JHARKHAND Rai University
UGC RECOGNISED UNIVERSITY
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PRACTICAL LAB MANUAL

BIOCHEMISTRY & CLINICAL PATHOLOGY

D. Pharm IInd Year

INDEX

Experiment: 1	Sl No.:	Date:

Aim: Qualitative analysis of carbohydrates (Glucose).

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali prakashan; 2020; 29th Edition; page no:5-6

Materials required: Beaker, water bath, test tubes, graduated pipettes, funnel, filter paper, watch glass, slide, microscope.

Chemical Required: Molisch's Reagent, Sulfuric Acid, water, Fehling's solution A, Fehling's solution B, Barfoed's reagent, Phenyl hydrazine hydrochloride, Acetate buffer, water.

Procedure:

Sl. No	Test	Observation	Inference
1	**Molisch's test:** Mix 2ml of Glucose sample with 5 drops of Molisch's Reagent in a test tube.Add gently through the side by tilting the tube, about 2 ml of concentrated H_2SO_4 so as to form a bottom layer.	Violet/purple ring at the junction of two liquids.	Glucose is present.
2	**Solubility test:** Mix the glucose sample with water properly.	It is Soluble in water.	Indicate the presence of glucose.
3	**Fehling's test:** 2ml of Fehling's solution A and 2ml of Fehling's solution B is added to the Glucose solution and boil it in water bath	Yellow or brick red precipitate observed.	Reducing sugar present (Glucose).

Sl. No	Test	Observation	Inference
4	**Barfoed's test:** Add 2 ml of Barfoed' reagent in 2ml solution of glucose sample and keep in boiling in water bath and cool for 2 minutes.	Brick red precipitate is observed at the bottom of test tube.	Reducing sugar present (Glucose).
5	**Osazone test:** Set up a boiling water bath. Take test tube; add 1 g of Phenyl hydrazine hydrochloride, 2ml of acetate buffer, pH5.0. Add 5ml of water mix well and warm gently. Filter it. To the filtrate add 5 ml glucose solution and keep in boiling water bath and cool. Mount the crystals under microscope and examine under microscope.	Greenish yellow needle shaped crystals observe.	Glucosazone i .e. glucose present

Observation:

Report:

Teacher's Signature

Experiment: 2	Sl No.:	Date:

Aim: Qualitative analysis of carbohydrates (Fructose).

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali prakashan; 2020; 29TH Edition; Page no:5-6

Materials required: Beaker, water bath, test tubes, graduated pipettes, funnel, filter paper, watch glass, slide, microscope.

Chemical Required: Fructose, Molisch's Reagent, Sulfuric Acid, Fehling's solution A , Fehling's solution B, Barfoed's reagent ,Phenyl hydrazine hydrochloride, Acetate buffer, fougler's reagent, water.

Procedure:

Sl. No	Test	Observation	Inference
1.	**Molisch's test:** Mix 2ml of Fructose sample with 5 drops of Molisch's Reagent in a test tube..Add gently through the side by tilting the tube, about 2 ml of concentrated H_2SO_4 so as to form a bottom layer.	Violet/purple ring at the junction of two liquids.	Fructose is present.
2.	**Solubility test:** Mix the Fructose sample with water properly.	It is soluble in water.	Indicate the presence of Fructose.
3.	**Fehling's test:** 2ml of Fehling's solution A and 2ml of Fehling's solution B is added to the Fructose solution and boil it in water bath.	Yellow or brick red precipitate observed.	Reducing sugar present (Fructose).

Sl. No	Test	Observation	Inference
4.	**Barfoed's test:** Add 2 ml of Barfoed' reagent in 2ml solution of Fructose sample and keep in boiling in water bath and cool for 2 minutes.	Brick red precipitate is observed at the bottom of test tube.	Reducing sugar present (Fructose).
5.	**Osazone test:** Set up a boiling water bath. Take test tube; add 1 g of Phenyl hydrazine hydrochloride, 2ml of acetate buffer, pH5.0. Add 5ml of water mix well and warm gently. Filter it. To the filtrate add 5 ml Fructose solution and keep in boiling water bath and cool. Mount the crystals under microscope and examine under microscope.	Greenish yellow needle shaped crystals observe.	Fructose is present.
6.	**Fougler's test:** To 3 ml fougler's reagent add 0.5 ml of Fructose solution. Boil for one minute.	Blue colour develops.	Fructose confirmed.

Observation:

Report:

Teacher's Signature

Experiment: 3	**Sl No.:**	**Date:**

Aim: Qualitative analysis of carbohydrates (Lactose).

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no:5-6

Materials required: Beaker, water bath, test tubes, graduated pipettes, funnel, filter paper, watch glass, slide, microscope.

Chemical Required: Molisch's Reagent, Sulfuric Acid, Fehling's solution A , Fehling's solution B, HNO_3, Ammonical silver nitrate , NaOH, Phenyl hydrazine hydrochloride, acetate buffer, water.

Procedure:

S. No	**Test**	**Observation**	**Inference**
1	**Molisch's test:** Mix 2ml of Lactose sample with 5 drops of Molisch's Reagent in a test tube..Add gently through the side by tilting the tube, about 2 ml of concentrated H_2SO_4 so as to form a bottom layer.	Violet/purple ring at the junction of two liquids.	Lactose is present
2	**Solubility test:** Mix the Lactose sample with water properly.	It is Soluble in water	Indicate the presence of Lactose
3	**Fehling's test:** 2ml of Fehling's solution A and 2ml of Fehling's solution B is added to the Lactose solution and boil it in water bath .	Yellow or brick red precipitate observed	Reducing sugar present(Lactose)
4	**Mucic acid test:** To 1 ml Lactose solution add 1 ml concentrated HNO_3, boil and cool.	Broken glass like crystals obtained which can be identified under microscope.	Lactose is present

5	**Silver Mirror test:**1 ml Lactose solution add 1 ml Ammonical silver nitrate solution add excess NaOH Warm.	Silver mirror is Observed.	Lactose is present
6	**Osazone test:** Set up a boiling water bath. Take test tube, add 1 g of Phenyl hydrazine hydrochloride 2 ml of acetate buffer, PH 5.0. add 5ml of water mix well and warm gently. Filter it. To the filtrate add 5 ml sugar solution and keep in boiling water bath and cool. Mount the crystals under microscope and examine under Microscope.	Badminton ball, powder puff shaped Crystals.	Lactosazone i.e. Lactose present.

Observation:

Report:

Teacher's Signature

Experiment: 4 **Sl No.:** **Date:**

Aim: Qualitative analysis of carbohydrates (Sucrose).

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; 5-6

Materials required: Beaker, water bath, test tubes, graduated pipettes, funnel, filter paper, watch glass, slide, microscope.

Chemical Required: Molisch's Reagent, Sulfuric Acid, wate, Seliwanoffs reagent, alpha- napthol Solution, HCL, sodium hydroxide solution.

Procedure:

Sl. No.	Test	Observation	Inference
1	**Molisch's test:** Mix 2ml of Sucrose sample with 5 drops of Molisch's Reagent in a test tube.Add gently through the side by tilting the tube, about 2 ml of concentrated H_2SO_4 so as to form a bottom layer.	Violet/purple ring at the junction of two liquids.	Sucrose is present
2	**Solubility test:** Mix the Sucrose sample with water properly.	It is Soluble in water.	Indicate the presence of Sucrose.
3	**Seliwanoff's Test:** To 3 ml of Seliwanoff's reagent add 1 ml of Sucrose solution and heat the mixture to boil for 2 minutes cool.	Red colour or red Precipitate is observed.	Ketoses like Sucrose present.
4	**Rapid furfural test:** To 2 ml of Sucrose solution adds 1 ml of alpha-napthol solution (1% in alcohol) and 5 ml concentrated HCL boil.	Deep purple colour is Observed.	Ketoses like Sucrose present.

5	**Inversion test**: To 25 ml of Sucrose solution add 5 ml of concentrated HCI boil for 3 minutes and cool under tap water. Neutralize with sodium hydroxide solution.	Red/yellow/green precipitate is Observed.	Sucrose is present.

Observation:

Report:

Teacher's Signature

Experiment : 5	**Sl No.:**	**Date :**

Aim: Qualitative analysis of Proteins (Albumin).

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; 12-16.

Materials required: Test tubes, Test tube holder, Dropper, Water bath, Stirrer.

Chemical Required: Copper sulphate, Sodium hydroxide, Nitric acid, Millon's reagent, Mercuric sulphate, Sodium nitrite, Sulphuric acid, pyridine solution, Ninhydrin reagent, Distilled water, lead acetate, mercuric nitrate, sulphosalicylic acid, Esbach's reagent, saturated ammonium sulphate.

Procedure:

Physical Test:

Sl. No	**Test**	**Observation**	**Inference**
1	Appearance of a solution	Turbid solution	Albumin, Globulin, Casein
2	Colour of solution	Opalescent of milky	Albumin, Casein
3	Smell	Egg smell	Albumin
4	Litmus Test	Neutral	Albumin, Gelatin

Precipitation test of Proteins

Sl. No.	**Test**	**Observation**	**Inference**
1.	**Precipitation by heavy metals**		
	2ml of alkaline sample + 2-3 drops of 2% lead acetate solution.	White ppt	Protein present
	2ml of alkaline sample + 2 drop of 5% mercuric nitrate solution.	Brownish ppt	Protein present
2.	**Precipitation by alkaloid reagents**		

	2 ml of sample solution + 3-4 drops of 20% sulphosalicylic acid.	White ppt	Protein present
	2 ml of sample solution + 1 ml Esbach's reagent.	Yellow ppt	Protein present

Note-For these test sample solution is made alkaline by using 1% Na_2CO_3 solution used.

Chemical Test:

Sl. No.	Test	Observation	Inference
1	**Biuret Test:** 1. Take the given sample to be tested in a clean test tube. 2. Add 2 ml of sodium hydroxide solution to it. 3. To that add 5 to 6 drops of copper sulfate solution to it.	Appearance of bluish violet colour indicates.	Presence of protein.
2	**Xanthoproteic Test:** 1. Take 2ml of given sample compound in a test tube. 2. Add a few drops of concentrated sulphuric acid and heat.	Yellow precipitate indicated.	Presence of protein is confirmed.
3	**Millions Test:** 1. Take 2ml of given sample solution in a clean test tube. 2. Add 2-3 drops of Millon's reagent and shake well. 3. Observe the change.	Brick red precipitate indicates.	Presence of protein.
4	**Ninhydrin Test:** 1. Take the sample solution to be tested in a clean test tube. 2. Add 1-2ml of ninhydrin solution to it. 3. Boil the mixture and observe the change.	Appearance of blue colouration.	Presence of protein.

Specification test for Albumin

Sl. No.	Test	Observation	Inference
1.	**Half saturation test:** 5ml of given sample with 5ml of saturated ammonium sulphate solution keep for 5 min than precipitate obtained and filter it. Filtreate add with 2ml of 40% NaOH and 5 drops of 1% $CUSO_4$ solution.	Violet colure observes.	Presence of albumin.
2.	**Heller's test:** 2ml of conc. HNO_3 with 2ml of given sample containing from the side of the test tube (without shaking the tube).	A white ring at the junction of two fluids observes.	Presence of albumin.

Observation:

Report:

Teacher's Signature

Experiment: 6	**Sl No.:**	**Date:**

Aim: Qualitative analysis of Proteins (Casein).

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no.12-16.

Materials required: Test tubes, Test tube holder, Dropper, Water bath, Stirrer.

Chemical Required: Copper sulphate, Sodium hydroxide, Nitric acid, Millon's reagent, Mercuric sulphate, Sodium nitrite, Sulphuric acid, pyridine solution, Ninhydrin reagent, Distilled water, lead acetate, mercuric nitrate, sulphosalicylic acid, Esbach's reagent, C.P.R. Indicator.

Procedure:

Physical test:

Sl. No.	**Test**	**Observation**	**Inference**
1	**Appearance of a solution**	Turbid solution	Casein, Albumin, Globulin
2	**Colour of solution**	Opalescent of milky	Casein, Albumin
3	**Smell**	Milk like smell	Casein
4	**Litmus Test**	Alkaline	Casein, Metaprotein

Precipitation reactions of Proteins:

Sl.No.	**Test**	**Observation**	**Inference**
1	**Precipitation by heavy metals**		
	2ml of alkaline sample + 2-3 drops of 2% lead acetate solution.	White ppt	Protein present
	2ml of alkaline sample + 2 drops of 5% mercuric nitrate solution.	Brownish ppt	Protein present
2	**Precipitation by alkaloid reagents**		

	2 ml of sample solution + 3-4 drops of 20% sulphosalicylic acid.	White ppt	Protein present
	2 ml of sample solution + 1 ml Esbach's reagent.	Yellow ppt	Protein present

Note-For these test sample solution is made alkaline by using 1% Na_2CO_3 solution used.

Chemical test:

Sl. No.	**Test**	**Observation**	**Inference**
1	**Biuret Test:** 1. Take the given sample to be tested in a clean test tube. 2. Add 2 ml of sodium hydroxide solution to it. 3. To that add 5 to 6 drops of copper sulphate solution to it.	Appearance of bluish violet colour indicates.	Presence of protein.
2	**Xanthoproteic Test:** 1. Take 2ml of given sample compound in a test tube. 2. Add a few drops of concentrated sulphuric acid and heat.	Yellow precipitate indicated.	Presence of protein is confirmed.
3	**Millions Test:** 1. Take 2ml of given sample solution in a clean test tube. 2. Add 2-3 drops of Millon's reagent and shake well. 3. Observe the change.	Brick red precipitate indicates.	Presence of protein.

Specific test for casein

Neumann's test		
5 ml. of sample solution + 3 drops of C.P.R. Indicator. Solution turns pink + 1% acetic acid drop wise till colour changes to yellow.	Ppt produces.	Casein present.

Above ppt + 3-4 drops of conc. H_2SO_4 + 10-12 drops of conc. HNO_3 .Heat until mixture is colourless.(Add more HNO_3) if necessary)cool + few ml of ammonium molybdate solution.	Shinnig yellow or cannary yellow colour is obtained.	Casein present.

Observation:

Report:

Teacher's Signature

Experiment: 7	**Sl No.:**	**Date:**

Aim: Qualitative analysis of amino acids (Tryptophan).

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no.12-16. **Materials required:** Test tubes, Test tube holder, Dropper, Water bath, Stirrer.

Chemical Required: Copper sulphate, Sodium hydroxide, Nitric acid, Millon's reagent, Sodium nitrite, Sulphuric acid, pyridine solution, Ninhydrin reagent, Distilled water.

Chemical Test:

Sl. No.	**Test**	**Observation**	**Inference**
1	**Biuret Test:** 1. Take the given sample to be tested in a clean test tube. 2. Add 2 ml of sodium hydroxide solution to it. 3. To that add 5 to 6 drops of copper sulphate solution to it.	Appearance of bluish violet colour indicates.	Presence of amino acid
2	**Xanthoproteic Test:** 1. Take 2ml of given sample compound in a test tube. 2. Add a few drops of concentrated sulphuric acid and heat.	Yellow precipitate indicated.	Presence of amino acid is confirmed.
3	**Aldehyde Test:** 2 ml of sample solution + 5 drops of Million's reagent + 5 drops formalin mix + 2 ml of conc. H_2SO_4 from the side of test tube.	Violet ring is formed at the junction.	Presence of amino acid.

4	**Ninhydrin Test:** 1. Take the sample solution to be tested in a clean test tube. 2. Add 1-2ml of ninhydrin solution to it. 3. Boil the mixture and observe the change.	Appearance of blue colouration.	Presence of amino acid.

Observation:

Results:

Teacher's Signature

Experiment: 8	**Sl No.:**	**Date:**

Aim: Qualitative analysis of amino acids (Tyrosine).

Reference: S. R. Kale; R. R. Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29th Edition; Page no.12-16.

Materials required: Test tubes, Test tube holder, Dropper, Water bath, Stirrer.

Chemical Required: Copper sulphate, Sodium hydroxide, Nitric acid, Millon's reagent, Sodium nitrite, Sulphuric acid, pyridine solution, Ninhydrin reagent, Distilled water.

Chemical Test:

Sl.No.	Test	Observation	Inference
1	**Biuret Test:** 1. Take the given sample to be tested in a clean test tube. 2. Add 2 ml of sodium hydroxide solution to it. 3. To that add 5 to 6 drops of copper sulphate solution to it.	Appearance of bluish violet colour indicates.	Presence of amino acid
2	**Xanthoproteic Test:** 1. Take 2ml of given sample compound in a test tube. 2. Add a few drops of concentrated sulphuric acid and heat.	Yellow precipitate indicated.	Presence of amino acid is confirmed.
3	**Millions Test:** 1. Take 2ml of given sample solution in a clean test tube. 2. Add 2-3 drops of Millon's reagent and shake well. Observe the change.	Brick red precipitate indicate	Presence of amino acid.
4	**Ninhydrin Test:** 1. Take the sample solution to be tested in a clean test tube. 2. Add 1-2ml of ninhydrin solution to it. 3. Boil the mixture and observe the change.	Appearance of blue colouration.	Presence of amino acid.

Observation:

Results:

Teacher's Signature

Experiment: 9	**Sl No.:**	**Date:**

Aim: Qualitative analysis of lipids (Triglycerides).

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no.17-18.

Materials required: Test tubes, Test tube holder, Water bath, Stirrer, hot plate.

Chemical Required: Water, Alcohole, NaOH, Nacl, Soap, $Cacl_2$, HCL, Triglyceride.

Procedure:

Physical test:

Fats are colourless, odourless and tasteless in chemically pure form. But in natural or rancid state they are associated with same colour or aroma.

Solubility test:

Sl. No.	**Test**	**Observation**	**Inference**
1	1)Triglyceride + water – Warm gently 2) Triglycerides + Organic solvents like chloroform, alcohol, ether etc.	Immiscible separate layers are formed. soluble	Triglycerides are insoluble in water. Triglycerides are soluble in organic solvents.
2	**Litmus test** Test the solution of Triglycerides with blue or red litmus.	No change in colour or red litmus paper.	Triglycerides are neutral in nature
3	**Specific gravity** Add small quantity of Triglycerides to a test tube full of water.	Triglycerides are float on water	Its specific gravity is less than one.

Chemical test:

S.No	Test	Observation	Inference
1	**Emulsification:** 1) 1 ml alcoholic solution of Triglycerides + 5ml of Distilled water shakes vigorously. 2) 1 ml. alcoholic solution of Triglycerides + 5ml. of 1 % bile salt solution + 5ml. distil Water, shake vigorously.	A white homogenous emulsion is formed which breaks on standing by separating fatty phase in the form of oily droplets. A white homogenous emulsion is formed which remains stable on standing	Triglycerides form emulsion in water, which breaks on Standing**.** Triglycerides form emulsion in water which can be stabilised by emulsifying agent like bile salt.

2	**Saponification:** 10 ml. of 20% NaOH + alcoholic solution of Triglycerides 10ml. Heat on water both, until a drop of this mixture do not separate oil drop when added To distil water. Add the mixture with equal quantities of distil water and divide in Three equal parts.		
	a) 2 mL of above mixture + 1) A large knife point of solid Nacl, shake vigorously.	Cake of soap floats on top separate this cake and makes two parts.	Triglycerides is saponified in to
	2) One part of above soap + 10, 20 ml water shake.	Nothing takes place. ppt dissolves.	Formation of soap conformed. Sodium soap is soluble in water.
	3) Second part of soap+ 5ml distils water.		
	b) 2 ml of above mixture + 3ml of 2% $Cacl_2$ solution shake.	A white flocculant ppt is formed insoluble in water	Calcium soap is insoluble in water.
	c) Above mixture 3ml + 2/3 drops of conc. Sulphuric or hydrochloride acid boil.	Triglycerides separates and floats on water	Soap salt of fatty acids is converted into fatty acids on treatment with HCL/H_2SO_4

Observation

Results:

Teacher's Signature

Experiment: 10	**Sl No.:**	**Date:**

Aim: Qualitative analysis of lipids (Cholesterol).

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no.19.

Requirement:
Materials required: Test tubes, Test tube holder, Stirrer, Microscope.

Chemical Required: Water, chloroform, H_2SO_4, acetic anhydride. **Procedure:**

Physical test: It forms white shining rhombic crystals having notched corners.

Chemical test:

Sl.No.	Test	Observation	Inference
1	**a) Solubility** i) Cholesterol + water ii) Cholesterol + organic solvent. **b) Microscopic appearance**	Insoluble. Soluble. White shining rhombic crystal.	Being lipid it is insoluble in water. Being lipid it is soluble in organic solvent.
2	**c) Salkiwaski's test:** 2 ml cholesterol solution in chloroform + slowly add 2 ml conc. H_2SO_4 wait for 3 min.	Upper chloroform layer shows red coloration while lower H_2SO_4 layer shows green Fluorescence.	Presence of cholesterol is confirmed.
3	**d)Libermann-Burchrdt's test:** 2ml of cholesterol solution in chloroform + 10 drops of acetic anhydride + 2 drops of conc. H_2SO_4.	A rose red colour develops which quickly changes to blue to green.	Presence of cholesterol is confirmed.

Observation:
Results:

Teacher's Signature

Experiment: 11	**Sl No.:**	**Date:**

AIM: Determination of constituents of urine (glucose).

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition

Requirement:
Material requirements: Flask (50ml), Pipette (1-10ml), Photoelectric Colorimeter.
Chemicals requirements:
Principle:
The urine glucose test involves taking a sample of urine. Once you provide your sample, a small cardboard device known as a dipstick will measure your glucose levels. The dipstick will change color depending on the amount of glucose in your urine.
A urine glucose test is a quick and simple way to check for abnormally high levels of glucose in your urine. Glucose is a type of sugar that your body requires and uses for energy. Your body converts the carbohydrates you eat into glucose.
Having too much glucose in your body can be a sign of a health problem. If you don't receive treatment and your glucose levels remain high, you can develop serious complications. The urine glucose test involves taking a sample of urine. Once you provide your sample, a small cardboard device known as a dipstick will measure your glucose levels.
The dipstick will change color depending on the amount of glucose in your urine. If you have a moderate or high amount of glucose in your urine, your doctor will perform further testing to determine the underlying cause.
The most common cause of elevated glucose levels is diabetes, a condition that affects your body's ability to manage glucose levels. It's important to monitor your glucose levels if you have already been diagnosed with diabetes, or if you show symptoms of prediabetes. These symptoms include:

- excessive thirst
- blurred vision fatigue

Procedure:

Benedict's Test

Materials required:

Test tube, test tube holder, urine sample, measuring cylinders, Benedict's solution and burner.
Procedure:

- Take 2 ml urine sample in a measuring cylinder from the urine sample bottle.

- Take a test tube and pour the urine sample in it.
- Take 5 ml Benedict’s reagent in a measuring cylinder.
- Add Benedict’s reagent to the test tube that contains urine sample.
- Using a test tube holder, hold the test tube firmly and heat it for 2 minutes on the burner.
- Keep shaking the test tube while heating.
- A yellow precipitate appears which indicates the presence of sugar in urine.
- Depending upon the concentration of sugar in the urine, either green, yellow, or brick red precipitates are formed.

Fehling's test

Materials required

Test tube, test tube holder, urine sample, measuring cylinders, Fehling’s solution A, Fehling’s solution B and burner.

Procedure

- Take 2 ml urine sample in a measuring cylinder from the urine sample bottle.
- Take a test tube and pour the urine sample in it.
- Take 2 ml Fehling’s solution A in a measuring cylinder.
- Add Fehling’s solution A to the test tube that contains urine sample.
- Take 2 ml Fehling’s solution B in a measuring cylinder.
- Add Fehling’s solution B to the test tube that contains urine sample.
- Using a test tube holder, hold the test tube firmly and heat it gently for 2 minutes on the burner.
- Keep shaking the test tube while heating.
- A green precipitate appears which indicates the presence of traces of sugar in urine.
- Depending upon the concentration of sugar in the urine, either green, yellow or brick red precipitates are formed. Simulator Procedure (as performed through the Online Labs)You can select the test from the ‘Select type of test’ drop down list.

Benedict's Test

- Drag the dropper containing Benedict’s reagent towards the test tube to pour the reagent into it.
- Click on the knob of the burner to turn it on.

- Drag the test tube towards the burner to heat it.
- Click on the information icon to see the inference.
- You can redo the experiment by clicking on the ‘Reset’ button.

Fehling’s Test

- Drag the dropper containing the Fehling’s reagent A towards the test tube to pour the reagent into it.
- Drag the dropper containing the Fehling’s reagent B towards the test tube to pour the reagent into it.
- Click on the knob of the burner to turn it on.
- Drag the test tube towards the burner to heat it.
- Click on the information icon to see the inference.
- You can redo the experiment by clicking on the ‘Reset’ button.

Observation:
Report:

Teacher’s Signature

Experiment: 12	Sl No.:	Date:

Aim: Determination of constituents of urine (Creatinine).

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no.38-39. **Requirement:**

Material requirements: Flask (50ml), Pipette (1-10ml), Photoelectric Colorimeter.

Chemicals requirements: Picric acid (1%), NaOH (10%), Standard creatinine solution.

Principle:

Creatine in urine is estimated by Polin modified method using "Photoelectric colorimeter". In this method creatinine present in the given sample of urine is estimated by Folin modified method. In this method, urine sample containing Creatinine is treated with picric acid in alkaline medium to obtain red coloured creatining *picrate.* Optical density of this red coloured solution is compared with that of standard solution similarly converted by picric acid to creatinine picrate. By using calorimetry principle concentration of creatinine in given sample of urine can be calculated.

In second part of the experiment urine sample is boiled with acid so that creatine present in urine gets converted into creatinine. Then total creatinine (creatinine present in urine + creatinine obtained by conversion of creatine) in urine is estimated by the same method.

So

Creatine obtained by conversion of creatine = Total creatinine - Creatinine present in the sample.

Creatine present in the sample ≡ Creatinine obtained by Conversion of creatine × Conversion factore of creatine to creatinine.

≡ Creatinine obtained by Conversion of creatine × 1.16 as

1mgof creatinine=1.16mg of creatine.

Procedure:

Step 1. Estimate the mg. % of creatinine in given sample of urine as explained in previous Experiment (suppose 'A' mg.%).

Step 2. In a 250 ml. flask pipette 0.5 ml of urine and l0 ml of picric acid. Add few porcelain

Pieces. Add 150 ml of water. Boil gently for 45 minutes and then rapidly till the volume is Reduced to about 10 ml. In this procedure creatine present in the urine sample is converted Into creatinine. Now estimate the mg % of total creatinine (creatinine present + creatinine Obtained from creatine) by similar method explained in previous method. (Suppose 'B' mg.%).

Calculation

Report

Patient's name:
Sample number:
Urine creatine value:
(Estimated)
Urine creatine value:
(Normal)
Date:
Sign:

Teacher's Signature

Experiment: 13	Sl No.:	Date:

AIM: To analyze the given sample of urine for its normal inorganic constituents.

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no.19.

Principle:

The principle inorganic constituents of urine are chlorides, phosphates, sulfates and ammonia. Sodium chloride is the predominant chloride and makes up about half of the inorganic substances.

Requirements

Sample: Human urine sample

Apparatus: Test strips, Beaker

Chemicals: Then add distilled water to make the total volume 1000 ml., Nitric acid solution – 2, Silver nitrate solution – 2N, sodium molybdate, dil. Hcl, of 5% barium chloride solution, Sodium hydroxide solution **Procedure:**

S. No.	Chemical	Test	Observation	Inference
1.	Chlorides	To about 5 ml of urine sample add 5 drops of 2N nitric acid and 2N silver nitrate solution.	Formation of white precipitate	Indicates the presence of chloride
2.	Phosphates	To about 5 ml of urine sample add nitric acid 5 ml and sodium molybdate 4 ml and heat it for about 5 minutes.	Appearance of yellow crystalline precipitate because of ammonium phosphor-molybdate	Indicates the presence of phosphate in urine.
3.	Sulfates	At first take 10ml of urine and to it add 1ml dil. Hcl and then add 4 drops of 5% barium chloride solution.	A white colour precipitate is formed because of barium sulfate	Indicates presence of sulphate.

4.	Ammonia	Take 5 ml of urine and to it add 10% NaOH solution of volume 1ml.	Now indicate the presence of evoluted ammonia by the help of red litmus paper.	Indicates presence of ammonia

Observation:

Report:

Teacher's Signature

Experiment: 14	**Sl No.:**	**Date:**

AIM: To analyze the given sample of urine for its normal organic constituents.

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no.19.

Requirements:
Sample: Human urine sample
Apparatus: Test strips, Beaker
Chemicals: Mercuric nitrate solution, Oxime reagent, picric acid solution, Benedict's reagent

Principle:
Urine is the waste product formed by the kidney that is excreted by body and helps in maintaining the electrolyte and pH level of the body.[1] Urine is composed of various organic and inorganic constituents. So the basic objective of this experiment is to study about various organic and inorganic constituents of urine through various tests.

Procedure:

Sl. no.	**Chemical**	**Test**	**Observation**	**Inference**
1.	Test for Urea a) Mercuric nitrate solution b) Oxime reagent			
2.	Creatinine (Alkaline picrate test)	Take about 5 ml of urine sample and add 1 or 2 drops of saturated picric acid solution. Then add sodium hydroxide solution.	When the solution becomes alkaline it changes to red colour or orange colour because of formation of creatinine picrate	It indicates the presence the creatinine

3.	Uric acid a) Schiff's test b) Phosphate tungstate reagent test	Take urine sample 2 ml and to it add 1 ml of Benedict's reagent and then for about three minutes heat it in hot boiling water bath	If a white precipitate is formed it	It indicates the presence of uric acid.

Observation:

Report:

Teacher's Signature

Experiment: 15	**Sl No.:**	**Date:**

Aim: **To analyze the given sample of urine for its abnormal constituents.**

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no.29-31

Requirement:
Material requirements: Flask (100ml), Burette (50ml), Pipette (10ml), Pieces of porcelain.

Chemicals requirements: Benedict's quantitive reagent, Anhydrous/Crystalline sodium carbonate, Distilled water.

Principle:

Benedict's solution (quantitive), which is copper sulphate in alkaline solution, is reduced by glucose. The benedict's quantitative reagent consists of copper sulphate, potassium thiocynate and other chemicals in alkaline media.

Copper sulphate is reduced to cuprous oxide by glucose. The potassium thiocynate reacts with cuprous oxide and forms a white ppt of cuprous thiocynate instead of usual red precipitate of cuprous oxide. The disappearance of blue colour /tint from solution indicates complete reduction of copper sulphate.

$$2CU(OH)_2 \xrightarrow[\text{reduction}]{-O} CU_2O + 2H_2O$$

Procedure:

Benedict's method

- Wash and clean the required apparatus.
- Pipet 10 ml of the benedict's quantitive reagent in 100ml flask with long narrow neck (So as to prevent oxidation, by atmospheric oxygen.
- Add 20 ml of water and 5gm of anhydrous sodium carbonate and few pieces of porcelain
- Heat the flask on a flame. It should be noted that the titration mixture must be kept boiling throughout the titration period.
- Fill the burette with urine (diluted). Adjust the meniscus.
- When the contents in the flasks begin to boil add urine ml wise rapidly until white precipitate appears.
- After this add urine from burette drop by drop at intervals of 10 seconds.
- Continue the addition of urine from burette until the last trace of blue colour (due to $CUS0_4$) disappears. **End point:**

- Allow the titration mixture to cool.
- The white ppt settles down.
- Complete disappearance of the blue colour of the benedict's reagent. ➢ The fluid in the titration mixture must show light green colour on cooling.

Calculation:

Reports:

Patient's name:
Sample number:
Urine glucose: (Estimated value) Urine glucose: (Normal value)
Date:
Sign:

Teacher's Signature

Experiment: 16	**Sl No.:**	**Date:**

Aim: **To analyze the given sample of urine for its abnormal constituents.**

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no.36-37. **Requirement:**

Material requirements: Flask (50ml), Pipette (1-10ml), Photoelectric Colorimeter.

Chemicals requirements: Picric acid (1%), NaOH (10%), Standard creatinine solution.

Principle:

Creatinine in urine is estimated by Folin modified method using photoelectric colorimeter. In this method, urine sample containing Creatinine is treated with picric acid in alkaline medium to obtain red coloured creatinine *picrate*. Optical density of this red coloured solution is compared with that of standard solution similarly converted by picric acid to creatinine picrate. By using colorimetry principle concentration of creatinine in given sample of urine can be calculated. **Procedure:**

Step 1. Label two flasks of 5O ml capacity as 'S' standard and 'U' unknown.

Step 2. Preparation of standard:

In standard flask (S) add followings:

- Standard creatinine solution - 0.5 ml
- Sodium hydroxide solution - 1.0 ml (NaOH 10%)
- Picric acid (1%)- 10.0 ml. Dilute by adding distil water to 5O ml. Mix and keep for 15 minutes.

Step 3. Preparation of unknown:

In unknown flask (U) add followings:

- Given urine sample- 0.5 ml.
- Sodium hydroxide solution - 1.0 ml. (NaOH 10%)
- Picric acid (1 %)- 10.0 ml. Dilute by adding distil water to make final volume 50 ml. Mix and keep for 15 minutes

. .

Step4. Record the colours (i.e. optical density) obtained of that of standard and unknown by using photoelectric colorimeter.

Calculation:

Reports:

Patient's name:
Sample number:
Urine creatinine value:
(Estimated)
Urine creatinine value:
(Normal) Date:
Sign:

Teacher's Signature

Experiment: 17	Sl No.:	Date:

Aim: Determination of constituents of blood/serum glucose in blood

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; NIRALI PRAKASHAN; 2020; 29TH Edition; Page no.41-44. **Requirement:**

Material requirements: Folins sugar tube, Pipette graduated, flasks, photoelectric colorimeter

Chemicals requirements: Alkaline copper sulphate solution, Phosphomolybdic acid, Sodium tungstate 10% , Sulphuric acid 2/3 N, Benzoic acid solution, Stock glucose solution, Standard glucose solution No. 1, Standard glucose solution No. 2, Fluoride oxalate solution.

Principle:
In this method protein free filtrate is obtained (Folin-wu filtrate) so that 10 ml of filtrate corresponds to 1 ml of blood sample. Protein free filtrate is obtained by precipitating the proteins of blood by tungstic acid. Then this protein free filtrate containing glucose is heated with alkaline copper sulphate solution. Thus glucose reduces copper sulphate to form equivalent quantity of cuprous oxide.

This cuprous oxide formed is reduced with phosphomolybdic acid to produce corresponding equivalent quantity of molybdenum blue. The molybdenum blue gives intense blue colour, the "intensity of which is directly proportional to cuprous oxide which corresponds to the amount of glucose present in given sample of ‘folin-wu" filtrate.

Reaction:

$C_6H_{12}O_6$ + CU^{++} $\rightarrow CU^{+}$ + Oxidation

Glucose cupric

$CU^{+}Na_2PO_4$: $12MoO_3$ $\rightarrow$ CU^{++} + CU^{++} 12MoO

Cuprous

The blue colour obtained with test blood sample is compared with standard solution by similar procedure and by using photoelectric calorimeter. The optical density of test and standard is measured and concentration of glucose in blood can be calculated using colorimetric principle.
Procedure:

Folin- Wu (modified)

- Wash clean, label three folin-wu tubes as:
  - unknown ... 'U
  - Standard I - Std I
  - Standard II - Std II

- To the folin wu tube labelled as "U" take 2 ml of "Folin wu filtrate".
- In a folin-wu tube labelled as "Std I" take 1 ml of standard sugar solution I (0.1 mg sugar). 4. In a folin-wu tube labelled as "Std II" take 1 ml of standard sugar solutjon Il (0.2 mg sugar).
- To all above tubes add 1 ml of alkaline copper sulphate solution.
- Keep the tubes in boiling water bath for 6 to 8 minutes.
- Remove from the water bath and add 1 ml of phosphomolybdic acid to all tubes.
- Keep the tubes again in boiling water bath for 2 minutes and after 2 minutes cool to room temperature.
- Add 25 ml of distil water to each tube mix well and record. Compare the optical densities by using photoelectric colorimeter by using tube filter 420 mμ.

Calculation:

Reports:

Patient's name:
Sample number:
Blood sugar:
(Estimated)
Blood sugar:
(Normal)
Date:
Sign:

Teacher's Signature

Experiment: 18	Sl No.:	Date:

Aim: Determination of constituents of blood/serum Cholesterol in blood.

Reference:S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no.55-57.

Requirement:
Material requirements: Centrifuge, Test tube, Graduate Pipettes, Photoelectric colorimeter.

Chemicals requirements: Standard cholesterol solution colour reagent.

Method used: Direct method of ferro and ham. **Principle:**

This is a direct method used for estimation of cholesterol in serum by ferro and hom. In this method is a mixture of acetic anhydride; glacial acetic acid, and sulphuric acid in appropriate proportion is used. The colour reagent gives bluish colour with cholesterol. In this method the colour is developed directly without the extraction of lipids.

In the "standard" preparation two drops of distil water addition is advised, as it hastens the reaction and develops color. This standard" color with which "unknown" color is compared by using photo-electric calorimeter.

Procedure:

Preparation of unknown sample:

i. In a test h1be labelled as "U" pipette out 0.2 ml of serum.
ii. Add 5 ml freshly prepared color reagent. **iii.** Mix well by shaking and keep the tube in dark for 10 minutes.
iv. Obtain a optical density for unknown by using photoelectric calorimeter at 660 mμ. Record and note it as "Eu".

Preparation of standard:

- In a tube labelled as 'S' take 0.2 ml of standard cholesterol solution.
- Add 2 drops of distil water and 5 ml of color reagent.
- Mix well by shaking and keep the tube in dark for 10 minutes.
- Obtain an optical density for standard by using photoelectric calorimeter at 660 mu.

Calculation:

Reports:

Patient's name:
Sample number:
Blood cholesterol:
(Estimated)
Blood cholesterol:
(Normal) Date:
Sign:

Teacher's Signature

Experiment: 19	Sl No.:	Date:

Aim: To estimation of Serum glutamate-oxaloacetate transaminase (S.G.O.T) in blood serum.

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; NIRALI PRAKASHAN; 2020; 29TH Edition; Page no.66-68.

Requirement:

Material requirements: Centrifuge, Test tube, Graduate Pipettes, Photoelectric colorimeter.

Chemicals requirements: Phosphate (pH 7.4), Stock pyruvate solution, Standard solution, DNPH solution, S.G.O.T. substrate, Sodium hydroxide.

Principle:

Transaminases are the enzymes which promotes the process of removal of α-amino groups of most of L amino acids to a α-keto acid. As a result number of alpha amino acids and alpha keto acids are formed.

One of these are serum asparate transminase i.e. which catalyses the reaction of glutamate oxalo acetate transaminase i.e.' G.O.T.

L - alpha - oxaglutrate + L asparare ⇆ L - glutamate + L –oxaloacetate.

This oxaloacetate formed in the reaction with glutamate oxalo acetate transaminase (GOT) decarboxylates spontaneously to pyruvate which is again measured by hydrazone formation. The colour obtained is measured in colorimeter at 510 mμ.(filter).

Procedure: [A] Preparation of unknown sample:

- In a tube labelled as "U" take asparate substrate 0.5 ml.
- Add 0.1 ml serum sample.
- Incubate the tube at 37° c for 30 minutes.
- Remove the tube and add 0. 5ml DNPH solution keep 20 minutes at room temperature.
- Add 5ml of 0.4N NaOH in the tube.
- Obtain the optical density for unknown by comparing the colours by using photoelectric colorimeter with green filter (520 mμ).

Note it as "Eu".

Preparation of control:

- In a tube labelled as "C" take · 0.5 ml of asparate substrate, 0.5 ml DNPH solution and 0.1 ml serum.
- Incubate the tube at 37° c for 30 minutes.
- After 30 minutes, remove from water bath and keep 20 minutes at room temperature.

- Add 5 ml of 0.4N NaOH to the tube compare the colour by using green filter.

Preparation of standard:

- In a tube labelled as "S" take 0.5 ml of asparate substrate and 0.5 ml of DNPH solution. Add 0.1 ml of standard-pyruvate.
- Incubate the tube at 37° C for 30 minutes.
- Remove after 30 minutes and keep at room temperature for 20 minutes.
- Add 5 ml of 0.4N NaoH solution.
- Compare the colour by using green filter. Note it as "Es". **Preparation of blank sample:**

  - In a tube labelled as "B" take:
  - 0.5 ml asparate substrate.
  - 0.5 ml DNPH solution.
  - 0.1ml distils water. Incubate at 37°C for 30 minutes.
  - After 30 minutes keep at room temperature for 20 minutes.
  - Add 5 ml NaoH (.4N) solution.
  - Compare the colour using green filter.
  - Prepare unknown control standard, blank as before [SGPT experiment} .
  - Incubate tube for 60 minutes at 37° c.

Calculation:

Reports:

Patient's name:
Sample number:
SGOT:
(Estimated) SGOT: (Normal) Date:
Sign:

Teacher's Signature

Experiment: 20	Sl No.:	Date:

Aim: To estimation of Serum glutamate-pyruvate transaminase (S.G.P.T) in blood serum.

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; NIRALI PRAKASHAN; 2020; 29TH Edition; Page no.64-66.

Requirement:

Material requirements: Centrifuge, Test tube, Graduate Pipettes, Photoelectric colorimeter.

Chemicals requirements: Phosphate (pH 7.4), Stock pyruvate solution, Standard solution, DNPH solution, S.G.P.T. substrate, Sodium hydroxide.

Principle:

Transaminases are the enzymes which promotes the process of removal of α-amino groups of most of L-amino acids to α-keto acid. As a result number of alpha amino acids and alpha keto acids are formed.

One of these are serum alanine-transminase (S.G.P.T.) this catalyses the reaction as follows.

L-alpha oxoglutrate + L alanine ⇆ L-glutamate + L-pyruvate.

This pyruvate produced by "glutamate-pyruvate-transminase" reacts with di-Nitrophenyl hydralazine (DNPH solution) in an alkaline medium which is measured at 510 mμ filter.

Note: In the estimation, the concentration of substrate is suboptimal, to reduce background colour produced by ketoglutarate in the reaction with Di-nitro-phenyl-hydrazine (DNPH).

Procedure:

Preparation of unknown sample:

- In a tube labelled as "U" take alanine substrate 0.5 ml.
- Add 0.1 ml serum sample.
- Incubate the tube at 37° C for 30 minutes.
- Remove the tube and add 0. 5ml DNPH solution keep 20 minutes at room temperature.
- Add 5ml of 0.4N NaOH in the tube.
- ·Obtain the optical density for unknown by comparing the colours by using photoelectric colorimeter with green filter (520 mμ).

Note it as "Eu".

[B] Preparation of control:

- In a tube labelled as "C' take 0.5 ml of alanine substrate, 0.5 ml DNPH solution and 0.1 ml serum. 2. Incubate the tube at 37°·c for 30 minutes.
- After 30 minutes; remove from water bath and keep 20 minutes at room temperature.
- Add 5 ml of 0.4 N NaOH to the tube compare the colour by using green filters. Note it as "Ec".

[C] Preparation of standard:

- In a tube labelled as "S" take 0.5 ml of alanine. Substrate and 0.5 ml of DNPH solution. Add 0.1 ml of standard pyruvate.
- Incubate the tube at 37°C for 30 minutes.
- Remove after 30 minutes and keep at room temperature for 20 minutes.
- Add 5 ml of 0.4 N NaoH solutions.
- Compare the colour by using green filter. Not it as "Es".

[D] Preparation of blank sample:

1. In a tube labelled as "B" takes:

    - 0.5 ml alanine substrate
    - 0.5 ml DNPH solution
    - 0.1 ml distil water

    Incubate at 37° c for 30 minutes
2. After 30 minutes keep at room temperature for 20 minutes.
3. Add 5 ml NaoH (.4N) solution.
4. Compare the colour using green filter. Note it as "EB"

Calculation:

Reports:

Patient’s name:
Sample number:
SGPT: (Estimated) SGPT: (Normal) Date:
Sign:

Teacher’s Signature