADP

Glycerophosphate oxidase

Glycerophosphate + O2 + dihydroxyacetone ⟶ H_2O_2

H_2O_2 is measured in a peroxidase catalyzed reaction that forms a colored dye. And then we measure by spectrophotometer**.**

HYPERLIPIDEMIA: Is the condition of abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. It considers a heterogeneous group of disorders

- **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.

- **Hyperlipidemia is a major**, modifiable risk factor for atherosclerosis and cardiovascular disease, including coronary heart disease; this is true both of disorders involving hypercholesterolemia and hypertriglyceridemia.

Lab practices:

Collect blood for serum preparation in an appropriate tube.

-Run lipid profile tests.

-Compare the results to the normal values.

METHOD of Triglyceride: (Enzymatic-colorimetric end point method)

Principle: Triglycerides in the sample originate, by means of the coupled reactions described below, a coloured complex that can be measure by spectrophotometer.

Standard = 200 mg/dl

Stability of reagents

To store at 2-8°C and protected from light.

Samples

Serum or plasma from fasting patients.

Procedure:

This reagent can be used on most analysers, semi-automated analyzers and manual methods. The applications are available on request.

Wavelength : 500 nm
Temperature : 37°C
Read against reagent blank.

	BLANK	STANDARD	SAMPLE
Reagent R	1000µL	1000µL	1000µL
Standard	--------	10µL	-------
Sample	--------	-------	10µ

Mix and incubate for 5 min at 37°C. Read the absorbance's of std and sample.

CALCULATION

A Sample / A Standard× n n = Standard Concentration

Lipoproteins

The largest lipoproteins, which primarily transport fats from the intestinal mucosa to the liver, are called chylomicrons. They carry mostly fats in the form of triglycerides and cholesterol. In the liver, chylomicron particles release triglycerides and some cholesterol. The liver converts unburned food metabolites into very low density lipoproteins (VLDL) and secretes them into plasma where they are converted to intermediate density lipoproteins(IDL), which thereafter are converted to low-density lipoprotein (LDL) particles and non-esterified fatty acids, which can affect other body cells.

LDL molecules, therefore, are the major carriers of cholesterol in the blood, and each one contains approximately 1,500 molecules of cholesterol ester. These LDL molecules are oxidized and taken up by macrophages, which become engorged and

form foam cells. These cells often become trapped in the walls of blood vessels and contribute to atherosclerotic plaque formation. These plaques are the main causes of heart attacks, strokes, and other serious medical problems, leading to the association of so-called LDL cholesterol (actually a lipoprotein) with "bad" cholesterol.

LDL Cholesterol

Optimal: Less than 100 mg/dL (2.59 mmol/L)

Near/above optimal: 100-129 mg/dL (2.59-3.34 mmol/L)

Borderline high: 130-159 mg/dL (3.37-4.12 mmol/L)

High: 160-189 mg/dL (4.15-4.90 mmol/L)

Very high: Greater than 190 mg/dL (4.90 mmol/L)

HDL (high density lipoprotein)

High-density lipoprotein (HDL) particles transport cholesterol back to the liver for excretion, but vary considerably in their effectiveness for doing this having large numbers of large HDL particles correlates with better health outcomes, and hence it is commonly called "good cholesterol".

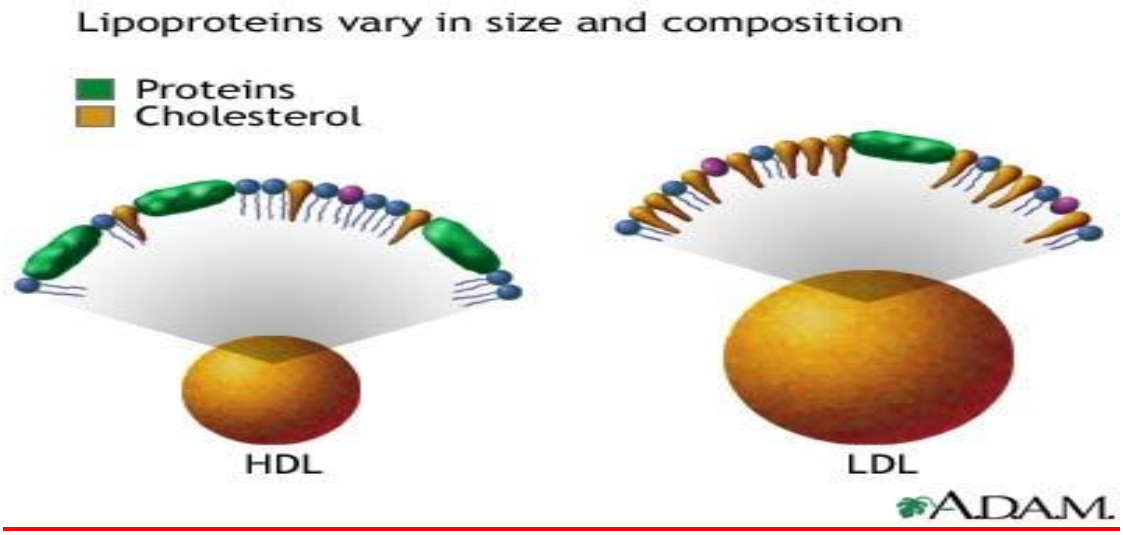
In contrast, having small amounts of large HDLparticles is independently associated with atheromatous disease progression within the arteries.

Recommended range:-

Interpretation	**Level** mmol/L	**Level** mg/dL
Low HDL cholesterol, heightened risk for heart disease	<1.03	<40 for men, <50 for women
Medium HDL level	1.03–1.55	40–59
High HDL level, optimal condition considered protective against heart disease	>1.55	>60

A low HDL level may also be associated with:

- Familial combined hyperlipidemia
- Noninsulin-dependent diabetes (NIDD)
- Use of certain drugs such as anabolic steroids, antipsychotics, beta blockers, corticosteroids, and protease inhibitors.



Calculation of LDL-cholesterol & VLDL cholesterol by Friedwald's formula:

LDL-c and VLDL-c can be calculated from the following formula:

LDL-c = TC – (HDL-c + VLDL-c)

VLDL-c = TG / 5

Risk factors include:

- Cigarette smoking.
- Age (if you are a male 45 years or older or a female 55 years or older)
- Low HDL cholesterol (less than 40 mg/dL (1.04 mmol/L))
- Hypertension (Blood Pressure of 140/90 or higher or taking high blood pressure medications)
- Family history of premature heart disease.
- Diabetes

Goals for Lipids

- LDL
  - < 100 →Optimal
  - 100-129 → Near optimal
  - 130-159 → Borderline
  - 160-189→ High
  - ≥ 190 → Very High
- Total Cholesterol
  - < 200 → Desirable
  - 200-239 → Borderline
  - ≥240 → High
- HDL
  - < 40 → Low
  - ≥ 60 → High
- Serum Triglycerides
  - < 150 → normal
  - 150-199 → Borderline
  - 200-499 → High
  - ≥ 500 → Very High

METHOD of HDL- Cholesterol (Enzymatic-colorimetric end point method)

Principle: This technique uses a speration method based on the selective precipitation of lipoproteins (VLDL , LDL , HDL, ..) by phosphotungstic acid/$MgCl_2$, sedimentation of the precipitant by centrifugation , and subsequent enzymatic analysis of HDL as residual cholesterol remaining in the clear supernatant.

Standard = 50 mg/dl

Stability of reagents

To store at 2-8°C and protected from light.

Samples

Serum or plasma obtained from the patient after an overnight fast.

Procedure:

This reagent can be used on most analysers, semi-automated analyzers and manual methods.

Wavelength : 500 nm
Temperature : 37°C

Sample or standard **Precipitating reagent**	**0.2 ml** **0.4 ml**

-Vortex and allow to stand for 10 minutes at room temperature.
-Centrifuge for 10 minutes at 4000 r.p.m.
- Separate off the clear supernatant within 2 hours.

Tubes	Blank	Sample supernatant	Standard supernatant
Monoreagent	**1.0 ml**	**1.0 ml**	**1.0 ml**
Supernatant	**-**	**50 µl**	
Standard	**-**	**-**	**50 µl**

-Mix and let the tubes to stand 10 minutes at room temperature or 5 minutes at 37°C

- Read the absorbance (A) of the supernatant and standard at 500 nm.

CALCULATION

A Sample / A Standard× n s n = Standard Concentration

THE END

LAB : 5

(RENAL FUNCTION TESTS)

Renal function, in nephrology, is an indication of the state of the kidney and its role in renal physiology. Glomerular filtration rate (GFR) describes the flow rate of filtered fluid through the kidney. Creatinine clearance rate (C_{Cr} or CrCl) is the volume of blood plasma that is cleared of creatinine per unit time and is a useful measure for approximating the GFR. Both GFR and C_{Cr} may be accurately calculated by comparative measurements of substances in the blood and urine.

Kidney function tests is a number of clinical laboratory tests that measure the levels of substances normally regulated by the kidneys can help determine the cause and extent of kidney dysfunction. These tests are done on urine samples, as well as on blood samples.

Physiology:

The liver produces urea in the urea cycle as a waste product of the digestion of protein. Normal human adult blood should contain between (15-40) mg/dl.
The most common cause of an elevated B.Urea(azotemia) is poor kidney function, although a serum creatinine level is a somewhat more specific measure of renal function .A greatly elevated BUN (>60 mg/dL) generally indicates a moderate-to-severe degree of renal failure. Impaired renal excretion of urea may be due to temporary conditions such as dehydration or shock, or may be due to either acute or chronic disease of the kidneys themselves.

The blood urea nitrogen (BUN) test is a measure of the amount of nitrogen in the blood in the form of urea, and a measurement of renal function. Urea is a substance secreted by the liver, and removed from the blood by the kidneys

A high BUN value can mean:

1-kidney disease

2-Blockage of the urinary tract (by a kidney stone or tumor)

3- Low blood flow to the kidneys caused by dehydration or heart failure.

4-Many medicines may cause a high BUN.

5-A high BUN value may be caused by a high-protein diet, tissue damage (such as from severe burns), or from bleeding in the gastrointestinal tract.

A low BUN usually has little significance:

1) Liver problems.
2) Malnutrition (insufficient dietary protein).
3) Excessive alcohol consumption.
4) Over hydration .
5) During pregnancy.

Kidney functions :

-Regulation of water and electrolyte balance.

-Regulation of acid base balance.

-Regulation of arterial blood pressure.

- Excretion of metabolic waste products and foreign chemicals.

-Metabolic Function : site for gluconeogenesis.

Renal diseases :

- Many diseases affect renal function.
- In some, several functions are affected.
- In others, there is selective impairment of glomerular function or one or more tubular function .

Signs and Symptoms of Renal Failure

- Symptoms of Uraemia (nausea, vomiting, lethargy)

- Disorders of Micturition (frequency, nocturia, dysuria)
- Disorders of Urine volume (polyuria, oliguria, anuria)
- Alterations in urine composition (haematuria, proteinuria, bacteriuria, leukocytouria, calculi)
- Pain
- Oedema (hypoalbuminaemia, salt and water retention)

Why Tests of Renal Function are Important?

- To identify renal dysfunction.
- To diagnose renal disease.
- To monitor disease progress.
- To monitor response to treatment.
- To assess changes in function that may impact on therapy (e.g. Digoxin, chemotherapy).

Blood tests:

There are several blood tests that can aid in evaluating kidney function. These include:

1-Blood urea nitrogen test (BUN).

2-Creatinine test.

3-Measurement of the blood levels of other elements regulated in part by the kidneys can also be useful in evaluating kidney function. These include sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, protein, uric acid, and glucose.

1- Blood Urea Nitrogen (BUN)

Urea is the characteristic and most abundant nitrogenous end product of protein catabolism in mammals. It is generated by the liver and excreted by the kidney. Urea filters easily through the glomerulus into the ultra-filtrate. It will diffuse passively into the blood as it passes down the renal tubules. Under conditions of normal flow

and normal renal function, about 40% of the filtered urea is reabsorbed; when the flow rate is decreased, the amount passively reabsorbed increases. As with creatinine, the serum concentration of urea nitrogen rises with impaired renal function.
The serum concentration of urea nitrogen is influenced by factors not connected with renal function or urine excretion as it is affected strongly by the degree of protein catabolism. A marked change in dietary protein consumption will be reflected in BUN values. The injection or ingestion of steroids produces a rise in BUN as do stressful situations that cause the adrenal gland to secrete additional cortisol. For these reasons, the measurement of serum creatinine is a better indicator of kidney status than is that of BUN although in many cases, they go up and down simultaneously. The various **prerenal**, **renal**, and **postrenal** factors that affect creatinine also influence the BUN.

Clinical Significance

The BUN test measures the amount of nitrogen contained in the urea. High BUN levels can indicate kidney dysfunction, but because blood urea nitrogen is also affected by protein intake and liver function, the test is usually done in conjunction with a blood creatinine, a more specific indicator of kidney function.

Expected Values

Normal range in serum or plasma of BUN (20 – 45 mg/dL)

BUN / Creatinine Ratio

Normal BUN / Creatinine ratio is 12 – 20 to 1

2-Serum Creatinine

Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass).

Chemically, creatinine is a spontaneously formed cyclic derivative of creatine. Creatinine is chiefly filtered out of the blood by the kidneys. 98% of the body creatine

is present in the muscles where it functions as store of high energy in the form of creatine phosphate.

About 1-2 % of total muscle creatine or creatine phosphate pool is converted daily to creatinine through the spontaneous, non enzymatic loss of water or phosphate.

Serum creatinine is a better indicator of renal function than either that of BUN or uric acid because is not reabsorbed by the renal tubules , creatinine is an endogenous substance not affected by diet and Plasma creatinine remains fairly constant throughout adult life.

Expected Results

Males = 0.6 – 1.4 mg /dl
Females = 0.5 – 1.2 mg /dl

- **Clearance** : Volume of plasma from which a measured amount of substance can be completely eliminated into urine per unit of time expressed in milliliters per minute.
- Function: Estimate the rate of glomerular filtration

Creatinine Clearance:

Used to **estimate** GFR (glomerular filtration rate) .Most sensitive measure of kidney function .Mathematical derivation taking into effect the serum creatinine concentration to the urine creatinine concentration over a 24- hour period.

- **Reference range**:
- **Male 97 - 137 mL/min**
  Female 8 -128 ml/min

What causes kidney failure?

Kidney failure can occur from an acute situation or from chronic problems.

In acute renal failure, kidney function is lost rapidly and can occur from a variety of insults to the body. The list of causes is often categorized based on where the injury has occurred.

Prerenal causes are due to decreased blood supply to the kidney. Examples of prerenal causes are:

- Hypovolemia (low blood volume) due to blood loss
- Dehydration from loss of body fluid (vomiting, diarrhea, sweating, fever
- Poor intake of fluids
- Medication, for example, diuretics ("water pills") may cause excessive water loss.
  - Increased Ratio- BUN is high/ creatinine is normal

Renal causes (damage directly to the kidney itself) include:

- Multiple Myeloma
- Acute glomerulonephritis or inflammation of the glomeruli, the filtering system of the kidneys. Many diseases can cause this inflammation.
- Systemic diseases (SLE-rheum. Arthritis)
  - Normal Ratio- both BUN and creatinine are **proportionally** elevated

Post renal causes are due to factors that affect outflow of the urine:

- Obstruction of the bladder or the ureters can cause back pressure when there is no place for the urine to go as the kidneys continue to work. When the pressure increases enough, the kidneys shut down.
- Prostatic hypertrophy or prostate cancer may block the urethra and prevents the bladder from emptying.
- Tumors in the abdomen that surround and obstruct the ureters.
- Kidney stones

  -Increased Ratio- BUN is high and creatinine also elevated.

Chronic renal failure develops over months and years. The most common causes of chronic renal failure are related to:

- Poorly controlled diabetes
- Poorly controlled high blood pressure
- Chronic glomerulonephritis

BUN: Methodology

— -**Kjeldahl** – a classical method for determining urea concentration by measuring the amount of nitrogen present

— -**Berthelot reaction** - Good manual method - that measures ammonia uses an enzyme (urease) to split off the ammonia

—-**Diacetyl monoxide** (or monoxime)

Popular method but not well suited for manual methods. **Because** uses strong acids and oxidizing chemicals

Determination of BUN

Principle

Determination of urea is by the indirect method using the urease-modified Berthelot reaction. Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide as in the following equation:

Urea + H_2O $\xrightarrow{\text{urease}}$ **2 NH_3 + CO_2**

In an alkaline medium, the ammonium ions react with the salicylate and hypochlorite to form a green colored dye the absorbance of which is proportional to the urea concentration in the sample.

Procedure:

Working reagent: Mix 1 volume of R1 with 24 volume of R2. The working reagent is stable for 30 days at 2-8 °C

Working reagent	Sample	standard
1 ml	**10 µl**	**-----**
1 ml	**-----**	**10 µl**

Mix and incubate at 37 °C for 5 minutes. Then add

R3	**1ml**

Mix and incubate at 37 °C for 5 minutes. Measure the absorbance For the sample and standard at 600 nm.

Calculation:

Concentration of BUN = Absorption of sample / Absorption Standard × Conc of Stan

Concentration of Standard is 50 mg/dl

Determination of Creatinine

Kinetic method (Modified Jaffe reaction):

Principle: Creatinine in protein-free filtrate of serum or diluted urine in alkaline solution reacts with picric acid to form a red-orange chromogen the absorbance of which is proportional to the creatinine concentration in the sample.

Procedure:

Working reagent: Mix 1 volume of R1 with1 volume of R2. The working reagent is stable for 30 days at 2-8 °C.

Working reagent	Sample	standard
1 ml	100 µl	-----
1 ml	-----	100 µl

Mix gently. Then insert the test tube in to the instrument and start stop watch. Read the absorbance after 30 sec (A1) and after 90 sec (A2). Read at 510 nm.

Calculation

(A2 – A1) sample / (A2 – A1) standard × Conc of Stan

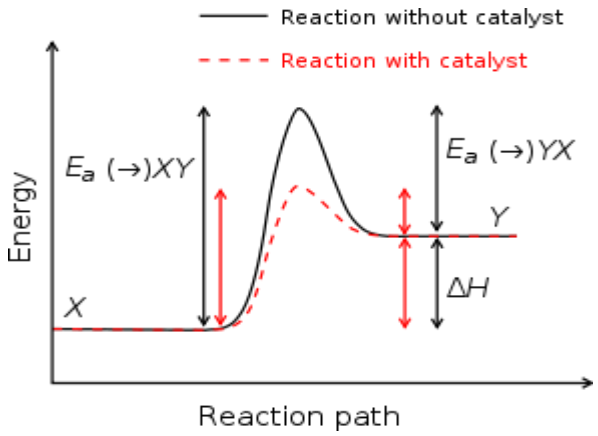
Concentration of Standard is 2 mg/dl

Enzyme specificity

Introduction - Enzyme Characteristics

A living system controls its activity through enzymes. An enzyme is a protein molecule that is a biological catalyst with three characteristics. First, the basic function of an enzyme is to increase the rate of a reaction. Most cellular reactions occur about a million times faster than they would in the absence of an enzyme. Second, most enzymes act specifically with only one reactant (called a substrate) to produce products. The third and most remarkable characteristic is that enzymes are regulated from a state of low activity to high activity and vice versa. Gradually, you will appreciate that the individuality of a living cell is due in large part to the unique set of some 3,000 enzymes that it is genetically programmed to produce. If even one enzyme is missing or defective, the results can be disastrous.

Catalysts: A catalyst is a substance that accelerates the rate of a chemical reaction but remains chemically unchanged afterwards. The catalyst increases rate reaction**.**



The presence of the catalyst opens a different reaction pathway (shown in red) with a lower activation energy. The final result and the overall thermodynamics are the same.

Enzyme Parts List:

The activity of an enzyme depends, at the minimum, on a specific protein chain. In many cases, the enzyme consists of the protein and a combination of one or more parts called cofactors. This enzyme complex is usually simply referred to simply as the enzyme includes:

- Apoenzyme:
- Cofactors: A cofactor is a non-protein substance which may be organic, and called a coenzyme.

The overall enzyme contains a specific geometric shape called the active site where the reaction takes place. The molecule acted upon is called the substrate

Urease occurs in many bacteria, several species of yeast and a number of higher plants. The enzyme is important in assaying for urea.

Specificity: Urease is specific for urea and hydroxyurea.

Factor Effect of Enzyme

(1) Enzyme Concentration

In order to study the effect of increasing the enzyme concentration upon the reaction rate, the substrate must be present in an excess amount;

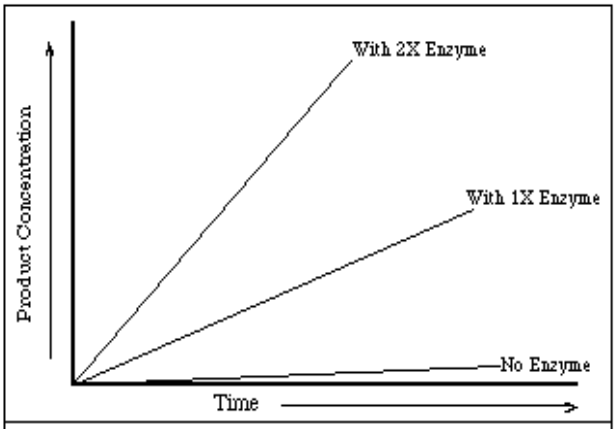


FIGURE 4
"Zero order" reaction rate is independent of substrate concentration.

These reactions are said to be "zero order" because the rates are independent of substrate concentration, and are equal to some constant k.

The formation of product proceeds at a rate which is linear with time. The addition of more substrate does not serve to increase the rate. In zero order kinetics, allowing the assay to run for double time results in double the amount of product.

Procedure

We take 4 test tubes and add the same amount of substrate for the first 3 test tubes but change the amount of enzyme leads to change of color

As follow:

1- Add (1) drop of phenol oxidase enz.(potato extract) + 14 drops of D.W. in order to complete the volume to 1 .5 ml.
2 - Add (3) drops of potato extract + 12 drops of D.W.

3 - Add (7) drops of potato extract +8 drops of D.W.

4 - Add (14) drops of potato extract

Then add 2 drops of 0 .01 M of catechol at 37C and wait for 5 minutes in water bath , the following changes can be noticed :

1 st yellow

2 nd. Light brown

3 rd. brown

4 th dark brown

So we can say when increasing the amount of enzyme, the rate of reaction is increasing also.

(2) Substrate Concentration

It has been shown experimentally that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity (delta A/delta T). This is represented graphically in Figure 2.

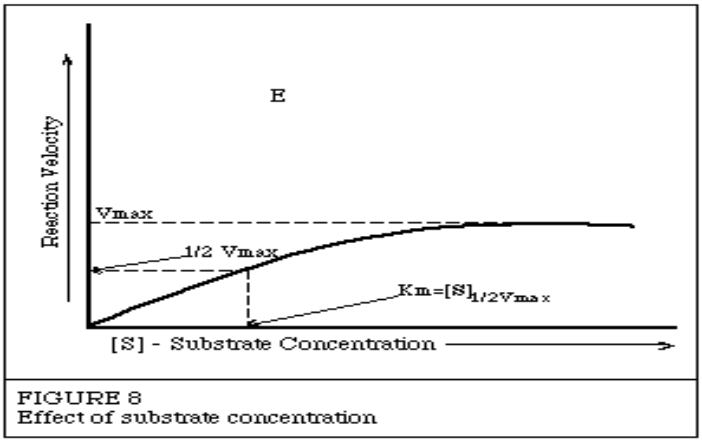


FIGURE 8
Effect of substrate concentration

Procedure:

- 0.5 ml of 0 .01M of catechol solution + 2.5 ml of D.W. in order to Complete the volume to 3 ml
- 1ml of 0 .01M of catechol solution + 2ml of D.W.
- 2ml of 0 .01M of catechol solution +1 ml of D.W.
- 3 ml of 0 .01M of catechol solution

Then add 0.5 ml of potato extract and put the test tubes at 37C and Wait for 5 minutes in the water bath, the following changes can be Noticed:

1 st . Very light brown

2 nd . light brown

3 rd . brown

4 th . Dark brown

So the activity increased as the amount of substrate is increased until We reach to the maximal velocity (V_{max}) at which any increase because there is no enzyme is available to react.

(3) Temperature Effects

Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised. A ten degree Centigrade rise in temperature will increase the activity of most enzymes by 50 to 100%. Variations in reaction temperature as small as 1 or 2 degrees may introduce changes of 10 to 20% in the results. In the case of enzymatic reactions, this is complicated by the fact that many enzymes are adversely affected by high temperatures. As shown in Figure 13, the reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature. Because most animal enzymes rapidly become denatured at temperatures above 40°C, most enzyme determinations are carried out somewhat below that temperature.

Over a period of time, enzymes will be deactivated at even moderate temperatures. Storage of enzymes at 5°C or below is generally the most suitable. Some enzymes lose their activity when frozen.

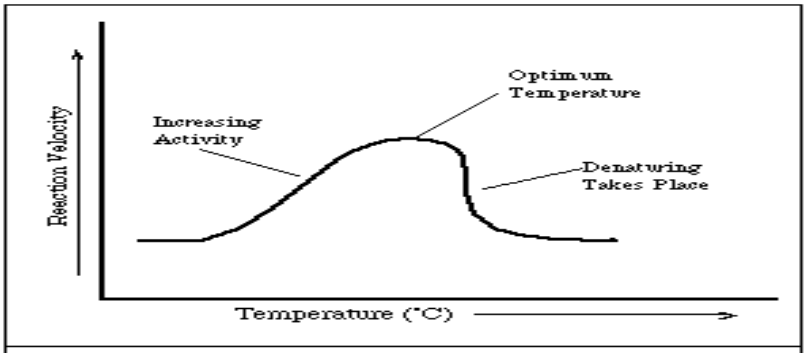


FIGURE 13
Effect of temperature on reaction rate

(4) Effects of pH

Enzymes are affected by changes in pH. The most favorable pH value - the point where the enzyme is most active - is known as the optimum pH. This is graphically illustrated in Figure.

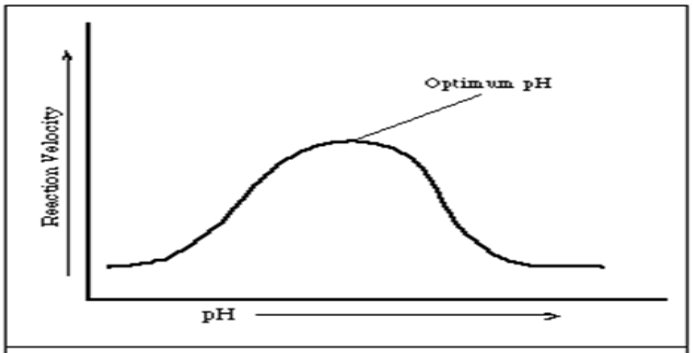


FIGURE 14
Effect of pH on reaction rate

Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes. As with activity, for each enzyme there is also a region of pH optimal stability.

MECHANISM OF ACTION OF ENZYMES

Enzymes increase reaction rates by decreasing the Activation energy:

Formation of Enzyme substrate complex by:

- Lock-and-Key Model
- Induced Fit Model

In the lock-and-key model of enzyme action:

- the active site has a rigid shape.

- only substrates with the matching shape can fit

- the substrate is a key that fits the lock of the active site, this is an older model, however, and does not work for all enzyme.

In the induced-fit model of enzyme action:

- the active site is flexible, not rigid , the shape of the enzyme, active site, and substrate adjust to maximumize the fit, which improves catalysis

- there is a greater range of substrate specificity ,this model is more consistent with a wider range of enzymes

Liver function tests LFTs

Liver function tests (LFTs or LFs), are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver. The parameters measured include albumin, bilirubin (direct and indirect) and others. According to some, liver transaminases (AST/ALT (SGOT/SGPT) are not liver function tests, but are

biomarkers of liver injury in a patient with some degree of intact liver function. Other sources include transaminases. Most liver diseases cause only mild symptoms initially, but it

is vital that these diseases be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. This testing is performed by a medical technologist on a patient's serum or plasma sample Some tests are associated with functionality (e.g., albumin); some with cellular integrity (e.g., transaminase) and some with conditions linked to the biliary tract (gamma-glutamyl transferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to (1) detect the presence of liver disease, (2) distinguish among different types of liver disorders, (3) gauge the extent of known liver damage, and (4) follow the response to treatment.

Some or all of these measurements are also carried out (usually about twice a year for routine cases) on those individuals taking certain medications- anticonvulsants are a notable example- in order to ensure that the medications are not damaging the person's liver.

Classification of LFTs

Group I: Markers of liver dysfunction:

- Serum bilirubin: total and conjugated
- Urine: bile salts and urobilinogen
- Total protein, serum albumin and albumin/globulin ratio
- Prothrombin Time

Group II: Markers of hepatocellular injury:

- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)

Group III: Markers of cholestasis

- Alkaline phosphatase (ALP)
- g-glutamyltransferase (GGT)

Alanine transaminase (ALT)

(ALT), also called serum glutamic pyruvate transaminase Alanine transaminase (SGPT) or alanine aminotransferase (ALAT) is an enzyme present in hepatocytes (liver cells).

Reference range :

Up to 40 U/L

Aspartate transaminase (AST)

(AST) also called serum glutamic oxaloacetic transaminase Aspartate transaminase (SGOT) or aspartate aminotransferase (ASAT) is similar to ALT in that it is another enzyme associated with liver parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells and cardiac and skeletal muscle and is therefore not specific to the liver. The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage. Elevated AST levels are not specific for liver damage, and AST has also been used as a cardiac marker.

Reference range : Up to 30 U/L

Alkaline phosphatase(ALP)

(ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP Alkaline phosphatase levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. ALP is also present in bone and placental tissue, so it is higher in growing children (as their bones are being remodeled) and elderly patients with Paget's disease.

In humans, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone, and the placenta. Humans and most other mammals contain the following alkaline phosphatase isozymes:

- ALPI – intestinal
- ALPL – tissue non-specific (liver/bone/kidney)
- ALPP – placental (Regan isozyme)

Diagnostic use

The normal range is 20 to 140 IU/L. High ALP levels can show that the bile ducts are blocked Levels are significantly higher in children and pregnant women. Also, elevated ALP indicates that there could be active bone formation occurring as ALP is a byproduct of osteoblast activity (such as the case in Paget's disease of bone). Levels are also elevated in people with untreated Celiac Disease. Lowered levels of ALP are less common than elevated levels.

Elevated levels

If it is unclear why alkaline phosphatase is elevated, isoenzyme studies using electrophoresis can confirm the source of the ALP. Heat stability also distinguishes bone and liver isoenzymes ("bone burns, liver lasts"). Placental alkaline phosphatase is elevated inseminomas and active form of Rickets.

Lowered levels

The following conditions or diseases may lead to reduced levels of alkaline phosphatase:

- Hypophosphatasia, an autosomal recessive disease.
- Postmenopausal women receiving estrogen therapy because of osteoporosis.
- Men with recent heart surgery, malnutrition, magnesium deficiency, hypothyroidism, or severe anemia.
- Wilson's disease.
- Oral contraceptives.

Gamma glutamyl transferase **(GGT)**

Although reasonably specific to the liver and a more sensitive marker for cholestasis damage than ALP, Gamma glutamyl trans peptidase (GGT) may be elevated with even minor, sub-clinical levels of liver dysfunction. It can also be helpful in identifying the cause of an isolated elevation in ALP (GGT is raised in chronic alcohol toxicity.

Reference range

(0 – 42) U/L

Determination of Liver function

Procedure:

General system parameter

	GPT	**GOT**
Mode of reaction	Kinetic	Kinetic
Wavelength	340 nm	340 nm
Factor	1745	1745
Reagent volume	1 ml	1m l

Working reagent: Mix 4 volume of R1 with the volume of R2. The working reagent is stable for 30 days at 2-8 °C.

Sample : serum or plasma (Free hemolysis)

Working reagent	Sample	Standard
1000	100 µl	-----
1000	-----	100 µl

Mix and incubate at 37 °C for 1 minute. Measure the change in absorbance per minute (Δ OD/ min) during (60, 120 and 180) Sec.

Calculation:

GPT, GOT = (Δ OD/ Sec) × 1745