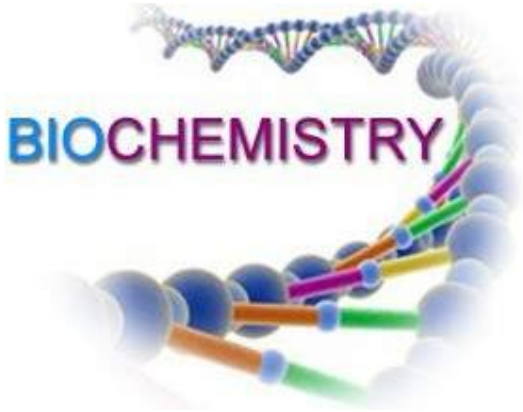
H.S.C (VOCATIONAL)

MEDICAL LABORATORY TECHNICIAN

STD: XII (PAPER-3)

Biochemistry

PRACTICALS



INDEX

Week No.	Week 1
Practical No.	1
Title/ Aim	**Preparation of molar solution**
Objectives	At the end of practical students shall be able to prepare 100 ml of 1M NaCl solution.
Principle	A molar solution is one litre solution of which contains 1 gm molecular of the substance. It is denoted as '1M'solution.When the weight of the solute dissolved in one litre of its solution is equal to its molecular weight it represents 1M solution. Molecular weight of a substance is obtained by adding the atomic weights of the elements in the proportion contained in the compound.Eg.NaCl. Atomic weight of Na=23 Atomic weight of Cl=35.5 Therefore molecular weight of NaCl=58.5
Requirements	NaCl powder, distilled water, analytical balance, volumetric flask
Environment	MLT laboratory
Procedure	1. Weigh out a clean and dry watch glass.Instead of watch glass butter paper can also be used. 2. Weigh 5.85 gms of sodium chloride by taking into account weight of watch glass/butter paper.some electronic balances can weigh after tearing and directly weigh the crystals. 3. Transfer the powder to 100 ml volumetric flask .With funnel addistilled/deionised

	water up to 3/4ths. Mix. Let the crystals dissolve completely. Add water up to 100 ml mark considering lower meniscus. 4. Stopper the flask. Swirl well. Transfer to reagent bottle.
Observations	• Weight to be taken =mol.wt.×strength reqd. ×vol. in litres. =58.5×1×1/10 =5.85 gms. • Exact solution volume and solute must be totally dissolved.
Result	One molar 100 ml solution is prepared.
Skills achieved	One molar solution is prepared
Skill evaluating criteria	Weighing of solute---3marks Solvent measurement---3 marks Checking for meniscus---2marks Dissolving solute completely-----2 marks.
FAQs	1. Define: Atomic weight, molecular weight, equivalent weight. 2. What do you understand by Molar solution?
Assignment/Activity	Prepare 5 different molar solutions?
Reference	Basic laboratory techniques

Week No.	Week 2
Practical No.	2
Title/ Aim	Preparation of Normal solutions:
Objectives	At the end of practical students shall be able to prepare Normal solution.
Principle	Exact normal solutions can be prepared by weight or by dilution only when a chemical is available in its purest form. Sodium carbonate and oxalic acid are available in pure form and so exact solutions of these is possible to prepare by weight. These are called primary standards.
Requirements	oxalic acid , sodium carbonate, distilled water, analytical balance, volumetric flask
Environment	MLT laboratory
Procedure	**<u>Preparation of primary standard sodium carbonate solution:</u>** Eg. To prepare 100 ml 0.1 N sodium carbonate solution. Mol.Wt of sodium carbonate=106 Its eq. wt.=106/2=53 Weight in gm. For 0.1 N ,100 ml solution=E x N xV =53x0.1x100/1000=0.53gms. *for procedure refer to molar solution preparation. **<u>Preparation of 100 ml oxalic acid 0.1 N solution</u>**. Mol.wt. of oxalic acid is=126.067 Its eq.wt.=126.067/2=63.033 Weight for 100 ml 0.1 N solution =ExNxV/1000 =63.033x0.1x100/1000=0.6303gm. *for procedure refer to molar solution preparation.
Observations	Exact solution volume and solute must be totally dissolved.

Result	Normal 100 ml solution is prepared.
Skills achieved	One normal solution is prepared
Skill evaluating criteria	Weighing of solute---3marks Solvent measurement---3 marks Checking for meniscus---2marks Dissolving solute completely----- 2 marks.
FAQs	1. Define: Atomic weight, molecular weight, equivalent weight. 2. What do you understand by Normal solution?
Assignment/Activity	Prepare 5 different normal solutions?
Reference	Basic laboratory techniques

Week No.	Week 3
Practical No.	3
Title/ Aim	**PREPARATION OF SATURATED SOLUTION**
Objectives	At the end of practical students shall be able to prepare saturated solution.
Principle	When a solid is dissolved in a liquid the solid is called as the solute and the liquid is called as the solvent. A saturated solution is one which holds as much solute it can.
Requirements	Sodium chloride, distilled water,beaker.
Environment	MLT laboratory
Procedure	<u>Preparation of saturated solution of sodium chloride.</u> 1. About 100ml of water is taken in a beaker. 2. Sodium chloride is added in small portion to this with constant stirring. 3. Continued the addition till some crystals are leftundissolved. Now the solution is saturated with sodium chloride, filled in a bottle and added one more spatula of the salt. 4. The bottle is labelled as "saturated sodium chloride solution" with date of preparation and name of the person prepared.
Observations	Exact solution volume. Some crystals must be undissolved.
Result	Saturated solution 100 ml is prepared.
Skills to be achieved	Students can prepare saturated solution accurately.

Skill evaluating criteria	Weighing of solute---3marks Solvent measurement---3 marks Checking for meniscus---2marks Dissolving solute completely-----2 marks.
FAQs	• Define: Atomic weight, molecular weight, equivalent weight. • What do you understand by Saturated solution?
Assignment/Activity	Prepare 5 different Saturated solutions?
Reference	Basic laboratory techniques

Week No.	Week 4
Practical No.	4
Title/ Aim	**Preparation of Percent solution**
Objectives	At the end of practical students shall be able to prepare percent solution.
Principle	A percent solution is one which contains a known weight of the substances in a specified volume of its solution. If the solute is a solid when it is percent solution wt/vol and if it is a liquid then it is vol/vol percent solution.
Requirements	NaCl crystals, deionised water, volumetric flask.
Environment	MLT laboratory
Procedure	Preparation of 5% (w/v)sodium chloride solution. Here 5 gms of NaCL are contained in 100 ml of its solution. 1. Take about 70 ml of deionised water in a beaker. 2. Exactly 5gms of sodium chloride are added to it. It is completely dissolved. 3. Transfer to a 100 ml volumetric flask or in a measuring cylinder with stopper. 4. The cylinder is stoppered, inverted and swirled several times for uniform mixing. 5. It is then filled to a reagent bottle and labeled properly
Observations	Exact solution volume and solute must be totally dissolved.
Result	5%percent solution (100 ml) is prepared.
Skills to be achieved	Students can prepare 5% percent solution accurately.

Skill evaluating criteria	Weighing of solute---3marks Solvent measurement---3 marks Checking for meniscus---2marks Dissolving solute completely-----2 marks.
FAQs	1. Define: Atomic weight, molecular weight, equivalent weight. 2. What do you understand by 5% percent solution?
Assignment/Activity	Prepare 5 different 5% percent solutions?
Reference	Basic laboratory techniques-handbook of biochemistry

Week No.	Week 5
Practical No.	5
Title/ Aim	**Preparation of buffer solution**
Objectives	At the end of practical students shall be able to preparebuffer solution.
Principle	A buffer solution is employed in some of the reactions which have to be carried at a particular pH. This is possible because buffer solution resists pH changes upon addition of small portions of acid or base. A buffer system consists of two chemicals in solution one of which is a weak acid and the other Na or K salt of the same acid.
Requirements	As per table.
Environment	MLT laboratory

Procedure

Acetate buffer is prepared by mixing sodium acetate and acetic acid. Buffer solutions are used in various enzymatic and other reactions. There are a variety of buffer mixtures like phosphate buffer, citrate buffer, carbonate-bicarbonate buffer, etc.

A buffer solution is labelled with the strength and the pH it maintains like, 0.2 M phosphate buffer, pH 7.8.

A table of preparation of different buffer mixtures is given below:

Phosphate buffer

Solution a - $\frac{M}{15}$ KH_2PO_4 *or* $\frac{M}{15}$ NaH_2PO_4

Solution b - $\frac{M}{15}$ Na_2HPO_4

Mix solution A and solution B in the volumes given below :

Phosphate buffer mixture

pH	ml of b	ml of a	pH	ml of b	ml of a

5.8	8	92	7.1	66.6	33.4
6.0	12.2	87.8	7.2	72.0	28.0
6.2	18.6	81.4	7.3	76.8	23.2
6.4	26.7	73.3	7.4	80.8	19.2
6.6	37.5	62.5	7.5	84.1	15.9
6.8	49.6	50.4	7.6	87.0	13.0
6.9	55.4	44.6	7.7	89.4	10.6
7.0	61.1	38.9	7.8	91.5	8.5

Note:

1. pK is the pH around (± 1 pK) which the buffer shows maximum buffering action. Further and farther away from pK buffer shows lesser and lesser buffering action.
2. The two stock solutions of desired Molarities are prepared and mixed in the same ratio as given above.

Observations	Exact volume of appropriate solution to be added.
Result	100 ml of phosphate buffer of required pH is prepared.
Skills to be achieved	Students can prepare buffer solutions of required pH.
Skill evaluating criteria	Weighing of solute---3marks Solvent measurement---3 marks Checking for meniscus---2marks Dissolving solute completely-----2 marks.
FAQs	Define: pH , Acid, Base. What do you understand by buffer solution?
Assignment/Activity	Prepare 5 different buffer solutions?
Reference	Basic laboratory techniques- Handbook of biochemistry

Week no.	Week 6
Practical no.	6
Title/ Aim	Creatinine clearance
Objectives	At the end of practical student shall be able determine creatinine clearance of urine
Introduction	The clearance of any substance is defined as the number of ml of plasma which contain the amount of that substance , excreted in urine in one minute. This is given by the formula Clearance = $\frac{UV}{S(P)} \times \frac{1.73}{A}$ In case of creatinine clearance U = mg/ml of urine creatinine S(P) = mg /ml of serum (or plasma) creatinine V = ml of urine excreted per minute 1.73 = standard average surface area of the normal individual A = surface area of patient
Requirements	Patient's serum, 24 hr.urine sample, reagents required for determination of serum creatinine , 3 gm/dl sulphosalicylic acid, 2/3 ml sulphuric acid , 10 gm/dl sodium tungstate.
Environment	MLT Laboratory
Procedures	PATIENT PREPARATION - The patient should be instructed to collect all urine passed during 24 hrs (collect at the beginning at 8 a.m in the morning until 8 O'clock the next morning The urine should be kept in a cool and dry place in a polythene container with 2-3 crystals of Thymol . LAB INVESTIGATION – Measure volume of the collected urine specimen. Determine serum creatinine. Determine urine creatinine. PROCEDURE **– For Urinary creatinine:** 1. Confirm presence of protein in urine by adding 2-3

drops of 3 gm/dl sulphosalicylic acid to 5 ml of urine .
If turbidity present deproteinize it by using following method

Distilled water	8 ml
Urine	1 ml
2/3 ml sulphuric acid	0.5 ml
10 gm/dl sodium tungstate	0.5 ml

Mix well . centrifuge at 1500 RPM for 10 min

2, If turbidity absent dilute urine 1:10 by using distilled water.

Now pipette in tubes

Reagents	Test	Std.	Blank
Deproteinized or diluted urine	0.2ml	-	-
Standard	-	0.2ml	-
Distilled water	4.8ml	4.8ml	-
Alkaline picrate reagent	1.0ml	1.0ml	1.0ml

Mix and keep at room temperature for 20 min. Take O.D using green filter (520nm) against blank .

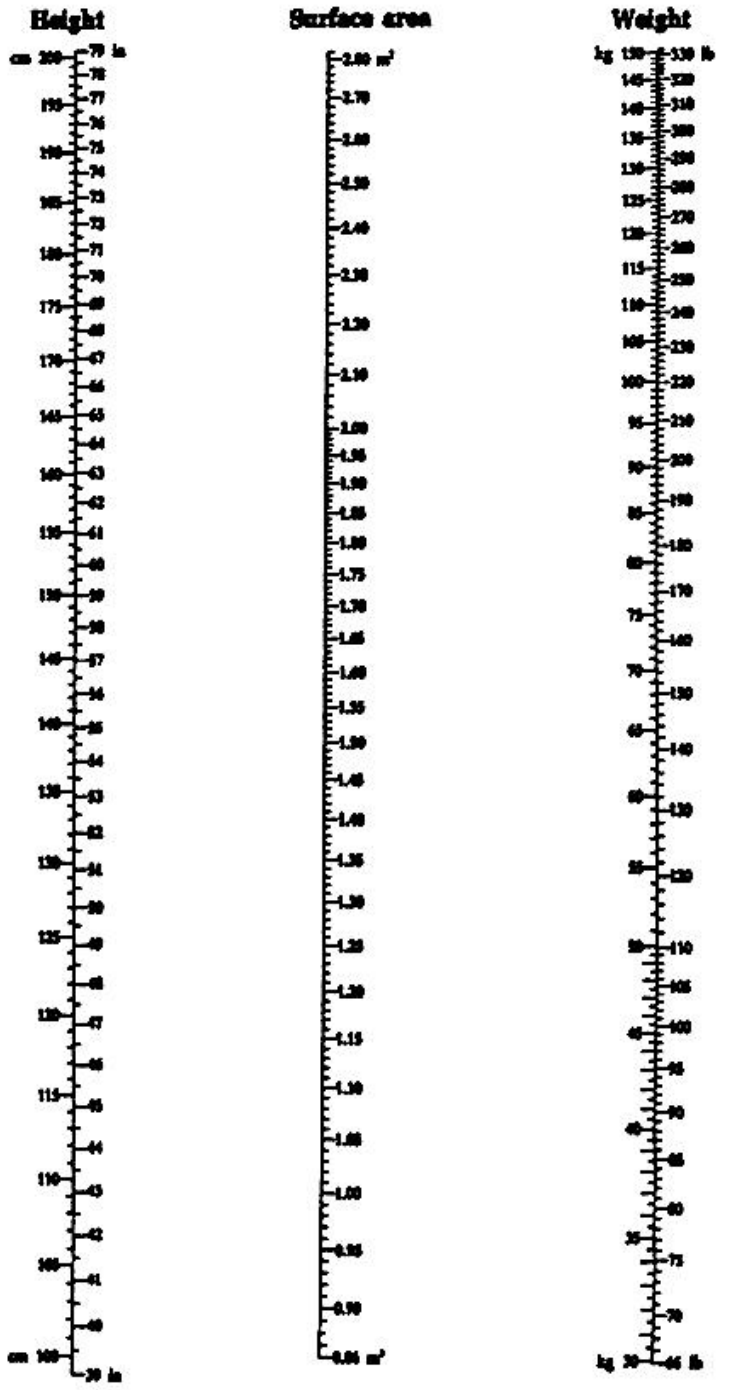
Calculations

Urine creatinine mg/dl = $\frac{O.D.of\ Test}{O.D.of\ Std}$ X 100

Find out the ratio $\frac{1.73}{A}$ by referring to the chart.

Calculate creatinine clearance using following formula-

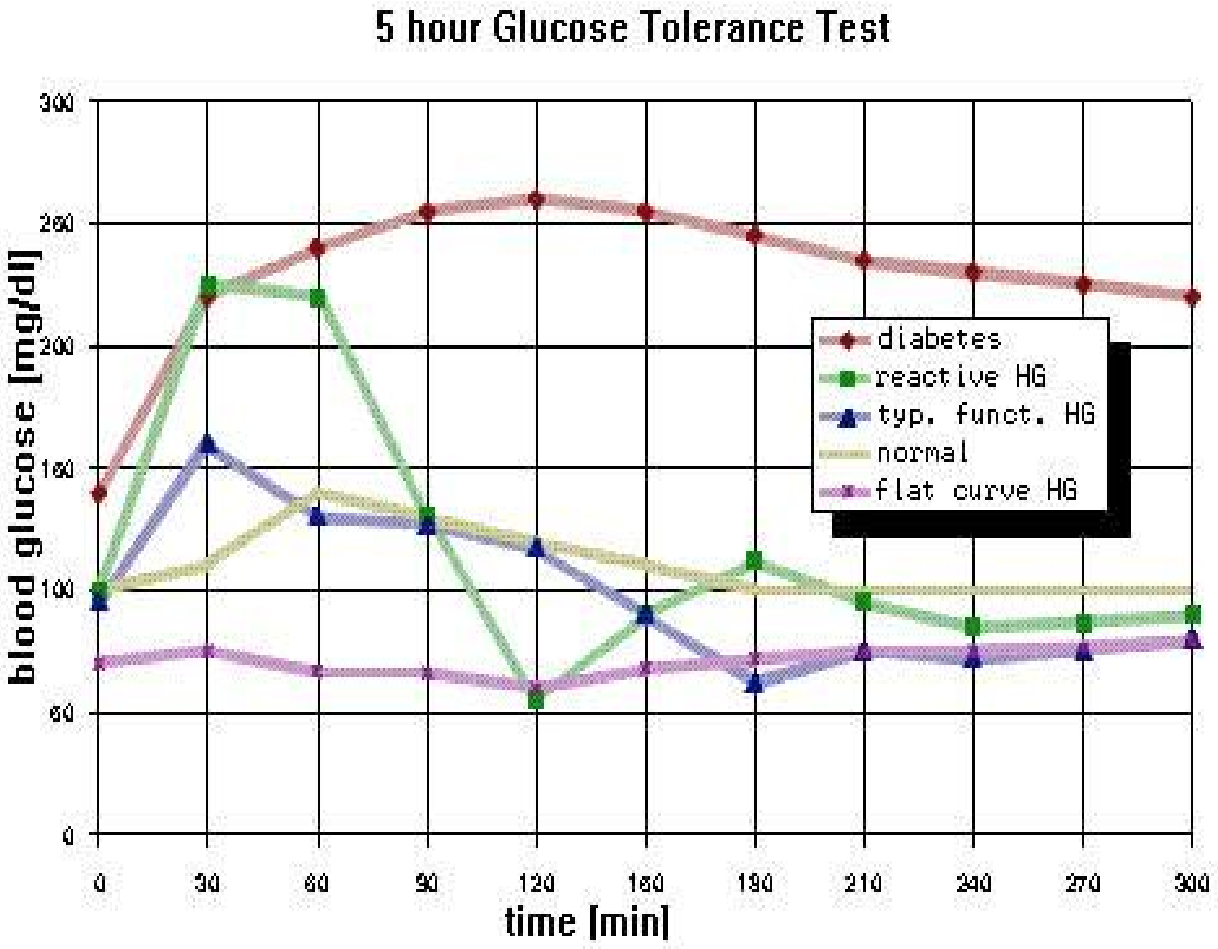
$$\text{Creatinine clearance} = \frac{urine\ creatinine\ \frac{mg}{dl}}{serum\ creatinine\ \frac{mg}{dl}} \ \frac{V}{\text{X min}} \text{ X } \frac{1.73}{A}$$

	
Normal Value	For male- 105±20 ml/min For female -95±20 ml/min
Clinical significance	This test gives relatively accurate and useful measure of the glomerular filtration rate and also excretory capacity of the kidney.
Result	Creatinine clearance of given patient
Skills to be achieved	Determination of creatinine clearance accurately.

Skill evaluation	patient instruction---3 marks Deprotinization of urine and serum ---3 marks Calculation ---3 marks Reporting --- 1 mark
FAQs	• What is creatinine • What is creatinine clearance?
Assignments	Perform creatinine clearance of 2 patients.
References/Link with Theory Topic	Protein metabolism

Week no.	Week 8
Practical no.	**8**
Title / aim	**Glucose tolerance test (GTT)** To determine the ability of the body to utilize glucose in blood
Objective	At the end of practical student shall be able to find out the ability of the body to utilize glucose in blood
Principle	Same as blood glucose
Requirement	Same as blood glucose **Preparation of patient** The patient should be on balanced diet at least for 2 to 3 days prior to the test. 1. Patient should report to the laboratory after fasting for 12-16 hrs. He can drink water. 2. He should bring fasting midstream sample collected in a clean &dry container or bottle. 3. Patient should be in position to wait at the laboratory for at least 2-3 hrs.,since 5 blood samples are collected at the interval of 30 minutes
Environment	**MLT Laboratory**
Procedure	1. First test the collected fasting urine specimen for glucose. If glucose is present, then do not perform Glucose tolerance test by giving glucose, instead a post prandial sample is collected. 2. Collect fasting blood sample, 2-3 ml in a fluoride bulb. If glucose is absent in the fasting urine , then follow the instruction as given below. 3. Give 75 gm or 100 gm of glucose dissolved in water to the patient. add lemon juce if necessary.

	4. Note the time 5. Collect 4 more sample at half hourly intervals for 2 hours after the glucose has been taken 6. Four urine samples are collected after collection of each blood sample (if patient is unable to collect four urine sample collect at least 2 sample one hour interval) 7. Determine blood and sugar by the specific method used in the lab 8. Prepare glucose tolerance curve by plotting on x axis and plasma glucose values on Y axis



Observation	<table><tr><td>time</td><td>Fasting</td><td>½ hour</td><td>1 hour</td><td>1 ½ hour</td><td>2 hour</td><td>2 ½ hour</td></tr><tr><td></td><td colspan="6">After taking glucose</td></tr><tr><td>Blood glucose mg/dl.</td><td>70</td><td>130</td><td>145</td><td>105</td><td>75</td><td>80</td></tr><tr><td colspan="7">Urine glucose absent throughout the test</td></tr></table> Normal renal threshold for glucose = 150 -170 mg/dl
Result	Type of glucose tolerance pattern shown by the patient.
Clinical	Glucose tolerance means ability of the body to utilize glucose in blood

significance	circulation. Glucose tolerance decreases in diabetes mellitus & in certain endocrine disorder like hyper thyroidism, hyperpituitarism& hyper adrenalism.
Skills achieved	Student can perform blood glucose& urine glucose and plot the tolerance graph.
Skill evaluation	Perfect quantity of reagents and sample.--- 3 marks Using correct wavelength filter.--- 1 mark Proper Calculation ---3 marks Plotting graph ---3 marks
FAQS	What is the principle of test? What is the normal value of blood glucose?
Activity	Perform glucose tolerance test
Assignment/ Links with theory topics-	Carbohydrate metabolism

Week no.	Week 9
Practical no.	**9**
Title/ Aim	Determination of serum urea To calculate blood urea level byDiacetyl-monoxime(DAM) method
Objectives	At the end of practical student shall be able to find out the blood urea level.
Method	**DAM method**
Principle	Urea reacts with diacetyl-monoxime in hot acidic medium and in the presence of thiosemicarbazide and in ferric ions to form pink colored compound which on green filter.
Requirements And Specimen	1. Test tubes (15 X125 mm) 2. 10 ml pipette, dispenser or burette. 3. Push button pipette or 0.1 ml serological pipette. 4. Measuring cylinder of 100ml. 5. Water-bath 6. Stopwatch 7. Photometer **Sample material** Serum, heparinized plasma or fluoride plasma **Preparation &stability of the reagent** 1. Reagent 1: (DMR): it contain 0.2 g/dl, directly l monoxime in distilled water . The reagent is stablem at room temperature ($25\ ^0c + \underline{5}^0$ C) for one year . 2. Reagent 2: (TSC): it contains 40 mg/dl, thiosemicarbazide in distilled water. The reagent is stable at room temperature ($25\ ^0 c + 5\ ^0 c$) for 6 months. 3. Regent 3: (Acid): it contains 60 ml of conc. Sulfuric acid, 10 ml of orthphosphoric acid and 10

	ml of 1 gm. /dl ferric chloride in orthophosphoric acid in one liter of reagent prepare in distilled water. This reagent is stable at room temperature for one year. 4. Urea nitrogen standard 20 mg \/dl: it contains 42.8 mg of urea in 100ml of 42.8 mg of urea in 100 of saturated benzoic acid. This standard is stable for one year when refrigerated. **Preparation of working reagent** It is prepared fresh by mixing one part of reagent 1, one part of reagent2, and two part of reagent3. This reagent should be prepared fresh for eachbatch of the determination.
Environment	MLT Laboratory

Procedures

Pipette in tubes labeled as follows

	Test	Standard	Blank
Working reagent ml	5.0	5.0	5.0
Serum / plasma ml	0.05	-	-
Standard 20 mg/dl, ml	-	5.0	-
Distilled water, ml	-	-	0.05

Mix the content of the tubes thoroughly and place them in a boiling, water bath for exactly 15 minutes. Cool immediately by using tap water and after 5 minutes measures the intensities of the test and standard against blank at 520 nm (green filter)

Clinical significance	Elevated levels of urea are observed in pre-renal, renal and post renal conditions. Pre- renal condition: diabetes mellitus, dehydration, cardiac failure, hematemesis, server burns, high fever etc. Renal conditions: disease of kidneys. Post-renal conditions: Enlargement of

	prostate, stones in urinary tract, tumor of the bladder. Decrease values have been reported in severe liver disease, protein malnutrition & pregnancy.
Calculation	Calculations Plasma (or serum) urea nitrogen, mg/dl =O.D test. X20 O.D std
Result	**Normal range (reference range)** • Birth to one year : 4-16 mg/dl (SI: 1.4-15.7mmol/L) • 1 to 40 years: 7-21 mg /dl (SI: 2.5-7.5mmol/L) • Gradual slight increase occurs over 40years of age.
Skills to be achieved	Student can estimate blood urea level
Skills evaluation	Perfect quantity of reagents and sample.--- 4 marks Using proper wavelength filter--- 1 mark Proper Calculation --- 3 marks Report --- 2 marks
FAQs	What is the principle of test? What is the normal value of blood urea?
Assignments	Process 10 samples for blood Urea determination
References/Link with Theory Topic	Protein metabolism

Week no.	Week 10
Practical no.	10
Title/ Aim	**Estimation of serum uric acid** Determination of uric acid by end point reaction – enzymatic method
Objectives	At the end of practical student shall be able to find out the serum uric acid concentration.
Method	
Principle	The enzyme uricase in the reagent acts on uric acid to catalyze the following reaction. Uric acid $+2H_2O$ $+O_2$ uricaseallanation $+H_2o+CO_2$ Peroxidase present in the reagent acts on H_2O_2 H_2O_2 peroxidase H_2O+ (O) The phenolic chromogens present in the reagent, 2 , 4- dichlorophenolsulfonate (DCFC) and 4-aminophenazone get oxidized to form red colored compound , intensity of which can be measured at 510(500-530 nm , green filter). The concentration of red coloured compound is proportional to the amount of uric acid in the specimen.
Requirements And Specimen	1) Stock reagent in lyophilized from contain the flowing: • Buffer , pH 7.5 : 100mMol/L • Uricase : 100 IU/L • Peroxidase : 140 IU/l • Chromogen : 2.5u Mol/L • Surfactants / stabilizers 2) Uric acid standard : 5.0 mg / dl Stability of reagents the reagents are able at 2-8 ^{0}C **Preparation of working reagent**- Working reagent is prepared by mixing contents of stock lyophilized reagent with distilled water. Working reagent is stable at 2-4 c for 60 days **Specimen** Serum
Environment	**MLT Laboratory**
Procedures	

Pipette in the tubes, labeled as followed:

	Test	Std	Blank
Working reagent ,ml	1.0	1.0	1.0
Serum ,ml	0.02	-	-
Uric acid std , ml	-	0.2	-
Distilled water	-	-	0.2

Mix well, keep the tubes at room temperature (25 ^{0}c $\pm$ 5 ^{0}c) for 10 min. Read absorbance of test and standard against blank at 510 nm (green filter)

Clinical significance	Uric acid is the end product of nucleoprotein metabolism. It is a low threshold excretory product. The serum uric acid is often raised in gout. The determination has diagnostic value in differentiating gout from non- gouty arthritis. Uric acid levels are also increased in renal failure, uremia and leukemia
Calculation	Calculations Serum uric acid , mg/ dl = $\frac{\text{O.D. test}}{\text{O.D STD.}}$ x 5
Result	**Normal range** Adults Male: 3-7 mg/dl (IU: 177 – 416 m Mol / L) Female: 2-6 mg / dl (IU: 118 – 357 m Mol/L)
Skills to be achieved	This method can be used on photometers, spectrophotometer, semi and fully – automated instruments. It is monostep method.
Skills evaluation	Perfect quantity of reagents and sample.--- 4 marks Using proper wavelength filter--- 1 mark Proper Calculation --- 3 marks Report --- 2 marks
FAQs	What is the principle ofserum uric acid test? What is the normal value of serum uric acid?
Assignments	Process 10 samples for serum Uric acid determination
References/Link with Theory Topic	Protein metabolism

Week no.	Week 11
Practical no.	**11**
Title/ Aim	Determination of creatinine by alkaline picrate method.
Objectives	At the end of practical student shall be able to find out the serum creatinine concentration.
Method	Alkaline picrate method
Principle	Creatinine reacts with picric acid in alkaline medium to form a reddish yellow complex , intensity of which is directly proportional to the concentration of creatinine in the specimen and can be measured at 520 nm (green filter).
Requirements And Specimen	1. Test tube:15 x 125 mm 2. 5.0 ml serological pipettes 3. 1.0&2.0 ml volumetric pipette 4. Test tube stand 5. Centrifuge tubes 6. Centrifuge 7. Photometer **Preparation of reagent-** 1. Picric acid reagent:0.91 gm ./dl 2. 10 gm/dl sodium hydroxide 3. Working creatinine standards,1 mg/dl, 5 mg/dl ,10mg/dl These standards are prepared in 0.01 N HCL by using stock creatinine standards 100mg/dl **Stability of the reagent-** Reagent 1& 2 are stable at R.T ($25^0C \pm 5^0C$) Working creatinine standard are stable at 2-8 ^{0} C **Preparation of alkaline picrate reagents** – it is prepared fresh by mixing 4 parts of reagents 1&1 part of reagent 2. This working reagent is stable for one day. **Specimen-** Serum or plasma
Environment	**MLT Laboratory**
Procedures	Pipette in the tube labeled as follows

	<table><tr><td></td><td>Test (in centrifuge tube)</td><td>Std.</td></tr><tr><td>Distilled water , ml</td><td>3.0</td><td>4.0</td></tr><tr><td>Serum , ml</td><td>1.0</td><td>-</td></tr><tr><td>Standard 1 mg / dl , ml</td><td>-</td><td>1.0</td></tr><tr><td>2/3 N Sulfuric acid , ml</td><td>0.5</td><td>-</td></tr><tr><td>10 g / dl sodium tungstate , ml</td><td>0.5</td><td>-</td></tr></table> Centrifuge the contents in the test and get clear filtrate. Pipette in the tube labeled as follows <table><tr><td></td><td>test</td><td>Std:1</td><td>blank</td></tr><tr><td>Distilled water,ml</td><td>3.0</td><td>3.0</td><td>3.0</td></tr><tr><td>Filtrate,ml</td><td>2.0</td><td>-</td><td>-</td></tr><tr><td>Diluted std. 1mg/dl,ml</td><td>-</td><td>2.0</td><td>-</td></tr><tr><td>Alkaline picrate reagent,ml</td><td>1.0</td><td>1.0</td><td>1.0</td></tr></table> Mix & keep at R.T.temperature ($25\ ^{0}c \pm 5\ ^{0}c$) for 20 min. Read absorbance of test and standard against blank at 510 nm (green filter)by setting blank to100% T.
Clinical significance	Serum creatinine is increased in renal failure. Increased serum creatinine concentration observed in congestive heart failure, shock and mechanical obstruction of the urinary tract.
Calculation	**Calculation-** Serum creatinine, mg/dl= $\frac{\text{O.D of test}}{\text{O.D. of std}} \times 1.0$

Result	**Normal range-** 0.7 to 1.7 mg/dl
Skills to be achieved	Student can perform serum creatinine
Skills evaluation	Perfect quantity of reagents and sample.--- 4 marks Using proper wavelength filter--- 1 mark Proper Calculation --- 3 marks Report --- 2 marks
FAQs	What is the principle ofserum creatinine test? What is the normal value of serum creatinine?
Assignments	Perform 2 serum creatinine
References/Link with Theory Topic	Renal function test

<table>
<tr><td>Week no.</td><td>Week 12</td></tr>
<tr><td>Practical no.</td><td>12</td></tr>
<tr><td>Title/ Aim</td><td>Determination of serum total proteins.</td></tr>
<tr><td>Objectives</td><td>At the end of practical student shall be able to find out serum protein concentration and AG ratio of given blood sample.</td></tr>
<tr><td>Method</td><td>Biuret method</td></tr>
<tr><td>Principle</td><td>cupric ions from biuret reagent react with proteins in an alkaline medium to form a violet coloured complex. The intensity of the produced colour is directly proportional to the protein concentration in the sample; and it is measured on colorimeter using green filter or at 530 nm .</td></tr>
<tr><td>Requirements And Specimen</td><td>Test tubes, serological pipettes, colorimeter commercially available kit

* Biuret reagent

* protein standard 6mg/dl
SPECIMEN- Serum</td></tr>
<tr><td>Environment</td><td>MLT Laboratory</td></tr>
<tr><td>Procedures</td><td>
<table>
<tr><th>Reagent</th><th>Test</th><th>Std</th><th>Blank</th></tr>
<tr><td>Biuret reagent</td><td>2ml</td><td>2ml</td><td>2ml</td></tr>
<tr><td>Serum</td><td>0.02ml</td><td>-</td><td>-</td></tr>
<tr><td>Standard</td><td>-</td><td>0.02ml</td><td>-</td></tr>
<tr><td>Distilled water</td><td>-</td><td>-</td><td>0.02ml</td></tr>
</table>
Mix well. Incubate at room temperature for exactly 10 min. Read the intensity of test and standard against blank using green filter(530nm).
</td></tr>
</table>

Clinical significance	Total serum protein values increase in multiple myloma. Decreased serum protein values are seen in Nephrotic syndrome , liver diseases like cirrhosis of liver , malnutrition , and when liver cells are severely damaged.
Calculation	Serum protein gm/dl = $\frac{\text{O.D of Test}}{\text{O.D of Std}}$ x 6
Result	**Normal Value-** 6-8 gm/dl. Write report.
Skills to be achieved	Determination of serum protein concentration in gm/dl
Skills evaluation	Perfect quantity of reagents and sample.---- 4 marks Using proper wavelength filter--- 1 mark Proper Calculation --- 3 marks Report --- 2 marks
FAQs	What is the principle of biuret test? What is the normal value of serum protein?
Assignments	Process 10 samples for serum protein determination
References/Link with Theory Topic	Protein metabolism

<table>
<tr><td>Week no.</td><td>Week 13</td></tr>
<tr><td>Practical no.</td><td>13</td></tr>
<tr><td>Title/ Aim</td><td>Determination of serum Albumin</td></tr>
<tr><td>Objectives</td><td>Student shall be able to determine albumin level of blood.</td></tr>
<tr><td>Principle</td><td>Albumin present in serum reacts with Bromocresol Green reagent to form green coloured complex. The intensity of the produced colour is directly proportional to the albumin concentration in the sample; and it is measured on colorimeter using red filter or at 640 nm .</td></tr>
<tr><td>Requirements and Specimen</td><td>Test tubes, serological pipettes, colorimeter commercially available kit-
* Bromocresol Green reagent

* Albumin standard 4mg/dl
SPECIMEN- Serum</td></tr>
<tr><td>Environment</td><td>MLT Laboratory</td></tr>
<tr><td>Procedures</td><td>
<table>
<tr><th>Reagent</th><th>Test</th><th>Std</th><th>Blank</th></tr>
<tr><td>Biuret reagent</td><td>2ml</td><td>2ml</td><td>2ml</td></tr>
<tr><td>Serum</td><td>0.02ml</td><td>-</td><td>-</td></tr>
<tr><td>Standard</td><td>-</td><td>0.02ml</td><td>-</td></tr>
<tr><td>Distilled water</td><td>-</td><td>-</td><td>0.02ml</td></tr>
</table>
Mix well. Incubate at room temperature for exactly 5 min. Read the intensity of test and standard against blank using red filter(640nm).</td></tr>
<tr><td rowspan="4">Calculation</td><td>❖ Serum albumin gm/dl = $\frac{\text{O.D of Test}}{\text{O.D of Std}} \times 4$</td></tr>
<tr><td>❖ Serum Globulin gm/dl = serum total proteins –serum albumin in gm/dl
NORMAL VALUE- 1.8-3.6 gm/dl</td></tr>
<tr><td></td></tr>
<tr><td>❖ AG Ratio = $\frac{\text{serum Albumin gm/dl}}{\text{serum Globulin gm/dl}}$
NORMAL VALUE- 1.2 : 1 to 2 : 1</td></tr>
<tr><td>Clinical significance</td><td>CLINICAL SIGNIFICANCE-
Total serum protein values increase in multiple myloma.</td></tr>
</table>

	Decreased serum protein values are seen in Nephrotic syndrome , liver diseases like cirrhosis of liver , malnutrition , and when liver cells are severely damaged
Result	**Normal Value** -3.3-4.8 gm/dl. Write report
Skills to be achieved	Determination of serum albumin concentration in gm/dl
Skills evaluation	Perfect quantity of reagents and sample.--- 4 marks Using proper wavelength filter--- 1 mark Proper Calculation --- 3 marks Report --- 2 marks
FAQs	What is the principle of BCG test? What is the normal value of serum albumin? What is A/G ratio?
Assignments	Process 10 samples for serum albumin determination.
References/Link with Theory Topic	Protein metabolism

Week no.	Week14
Practical no.	14
Title/ Aim	Determination of serum chlorides
Objectives	At the end of practical student shall be able to find out serum chloride concentration of given blood sample
Principle	When protein free filtrate of a specimen is titrated against mercuric nitrate in presence of indicator diphenylcarbazone ; the free mercuric ions combine with chloride ions to form soluble mercuric chloride. $2Cl^- + Hg^{++} = HgCl_2$ After all chloride ions have reacted with mercuric ions , the excess mercuric ions react with the indicator diphenylcarbazone to form ablue violet coloured complex .The point at which colour changes is considered as end point of reaction .
Requirements	Test tubes, graduated pipettes, burette , centrifuge **For titration** – mercuric nitrate sol. Diphenylcarbazone indicator, chloride std 100mEq/L, **For deprotenization**- 2/3 N sulphuric acid,10 gm/dl sodium tungstate Specimen – serum
Environment	MLT Laboratory
Procedures	STEP 1: **Preparation of protein free filtrate**- • In a centrifuge tube take 4.0ml distilled water , add 0.5 ml serum . • Add 0.25 ml of 2/3 N H2SO4 and 0.25 ml 10 gm/dl sodium tungstate. Mix well. • Centrifuge at 3000 RPM for 10 min. Separate supernatant. STEP2: **Titration -** • Take 2ml supernatant in a test tube. Add

	one drop of indicator diphenylcarbazone. • Titrate against mercuric nitrate solution till the end point reaches (i.e. when the solution turns from colourless to violet blue) • Note the reading for test on burette. 'X'ml • Dilute standard 1:10 using glass distilled water. • Take 2ml diluted standard in a test tube and titrate it same as for test. • Note the reading for standard on burette. 'Y'ml
Calculations	Serum chlorides mEq/L = $\frac{\text{X ml}}{\text{Y ml}} \times 100$
Result	**Normal value** serum Chlorides: 95-106mEq/L
Clinical significance	Low chloride values are seen in prolonged vomiting, burns , over dehydration , salt losing renal diseases High chloride values are seen in renal tubular diseases , dehydration
Skills to be achieved	Determination of serum chloride concentration in mEq/L
Skill evaluation	Perfect quantity of reagents and sample.--- 4marks Achieving perfect end point.---4 marks Proper Calculation--- 2 marks
FAQs	What is the principle of serum chloride test? What is the normal value of serum chloride?
Assignments	Process 10 samples for serum chloride determination
References/Link with Theory Topic	Water and mineral metabolism

Week No.	Week 15
Practical No.	15
Title/ Aim	**Determination of Inorganic phosphorus.**
Objectives	The student shall be able to estimate inorganic phosphorus from given sample colorimetrically
Principle	**Method: - (**MODIFIED METOL METHOD FOR IN VITRO ESTIMATION Ammonium molybdate under acidic conditions reacts with phosphorus to form phosphomolybdate complex which is reduced to blue colored complex by metol. The absorbance of color developed is proportional to the inorganic phosphorus concentration.
Requirements	Charts,ppt, you tube **REAGENTS PROVIDED:** 1. Catalyst Reagent 50 ml 2. Molybdate Reagent 50 ml 3. Metol Reagent 50 ml 4. Standard (5 mg%) 2 ml **SPECIMEN:** Serum: Hemolysed / lipemic sera should not be used. Inorganic phosphorus is stable upto 4 days at room temperature and for one week at 2-8°C. Urine: 24 hours urine collection; dilute the urine sample 1:10 with deionized water before use.
Environment	MLT laboratory
Procedure	**PROCEDURE:** Pipette into clean, dry test tubes labelled Blank (B), Standard (S) and Test (T). (B) (S) (T)

	Catalyst Reagent	(1)	1.0 ml	1.0 ml	1.0 ml
	Molybdate Reagents	(2)	1.0 ml	1.0 ml	1.0 ml
	Deionized water		0.1 ml	-	-
	Standard	(4)	-	0.1 ml	-
	Serum/Diluted Urine		-	-	0.1 ml
	Metol-Reagent	(3)	1.0 ml	1.0 ml	1.0 ml

Mix well and allow them to stand at room temperature for 5 minutes. Measure absorbance of Test (T) and Standard (S) against Blank (B) on a photocolorimeter with a red filter or on a spectrophotometer at 680 nm, within 30 minutes.

Observations

absorbance of Test (T) and Standard (S) against Blank (B)

Result

CALCULATIONS:

a) Serum Phosphorus in mg% = $\frac{\mathbf{A\ of\ (T)}}{\mathbf{A\ of\ (S)}} \times 5$

b) Urine Phosphorus in gms/Lit = $\frac{\mathbf{A\ of\ (T)}}{\mathbf{A\ of\ (S)}} \times 0.5$

c) Urine Phosphorus in gms/24 hours =(b) ×24 hours Urine volume in litres.

Normal values:

1. Serum Phosphorus : 2.5 – 4.8 mg%

	2. Urine Phosphorus : 0.4 – 1.3 gms/24 hours urine
Clinical significance	Decrease levels are seen in hyperparathyroidism,rickets.
Skills achieved	Performing serum/urine phosphorus with accuracy independently
Skill evaluating criteria	Dilution technique---3marks Accurate pippetting---3 marks Taking correct O.D.values----2marks Correct calculation---2 marks
FAQs	What is the principle of serum phosphorus test? What is the normal value of serum phosphorus.
Assignment/Activity	Process 10 samples for serum phosphorus determination
Reference	Water and mineral metabolism

Week No.	Week 16
Practical No.	16
Title/ Aim	Determination of Calcium .
Objectives	The student should be able to do serum calcium assay.
Principle	**METHOD:**o-Cresolphthaleincomplexone Method(OCPC) **PRINCIPLE:** The metal complexing dye o-cresolphthaleincomplexone [CPC] forms a red complex with calcium in alkaline solution.
Requirements	**REAGENTS:** • Conc HCL (6 M) • AMP Buffer pH 10.7 **Specimen**:serum 1. Do not use grossly hemolyzed or lipemic serum 2. Glassware used must be free from Calcium. Before use all glassware should be soaked in 3N HCl and thoroughly rinsed 5-6 times with deionized water and dried. 3. As the reagents are very sensitive, handling must be done carefully to avoid contamination of reagents. 4 .Use of plastic tubes instead of glass tubes is recommended for accurate and reproducible results
Environment	MLT laboratory

Procedure

For Colorimeter/Spectrophotometer

	Blank(B)	Standard(S)	Test(T)
Reagents 1 : Calcium Color Reagent	3.0 ml	3.0 ml	3.0 ml

Reagents 2 : AMP Buffer	1.0 ml	1.0 ml	1.0 ml
Serum Sample	-	-	0.05 ml
Reagents 3: Working Calcium Standard, 10 mg%	-	0.05 ml	-
Mix well and allow to stand at R.T. for 5 minutes.			

Measure the O.D. of Blank (B), Standard (S) and Test (T) against distilled water at 570nm on Spectrophotometer or on the Colorimeter with a yellow filter

Observations	O.D. of Blank (B), Standard (S) and Test (T)
Result	**CALCULATION:** Serum Calcium = $\frac{\text{O.D. Test} - \text{O.D. Blank}}{\text{O.D. std} - \text{O.D. Blank}} \times 5$ mEq/litre d) Or $\frac{\text{O.D. Test} - \text{O.D. Blank}}{\text{O.D. Std} - \text{O.D. Blank}} \times 10$ mg/100 ml **Normal Values:** Adults : 4.5 – 5.3 mEq/litre (9 – 10.6 mg/100 ml) Children : Values are slightly elevated.
Clinical significance	Increase in serum Calcium levels are observed in primary Hyperparathyroidism, Hypervitaminosis D, Multiple myeloma and some Neoplastic diseases of bone. Low values are observed in Hypoparathyroidism, Rickets, Osteomalacia, Steatorrhoea, Renal failure, Tetany and sometimes during an attack of acute pancreatitis
Skills achieved	Determination of serum calcium level.
Skill evaluating criteria	Exact quantityof reagents &sample---4 marks.

	Using proper wavelength filter---4 marks Proper Calculation------2 marks
FAQs	What is the principle of serum calcium test? What is the normal value of serum calcium.
Assignment/Activity	Process 10 samples for serum calcium determination
Reference	Water and mineral metabolism

Week No.	Week 17
Practical No.	17
Title/ Aim	**Determination of transaminase – SGOT and SGPT**
Objectives	At the end of practical students shall be able to perform SGOT and SGPT
Principle	**Method:** DNPH method **Principle:** The activity of transaminases (aminotransferases) is determined by measuring the colour of the hydrazone (brown) which is formed by the reaction between 2.4-dinitrophenyl hydrazine (DNPH) and the ketoacid which is one of the products of transaminase reaction. The DNPH reacts with all oxoacids. These include oxoglutarate and oxaloacetate, as well as Pyruvate; DNPH gives more colours with oxalacetate, as well as Pyruvate than with oxoglutarate, thus making the method feasible with an acceptable limit of error. In both estimations- ALT and AST, the substrates are suboptimal, to reduce the background colour given by the alpha-ketoglutarate (or oxoglutarate) in the reaction with DNPH**.**
Requirements	**Reagents, equipment and supplies** • Phosphate buffer, pH 7.0, 0.1 M: • Substrate for ALT-DL-alanine (200 mM/L) and alpha-ketoglutrate (2 mM/L): • Substrate for AST-DL-aspartate (200 mM/L) and alpha-ketoglutrate (2 mM/L) • Pyruvate standard: a) Stock standard (20 mM/L): b) Working standard (2 mM/L or 2 µmole/mL): Dilute 10 mL of the stock standard to 100 mL with phosphate buffer. Dispense small amount (approximately 2 mL) of the working standard in test tubes (5 mL), stopper them and keep them in the freezer. Use one tube at a time for preparing the calibration curve. Any remaining solution in the tube should be discarded. Sodium Pyruvate is not stable in the solution and care should be taken to ensure that the standard solution has not deteriorated. • 2.4-dinitrophenylhydrazine or DNPH (1 mM/L): • Sodium hydroxide solutions (1 N and 0.4 N): 1 N: Dissolve 40g of sodium hydroxide in 500 mL of

	water and make up to 1000 mL in a volumetric flask. 0.4 N: Dilute 40 mL of 1 N NaOH to 100 mL volumetric flask. Specimen: serum
Environment	MLT laboratory
Procedure	This procedure describes the simultaneous determinations of ALT and AST 1. Label 2 test tubes as 'T' and 'B', corresponding to test and blank. If both enzymes are to be determined simultaneously, use 4 test tubes and label them as 'AL_T', 'AL_B' and 'AS_T', 'AS_B' corresponding to the ALT and AST- test and blank respectively. 2. Pipette 1.0 mL of the desired substrate into the respective tubes-ALT substrate for 'AL_T' and 'AL_B' tubes and AST substrate for 'AS_T' and 'AS_B' tubes. Place all the test tubes in a 37°C water bath for about 5 min for temperature equilibration. 3. To the tubes marked 'AL_T' and 'AS_T' add 0.2 mL serum, set the timer concurrently. 4. Mix rapidly by swirling and place the tubes back in the water bath. If there are more specimens, space the timing accordingly. 5. After exactly 30 min remove the tubes marked ALT and ALB and immediately add 1.0 mL of 2.4-dinitrophenylhydrazine and mix, thereby stopping the reaction and developing the colour. Leave the tubes at room temperature until the 'AS_T' and 'AS_B' tubes are ready in the following step. 6. Continue incubation (37°C) for the tubes marked 'AS_T' and 'AS_B' until exactly 60 min after adding the serum. Add 1.0 mL of hydrazine and mix, as done in the previous step for AL_T. 7. Remove the tubes from the water bath and add 0.2 mL of serum to the tubes for the serum blank, marked 'AL_B' and 'AS_B'. 8. After 20 min at room temperature, following theaddition of hydrazine, add 10 mL of 0.4 N NaOH to all the tubes, stopper them and mix by inversion. 9. Leave the reaction to continue for at least 5 min but no longer than 30 min. 10. Read the absorbance of the test solutions ('AL_T' and 'AS_T') and the serum blanks ('AL_B' and 'AS_B') against

water at 505 nm or use a green filter (490-520 nm).

11. Determine the changes in absorbance (AA) by subtracting the blank readings from the corresponding test readings.
    a) For $AL_T = AL_T - AL_B$
    b) For $AS_T = AS_T - AS_B$
12. Refer to the calibration curve for reporting the enzymes activity in International Units (U/L).

Preparation of calibration curve

1. Take five test tubes and mark them #1 to #5.
2. Add the substrates and water according to the following table.

Note: The total quantity of the serum, substrate and Pyruvate solutions is 1.2 mL, the same quantity as the reaction mixture in the test.

3. Add 1 mL of hydrazine reagent to each tube, mix, leave for 20 min at room temperature, and add 10 mL of 0.4 N sodium hydroxide. Mix thoroughly. Leave for at least 5 min.
4. Read the absorbance within 5-30 min after addition of NaOH, at 505 nm or use a green filter. Use tube 1 (without the standard) as a blank to set the zero absorbance.

5. Tabulate the results with varying enzyme units against the correspondingabsorbances.

Tube No.	Pyruvate standard (mL)	Water (mL)	Substrates* (mL)	ALT U/L	AST
1	0	0.2	1.0	0.0	0.0
2	0.1	0.2	0.9	13.4	11.5
3	0.2	0.2	0.8	27.4	29.3
4	0.3	0.2	0.7	46.6	54.7
5	0.5	0.2	0.6	72.0	91.2

6. Plot a calibration curve of absorbance against transaminase units as given in the table for ALT and

	*Use specific substrate- ALT or AST (2 µmole/mL).
Observations	Take absorbance of 5 tubes of standard
Result	Extrapolated test value from the graph **Normal ranges:** The adult reference range for both AST and ALT is roughly 10-40 U/L when measured at 37°C. Although men have higher values than women do, most laboratories use a single range for both genders
Clinical significance	Increased ALT in hepatocellular damage. Both AST and ALT increase in hepatitis. Increased AST in myocardial necrosis.
Skills achieved	Determination of transaminases – SGOT and SGPT
Skill evaluating criteria	Perfect quantity of reagents and sample---4 marks Using proper wavelength filter---2 marks Plotting proper curve ---4 marks
FAQs	What is the principle of AST test? What is the normal value of AST and ALT. What is the principle of ALT test?
Assignment/ Activity	Process 10 samples for SGOT and SGPT determination
Reference	Enzymes

Week No.	Week 18
Practical No.	18
Title/ Aim	Determination of ACID PHOSPHATASE (ACP
Objectives	The student shall be able to do ACID PHOSPHATASE (ACP)
Principle	**Method:** KING'S Method **PRINCIPLE:** Serum ACP hydrolyzes disodium phenyl phosphate to phenol and disodium phosphate at pH 5.0. The phenol so formed, reacts with 4-aminoantipyrine in alkaline medium, in the presence of oxidizing agent potassium ferricyanide to form a red colored complex whose absorbance is proportional to the enzyme activity. Prostatic ACP activity is inhibited by tartarate. Hence prostatic ACP is assayed using 1M tartarate as inhibitor. The ACP assay in presence of 1M tartarate measures non-prostatic fraction. The difference between total ACP and non-prostatic fraction measures prostatic ACP activity.
Requirements	**REAGENTS:** 1. Substrate 10vials. 2. Buffer solution pH 5.0 x 60 ml. 1 3. Color Reagent x 100 ml. 1 4. Tartarate solution 1M x 2.0 ml. 1 5. Phenol standard 10 mg% x 2.0 ml. 1 **SPECIMEN:** Freshly drawn serum should be separated soon after collection. Serum should be unhemolysed. ACP is

	unstable. Hence assay should be carried out immediately or the specimen should be stored at -20°C. ACP in serum can be stabilized by making the serum acidic by adding 10 ml of Acetic acid. **REAGENT PREPARATION:** Reconstitute Reagent (1) Substrate with 5.5 ml Buffer solution (2) Mix well.
Environment	MLT laboratory

Procedure

Pipette into five test tubes labeled Blank (B), standard (S), Internal Blank (IB), Test (T) and Tartarate stable (-Ts) as follows:

	(B)	(S)	(IB)	(T)	(Ts)
Buffered substrate	1.0 ml	1.0 ml	1.0 ml	1.0ml	1.0ml
Deionized Water	3.2 ml	3.1 ml	3.0 ml	3.0ml	3.0 ml
Mix well and incubate at 37° for 3 minutes					
TartarateSolutio(3)	___	___	___	___	One drop
Serum	___	___	___	0.2ml	0.2 ml
Phenol Standard (4)	___	0.1 ml	___	___	___
Mix well and incubate at 37° for 60 minutes					
Color Reagent (2)	2.0 ml	2.0 ml	2.0 ml	2.0ml	2.0 ml
Serum	___	___	0.2ml	___	___

Mix well after each addition of reagent and measure absorbance (A), for Blank

(B), Standard (S), Internal Blank (IB), Test (T) and Tartarate Stable (Ts) against distilled water on photocolorimeter using a green filter or on spectrophotometer at 510 nm.	
Observations	measure absorbance (A), for Blank (B), Standard (S), Internal Blank (IB), Test (T) and Tartarate Stable (Ts)
Result	CALCULATION: Total serum ACP activity in KA units = $\frac{A(T)-A(IB)}{A(S)-A(B)} \times 5$ NORMAL RANGE: Total ACP activity = 1.0-4.0 KA units
Clinical significance	Increase in serum ACP activity is observed in Paget's disease of bone, hyperparathyroidism, Gaucher's disease and prostatic Carcinoma.
Skills achieved	Skillfully perform Acid phosphatase test.
Skill evaluating criteria	Perfect quantity of reagents and sample---4 marks Using proper wavelength filter---2 marks Plotting proper curve ---4 marks
FAQs	What is the principle of ACP test? What is the normal value of serum ACP.
Assignment/Activity	Process 10 samples for serum ACP determination
Reference	Water and mineral metabolism.

<table>
<tr><td>Week No.</td><td>Week 19</td></tr>
<tr><td>Practical No.</td><td>19</td></tr>
<tr><td>Title/ Aim</td><td>Determination of Alkaline phosphatase</td></tr>
<tr><td>Objectives</td><td>The student shall be able to do alkaline phosphate assay</td></tr>
<tr><td>Principle</td><td>METHOD: kind &Kings method.

PRINCIPLE:
Alkaline phosphatase from serum converts Phenyl Phosphate to Inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts with alkaline medium with 4-Aminoantipyrine in presence of the oxidizing agent potassium ferricyanide and forms an orange-red coloured complex, which can be measured colorimetrically. The color intensity is proportional to the enzyme activity.</td></tr>
<tr><td>Requirements</td><td>SAMPLE:
Serum: Various blood Collected aspectically in a clean; tube/vial

REAGENTS (supplied in the Kit)

Reagent 1: Buffered Substrate, pH 10.0.
Reagent 2: Chromogen Reagent
Reagent 3: Phenol standard, 10 mg%

PREPARATION OF WORKING SOLUTION

Reconstitude one vial of Reagent 1, Buffered substrate with 4.5 ml of Distilled water. Reagents 2 & 3 are ready for use.</td></tr>
<tr><td>Environment</td><td>MLT laboratory</td></tr>
<tr><td>Procedure</td><td>A. For colorimeter
<table>
<tr><td></td><td>Blank(B)</td><td>Standard(S)</td><td>Control(C)</td><td>Tests(T)</td></tr>
<tr><td>Working Buffered Substrate</td><td>1.0 ml</td><td>1.0 ml</td><td>1.0 ml</td><td>1.0 ml</td></tr>
<tr><td>Distilled</td><td>3.1 ml</td><td>3.0 ml</td><td>3.0 ml</td><td>3.0 ml</td></tr>
</table></td></tr>
</table>

<table>
<tr><td></td><td>
<table>
<tr><td>water</td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="5">Mix well and incubate for 3 minutes at 37°C</td></tr>
<tr><td>Serum</td><td>-</td><td>-</td><td>-</td><td>0.1 ml</td></tr>
<tr><td>Phenol Standard 10 mg %</td><td>-</td><td>0.1 ml</td><td>-</td><td>-</td></tr>
<tr><td colspan="5">Mix well and incubate for 15 minutes at 37°C</td></tr>
<tr><td>Chromogen Reagent</td><td>2.0 ml</td><td>2.0 ml</td><td>2.0 ml</td><td>2.0 ml</td></tr>
<tr><td>Serum</td><td>-</td><td>-</td><td>0.1 ml</td><td>-</td></tr>
</table>

Mix well after the addition of each reagent and measure the O.D. of Blank (B), Standard (S), Control (C) and Test (T) against distilled water using a green filter.
</td></tr>
<tr><td>Observations</td><td>measure the O.D. of Blank (B), Standard (S), Control (C) and Test (T)</td></tr>
<tr><td>Result</td><td>CALCULATION:
Serum Alkaline Phosphatase activity in KA units =
$\frac{O.D.\,Test - O.D.\,Control}{O.D.\,Std - O.D.\,Blank} \times 10$

Normal Values:
Adults: 4-11 KA Units
Children: Higher values are found.</td></tr>
<tr><td>Clinical significance</td><td>Increased levels are seen in diseases of bone , liver and in pregnancy.</td></tr>
<tr><td>Skills achieved</td><td>Determination of Alkaline phosphatase</td></tr>
<tr><td>Skill evaluating criteria</td><td>Perfect quantity of reagents and sample---4 marks
Using proper wavelength filter---2 marks
Plotting proper curve ---4 marks</td></tr>
<tr><td>FAQs</td><td>What is the principle of serum alkaline phosphatase test?
What is the normal value of serum alkaline phosphatase.</td></tr>
<tr><td>Assignment/Activity</td><td>Process 10 samples for serum calcium determination</td></tr>
</table>

Reference	Enzymes

Week no.	**Week 20**
Practical no.	**20**
Title / aim	**Serum bilirubin test** Determination of serum bilirubin by-Malloy and EvelynMethod
Objective	Students shall be able to estimate serum bilirubin by-Malloy and EvelynMethodcolorimetrically.
Principle	The method is based on Van den Bergh reaction. When bilirubin reacts with diazo reagent, purple colouredazobilirubin is formed. Methanol is used as reaction accelerator. Since total bilirubin (indirect & direct type) is soluble in it.by using only distilled water,direct bilirubin is determined. The difference between total bilirubin&direct bilirubin gives measure of indirect bilirubin. The optical densities of total test & direct test are measured against respective blanks at 540 nm(green filter,510-560 nm)
Requirement	1. Test tube:15 x 125 mm 2. 5.0 ml 0.2 ml serological pipettes 3. Test tube stand 4. stopwatch 5. Photometer **Preparation of reagents** 1. Diazo 'A': it is prepared by mixing 0.1 gm. of sulfanilic acid in 100 ml of 1.5 %(v/v)HCL 2. Diazo 'B': it is prepared by mixing 0.5 gm. of sodium nitrate in 100 ml of distilled water. 3. Diazo blank reagent: (1.5% HCL) it is prepared by adding 1.5 ml of conc. HCL to about 90 ml of distilled water in a 100 ml of volumetric flask.Distilled water is added up to mark. 4. Methanol 5. 10 mg/dl artificial bilirubin standard: it is prepared as follows- a) Stock standard: it is prepared by mixing

<table>
<tr><td></td><td>0.29 gm. Of methyl red in 100 ml of glacial acetic acid.
b) Working standard: 0.1 ml of Stock standard 0.5 ml ofglacial acetic acid. & 1.44 gm. of sodium acetate are mixed in distilled water & diluted to 100 ml by adding distilled water.
Stability of reagent-
Reagents 1,2,3,4 & 5 are stable at R.T. (25^0 c $\pm$ 5^0c) for 1 year. Reagent 2 is stable at 2-8 ^{0}c in amber coloured bottle.
Specimen-
Serum or heparinized plasma (free from hemolysis).
If not tested immediately, store in amber coloured bottle at 2-8^0c for not more than 24 hours.</td></tr>
<tr><td>Environment</td><td>MLT Laboratory</td></tr>
<tr><td>Procedure</td><td>Prepare fresh diazo mixture by mixing 5.0 ml of Diazo A & 0.15 ml of Diazo B. this mixture is stable for a day.
Pipette in tube labeled as follows:
<table>
<tr><th></th><th>Total test</th><th>Total blank</th><th>Direct test</th><th>Direct blank</th></tr>
<tr><td>Distilled water ,ml</td><td>1.8</td><td>1.8</td><td>1.8</td><td>1.8</td></tr>
<tr><td>Serum ,ml</td><td>0.2</td><td>0.2</td><td>0.2</td><td>0.2</td></tr>
<tr><td>Diazo mixture ,ml</td><td>0.5</td><td>-</td><td>0.5</td><td>-</td></tr>
<tr><td>Diazo blank reagent,ml</td><td>-</td><td>0.5</td><td>-</td><td>0.5</td></tr>
<tr><td>Methanol ,ml</td><td>2.5</td><td>2.5</td><td>-</td><td>-</td></tr>
<tr><td>distilled water</td><td>-</td><td>-</td><td>2.5</td><td>2.5</td></tr>
</table>
Keep in dark for 30 minutes. Read the intensities at 540 nm (green filter)
Read O.D. of the artificial bilirubin standard (undiluted) by transferring the standard solution in a dry cuvette at 540 nm (green filter).</td></tr>
</table>

Observation	**Calculation-** **Total bilirubin ,mg/dl** = O.D. of total test -O.D. of total blankX10 O.D. of std. **Direct bilirubin, mg/dl** = O.D. of direct test - O.D. of direct blankX10 O.D. of std. **Indirect bilirubin, mg/dl** = (total bilirubin, mg/dl) - (direct bilirubin, mg/dl)
Result	**Normal range-** • Total bilirubin up to 1.0 mg/dl • Direct bilirubin up to 0.5mg/dl • Indirect bilirubin up to 0.5mg/dl
Clinical significance	Bilirubin estimation is performed in liver function test. An increase in total bilirubin is observed in jaundice. The estimation of direct (conjugated)&indirect(unconjugated)bilirubin may help in diagnosis of various types of jaundice-prehepatic(haemolytic),hepatic&post hepatic
Skills achieved	Student can perform serum bilirubin
Skill evaluation	Perfect quantity of reagents and sample---4 marks Using proper wavelength filter---2 marks Plotting proper curve ---4 marks
FAQS	What is the principle of serum Bilirubin test? What is the normal value of serumBilirubin.
Activity	Perform at least 10 serum bilirubin
Assignment/ Links with theory topics-	**Liver function test**

<table>
<tr><td>Week No.</td><td>Week 21</td></tr>
<tr><td>Practical No.</td><td>21</td></tr>
<tr><td>Title/ Aim</td><td>Cholesterol estimation.</td></tr>
<tr><td>Objectives</td><td>Determination of serum bilirubin by-Malloy and EvelynMethod</td></tr>
<tr><td>METHOD:</td><td>Wybenga and Pileggi</td></tr>
<tr><td>Principle</td><td>In hot acidic medium, cholesterol oxidizes ferric ions to a brown coloured complex which absorbs are 530 nM and is directly proportional to cholesterol</td></tr>
<tr><td>Requirements</td><td>REAGENTS :
Composition
1. Cholesterol Reagent 250 mL
Acetic Ethyl Acetate 6.5 Mol/L
Sulphuric Acid 3.8 mMol/ L
Ferric ion 306 µMol/L
2. Cholesterol Standard 5.0 mL
Cholesterol 200 mGs/dL
Acetic Acid q.s.
3. HDL Precipitation Reagent 5.0 mL
Phosphotungstate 13.9 mMol/L
MgCl 490 mMol/L
Preparation of Working Reagent
All Reagents are ready to use.
SAMPLE:
Sample can be serum or plasma which has no sign of heamolysis. Cholesterol is affected by food intake. Hence, keep the patients fasting for atleast 8 hrs, prior to sample collection.</td></tr>
<tr><td>Environment</td><td>MLT laboratory</td></tr>
<tr><td>Procedure</td><td>Procedure:
<u>Manual method: for total cholesterol:</u>
<table>
<tr><th>Reagent</th><th>Blank ml</th><th>Test ml</th><th>Standard ml</th></tr>
<tr><td>Cholesterol reagent</td><td>5.0</td><td>5.0</td><td>5.0</td></tr>
<tr><td>Distilled water</td><td>0.05</td><td>-</td><td>-</td></tr>
<tr><td>Cholesterol standard</td><td>-</td><td>-</td><td>0.05</td></tr>
</table></td></tr>
</table>

Sample	-	0.05	-

Mix well for 20 secs. Keep in a boiling water bath immediately exactly 90 seconds 1min and 30 secs). Cool immediately for 5 minutes under running tap-water. Read at 520-540 nM or GREEN filter against Blank. The final colour is stable for 30 minutes.

Observations

STEP 1: FOR HDL – CHOLESTROL:
(Precipitation):

SERUM……0.2 mL
HDL Reagent No. 3……. 0.2 mL
Mix well. Keep for 10 minutes and centrifuge. Separate clear supernatant and estimate Cholesterol level of the supernatant as per STEP II.

STEP 2: FOR HDL – CHOLESTEROL:

Reagents	**Blank HDL ml**	**Standard HDL ml**	**Test HDL ml**
Cholesterol Reagent No.1	5.0	5.0	5.0
HDL Reagent No.3	0.2	0.2	-
Supernatant from STEP 1	-	-	0.02
Cholesterol Std (200 mGs/dL)	-	0.02*	-

*Standard (200 mGs/dL) volume is only 0.02 mL whereas the sample volume is 0.2 mL.

1. Mix well for 20 secs. Immediately keep in a boiling water bath for exactly 90 seconds (1min and 30 secs). Cool immediately for 5 minutes under running tap-water. Read at 520-540 nM or GREEN filter against Blank. (Final colour is stable for 30 minutes).

Result	**CALCULATION**: $\text{Total Cholesterol} = \frac{\text{O.D. Test}}{\text{O.D. Std}} \times 200$ $\text{HDL Cholesterol} = \frac{\text{O.D. Test HDL}}{\text{O.D. Std HDL}} \times 40$ Expected Values: Total Cholesterol: 150 to 250 mGs/dL (3.88 to 6.47 mMol/L) HDL Cholesterol: Men: 30 to 60 mGs/dL (0.78 to 1.55 mMol/L) Women: 40 to 70 mGs/dL (1.03 to 1.81 mMol/dL)
Clinical significance	**Total Cholesterol:** Increased levels are associated with atherosclerosis, nephrosis, diabetes mellitus, myxoedema, obstructive jaundice. Decreased levels are observed in cases of hyperthyroidism, certain anamias, malabsorption and wasting syndrome. **HDL Cholesterol:** Decreased levels are associated with increased risk of developing coronary artery diseases and other atherosclerotic diseases
Skills achieved	Determination of serum cholesterol
Skill evaluating criteria	Perfect quantity of reagents and sample---4 marks Using proper wavelength filter---2 marks Plotting proper curve ---4 marks
FAQs	What is the principle of serum Cholesterol test? What is the normal value of serumCholesterol.
Assignment/Activity	Process 10 samples
Reference	Lipid metabolism

Week No.	Week 22
Practical No.	22
Title/ Aim	**Serum triglycerides**
Objectives	The student shall be able to do serum triglycerides estimation colorimetric Method.
Principle	**METHOD:** Acetyl-acetone **PRINCIPLE:** The serum lipids are extracted by isopropanol, which also precitates serum proteins. The interfering phospholipids, containing glycerol as integral part, are removed by adsorption on alumina. Filtrate is used for saponification and glycerol is separated from triglycerides. Action of metaperiodate converts glycerol into glyceraldehydes, which forms a yellow coloured complex with acetyl acetone. The intensity of the coloured complex is measured at 410 nm. (Violet filter).
Requirements	1) Test tubes (15 x 125 mm) 2) Centrifuge tubes 3) 0.1 ml & 5.0 ml graduated pipettes 4) Stop watch 5) Centrifuge 6) Photometer. **Reagents/Chemicals** 1) Alumina: (active grade ; 1, for chromatography): It is washed with distilled water & dried in an overnovernight at 100°C. 2) Isopropanol : AR, grade. 3) Alcoholic KOH : It is prepared by dissolving 50 g of potassium hydroxide in 600 ml of distilled water and 400 ml of isopropanol. 4) Metaperiodate: It is prepared by dissolving 77 g of ammonium acetate & 650 mg of sodium metaperiodate in 940 ml of distilled

<table>
<tr><td></td><td>water and 60 ml of glacial acetic acid.
5) Acetyl-acetone: It is prepared by mixing 7.5 ml of acetyl acetone and 200 ml of isopropanol in 800 ml of distilled water.
6) Triglyceride standard: 100 mg/dl (It is prepared by dissolving tripalmitine (or triolein) in chloroform.</td></tr>
<tr><td>Environment</td><td>MLT laboratory</td></tr>
<tr><td>Procedure</td><td>1) Take two test-tubes labeled as test and standard.
2) Put alumina approximately 0.5 gms in both the tubes.
3) Add 4.0 ml of isopropanol to both the tubes.
4) Add 0.1 ml serum in the tube labeled as test.
5) Add 0.1 ml of triglyceride standard 100 mg/dl in the tube labeled as standard.
6) Mix the contents of test thoroughly and also mix the contents of standard.
7) Keep exactly for 15minutes at room temperature (25°C ± 5°C) with intermittent mixing.
8) Transfer the contents of test and standard to respective centrifuge tubes and centrifuge at 3000 RPM for 10 minutes. Now pipette in the tubes labelled as follows;

Table 15.14
<table>
<tr><td></td><td>Test</td><td>Std</td><td>Blank</td></tr>
<tr><td>Filitrate (Test). Ml</td><td>2.0</td><td>-</td><td>-</td></tr>
<tr><td>Filitrate (Std). ml</td><td>-</td><td>2.0</td><td>-</td></tr>
<tr><td>Isopropanol. Ml</td><td>-</td><td>-</td><td>2.0</td></tr>
<tr><td>Alcoholic KOH. Ml</td><td>0.6</td><td>0.6</td><td>0.6</td></tr>
<tr><td colspan="4">Mix thoroughly and keep at 60-70°C for 15 minutes</td></tr>
<tr><td>Metaperiodate. Ml</td><td>1.5</td><td>1.5</td><td>1.5</td></tr>
<tr><td colspan="4">Mix thoroughly and keep at room temperature for 5 min</td></tr>
<tr><td>Acetyl acetone. Ml</td><td>1.5</td><td>1.5</td><td>1.5</td></tr>
</table></td></tr>
</table>

	Mix, thoroughly and keep at 70°C for 15minutes. Cool the tubes and read intesities of test and standard against blank at 420 nm
Observations	Read intensities of test and standard against blank
Result	**CALCULATION:** Serum triglycerides, mg/dl = $\frac{\text{O.D. Test}}{\text{O.D. Std}}$ × 100 **Normal range** 10-190 mg/dl.
Clinical significance	Elevated levels of triglycerides in piasma have been considered as risk factors related to atherosclerotic diseases. The hyperlipidemias can be inherited trait or they can be secondary to a variety of disorders of diseases of diseases including nephrosis, diabetes mellitus, biliary obstruction and metabolic disorders associated with endocrine disorders
Skills achieved	Determination of serum triglycerides.
Skill evaluating criteria	Perfect quantity of reagents and sample---4 marks Using proper wavelength filter---2 marks Plotting proper curve ---4 marks
FAQs	What is the principle of serum Triglycerides test? What is the normal value of serumTriglycerides.
Assignment/Activity	Process 10 samples.
Reference	Lipid metabolism