مفردات الدراسة

اسم المادة	لغة التدريس	السنة الدراسية	عدد الساعات الاسبوعيـــــــــــــــــــة			
			نظري	عملي	المجموع	عددالوحدات
اضطرابات الايض	الانكليزية	الثالثـــة				
Metabolicdisorder		الكورس الثاني	2	4	6	4

المفردات العمليه

اسم المادة : اضطرابات الايض

First semester	
WEEK	تفاصيل المفردات
1	Estimation of molarity, molality,osmolarity, osmolalityand Equivalent
2	Estimation of Na^{++}, K^{+},$P^{++}$and Ca^{++}
3	Estimation of blood gases
4	Estimation of Ze and I
5	Estimation of urea creatinine, andcreatinine clearance
6	Estimation of GOT, GPT and ALP
7	Estimation of Bilirubin (direct and indirect)
8	Estimation of amylase enzyme
9	Estimation of triglyceride and cholesterol
10	Estimation of HDL and LDL and VLDL
11	Estimation of total protein andAlbumin/Globulin Ratio
12	Estimation of uric acid
13	Estimation of Total iron and Iron binding capacity
14	Estimation of HbA1c and BMI
15	Estimation of LDH, CK, cardiac troponin andD-Dimers

Estimation of molarity, molality,osmolarity, osmolalityand Equivalent

Concentration refers to the amount of a substance (solute) present in a given amount of solution or solvent. It expresses how "strong" or "dilute" a solution is and is essential in chemistry, physiology, pharmacology, and clinical practice.

Common Types of Concentration

1. **Molar Concentration (Molarity, M)**
2. **Molal Concentration (Molality, m)**
3. **Osmotic Concentration**
   - **Osmolarity**
   - **Osmolality**
4. **Equivalent Concentration**
5. **Percentage Concentration**
   - **Weight/volume (% w/v): g per 100 mL**
   - **Weight/weight (% w/w): g per 100 g**
   - **Volume/volume (% v/v): mL per 100 mL**
6. **Mass Concentration**

Molarity (M): Number of **moles of solute per liter of solution**.

Formula:

$$\text{Molarity (M)} = \frac{\text{moles of solute}}{\text{volume of solution (L)}}$$

Example:
5 g NaCl (MW = 58.5) dissolved in 500 mL solution

$$\text{moles} = \frac{5}{58.5} = 0.085$$
$$M = \frac{0.085}{0.5} = 0.17\ M$$

Molality (m): Number of **moles of solute per kilogram of solvent**.

Formula:

$$\text{Molality (m)} = \frac{\text{moles of solute}}{\text{mass of solvent (kg)}}$$

Example:
0.1 mol glucose in 1 kg water

$$m = \frac{0.1}{1} = 0.1\ m$$

Osmolarity (Osm/L): Number of **osmoles of solute per liter of solution**.

$$\text{Osmolarity} = \text{Molarity} \times \text{number of particles formed}$$

Examples:

- NaCl → dissociates into **Na^+ + Cl^- = 2 particles**

$$1\ M\ NaCl = 2\ Osm/L$$

- Glucose (no dissociation):

$$1\ M = 1\ Osm/L$$

Osmolality (Osm/kg): Number of **osmoles of solute per kilogram of solvent**.

$$\text{Osmolality} = \text{Molality} \times \text{number of particles}$$

Example:
1 m NaCl →

$$1 \times 2 = 2\ Osm/kg$$

Equivalent (Eq): Amount of substance that **reacts with or supplies 1 mole of charge (H^+ or e^-)**.

$$\text{Equivalents} = \text{moles} \times \text{valency}$$

Example:

- 1 mol Na^+ (valency = 1) → **1 Eq**
- 1 mol Ca^{2+} (valency = 2) → **2 Eq**

Normality (N)

$$N = \frac{\text{Equivalents}}{\text{Liter of solution}}$$

Estimation of Na^{++}, K^{+},$P^{++}$and Ca^{++}

Sodium

CLINICAL SIGNIFICANCE

Sodium is the major cation of extracellular fluid. It plays a central role in the maintenance of the normal distribution of water and the osmotic pressure in the various fluid compartments. The main source of body sodium is the sodium chloride contained in ingested foods. Only about one-third of the total body sodium is contained in the skeleton since most of it is contained in the extracellular body fluids. Hyponatremia (low serum sodium level) is found in a variety of conditions including the following: severe polyuria, metabolic acidosis, Addison's disease, diarrhea, and renal tubular disease. Hypernatremia (increased serum sodium level) is found in the following conditions: hyperadrenalism, severe dehydration, and diabetic coma after therapy with insulin, excess treatment with sodium salts.

PRINCIPLE

The method is based on reaction of sodium with a selective Chromogen producing a chromophore whose absorbance is directly proportional to sodium concentration in the sample

REAGENT COMPOSITION

Reagent 1: Sodium Reagent

Reagent 2: Sodium Standard 150 mEq/L

NORMAL RANGE

Serum/Plasma: 135 - 155 mEq/L.

It is recommended that each laboratory establish its own normal range representing its patient population.

ASSAY

Wavelength = 630 nm

Reaction type = Endpoint

Cuvette = 1 cm light path

Temperature = Room temp.

Measurement = Against reagent blank

PROCEDURE

Pipette in to cuvettes	Blank	Standard	Sample
Sodium Reagent	1000 µL	1000 µL	1000 µL
Standard	--	10 µL	--
Sample	--	--	10 µL
Mix and incubate for 5 min at room temp. and measure the absorbance of the sample (As) and the standard (A.std) against the reagent blank.			

CALCULATION

$$\text{Concentration of Sodium (mEq/L).} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} X\ 150\ (\text{Std. conc.})$$

Potassium

CLINICAL SIGNIFICANCE

Potassium is an electrolyte that is vital to cell metabolism. It helps transport nutrients into cells and removes waste products out of cells. It is also important in muscle function, helping to transmit messages between nerves and muscles, Elevated potassium levels (hyperkalemia) are often associated with renal failure, dehydration shock or adrenal insufficiency. Decreased potassium levels (hypokalemia) are associated with malnutrition, negative nitrogen balance, gastrointestinal fluid losses and hyperactivity of the adrenal cortex, this test measures the amount of potassium in the blood

PRINCIPLE

Potassium reacts with sodium tetra phenol boron in a specially prepared buffer to form a colloidal suspension. The amount of the turbidity produced is directly proportional to the concentration of potassium in the sample.

REAGENT COMPOSITION

Reagent 1: Potassium Reagent

Reagent 2: Potassium Standard 5 mEq/L

ASSAY

Wavelength = 630 nm

Reaction type = Endpoint

Cuvette =1 cm light path

Temperature = Room temp.

Measurement = Against reagent blank

PROCEDURE

Pipette in to cuvettes	Blank	Standard	Sample
Potassium Reagent	1000 µL	1000 µL	1000 µL
Standard	--	20 µL	--
Sample	--	--	20 µL
Mix and incubate for 5 min at room temp. And measure the absorbance of the sample (As) and the standard (A.std) against the reagent blank.			

CALCULATION

$$\text{Concentration of Potassium (mEq/L).} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} X\ 5\ (\text{Std. conc.})$$

Calcium

Serum calcium refers to the concentration of calcium in the blood and is essential for normal physiological functions such as neuromuscular activity, blood coagulation, and bone metabolism.

The normal range of total serum calcium is approximately 8.5–10.5 mg/dL (2.1–2.6 mmol/L).

In the circulation, calcium exists in three forms:

A. ionized (free),
B. protein-bound—mainly to albumin—
C. and complexed with anions, with ionized calcium being the biologically active fraction.

Serum calcium levels are tightly regulated by

1. parathyroid hormone,
2. vitamin D, and
3. calcitonin through their actions on the bones, kidneys, and gastrointestinal tract.

Abnormal serum calcium levels have significant clinical implications;

hypocalcemia may result in neuromuscular irritability, tetany, or cardiac arrhythmias,

whereas **hypercalcemia** may cause renal stones, skeletal pain, and neuropsychiatric disturbances. Measurement of serum calcium is therefore important in the assessment of bone, renal, and endocrine disorders.

Phosphate

Serum phosphate refers to the level of inorganic phosphate circulating in the blood and plays a critical role in numerous physiological processes, including bone and teeth mineralization, energy production through ATP, nucleic acid synthesis, and intracellular signaling pathways.

Phosphate homeostasis is tightly regulated by intestinal absorption, renal excretion, and exchange with bone, under the control of parathyroid hormone (PTH), active vitamin D (calcitriol), and fibroblast growth factor-23 (FGF23).

Normal adult serum phosphate levels range from about 2.5 to 4.5 mg/dL, with higher values seen in children due to increased bone growth.

Abnormal serum phosphate levels have important clinical implications: hyperphosphatemia is most commonly associated with chronic kidney disease, hypoparathyroidism, and massive tissue breakdown, while hypophosphatemia may result from inadequate intake, malabsorption, vitamin D deficiency, hyperparathyroidism, or intracellular shifts. Persistent disturbances in serum phosphate can lead to skeletal disorders, muscle weakness, and cardiovascular complications.

Estimation of blood gases

An arterial blood gas (ABG) test is a blood test that requires a sample from an artery in your body to measure the levels of oxygen and carbon dioxide in your blood. The test also checks the balance of acids and bases, known as the pH balance, in your blood.

Your body normally tightly regulates the amount of oxygen and carbon dioxide in your blood, because low blood oxygen levels (hypoxemia) can lead to many serious conditions and damage to individual organ systems, especially your brain and heart.

Other common names for an arterial blood gas test include:

- Blood gas test.
- Arterial blood gases.
- ABG.
- Blood gas analysis.

An arterial blood gas test usually includes the following measurements:

- ✓ Oxygen content (O2CT): This measures the amount of oxygen in your blood.
- ✓ Hemoglobin: This measures the amount of hemoglobin, the protein responsible for carrying oxygen to your cells, in your blood.
- ✓ Oxygen saturation (O2Sat): This measures how much hemoglobin in your blood is carrying oxygen. Hemoglobin is a protein in your red blood cells that carries oxygen from your lungs to the rest of your body.
- ✓ Partial pressure of oxygen (PaO2): This measures the pressure of oxygen dissolved in your blood. It helps show how well oxygen moves from your lungs to your bloodstream.
- ✓ Partial pressure of carbon dioxide (PaCO2): This measures the amount of carbon dioxide in your blood and how well carbon dioxide can move out of your body.
- ✓ pH: This measures the balance of acids and bases in your blood, known as your blood pH level. The pH of blood is usually between 7.35 and 7.45. If it's lower than that, your blood is considered too acidic. If it's higher than that range, your blood is considered too basic (alkaline).
- ✓ Bicarbonate (HCO3): This is calculated using the measured values of pH and PaCO2 to determine the amount of the basic compound made from carbon dioxide (CO2.)

Healthcare providers frequently order arterial blood gas (ABG) tests:

- ✓ Emergency medicine: Emergency medicine is the area of medicine that's concerned with the care of illnesses or injuries requiring immediate medical attention.
- ✓ Anesthesiology: Anesthesiology is the area of medicine that's concerned with the care of people before, during and after surgery. It encompasses anesthesia, intensive care medicine, critical emergency medicine and pain medicine.
- ✓ Pulmonology: Pulmonology is the area of medicine that deals with diseases involving your respiratory system.

In general, normal values at sea level include:

pH: 7.35-7.45.

Partial pressure of oxygen (PaO2): 75 to 100 millimeters of mercury (mmHg).

Partial pressure of carbon dioxide (PaCO2): 35 to 45 mmHg.

Bicarbonate (HCO3): 22 to 26 milliequivalents per liter (mEq/L).

Oxygen saturation (O2Sat or SaO2): 95 to 100%.

Estimation of Zn and I

Zn

An essential trace element and plays many key roles in metabolism. It is required for the activity of more than 300 enzymes, the structure of many proteins, and control of genetic expression. Zinc status affects basic processes of cell division, growth, differentiation, development, performance and aging through its requirement for synthesis and repair of DNA, RNA and protein. The common causes of zinc deficiency are low dietary intakes and low bioavailability. Clinical signs of zinc deficiency include acrodermatitis, low immunity, diarrhea, poor healing, stunting, hypogonadism, fetal growth failure, teratology and abortion. Zinc deficiency has now been recognized to be associated with many diseases such as malabsorption syndrome, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses.

Simple, direct and automation-ready procedures for measuring zinc concentration in biological samples are highly desirable in Research and Drug Discovery. Biochain's zinc assay kit is designed to measure zinc directly in biological samples without any pretreatment. The present method utilizes a chromogen that forms a colored complex specifically with zinc. The intensity of the color, measured at 425 nm, is directly proportional to the zinc concentration in the sample.

PROCEDURES

Sample preparation: serum and plasma samples should be clear and free of turbidity or precipitates. If present, precipitates should be removed by filtration or centrifugation on a table centrifuge. Prior to assay, dilute serum or plasma samples 5-fold (n = 5) in deionized water. Reagent preparation: equilibrate all reagents to room temperature. Vortex Reagents B and C before assay. Prepare enough Working Reagent: for each assay well, mix 200 mL Reagent A, 4 mL Reagent B and 4 mL reagent C.

Procedure using 96-well plate:

1. Prepare standards in deionized water. Transfer 50 µL of the Zn^{2+}

No	STD + H_2O	Vol (µL)	Zn^{2+} (µM)
1	20µL + 80µL	100	10.0
2	15µL + 85µL	100	7.5
3	10µL + 90µL	100	5.0
4	5µL + 95µL	100	2.5
5	0µL + 100µL	100	0

standards into wells of a clear flat-bottom 96-well plate.

Transfer 50 µL Sample and Sample Blank (50 µL sample + 2 µL EDTA) into wells of a Add 200 µL working reagent and tap plate lightly to mix.

2. Incubate 30 min at room temperature and read optical density at 425 nm (range 420 - 426 nm).

Procedure using cuvette:

Transfer 200 mL standards, sample and sample blank (200 mL Sample + 8 mL EDTA) to appropriately labeled tubes. Add 800 mL working reagent and tap lightly to mix. Incubate 30 min and read optical density at 425 nm.

CALCULATION

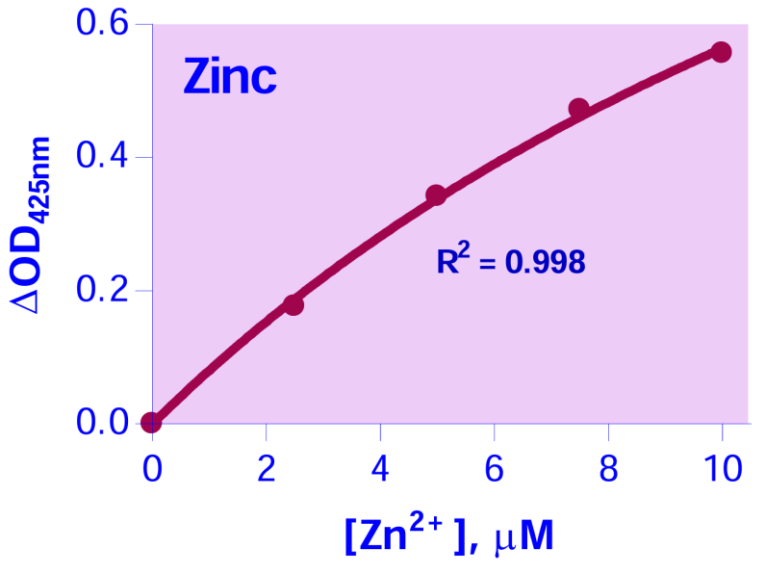
Subtract blank OD (water, #5) from the standard OD values and plot the ΔOD against Zn^{2+} standard concentrations.

ΔOD for the Sample = $OD_{SAMPLE} - OD_{SAMPLE\ BLANK}$).

Determine the Sample Zn^{2+} concentration from the standard curve by non-linear regression fitting with a single-site saturation binding function

($\Delta OD = a \times [Zn^{2+}]/(b+[Zn^{2+}])$

If the Zn^{2+}, concentration is higher than 10 mM, dilute sample in deionized water. Repeat the assay and multiply the results by the dilution factor.



Iodine

The serum iodine test measures the concentration of iodine in the blood and is used to assess iodine exposure and, less commonly, iodine status related to thyroid function.

Iodine is an essential trace element required for the synthesis of thyroid hormones (T_3 and T_4), which regulate metabolism, growth, and development.

Serum iodine reflects recent iodine intake rather than long-term body stores, so it is not the preferred test for population iodine status (urinary iodine is more reliable for that purpose). Elevated serum iodine levels may be seen after exposure to iodinated contrast media, iodine-containing medications (e.g., amiodarone), or excessive dietary supplementation, and can precipitate thyroid dysfunction.

Low serum iodine levels are uncommon but may occur in severe iodine deficiency or prolonged malnutrition and can contribute to hypothyroidism and goiter.

Estimation of urea, creatinine, and creatinine clearance

Urea

CLINICAL SIGNIFICANCE

Urea is the end product of the protein metabolism. It is synthesized in the liver from the ammonia produced by the catabolism of amino acids. It is transported by the blood to the kidneys from where it is excreted. Increased levels are found in renal diseases, urinary obstructions, shock, congestive heart failure and burns. Decreased levels are found in liver failure and pregnancy

PRINCIPLE

Urease catalyses the conversion of urea to ammonia. In a modified Berthelot reaction, the ammonium ions react with a mixture of salicylate, hypochlorite and nitroprusside to yield a blue-green dye (Indophenol.) The intensity of this dye is proportional to the concentration of urea in the sample.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

$$NH_3 + \text{salicylate} + \text{hypochlorite} \xrightarrow{\text{Nitroprusside}} \text{2, 2-Dicarboxyindophenol}$$

REAGENT COMPOSITION

UREA REAGENT 1

Phosphate buffer 120 mmol/L

Sodium Salicylate 60 mmol/L

Sodium nitroprusside 5 mmol/L

EDTA 1 mmol/L

Urease 5 KU/L

UREA REAGENT 2

Phosphate buffer 120 mmol/L

Sodium Hydroxide 400 mmol/L

Sodium Hypochlorite 10 mmol/L

UREA STANDARD

Urea standard concentration 80 mg/dL or 13.3mmol/L

PROCEDURE

Pipette into cuvettes	Blank	Standard	Sample
Reagent-1	1000 µL	1000 µL	1000 µL
Sample	--	--	10 µL
Standard	--	10 µL	--
Mix and incubate for 5 minutes at 20-25°C or 3 minutes at 37°C			
Reagent-2	1000 µL	1000 µL	1000 µL
Mix and incubate for 10 minutes at 20-25°C or 5 minutes at 37°C Measure the absorbance of the sample (As) and the standard (Astd) against the reagent blank			

CALCULATION

$$\text{Urea Conc. (mg/dL)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 80 \text{ (Std.conc.)}$$

Urea (g/24 urine) = mg/dL X volume of 24-hour urine

To convert mg/dL to mmol/L divide by 6.01

Creatinine

CLINICAL SIGNIFICANCE

Creatinine is formed in muscles from Phospho Creatinine. It is an important form of energy, being a store of high-energy phosphate. Creatinine determinations have one advantage over Urea determination that it is not affected by a high protein diet. Serum Creatinine is more specific & sensitive indicator of renal function. Simultaneous estimations of serum Urea & Creatinine provide better information. Serum Urea nitrogen, Creatinine ratio is > 15 in pre renal failure, & < 10 in renal failure. Decreased levels are found in muscle dystrophy. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE

In the Jaffe reaction, Creatinine react with alkaline picrate to produce a reddish - orange color the intensity of which at 520 nm is directly proportional to the Creatinine concentration.

Alkali

Creatinine + sodium picrate ----------> Creatinine - picrate complex (reddish orange color)

REAGENT COMPOSITION

Creatinine R1= Picric acid 35 mmol/L

Creatinine R2 = Sodium Hydroxide 320 mmol/L

Creatinine R3 = Creatinine Standard 2 mg/dL or 177 µmol/L

Creatinine R4 = Trichloroacetic Acid 1.2 mol/L

PROCEDURE

Pipette into cuvettes	Blank	Standard	Sample
Distilled Water	1000 µL	---	---
Standard	---	1000 µL	---
Sample	---	---	1000 µL
TCA	1000 µL	1000 µL	1000 µL
Mix well and centrifuge at 5000 rpm X 10 minutes			
supernatant	1000 µL	1000 µL	1000 µL
Working reagent	1000 µL	1000 µL	1000 µL
Mix well and wait exactly 20 minutes after adding working reagent, read absorbance against reagent blank.			

CALCULATION

$$\text{Serum Creatinine (mg/dL)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 2 \text{ (Std.conc.)}$$

To convert mg/dL to µmol/L multiply by 88.4

NORMAL RANGE

Serum Creatinine

Male	0.7-1.4 mg/dL	62-124 µmol/L
Female	0.7-1.2 mg/dL	62-106 µmol/L

Creatinine Clearance

The relationship between creatinine and kidney function is curvilinear with a greater decline in kidney function occurring as serum creatinine rises from 1 to 2 mg/dL as compared to from 4 to 5 mg/dL.

The curvilinear nature of this relationship results in the need to estimate kidney function using an equation that takes into account factors that reflect underlying muscle mass (and

subsequently creatinine generation) that have been developed where kidney function has been actually measured.

Patients with decreased eGFR have kidney disease and are at higher risk of both acute kidney injury and progressive kidney disease. Management of modifiable risk factors, such as blood sugar and blood pressure control in diabetes mellitus and hypertension, is critical to slowing progression.

Medications should be dose-adjusted for the most recent available estimate of kidney function. In this setting, eGFR and creatinine clearance may be calculated and used similarly depending on the equation utilized when the medication was being developed, though they are physiologically different terms. Cutoffs for many medications are <60, <45 and <30 mL/min/1.73m^2, as well as adjustments for advanced kidney disease and dialysis patients.

Cockcroft-Gault Equation

The Cockcroft-Gault formula estimates creatinine clearance to clinically approximate glomerular filtration rate (GFR). Creatinine clearance, however, may over-estimate GFR by 10-20% and has largely been replaced clinically by equations to more directly estimate GFR.

Further, the Cockcroft-Gault equation uses body weight and is even less accurate in weight extremes (underweight and particularly overweight/obesity).

CrCl, mL/min = (140 – age) × (weight, kg) × (0.85 if female) / (72 × Cr, mg/dL)

CrCl=Creatinine clearance, Cr=creatinine

Estimation of GOT, GPT and ALP

GOT

CLINICAL SIGNIFICANCE

The Aspartate aminotransferase (AST/GOT) a cellular enzyme, it is present in most of the tissues. Especially in cardiac muscle, liver cells, skeletal muscle & kidneys. Injury to these tissues results in the release of the enzyme in blood stream. Increased levels are found in myocardial infarction. The duration & extent of increase is related to the infract. GOT determination is of considerable value to differentiate myocardial infraction from other cardiac disorders. Increased levels are also found in various types of liver disease, skeletal muscle trauma & in renal diseases. Decreased levels may be found in pregnancy, Beriberi & Diabetic ketoacidosis.

PRINCIPLE

The AST/GOT catalyzes the transfer of the amino group from L-aspartate to 2-Oxoglutarate to yield oxaloacetate and L-glutamate. The oxaloacetate undergoes reduction with simultaneous oxidation of NADH to NAD+ in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly

proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

$$\text{L-Aspartate + 2-Oxoglutarate} \xrightarrow{\text{AST/GOT}} \text{Oxaloacetate + L-Glutamate}$$

$$\text{Oxaloacetate + NADH + } H^+ \xrightarrow{\text{MDH}} \text{L-Malate + } NAD^+$$

REAGENT COMPOSITION

REAGENT 1 (ENZYME REAGENT)	
Tris pH 7.8	80 mmol/L
L-Aspartate	240 mmol/L
MDH	> 600 U/L
LDH	≥ 600 U/L
REAGENT 2 (SUBSTRATE)	
2-Oxoglutarate	12 mmol/L
NADH	0.18 mmol/L

PROCEDURE

SUBSTRATE START

Temperature	25°C or 30°C	37°C
Reagent 1 Buffer	1000 µL	1000 µL
Sample	200 µL	100 µL
Mix incubates for approx... 1 min, then add,		
Reagent 2 Substrates	250 µL	250 µL

SAMPLE START

Mono reagent (R1+R2)	1000 µL	1000 µL
Sample	200 µL	100 µL

READING FOR BOTH

Mix and read absorbance after 1 min and start stop watch.
Read absorbance again after 1, 2 and 3 min.

CALCULATION

Multiply factor from table below with ΔA/min,

Substrate start	**25°C / 30°C**	**37°C**
340 nm	1151	2143
334 nm	1173	2184
365 nm	2132	3971
Sample start	**25°C / 30°C**	**37°C**
340 nm	952	1745
334 nm	971	1780
365 nm	1765	3235

GPT

CLINICAL SIGNIFICANCE

Alanine Aminotransferase (ALT or GPT) is present in high concentrations in the liver and to a lesser extent in kidney, heart and skeletal muscle, pancreas, spleen and lung. Increased levels of ALT however are generally a result of liver disease associated with some degree of hepatic necrosis such as cirrhosis, carcinoma, viral or toxic hepatitis and obstructive jaundice. Characteristically ALT is generally higher than AST in acute viral or toxic hepatitis, whereas for most patients with chronic hepatic disease, ALT levels are generally lower than AST levels. Elevated ALT levels have also been found in extensive trauma and muscle disease, circulatory failure with shock, hypoxia, myocardial infarction and haemolytic disease.

PRINCIPLE

The amino group is enzymatically transferred by ALT present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate. Pyruvate is reduced to lactate by LDH present in the reagent with the simultaneous oxidation of NADH to NAD+. The reaction is monitored by measuring the rate of decrease in absorbance at 340nm due to the oxidation of NADH.

$$\text{L-Alanine} + \text{2-Oxoglutarate} \xrightarrow{\text{ALT/GPT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{D-Lactate} + \text{NAD}^+$$

REAGENT COMPOSITION

REAGENT 1 (enzyme reagent)	
Tris pH 7.5	100 mmol/L
L-Alanine	500 mmol/L
LDH	≥ 1200 u/L
REAGENT 2 (substrate)	
2-Oxoglutarate	15 mmol/L
NADH	0.18 mmol/L

PROCEDURE

SUBSTRATE START

Temperature--→	25°C or 30°C	37°C
Reagent 1 Buffer	1000 µL	1000 µL
Sample	200 µL	100 µL
Mix incubates for approx... 1 min, then add,		
Reagent 2 Substrates	250 µL	250 µL

SAMPLE START

Mono reagent (R1+R2)	1000 µL	1000 µL
Sample	200 µL	100 µL

CALCULATION

Multiply factor from table below with ΔA/min,

Substrate start	**25°C / 30°C**	**37°C**
340 nm	1151	2143
334 nm	1173	2184
365 nm	2132	3971
Sample start	**25°C / 30°C**	**37°C**
340 nm	952	1745
334 nm	971	1780
365 nm	1765	3235

ALP

CLINICAL SIGNIFICANCE

Alkaline Phosphatase (ALP) is widely distributed throughout the body, but clinically important for diagnostic reasons are in bone, liver, placenta & intestine. Growing bone is associated with the release of ALP and so in childhood the level of ALP is around 3 times of that of adult. During pregnancy in 2nd & 3rd trimester the enzyme rises considerably due to placenta releasing ALP. It can be used to examine placental function. Elevated levels are seen in bone diseases, E.g., pagets disease, rickets, osteoblastic metastatic & in obstructive disease of biliary tract. Decreased levels are rarely seen. E.g., in Vitamin A resistant rickets.

PRINCIPLE

Alkaline Phosphatase (ALP) catalyses the hydrolysis of P-Nitrophenyl phosphate (p-Npp) at pH 9.8, liberating p-Nitrophenyl and phosphate, according to the following reaction:

$$\text{p-Npp} + H_2O \xrightarrow{\text{ALP}} \text{P-Nitrophenol} + \text{Phosphate}$$

The rate of p-Nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of alkaline Phosphatase present in the sample ز

REAGENT COMPOSITION

ALP BUFFER R1	
Diethanolamine	1.0 mol/l
Magnesium chloride	0.5 mmol/l
ALP SUBSTRATE R2	
P-Nitrophenylphosphate	10 mmol/l

ASSAY

Wavelength	:	405 nm
Cuvette	:	1 cm light path
Temperature	:	25°C/ 30°C/37°C
Measurement	:	Against distilled water or air

PROCEDURE:

- **SUBSTRATE START**

Reagent 1 Buffer	1000 µL
Sample	20 µL
Mix incubates for approx... 1 min, then add	
Reagent 2 Substrates	250 µL

- **SAMPLE START**

Mono reagent (R1+R2)	1000 µL
Sample	20 µL

READING FOR BOTH

Mix and read absorbance after 1 min and start stop watch.
Read absorbance again after 1, 2, 3 min.

SUBSTRATE START

ALP activity U/L = ΔA/min. X 3433

SAMPLE START

ALP activity U/L = ΔA/min. X 2757

Estimation of Bilirubin (direct and indirect)

Total Bilirubin

CLINICAL SIGNIFICANCE

Bilirubin is a breakdown product of hemoglobin. It is transported from the spleen to the liver and excreted into bile. Hyperbilirubinemia results from the increase of bilirubin concentrations in plasma. Causes of hyperbilirubinemia: Total bilirubin: Increase hemolysis, genetic errors, neonatal jaundice, ineffective erythrpoiesis, and drugs. Direct bilirubin: Hepatic cholestasis, genetic errors, hepatocellular damage1,6,7. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions presents in serum, bilirubin-glucuromide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulphoxide

(DMSO) to react (bilirubin indirect). In the determination of indirect bilirubin, the direct is also determined, the results correspond to total bilirubin. The intensity of the color formed is proportional to the bilirubin concentration in the sample.

Total Bilirubin Reagent (R1)

Sulphanilic Acid = 30 mmol/L

Hydrochloric = Acid 50 mmol/L

Dimethylsulphoxide (DMSO) = 7 mol/L

Total Bilirubin, Nitrite Reagent (R2)

Sodium Nitrite = 29 mmol/L

PROCEDURE

1. Assay condition:

Reaction type	End point
Wavelength	555 nm (530-580)
Temperature	15-25 °C
Measurement	Against sample blank (without nitrite)

2. Adjust the instrument to zero with distilled water.

3. pipette into a cuvette:

	Blank	Sample
Total Bilirubin Reagent (R1)	1.5ml	1.5ml
Total Bilirubin, Nitrite reagent (R2)	--	50 µL
Sample	100 µL	100 µL

4. Mix and stand for exactly '10' minutes at room temperature.

5. Measure the absorbance of sample Blank (As).

CALCULATION

- **WITH CALIBRATOR:**

$$\frac{(\mathrm{A})\ Sample - (A)Sample\ Blank}{(\mathrm{A})\ Calibrator - (A)Calibrator\ Blank} x\ Conc.Calibrator = mg/dl\ bilirubin$$

-With Factor:

$$(\boldsymbol{A})\boldsymbol{Sample} - (\boldsymbol{A})\ \boldsymbol{Sample\ Blank\ x\ Factor = mg\ /\ (dl\ bilirubin\ in\ the\ sample)}$$

$$Factor = \frac{\text{Concentration of Calibrator}}{(\mathrm{A})\ Calibrator - (A)Calibrator\ Blank}$$

$$Conversion\ factor: mg/dl\ x\ 17.1\ \mu mol/L$$

Direct Bilirubin

CLINICAL SIGNIFICANCE

Bilirubin is a breakdown product of hemoglobin. It is transported from the spleen to the liver and excreted into bile. Hyperbilirubinemia results from the increase of bilirubin concentrations in plasma. Causes of hyperbilirubinemia: Total bilirubin: Increase hemolysis, genetic errors, neonatal jaundice, ineffective erythrpoiesis, and drugs. Direct bilirubin: Hepatic cholestasis, genetic errors, hepatocellular damage1,6,7 Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions presents in serum, bilirubinglucuromide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulphoxide (DMSO) to react (bilirubin indirect). In the determination of indirect bilirubin, the direct is also determined; the results correspond to total bilirubin. The intensity of the color formed is proportional to the bilirubin concentration in the sample1,2,3

REAGENT COMPOSITION

Direct Bilirubin Reagent (R1)	
Sulphanilic Acid	30 mmol/L
Hydrochloric acid (HCl)	150 mmol/L
Direct Bilirubin, Nitrite Reagent (R2)	
Sodium nitrite	29 mmol/L

PROCEDURE

1.ASSAY Condition

Wavelength	555 nm (530-580)
Temperature	15-25 °C
Measurement	Against sample blank (without nitrite)

2. Adjust the instrument to zero with distilled water.

3.Pipette into a cuvette

	Blank	Sample
direct Bilirubin Reagent (R1)	1000 µL	1000 µL
direct Bilirubin, Nitrite reagent (R2)	--	50 µL
Sample	100 µL	100 µL

4.Mix and stand for exactly '10' minutes at room temperature.

5. Measure the absorbance of sample Blank (As).

CALCULATION

-With Calibrator:

$$\frac{(\mathrm{A})Sample - (A)Sample\ Blnk}{(\mathrm{A})Calibrator - (A)Calibrator\ Blank}\ x\ Conc.Calibrator = mg/dl\ of\ bilirubin\ in\ the\ sample$$

-With Factor :

$$\big((A)\boldsymbol{Sample} - (A)\boldsymbol{Sample\ \ Blank}\big)\boldsymbol{x\ Factor} = \boldsymbol{mg/dl}\ \ of\ bilirubin\ in\ the\ sample$$

$$Factor = \frac{\text{Concentration of Calibration}}{(\text{A})Calibrator - (A)Calibrator\ \ Blank}$$

Conversion Fctor : $\boldsymbol{mg/dl\ x\ 17.1\ \mu\ mol.}$

Estimation of amylase enzyme

PRINCIPLE

In this direct method α-amylase catalyzes the hydrolysis of 2chloro-p-nitrophenyl-α-Dmaltotrioside (CNP-G3) substrate at pH 6.0 forming 2-chloro-p-nitrophenol (CNP) and free glycosides. The reaction is monitored kinetically at 405 nm by the rate of formation of the colored CNP produced, proportional to the activity of the αamylase in the sample.

$$10\ \text{CNP-G3} \xrightarrow[\text{pH 6.0}]{\alpha\text{-AMYLASE}} 9\ \text{CNP} + 1\text{CNP-G2} + \text{G3} + \text{G}$$

REAGENT COMPOSITION

R1 Monoreagent:

MES buffer	100 mmol/L (pH 6.0)
Calcium acetate	6 mmol/L
Sodium chloride	350 mmol/L
Potassium thiocyanate	900 mmol/L
CNP-G3	2.27 mmol/L.
Stabilizers and detergents	<0.1%

PROCEDURE

1. Preincubate working reagent, samples and controls to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Reaction temperature	37ºC	
	Blank	Sample
R1.Monoreagent	1.0 mL	1.0 mL
Dist. Water or saline	25 µl	-
Serum/plasma	-	25 µl

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
   Incubate at 37ºC for 1 minute and record initial absorbance reading.
5. Read the absorbance (at 405nm) exactly after 1, 2 and 3 minutes.
6. Calculate the difference between absorbances.
7. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

CALCULATIONS

Serum, plasma

U/L = ΔA/min x 3178

If results are to be expressed as SI units apply:
U/L x 0.01667 = µkat/L.

Estimation of triglyceride and cholesterol

Triglyceride

CLINICAL SIGNIFICANCE

Triglycerides are fats that provide energy for the cell. Like cholesterol, they are delivered to the body's cells by lipoproteins in the blood. A diet with a lot of saturated fats or carbohydrates will raise the triglyceride levels. The increases in serum triglycerides are relatively non-specific. For example liver dysfunction resulting from hepatitis, extra hepatic biliary obstruction or cirrhosis, diabetes mellitus is associated with the increase3,6,7 . Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE

Sample triglycerides incubated with lipoproteinlipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3- phosphate (G3P) and adenosine-5-diphosphate

(ADP) by glycerol kinase (GK) and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate oxidase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H2O2). In the last reaction, hydrogen peroxide (H2O2) reacts with 4-aminophenazone (4-AP) and pchlorophenol in presence of peroxidase (POD) to give a red colored dye:

Triglycerides + H2O $\xrightarrow{LPL}$ Glycerol + free fatty acids

Glycerol + ATP $\xrightarrow{Glycerolkinase}$ G3P+ ADP

G3P + O2 $\xrightarrow{GPO}$ DAP + H2O2

H2O2 + 4-AP + p-Chlorophenol $\xrightarrow{POD}$ Quinone + H2O

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

REAGENT COMPOSITION

R	GOOD PH 6.3 p-Chlorophenol Lipoprotein(LPL) Glcerol kinase (GK) Glycerol – 3- oxidase (GPO) Peroxidase (POD) 4-Aminophena\one (4 – AP) ATP	50 mmol/L 2 mmol/L 150000 U/L 500 U/L 3500 U/L 440 U/L 0.1 mmol/L 0.1 mmol/L
TRIGLYCERIDE CAL	Aqueous primary standard	200 mg/dl

PROCEDURE

1.ASSAY CONDITION :

Wavelength 505 NM (490-550)

Temperature 15-25 °C/ 37°C

2. Adjust the instrument to zero with distilled water.

3. Pipette into acuvette

	Blank	standard	Sample
R(ml)	1	1	1
Standard (μL)	--	10	--
Sample	--	--	10

4. Mix and incubate for 5 min at 37ºC or 10 min at 15-25ºC.

5. Read the absorbance (A) of the samples and standard, against the Blank. The colour is stable for at least 30 minutes.

CALCULATION

$$\frac{(\mathrm{A})\ Sample - (A)Sample\ Blank}{(\mathrm{A})\ Calibrator - (A)Calibrator\ Blank}\ x\ Conc.Calibrator = mg/dl\ triglycerides$$

$Conversion\ factor{:}mgdl\ x\ 0.0113\ \mu mol/L/$

Cholesterol

CLINICAL SIGNIFICANCE

Cholesterol is a fat-like substance called a lipid that is found in all body cells. The liver makes all of the cholesterol the body needs to form cell membranes and to make certain hormones. The determination of serum cholesterol is one of the important tools in the diagnosis and classification of lipemia, High blood cholesterol is one of the major risk factors for heart disease.

PRINCIPLE

Cholesterol Esterase (CHE) catalysis the hydrolysis of cholesterol esters, to produce cholesterol, which is oxidized by Cholesterol Oxidase (CHO) to yield Hydrogen Peroxide (H2O2), In a coupled reaction catalyzed by peroxidase (POD), Quinonimine dye (red) is formed from (H2O2), 4 Amino Antipyrine (4-AA) and phenol. The absorbance of the dye at 546nm is proportional to the concentration of cholesterol in the sample.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acid.}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholesterol -3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

REAGENT COMPOSITION

Cholesterol Reagent	
Pipes buffer, (pH 6.80)	50 mmol/L
Phenol	5 mmol/L
4-Aminoantipyrine	0.25 mmol/L
Cholesterol Esterase	>350 U/L
Cholesterol Oxidase	>140 U/L
Peroxidase	>10 U/L
Cholesterol Standard	
Cholesterol standard concentration	200 mg/dL or 5.14 mmol/L

PROCEDURE

Pipette into cuvettes	Blank	Standard	Sample
Cholesterol reagent	1000 µL	1000 µL	1000 µL
Standard	--	10 µL	--
Sample	--	--	10 µL
Mix and incubate for 10 minutes at 20-25°C or 5 minutes at 37°C Measure the absorbance of the sample (As) and the standard (Astd) against the reagent blank			

CALCULATION

$$\text{Cholesterol Conc. (mg/dL)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \text{ X 200 (Std.conc.)}$$

To convert mg/dL to mmol/L divide by 38.9

Estimation of HDL and LDL and VLDL

HDL

CLINICAL SIGNIFICANCE

Cholesterol is a fatty substance found in blood, bile and brain tissue, it serves as precursor to bile acids, steroids and vitamin D. The concentration of total cholesterol in serum has been associated with metabolic, infectious and coronary diseases. In the plasma, cholesterol is transported by three lipoprotein, high density lipoprotein (HDL-Cholesterol), low density lipoprotein (LDL-Cholesterol) and very low-density lipoprotein (VLDLCholesterol). Castelli and co-workers have indicated that an inverse relationship exists between serum HDL-cholesterol and the risk of coronary heart disease. The measurement of total and HDL cholesterol and triglycerides provide valuable information for the prediction of coronary heart disease and for the lipoprotein phenotyping.

PRINCIPLE

Phosphotungstic acid and magnesium ions specifically precipitate low and very low-density lipoproteins (LDL and VLDL). After centrifugation the cholesterol content of the high-density lipoproteins (HDL) in the supernatant can be determined using Bio Research Cholesterol test kit (Product Code:CS005).

REAGENT COMPOSITION

HDL Cholesterol (Liquid) Reagent

Phosphotungstic acid	0.55 mmol/L
Magnesium Ion	25 mmol/L

PROCEDURE (Step 1):

	MACRO	SEMI MACRO
Sample	**500 µL**	**200 µL**
HDL reagent undiluted	**1000 µL**	**--**
HDL reagent diluted	**--**	**500 µL**
Mix and allow to stand for 10 minutes. Centrifuge for 10 minutes at 4000rpm. Determine the cholesterol content of the HDL supernatant by **using Bio Research Cholesterol test kit (Product Code: CS005).**		

ASSAY

Wavelength : 546nm
Cuvette : 1 cm light path
Temperature : 20-25°C or 37°C
Measurement Against reagent blank

PROCEDURE (Step 2):

Pipette into cuvettes	Blank	Sample
Distilled H_2O	100 µL	---
HDL supernatant	---	100 µL
Cholesterol reagent	1000 µL	1000 µL
Mix and incubate for 20 minutes at 25°C or 10 minutes at 37°C Measure the absorbance of sample against the reagent blank within 30 minutes (ΔA).		

CALCULATION

HDL Cholesterol Conc. (mg/dL) = ΔA X Factor

FACTOR

MACRO	SEMI-MACRO
274 mg/dL	**320 mg/dL**
7.05 mmol/L	**8.23 mmol/L**

To convert mg/dL to mmol/L divide by 38.9

LDL

CLINICAL SIGNIFICANCE

Blood total cholesterol levels have long been known to be related to coronary heart disease (CHD). In recent years, in addition to total cholesterol, low density lipoprotein cholesterol (LDL-C) has become an important tool used to assess an individual risk of developing CHD since a strong positive relationship between LDL-C concentration and the incidence of CHD was reported. LDL Cholesterol acts as a key factor in the pathogenesis of atherosclerosis and coronary artery disease

PRINCIPLE

The Cholesterol content of low-density lipoproteins (LDL) can be extrapolated using the Friedwald equation.

LDL Cholesterol (mg/dL) = Total Cholesterol- (Triglyceride) – HDL
5

LDL Cholesterol (mmol/L) = Total Cholesterol- (Triglyceride) – HDL
2.2

5: 1 ratio exists between plasma triglycerides & VLDL Cholesterol over a broad range. If the total triglyceride is greater than 400 mg / dl, this approximation for VLDL Cholesterol is no longer valid and the sample will need to be diluted.

PROCEDURE

Pipette into cuvettes	Blank	Standard	Sample
Cholesterol reagent	1000 µL	1000 µL	1000 µL
Standard	--	10 µL	--
Sample	--	--	10 µL
Mix and incubate for 10 minutes at 20-25°C or 5 minutes at 37°C Measure the absorbance of the sample (As) and the standard (Astd) against the reagent blank			

CALCULATION

$$\text{Cholesterol Conc. (mg/dL)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \text{ X 200 (Std.conc.)}$$

To convert mg/dL to mmol/L divide by 38.9

VLDL

Serum VLDL estimation is usually done indirectly, rather than by direct measurement, as part of a routine lipid profile.

The most common method is based on the Friedewald formula, which assumes that VLDL cholesterol carries most of the circulating triglycerides in the fasting state:

VLDL-C (mg/dL) = Triglycerides / 5

or VLDL-C (mmol/L) = Triglycerides / 2.2

This estimation is valid only when the patient is fasting and when serum triglycerides are < 400 mg/dL, because chylomicrons and very high triglyceride levels distort the relationship. Elevated VLDL levels are typically associated with hypertriglyceridemia, metabolic syndrome,*type 2 diabetes mellitus, and cardiovascular risk. Direct VLDL measurement using ultracentrifugation or advanced lipid testing is reserved for research or specialized clinical settings.

Estimation of total protein and Albumin/Globulin Ratio

Total Protein

CLINICAL SIGNIFICANCE

Proteins form the major portion of dissolved substances in the plasma. They form the basic structural components of the body. They constitute the enzymes present in our body & also act as secondary source of energy. The other functions include distribution of water, buffering, transport of various components, defense & coagulation of blood in our body. Increased levels are found in dehydration & myeloma. Decreased levels are found in liver disorders, Nephrotic syndrome, malnutrition & protein due to haemorrhage.

PRINCIPLE

Protein in serum or plasma forms a blue/violet complex when mixed with copper ions in alkaline solution (Biuret reaction) each copper ion binding with 5 or 6 peptide bonds. Tartrate is added as a stabilizer and iodide is used to prevent auto reduction of the alkaline copper complex. The absorbance of this complex at 546 nm is proportional to the protein concentration.

REAGENT COMPOSITION

TOTAL PROTEIN (SL)

Sodium hydroxide	200 mmol/L
Potassium sodium tartrate	32 mmol/L
Copper Sulphate	18 mmol/L
Potassium Iodide	30 mmol/L

Protein Standard

Protein standard concentration	8 g/dL

ASSAY

Wavelength	546nm
Cuvette	1 cm light path
Temperature	20-25°C
Measurement	Against reagent blank

PROCEDURE

Pipette into cuvettes	Blank	Standard	Sample
T. Protein reagent	1000 µL	1000 µL	1000 µL
Standard	--	20 µL	--
Sample	--	--	20 µL
Mix and incubate for 5 minutes at 20-25°C (RT). Measure the absorbance of the sample (As) and the standard (Astd) against the reagent blank within 30 minutes.			

CALCULATION

$$\text{Total Protein Conc. (g/dL)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 8 \text{ (Std.conc.)}$$

Albumin/Globulin Ratio

The albumin globulin ratio expresses the balance between albumin and globulins in serum. Albumin acts as the main carrier protein and helps maintain oncotic pressure. Globulins include antibodies and transport proteins that support immunity and other functions.

Clinicians use the ratio as a quick screen to detect shifts in protein production or loss. The result does not diagnose a specific disease alone. Instead, clinicians treat it as a clue that guides further testing.

The **albumin–globulin (A/G) ratio** is a calculated parameter used to evaluate the relative proportions of albumin and globulins in serum and provides insight into liver function, nutritional status, and immune activity.

It is calculated as:
A/G ratio = Serum albumin ÷ Serum globulin,

where globulin is obtained by subtracting albumin from total protein.

The normal A/G ratio is approximately **1.0–2.0**, as albumin normally exceeds globulins in concentration.

A **decreased A/G ratio** may result from reduced albumin synthesis (e.g., liver disease), increased globulin production (e.g., chronic infections, autoimmune disorders, multiple myeloma), or protein loss.

An **increased A/G ratio** is less common and may be seen in conditions associated with low globulin levels, such as immunodeficiency states or leukemia.

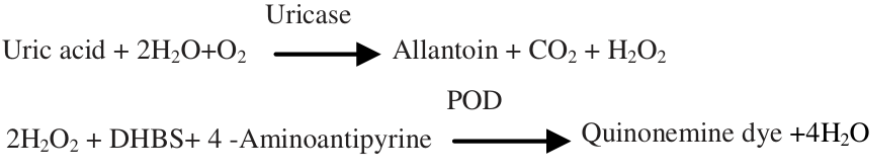
Estimation of uric acid

CLINICAL SIGNIFICANCE

Uric acid measurements are used in the diagnosis and treatment of numerous renal and metabolic disorders, including renal failure, gout, leukemia, psoriasis, starvation or other wasting conditions, and of patients receiving cytotoxic drugs.

PRINCIPLE

Uric Acid is the end product of purine metabolism. Its quantitation aids in the diagnosis of gout, renal dysfunction, diabetes and other condition, Uricase catalyze the oxidation of uric acid to Allantoin and H2O2. In the presence of Peroxidase (POD), H2O2 reacts with 4Aminoantipyrine and 3, 5, Dichloro-2-Hydroxybenzensulphonate (DHBS) to from Quinonemine dye, the concentration of which at 546 nm is directly proportional to the Uric Acid concentration.



REAGENT COMPOSITION

Uric Acid Reagent

Phosphate Buffer (pH7.5)	50 mmol/L
4-Aminoantipyrine	0.3 mmol/L
DHBS	4.0 mmol/L
Uricase	400 U/L
Peroxidase	100 KU/L

Uric Acid Standard

Uric Acid standard concentration	8 mg/dL or 476 µmol/L

ASSAY

Wavelength	546nm
Cuvette	1 cm light path
Temperature	20-25°C or 37°C
Measurement	Against reagent blank

PROCEDURE

Pipette into Cuvettes	Blank	Standard	Sample
Uric acid reagent	1000 µL	1000 µL	1000 µL
Standard	--	20 µL	--
Sample	--	--	20 µL
Mix and incubate for 10 minutes at 20-25°C or 5 minutes at 37°C Measure the absorbance of the sample (As) and the standard (Astd) against the reagent blank immediately.			

CALCULATION

$$\text{Uric acid Conc. (mg/dL)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \text{ X 8 (Std.conc.)}$$

To convert mg/dL to µmol/L multiply by 59.60

Estimation of Total iron and Iron binding capacity

Total iron

CLINICAL SIGNIFICANCE

Following intestinal absorption of iron or erythrocyte destruction, iron ions are released into the plasma where they bind to either apotransferrin or apoferritin proteins to form transferrin and ferritin, respectively. The former helps transport iron to bone marrow for erythropoiesis; the latter stores iron in tissues, until is needed. An increase in the iron level in plasma due to rapid destruction of erythrocytes or excesive uptake of iron may also lead to iron overload. The latter causes iron deposition disorders in tissue known as hemosiderosis or hemochromatosis. Conversely, a decrease in the iron level in plasma due to malnutrition or malapsorption may lead to excesive depletion in iron storage, resulting in anemia such as irondeficiency anemia.

PRINCIPLE

The Fe+3 bound to serum ferritine once dissociated in a week-acid medium by Teepol and guanidium chloride, is reduced by hydroxylamine to Fe+2, forming the ferrous ion a colored complex with FerroZine® proportional to the concentration of iron present in the sample.

$$\boldsymbol{Transferrin} - Fe^{2} \xrightarrow[\text{Teepol}]{PH\ <5} Apotransferrin$$

$$\boldsymbol{Fe^{3} + Hydroxylamine} \longrightarrow \boldsymbol{Fe^{2}}$$

$$\boldsymbol{Fe^{2} + FerroZine} \longrightarrow \textbf{Fe (FerroZine)}\boldsymbol{3^{2}}\ \textbf{complex}$$

REAGENT COMPOSITION

Iron REAGENT 1 (Buffer)

Guanidine chloride	1.0 mol/L
hydroxylamine	0.6 mol/L
acetate buffer	400mmol/L
pH	4.0

Iron REAGENT 2

(Chromgen)	
FerroZine	8 mmol/L
Sodium acetate	400 mmol/L

PROCEDURE

1. Bring the Reagent to room temperature.
2. Pipette into cuvettes

Tubes	Reagent Blank	Sample blank	Sample	CAL Standard
Distilled water	200 µL			
Standard				200 µL
Sample	--	200 µL	200 µL	-
Reagent(1)	--	1000 µL	--	--
working reagent	1000 µL		1000 µL	1000 µL

3. Mix and let the tubes stand 5 minutes at room temperature.
4. Read the absorbance (A) of the sample blank at 560 nm against distilled water.
5. Read the absorbance (A) of the samples and the standard at 560 nm against the reagent blank.

CALCULATION

The iron concentration in the sample is calculated using the following general formula:

$$\frac{\text{A Sample} - \text{A Sample blank}}{\text{A Standard}} \; x\; C\; Standard = \mu g/dl\; iron$$

Samples with concentrations higher than 1000 µg/dL should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

If results are to be expressed as SI units apply: µg/dL x 0.179 = µmol/L

Iron binding capacity

BACKGROUND

The serum total iron-binding capacity (TIBC) represents the maximum concentration of iron that can be bound by an individual's serum protein. Determination of TIBC is one of several commonly used assays in assessment of iron status and TIBC is highly correlated with serum transferrin (the primary serum iron transport protein) because > 95% of serum nonheme iron is bound by transferrin. Usually, only 30 % of the available serum iron-binding sites are occupied, and changes in ratio of serum iron to TIBC reflec changes in the body iron stores.

ASSAY PRINCIPLE

In the first step, the serum sample is added to reagent 1 (R1). R1 contains iron as ferric ion in sufficient quantity to saturate the highest anticipated TIBC in a complex with an excess of chromazurol B in acetate buffer ar pH 4.8. When the serum sample is added, the serum iron is released from transferrin because of the low pH. The iron from sample then forms a complex with the remaining excess of chromazurol B,increasing the absorbance. In the second step, reagent 2 (R2) which is strongly buffered is added. The affinity of transferrin for iron increases and the transferrin extracts iron from the iron-dye complex, decreasing the absorbance. The decrease in absorbance is directly proportional to TIBC.

Reagents

Reagent 1 (R1)

Acetate Buffer = pH 4.8 0.4 mol/L

Chromazurol B = 300 □mol/L

Surfactant = 0.1 %

Non active ingredients.

Reagent 2 (R2)

MOPs buffer pH 8.0 = 100 mmol/L

Procedure

	Calibrator Blank	Calibrator	Sample Blank	Sample
Reagent1	500 µl	500 µl	500 µl	500 µl
Calibrator	40 µl	40 µl	-----	-----
Sample	-----	-----	40 µl	40 µl

Mix and incubate for 5 min, at 37 °C, then add R2

Reagent 2	-----	150 µl	-----	150 µl

Mix and incubate for 7 minutes then read the absorbance of the Calibrator against Calibrator Blank and absorbance of sample against sample Blank.

Calculation

$$\text{Total iron binding capacity} = \frac{A_{sample}}{A_{calibrator}} \times \text{calibrator Conc.}$$

Estimation of HbA1c and BMI

HbA1c

CLINICAL SIGNIFICANCE

Throughout the circulatory life of the red cell, Hemoglobin A1c is formed continuously by the adduction of glucose to the N-terminal of the hemoglobin beta chain. This process, which is non-enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period. In a classical study, Trivelli et al1 showed Hemoglobin AB1c in diabetic subjects to be elevated 2-3 fold over the levels found in normal individuals. Several investigators have recommended that Hemoglobin A1c serve as an indicator of metabolic control of the diabetic, since Hemoglobin A1c levels approach normal values for diabetics in metabolic control.2,3,4 Hemoglobin A1c has been defined operationally as the "fast fraction" hemoglobins (Hb1A, A1B, A1c) that elute first during column chromatography with cation-exchange resins. The non-glycosylated hemoglobin, which consists of the bulk of the hemoglobin has been designated HbA0. The present procedure utilizes an antigen and antibody reaction to directly determine the concentration of the HbAB1c.

PRINCIPLE

This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbAB1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbAB1c monoclonal antibody is added (R2), latex HbA1cmouse anti human HbAB1c antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of latex particles. The amount of agglutination is measured as absorbance. The HbAB1c value is obtained from a calibration curve.

REAGENTS

(R1)	Latex 0,13%, Buffer, stabilizer.
(R2)	Mouse anti-human HbA1c monoclonal antibody 0,05mg/ml, goat anti-mouse IgG polyclonal antibody 0,08mg/dl, Buffer, stabilizers
(R3) (Hemolysis Reagent)	Water and stabilizers

PROCEDURES

1. Assay conditions:
   Wavelength: 660 nm (600 - 660)
   Temperature: 37ºC
   Cuvette ligth path: 1 cm
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette: (Note 2)

R1 (µl)	360
Calibrator or Sample (µl)	10

4. Mix and incubate 5 minutes.
5. Pippete into the cuvette:

R2 (µl)	120

6. Mix and read the absorbance after 5 minutes (A) of the R2 addition.

CALCULATIONS

HbA1c concentration (%): Plot (A) obtained against the HbA1c concentration of each calibrator (1 to 4 Level). HbA1c percentage in the sample is calculated by interpolation of its absorbance (A) in the calibration curve.

BMI

Body Mass Index (BMI) is a simple anthropometric measure used to assess body weight relative to height and to classify nutritional status in adults. widely used in clinical and epidemiological settings to estimate obesity-related health risk, but it does not distinguish between fat and lean mass and may misclassify muscular individuals, the elderly, or those with altered body composition.

It is calculated as:
BMI = weight (kg) ÷ height² (m²)

According to WHO classification:

- **Underweight:** < 18.5 kg/m²
- **Normal weight:** 18.5–24.9 kg/m²
- **Overweight:** 25.0–29.9 kg/m²
- **Obesity:** ≥ 30.0 kg/m²

Estimation of LDH, CK, Cardiac Troponin and D-Dimers

LDH

CLINICAL SIGNIFICANCE

Lactate dehydrogenase (LDH) is an enzyme with wide tissue distribution in the body. The higher concentrations of LDH are found in liver, heart, kidney, skeletal muscle and erythrocytes. Increased levels of the enzyme are found in serum in liver disease, myocardial infarction, renal disease, muscular dystrophy and anemia. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE

Lactate dehydrogenase (LDH) catalyses the reduction of pyruvate by NADH, according the following reaction:

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-lactate} + NAD^+$$

REAGENT COMPOSITION

REAGENT 1 (BUFFER REAGENT)	
Tris buffer	80 mmol/L
Pyruvate	1.6 mmol/L
Sodium Chloride	200 mmol/L
REAGENT 2 (SUBSTRATE)	
NADH	0.15 mmol/L

ASSAY

Wavelength	:	340 nm, Hg 365 nm, Hg 334 nm
Cuvette	:	1 cm light path
Temperature	:	25°C/ 30°C/37°C

Adjust the instrument to zero with distilled water or air

PROCEDURE

SUBSTRATE START

Temperature--→	25°C or 30°C	37°C
Reagent 1 Buffer	1000 µL	1000 µL
Sample	20 µL	10 µL
Mix incubates for approx... 1 min, then add,		
Reagent 2 Substrates	250 µL	250 µL

SAMPLE START

Mono reagent (R1+R2)	1000 µL	1000 µL
Sample	20 µL	10 µL

READING FOR BOTH

Mix and read absorbance after 1 min and start stop watch.
Read absorbance again after 1, 2 and 3 min.

CALCULATION

Multiply factor from table below with ΔA/min,

Substrate start	**25°C / 30°C**	**37°C**
340 nm	10080	20000
334 nm	10275	20390
365 nm	18675	37060
Sample start	**25°C / 30°C**	**37°C**
340 nm	8095	16030
334 nm	8250	16345
365 nm	15000	29705

CK

CLINICAL SIGNIFICANCE

CK-MB is an enzyme formed by the association of two subunits from muscle (M) and nerve cells (B). CK-MB is usually present in serum at low concentration; it is increased after an acute infarct of myocardium and later descends at normal levels. Also is increased, rarely, in skeletal muscle damage5,6,7,8 . Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE

The procedure involves measurement of CK activity in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity of CKMM and half of the activity of CK-MB while not affecting the B subunit activity of CK-MB and CK-BB. Then it's used

the CK method to quantitatively determine CKB activity1,2. The CKMB activity is obtained by multiplying the CK-B activity by two.

REAGENT COMPOSITION

R1	**Imidazol, PH 6.7** **D-Glcose** **N-Acetyl- Cysteine** **Magnesium acetate** **NADP** **EDTA** **Heaokinase**	**125 mmol/L** **25 mmol/L** **25 mmol/L** **12.5 mmol/L** **2.52 mmol/L** **2.02 mmol/L** $\geq 6\ \ 800\ U/L$
Anti – human polyclonal CK -M antibody (sheep|) sufficient to inhibit up to 2000 U/L of CK-MM		
R2	**ADP** **AMP** **di-Adenosine -5- peentaphosphate** **Glucose – 6- phosphate dehydrogenase** **Creatine phosphate**	**15.2 mmol/L** **25 mmol/L** **103 mmol/L** $\geq 8\ \ 800\ U/L$ **250 mmol/L**

PROCEDURE

1.Assay condition:

Wavelength 340 nm
Cuvette 1 cm light path
Constant temperature 25°C / 30 °C/ 37 °C

2. Adjust the instrument to zero with distilled water or air.

3. Pipette into a cuvette:

WR (ml)	1.0
Sample (μL	40

4. Mix and incubate 10 minutes.

5. Read initial absorbance (A) of the sample, start the stopwatch and read again after 5 minutes (A2). 6. Calculate the difference between absorbances ΔA= A2 – A1.

CALCULATION

$\Delta A\ X\ 825 = U/L\ of\ CK-MB$ $\Delta A\ X\ 1651 = \frac{U}{L} of\ CK-MB$

Calculating factor in automatic analyzers by kinetic method (ΔA/min.) is 8255.

Units: One international unit (IU) is the amount of enzyme that transforms 1µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors

To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25 °C	30°C	37°C
25 °C	1.00	1.53	2.38
30 °C	0.65	1.00	1.56
37 °C	0.42	0.64	1.00

Cardiac Troponin

PRINCIPLE

The Troponin I Rapid Test Device (Whole Blood/Serum/Plasma) has been designed to detect cardiac Troponin I through visual interpretation of color development in the strip. The membrane was immobilized with anti-cTnI antibodies on the test region. During the test, the specimen is allowed to react with colored anti-cTnI antibodies colloidal gold conjugates,

which were precoated on the sample pad of the test. The mixture then moves on the membrane by a capillary action, and interact with reagents on the membrane. If there were enough cTnI in specimens, a colored band will form at the test region of the membrane. Presence of this colored band indicates a positive result, while its absence indicates a negative result. Appearance of a colored band at the control region serves as a procedural control. This indicates that proper volume of specimen has been added and membrane wicking has occurred.

PROCEDURE

Allow test device, specimen, buffer and/or controls to equilibrate to room temperature (15-30°C) prior to testing.

1. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. To obtain a best result, the assay should be performed within one hour.

2. Transfer 2 drops of serum or plasma to the specimen well of the device with a disposable pipette provided in the kit, and then start the timer.

OR Transfer 3 drops of whole blood specimen to the specimen well of the device with a disposable pipette provided in the kit, then add 1 drop of buffer, and start the timer. OR Allow 3 hanging drops of finger stick whole blood specimen to fall into the centre of the specimen well (S) on the device, then add 1 drop of buffer, and start the timer. Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window. As the test begins to work, you will see colour move across the membrane. Wait for the colored band(s) to appear. The result should be read at 10 minutes. Do not interpret the result after 20 minutes.

RESULTS

POSITIVE: Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

NEGATIVE: Only one colored band appears in the control region (C). No apparent colored band appears in the test region (T).

INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified reading time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

D-Dimers

CLINICAL SIGNIFICANCE

During blood coagulation, fibrinogen is converted to fibrin by the activation of thrombin. The resulting fibrin monomers polymerize to form a soluble gel of non-cross-linked fibrin. This fibrin gel is then converted to cross-linked fibrin by thrombin activated Factor XIII to form an insoluble fibrin clot. Production of plasmin, the major clot-lysing enzyme, is triggered when a fibrin clot is formed. Fibrinogen and fibrin are both cleaved by the fibrinolytic enzyme plasmin to yield degradation products, but only degradation products

from cross-linked fibrin contain D-Dimer. Therefore, cross- linked fibrin degradation products (XL-FDP) are a specific marker of fibrinolysis.

PRINCIPLE

D-Dimer Latex is a rapid agglutination assay utilizing latex beads coupled with a highly specific D-Dimer monoclonal antibody. XL-FDP present in a plasma sample bind to the coated latex beads, which results in visible agglutination occurring when the concentration of D-Dimer is above the threshold of detection of the assay.

PROCEDURE

 Equilibrate reagents to room temperature (20°C to 25°C) before use.

 Latex Reagent should be mixed by inversion immediately prior to use.

Qualitative Method

1. Bring reagents and specimens to room temperature before use.

2. Place 15 µL of the reagent within a well on a reaction slide. AVOID touching the surface of the Reaction slide.

3. Accurately pipette 15µL of undiluted plasma or of control solution inside the same well next to the drop of Latex Reagent.

4. Mix the Latex Reagent and sample with a stirrer until the Latex is uniformly distributed.

5. Rock the reaction slide gently by hand for exactly 3 minutes.

6. At exactly 3 minutes, check for agglutination under a strong light source.

NOTE

If test reading is delayed beyond 3 minutes, the latex suspension may dry out giving a false agglutination pattern. If this is suspected, the specimen must be retested.

Semi quantitative Method

1. Prepare serial dilutions of the test plasma with Buffer as follows:

1:2 dilution 100 µL plasma plus 100 µL Buffer solution

1:4 dilution 100 µL

1:2 dilution plus 100 µL Buffer solution

1:8 dilution 100 µL

1:4 dilution plus 100 µL Buffer solution

2. Test each dilution as described in the qualitative method.

RESULTS

1. Qualitative Assay

For the qualitative assay protocol, the following pattern of results should be obtained:

Undiluted Plasma D-Dimer (XL-FDP) concentration

Negative Less than 0.20 mg/L (200ng/mL) Positive
Greater than 0.20 mg/L (200ng/mL)

Note: All values in mg/L (ng/mL) are approximate

2. Semi quantitative Assay

Approximate levels of XL-FDP, containing the D-Dimer domain, for specimen dilutions are shown in Table below. As with all semi quantitative tests, some variability in dose-response can be expected.

Approximate Range of D-Dimer (XL-FDP) mg/L (ng/m)	**Sample Dilution**			
	Undil.	1:2	1:4	1:8
< 0.2 (< 200)	-	-	-	-
0.2 – 0.4 (200 – 400)	+	-	-	-
0.4 – 0.8 (400 – 800)	+	+	-	-
0.8 – 1.6 (800 – 1600)	+	+	+	-
1.6 – 3.2* (1600 – 3200*)	+	+	+	+

"+" = agglutination, "-" = no agglutination

* Levels of XL-FDP greater than 3.20 mg/L (3200 ng/mL) can be estimated by further dilutions beyond 1:8.