JHARKHAND Rai University
RANCHI

PRACTICAL LAB MANUAL

CLINICAL CHEMISTRY -I

B.Sc. MLT (IInd Semester)

INDEX

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1.	Physical examination of Urine Colour & Odour, pH of Urine, Volume, Specific gravity of Urine		
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3.	Estimation of Blood Urea Uric acids		
4.	Determination of Uric acids		
5.	Determination of serum protein		
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7.	Determination of Chlorides in urine Calcium, Phosphate, ketones		
8.	Determination of Microscopic Structure of urine Calcium chlorides		

Experiment: 1	Sl No.:	Date:

Aim: Physical examination of Urine Colour & Odour, pH of Urine, Volume, Specific gravity of Urine

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

Materials required: Beaker, water bath, test tubes, graduated pipettes, litmus paper.

Procedure: 1. Collection of Urine Sample

- **Type of sample**: Midstream clean-catch is most common.
- **Container**: Use a clean, dry, and transparent container with a secure lid.
- **Volume**: At least 10–15 mL is typically sufficient.

2. Observations Made During Physical Examination

A. Colour

- **Normal**: Pale yellow to amber.
- **Abnormal**:
  - Colourless: Dilute urine.
  - Dark yellow or amber: Dehydration.
  - Red/pink: Haematuria, haemoglobinuria, or beets/medication.
  - Brown/tea-coloured: Myoglobin, bile pigments.
  - Green/blue: Certain medications or infections (e.g., Pseudomonas).

B. Clarity (Turbidity)

- **Normal**: Clear to slightly cloudy.
- **Abnormal**: Cloudy or turbid, which may suggest:
  - Presence of pus (pyuria)
  - Crystals
  - Mucus
  - Bacteria
  - Epithelial cells

C. Odor

- **Normal**: Slightly aromatic.
- **Abnormal**:
  - Foul-smelling: UTI.
  - Sweet/fruity: Diabetes mellitus (ketones).
  - Ammonia-like: old sample or infection.
  - Musty: Phenylketonuria.

D. Volume (if measured over time)

- Not part of single-sample exams, but relevant in timed collections.
  - **Normal daily output**: ~800 to 2000 mL.
  - **Polyuria**: >2500 mL/day (e.g., diabetes).
  - **Oliguria**: <400 mL/day (e.g., dehydration, renal failure).
  - **Anuria**: <100 mL/day (severe renal failure).

3. Documentation

- Record all findings accurately, including:
  - Time and date of collection
  - Physical characteristics observed
  - Any abnormal findings

Urine Specific Gravity (SG)-

Normal Range: **1.005 – 1.030**

Interpretation:

- **Low SG (<1.005)**: May indicate overhydration, diabetes insipidus, or renal tubular damage.
- **High SG (>1.030)**: May indicate dehydration, glycosuria, or proteinuria.

Urine pH

- **Definition**: Measures the acidity or alkalinity of urine.
- **Normal Range**: **4.5 – 8.0**
- **Result:**
  - **Acidic urine (pH < 6)**: Seen in high-protein diets, metabolic acidosis, or uncontrolled diabetes.
  - **Alkaline urine (pH > 7)**: Can occur with vegetarian diets, urinary tract infections (with urea-splitting bacteria), or metabolic alkalosis.

Teacher signature

Experiment: 2	Sl No.:	Date:

Aim: Chemical examination of Urine, (Glycosuria or Benedicts test)

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, urine sample

Chemical Required: • Copper **(II) sulphate ($CuSO_4$)** – provides Cu^{2+} ions. **Sodium carbonate (Na_2CO_3)** – creates an alkaline environment. **Sodium citrate ($Na_3C_6H_5O_7$)** – complexes with copper (II) ions to keep them in solution

1. **Procedure: Prepare the sample:**
   - Place about **2 mL of the test solution** in a clean test tube.
2. **Add Benedict's reagent:**
   - Add about **2 mL of Benedict's reagent** to the test tube containing the sample.
3. **Heat the mixture:**
   - Gently heat the mixture in a **boiling water bath** (or over a Bunsen burner using a test tube holder) for about **2–5 minutes**.
   - **Observation:** As the solution heats, observe any **color changes** that occur.

Report: Interpretation of Results:

Colour Change	Indication
Blue (no change)	No reducing sugar present
Green	Trace amounts of reducing sugar
Yellow	Low concentration of reducing sugar
Orange	Moderate concentration
Brick-red precipitate	High concentration of reducing sugar

Teacher's Signature

Experiment: 3	Sl No.:	Date:

Aim: Chemical examination of urine (Proteinuria) by heat acetic acid test

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, urine sample

Chemical Required: Acetic Acid (Glacial or 5%) – to acidify the urine sample and prevent precipitation of phosphates.

Distilled Water – for dilution if necessary

Proteinuria: The **"heat and acetic acid test"** is a **qualitative test for protein in urine**, commonly used in basic urinalysis. It's based on the principle that **proteins, especially albumin, coagulate when heated** in the presence of **acetic acid**, which helps to rule out false positives due to phosphates.

Theory of Acetic Acid Test:

1. **Proteins denature (coagulate) on heating**:
   - Albumin and other proteins unfold and precipitate when exposed to heat.
2. **Phosphates and carbonates can also precipitate** upon heating:
   - These may form turbidity similar to protein, potentially giving false positives.
3. **Addition of acetic acid**:
   - Helps dissolve phosphates and carbonates.
   - Does **not** dissolve protein precipitates.
   - Thus, persistent turbidity after acetic acid addition confirms protein presence.

Procedure:

1.Fill 2/3 of a test tube with the urine sample.

2.Heat the **upper third** of the urine in the test tube gently.

3.Observe for any **cloudiness or turbidity**.

4. Add a few drops of **acetic acid**:

- If the turbidity persists or increases, it confirms the **presence of protein (albumin)**.
- If the turbidity disappears, it was likely due to **phosphates or carbonates**.

Observation: cloudiness or turbidity.

Report: **Negative**: Clear after heating and adding acetic acid – no protein.

Positive: Persistent turbidity after heating and acid addition – indicates proteinuria.

Teacher's Signature

Experiment: 4	Sl No.:	Date:

Aim: Determination of chemical examination of urine (Heller test for proteinuria)

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; 5-6, Harsh mohan practical manual 2025 5th Edition

Materials required: Urine sample – Freshly collected, preferably midstream., Test tube, beaker filter paper

Chemical Required: Concentrated Nitric Acid (HNO_3)

Theory: When concentrated nitric acid is added carefully to urine, **proteins (if present)** will **precipitate** at the junction of the acid and urine, forming a **white ring**. This happens due to protein denaturation in the strongly acidic environment.

Procedure: •

1.Take **2–3 mL** of concentrated nitric acid in a test tube.

2.Slowly and carefully **layer an equal volume of urine sample** over the acid using a pipette or dropper (along the side of the test tube).

3.Observe the **junction** of the two liquids.

Observation: junction of the two liquids.

Report:

White ring at the interface → Protein present (positive test).

No ring → Protein absent (negative test).

Teacher's Signature

Experiment : 5	Sl No.:	Date :

Aim: Determination of bile salts in urine (Hay test)

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; 12-16, Harsh Mohan practical pathology 2025 5th Edition

Materials required: Test tubes, Test tube holder, Dropper, urine sample beaker, urine tube.

Chemical Required: Sulfur powder

Theory: The **Hay test** (also known as the **Hay's Sulphur powder test**) is a qualitative test used to detect the presence of **bile salts** in urine. It is often used in the diagnosis of conditions like **obstructive jaundice**, where bile components may spill into the urine.

Procedure:

1. Place the urine sample in a test tube.

2 Sprinkle sulphur powder on the surface.

3 If sulphur sinks, **bile salts are present** (positive test).

4. If sulphur floats, test is negative.

Observation:

Bile salts reduce the surface tension of urine, causing sulphur powder to sink. This indicates the **presence of bile salts** in the urine.

If the powder floats, bile salts are **absent**.

Report: bile salts are present (positive test).

sulphur floats, test is negative.

Teacher's Signature

Experiment: 6	Sl No.:	Date:

Aim: Estimations of urobilinogen in urine using Ehrlich aldehyde reagent

Reference: S.R.Kale; R.R.Kale: Practical Biochemistry & Clinical Pathology; Nirali Prakashan; 2020; 29TH Edition; Page no.-112

Materials required: Test tubes, Test tube holder, Dropper, urine sample, urine container

Chemical Required: p-Dimethylaminobenzaldehyde (DMAB), Hydrochloric acid (HCl), Ethanol or methanol

Standard Preparation:

A typical formulation:

- **1 g** of p-dimethylaminobenzaldehyde
- **50 mL** of concentrated hydrochloric acid
- **50 mL** of ethanol or methanol

Theory: The **Ehrlich Aldehyde Reagent Test** is a **chemical test used to detect indole derivatives**, especially **tryptophan and tryptamine structures**, commonly found in compounds like **tryptophan, serotonin, and LSD**.

Principle of the Ehrlich Test:

The **Ehrlich reagent** contains **p-dimethylaminobenzaldehyde (DMAB)** dissolved in an acid, typically **hydrochloric acid (HCl)** or **ethanol with HCl**.

- When this reagent is added to a compound containing an **indole ring**, it reacts with the **indole nitrogen** to form a **coloured complex**, typically **violet, purple, or blue**.
- The colour formation is due to the **electrophilic aromatic substitution** reaction between the aldehyde group in DMAB and the electron-rich indole ring.

Reaction Summary:

Indole-containing compound + DMAB (in acid) → **Coloured complex** (violet/purple)

Procedure:

1. Take 5 mL of freshly collected urine in a test tube.

2. Add 1–2 mL of Ehrlich's reagent to the urine sample.

3. Mix gently.

Observation:

Observe the colour change within a few minutes.

Report:

Positive Result:

- A **cherry-red or pink colour** develops.
- This usually indicates the presence of:
  - **Urobilinogen** (increased in conditions like haemolytic anaemia or liver disease)
  - **Porphobilinogen** (increased in acute intermittent porphyria)
  - Some **tryptophan metabolites**, such as in **carcinoid syndrome**

2. Negative Result:

- No significant colour changes.
- Suggests absence or low levels of urobilinogen/porphobilinogen.

Teacher's Signature

Experiment: 7	Sl No.:	Date:

Aim: Estimation of chemical examination of urine for ketonuria using Rothera, s test

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: • **Sodium nitroprusside** – the key reagent that reacts with ketone bodies.

• **Ammonium sulphate** – used to saturate the urine sample.

• **Strong ammonia solution (concentrated ammonium hydroxide)** – added to create an alkaline environment.

Theory: Rothera's Test is a qualitative chemical test used primarily to detect the presence of **ketone bodies** (especially **acetoacetic acid** and **acetone**) in **urine**. It is commonly used in clinical settings to help diagnose conditions like **diabetic ketoacidosis** and **starvation ketosis**.

Principle: Rothera's test is based on the principle of **nitroprusside reaction**. When ketone bodies such as **acetoacetic acid** or **acetone** are present in urine, they react with **sodium nitroprusside** in the presence of **ammonia** (alkaline medium) to produce a **purple or permanganate-coloured complex**.

Chemical Reaction Involved:

- **Acetoacetic acid** or **acetone** + **Sodium nitroprusside** + **Ammonia** → **Purple-coloured complex**

(Note: **β-hydroxybutyric acid** is not detected by this test because it does not react with sodium nitroprusside.)

Procedure:

1.Take a clean test tube and add about **5 mL of urine**.

2.Add about **1 gram of solid ammonium sulphate** to saturate the urine.

3.Add a **few crystals of sodium nitroprusside** to the test tube.

4.Mix the solution gently to dissolve the chemicals.

5. **Carefully add strong ammonia** (liquor ammonia) along the side of the test tube so that it forms a layer top of the urine mixture.

6.Allow the test tube to stand undisturbed for a few minutes.

Observation: A **purple or permanganate-colored ring** at the junction of the two layers indicates the **presence of ketone bodies** (especially **acetoacetic acid**).

Results: Positive **Rothera's Test**

- **Appearance**: A **purple or violet ring** at the junction of the two liquids (ammonium sulphate + sodium nitroprusside with urine and added ammonia).
- **Meaning**: **Ketone bodies are present** in the urine. This can indicate:
  - Diabetic ketoacidosis (DKA)
  - Starvation
  - High-fat/low-carbohydrate diet
  - Prolonged fasting or vomiting
  - Alcoholic ketoacidosis

- Negative **Rothera's Test**

- **Appearance**: **No colour change** or no purple ring.
- **Meaning**: **No detectable ketone bodies** in the urine.

Teacher's Signature

Experiment: 8	Sl No.:	Date:

Aim: To study the microscopic examination of urine

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

Materials required: clean, dry, sterile container**,** Centrifuge, Test tubes, Pasteur pipette or dropper, Glass slides and coverslips, Light microscope

Principle

1. **Centrifugation**:
   A fresh urine sample is centrifuged to separate the solid components (sediment) from the liquid (supernatant).
2. **Decanting**:
   The supernatant is discarded, and the sediment is resuspended in a small volume of urine.
3. **Microscopic Analysis**:
   A drop of the resuspended sediment is placed on a glass slide and examined under a microscope, typically at low power (10x) for casts and high power (40x) for cells, crystals, and bacteria.
4. **Identification**:
   The formed elements such as:
   - Cells (e.g., red blood cells, white blood cells, epithelial cells),
   - Casts (e.g., hyaline, granular, cellular),
   - Crystals (e.g., uric acid, calcium oxalate),
   - Microorganisms (e.g., bacteria, yeast, parasites), are identified based on their morphological characteristics.

Clinical Significance:

This examination helps in diagnosing conditions such as:

- Urinary tract infections
- Kidney diseases
- Haematuria (blood in urine)
- Proteinuria-related disorders
- Crystalluria or stone formation

Procedure:

Collection of Urine Sample

- Use a clean, dry container.
- Preferably a midstream, clean-catch specimen is collected.
- Sample should be fresh (ideally examined within 1–2 hours).

2. Mixing and Aliquoting

- Mix the urine sample thoroughly to distribute sediment evenly.
- Pour about 10 mL of the urine into a conical centrifuge tube.

3. Centrifugation

- Centrifuge the urine at **1,500–2,000 rpm for 5 minutes**.
- Carefully decant the supernatant without disturbing the sediment.

4. Resuspension of Sediment

- Tap the bottom of the tube gently to resuspend the sediment in the remaining fluid (usually ~0.5 mL).

5. Slide Preparation

- Place 1 drop of the resuspended sediment on a clean glass slide.
- Cover with a coverslip (avoid air bubbles).

6. Microscopic Examination

- Examine under low power (10x) to locate elements.
- Then use high power (40x) for detailed observation.
- If available, use phase-contrast microscopy to enhance visibility of unstained elements.

Observation: • Red blood cells (RBCs)

White blood cells (WBCs)

Epithelial cells

Casts (e.g., hyaline, granular, cellular)

Crystals

Microorganisms (bacteria, yeast, parasites)

Others (sperm, mucus, artifacts)

Results: **Red Blood Cells (RBCs)**

- **Normal:** 0–4 RBCs/hpf (high power field)
- **Elevated:** May indicate bleeding in the urinary tract (e.g., infection, kidney stones, trauma, or glomerulonephritis)

2. White Blood Cells (WBCs)

- **Normal:** 0–5 WBCs/hpf
- **Elevated:** May suggest infection or inflammation (e.g., urinary tract infection or interstitial nephritis)

3. Epithelial Cells

- **Normal:** Few
- **Increased:** May indicate contamination (e.g., skin cells) or tubular damage (renal epithelial cells)

4. Casts

- **Hyaline casts:** Can be normal in small numbers
- **Red blood cell casts:** Suggest glomerulonephritis
- **White blood cell casts:** Suggest pyelonephritis or interstitial nephritis
- **Granular or waxy casts:** May indicate chronic kidney disease

5. Crystals

- **Calcium oxalate, uric acid:** Can be normal or indicate risk for kidney stones
- **Cystine, struvite, or other abnormal crystals:** May indicate metabolic issues or infections

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.	Formation of soap conformed.
	3) Second part of soap+ 5ml distils water.	ppt dissolves.	Sodium soap is soluble in 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: 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: 14	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: 15	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: 16	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