NUTRITIONAL BIOCHEMISTRY & FOOD REGULATION AND QUALITY CONTROL

PRACTICAL

M.Sc. FOOD AND NUTRITION SCIENCE
SEMESTER-II, PAPER-V

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M.Sc. FOOD AND NUTRITION SCIENCE: NUTRITIONAL BIOCHEMISTRY & FOOD REGULATION AND QUALITY CONTROL (PRACTICAL)

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This book is exclusively prepared for the use of students of M.Sc. (Food and Nutrition Science), Centre for Distance Education, Acharya Nagarjuna University and this book is meant for limited circulation only.

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

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M.Sc. FOOD AND NUTRITION SCIENCE
SEMESTER-II, PAPER-V
PRACTICAL-I
205FN24-NUTRITIONAL BIOCHEMISTRY & FOOD REGULATION AND QUALITY CONTROL

SYLLABUS

I

1. Quantitative Analysis of Serum / Blood Constituents

- Blood Glucose
- Serum Proteins
- Serum Cholesterol
- Serum Iron
- Serum Phosphorous
- SGOT (Serum Glutarnic Oxaloacetate Transaminase)
- Serum Alkaline Phosphatase

2. Urinary Estimations

- Normal and Abnormal Constituents
- Creatinine and Urea

II

1) Determination of Threshold Value for Basic Tastes
2) Odour Recognition
3) Determination of Threshold Value for Various Odours
4) Perform Preference Tests: Paired Comparison
5) Perform Discrimination Tests: Duo-trio
6) Perform Discrimination Tests: Triangle
7) Perform Discrimination Tests: Ranking Test
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9) Training of Judges, for Recognition of Certain Common Flavour and Texture Defects Using Different Types of Sensory Tests
10) Descriptive Analysis Methodology-Perform Descriptive Sensory Test
11) Sensory Evaluation of Various Food Products Using Different Scales, Score Cards etc.
12) Texture Profile Analysis of Selected Food Product
13) Estimation of Color of Food Product

I - QUANTITATIVE ANALYSIS OF SERUM / BLOOD CONSTITUENTS

EXP: 1

DETERMINATION OF BLOOD GLUCOSE

AIM: To estimate the amount of glucose, present in blood.

PRINCIPLE:

Glucose reacts with o-toluidine is glacial acetic acid in the presence of heat to yield a blush green. N-glucosylamine Proteins in blood are precipitated with trichloroacetic acid. Equal volume of protein-free filtrate containing glucose and a standard solution and blank containing trichloroacetic acid are separately treated with the reagent and optical density values are read in a photoelectric colorimeter using a red Alter (625).

REAGENTS:

1) 0-Toluidine reagent - To 5.0g thiourea (reagent grade) adds 90.0ml of o-toluidine and dilute to I litre with glacial acetic acid-Store in a brown or amber coloured bottle and keep the reagent in a refrigerator.
2) 10% Trichloroacetic acid.
3) Glucose standard-Dissolve 10mg glucose in about 50ml of distilled water in 100ml of volumetric flask 50ml of 10%trichloroacetic acid. Make up to 100ml mask with distilled water.
4) Blank solution: - Dilute 30ml of 10% trichloroacetic acid to 100ml.

PROCEDURES:

Preparation of Protein-Free Filtrate: Into a dry test-tube, pipette 50ml of distilled water and 0.5ml of blood. Mix well. Add 1.5ml of 10% trichloroacetic acid and mix thoroughly. Let stand for 10 minutes and filter into a day test tube. Development of Colour.

Label three test tubes as T, s and B Pipette 1.0ml of protein-free filtrate into T.1.0ml of standard into s and 10 ml of blank solution into B. To all the tubes add 5.0ml of O-toluidine reagent and mix thoroughly. Keep the tubes in a boiling water bath for lo minutes. Cool and read the optical densities at 630 mm.

Standard Concentration/volume of Samplex100 = glucose in mg/%
For s1, 0.05/0.1×100=51 = 50mg%.

Determination of Blood Glucose:

ADDITIONS	B(ML)	S(ML)	T(ML)
DISTILLED WATER	1	-	-
GLUCOSE STANDARD	-	1	-
PROTEIN FREE FILTRATE	-	-	1
Θ-TOLUIDINE REAGENT	5	5	5

Calculations:

Glucose (mg) in 100ml of blood =

=O. D of test – O D of blank ÷ O.D of standard – O.D of blank × 0.1÷0.1 ×100

= T-B ÷ S-B × 1×100

Result:

Clinical aspect:

The normal level of blood glucose in the fasting state varies between 70 and 110mgldl. The level rises post-prandially i.e., following the ingestion of a regular meal to not more than 140mg%.

REFERENCE:

1) Tietz NW, ed. Clinical Guide to Laboratory Test, 3r ed. Philadelphia, Pa: W.B. Saunders, 1995: 76-77.
2) Biswajit Mohanty and Sharbari Basu (2007). Textbook of Fundamental of Practical Clinical Biochemistry, B1 Publications Pvt. Ltd 54. Janpath, New Delhi.
3) Sadasivam S and Theymoli Balasubramanian (1985). Practical Manual (Under Graduate) Tamil Nadu Agricultural University, Coimbatore.2.
4) Sadasivam S and Manickam A (1997). Biochemical Methods. New Age International (p) Limited. Ansari road, Daryaganj, New Delhi. 1-5.

EXP: 2

DETERMINATION OF SERUM PROTEINS

AIM: To estimate the amount of protein present in serum

PRINCIPLE:

The peptide bonds (-co-NH) present in the protein react with copper sulphate in an alkali medium (biuret reagent) blue/purple to give a blue/purple colour. The optical density of this solutionat 540mm is compared with the colour developed by standard protein solution similarly treated to get serum protein level.

Total protein estimation by the biuret method can be further extended to determine the number of Globulins (G) and albumin (A) and determine A/G ratio. The globulins are precipitated using a sulphate-sulphite solution. The albumin present in the globulin-free solution is estimated using biuret procedure. Globulin - Total Protein - Albumin the

REAGENTS:

1) **Sulphate-Sulphite Solution:** Dissolve 208gof Sodium sulphate and 70g of sodium sulphite in 900ml of water, add 2ml of conc. H2so4 and make up the volume to 1 litre, pH> 7.0.
2) **Biuret Reagent:**

   Stock solution: -Take 4.5g of Rochelle salt in 400ml of 0.21 NaOH, and add 15g of CuSo4 while stirring into it, pour 5gKI and make up volume to 1 litre with o.2N NaOH.

   Working Standard: -Prepare the working solution by 5-fold dilution of the stock solution with 0.2NNaOH Containing 0.59% KI
3) **Tartrate Iodine Solution:** Take 9g of Rochelle salt in 1 litre of 0.2NaOH containing 6.5 9% KI.
4) **Ether**
5) **Standard Solutions:** -stock solution 6g%.
6) **Working Standard:** 625 mg% (6.25mg/ml) Dilute 0.66ml of stock solution in 6.4ml of sulphate – sulphite solution for preparing standard curve. For one point calibration, dilute the stock 1:16 times, 1 ml = 375mg.

Procedure:

To 6ml of sulphate sulphite solution, add 0.4 ml of plasma white stirring. Pipette out 2ml of mixture and 5ml biuret reagent. The OD of the colour developed is measured. This reading gives the value of total protein. In another test tube take 2ml of the above mixture and add 3ml of ether. The tube is stoppered and shaken by inversion 40 times. The solution is then centrifuged at 3000rprn for 5 minutes. The tube is then tilted, taking care not disturb the precipitate, 2ml of the clear solution below the globulin layers is pipette out and 5ml of biuret reagent is added. For blank, add 2ml of sulphate-sulphite solution to 5ml of biuret reagent.

Mix the test tubes properly and place at 87°c for10 minutes in a water bath. Then cool it at room temperature. Read the final colorimeter reading (OD) at 540mm.

Calculation:

Amount of total protein present in g% =

= ODT ÷ ODS × Conc. Od S ÷ vol of sample × 100

= ODT ÷ ODA × Conc.S ÷ vol of sample × 100

CLINICAL ASPECTS:

The total protein in adults ranges from 60 to 8.09% and albumin varies from 3.5 to 5.0g/8. The normal A/F ratio is 1.7:1, Liver synthesizes albumin and most of globulins except the immune globulins, globulins, which are synthesized by the plasma cells. Very minute quantity of albumins is excreted (<100mg / dayin urine), which cannot be detected by normal heat coagulation. Test. Thus, diseases of the liver, kidney and the state of hydration affect the serum protein level. In acute conditions of liver diseases, the albumin concentration remains apparently normal because of its long half-life, whereas in chronic liver disease, like cirrhosis of liver, albumen concentration starts falling. This in turn leads to reversal of AIG ratio. Therefore, in case of cirrhosis, reversal of A/Gratio is a bad prognostic sign as it depicts total parenchymal damage. Similarly, nephritic syndrome is associated with massive proteinuria, albumin being a lower molecular weight protein leaks out early boding to reversal of A/G ratio. In dehydration, because of haemo concentration there is an apparent increase in serumprotein. In monoclonal diseases like multiple myelorna (abnormal Proliferation of plasma cells), serum protein concentration is found to be increased.

RESULT:

REFERENCE:

1) Tietz NW, ed. Clinical Guide to Laboratory Test, 3rd ed. Philadelphia, Pa: W.B. Saunders, 1995: 76-77.

2) Biswajit Mohanty and Sharbari Basu (2007). Text book of Fundamental of Practical Clinical Biochemistry, B1 publications Pvt. Ltd 54. Janpath, New Delhi.

3) Sadasivam S and Theymoli Balasubramanian (1985). Practical Manual (Under Graduate) Tamil Nadu Agricultural University, Coimbatore.2.

4) Sadasivam S and Manickam A (1997). Biochemical Methods. New age International (p) limited. Ansari Road, Daryaganj, New Delhi. 1-5.

EXP: 3

DETERMINATION OF SERUM CHOLESTEROL

AIM: To estimate the amount of cholesterol, present in serum

PRINCIPLE:

Cholesterol reacts with acetic anhydride and concentrated sulphuric acid, producing a green-colored complex. The intensity of this green colour, measured at 540-600mm, is directly proportional to the cholesterol concentration.

REAGENTS:

1) **FeCl3-Acetic Acid reagent: -**10g Ferric chloride in 1000ml glacial acetic acid.
2) **Colour Reagent:** Concentrated sulfuric acid (H2SO4).
3) **Standard cholesterol solution: -**0.3g of cholesterol in ml of ethanol.

PROCEDURE:

1) Label test tubes - Blank (B) Standard(s) and test (T).
2) Add 5 ml of FeCl2 -Acetic Acid in all three test tubes
3) Add 0.5ml of distilled water in Blank (B).
4) Add 0.5 ml of standard cholesterol solution in Standard (s)
5) Add 0.5 ml of serum sample in Test(T).
6) Add 3ml of concentrated H2So4 slowly, in test tubes.
7) Incubate for 15-20 minutes at room temperature-
8) Measure absorbance at 560mm.

COMPONENT	BLANK (B)	STANDARD (S)	TEST (T)
FECL3 - ACETIC ACETIC ACID REAGENT	5ML	5ML	5ML
DISTILLED WATER	0.5ML	-	-
STANDARD SOLUTION	-	0.5ML	-
SERUM SAMPLE	-	-	0.5 ML

Determination of Serum Cholesterol:

CLINICAL ASPECTS:

Normal level of cholesterol is 130-200 mg/dl. However, it is desirable that level should not exceed 200mg/dl. The serum cholesterol concentration is low at birth but it Increases with the growth of the child. At the time of growth of the child it reaches slightly above the adult volume and then comes back with in normal limits is by the end of second decade. The cholesterol value is slightly lowered in women till they attain menopause.

Hyper cholestrolemia is seen in the case of severe diabetes mellitus, biliary obstruction. Mysedumanephritic syndrome, chronic renal failure and familiar hypercholestrolemia. A lower observed in case of malabsorption cirrhosis of liver hyperthepeliodsanemia and starvation.

RESULT:

CALCULATION:

Cholesterol (mg /dl) =

(Absorbance of test / Absorbance of standard) ×Concentration of standard

REFERENCE:

1) Tietz NW, ed. Clinical Guide to Laboratory Test, 3rd ed. Philadelphia, Pa: W.B. Saunders, 1995: 76-77.

2) Biswajit Mohanty and Sharbari Basu (2007). Textbook of Fundamental of Practical Clinical Biochemistry, B1 publications Pvt. Ltd 54. Janpath, New Delhi.

3) Sadasivam S and Theymoli Balasubramanian (1985). Practical Manual (Under Graduate) Tamil Nadu Agricultural University, Coimbatore.2.

4) Sadasivam S and Manickam A (1997). Biochemical methods. New age International (p) limited. Ansari road, Daryaganj, New Delhi. 1-5.

EXP: 4

DETERMINATION OF SERUM IRON

AIM: To determine the amount of Iron present in scrum.

PRINCIPLE:

Ferrous iron gives a pink colour with 2-Dipyridyl.A solution of dipyridyl to acetic acid is added to serum followed by reducing agent. Proteins are removed by heating in boiling Water and then centrifuging (0) filtering. (ox)

REAGENTS: -

1) **2-3 dipyridyl:** 0.8% in acetic acid 3% o/v.
2) **Sodium Sulphite:** 0.1m dissolved 1.85 gm of anhydrous Sulphite (or) 2.52gm of NO2SO3, 7H2O in water and make upto 100ml prepare freshly every few days.
3) **Chloroform**
4) **Standard solution**- Dilute 3ml of stock solution to 100mlWith water to obtain solution containing 3mg/ml.

PROCEDURE:

Mix equal volumes of serum, 0.1m sodium sulphate and dipyridyl reagent in a glass. Stoppered tube which can be centrifuged. Heat in boiling water bath for 5 minutes. Cool and 1 ml of chloroform stopper and shake vigorously for 30 of seconds. Remove the stopper and centrifuge for 5 minutes at 300 rpm. If the supernatant fluid is not completely clean, repeat the shaking and centrifuge. Read at 520nm on using a green filter. As blank use water instead of serum for the standard put through the working in the same way. Clean the tubes used by placing them in boiling 5NHCl.Then wash with glass dissolved in water and keep aside.

CLINICAL ASPECTS:

Nearly $2/3^{rd}$ of the body's iron 4.5g is present in haemoglobin of which about 90% is red cell. The greater part of the remaining iron is stored, apparently combined with protein as ferritin, the lives being the tissue richest in Iron. This is readily available when required in addition. Iron is present in most tissues in protein such as cytochrome and catalase which iron is thus concentrated with the transport of oxygen by the blood and in Cellular oxidations.

The blood iron is almost entirely present in the red cell haemoglobin but there is a small amount in the plasma as Ferric iron forming a pink complex with a protein, which regarded as in iron, which is being transported in concentration with the metabolism of haemoglobin. Accordingly, determinations of serum iron are used in investigating some cases of iron anemia.

CALCULATION:

Micrograms iron per 100ml of serum

= OD of Test ÷ OD of standard × 300

RESULT:

REFERENCE:

1) Tietz NW, ed. Clinical Guide to Laboratory Test, 3rd ed. Philadelphia, Pa: W.B. Saunders, 1995: 76-77.

2) Biswajit Mohanty and Sharbari Basu (2007). Text Book of Fundamental of Practical Clinical Biochemistry, B1 Publications Pvt. Ltd 54. Janpath, New Delhi.

3) Sadasivam S and Theymoli Balasubramanian (1985). Practical Manual (Under Graduate) Tamil Nadu Agricultural University, Coimbatore.2.

4) Sadasivam S and Manickam A (1997). Biochemical Methods. New Age International (p) Limited. Ansari Road, Daryaganj, New Delhi. 1-5.

EXP: 5

DETERMINATION OF SERUM PHOSPHOROUS

AIM: To determine the amount of phosphorous present in serum

PRINCIPLE:

The method for serum phosphorus estimation is based on the formation of a phoshomolybdate complex. Inorganic phosphate reacts with ammonium molybdate in an acidic medium to form a phosphomolybdic acid complex. This complex is colourless but can be measured calorimetrically. A reducing agent is added to produce a blue-colored Complex, enhancing the sensitivity. The intensity of the blue colour is directly proportional propos to the Concentration of inorganic phosphate in the sample and is measured at 340-380 m.

Reagents:

1) **Ammonium Molybdate Reagent:** Dissolve 2.5g of ammonium molybdate in 100ml of 5N sulfuric acid.
2) **Phosphate Standard Solution:** Dissolve 0.439g of KH1PO4 in distilled water and make up to 1 litre.
3) **Reducing Agent:** Use freshly prepared ascorbic acid solution if reduction to a blue complex is required 4.10% Trichloroacetic Acid.

PROCEDURE

1) Collect 2-3 ml of blood, allow it to clot and centrifuge to obtain serum
2) Mix 1ml of serum with 1ml of TCA, centrifuge and usethe supernatant.
3) Label three test tubes as Blank (B), Standard (s) and Test (T).
4) Add 1ml of molybdate reagent to three test tubes.
5) Add 1ml of Distilled water in Blank (B).
6) Add 1ml of phosphate standard in standard (s).
7) Add 1ml of serum sample In Test (T).
8) Mix well and incubate at room temperature for 10minutes.
9) Measure absorbance at 340-380nm using a using a spectrophotometer.

CLINICAL ASPECTS:

Serum phosphorus plays a crucial role in maintaining bone health, nerve function and overall metabolism. Normal values of serum phosphorus 2.5 to 4.5 mg/dl in adults. Phosphorus along with calcium, forms the mineral matrix of bones and teeth.

Phosphorus is a key component of ATP, which is the primary energy currency of cells, it is also involved in nerve Impulse transmission.

Low serum phosphorus levels lead to hypophosphatemia which leads to muscle weakness, paralysis, seizures, respiratory failure and death. High serum phosphorus levels lead to Hypophosphatemia which causes soft tissue calcification, cardiovascular disease and Increased risk of Fractures.

CALCULATION:

Serum phosphorus (mg/dl):-

= Absorbance of test / absorbance of standard × concentration of standard

RESULT:

REFERENCES:

1) Tietz NW, ed. Clinical Guide to Laboratory Test, 3rd ed. Philadelphia, Pa: W.B. Saunders, 1995: 76-77.

2) Biswajit Mohanty and sharbari Basu (2007). Textbook of fundamental of practical clinical biochemistry, B1 publications Pvt. Ltd 54. Janpath, New Delhi.

3) Sadasivam S and Theymoli Balasubramanian (1985). Practical Manual (Under Graduate) Tamil Nadu Agricultural University, Coimbatore.2.

4) Sadasivam S and Manickam A (1997). Biochemical methods. New Age International (p) limited. Ansari Road, Daryaganj, New Delhi. 1-5.

EXP: 6

SGOT/AST

AIM: To determine the amount of SGOT present in serum

PRINCIPLE

Aspartate amino transferase (AST) catalyzes the transfer of the amino group from L-aspartate to α-ketoglutarate to yield oxaloacetate and L-glutamate. Malate dehydrogenase (MDH) catalyzes the reduction of oxaloacetate with simultaneous oxidation of NADH+ to NAD. The resulting rate of decrease in absorbance at 340 nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

$$\text{L-aspartate} + \alpha\text{-ketoglutatrate} \xrightleftharpoons{\text{AST}} \text{Oxaloacetate} + \text{NADH} + \text{H}^+$$

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{MDH}} \text{L-malate} + \text{NAD}^+$$

Concentrations in the Test

Reagent R1		
Buffer Tris, pH = 7.5	125	mmol/L
L-aspartate	290	mmol/L
LDH	≥ 0.9	U/L
MDH	≥ 0.6	U/L
Detergent, preservative.		
Reagent R2		
NADH	0.18	mmol/L
α-ketoglutarate	15	mmol/L
Detergent, preservative.		

Kit Components:

Reagent 1: SGOT Reagent 1

Reagent 2: SGOT Reagent 2

REAGENT PREPARATION, STORAGE & STABILITY:

Reagent R1 and R2 are ready to use liquid reagents. Mix the reagent R1 and R2 in the ratio of 4:1 respectively to prepare the desired volume of working reagent prior to use. Do not shake vigorously. The working reagent is stable for 20 days at 2-8 °C. The reagent kit should be stored at 2-8 °C and is stable till the expiry date indicated on the label.

PRECAUTIONS & HANDELING: The reagents/samples should be handled by qualified personnel only. Discard reagent/sample as per good laboratory practices and local regulatory

requirements. Read the instructions given on the labels and instructions for use carefully before using the kit. The kit is intended for in-vitro diagnostic use only. Don't freeze the reagent. Do not shake the reagent vigorously. Discard the reagent if the absorbance of the reagent goes below 0.900 O.D. against D/W at 340 nm. Contamination of the reagent should be avoided.

TEST PARAMETERS

Name	SGOT
Reaction Type	Kinetic (↓)
Wavelength Primary	340 nm
Flow Cell Temp.	37 °C
Blank setting	D.W.
Blank Abs Limit	>0.900
Linearity	1700 IU/L

Reagent Volume	1000 µl
Sample Volume	100 µl
Incubation Temp.	37 °C
Delay Time	60 sec.
Read Time	30 sec.
Factor	1746
Standard Conc.	-

MATERIALS REQUIRED BUT NOT PROVIDED:

Test tubes, Micropipette with tips, Analyser, Controls, Incubation chamber.

SPECIMEN COLLECTION & PRESERVATION:

Blood should be collected in a clean dry container. Serum is preferred. Heparinized or EDTA - Plasma can be used. Specimen with any visible haemolysis is not acceptable. SGOT in serum / plasma is stable for 7 days at 2-8 °C and for 30 days at -10 °C.

COMPONENTS OF REAGENT:

Component	**Concentration**
Tris Buffer, pH 7.7	80 mmol/l
L-Aspartate	200 mmol/l
NADH	0.15 mmol/l
LDH	>1000 IU/L
MDH	>500 IU/L
Ketogluterate	10 mmol/l
Stabilizers, inactive ingredients and surface-active agents.	

ASSAY PROCEDURE:

	Test
Reagent	1000 µl
Serum / Plasma	100 µl
Mix the reagent and sample in the above-mentioned ratio and start the stop watch.	
Aspirate the reaction mixture into the flowcell and record the absorbance at 60th and 90th sec.	

CALCULATION:

SGOT Activity (IU/L) = Abs. per min x 1746.

REFERENCE VALUES FOR NORMAL PEOPLE: Upto 40 IU/L at 37 °C.

PERFORMANCE CHARACTERISTICS

Measuring Range: The assay is linear between 09 - 1700 IU/L. If the SGOT value exceeds linearity limit (above 1700 IU/L), dilute the specimen suitably with normal saline and repeat the assay. In that case, assay value should be multiplied with the dilution factor to obtain correct SGOT value of the specimen.

Interference: There is no significant interference in samples containing Bilirubin upto 20 mg/dL. Haemolysis interferes due to SGOT activity from erythrocytes.

Precision: Precision studies has been carried out using quality control sera as shown below:

(n=10)	Within Run			Between Run		
Specimen Material	Mean (IU/L)	SD (IU/L)	CV %	Mean (IU/L)	SD (IU/L)	CV %
Low Value Serum	39.4	0.45	1.1	34.8	0.52	1.5
High Value Serum	196.2	0.95	0.5	181	1.25	0.7

QUALITY CONTROL:

Inclusion of a normal value and abnormal value chemistry control serum in each test run ensures optimum quality control. Consistent use of same type and methodology of control serum provides between run precision and accuracy data for SGOT. We recommend to produce such data on daily basis for greater accuracy in assay system which include reagents, instrument, apparatus and operator.

PRECAUTIONS

1) Discard the working reagent if its absorbance is less than 0.900 at 340 nm against distilled water.
2) Haemolysis must be avoided because erythrocytes contain 10 times the normal concentration of GOT found in serum.
3) If the SGOT value exceeds 1700 IU/L then dilute the specimen suitably with normal saline and repeat the assay. In such case multiply the result obtained with the dilution factor to obtain correct SGOT value.

REFERENCE:

1) Tietz NW, ed. Clinical Guide to Laboratory Test, 3rd ed. Philadelphia, Pa: W.B. Saunders, 1995: 76-77.
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3) Fischbach F, Zawta B. Age dependent Reference Limits of Several Enzymes in plasma at different measuring temperatures. Klin. Lab. 1992; 38:556-561.
4) Penttila, I.M., et al., Scand, J.Clin, Lab. Invest 35, 275/ (1975). 5. Hafken shield, J.C, et al. J.ClinChem.Clin.Biochem.17, 219 (1979).

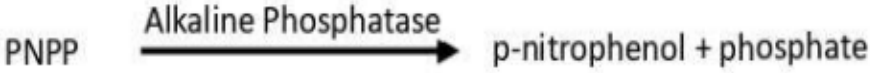
EXP: 07

SERUM ALKALINE PHOSPHATASE

AIM: Estimation of Alkaline Phosphatase activity in serum / plasma using PNPP kinetic method.

PRINCIPLE:

Serum Alkaline Phosphatase levels are of interest in the diagnosis of hepatobiliary disorders and bone disease associated with increased osteoblastic activity. Moderate elevations of Alkaline Phosphatase may be seen in Hodgkin's disease, congestive heart failure, ulcerative colitis, regional enteritis, and intra-abdominal bacterial infections. Alkaline Phosphatase is a reagent set for determination of Alkaline Phosphatase activity based on kinetic method using P-nitrophenylphosphate. Alkaline Phosphatase is a single reagent system with one step reconstitution.



KIT COMPONENTS

Reagent 1: Substrate Reagent

Reagent 2: Diluent

REAGENT PREPARATION, STORAGE & STABILITY

Reconstitute each substrate tablet with diluent as per the instruction indicated on the substrate bottle. The working reagent is stable for 21 days at 2-8 °C. The reagent kit should be stored at 2-8 °C and is stable till the expiry date indicated on the label.

PRECAUTIONS & HANDELING

The reagents/samples should be handled by qualified personnel only. Discard reagent/sample as per good laboratory practices and local regulatory requirements. Read the instructions given on the labels and instructions for use carefully before using the kit. The kit is intended for in-vitro diagnostic use only. Don't freeze the reagent. Do not shake the reagent vigorously. Discard the reagent if the absorbance of the reagent exceeds 0.700 O.D. against D/W at 405 nm. Contamination of the reagent should be avoided.

TEST PARAMETERS

Name	ALK.PHOS.
Reaction Type	Kinetic (↑)
Wavelength	405 nm
Flow Cell Temp.	37 °C
Blank setting	Reagent
Blank abs. limit	< 0.700
Linearity	2000 IU/L

Reagent Vol	1000 µl
Sample Vol	20 µl
Temperature	37 °C
Delay Time	60 sec.
Read Time	30 sec.
Factor	2720
Standard Conc.	-

MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes, Micropipette with tips, Analyser, Controls, Incubation chamber.

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Haemolysed specimen should be avoided as it may falsely elevate results. EDTA, Citrate and Oxalate inhibit Alkaline Phosphatase activity and should not be used as anti-coagulant. Alkaline Phosphatase in the serum/plasma is stable for 4 days when stored at 2-8 °C and several months when stored at -10° C.

COMPONENT OF REAGENT:

Component	Concentration
Diethanolamine Buffer, pH 9.8	1 Mol/l
p-nitrophenyl phosphate	10 mmol/l
Magnesium chloride	0.5 mmol/l
Stabilizers and inactive ingredients.	-

ASSAY PROCEDURE

	Blank	Test
Reagent	1000 µl	1000 µl
Serum / Plasma	-	20 µl
Mix the reagent and sample in the above-mentioned ratio and start the stop watch.		
Aspirate reaction mixture into flow cell.		
Record absorbance at 60^{th}, 90^{th}, 120^{th} & 150^{th} sec. (30 sec. interval).		

CALCULATION

Calculate average Abs/min = Abs /30 sec x 2

Alkaline Phosphatase (IU/L) = Abs / min x 2720.

REFERENCE VALUES FOR NORMAL PEOPLE:

	25 °C	30 °C	37 °C
Adult (>15 years)	60-170 IU/L	70-207 IU/L	108-306 IU/L
Children (<15 years)	150-450 IU/L	195-585 IU/L	210-810 IU/L

PERFORMANCE CHARACTERISTICS

Measuring Range: The assay is linear between 27 - 2000 IU/L. If the Alkaline Phosphatase value exceeds linearity limit (above 2000 IU/L), dilute the specimen suitably with normal saline and repeat the assay. In that case, assay value should be multiplied with the dilution factor to obtain correct Alkaline Phosphatase value of the specimen.

Interference: There is no significant interference in samples containing Bilirubin upto 20 mg/dL and Haemoglobin upto 400 mg/dL.

QUALITY CONTROL:

Inclusion of a normal value and abnormal value chemistry control serum in each test run ensures optimum quality control. Consistent use of same type and methodology of control serum provides between run precision and accuracy data for Alkaline Phosphatase. We recommend to produce such data on daily basis for greater accuracy in assay system which include reagents, instrument, apparatus and operator.

PRECAUTIONS

1) Discard the reagent if its absorbance exceeds 0.700 at 405 nm against distilled water.
2) If Alkaline Phosphatase activity exceeds 2000 IU/L then dilute the specimen suitably with normal saline & repeat the assay. In such case the results obtained should be multiplied by dilution factor to obtain the correct Alkaline Phosphatase activity.

REFERENCE:

1) Henry R.J.,"Enzymes" in Clinical Chemistry Principle and Techniques, Harper & row Publishers, New York, 815 (1974).
2) Young D.S. et. al, Clin Chem.18,1041, (1972).

URINARY ESTIMATIONS

NORMAL AND ABNORMAL CONSTITUENTS

OBJECTIVE:

To identify the normal chemical constituents in urine. To identify the abnormal constituents in urine.

INTRODUCTION:

Many kidney and other medical problems can be detected by routine urine analysis, which includes chemical PH measurements tests to detect protein, sugar and ketones and microscopic examination to detect red and white blood cells in a doctor office, strips (or) dipsticks are used in routine testing of urine. The chemicals in the strip least with substances in the wine and change colour. Protein present in urine usually a sign of kidney disease, but if may occur normally after strenuous exercises, Glucose in the urine may appear as a result of Starvation uncontrolled diabetes of and alcohol intoxication ketones are formed, when the body breaks down fat. In some instances, doctors prescribe specialized test for the levels of inorganic ions, such as chloride sulphate, calcium, ammonium and phosphate. Although normal urine contains these ions some medical disorders resulting from biochemical function may occurs either lower (or) higher concentration of some of these ions may increases.

MATERIAL REQUIREMENTS:

1) Test Tube
2) Test Tube Holders
3) Water Bath
4) Spatula
5) Dropper

EXP: 8

A) NORMAL CONSTITUENTS IN WINE TEST FOR INORGANIC COMPOUNDS.

- **Chloride Tests:**

Usually, excretion of wine may be decreased in some fevers, chronic nephritis, and fastingand diarrhoeasilver nitrate test. Take 5 ml of urine in a Test tube and add few drops of nitric acid to acidify it then add a few drops of silver nitrate. A white precipitate of silver chloride is produced which darkness on standing, the precipitate is soluble in ammonium hydroxide.

- **Calcium and phosphorus:**

To 20ml of wine add few drops of liquid ammonia and boil. A white flaky precipitate indicates phosphate of calcium & magnesium.

Filter and dissolve the precipitate in 5 ml of dilute acetic acid, to one half of this solution, add potassium oxalate solution. A white precipitate of calcium oxalate is formed to the other half of solution add equal volume of conc. Nitric acid and 5 ml of ammonium hydroxide.

- **Sulphuric Tests:**

1) Inorganic sulphate.
2) Ether & sulphate: There are ethers of sulphuric acid with phenolic substances like phenol, cresol.
3) Organic sulphur traces a sulphur containing amino acids crystalline are also present but not usual methods to ml of urine a few crops of concentration hydrochloric acid and 8 ml of solutions of husky precipitate indicate the presence of inorganic sulphate.

Tests for Organic Compounds.

Test for Urea:

1) **BiureticTest**: Take a small amounts urine in a test tube & heat gently in a low flame urea meets and liberates ammonia. Continue heating until the mass begins to solidify cool the tube and dissolve the residue in dilute NaOH Solution. A purple colour is formed due to the Presence of biuret.
2) **Decomposition Test:** Urea, can be decomposed by the enzyme urea liberating ammonia. Take 5 ml of fresh wine and add 1ml of urea solution in a test tube. Keep it for 10 minutes for the reaction to proceed then boil the contents, odours of the ammonia come through the mouth of the tube.

Test for Creatinine:

Creatinine is the anhydride of creatine and under normal conditions about 0.1g of creatinine100 ml of wine is present.

1) **Nitroporous Side Test:** Fake 5 ml of urine in a test tube and add few drops of NaOH solution test tube and add few drops of NaOH solution. Make the content of the tube alkaline by adding a few drops of sodium hydroxide solution. A ruby red colour will develop which soon turns yellow.

2) **Picric Acid Test:** Take 5 ml of urine in a test tube and add few drops of sodium aqueous solution of picric acid and make the mixture alkaline with NaOH solution a red colour is produced which turns yellow if the solution is acidified.

Test for Glucose:

About 0.15% of reducible sugar are present in urine, which may include pentose, lactose & other types of carbohydrates. Normal urine doesn't contain glucose. However, in glycosuria and diabetes 3-10% (or) more glucose may be present in urine, tranceglycose, glycosuria has to be detected in urine by conventional methods.

PROCEDURE	OBSERVATION	INFLUENCE
ADD ABOUT 5ML OF BENEDICT REAGENT INTO 0.5 ML OF URINE AND BOIL FOR 2MIN	BLUE COLOUR APPEARS	SUGAR ABSENT
ADD ABOUT 5ML OF BENEDICT REAGENT INTO 0.5 ML OF URINE AND BOIL FOR 2MIN	LIGHT GREEN PRECIPITATE APPEARS	0.1-0.5% OF REDUCING SUGAR PRESENT
ADD ABOUT 5ML OF BENEDICT REAGENT INTO 0.5 ML OF URINE AND BOIL FOR 2MIN	GREEN PRECIPITATE APPEARS	0.5 TO 10% REDUCING SUGAR.
ADD ABOUT 5ML OF BENEDICT REAGENT INTO 0.5 ML OF URINE AND BOIL FOR 2MIN	YELLOW PRECIPITATE APPEARS	1-2 % REDUCING SUGAR PRESENT
ADD ABOUT 5ML OF BENEDICT REAGENT INTO 0.5 ML OF URINE AND BOIL FOR 2MIN	BRICK RED PRECIPITATE APPEARS	ABOVE 2% REDUCING SUGAR PRESENT

Benedict's Test: Take 5 me of benedict's reagent in test tube and add 8 drops of urine which to be tested. Boil the content for 1-2 minutes and then cool slowly if glucose is present the solution will be filled with greenish yellow red precipitate depending upon the quantity of glucose present. Greenish precipitate would intake very small amount of glucose it no glucose is present. The solution will remain clear.

Acetone Bodies:

Acetone Bodies (or) ketones are compounds that appear in body during fatty acid oxidation as intermediate products. In normal conditions these appear in the wine, these compounds are auto acetic acid acetone & B- hydroxyc butyric acid. During acid acetone and B-hydroxyl butyric. During starvation more of these bodies accumulates in the body.

Nitroprusside Tests: Take 2 me of urine in the test tube & add few drops of 5% aqueous NaNitroprusside solution make it alkaline by adding few chops of NaOH solution. A persistent red colourindicates the presence of acetone bodies.

Another test can be performed. Take 5 me of urine and add few drops of sodium Nitroprusside solution mix well and add cone. NH4OH solution down the sides of the tube to form a layer.

A purple ring at the zone of contact indicates the presence of acetone bodies.

EXP: 8

B). ABNORMAL CONSTITUENTS

- **Protein in urine:**

Take about 10 ml of urine in a clean & dry test tube, that the upper position of the test tube lower position is used as control. Adel 2-3 drops of 5% acetic acid. Its coagulum is formed it indicates the presence of protein in the wine.

- **Blood pigments in urine:**

1) **Benxidine Test:** Take a clean and dry test tube mix two days, each of Benxidine and hydrogen peroxide. Add a drop of these mixture to the urine, if blue green colour is formed it indicates the presence of blood in the wine sample. This test can be used to detect the presence of blood in the urine is called haematuria. The common cause of haematuria is caused by injury of urethra trait, Infection (or) stones in the urinary tract etc.

Principal: Copper sulphate of benedict's qualitative solution is reduced by reducing substances on boiling to form the colored precipitate of copper oxide. Normal urine also contains a trace of glucose and gluconate but their amount is too small to cause reduction in Benedict's test in diabetes mellitus. And in renal glycosuria, glucose is found in urine. This gives a benedict's test positive.

BILE SALT (HAYS TEST) AND BILE PIGMENTS FOUCHET'STEST:

Hay's Test:

Take 3 ml of urine & sprinkle a little of sulphur powder. It sulphurs sinks to the bottom of the test tube it indicates the presence on bile salts on the given urine sample bile salt reduce the surface tension. Sosulphurssink to bottom of the tube.

Fouchet's Test:

To 5 ml of wine add 2me of serum Chloride and little of magnesium sulphate and mix well. Barium sulphate is precipitate. The bile pigment get absorbed to barium sulphate & are present in the precipitate filter, the precipitate by using a filter paper, ad a few drops of faucet's reagent.

Result (for Benedict's Test): Yellow coloured precipitate is observed in the given urine sample indicates the presence of reducing sugars.

Result (for Nitro peroxide Test): A purple colour ring is observed in the given urine sample inculcates the presence of acetone bodies.

(B) ABNORMAL CONSTITUENT IN WINE

Result: (Heat coagulation protein Test): No coagulation is formed, indicates the absence of proteins in the given urine sample.

Result (Benzidine Test): No blue-green colour is formed indicates the absence of proteins in the given urine sample.

Result (Benedict's test): Light Green colour precipitate is observed indicates the presence of sugar in the given sample.

Result: (Hay's Test): Sulphur in sinked at bottom of the tube indicates the presence of bile salts in given sample.

Result (Faucet's Test): No blue (or) green colour is observed indicates to absence of bile pigments in given Sample.

To this precipitate green (or) blue colour formation. Indicates the presence of bile pigment faucet's reagent oxalated the mile to form blue coloured bilicyanin (or) green colored biliverdin.

Bile salts and bile pigments are presents. In the urine in case of abstructure jaundice and hepatic jaundice.

Result (For A): A yellow colour crystalline precipitate is observed indicates the presence of phosphatein given urine sample.

Result (For Sulphur Test): A bulky precipitate indicates the presence of inorganic sulphate in the given urine sample.

Result (For Organic Compounds): Ammonia odour is detected and indicates. The presence of urea in given sample.

Result (For Creatinine Test): The appearance of the yellow colour in the given sample indicates the presence of creatine.

Result (For Picric Acid Test): The appearance of red colour in the given wine sample indicates the presence of creatine

REFERENCE:

1) Tietz NW, ed. Clinical Guide to Laboratory Test, 3rd ed. Philadelphia, Pa: W.B. Saunders, 1995: 76-77.

2) Biswajit Mohanty and sharbari Basu (2007). Textbook of fundamental of practical Clinical Biochemistry, B1 publications Pvt. Ltd 54. Janpath, New Delhi.

3) Sadasivam S and Theymoli Balasubramanian (1985). Practical Manual (Under Graduate) Tamil Nadu Agricultural University, Coimbatore.2.

4) Sadasivam S and Manickam A (1997). Biochemical methods. New Age International (p) limited. Ansari road, Daryaganj, New Delhi. 1-5.

EXP: 9

ESTIMATION OF CREATININE IN URINE

Objective:

- To estimate the amount of creatinine in urine

Principle:

The method makes use of the jaffee's reaction, the production of a mahogany red colour with an alkali picrate solution. The intensity of the colour developed is compared in the colorimeter against a reagent blank at 520nm.

Reagents:

1) Picric acid (0.04 molar solutions): 9.16g of crystalline picric acid is dissolved in 100ml of water.
2) 0.75 Sodium hydroxide: 30g of sodium hydroxide in liter of water.
3) Stock solution of creatinine: Dissolve 100mg of creatinine in 0.1N hydrochloric acid and made to 100ml with the same.
4) Working standard: Diluted 2ml of the stock solution to 100ml with water. This contains 20 micro grams of creatinine per ml.

Procedure:

Dilute 5 ml. of urine to 500 nil, in a volumetric flask. Pipette 3 ml. of the diluted urine into a test tube and add 1 ml. of 004 M picric acid followed by 1 ml. of 075 N sodium hydroxide. Treat 3 ml. (= 0.03 mg.) of the standard solution for use in the same way and put up a blank consisting of 3 ml. water, and 1 ml. of each of the reagents. Allow to stand for fifteen minutes and then read in the colorimeter during the next half hour with a blue-green filter, or transmission at 500 milli microns.

Calculations:

Since the standard contains 003 rug. creatinine, and 3 ml. of diluted urine corresponds to 003 ml. of the original urine.

Grams Creatinine per Litre of Urine

$$\frac{\text{OD of the Test}}{\text{OD of the Standard}} \times 0.03 \times \frac{1000}{0.03} \times \frac{1}{1000}$$

$$= \frac{\text{OD of the Test}}{\text{OD of the Standard}}$$

Standard Curve:

Since Beer's law may not he followed sufficiently closely, prepare a standard curve to check this. For this curve dilute 2 ml. of the stock standard to 100 ml. to obtain a solution which contains 002 mg. per ml. Set up tubes as follows: For the preparation of standard curve, follow the procedure listed in table below

REAGENTS	**B**	**S_1**	**S_2**	**S_3**	**S_4**	**S_5**
GRAMS PER LITRE OF URINE	0.0	0.4	0.8	1.2	1.6	2.0
MG. PER 100 ML. URINE	0.0	40	80	120	160	200
ML. OF STANDARD SOLUTION(0.02 MG. PER ML)	0.0	0.6	1.2	1.8	2.4	3.0
ADD DISTILLED WATER (ML)	3.0	2.4	1.8	1.2	0.6	0
ADD PICRIC ACID AND SODIUM HYDROXIDE AS ABOVE						

Clinical aspects:

Urine creatine is necessary in the process of muscle contraction, which is related to the amount of phosphor creatine broken down, resynthesize occurring after contraction. Creatinine is derived from creatine and is a waste product. The normal daily excretion of creatinine ranges from 1 to 2 grams, being as a rule nearer to the higher limit in men and to the lower in women. Creatinine is largely endogenous in origin, so that the amount excreted in the urine is little influenced by the diet, provided this is a normal one and does not contain considerable amounts of creatine- or creatinine-rich foods such as meat. Such endogenous creatinine is related rather to the amount of muscle tissue and so of phosphor creatine in the body. This remains relatively constant, for which reason it can be used to check the reliability of 24 hour urine collections. It is particularly useful in this respect when a series of daily specimens is being collected.

REFERENCE:

1) Harold Varley (2005). Practical clinical biochemistry, chapter 11 Determination of creatinine in urine, CBS publishers and distributors 11, Daryaganj, New Delhi, India. 197-198.

EXP: 10

UREA (COLORIMETRICMETHOD: REAGENTFORQUANTITATIVEDETERMINATIONOFUREAINHUMANSERUMA NDPLASMAORURINES)

AIM: Quantitative determination of urea in human serum and plasma or urines to screen its level.

GENERALITIES:

More than 90% of urea is excreted through the kidneys in urines. Measurement of the plasma or serum urea concentration is widely regarded as a test of renal function. However, a number of non renal factors also influence the circulating urea concentration: Urea increased level occurs when proteins catabolism is accelerated, burns, stress, myocardial infarction... Urea is decreased in acute liver destruction and is accompanied with increased ammonium level. Urea level is generally studied in conjunction with creatinine level (urea/creatinine ratio) to refine post-renal or pre-renal diagnosis.

PRINCIPLE:

Enzymatic and colorimetric method based on the specific action of urease which hydrolyses urea in ammonium ions and carbon dioxide. Ammonium ions then form with chloride and salicylate a blue-green complex. This coloration, proportional to urea concentration in the specimen, is measured at 600 nm.

REAGENTS

R1	**UREA**	**Salicylate**
Salicylate		31mmol/L
Nitroprussiate		1.67mmol/L

Warning: Eye Irrit. 2: H319 - causes serious eye irritation

P280: Wear Protective gloves / eye protection / face protection

P305+P351+P338: IFINEYES, Rinse cautiously with water several minutes;

Remove contact lenses, if present and easy to do. Continue rinsing.

Classification due to sodium salicylate 1-<2.5%.Formore details, refer to safety data sheet (SDS).

R2	**UREA**	**Urease**
Urease		≥15KUI/L

Working reagent (R1+R2) is not classified as dangerous.

R3	**UREA**	**Base**
Sodium hypochlorite		7mmol/L
Sodium hydroxide		62mmol/L

Before Dilution: Danger: Met.Corr. 1: H290 –may be corrosive to metals Skin CORR. 1B; H314 - causes severe skin burns and eye damage.

P280: wear protective gloves/eye protection/face protection P305+P351+P338: IFINEYES, R in secautiously with water several minutes; Remove contact lenses, if present and easy to do. Continue rinsing.

Classification due to sodium hydroxide, sodium hypochlorite 1- <2.5%.
For more details, refer to safety data sheet (SDS).

Once diluted, reagent is not classified as dangerous according to regulation1272/2008/EC.

R4 UREA Standard Urea40mg/dL (6.66mmol/L)

Reagents R2 and R4 are not classified as dangerous according to regulation 1272/2008/EC.

REAGENTS:

R1 UREA Salicylate

Salicylate 31mmol/L

Nitroprussiate 1.67mmol/L

Warning: Eye Irrit. 2: H319 - causes serious eye irritationP280: wear protective gloves / eye protection / face protection.

Remove contact lenses, if present and easy to do. Continue rinsing. Classification due to sodium salicylate 1-<2.5%.Formore details, refer to safety data sheet (SDS).

R2 UREA Urease

Urease ≥15KUI/L

Workingreagent (R1+R2) isnotclassified as dangerous.

R3 UREA Base

Sodium hypochlorite 7mmol/L

Sodium hydroxide 62mmol/L

Before Dilution: Danger: Met.Corr. 1: H290 –may be corrosive to metals Skin CORR. 1B ; H314 – causes severe skin burns and eye damage.

P280: wear protective gloves/eye protection/face protectionP305+P351+P338: IFINEYES, R in secautiously with water several minutes; Remove contact lenses, if present and easy to do. Continue rinsing.

Classification due to sodium hydroxide, sodium hypochlorite 1-<2.5%. For more details, refer to safety data sheet (SDS).

Once diluted, reagent is not classified as dangerous according toregulation1272/2008/EC.

R4 UREA Standard Urea40mg/dL (6.66mmol/L)

Reagents R2 and R4 are not classified as dangerous according to regulation 1272/2008/EC.

REAGENTS PREPARATION

Working reagent: Add contents of vial R2intovial R1 (Salicylate). Mixgently by inversion.

Base (vial R3): Dilute (1 +3) with demineralised water in some cases (automated procedure), may be used pur

Standard (vialR4): ready for use.

SPECIMEN COLLECTION AND HANDLING

Unhemolysed serum or heparinised plasma. Avoid fluoride or ammonium as anticoagulant which interfere with the assay.

Urea is stable in serum or plasma for:

- 24hat room temperature.
- Several days at 2-8°C.
- atleast 2-3 months frozen.

24h Urine: diluted (1+19) with demineralized water before assay. Urea is stable in urines for: 4 days at 2-8°C.

Add antibacterial agent as Thymolto improve the stability.

REFERENCE INTERVAL:

In serum and plasma	mg/dL	[mmol/L]
In cord	45-86	[7.5-14.3]
Premature	6-54	[1.1-8.9]
< 1 year	9-41	[1.4-6.8]
Children	11-39	[1.8-6.4]
18-60 years	13-43	[2.1-7.1]
60-90 years	17-49	[2.9-8.2]
> 90 years	21-66	[3.6-11.1]
In urines	26-43 g/24 h	[0.43-0.71 mol/24 h]

Each laboratory should establish its own normal ranges for the population that it serves.

PROCEDURE:

Manual Procedure: Let stand reagents and specimens at room temperature.

Pipette into test tubes	Blank	Standard	Assay
Working reagent (R1+R2)	1 mL	1 mL	1 mL
Demineralised water	5 µL		
Standard		5 µL	
Specimen (Note 1)			5 µL
Mix and wait for 4 minutes at room temperature or 2 minutes at 37°C			
Base (vial R3) diluted ¼	1 mL	1 mL	1 mL
Mix. Let stands for 8 minutes at room temperature or 5 minutes at 37°C. Read absorbance at 600 nm (590-610) against blank (Note 3). Reaction coloration is stable for 2 hours.			

1) Performances with manual procedure should be validated by user.
2) For better sensitivity, specimen volume may be enhanced to10 µL, with lowest linearity at 600 nm
3) Sensitivity is higher at upper wave length and lower at inferior wavelength
4) On Kenza Max at 578 nm, specimen volume is 10 µL to optimize the couple sensitivity/linearity
5) Above linearity limit dilute specimen with saline solution and re-assay considering dilution factor.
6) Specific applications for automatic analyzers are available on request.

CALCULATION

Manual Procedure:

Serum and Plasma:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Urines diluted (1+19): Multiply the result by 20 (dilution factor).

To calculate blood urea nitrogen (BUN): multiply the value of urea (mg/dL) by 0.467.

Automatic Biochemistry Analyzer:

The analyzer provides directly calculated result.

For more details about calibration and calculation of results, refer to User's manual and specific application.

REFERENCE:

1) TIETZ N.W. Text Book of Clinical Chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1239-1241.
2) Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006)p. 1096-1099.
3) YOUNGD.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed.(1990) p. 3-599 to3-609
4) SEARCYR.L., REARDONJ.E., FOREMANJ.A., Amer. J.Méd. Techn.1967, 33, 15-20.
5) Bernard S.Bioch. clin. Diagnostics médica uxchirurgicau x2ème éd.p.143-144.Ed. Maloine PARIS(1989).
6) SRM: Standard Reference Material.

EXP: 11

CALCIUM

AIM: To quantify calcium in urine for assessment of hypercalciuria / hypocalciuria, renal calcium handling, metabolic bone disease, kidney stone workup, or for research.

PRINCIPLE (TWO COMMON METHODS)

A. Colorimetric method (o-cresolphthale in complex one - OCPC):

Calcium reacts with o-cresolphthale in complex one in alkaline medium to form a purple complex. The absorbance (≈570–575 nm) is proportional to calcium concentration. A magnesium masking agent (e.g., 8-hydroxyquinoline) or kit buffer prevents interference from Mg.

B. Atomic Absorption Spectroscopy (AAS):

Flame AAS measures atomic absorption of calcium at its resonance line (≈422.7 nm). Samples are diluted and often mixed with a releasing/matrix modifier (e.g., lanthanum nitrate) to minimize phosphate/salt interference.

Specimen collection & handling

A. 24-hour urine (preferred for total excretion)

1) Provide patient with a clean, acid-washed container and written instructions.
2) Instruct patient to discard first morning void at start time, then collect all urine for 24 hours including first void next morning.
3) Note start and end times.
4) Keep the container refrigerated (2-8 °C) during collection or add preservative if indicated (e.g., thymol or small amount of HCl) - follow institutional policy. If preservative used, note it on the request.
5) On receipt: mix thoroughly and measure total urine volume (mL). Aliquot and store refrigerated (2–8 °C) for immediate testing, or freeze (-20 °C) for longer storage.

B. Spot urine (random) or first morning

Used for Ca/creatinine ratio. Collect single sample, refrigerate and test promptly.

Reagents & materials (general)

- OCPC calcium reagent kit (includes reagent, standard/calibrators, controls) or AAS grade reagents if using AAS.
- Distilled/deionized water.
- Standard calcium solution (certified) — for calibration (e.g., 1.0 mmol/L or 100 mg/L depending on kit).

- Mg-masking agent (often included in OCPC kit).
- Lanthanum nitrate (1% w/v) as matrix modifier for AAS (if used).
- Automated photometer/ spectrophotometer capable of 570–575 nm (OCPC) OR flame AAS instrument (422.7 nm).
- Glassware, pipettes, cuvettes, centrifuge tubes.
- Calibrators and control materials (low, normal, high).
- Personal protective equipment (PPE).

OCPC Colorimetric Assay - Step-By-Step (Typical)

(Adapt incubation times/volumes to your kit or instrument; below is a commonly used manual procedure.)

Preparation

1) Bring samples to room temperature. Mix well.
2) Centrifuge turbid urine at 1,500-3,000 × g for 5 minutes and use supernatant.
3) Prepare calibrators (zero, low, mid, high) per kit, and QC controls.

Assay (manual)

1) Pipette into cuvettes or microplate wells:
   - Blank / reagent blank: Buffer + reagent (no sample)
   - Calibrators: X µL standard + buffer + reagent (per kit volumes)
   - Controls: as calibrators
   - Samples: usually 10–50 µL urine + buffer + reagent (may require dilution)
2) Mix, incubate at room temperature (commonly 5–10 minutes; follow kit).
3) Read absorbance at 570-575 nm against reagent blank.
4) Construct calibration curve (absorbance vs concentration). Determine sample concentration from curve.

Dilution

- If urine Ca > upper calibrator, dilute sample (e.g., 1:5 with deionized water), re-assay and multiply result by dilution factor.

AAS method - step-by-step (typical)

Preparation

1) Dilute urine samples with deionized water (common dilution 1:10 to 1:100 depending on expected concentration).

2) Add lanthanum nitrate solution (final ~1% w/v lanthanum) as matrix modifier to standards, controls and samples to a constant final concentration to eliminate interferences from phosphate/salts.
3) Prepare calibration standards (e.g., 0, 1, 2, 5, 10 mg/L Ca or in mmol/L depending on instrument).

Measurement

1) Set AAS to calcium line (≈422.7 nm). Warm up lamp and nebulizer per manufacturer.
2) Aspirate blank, calibrators, controls and samples in sequence.
3) Use calibration curve to compute Ca concentration in diluted sample; multiply by dilution factor.

Calculations & reporting

A. Concentration units

- OCPC often reports mg/dL or mg/L; AAS can report mg/L (which is mg per liter = mg/L = µg/mL ×1000). Convert as needed.

B. 24-hour urinary calcium (mg/day)

1) Determine urine calcium concentration (mg/L).
2) Measure total 24-hour urine volume (L).
3) Multiply:

Calcium excretion (mg/day) = [Ca] (mg/L) × urine volume (L/24 hr)

Example (step-by-step arithmetic):
If [Ca] = 160 mg/L and total volume = 1.8 L, then

1) Multiply 160 × 1.8 = (160 × 1) + (160 × 0.8) = 160 + 128 = 288 mg/day**.**

C. Convert mmol ↔ mg

- Molar mass Ca = 40.08 mg/mmol.

mg = mmol × 40.08
mmol = mg / 40.08

Example: If excreted = 8.0 mmol in 24 h, then mg = 8.0 × 40.08 = 320.64 mg/day. (Compute step: 40.08 × 8 = 320.64.)

D. Spot urine - Calcium/Creatinine Ratio

- Report as mg Ca / g creatinine (or mmol/mmol). Useful cutoffs: in adults, Ca/Cr ratio <0.2 (mg/mg) often normal; values vary by lab — see reference ranges below. (Convert units carefully: if Ca in mg/L and creatinine in mg/dL etc., align units before ratio.)

Reference ranges

24-hour urinary calcium (adult): ~100–300 mg/day (some labs use <250 mg/day or <300 mg/day as normal).

- **Children:** age-dependent — much lower; consult pediatric references.
- **Spot urine Ca/Cr ratio:** adult normal often <0.2 (mg/mg) but laboratories differ.

Always report with the laboratory's own reference interval established by method and population.

REFERENCE:

2) TIETZN.W. Text book of clinical chemistry,3rd Ed.C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1239-1241.
3) Clinical Guideto Laboratory Test, 4th Ed., N.W. TIETZ (2006)p.1096-1099.
4) YOUNGD.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed.(1990) p. 3-599 to3-609
5) SEARCYR.L., REARDON J.E.,FOREMAN J.A., Amer.J. Méd. Techn.1967, 33, 15-20.
6) Bernard S.Bioch.clin. Diagnostics médica uxchirurgicaux 2èmeéd.p.143-144.Ed. Maloine PARIS (1989).
7) SRM: Standard Reference Material.

EXP: 12

PHOSPHOROUS

AIM: To quantify inorganic phosphate (PO_4^{3-}) in urine for assessment of renal phosphate handling, hypophosphatemia/hyperphosphatemia evaluation, rickets/osteomalacia workup, or research (24-hour excretion or spot Ca/PO_4 and PO_4/creatinine ratios).

Principle (common colorimetric methods)

A. Molybdate-ascorbic acid (molybdenum blue) method:

Phosphate reacts with ammonium molybdate in an acidic medium to form phosphomolybdate. This is reduced (commonly by ascorbic acid) to a blue complex (molybdenum blue). Absorbance is measured typically at 340-880 nm depending on variant; a frequent wavelength is 650-700 nm (check reagent specs). Absorbance is proportional to phosphate concentration.

B. Malachite green method:

A variant that forms a green complex (malachite green–phosphomolybdate) with high sensitivity; commonly used in microplate assays for low concentrations.

Specimen collection & handling

24-hour urine (preferred for total excretion)

1) Provide clean, acid-washed collection container and written instructions.
2) Discard first morning void at start time; collect all urine for 24 hours including first void next morning.
3) Record start and end times and total volume.
4) Keep refrigerated (2–8 °C) or add preservative per institutional policy (if used, record it). Many phosphate assays tolerate refrigeration; strong acids as preservative will change pH and may affect colorimetric assays - avoid unless validated.
5) Mix thoroughly on receipt, record volume, aliquot for assay and for storage (2-8 °C short term, -20 °C longer).

Spot urine (random or first morning)

Use for phosphate/creatinine ratio. Collect single sample, refrigerate and test promptly.

Reagents & equipment (general)

- Ammonium molybdate reagent (or commercial kit reagents).
- Reducing agent (ascorbic acid) OR malachite green reagent if using that method.
- Acid solution (e.g., sulphuric acid) as specified by method.

- Distilled/deionized water.
- Certified phosphate standards (calibrators) covering expected range.
- Quality control materials (low and high).
- Spectrophotometer or microplate reader (wavelength per reagent, commonly 650–820 nm for molybdenum blue variants; malachite green often read at 620-660 nm) - follow reagent instructions.
- Glassware, micropipettes, cuvettes or 96-well plates, centrifuge.
- PPE and waste containers.

Always use reagent-grade chemicals and observe manufacturer kit instructions when available.

Example manual microplate protocol

Adapt volumes to your plate format and kit. These volumes are illustrative and commonly used for a sensitive microplate assay.

Preparation

1) Bring samples to room temperature; centrifuge turbid samples at 1,500–3,000 × g for 5 min and use supernatant.
2) Prepare standards (e.g., 0, 0.5, 1.0, 2.0, 5.0 mg/dL PO_4) in the same matrix if possible. Prepare QCs.

Assay

Pipette into wells:

- Standards and QCs: 20 µL each.
- Samples: 20 µL each (dilute urine if expected concentration > top standard; common dilutions 1:2 to 1:10 with deionized water).

1) Add 80 µL malachite green reagent (or molybdate reagent + reductant per kit) to each well.
2) Mix gently (pipette up/down), incubate at room temperature for 10–15 min (time depends on reagent; read color development when stable).
3) Read absorbance at 620–660 nm on microplate reader.
4) Construct calibration curve (absorbance vs concentration). Determine sample concentration; apply dilution factor if used.

Manual cuvette method (larger volumes)

Use proportionally larger volumes (e.g., 0.1–1.0 mL sample + reagent) and the same principles. Incubate and read in cuvette.

Calculations & reporting

Units & conversions

- **If assay reports as phosphate (PO_4):** units often mg/dL or mmol/L.
  - Molar mass PO_4^{3-} = 94.971 g/mol (≈ 94.97 mg/mmol).
  - mmol/L = (mg/L) / 94.97
  - mg/L = mmol/L × 94.97
- **If assay reports as elemental phosphorus (P):** molar mass P = 30.974 mg/mmol.
  - mg (as P) = mmol × 30.974
  - Conversions between PO_4 and P: 1 mmol PO_4 = 1 mmol P (same mole), but mg differ because of different molar masses. To convert mg PO_4 to mg P: mg P = mg PO_4 × (30.974 / 94.97) ≈ mg PO_4 × 0.3261
  - To convert mg P to mg PO_4: mg PO_4 = mg P × (94.97 / 30.974) ≈ mg P × 3.063

24-hour urinary phosphate (mg/day)

1) Determine urine phosphate concentration (mg/L).
2) Measure total 24-hour urine volume (L).
3) Multiply:

Phosphate excretion (mg/day) = [PO_4] (mg/L) × urine volume (L/24 h)

Example calculation (step-by-step arithmetic):
Measured urine phosphate = 6.5 mg/dL (reported by assay as PO_4).

Convert to mg/L: 6.5 mg/dL × 10 = 65 mg/L.
Total 24-hr volume = 1.5 L. Multiply 65 × 1.5. Compute: 65 × 1 = 65; 65 × 0.5 = 32.5; sum = 65 + 32.5 = **97.5 mg/day**.

Spot urine phosphate/creatinine ratio

- Express as mg PO_4 per g creatinine (or mmol/mmol). Useful for screening; labs must provide appropriate cutoffs.

Reference Ranges (Typical - Verify With Method & Population)

- **24-hour Urinary Phosphate (Adult):** roughly 400–1300 mg/day (wide physiologic range; depends on diet, age, renal function). *[Note: ranges vary by lab; confirm and use lab-specific reference intervals.]*
- **Spot Urine Phosphate/Creatinine Ratio:** Age- And Lab-Dependent.

REFERENCE:

1) TIETZ N.W. Textbook of Clinical Chemistry, 3rd Ed.C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1239-1241.

2) Clinical Guideto Laboratory Test, 4th Ed., N.W.TIETZ (2006) p.1096-1099.

3) YOUNGD.S., Effect of Drugs on Clinical Laboratory Tests, 4th Ed.(1990) p. 3-599 to 3-609.

FOOD CHEMISTRY

EXPERIMENT: II - 1

DETERMINATION OF THRESHOLDVALUE FOR BASIC TASTES

AIM: To determine the sensitivity threshold value for basic tests.

INTRODUCTION: -A threshold is a point on a stimulus scale at which a difference is detected. Sugar is the generic name for sweet tasting, soluble, CHO many of which are used to food. Simple sugars also called mono saccharides include glucose, Fructose and galactose. Salt is a mineral composed primarily of sodium chloride. Salt in the form of a natural crystalline mineral is known as rock salt (or) halite. The lemon is a species of small ever green there in the following plant family. Rutaceae the soluble dietary fibre in lemon aids in healthy digestion. Bitter guard has a strongbitter taste and no sweetness. In for fact, it gets more bitter as it.

PROCEDURE:

1) **Sweet:**
   - Sugar was taken using concentrations i.e. 0, 0 5, 1, 1.5.
   - Mixed in water at equal quantities.
   - And were kept in random order.
   - The panellist was made to identify the concentration in ascending order
2) **Salt:**
   - Salt was taken to observe saltiness using concentrations i.e. 0,0.5, 111.5
   - Mixed in water at equal quantities.
   - And were kept in random order.
   - The panelist was made to identify ascending order the concentration in ascending order.
3) **Sour:**
   - Lemon was taken to observe sour taste using Concentration le 0,0.5, 1, 1.5
   - Mixed in water at equal quantities.
   - And were kept in equal random order
   - The panelist was made to identify the concentration i.e. in ascending order.
4) **Bitter:**
   - Bitter guard was taken to observe bitter taste using concentration i.e. 0, 0.5, 1, 1.5.
   - Mixed in water accordingly and were placed in a random order.
   - The member was made to identify and were asked to place them in ascending order.

Determination of threshold value for basic tests procedure table:

S.NO	WATER LEVEL	SAMPLE	CONCENTRATION	SCORE	TASTE
1	5ML	A	0	-	NO TASTE
2	5ML	B	0.5	2	MILD TASTE
3	5ML	C	1	3	MODERATE TASTE
4	5ML	D	1.5	4	STRONG TASTE

DISCUSSIONS:

1) **Sweet:** Sweet concentrations were identified and placed accordingly in the ascending order.
2) **Salt**: Salt concentration was identified wrongly with moderate and strong taste, remaining both are identified correctly.
3) **Sour:** Sour concentration was identified and placed accordingly in the ascending order.
4) **Bitter:** Bitter concentration was identified and placed accordingly in the ascending order.

RESULT:

SUGAR:

S.NO	SAMPLE	SCORE	TASTE
1	A		
2	B		
3	C		
4	D		

SALT:

S.NO	SAMPLE	SCORE	TASTE
1	A		
2	B		
3	C		
4	D		

SOUR:

S.NO	SAMPLE	SCORE	TASTE
1	A		
2	B		
3	C		
4	D		

BITTER:

S.NO	SAMPLE	SCORE	TASTE
1	A		
2	B		
3	C		
4	D		

CONCLUSION:

REFERENCE:

1) B.Srilakshmi, Food Science, 8th Edition, Newage International Publishers.

EXPERIMENT: II - 2

ODOUR IDENTIFICATION TEST

AIM: To identify the ability of distinguishing order.

INTRODUCTION:

The odour of food immeasurably contributes to the pleasure of eating. It may be valuable index to the quality of food ever to its whole some edibility. Information regarding to odour of food is obtained through the olfactory epithelium a yellow pigmented area. The size of portal upper part of nasal cavity and the above tubinate bones. Olfactory cells are located in the area through these cells the odour were detected. Each olfactory cells terminate in a no. of hair. Like projections (or) extended into the cells mucous lining of the Olfactory epithelium nerve ending from the first cranial nerve makes contact with nerve fibre from the olfactory cells by way of the olfactory teeth which is the above the odour detecting area.

The concentration of which substance can be detected can be sensed when the concentration is only 2.%. log/ lit of air, allow concentration induced.

PROCEDURE:

We took some spices like ginger, garlic, onion, cloves, pepper and cinnamon. We crushed them and tied them individually in muslin cloth. These were placed in random sequences. The panelist will identify the spices and placed them accordingly.

RESULT:

S.NO.	NAME OF THE ITEM	ODOUR		
		MILD	STRONG	NO SMELL
1	CINNAMON			
2	PEPPER			
3	GARLIC			
4	GINGER			
5	CLOVES			
6	ONION			

DISCUSSIONS:

CONCLUSIONS:

REFERENCE:

1) B. Srilakshmi, Food Science, 8th Edition, Newage International Publishers.

EXPERIMENT: II - 3

PAIRED COMPARISON TEST

AIM: To test the difference between a stimulus as two among several stimuli by conducting difference testi e-paired comparison fest.

INTRODUCTION:

Most information a human response to physical and chemical stimuli has been obtained in laboratories under very specific condition. Laboratory panels can provide to the provide answer in 2 general questions, relative to the sensoryproperties of food preferences is much clearly Identifying difference which may be regards of its nature of direction. Difference may be: -

1) Simple difference.
2) Directional difference of a defined criteria.
3) Quality preference difference.
4)

Simple difference tube is used effectively for obtaining information in several fields of inventors. The physiological and psychological basic information of human responses to stimuli. Isolate and combine isobtained through there procedures.

Comparison of two samples is commonly referred to as the physical stimuli (or) pairedcomparison test. In two differences test the judge is permitted with & stimuli and asked to indicate whether there Is a difference between them. The presentation two sample simultaneously is referred to as the paired comparison test.

FIELD OF APPLICATIONS:

This test help to prepare foods of good taste, texture, acceptance, colour difference in Consumer analysis of food.

PROCEDURE:

The panel members receive several pairs of samples. These may be different or the same samples in each pair. Samples are always given in code numbers.

Different samples are given in each pair which differ in the intensity of one Characteristics, **e.g.:** Sweetness, bitterness or rancidity. In each pair, the sample with more or less intense taste will have to be picked out.

DISCUSSION:

In this test 5 pairs of samples like lays – Bingo, Fizz-Apple juice, Mirinda-Orange juice, perk-munch and Tang-Rasana are tested. Among these sample T Couldn’t identify the difference between Fizz and Apple juice. I am able to identify remaining all the samples.

OBSERVATION: Paired Comparison Test.

SAMPLES		**IDENTIFICATION**	
A	**B**	**YES**	**NO**
LAYS	BINGO		
FIZZ	APPLE JUICE		
MIRINDA	ORANGE JUICE		
PERK	MUNCH		
TANG	RASANA		

DISCUSSIONS:

CONCLUSIONS:

REFERENCE:

1) B. Srilakshmi, Food Science, 8th Edition, Newage International Publishers, Pg. No. 344.

EXPERIMENT: II - 4.

DUO-TRIO TEST

AIM: To identify the sample which & identical with the reference sample in triplet sample given i.e. duo-trio test.

INTRODUCTION:

The duo-trio was described by peryam and Swarty in the year 1950. It is a sensory evaluation method commonly used to determine whether there is a perceivable difference between two samples, by presenting a reference sample and two coded samples, one matching the reference and asking panelist to identity the maten.

FIELD OF APPLICATION:

- Determining of product differences result from changes in ingredients, processing, packaging or storage.
- Evaluating whether an overall difference exists, where Specific attributes are not known
- Quality control and product development.

PROCEDURE:

This test employs three samples, two identical and one different. The panel is first given one of the pair of identical samples as known reference sample R and then the other two successively in random order, and asked to match one of these with the first of positive answer is required.

Observation: 2 samples, 1 reference

SAMPLES A	SAMPLES B	REFERENCE	IDENTIFICATION	
			YES	NO
LAYS	BINGO	LAYS		
FIZZ	APPLE JUICE	FIZZ		
ORANGE JUICE	MIRANDA	ORANGE JUICE		
PERK	MUNCH	PERK		
TANG	RASNA	TANG		

DISCUSSION:

CONCLUSION:

REFERENCE:

1) B. Srilakshmi, Food Science, 8th Edition, Newage International Publishers, Pg. No. 344.

EXPERIMENT: II - 5.

TRIANGLE TEST

AIM: To identify the add samples from the given sample.

INTRODUCTION:

The triangle test was first suggested by Beg Lencon (1943) was used by him testers method of selecting except Been testers. Pyram and Joseph quality control and research and whisky since it appearance. The method and we has been used by most labs in measuring the sensory properties of food become of its extensive applications to test has been the most thoroughly studied and Criteria of all, test design. It is applied as frequently for determining the difference as for determining directional simultaneously allowing for its comparison. They could also be presented successively. The triangle test was first application of food is true different tests. The judge informed that 2 of stimuli are identical and one is different and must select the odd sample-

The triangle test can be used in the same diffraction as the two samples different test under the sample are different tests when the sample are homogenous and especially when the distion of different is not known is to complex for all slides to comprehended. A like person and also found the power of difference discrimination, quite similar in the two taste and Slightly infusion of the trait design.

PROCEDURES:

This test employs three samples, two identical and one different, presented simultaneously to the panel. The judge is asked to determine which of the three is the odd sample. A positive answer is required even if it is a guess. In general, it is preferable to give the sample which is strong in odour and taste. Two samples A and B can be presented in two combinations AAB and BBA and for replication in six different arrangements-AAB, ABA, BAA, BAB, ABB and BBA.

IDENTIFICATION OF ODD SAMPLE BY TRIANGLE TEST

SAMPLES			**IDENTIFICATION OF ODD SAMPLE**	**DECISION OF PANELISTS**
A	**B**	**C**		
MIRANDA	MIRANDA	ORANGE JUICE		
PERK	MUNCH	MUNCH		
MUNCH	MUNCH	MUNCH		
RASNA	TANG	TANG		

DISCUSSION:

CONCLUSION:

REFERENCE:

1) Food Science, B. Srilakshmi, 8th Edition, New Age International Publishers, Pg No-345.

EXPERIMENT: II - 6.

RANKING TEST

AIM: To rank and study the shelf life of prepared Food.

INTRODUCTION:

Ranking is a process of arranging three or more product samples to increasing or decreasing order of a Specified attribute (or) overall quality. Many times, repeated tasting is required to arrive at the accurate results because the samples are not evaluated independently but these are evaluated relation to each other. Ranking tests are used in storage life evaluation of products. Product development, consumer preferences, training of panel members and studies on quality. A shelf-life studyis most effective way to determine the durable life (or) text before date of pre packaged food and obtain evidence showing that the food will remain wholesome, palatable and nutritional until the end of durable life.

PROCEDURE:

These tests give more quantitative data than difference tests can be used for the analysis of more than 2 samples at the same time.

This test is used to determine how several samples differ on the basis of a single characteristic. A control need not be identified. Panelist are presented all samples simultaneously with code numbers and are asked to rank all samples according to the intensity of the specified characteristics. In consumer analysis, the panellists are asked to rank the coded samples ask to their preference. Here we have taken orange quash and 1 glass of water. Those two are mixed in 10 different proportions and panellists are allowed to rank, them according to their intensity of taste.

Observation:

S.NO.	SAMPLE	SCORE
1	1 GLASS WATER + 1/2 SPOON OF ORANGE SQUASH	0
2	1 GLASS WATER + 1½ SPOON OF ORANGE SQUASH	2
3	1 GLASS WATER + 2½ SPOON OF ORANGE SQUASH	4
4	1 GLASS WATER + 3 SPOONS OF ORANGE SQUASH	5
5	1 GLASS WATER + 4 SPOONS OF ORANGE SQUASH	7
6	1 GLASS WATER + 3 SPOONS OF ORANGE SQUASH	3

7	1 GLASS WATER + 3½ SPOONS OF ORANGE SQUASH	6
8	1 GLASS WATER + 5 SPOONS OF ORANGE SQUASH	9
9	1 GLASS WATER + 4½ SPOONS OF ORANGE SQUASH	8
10	1 GLASS WATER + 1 SPOON OF ORANGE SQUASH	1

DISCUSSION:

CONCLUSION:

REFERENCE:

1) Food Science, B. Srilakshmi, 8th Edition, New Age International Publishers, Pg No-345.

EXPERIMENT: II - 7

SELECTION OF JUDGING PANEL

AIM: To select the judging panel

TRAINED PANEL MEMBERS:

The sensory qualities, particularly the Flavour Attributes are essentially to be measured subjectively. From early times this judging has been the preserve of experts who used to evaluate tea, coffee and wine. Wither the development of sensory evaluation techniques on scientific lines, the experts are being replaced by panels whose sensitivity and consistency have been established by training and repeated tests. The panel members analyse food products through properly. Planned experiments and their judgements are quantified by appropriate statistical analysis.

SELECTION OF PANEL OF JUDGES:

Actually, one extremely discriminating pain taking and unbiased individual would suffice for fasting. Further one individual may not be able to discriminate different aspects of food quality. Hence a panel of judges may be used. Members of the panel should be carefully selected and trained to find out difference in specific quality characteristics between different stimuli and also direction and intensity of difference.

The requirements for an ideal panel member are as follows.

- They should be able to discriminate easily between samples and should be able to distinguish appreciable differences in taste and smell.
- He should have good health. If he is suffering from cold his sensitivity may be affected. A sick patient cannot judge the food correctly. He should not be habituated to chewing pan or supari.
- He should be experienced in the particular field.
- They should have high personal integrity. He should not be prejudiced. He should be able to evaluate objectively.
- Willingness to spend time for the sensory evaluation work is required.
- He should have interestin sensory analysis of samples and intellectual curiosity.
- He should have ability to concentrate and derive proper Conclusion.
- He should have ability to concentrate and should be available and willing to submit to periodic test to get consistent results.

TRAINED PANEL:

Laboratory panels must then be carefully trained for specific products or purposes. These tests aim of funding differences in specific quality characteristics between different stimuli

and also direction and/or intensity of the difference. Periodically the panel is given refresher training and tests. The number of members in the trained panel should be small varying from 5 to 10.

DISCRIMINATIVE, COMMUNICATIVE ORSEMI-TRAINED PANELS:

These panels are constituted of technical people and their families, who are normally familiar with the qualities of different types of food. They are capable, with few preliminary test runs, of following instructions for tests given, discriminating differences and communicating their reactions. Such panels of 25-30 are used to find the acceptability of preference of final experimental products prior or large-scale consumer trial.

CONSUMER PANELS:

Such panels are made up of untrained people chosen at random to represent a cross-section of the population for which the product is intended. The greater the number, the greater the dependability of the result. A group of not bees than 100 is considered the minimum.

DISCUSSION:

CONCLUSIONS:

REFERENCE:

1) Food Science, B. Srilakshmi, 8th Edition, New Age International Publishers, Pg No-345.

EXPERIMENT: II -8

TRAINING OF JUDGES

AIM: To train the selected panelist, to acquire higher accessory to taste, odour and aroma perception and make judgemental on intensity and qualify when odour in normal foods.

INTRODUCTION:

Systematic analysis of the sensory properties of food involves the use of human subjected and the productibility of the analysis food greatly influence the dissection and Validity the judgement are obtained also influence the data

In some case of the result to find, difference between trained enable to discriminate had its origin in methodological and statistical had deficiency, proves and intes (1916) believe that following considerations are important in selecting Judges between Flavours difference teeth.

- Precision or internal sensitivity for a particular flavour.
- A tolerance (or) internal ability to produce a complicate difference judgement.

The main purpose is to decide on the magnitude to importance on the magnitude of difference variable in taste and odour.

To train them following test must be understand:

Threshold Test for Basic Test:

Procedure:

Given into the beginning of each series given in table 2 for threshold test. Give 5-8 because depending on the quality of taste. Ask thepanelist to evaluating threshold scoring code.

Direction Threshold:

It is different from water, whereas the Solution in concentration series but the basic taste can be recognized.

Recognition Threshold:

By containing tasting on Concentration in each where basic taste recognised correctly.

Juminal threshold:

Above certain concentration is reached, where basic taste is recognized correctly.

Above certain concentration increases; concentration cannot be differentiated of different threshold is the concentration of at which the difference is recognised.

DISCUSSION:

CONCLUSION:

REFERENCES:

1) American try and Rarinari C, Pang test Rosalc. The score of taste laboratory studies principles of sensory evaluation of food ocedary, chapter No-4.6. pg noi-177-189.

EXPERIMENT: II - 9

DESCRIPTIVE SENSORY TEST

AIM: To conduct descriptive test (favour profile test) for developed function al food.

INTRODUCTION:

A descriptive sensory evaluation provides a of a food products, sensory attributes as well detailed profile of a as qualitative measurements of each attribute intensity.

In the descriptive sensory evaluation test some terminology is used to describe (or) quantity the difference between Sample.

E.g.: Excellent, very good, good and fair in appearance or very desirable, moderately, slightly desirable in juiciness.

FLAVOUR PROFILE METHOD: -

The flavour profile method describes flavour in terms of fibre major components such as character attributes, attribute intensity, order of attribute appearance after taste and amplitude. The advantage is small, trained panel can focus specifically on flavour. Disadvantage is It is potential for bias or influence by dominant panel member.

RECIPE:

Tomato Pickle

Ingredients:

- Tomatoes
- Tamarind
- Red chilli powder
- Salt
- Mustard seeds
- Fenugreek seeds
- Garlic

Procedure:

- Heat a pan, add chopped tomatoes and oil, cook on medium flame until they turn soft. Add tamarind and cook for 5 mins.
- Add red chilli powder and salt, then sauté for 5 minutes and then turn off the stove.
- Once cooled, grind the cooked tomatoes into a slightly coarse paste.
- Heat oll in a pan, add mustard seeds. Once they splutter, add crushed garlic, red chilliand sauté until golden, then to this add the ground tomato paste, stir well and cook on low heat until oil separates.

- Roast fenugreek seeds in a pan until golden brown and then. Grind them into a fine powder.
- Atlast and fenugreek powder, stir well and turn off the stove. store it in an airtight container.
-

DESCRIPTIVE SENSORY TEST

Sensory Evaluation for Tomato Pickle

S.NO.	COLOUR	TASTE	TEXTURE	FLAVOUR	OVERALL ACCEPTABILITY
1					
2					
3					
4					
5					

- Like extremely - 9
- Like very much - 8
- Like moderately - 7
- Like slightly - 6
- Neither like or dislike - 5
- Dislike slightly - 4
- Dislike moderately - 3
- Dislike very much - 2
- Dislike extremely - 1

DISCUSSION:

CONCLUSION:

REFERENCE:

1) B. Srilakshmi, Food Science, 8th Edition, New Age International Publishers, Pg no-353.

EXPERIMENT: II -10

SENSORY-EVALUATION OF VARIOUS FOOD PRODUCTS USING SCALES AND SCORE CARDS

AIM: To conduct sensory evaluation of various food products using different scales and scorecards

INTRODUCTION:

A score card is a tabulated list of sensory attributes contributing toward the quality of a product with a numerical value assigned to each attribute. These attributes are usually arranged on the card in the order of their importance i.e., flavour, appearance, colour, texture. Every time food is eaten, a judgement is made.

SENSORY CHARACTERISTICS OF FOOD: -

Appearance:

Surface characteristics of food products contribute. To the appearance. Interior appearance can also be evaluated.

Colours:

In addition to giving pleasure, the colour of food is giving associated with other attributes. Ripeness of fruits like banana, tomato, mango, guava, papaya and plum can be assessed by the colour.

Flavour:

The Flavour of food has three components like odour, taste and mouthfeel.

Odour:

A substance which produces odour must be volatile and the molecules of the substance must come in Contact with receptors in the epithelium of olfactory organ.

Taste: -

We value foods for its taste sensation which the taste buds register is categorised as sweet, salt, sour or bitter.

Texture:

Texture in ice-cream depends upon the size of the crystals. The bitterness of food is another aspect of texture. It impacts the overall perception and acceptance of a product. It includes hardness, viscosity, roughness, elasticity and adhesiveness of the food product.

Score Cards:

Score card is a guide to the improvement of quality. The uniformity in the score card system Involves 3 points, 5 points and 7 points. The use of the score card enables the mature judge to establish accurate judging habits by following a definite Orderly rating.

PROCEDURE: In this experiment we conduct sensory evaluation for lemon pickle and makhana laddoo based on that we have to prepare score cords.

Lemon Pickle:

Wash the lemons and wipe them dry with a paper towel. Now take to lemons, cut them into halves, then cut the half's into quarts. Add this to a bowl. Take the remaining 5 lemons and juice them. Add the juice to the lemon pieces bowl. Now add salt, turmeric powder to the lemon pieces and transfer them to a clean dry air tight glass jar and set them in a day place for 4 days. After 4 days add chilli powder to the pieces and mix well. Again, marinate for 4-5days till the place becomes soft and well coated with chillipowder.

Score Card of Lemon Pickle:

S.NO	ATTRIBUTE	RATING	PANEL					AVERAGE FINAL
			1	2	3	4	5	
1	**COLOUR**							
	LIGHT RED	**7**						
	TOMATO RED	**6**						
	BRIGHT RED	**5**						
	ORANGE	**4**						
	MAROON	**3**						
	BRICK RED	**2**						
	DARK RED	**1**						
	BROWN	**0**						
2	**TEXTURE**			—	—	—	—	
	COMPLETELY SOFT	**7**						
	SLIGHTLY SOFT	**6**						
	JUICY, DELICATE	**5**						
	SLIGHTLY HARD	**4**						
	CRISPY	**3**						
	DRY	**2**						
	COMPLETELY HARD	**1**						
	MUSHY	**0**						

3	**FLAVOUR**	**—**						
	SPICY & SOUR	**7**						
	SPICY	**6**						
	SOUR	**5**						
	SLIGHTLY SALTY	**4**						
	SALTY	**3**						
	OVER SPICY	**2**						
	BITTER	**1**						
	SOUR BITTER	**0**						
4	**TASTE**							
	SPICY & SOUR	**7**						
	SOUR	**6**						
	SPICY	**5**						
	SALTY	**4**						
	BITTER& SOUR	**3**						
	BITTER	**2**						
	OVER SPICY	**1**						
	OVER SALTY	**0**						
5	**OVERALL ACCEPTABILITY**							
	LIKE EXTREMELY	**7**						
	LIKE VERY MUCH	**6**						
	LIKE SLIGHTLY	**5**						
	NEITHER LIKE NOR DISLIKE	**4**						
	DISLIKE SLIGHTLY	**3**						
	DISLIKE MODERATELY	**2**						
	DISLIKE VERY MUCH	**1**						
	DISLIKE EXTREMELY	**0**						

Ragi Date Cookies: -Take Dates and raisins, soak them for 5 mins in hot water and grind them into a smooth paste and keep it aside. Takeragi flour in equal amounts and mix them together. Add some baking powder and cardamom powder to the flows and mix them well. Add the grinded dates-raisinyeast to the dry mixture and add ghee along with them and mix well. Add Ghee according to the required consistency Grease the baking tray with butter paper and pre heat and fore heat the oven. Place the cookies in the oven and bake for 30 minutes at 130 DC.

Score Card of Ragi Dates:

S.NO	ATTRIBUTES	RATING	PANEL -1	P- 2	P -3	P-4	P-5	AVERAGE SCORE
1.	**COLOUR** LIGHT RED TOMATO RED BRIGHT RED ORANGE MAROON BRICK RED DARK RED BROWN	 7 6 5 4 3 2 1 0						
2.	**TEXTURE** COMPLETELY SOFT SLIGHTLY SOFT JUICY, DELICATE SLIGHTLY HARD CRISPY DRY COMPLETELY HARD MUSHY	 7 6 5 4 3 2 1 0						

3	**FLAVOUR** SPICY & SOUR SPICY SOUR SLIGHTLY SALTY SALTY	 7 6 5 4 3 2						

DISCUSSION:

CONCLUSION:

REFERENCE:

1) B. Srilakshmi, Food Science, 8th Edition, New Age International Publishers Pg No-353.

EXPERIMENT: II - 12

TEXTURE PROFILE ANALYSIS OF SELECTED FOOD PRODUCT

AIM: Analyse of texture profile of different food products.

INTRODUCTION:

Various instruments are used to measure the texture of liquids, semisolids and solids. Rheology is defined as the science of deformation and flow of matter.

It has three factors: - elasticity, viscous flow and plastic flow. The science of rheology deals with the measurement of various mechanical properties of food Is important for two reasons.

1) To determine the flow properties of liquid pad stuffs.
2) To ascertain the mechanical behaviour of solid foods when consumed and during processing.

PHYSICAL TEXTURE ANALYSIS

1) Bread: Tested by Compression.

Press down on the slice of bread with your finger and observe how it deforms and recovers. Firmer bread will reset compression faster than softer bread.

2) Fruits (Apple, Banana):Tested by penetration

Use fork or knife to penetrate the fruit. Measure the force required to break the skin and enter the flesh. Crisper fruits required more force.

3) Cheese: Tested by compression and tensile.

Compress a piece of cheese with your finger to test firmness. Starch a slice of mozzarella to observe it's Stretch ability. Firmer cheese will be harder to compress and stretch

4) Yogurt: Tested by viscosity

Use a spoon to stir the yogurt and observe its thickness and flow. Thicker yogurt will have high viscosity and be more resistant to stirring

5) Chocolate: Scooptest

Break a piece of chocolate and listen for a snapping sound. Higher quality chocolate will produce a clear, sharp snap.

6) Raw vegetables [carrot, cucumber): Shear and penetration

Cut through the vegetable with a knife and observe the vegetable with or force needed Crisp vegetables will require more force to cut through.

7) Cookies / Biscuits: Break test

Break a cookie or biscuit and observe the break ease with which it breaks and the texture of the break.

We can test viscosity of milk, honey and curd. But physical texture analysis we can identify its appearance, texture and quality of food materials. Some other instrumental methods are:

Mean score of orange jelly = $\frac{7+7+8+6+7}{5}$

$=35/5 = 7.$

Christal Texture Meter: (Cutting)

This is designed with series of rod which are pushed into the meat sample. The harder the meat more force is required to penetrate

Grinding and Extensibility: -

The power used by a household food grinder is measured. Increased toughness would increase the current Consumption of the grinder extensibility has proved to be inversely related to tenderness.

Kramer-Shear Press:

This is a multi-purpose instrument with some until and with different test all assemblies This instrument is widely used. Storms viscometer It is used to measure the viscosity or consistency of certain food products and to give an index of the resistance of the sample to flow the number of seconds required for the motor to make 100 revolutions .It measures the time necessary for a quality of fluid to pass through an orifice or capillary under standard pressure.

Ex: Tomato puree.

Shortometer:

This device consists a platform containing two parallel dull blades on which the sample cust a third blade an which the sample crist is accelerated by a motor to press down on this sample until the sample snaps. The force required to break the sample is the measure of the tenderness of the product.

Procedure:

In this experiment we select 5 judges and they give the texture analysis of jelly and tutti-frutti

Jelly:

They observe the jelly's appearance and flow characteristics. Most of the panel members conclude that the appearance of jelly is semisolid and they also examine the flow characteristics. This can be done by placing jelly on across surface and measure how easily it flows and record the time: The judges used their fingers to examine the texture. Based on how easily it spreads on surface they measure the viscosity of jelly. Based on above observation they give overall score.

TEXTURE ANALYSIS OF JELLY

QUALITY	TIME	TEXTURE	FLAVOUR	APPEARANCE	VISCOSITY	SCORE
GOOD	47.3 SEC	SMOOTH	SWEET	SEMISOLED	MEDIUM	7
GOOD	23.5 SEC	SMOOTH	SWEET	SEMISLID	MEDIUM	7
FAIR	34.5 SEC	SOFT	SWEET	SEMISLID	MEDIUM	8
GOOD	42 SEC	SOFT	MILD SWEET	SEMISLID	MEDIUM	6
FAIR	30.6 SEC	SOFT	MILD SWEET	SEMISLID	MEDIUM	7

Tutti Frutti:

Tutti Frutti is kept for observation to analyse the texture, the panel members are allowed to analyse the texture. The panel members are allowed to analyse the quality whether it is good or fails. Now the panelists are allowed to analyse the texture whether it is hard or soft. This can be done by using fingers. Note the time in how much time the spice & piece is dropped. To analyse the flavour, they need to be sensorily evaluated. Overall score is given by observing.

TEXTURE ANALYSIS OF TUTTI-FRUTTI

S.NO	QUALITY	TIME	TEXTURE	FLAVOUR	APPEARANCE	VISCOSITY	SCORE
1	GOOD	1:30 SEC	SEMI SOFT	SWEET	SEMI GLOSSY	-	9
2	GOOD	1:25 SEC	SEMI SOFT	SWEET	SEMI GLOSSY	-	8
3	GOOD	1:20 SEC	SEMI HARD	SWEET	STICKY	-	8
4	GOOD	1:27 SEC	SLIGHTLY SOFT	SWEET	STICKY	-	8
5	GOOD	1:29 SEC	SEMI HARD	SWEET	GLOSSY	-	8

Mean score of tutti-frutti = $\frac{9+8+8+8+8}{5}$

$41/5 = 8.2$

Discussion:

Conclusion:

References:

1) B. Srilakshmi, Food Science, 8th Edition, New Age International Publishers Pg no-353.

EXPERIMENT: II -13

ESTIMATION OF COLOR OF FOOD PRODUCT

AIM: To determine chlorophyll content of given sample (gogu).

INTRODUCTION:

The chlorophyll are the essential components for photosynthesis and occur in chloroplast as green pigments in all the photosynthetic plant tissue. There are at least five types of chlorophylls in plants: chlorophyll a and b occur in higher plants, ferns and mosses. Chlorophyll c, d and e are found in algae and in certain bacteria.

INSTRUMENTS USED FOR COLOUR ESTIMATION:
COLOUR DICTIONARIES:

The dictionary of Abney and Paul are most commonly used. The dictionary consists of 56 charts. Several main groups of hues are presented in order of their spectra. For each group there are 9 planes. In place of colour dictionary colour reproduced on secondary standards such as painted test panels, strips, dishes or plastic models may be used.

A mask of neutral grey having two openings is used. The size of each opening should be equal to the size of the individual colour patch in the sheet. An opening should be placed over the sample and the other over different patches on the chart until a match is achieved and the colour is noted.

Disc Colorimeters:

Here the discs have radial slits, so that a no. of them may be slipped together with varying portions of each showing. The discs are spun on a spindle at about 2700 rpm so that the colours merge into a single hue without flickering. The test sample is placed adjacent to the spinning disc under controlled illumination and both viewed simultaneously.

Coloured Chips:

A simple method is to match the colour of the food with the colour chips or colour glass chart or colour tube. This method is not very satisfactory as it is difficult to match the food with one small block of colour as the chart. The data are difficult to tabulate and analyse also.

Spectrophotometer:

Visual matching of colour is subject to short comings of human observers. To overcome this, spectrophotometer can be used. In this tube with the liquid is placed in a slot and light of selected wavelength is passed through the tube. This light will be differentially absorbed depending upon the colour of the liquid and the intensity of the colour. Two liquids of exactly the same colour and intensity will transmit equal fractions of the light directed through them. If one of the liquids is a juice and other is the juice diluted with water, the later sample will transmit a greater fraction of the incoming light and this will cause a proportionately greater deflection of the sensing needle on the instrument. Such an instrument can also measure the clarity/cloudiness of a liquid depending on the amount of light the liquid allowed to pass.

Procedure for Estimating Chlorophyll Present in Goya Leaves

Principle:

Chlorophyll is extracted in 80% acetone and the absorbances at 620 nm and 680 nm are read on a spectrophotometer. Using the absorption coefficient, the amount of chlorophyll is calculated.

Materials Required: 80% Acetone, Goya leaves, spectrophotometer

Procedure:

1) Weigh accurately 1g of finely cut and well mixed representative goya leaf sample into a clean mortar & pestle.
2) Grind the tissues to a fine pulp with addition of 20 ml of acetone.
3) Centrifuge the content at 5000 rpm for 5 mins and transfer the supernatant to a 100 ml volumetric flask.
4) Rinse the residue with 20 ml of 80% acetone, centrifuge and transfer the supernatant to the volumetric flask.
5) Repeat this procedure until the residue becomes colourless.
6) 80% acetone and collect the clear washings in the flask.
7) Make up the volume to 100 ml with 80% acetone.
8) Read the absorbance of the solution at 620 and 680 nm against the solvent (80% acetone) as blank.

Calculation for Estimating Amount of Chlorophyll:

Chlorophyll (mg/g) = $20.2(A_{620}) - 8.02(A_{680}) \times V/1000 \times 1/W$

Amount of Chlorophyll Present in Gogu Leaves = 0.223 mg/g

Where:
A = Absorbance at specific wavelengths
V = Final volume of chlorophyll extract in acetone
W = Weight of the fresh tissue extracted

Chlorophyll a (mg/g) = $12.7(A_{680}) - 2.69(A_{620}) \times V/1000 \times 1/W$

Chlorophyll b (mg/g) = $22.9(A_{620}) - 4.68(A_{680}) \times V/1000 \times 1/W$

Discussion:

Conclusion:

REFERENCE:

1) B. Srilakshmi, Food Science, 8th Edition, New Age International Publishers.