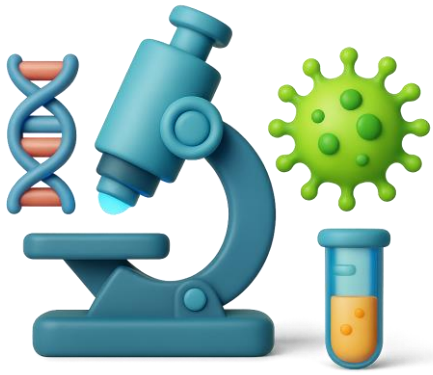
AL-Zahraa University for Women – College of Pharmacy

Manual for the practical part of:



Clinical Chemistry

5nd Stage

2024-2025

Al-Zahraa University for Women
College of Pharmacy

Scientific Manual Evaluation Certificate (Practical Part)

Based on a thorough and comprehensive evaluation of the content of the scientific manual for the practical part of **"Clinical Chemistry"**

After a comprehensive review, that the manual meets the scientific and academic standards approved by the College of Pharmacy. We confirm that the manual meets the required standards.

Signature:

Head of Department

Signature:

Prof. Dr Noor Hatef Aldabagh
Dean of the College of Pharmacy

Lab:1

1. Specimen collection and preservation

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

1.1. 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.

1.2. 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)

1.2.1 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.

1.2.2 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.

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

Urine has a long, rich history as a source for measuring health and well-being and remains an important tool for clinical diagnosis.

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.

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.

1.4 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

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.

1.5 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+, Ca2+, 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:

- Hemolysis: 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.

1.6 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 monochromatic.

Lab:2

1. Estimation of blood glucose (enzymatic method)

Normal values

Fasting	70-99 mg/dL (3.9-5.5 mmol/L)
Post-prandial	>140 mg/dL (7.8 mmol/L)

- Glucose is the major carbohydrate present in blood. Its oxidation
- in cells is the source of energy for the body.
- Insulin facilitates glucose entry into the cells.
- Increased levels of glucose (hyperglycemia): are found in
- diabetes mellitus, hyperparathyroidism, pancreatitis, renal failure.
- Decreased levels (hypoglycemia): are found in insulinoma, hypothyroidism, hypopituitarism and extensive liver disease.

Diabetes mellitus can be classified into the following general categories:

- **Type 1 diabetes mellitus (Insulin dependent diabetes mellitus (IDDM):** which characterized by absolute insulin deficiency (due to auto immune beta-cell destruction which occur by contribution of one or more environmental factors).
- **Type 2 diabetes mellitus (Non-insulin dependent diabetes mellitus (NIDDM)**: is a complex disorder of glucose metabolism, which results from combination of peripheral insulin resistance and impaired insulin secretion, and accounts for most cases of diabetes mellitus worldwide.

- **Gestational diabetes mellitus (GDM)** (diabetes diagnosed in the second

or third trimester of pregnancy).

- **Specific types of diabetes due to other causes,** (such as neonatal diabetes and maturity-onset diabetes of the young (MODY).
- **Latent autoimmune diabetes in adults (LADA).**

1.2 Principle

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

$$H_2O_2 + \text{Phenol} + \text{4-AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + H_2O$$

Stability and preparation of reagents

R1a. Buffer

Contents ready for use. Stable up to the expiry date when stored at +2 to +8C.

R1b. GOD-PAP Reagent

Reconstitute the contents of one vial of Reagent R1b with a portion of Buffer R1a and then transfer entire contents to bottle R1a, rinsing bottle R1b several times. The working reagent is stable for 3 months at +2 to +8C or 5 days at +15 to +25C.

CAL. Standard

Contents ready for use. Stable up to the expiry date at +2 to +8C

1.3 Procedure

Samples: Venous blood (serum or plasma, free from hemolysis).

*Individuals should be fasting (8 hours) before sample collection

•Use anticoagulants: Heparin, EDTA, and flouride are the only accepted anticoagulants

Stability of the sample: 3 days at 2-8ºC.

1.Assay conditions:

Wavelength: (492-550) nm

Cuvette: 1 cm. light path

Temperature 37ºC or 20-25ºC

2. Adjust the instrument to zero with reagent blank.

3. Pipette into a cuvette:

	Blank	Standard	Sample
Reagents	**1000 µl**	**1000 µl**	**1000 µl**
Standard	**-**	**20 µl**	**-**
Sample	**-**	**-**	**20 µl**

Mix, incubate for 25 min at 15 - 25C or 10 min at 37C. Measure the absorbance of the standard (A standard) and the sample (A sample) against the reagent blank within 60 minutes.

1.4 Calculation

$$\text{Glucose concentration (mmol/l)} = \frac{\text{A sample}}{\text{A standard}} \times 100$$

Wavelength: 500 nm, Hg 546 nm

Cuvette: 1 cm path length

Temperature: 15 - 25C or 37C

Measurement: against reagent blank

Lab:3

1. Oral glucose tolerance test (OGTT)

Normal Values

Fasting: **Adult** **Child**	 **3.9 -6.1mmol / L or 70 -110 mg / dL** **< 7.2 mmol / L or < 130 mg / dL**
30 min **Adult**	6.1 – 9.4 mmol / L or 110 -170 mg / dL
60 min (1hour) **Adult** **Child**	 6.7 – 9.4 mmol / L or 120 – 170 mg / dL < 7.8 mmol /l or < 140 mg / dL .
120 min (2. Hour)	3.9 – 6.7 mmol / L or 70 -120 mg /dL
3 hours	3.9 – 6.7 mmol / L or 70 – 120 mg / dL

1.1 Sample collection

- Collect fasting blood and urine specimens
- Collect approximately 5 ml of venous blood at 30 min. and at hourly periods
- Collect urine specimens at hourly periods
- Mark the tubes with the time that the specimens are collected.

1.2 Explanation of the Test:

If fasting and postprandial glucose test results are borderline, the GTT can support or rule out a diagnosis of diabetes mellitus, it can also be part of

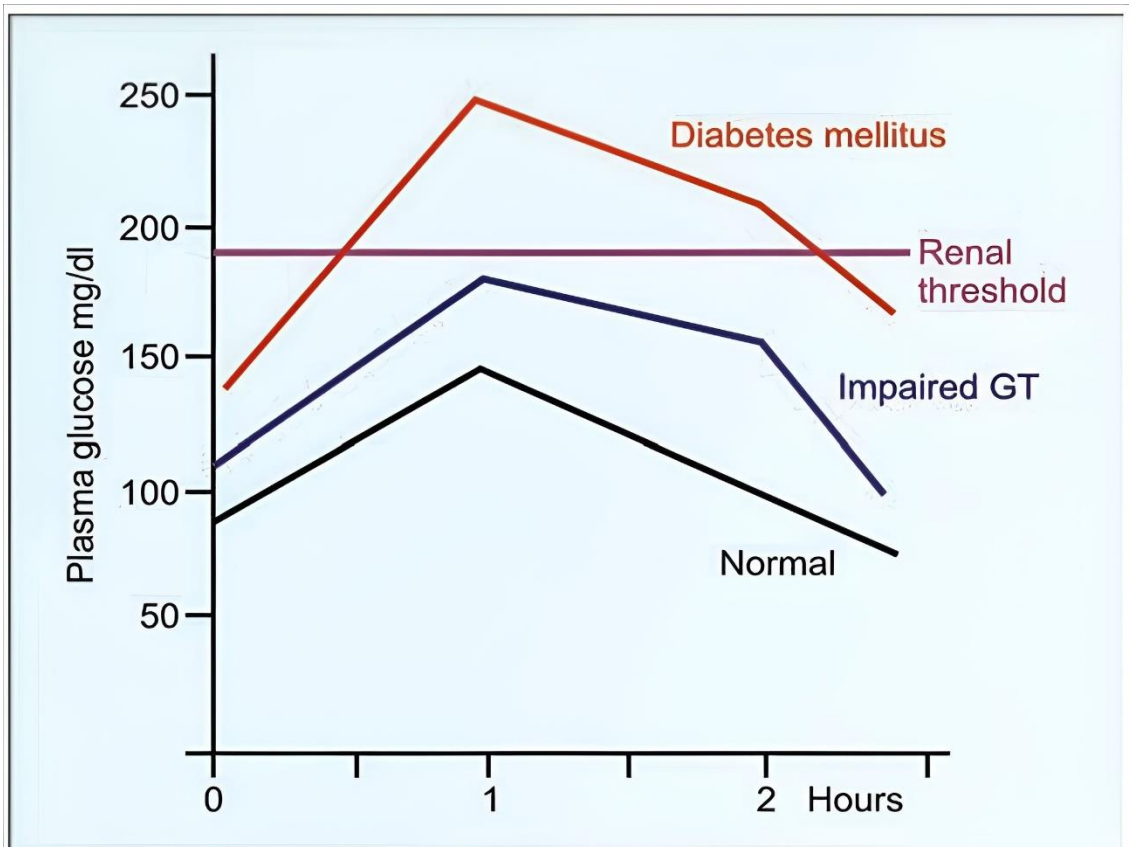
workup for un explained hypertriglyceridemia, neuropathy, renal diseases, or retinopathy. This test may be ordered when there is glucose in urine or when the fasting blood glucose level or 2 – hour postprandial blood sugar level is significantly elevated. GTT is also suggested for the following conditions:

1- Patients with a family history of diabetes
2- Patient who are massively obese
3- Patients with a history of recurrent infections
4- Patient with delayed healing of wounds (especially on the lower legs or feet)
5- Women who have a history of delivering large babies
6- Patients who have transient glycosuria or hyperglycemia during pregnancy or following myocardial infraction, surgery, or stress

1.3 Test Performance

In the GTT, the patient's ability to tolerate a standard oral glucose load is evaluated by obtaining serum and urine specimen for glucose level determination before glucose load administration and then at 30 minutes, 1 hour, 2 hours, 3 hours, and sometime 4 hours after wards. Normally, there is a rapid insulin response to the ingestion of large oral glucose load. peak response occurs in 30-60 min and returns to normal in about 3 hours.

Normally, subjects with appropriate insulin response are able to tolerate the glucose load quite easily, with only a minimal and transient rise in serum glucose levels within 1 -2 hours after ingestion and glucose does not spill over into the urine.



1.5 Indications for OGTT

- Patient has symptoms suggestive of diabetes mellitus; but fasting blood sugar value is inconclusive (between 100 and 126 mg/dl).
- During pregnancy, excessive weight gaining is noticed, with a past history of big baby (more than 4 kg) or a past history of miscarriage.
- To rule out benign renal glycosuria

1.6 Potential complications

1. Dizziness, tremors, anxiety, sweating, fainting during testing.
2. If these symptoms occur, a blood specimen is obtained, if the glucose

levels too high, stop the test and start the administration of insulin.

3. Patients with concurrent infections or endocrine disorders, because glucose intolerance will be observed even though these patients may not have diabetes.

1.6 Interfering factors

1. Smoking during test stimulates glucose production because of Nicotine
2. stress (eg. from surgery, infection) can increase glucose levels
3. Exercise during the test can affect glucose levels
4. Reduced caloric intake before GTT can cause glucose intolerance.
5. Drugs that may cause glucose intolerance include antihypertensive anti-inflammatory drugs; aspirin, B- blockers, furosemide, nicotine oral contraceptives, psychiatric drugs, steroids and thiazide diuretics
6. If the patient vomits the glucose solution, the test is declared invalid, it can be repeated in 3 days.

1.7 Patients Preparation for GGT

1. Patient should have a high carbohydrates diet (150 gm) for 3days preceding the test. Instruct the patient to abstain from alcohol
2. Patient should fast at least 12 h but not more than 16 hours before the test. Only water may be ingested during the fasting time and test time. (smoking is not permitted during testing).
3. Patient: should rest or walk quietly during the test period. They may feel weak, faint or nauseated during the test. Vigorous exercise should be avoided during testing.
4. Collect blood specimens at the prescribed times (at 30 min. interval and

record exact times collected and urine specimens at hourly periods.

5. Record the patient's weight.
6. This is a timed test. A 2-hour test done for detecting diabetes in individuals other than pregnant women, the 3-hour test done for pregnant women and the 5-hour test evaluates possible hypoglycemia.
7. For IV-GTT administer glucose load intravenously over 3-4 minutes

1.8 glucose load

- Pediatric doses of glucose are based on body weight calculate as 1.75 gm/kg of body weight up to 75 gm.
- Pregnant women: 100 gm glucose.
- Non-pregnant adult: 75 gm glucose.

Lab: 4

1. Determination of blood urea nitrogen

Normal values

Adult:	**2.5-6.4 mmol/L or 7-18 mg/dl**
Elderly (>60 yr.)	**2.9- 7.5 mmol/l or 820mg/dl**
Child	**1.8-6.4 mmoll ot 5-18 mg/ dl)s**

Critical values: > 36 mmol/L (>100 mg/dl) indicates serious impairment of renal function.

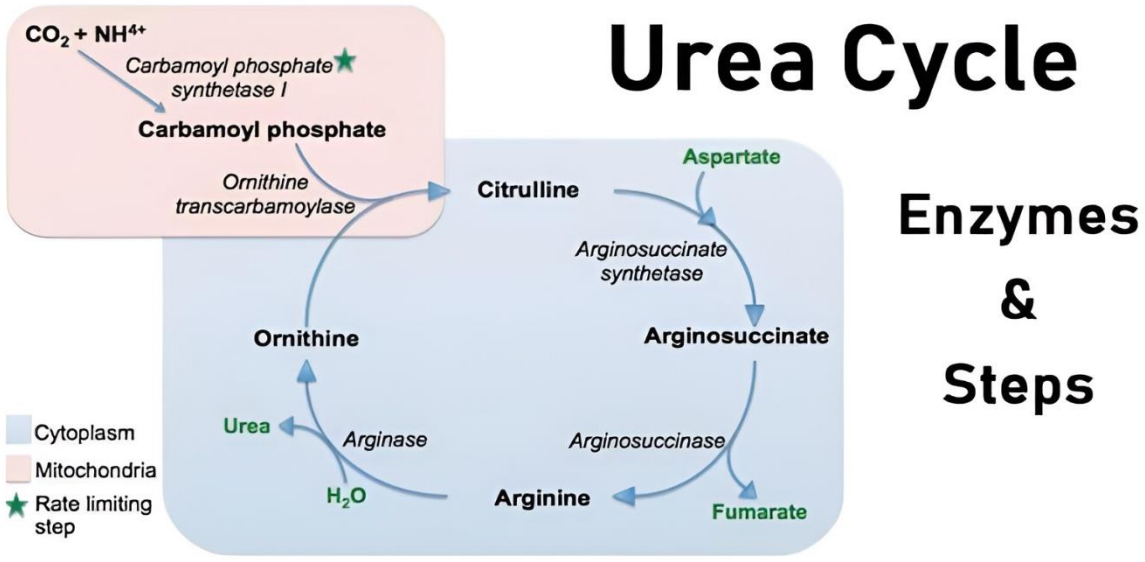
Urea is the major end product of protein nitrogen metabolism It is synthesized by the urea cycle in the liver and excreted through the kidneys The circulating levels of urea depend upon protein intake, protein catabolism and kidney function Elevated urea levels can occur due to renal impairment or in some diseases such as diabetes, infections, congestive heart failure and during different liver diseases. Determination of blood urea nitrogen is the most widely used screening test for renal function together with serum creatinine.

1.1 Interfering Factors

1. Changes in protein intake may affect BUN levels.
2. Advanced pregnancy may cause increased levels.
3. Over hydration and dehydration will affect levels.

4. Drugs that may cause increased BUN levels include: Allopurinol, aminoglycosides, cephalosporines, chloralhydrate, Furosemide. guanethidine, indomethacin, methotrexate, methyldopa, nephrotoxic drugs (eg, amphotericin B, Bacitracin, carbamazepine, gentamicin, neomycine, pencillamine, polymyxin B, aspirin, probencid, vancomycin), propranolol, rifampin, spiranolactone, tetracyclines, thiazide diuretics and triameterne.

Drugs that may cause decrease BUN levels include chloramphenicol and streptomycin.



1.2 Clinical significance

Normal renal function depends on a normal filtration rate normal tubular function and normal blood supply A low GFR glomerular filtration rate lead to uremia.

1.2.1 Causes lead to increased serum urea concentration

1- Increase production High protein diet increase catabolism of cellular protein of the body as in fever and infection

2-Decrease GFR may be due to

A. Reduction of blood volume, which could be associated with gastrointestinal loss, reduced intake and dehydration
B. Reduced pressure due to shock as a result for example MI or intravascular hemolysis

3- Post renal uremia as a result for example renal calculi or kidney diseases.

1.2.2 Causes lead to decreased serum urea concentration

1-Malnutrition

2-Very sever liver disease

3-Inborn error of urea cycle

4-Inappropriate ADH secretion

1.3 Principle

Urea is hydrolyzed by urease forming ammonia and carbamic acid. Carbamic acid spontaneously decomposes into ammonia and carbon dioxide. The released ammonium, in the presence of salicylate and nitroferricyanide reacts in alkaline solution of sodium hypochlorite, to form a green dye compound. The intensity of the green color produced is directly proportional to the urea concentration.

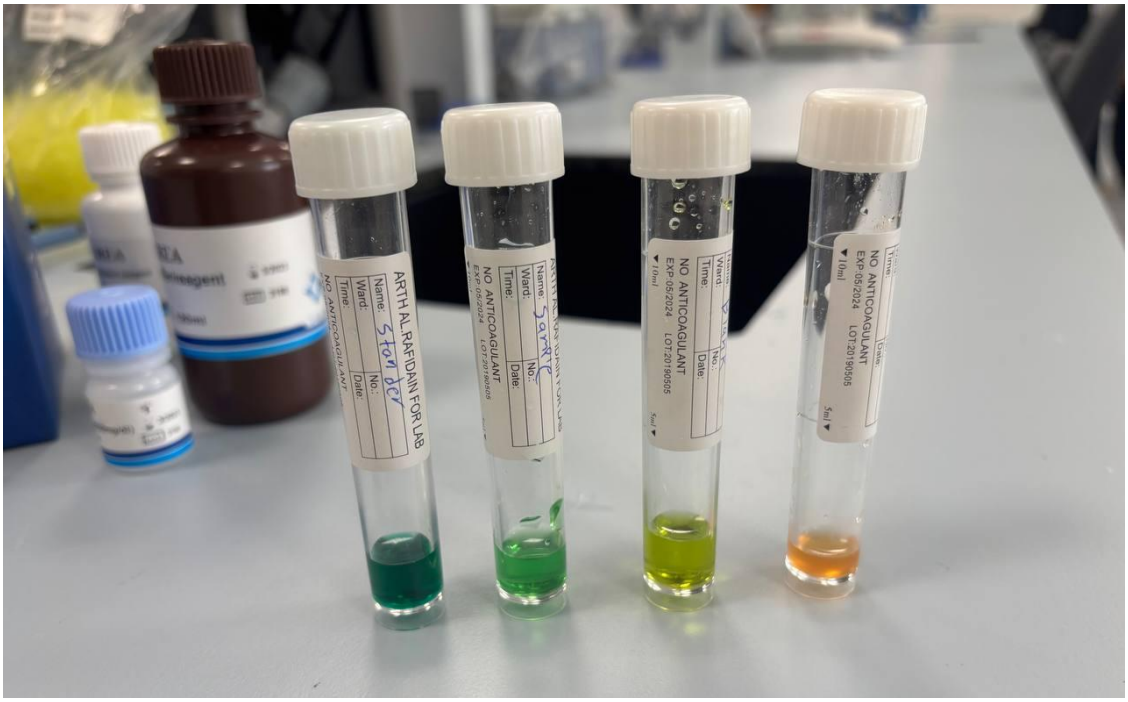
1.4 Procedure

Pipetting in tubes

	BLANK	STANDARD	SAMPLE
Reagent (R3)	1000µl	1000µl	1000µl
R2	50 µl	50 µl	50 µl
Standard		10 µl	
Sample			10 µl

Mix and incubate for 3 min at 37 C or 5 min at 20-25C

Reagent (R4)	**200 µl**	**200 µl**	**200 µl**



Notes:

Wave length: 578nm (578-630)

Optical Path: 1 cm light path

Temperature: 20-25 / 37 C

Reading: A gainst reagent blank

1.5 Calculation

$$\text{UREA mg/dl} = \frac{\text{Sample}}{\text{Standard}} \times 50$$

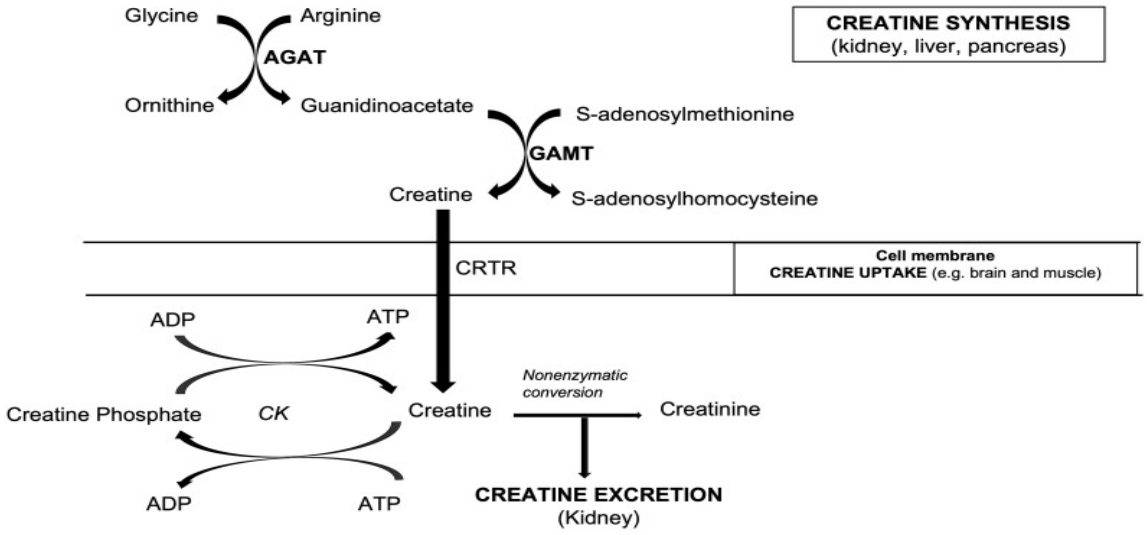
Unit conversion

mg/dl x 0.166 = mmol/l

Lab: 5

1. Determination of creatine and creatinine

Creatine is synthesized in the liver, pancreas, and kidneys from the amino acids arginine, glycine, and methionine Creatine is transported through the circulatory system to muscle where it is converted to phosphocreatine and acts as an energy reservoir much like ATP Creatinine is a waste product produced in your muscles from the breakdown of creatine (Creatinine is formed by the hydrolysis of creatine Almost all creatinine is excreted by the kidneys, so blood levels are a good measure of how well your kidneys are working.



1.1 Role of Creatinine in the body

1. Indicator of Kidney Function: High creatinine levels suggest impaired kidney function, as the kidneys are responsible for removing creatinine from the blood.

2. Muscle Health Marker: Creatinine levels can provide insights into muscle mass and overall muscle health, making it especially relevant for athletes.

3. Stability of Metabolic Processes: Stable creatinine levels indicate a healthy balance of muscle metabolism and waste elimination.

Specimen: Collect 5 ml venous blood sample. Serum is preferred, but heparinized blood can be used.

1.2 Causes of High Creatinine Levels

1. **Kidney Disease:** Conditions like chronic kidney disease or acute kidney injury can lead to impaired kidney function and elevated creatinine levels.
2. **Dehydration:** Inadequate fluid intake can result in concentrated blood, leading to higher creatinine levels.
3. **Medications:** Certain medications, such as nonsteroidal anti-inflammatory drugs NSAIDs), can cause temporary elevation in creatinine levels.

1.3 Causes of low creatinine Levels

Low levels of creatinine are not common and are not usually a cause for concern.

Low blood creatinine levels can indicate:

1. A diet very low in protein.
2. A decrease in muscle mass caused by a disease, such as muscular dystrophy.
3. Pregnancy can also cause low blood creatinine levels

1.4 Measuring Creatinine Levels

1.4.1 Blood Test

A simple blood test is performed to measure the amount of creatinine present in the bloodstream.

1.4.2 Urine Test

Creatinine clearance can be measured by collecting a 24-hour urine sample and comparing it with blood creatinine levels.

1.5 Interfering factors

1. High levels of ascorbic acid and cephalosporin antibiotics can cause a falsely increased creatinine level. These agents also interfere with BUN/ creatinine ratio.
2. A diet rich in meat can cause increased creatinine levels.
3. Creatinine is falsely decreased by bilirubin, glucose, histidine and quinidine compounds.
4. Ketoacidosis may increase serum creatinine substantially.

1.6 Principle

Creatinine reacts with picric acid in alkaline conditions to form a yellow-orange color complex. The rate of formation of color is proportional to the creatinine quantity in the sample.

Reagent preparation

Mix reagents (R 2) and (R 3) in the ratio 1:1

Working reagent is stable 2 days at room temperature.

Wavelength: 492 490 500

Optical path: 1 cm Light path

Temperature: 25 C

Reading: Against distilled water

1.7 Procedure

Pipetting in cuvette

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

Mix, and after 30 sec read the absorbance A1 of the standard orbsample.

Exactly 2 min later, read absorbance A2 of standard or sample.

1.8 Calculation

A2-A1= A standard or A sample

SERUM: Creatinine mg/dl = A (sample) / A (standard) x 2

URINE: Creatinine mg/dl = A (sample) / A (standard) x 2 x50

1.8 Abnormal findings

Increased levels ▲

Glomerulonephritis

Pyelonephritis

Acute tubular necrosis

Urinary tract obstruction

Reduced renal blood flow

(eg, shock, dehydration, CHF, atherosclerosis)

Diabetic nephropathy

Nephritis Rhabdomyolsis, Acromegaly, Gigantism

Decreased levels▼

Debilitation

Decreased muscle mass (eg

muscular dystrophy,

myasthenia gravis)

Lab: 6

1. Estimation of serum uric acid

Normal values

Men	**0.21-0.42mmol/l or 3.5-7.2mg/dl**
Women	**0.15-0.35mmol/l or 2.6-6 mg/dl**
Children	**0.12-0.32mmol/l or 2-5.5mg/dl**
Urine	**250-750mg/24hr**

Uric acid is a nitrogenous compound that is a product of purine (deoxy ribonucleic acid (DNA) building block) catabolism. Lack of the enzyme uricase allows this poor soluble substance to accumulate in body fluids. Two thirds of the uric acid produced daily is excreted by kidneys whereas the remaining one third exits by the stool. Over production of uric acid occurs when there is excessive cell break down and catabolism of nucleic acids (as in gout), excessive production and destruction of cells (as in leukemia), or inability to excrete the amount produced (as in renal failure).

Measurement of uric acid is used most commonly in the evaluation of renal failure, gout and leukemia.

Specimen: Obtain a 5 ml venous blood sample (serum is preferred) heparinized blood is acceptable, urine (diluted 1 to 10 in DW).

1.1 Interfering Factors

1. Stress and strenuous exercise will falsely elevate uric acid.
2. Many drugs cause increase or decrease of uric acid eg, allopurinol, acetazolamide, ascorbic acid, diuretics (eg, furosemide), niacin,

salicylate.

3. Purine-rich diet (eg, liver, kidney) increases uric acid levels.
4. High doses of aspirin decrease uric acid

1.2 Principle

Uric acid is oxidized by uricase to allontoine and hydrogen peroxide:

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoine} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + H_2O$$

1.3 Procedure

	Blank	Standard	Sample
Working reagent (R1 + R2)	**1 ml**	**1 ml**	**1 ml**
Std. Solution uric acid (60 mg/L or 357 µmol/L)	**-**	**20 µl**	**-**
Sample	**-**	**-**	**20 µl**

Mix then incubate for-5 min at 37 °C or 10 min at 20-25 °C read at 510 nm. The color is stable for 30 min.

1.4 Reagents

Reagent 1	Phosphate buffer pH7.4 2-4 DHBS	50 mmol/L 4 mmol/L
Reagent	Uricase Peroxidase 4-aminophenazone	70 U/L 660 U/L 1 mmol/L

1.5 Calculation

$$\text{Uric acid con.} = \frac{\text{A sample}}{\text{A standard}} \times n$$

Where: n = 6---> mg/ dl

n = 357 ---> µmol/L

For urine multiply result by 10

Lab: 7

1. Estimation of serum bilirubin

• Bilirubin is a degradation product of the heme portion of hemoglobin.

• Heme is degraded in cells of the reticuloendothelial system, mainly the spleen.

• The protoporphyrin ring of the heme is opened to the biliverdin form and iron is released. Biliverdin is reduced to produce the yellow-pigmented molecule bilirubin.

• The bilirubin molecule, a tetrapyrole, has low solubility in water or plasma. When it is released into blood, it is bound to albumin for transport

1.1 Forms of Bilirubin

unconjugated and conjugated.

Unconjugated bilirubin is insoluble in water. This means it can only travel in the bloodstream if bound to albumin and it cannot be directly excreted from the body.

In contrast, **conjugated bilirubin** is water soluble. This allows it to travel through the bloodstream without requiring transport proteins like albumin, which means that it can also be excreted out of the body.

Hyperbilirubinemia (Jaundice)

Bilirubin levels in the blood are increased as the result of several disorders or conditions. These disorders or conditions are categorized into three phases of bilirubin metabolism, prehepatic, hepatic, and posthepatic

1. Prehepatic hyperbilirubinemia is caused by increased hemolysis and increased degradation of heme. Prehepatic hyperbilirubinemia occurs in patients with sickle cell anemia and other hemolytic diseases that cause increased destruction of red blood cells and release of hemoglobin. The typical serum bilirubin pattern of prehepatic hyperbilirubinemia is increased unconjugated bilirubin and normal conjugated bilirubin.
2. Hepatic hyperbilirubinemia is generally due to defective transport to the liver or conjugation of bilirubin in the hepatocytes. Disorders of transport into the hepatocytes or conjugation disorders result in increased unconjugated bilirubin.

Examples of these conditions include:

A- Gilbert's syndrome: is a genetic disorder in which there is decreased bilirubin transport into the hepatocytes.

B- Crigler-Najjar syndrome: results from a genetic deficiency of the UDPG-transferase enzyme.

- Damage to hepatocytes by hepatitis, cirrhosis, toxic substances, and other disorders can inhibit conjugation as well.

- The typical serum bilirubin pattern of hepatic hyperbilirubinemia is increased unconjugated and conjugated bilirubin.

- Serum enzymes that indicate hepatocellular inflammation and cellular damage within the liver, including ALT and AST, are also often elevated.

3- Post-hepatic hyperbilirubinemia is generally due to a defect in transporting conjugated bilirubin and bile out of the liver. It can involve obstruction of the

small canaliculi within the liver, the hepatic bile duct, and the common bile duct leading to the duodenum of the small intestine. Posthepatic hyperbilirubinemia is often called obstructive jaundice.

Obstruction of the bile flow can be due to gallstones or to scarring and nodules, such as from cirrhosis or hepatic tumours and pancreatic tumours

• The typical serum bilirubin pattern of posthepatic hyperbilirubinemia is increased conjugated bilirubin but normal unconjugated bilirubin.

• Serum enzymes that indicate biliary cell damage, including alkaline phosphatase and GGT, are also often elevated.

-Neonatal jaundice is caused by the inability of the immature liver of the newborn to produce UDPG-transferase. A slight increase in bilirubin in the

second and third days of life is a normal response.

-There is accumulation of unconjugated bilirubin in blood circulation, but the serum bilirubin level generally does not increase above 5 mg/dL.

• Assay: Colorimetric method

• Sample material: Serum

Note: Hemolysis may falsely decrease bilirubin measurement.

Lipemia may falsely elevate bilirubin measurement. Bilirubin may be broken down by light or heat and should be protected from these environmental conditions.

Wavelength: 546 nm

Reference Ranges:

Adult bilirubin 0 – 1 mg/dL

Adult direct bilirubin 0 – 0.2 mg/d

1.3 Principle

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. in the presence of dimethyl sulfoxide, total bilirubin reacts with diazotized sulfanilic acid to form azobilirubin. in the absence of dimethyl – sulfoxide, only direct bilirubin reacts with diazotized sulfanilic acid to form azobilirubin.

1.4 Procedure

	Std. blank	Std	Test blank	test
Reagent R4	**50 µl**	**50 µl**	**-**	**-**
Sample	**-**	**-**	**50 µl**	**50 µl**
Reagent R1	**1 ml**	**-**	**1 ml**	**-**
Working	**-**	**1 ml**	**-**	**1 ml**

Mix well and intubate exactly for 50 min. at 37 C read the absorbance of Std. and testing against their blank at 555 nm.

	Std. blank	Std	Test blank	test
Reagent R4	**50 µl**	**50 µl**	**-**	**-**
Sample	**-**	**-**	**50 µl**	**50 µl**
Reagent R2	**1 ml**	**-**	**1 ml**	**-**
Working	**-**	**1 ml**	**-**	**1 ml**

Mix well and intubate exactly for 5 min. at 37 C read the absorbance of Std. and testing against their blank at 555 nm stable in the dark for 6hs at 20- 25 C.

Working Solution for Total Bilirubin: mix 20 volume of R1 with I volume of R3

Working Solution for Direct Bilirubin: mix 20 volume of R2 with 1 vol. R3

1.5 Reagents

Reagent 1	**Sulfanilic acid** **Hydrochloric acid** **Dimethylsulfoxide**	**30mmol\L** **150mmol\L.** **7mmol\L**
Reagent 2	**sulfanlic acid** **Hydrochloric acid**	**30mmol\L** **50mmol\L**
Reagent 3	**Sod. Nitrite**	**20mmol\L**
Reagent 4	**standard**	

Lab: 8

1. Estimation of serum calcium and phosphate

1.1 Calcium

Normal Values

Children 3-9 years	**2.2-2.7 mmol\L (8.8-10.1 mg\dL)**
13-15 years	**2.10-2.55 mmol\L (9.2-10.7 mg\dL)**
Adult	**2.25-2.75 mmol\L (8.4-10.2 mg\dL)**

The bulk of body calcium (98-99%) is found in the skeleton and teeth About 50% of blood-calcium in ionized, the rest is protein-bound. Only ionized calcium can be used by the body in vital processes a muscular contraction, cardiac function, transmission of nerve impulses and blood clotting.

The amount of protein in blood also affects calcium levels, because 50% of blood calcium is protein-bound, however, ionized form of calcium is not affected. Parathyroid hormone calcitonin, vitamin D, estrogens, androgens, carbohydrates and lactose all are factors that could influence calcium levels.

Note: Obtain 5 ml venous blood sample to get serum for calcium estimation. Heparinized plasma is preferred for ionized calcium studies. (EDTA or oxalate cause decreased calcium levels.

-Avoid prolonged tourniquet use (otherwise falsely high calcium levels)

1.1.2 Interfering Factors:

- Vitamin D intoxication may cause increased serum calcium levels.

- Excessive ingestion of milk may cause increased level.
- Serum pH can effect calcium levels. A decrease in pH causes increased calcium levels.
- Hypoalbuminimia artifactually associated with decreased levels of total calciam.
- Drugs that may cause inecrased serum levels include: calcium salts, hydralazine, thiazide diuretics, parathyroid hormone, thyroid hormone, alkaline antacids ergocalciferol, androgens and vitamin D.
- Drugs that may cause decrased serum levels include: acetazolamide, diuretics, Mg salts, estrogen and oral contraceptives.

1.1.3 Principle

Colorimetric determination of calicum without deproteinization using O-cresol phthalein complex. Interference due to Mg is by eliminated B-hydroxyquinoline (up to 4 mmol/L 0.10mg/L).

1.1.4 Procedure

	Reagent blank	Standard	Sample
Sample	**_**	**_**	**20 ml**
Reagent 1 (std)		**20 ml**	
Working solution (R2+R3)	**1 ml**	**1 ml**	**1 ml**

mix, read absorbance after 5 min. at 572nm against blank. The color intensity is stable for 1 hour.

1.1.5 Calculation

$$\text{Calcium conc.} = \frac{\text{A sample}}{\text{A standard}} \times n$$

n= con. of std

1.1.6 Reagents

Reagent 1 (std)	**Ca 2.5 mmol/l**	**100 mg/l**
Reagent 2 colour reagent	**O-cresolphtalein complexon** **B-hydroxyquinoline**	**104mg/L** **1.5 g/L**
Reagent 3 alkaline reagent	**2-amino,2-methyl-propanol** **pH > 11**	**43.25g/L**

2.1 Phosphate

Adult:	**0.87-1.45**
Child:	1.45-1.78
Newborn: 1.45-2.91	mmol/L

In the human body, about 85% of total phosphorus is found combined with calcium in the bones, while the rest is present in cells. Most phosphorus in the bloodstream exists as phosphates or esters. Phosphate is crucial for forming bone tissue and plays a role in glucose and lipid metabolism, maintaining acid-base balance, and facilitating the storage and transfer of energy throughout the body. Additionally, phosphorus enters red blood cells alongside glucose, leading to a decrease in plasma phosphorus levels after carbohydrate consumption or infusion.

2.1.2 Interfering Factors

- Laxatives or enemas containing sodium phosphate can elevate phosphorus levels.
- Recent carbohydrate intake, such as intravenous glucose administration, leads to lower phosphorus levels because phosphorus enters cells along with glucose.
- Certain medications, including excessive vitamin D and methicillin, can increase phosphorus levels, while antacids and mannitol may contribute to decreased levels.
- Phosphorus levels are generally higher in children.
- Additionally, seasonal variations may occur, with peak levels in May and June and the lowest levels in winter.
- It's also important to consider the time of day when samples are collected, as levels tend to be highest in the morning and lowest in the evening.

2.1.3 Principle

Colorimetric determination, without deprotinization, of serum phosphonus using a single reagent which forms a phosphomlybdate complex in the presence of a reducing agent (ferrous sulphate).

2.1.4 Procedure

	Reagent blank	Standard	Sample
Sample	-	-	100 ml
Reagent (std)	-	100 ml	-
D.W	100 ml	-	-
Working solution	2.5 ml	2.5 ml	2.5 ml

2.1.5 Calculation

$$\text{Inorganic phosphate conc.} = \frac{A\ \text{sample}}{A\ \text{standard}} \times n$$

Where n= 1.61 mmol/L or n= 5 mg /dL

2.1.6 Reagents

Reagent 2 (reducing reagent)	**sulfuric acid** **ferrous ammonium sulphate** **Ferrous nitrate**	**1.06 N** **100 g/L** **2 g\L**
Reagent 3 (colour reagent)	**sulfuric acid** **ammonium hepta-molybdate**	**1.05 N** **4.5g\L**

Lab: 9

1. Total lipid profile: Estimation of serum cholesterol

Normal values

Adult	< 5.20 m mol /L or 200 mg / dl
Child and Adolescent (12 – 18 yr)	3.12 – 5.20 m mol /L or 120 – 200 mg /dl

Cholesterol is a steroid alcohol formed in animal fats. It is widely distributed throughout the body, especially in the blood, brain, liver, kidneys and never fiber myelin sheaths and it is an essential component of cell membrane development and production of bile acids, adrenal steroids and sex hormones. Most of the cholesterol we eat comes from foods of animal origin. The liver metabolizes the cholesterol to its free form and cholesterol is transported in blood stream by lipoproteins. Nearly 75 % of the cholesterol is bound to low – density lipoproteins (LDLs), and 25 % is bound to high – density lipoproteins (HDLs). LDL is the most directly associated with increased risk of coronary heart disease (CHD). Cholesterol testing is usually done as a part of lipid profile testing, which also evaluates lipoprotein.

1.1 **Specimen collection**

Obtain 5 ml venous blood sample. Fasting is required. Instruct the patient to fast 12 -14 hr after eating a low fat diet before testing. Only water is permitted also no alcohol should be taken 24 hr before the test.

1.2 Interfering Factors

- Estrogen decreases plasma cholesterol levels, pregnancy increases these levels.

- Positional variation occur, levels are lower when sitting, versus standing and lower when recumbent versus sitting.
- Seasonal variation in cholesterol levels, higher levels were observed in winter and lower in spring and summer.
- Drugs that may cause increased levels include adrenocorticotropic hormones, epinephrine, oral contraceptives, phenytoin, sulfonamides, thiazide diuretics, cyclosporine and vitamin D.
- Other drugs may cause decreased levels including allopurinol, bile salt binding agents. captopril, chlorpropmide, clofibrate, colchicine, erythremyem isoniazide. lovastatin, monoaminoxidase inhibitors, neomycin (oral), niacin and nitrates.

1.3 Principle

Cholesterol is determined according to the following reaction:

Cholesterol esterase

Cholesterol ester ----------> cholesterol + fatty acids

Cholesterol oxidase

Cholesterol ----------> cholest-4-en-3 one + H2O2

Peroxidase

2H2O + Phenol + 4- aminoantipyrine ----------> quinonimine +4 H2O

1.4 Procedure

	Blank	Standard	Sample
Standard (2 g/L cholesterol)	**-**	**10 µL**	**-**
Sample	**-**	**-**	**10 µL**
Working solution (R1+R2)	**1 mL**	**1 mL**	**1 mL**

Mix and measure absorbance after incubation at 37 °C for 5 min, or incubate for 10 min at (20-25 °C). The color intensity is stable for 30 min.

1.5 Reagents

Reagent 1 (Buffer) phosphate buffer	**Phenol** **Sod. Cholate** **Surfactant**	**0.1 mol/L** **15 mmol/L** **3.74 mmo/L**
Reagent 2 **(enzymes)**	**4- aminoantipyrine** **peroxidase** **Cholesterol oxidase** **Cholesterol esterase**	**0.5mmol/L** **≥ 1000U/L** **≥ 200 U/L** **≥ 124 U/L**

1.6 Calculation

$$\text{Cholesterol conc.} = \frac{\text{A sample}}{\text{A standard}} \times n$$

Where n= 5.17 mmol/l or n=200 mg/dl

Lab: 10

1. Total lipid profile: Estimation of HDL

Normal values

HDL male	**> 45 mg/dL or > 0.75 mmol/L**
female	**> 0.91 mmol/L or > 55mg/dL**

Lipoproteins are complexes of lipids (fats) and proteins that serve as transport vehicles for fats in the bloodstream. Since lipids are not water-soluble, they need to be carried by proteins to move through the blood. Lipoproteins play a crucial role in lipid metabolism and are essential for the transport of cholesterol, triglycerides, and other fats to and from cells.

Understanding lipoproteins and their function is essential in managing and preventing cardiovascular diseases, and they are often targeted in lipid-lowering therapies. The lipid profile usually includes total cholesterol, triglycerides, HDL, LDL and VLDL.

HDLs are carries of cholesterol that are produced in liver. It is main function is to remove cholesterol for excretion. Also HDLs Prevent cellular uptake of cholesterol and lipids. These potential actions maybe the cause of their protective cardiovascular characteristics associated with HDLs (good cholesterol) within the blood.

LDLs are cholesterol rich. Cholesterol carried by LDLs can be deposited into the peripheral tissues and is associated with increased risk of arteriosclerotic heart and vascular diseases

1.1 Interfering factors

1. Smoking and alcohol consumption can lead to a reduction in HDL levels.

2. HDL values vary based on age and sex.

3. Similar to cholesterol, HDL levels tend to significantly decrease for up to three months following a myocardial infarction (heart attack).

4. HDL levels are elevated in individuals with hypothyroidism and reduced in those with hyperthyroidism.

5. Higher LDL levels are often observed during pregnancy, while women on oral estrogen therapy tend to show lower LDL levels and higher HDL levels.

6. Certain drugs, including steroids, progesterone (from oral contraceptives), androgens, phenothiazines, and sulfonamides, can cause an increase in lipoprotein levels.

7. Some medications, such as steroids, diuretics, and beta-blockers, are known to lower HDL levels.

1.2 Sample collection

- Collect 5-10 ml of venous blood.
- Instruct the patient to fast for 12-14 hours before sampling only water is permitted.
- No alcohol should be consumed for at least 24 hours.
- If possible, all medications should be withheld for at least 24 hours.

1.3 Principle

The chylomicrons VLDL and LDL –lipoproteins that are contained in the sample are precipitated by the addition of phosphotungestic acid in the presence of Mg+. the supernatant obtained after centrifugation contains high density lipoproteins(HDL) from which the cholesterol can be determined by using the cholesterol enzymatic kit.

1.4 Procedure

Serum 500 micro liter +50 micro liter R1

Mix and stand for 10 min centrifuge for 15 min at 500rpm. for the determination of HDL –Cholesterol use the same procedure used for cholesterol estimation using the resultant supernatant from the above mixture.

	Blank	**Standard**	**Sample**
D.W	50 micro liter		
HDL –cholesterol Calibrating Sol. 1.3 mmol/L (50mg /dl)	-	50 micro liter	-
Supernant	-	-	50µL
Working sol.	1ml	1ml	1ml

(Reagent 1+reagent 2 of cholesterol kit)

Mix and incubate for 5min at 37 C measure at 500nm, the color is stable for 30 min.

1.5 Reagent

Reagent 1	**phosphotungstic acid** **MgCl2.6H2O,** **Ph 6.2**	**40g/L** **100g/L**

1.6 Calculation

$$\text{HDL-Cholesterol conc.} = \frac{\text{A sample}}{\text{A standard}} \times n$$

mmol / L n =1.42

mg/ dl n =55

Lab: 11

1. Total lipid profile: Estimation of Triglycerides

Normal values

Adult Male	**0.45-1.81 mmol/L or 40-160 mg/dL**
Female	**0.40-1.52 mmol/L or 35-135 mg/dL**
Child(6-11 yr.)	**33-112 mg/dL**

Triglycerides are a type of fat (lipid) found in your blood, and they are the most common form of fat in the body. When you eat, your body converts excess calories, especially from carbohydrates and fats, into triglycerides. These triglycerides are stored in fat cells and released between meals to provide energy. While triglycerides are essential for storing energy, having high levels of triglycerides can increase the risk of cardiovascular diseases.

1.1 Interfering Factors

1- Ingestion of fatty meals may cause elevated TG levels.

2- Ingestion of alcohol may cause elevated TG levels.

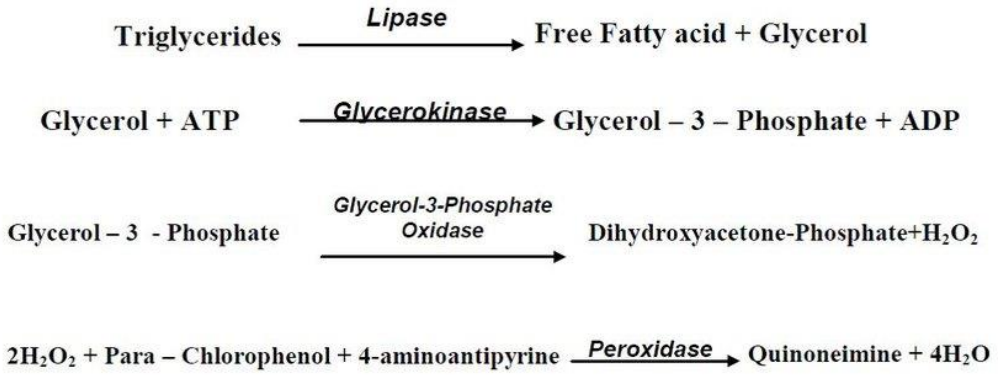
3- Pregnancy may cause increased levels.

4- Drugs that May cause increased TG levels include cholesytramine, estrogens, and oral contraceptives.

5- Drugs that may cause decrease levels include ascorbic acid, clofibrate, and colestipol.

Collect 5 to 10 ml of venous blood. Instruct the patient as with lipoproteins est imation to obtain serum or plasma (collected in heparin, or oxalate or citrate salt or EDTA) Hemolysis will interfere.

1.2 Principle



1.3 Procedure

	Blank	Standard	Sample
Standard	–	**10** µl	–
Sample	–	–	**10** µl
Working solution (R2+R3)	**1000** µl	**1000** µl	**1000** µl

Mix, incubate for 5 min at 37 °C or 10 min. at 20-25 °C measure absorbance at 505 nm, the color intensity is stable for 30 min.

1.4 Calculation

TG Conc. = A sample / A standard x n

mmol/L = 2.29 mg/dl: n= 200

1.5 Reagents

Reagent 1 (Std. Glycerol)	**2.29 mmol/L or 200 mg/ dl tris**
Reagent 2	Buffer pH7.6 100 mmol/L Parachlorophenol 2.7 mmol/L
Reagent 3 (enzymes)	Magnesium 4 mmol/L amino- antipyrine > 0.4mmol/L Lipase 1000 u/L glycerokinase ≥ 200 u/L glycerol 3 phosphate oxides ≥ 2000 u/L Peroxidase ≥ 200 u/L ATP 0.8 mmol/L

Lab: 12

1. Total lipid profile: Estimation of LDL

Normal values

LDL	< 3.3 mmol/L or 60-180 mg/dL
VLDL	< 30 mg/dL (0.77 mmol)

LDLs are cholesterol rich. Cholesterol carried by LDLs can be deposited into the peripheral tissues and is associated with increased risk of arteriosclerotic heart and vascular diseases. Therefore, high levels **of LDL (bad cholesterol)** are atherogenic. LDL is very difficult to isolate and measure. Hence LDL is most usually derived by the **friedwald formula** as follows:

LDL =TC – HDL -(TG/5) VLDL
LDL = TOTAL CHOLESTEROL – (HDL + TG/5)
VLDL= TG/5

VLSLs although carrying a small amount of cholesterol these are the predominant caries of blood triglycerides. The VLDL value is usually expressed as a percentage of total cholesterol levels in excess of 25-50% are associated with increased risk of coronary heart disease.

1.1 Interfering factors

1-smoking and alcohol ingestion decrease HDL level

2-HDL values are age and sex dependent

3-HDL values like cholesterol, tend to significantly decreased for as long as 3 month following MI

4- HDL level is elevated in hypothyroidism and diminished in hyperthyroidism.

5-Increased LDL levels are associated with pregnancy while women taking oral estrogen therapy show decreased LDL and increased HDL levels.

6- Drugs may cause increased lipoprotein levels include steroids progesterone (oral contraceptive) androgens, phenothiazine and sulfonamides

7- some drugs decrease HDL levels such as: steroids, diuretics, B - blocker.

1.2 Specimen

collect 5-10 ml of venous blood.

- Instruct the patient to fast for 12-14 hours before sampling only water is permitted.
- No alcohol should be consumed for at least 24 hours.
- If possible, all medications should be withheld for at least 24 hours.

Lab: 13

1. Estimation of AST activity

Normal Values

Adult	up to 12 u|L
Elderly	values slightly higher than adult.
Child	values similar to adult

Aspartate Aminotransferase (AST), also known as serum glutamic-oxaloacetic transaminase (SGOT), is an enzyme found primarily in the liver, heart, muscles, and other tissues. AST plays a role in amino acid metabolism, particularly in the conversion of aspartate and α-ketoglutarate into oxaloacetate and glutamate.

Function: AST is involved in the process of transamination, which is essential for the production and breakdown of amino acids.

Location: The highest concentrations of AST are found in the liver and heart, but it is also present in the muscles, kidneys, brain, and red blood cells.

1.1 Interfering factors

1- Exercise may cause increased levels.

2- Drugs that may cause increased levels include: antihypertensive, cholinergic agent, coumarin – type anticoagulants, digitalis preparation erythromycin, methyldopa. oral contraceptives, salicylates, hepatotoxic medications.

3- Decreased levels could be seen in pyridoxine deficiency (beriberi or pregnancy) sever long standing liver disease, uremia or diabetic ketoacidosis.

1.2 Sample collection

Collect a venous sample of blood. This is usually done daily for three days and then again after 1 week. (in case of suspected MI for emergency room patients) It is preferred to instruct the patient to hold drugs that could interfere with test results for 12 hrs before the test. Avoid hemolysis.

1.3 Principle

1.4 Procedure

	Reagent blank	**sample**
Sample	-	0.1 ml
GOT buffer	o.5 ml	0.5 ml
D.W	o.1 ml	-

Mix, incubate for exactly 30 min. at 37°C.

2,4-dinitrophneyl hydrazine	**0.5 ml**	**0.5 ml**

Mix, allow to stand for exactly 20 min at 20 to 25 °C.

sod. Hydroxide	**5 ml**	**5 ml**

Mix, read the absorbance of sample at 530 nm against the reagent Blank after 5 min. Obtain the activity of GOT in the serum from the table:

Absorbance	U/L	Absorbance	U/L
0.020	**7**	**0.100**	**38**
0.030	**10**	**0.110**	**41**
0.040	**13**	**0.120**	**47**
0.050	**16**	**0.130**	**52**
0.060	**19**	**0.140**	**59**
0.070	**23**	**0.150**	**67**
0.080	**27**	**0.160**	**76**
0.090	**31**	**0.170**	**89**

1.5 Reagents

A-GOT buffer	
Phosphate buffer	**100 mmol/L, pH 7.4**
L- aspartate	**100 mmol/L**
A-oxoglutarate	**2 mmol/L**
B-2,4- dinitrophenylhydrazine	**2.0 mmol/L**
C-Sodium hydroxide	

D-Pyruvate standard	
Pyruvate	**2 mmol/L**

Lab: 14

1. Estimation of ALT activity

Normal values

Adult/ child	Up to 12 U/L
Elderly	May be slightly higher than adult
Infant	May be twice as high as adult

Alanine Aminotransferase (ALT), also known as serum glutamate-pyruvate transaminase (SGPT), is an enzyme primarily found in the liver. It plays a key role in converting alanine, an amino acid, into pyruvate, which is involved in energy production. ALT is crucial for protein metabolism, and it is commonly used as a marker for liver health.

Function: ALT helps in the transamination process, which converts amino acids for use in energy production and biosynthesis.

Location: ALT is most concentrated in the liver, but smaller amounts are also found in the heart, kidneys, muscles, and pancreas.

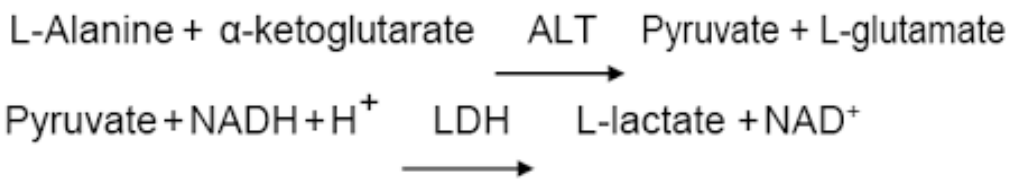
1.2 Interfering Factors

- Previous intramuscular injections may cause elevated levels.
- Drugs that may cause increased ALT levels include: acetaminophen, allopurinol, aminosalicylic acid, ampicillin, carbamazepine, cephalosporins, chlorpropamide, clofibrate, cloxacillin, codien, indomethacin, isoniazid, methyldopa.

1.3 Sample collection

Obtain 7-10 ml of venous blood to obtain serum.

1.4 Principle



1.5 Procedure

	Blank	Sample
Sample	**-**	**0.1 ml**
Buffer	**0.5 ml**	**0.5 ml**
D.W	**0.1 ml**	**-**

Mix. Incubate for exactly 30 min at 37 C°

2,4-DNP	**0.5 ml**	**0.5 ml**

Mix. Allow to stand for 20 min at 20-25 C°

Sod. Hydroxide	**5 ml**	**5 ml**

Mix. Read the absorbance of sample at 550nm using reagent blank to adjust the spectrophotometer to read the absorbance to estimate the activity of ALT in serum.

See the following table

Absorbance	U/L	Absorbance	U/L
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.350	62
0.100	17	0.375	67
0.125	21	0.400	72
0.150	25	0.425	77
0.175	29	0.450	83
0.200	34	0.475	88
0.225	39	0.500	94
0.250	43		

Lab: 15

1. Estimation of CK activity

Normal values

Adult	**Male**	55-170U/l
	Female	30-135U/L
Newborn		68-580U/L

Creatine Kinase (CK), also known as creatine phosphokinase (CPK), is an enzyme found in various tissues, including the muscles, brain, and heart. CK plays a crucial role in energy metabolism, facilitating the conversion of creatine and adenosine triphosphate (ATP) into phosphocreatine, which is used for energy storage in cells. CK is particularly important in tissues with high energy demands, such as skeletal muscles and the heart.

There are three main isoenzymes of CK, which are found in different tissues:

1. CK-MM: Found primarily in skeletal muscle.

2. CK-MB: Found in the heart muscle, commonly used to diagnose myocardial infarction (heart attack).

3. CK-BB: Found in the brain and other smooth muscles.

1.2 Interfering Factors

1- IM injections can cause elevated CPK levels.

2- Strenuous exercise and recent surgery may cause increased levels.

3- Early pregnancy may produce decreased levels

4- Drugs that may cause increased level include amphotericin B, ampicillin, some anesthetics, anticoagulants, aspirin, clofibrate, dexamethasone, furosemide, captopril, alcohol, propanol and morphine.

1.3 Sample collection

Collect venous blood sample (5-7 ml) to separate serum, avoid hemolysis. This is usually done daily for 3 days and then at 1 week when cardiac disease is suspected. Record the exact time and date of venipuncture on laboratory slip, to aid in the interpretation of pattern of enzyme elevation.

1.4 Principle

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{CK} \text{creatine} + \text{ATP}$$

$$\text{Glucose} + \text{ATP} \xrightarrow{HK} \text{Glucose-6-P} + \text{ADP}$$

$$\text{Glucose-6-P} + \text{NADP}^+ \xrightarrow{G6P\text{-}DH} \text{Gluconate-6-P} + \text{NADPH} + \text{H}^+$$

The catalytic activity of CPK is determined by the measurement of NADPH formation.

1.5 Procedure

Pipette into cuvettes:

Working solution 1000 microLiter

Then add 40 microliter of sample for 5 min. at 37

mix and incubate at 370C for 2 min. read the initial absorbance at 365 nm and started timer immediately. Read again at constant intervals for 3min.

1.6 calculation

Calculate the average value of variations of absorbance per min OD\min then apply the following formula:

CK(U\L) = △ OD nm\min x 7429

1.7 Reagents

Reagent 1	**Imidazol acetate buffer PH 6.5** **Magnesium acetate**
Reagent 2 (substrate)	D-glucose Creatine phosphate EDTA Nacetyl cysteine ADP AMP Diadenosine pentaphosphate NADP Hexokinase (HK) G-6-PDH

References

1. Clinical Chemistry & Metabolic Medicine, Crook, 2006.
2. Clinical Chemistry, Kaplan, 2003.
3. Lippincott's Illustrated Reviews of Biochemistry.
4. Clinical Chemistry by Bishop and Fody.