Bachelor of Science in Medical Laboratory Technology (BMLT)

CLINICAL CHEMISTRY - II

Semester: Third (IIIrd)

PRACTICAL LABORATORY MANUAL

INDEX

S. No.	Name of Experiments	Expt. No.	Page
1	Determination of Blood glucose Test by Folin-Wu method	01	3-4
2	Determination of Blood glucose Test by GOD – POD method	02	5-6
3	Glucose Tolerance Test (GTT)	03	7
4	Determination of Serum Bilirubin Test	04	8-9
5	Principal and procedure of SGPT test	05	10-11
6	Principal and procedure of SGOT test	06	12-13
7	Principal and procedure of Serum protein test	07	13-14
8	Principal and procedure of Alkaline phosphatase test	08	14-15
9	Determination of Serum Urea Test	09	16-18
10	Determination of Uric Acid Test	10	18-19
11	Determination of Serum Creatinine Test	11	20-21
12	Determination of Serum total cholesterol	12	22-23

EXPERIMENT - 01

Diabetic Profile Test

Aim: - Determination of Blood glucose Test by Folin-Wu method.

Principal: The Folin-Wu method is based on the reduction of cupric ions (Cu^{2+}) **to** cuprous ions (Cu^{+}) by glucose in an alkaline medium. The cuprous ions then react with phosphomolybdic acid (Folin-Wu reagent) to form a blue-colored complex. The intensity of the blue color is proportional to the amount of glucose and is measured calorimetrically at 620 nm**.**

1. Reagents Required: Folin-Wu Reagent (Phosphomolybdic acid reagent)
2. Folin-Wu Protein Precipitating Reagent (10% Sodium tungstate + 2/3N Sulfuric acid)
3. Standard Glucose Solution (100 mg/dL)
4. Distilled Water

Apparatus:

- Test tubes
- Pipettes
- Centrifuge
- Colorimeter/Spectrophotometer
- Water bath.

Procedure:

Test Tube	Protein-Free Filtrate	Distilled Water	Glucose Standard	Distilled Water	Folin-Wu Reagent
Test (T)	1.0 mL blood sample (after deproteinization)	—	—	1.0 mL	1.0 mL
Standard (S)	—	1.0 mL	1.0 mL (from standard glucose)	—	1.0 mL
Blank (B)	—	1.0 mL	—	1.0 mL	1.0 mL

Steps:

1. **Deproteinization:**

Add 1.0 mL of blood to 1.0 mL of 10% sodium tungstate and 1.0 mL of sulfuric acid.

Mix well and centrifuge to get the clear supernatant (protein-free filtrate).

2. Take 1 mL of this filtrate for the test.
3. Add reagents to the respective tubes as per the table above.
4. Mix well and place the tubes in a boiling water bath for 8 minutes, then cool to room temperature.
5. Measure the absorbance of the test and standard against the blank at 620 nm.

Calculation:

Blood Glucose (mg/dL) = (Absorbance of Standard/Absorbance of Test) ×100

Normal Range:

- **Fasting blood glucose:** 70–110 mg/dL
- **Postprandial (after meal):** up to 140 mg/dL

Precautions:

- Avoid haemolysis of the blood sample.
- Ensure proper deproteinization for accuracy.
- Use freshly prepared reagents.
- Follow safety guidelines while using sulfuric acid and heating.

REFERENCE BOOKS:

1. Clinical Biochemistry, Nanda Maheshwari, Jaypee Brothers
2. Textbook of Clinical Biochemistry, Ramnik Sood, CBS Publisher
3. Practical Clinical Biochemistry, Ranjana Chawla, Jaypee Brothers.

EXPERIMENT - 02

AIM: -. Determination of Blood glucose Test by GOD – POD method.

Principle:

The **GOD-POD** method is based on enzymatic reactions:

1. **Glucose Oxidase (GOD)** catalyses the oxidation of **glucose** to **gluconic acid** and **hydrogen peroxide (H_2O_2)**.
2. **Peroxidase (POD)** uses the hydrogen peroxide to oxidize a chromogenic substrate (like **4-aminoantipyrine and phenol**), producing a **coloured compound**.
3. The **intensity of the colour** is directly proportional to the **glucose concentration** and is measured **spectrophotometrically** at **505 nm**.

Requirements:

- Reagents: GOD-POD reagent (contains glucose oxidase, peroxidase, phenol, and 4-aminoantipyrine)
- Standard glucose solution
- Blood sample (serum or plasma)
- Spectrophotometer or colorimeter (at 505 nm).

Reaction:

Procedure:

Test Tube	Blank	Standard	Test
GOD-POD Reagent	1.0 mL	1.0 mL	1.0 mL
Distilled Water	0.01 mL	–	–
Standard Glucose	–	0.01 mL	–
Sample (Serum/Plasma)	–	–	0.01 mL

1. Mix the contents of each tube well.
2. Incubate all tubes at **37°C for 15 minutes** (or room temperature for 30 minutes).
3. Measure the **absorbance** of the **Test** and **Standard** against the **Blank** at **505 nm**.

Calculation:

$$\textbf{Glucose (mg/dL)} = \frac{\text{(Abs of Test)}}{\text{(Abs of Std)}} \times \text{Concentration of Standard}$$

Normal Reference Range:

- **Fasting blood glucose:** 70 – 110 mg/dL
- **Postprandial (after meal):** <140 mg/dL

Experiment - 03

AIM: Glucose Tolerance Test (GTT)

Principle: The Glucose Tolerance Test (GTT) measures the body's response to a glucose load. After administering a known quantity of glucose orally, blood samples are collected at intervals to evaluate how effectively glucose is cleared from the blood.

Requirements:

Subject Preparation:

- Overnight fasting (8–12 hours)
- No smoking or heavy exercise before the test
- Avoid medications that affect glucose metabolism unless advised.

Reagents & Materials:

- Glucose solution (usually 75g in 300ml water for adults)
- Antiseptic swabs, tourniquet, disposable syringes/needles
- Fluoride oxalate tubes (for blood collection)
- Glucometer or biochemical analyser
- Timer or stopwatch

Procedure:

1. **Fasting Sample:**
    - Collect baseline (fasting) blood sample.
2. **Administer Glucose:**
    - Ask the patient to drink a glucose solution (typically 75g glucose in adults).
3. **Post-Glucose Blood Sampling:**
    - Collect blood samples at 30 minutes, 1 hour, 1.5 hours, and 2 hours post-glucose intake.
4. **Optional:**
    - Urine samples may also be collected simultaneously to check for glycosuria.
5. **Glucose Estimation:**
    - Blood glucose concentration is estimated using:
        - GOD-POD method
        - Glucometer
        - Autoanalyzer (for lab tests)

Interpretation (WHO Criteria for 75g OGTT in adults):

Condition	Fasting Glucose	2-hour Post Load Glucose
Normal	< 100 mg/dL	< 140 mg/dL
Impaired Glucose Tolerance (IGT)	100–125 mg/dL	140–199 mg/dL
Diabetes Mellitus	≥ 126 mg/dL	≥ 200 mg/dL

EXPERIMENT-4

Liver Function Test

AIM- Determination of Serum Bilirubin Test.

Introduction:

Bilirubin is a yellow pigment formed from the breakdown of haemoglobin. It exists in two forms:

- **Direct (Conjugated) Bilirubin**: Water-soluble, excreted in bile.
- **Indirect (Unconjugated) Bilirubin**: Fat-soluble, carried in the blood bound to albumin.

Elevated bilirubin levels may indicate liver dysfunction, haemolysis, or bile duct obstruction.

Principle (Malloy-Evelyn Method):

Bilirubin reacts with **diazotized sulfamic acid** to form a **coloured azobilirubin complex**:

- **Direct bilirubin** reacts in aqueous solution.
- **Indirect bilirubin** requires a solubilizing agent (e.g., methanol or caffeine-benzoate) to react.

The intensity of the colour developed is proportional to the bilirubin concentration and is measured spectrophotometrically at **540 nm**.

Reagents Required:

1. **Diazo reagent** (Sulfamic acid + Sodium nitrite)
2. **Accelerator** (Methanol or caffeine-benzoate solution)
3. **Standard bilirubin solution**
4. **Buffer solution (pH 7.4)**
5. **Distilled water**

Specimen:

- Fresh **serum** (no haemolysis).
- Protect sample from light to prevent degradation of bilirubin.

Procedure:

A. Total Bilirubin Estimation:

1. Pipette the following into test tubes:

Content	Test (T)	Blank (B)
Serum	0.2 mL	–
Distilled Water	–	0.2 mL
Accelerator Reagent	2.0 mL	2.0 mL
Diazo Reagent	0.5 mL	0.5 mL

2. Mix well and incubate at **room temperature for 30 minutes**.
3. Measure absorbance at **540 nm** against the blank.

B. Direct Bilirubin Estimation:

1. Follow the same procedure **without accelerator** reagent.
2. Add:
   - Serum: 0.2 mL
   - Water: 2.0 mL
   - Diazo reagent: 0.5 mL
3. Incubate for **5 minutes**, measure absorbance at **540 nm**.

Calculation:

Bilirubin (mg/dL) =Absorbance of test/Absorbance standardXConcentration of standard

Indirect (Unconjugated) Bilirubin = Total Bilirubin – Direct Bilirubin

Clinical Significance:

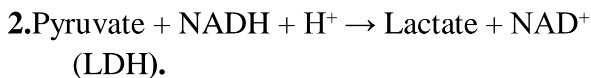
- **Elevated Total Bilirubin:** May indicate liver disease, haemolysis, or bile duct obstruction.
- **Elevated Direct Bilirubin:** Suggests obstructive jaundice or hepatocellular damage.
- **Elevated Indirect Bilirubin:** Indicates haemolysis or impaired bilirubin conjugation (e.g., Gilbert's syndrome).

Experiment-5

AIM- Principal and procedure of SGPT test

Principal: SGPT (ALT) is an enzyme found primarily in the liver. It catalyzes the transamination reaction between **L-alanine and α-ketoglutarate**, forming **pyruvate and glutamate**.

The **pyruvate** formed is then measured. In the commonly used **UV kinetic method**, pyruvate reacts with NADH in the presence of **lactate dehydrogenase (LDH)**:



2. Procedure of SGPT Test (UV Kinetic Method)

Reagents Required:

1. **Buffer reagent**: Contains Tris buffer, α-ketoglutarate, LDH
2. **Substrate reagent**: L-alanine
3. **Cofactor**: NADH
4. **Sample**: Serum

Steps:

1. Preparation of working reagent: Mix buffer, substrate, and NADH as per kit instructions.
2. Take 1.0 mL of working reagent in a test tube or cuvette.
3. Add 0.1 mL (100 µL) of serum sample.
4. Mix well and incubate at 37°C for 1 minute.
5. Measure the decrease in absorbance at 340 nm for 1–3 minutes at regular intervals using a spectrophotometer

3. Calculation:

The enzyme activity is calculated using the formula provided by the kit manufacturer, generally in **U/L**.

SGPT (U/L) =ΔA/min×Factor\text {SGPT (U/L)

Normal Reference Range:

- Adults: **7 to 56 U/L**
- May vary slightly with lab and method.

Experiment-6

AIM: Principal and procedure of SGOT test

Principal: SGOT (AST) is an enzyme found in high concentrations in the liver, heart, muscles, and kidneys. When these tissues are damaged, SGOT is released into the bloodstream.

The **biochemical principle** involves:

- **Enzymatic Reaction**:
  Aspartate + α-Ketoglutarate → (SGOT) → Oxaloacetate + Glutamate
- **Coupled Reaction** (for colorimetric detection):
  Oxaloacetate is reduced to malate-by-malate **dehydrogenase (MDH)** using **NADH**, converting

NADH to NAD^+. The decrease in absorbance at **340 nm** due to NADH oxidation is measured spectrophotometrically, and is proportional to the SGOT activity.

Reagents Required:

- Buffered substrate (containing L-aspartate and α-ketoglutarate)
- NADH solution
- MDH (malate dehydrogenase) enzyme
- Sample (serum)
- Distilled water

Equipment:

- Spectrophotometer
- Cuvettes
- Micropipettes
- Incubator or water bath at 37°C

Procedure:

1. Label 2 cuvettes: Test and Blank.
2. Pipette into each cuvette**:**

Reagent	Test	Blank
Buffered substrate	1000 µL	1000 µL
NADH + MDH reagent	250 µL	250 µL
Serum (sample)	100 µL	—
Distilled water	—	100 µL

3. Mix well and incubate at 37°C for 1 minute.
4. Measure the absorbance at 340 nm at 1-minute intervals for 3 minutes.
5. Calculate the change in absorbance per minute (ΔA/min).

Calculation:

SGOT Activity (U/L) =ΔA/min×F\text {SGOT Activity (U/L)}

Normal Range:

- **SGOT/AST**: 8 – 40 U/L (may vary by lab)

Clinical Significance:

Elevated SGOT levels seen in:

- Hepatitis
- Myocardial infarction
- Liver cirrhosis
- Muscle injury
- Homolysis

Experiment-7

AIM- Principal and procedure of Serum protein test

PRINCIPAL: The Biuret method is based on the reaction of proteins with copper ions in an alkaline medium. When proteins are present, the peptide bonds form a violet-coloured complex with cupric ions (Cu^{2+}). The intensity of the colour produced is directly proportional to the concentration of total protein in the serum.

Reaction:
Proteins + Cu^{2+} (in alkaline medium) → Violet-coloured complex.

Reagents Required:

1. Biuret Reagent (contains copper sulphate, sodium potassium tartrate, and sodium hydroxide)
2. Distilled water
3. Standard protein solution
4. Serum sample

PROCEDURE:

1. Label three test tubes as **Blank**, **Standard**, and **Test**.
2. Add 1.0 mL of Biuret reagent to each tube.
3. Add reagents as follows:
   - Blank: 20 µL distilled water
   - Standard: 20 µL standard protein solution
   - Test: 20 µL serum sample.
4. Mix well and incubate at room temperature for 15-30 minutes.
5. Measure absorbance at **540 nm** using a colorimeter or spectrophotometer.

Calculation:

Total Protein (g/dL) = (Absorbance of Standard/Absorbance of Test) ×Concentration of Standard (g/dL)

Normal Reference Range:

- Total Serum Protein**:** 6.0 – 8.0 g/dL

Experiment-8

AIM: Principal and procedure of Alkaline phosphatase test

Principal: Alkaline phosphatase (ALP) is an enzyme that catalyses the hydrolysis of phosphate esters at an **alkaline pH (around 10)**. In the test, ALP acts on a substrate such as **p-nitrophenyl phosphate (pNPP)** and converts it into **p-nitrophenol (pNP)** and **inorganic phosphate**.

- **p-Nitrophenyl phosphate (colourless) →** *(ALP)* **→ p-Nitrophenol (yellow in alkaline pH)**

The intensity of the **yellow colour** formed (due to p-nitrophenol) is directly proportional to the **ALP activity** and is measured **spectrophotometrically at 405 nm**.

Reagents Required:

- ALP reagent (containing pNPP substrate in an alkaline buffer)
- Calibrator or standard (optional)
- Distilled water (if dilution is required)

Equipment:

- Spectrophotometer or colorimeter
- Test tubes or cuvettes
- Water bath (if temperature control is needed)
- Micropipettes

Procedure:

Steps:

1. **Label** test tubes as Blank, Standard, and Test.
2. **Pipette the following** (example volumes):

Component	Blank	Standard	Test
ALP reagent	1.0 mL	1.0 mL	1.0 mL
Distilled Water	0.1 mL	-	-
Standard	-	0.1 mL	-
Serum sample	-	-	0.1 mL

3. **Mix well** and incubate all tubes at **37°C for 3–5 minutes**.
4. Measure the **absorbance at 405 nm** against the blank.
5. Calculate ALP activity using standard concentration or using the instrument's programmed method.

Normal Reference Range:

- Adults: **44 – 147 IU/L** (varies by lab)
- Higher in children and pregnant women (due to bone growth or placental activity)
- **Clinical Significance:**

- **Increased ALP**: Liver disease, bone disorders (e.g., rickets, osteocalcin), bile duct obstruction
- **Decreased ALP**: Malnutrition, hypothyroidism, certain genetic conditions.

Experiment-9.

Liver Function Test

AIM: Determination of Serum Urea Test

Principal: Urea in the serum is hydrolyzed by the enzyme **urease** to produce **ammonia (NH_3)** and **carbon dioxide (CO_2)**. The liberated ammonia then reacts with **hypochlorite** and **phenol** in the presence of **sodium nitroprusside** to form a **blue-colored complex** (Berthelot's reaction). The intensity of the color is directly proportional to the amount of urea present in the sample and is measured **spectrophotometrically**.

Reaction:

Urea + H_2O → (Urease) → $2NH_3$ + CO_2

NH_3 + Phenol + Hypochlorite → Blue-coloured indophenol (measured at 578–600 nm).

2. Requirements:

- **Reagents:**
  - Urease enzyme reagent
  - Phenol reagent
  - Hypochlorite reagent
  - Sodium nitroprusside
  - Standard urea solution
- **Apparatus:**
  - Colorimeter or spectrophotometer

- Test tubes
- Micropipettes
- Incubator or water bath

Procedure:

3. Procedure:

1. **Take 3 clean test tubes** and label them as:
   - **Blank**
   - **Standard**
   - **Test**
2. **Add the following:**

Reagent	Blank	Standard	Test
Distilled water	1.0 mL	–	–
Urea standard (20 mg/dL)	–	1.0 mL	–
Serum sample	–	–	1.0 mL
Urease reagent	1.0 mL	1.0 mL	1.0 mL

3. Mix well and **incubate at 37°C for 10 minutes**.
4. Add the **colour reagent** (Phenol + Hypochlorite + Nitroprusside) to each tube (e.g., 1 mL), mix, and **incubate again at 37°C for 20 minutes**.
5. Cool and read the absorbance at **578–600 nm** against the blank.

. Calculation:

Serum Urea (mg/dL) = (**Absorbance of Test/Absorbance of Standard) ×20**

5. Normal Range:

Serum Urea: 15–40 mg/dL (may vary slightly by laboratory).

Clinical Significance:

- ↑ **Increased levels (Uraemia):**
  - Kidney failure
  - Dehydration
  - Gastrointestinal bleeding
  - High protein diet
- ↓ **Decreased levels:**
  - Severe liver disease
  - Malnutrition
  - Overhydration

Experiment-10

AIM: Determination of Uric Acid Test

Principal: Uric acid is oxidized by the enzyme **uricase** to **allantoin** and **hydrogen peroxide (H_2O_2)**. The generated H_2O_2 reacts with a chromogen (usually 4-aminophenazone and TOOS or phenol) in the presence of **peroxidase** (POD), producing a colored complex. The intensity of the color formed is directly proportional to the uric acid concentration and is measured spectrophotometrically.

Chemical Reaction:

1. Uricase reaction:
   Uric acid + O_2 + H_2O ⟶ Allantoin + CO_2 + H_2O_2
2. Peroxidase reaction:
   H_2O_2 + Chromogen (e.g., TOOS + 4-AAP) ⟶ coloured quinonimine dye + H_2O

Materials Required:

- Serum sample
- Uric acid reagent kit (containing uricase, peroxidase, chromogens)
- Standard uric acid solution
- Test tubes or cuvettes
- Pipettes
- Colorimeter or spectrophotometer (usually at 520–546 nm).

Procedure:

Tube	Blank	Standard	Test
Reagent	1.0 mL	1.0 mL	1.0 mL
Standard	—	0.02 mL	—
Sample	—	—	0.02 mL

1. Mix well and incubate at 37°C for 10 minutes (or as per kit instructions).
2. Measure absorbance of **standard** and **test** against **blank** at 520–546 nm.

Calculation:

Uric Acid (mg/dL) =Absorbance of Test/Absorbance of Standard Concentration of Standard (mg/dL)

Normal Reference Range:

- **Men:** 3.4 – 7.0 mg/dL
- **Women:** 2.4 – 6.0 mg/dL

Clinical Significance:

- **Increased Uric Acid:** Gout, renal failure, leukaemia, polycythaemia, starvation, high protein diet.
- **Decreased Uric Acid:** Wilson's disease, Fanconi syndrome, SIADH.

Experiment-11

AIM: Determination of Serum Creatinine Test

Principal: The most commonly used method is the **Jaffe's reaction**. In this method, creatinine reacts with picric acid in an alkaline medium to form a reddish-orange coloured complex called creatinine-picrate. The intensity of this colour is directly proportional to the creatinine concentration and is measured spectrophotometrically.

Reaction: Creatinine + Picric acid (in alkaline medium) → Creatinine-picrate (reddish-orange complex)

Reagents Required:

1. Picric acid solution
2. Sodium hydroxide (NaOH) solution
3. Standard creatinine solution
4. Distilled water
5. Serum sample

Procedure:

Tube Type	Standard (S)	Test (T)	Blank (B)
Serum	-	0.5 ml	-
Standard	0.5 ml	-	-
Distilled water	-	-	0.5 ml
Picric acid	2.5 ml	2.5 ml	2.5 ml
NaOH	0.5 ml	0.5 ml	0.5 ml

1. Mix all contents well.

2. Incubate at room temperature for **20 minutes**.

3. Measure absorbance at **520 nm** against the blank.

Calculation:

Serum Creatinine (mg/dL) = (Abs. of T/Abs. of S) ×Concentration of Standard

Normal Values:

Gender	Normal Range
Male	0.7 – 1.3 mg/dL
Female	0.6 – 1.1 mg/dL

Clinical Significance:

- Elevated levels may indicate renal dysfunction, dehydration, or muscle disease.
- Decreased levels may be seen in muscle wasting or liver disease.

Experiment-12

AIM: Determination of Serum total cholesterol
Principle: Serum total cholesterol is commonly determined using the enzymatic CHOD-PAP method (Cholesterol Oxidase - Phenol Amino phenazone).

1. **Cholesterol esterase** hydrolyses cholesterol esters to free cholesterol and fatty acids.
2. **Cholesterol oxidase** then oxidizes the free cholesterol to cholest-4-en-3-one and hydrogen peroxide (H_2O_2).
3. **Peroxidase** uses the generated H_2O_2 to oxidize phenol and 4-aminoantipyrine to form a **red quinonimine dye**.
4. The intensity of this coloured compound is directly proportional to the total cholesterol and is measured **spectrophotometrically at 500 nm**.

Reagents Required:

- Enzyme reagent (containing cholesterol esterase, cholesterol oxidase, peroxidase, phenol, and 4-aminoantipyrine)
- Cholesterol standard solution
- Distilled water

Specimen:

- **Serum** obtained from blood after clotting and centrifugation.

Procedure:

Test Tube	Blank	Standard	Test
Reagent	1.0 mL	1.0 mL	1.0 mL
Distilled Water	0.01 mL	—	—
Cholesterol Standard	—	0.01 mL	—
Serum Sample	—	—	0 .01 mL

3. Mix well and incubate all tubes at **37°C for 10 minutes** or at **room temperature for 15 minutes**.

4. Measure absorbance of **Standard and Test** against the **Blank** at **500–520 nm** using a spectrophotometer.

Calculation:

Total Cholesterol (mg/dL) =(Abs of TestAbs of Standard)×Concentration of Standard

- If standard = 200 mg/dL:

Total Cholesterol (mg/dL) = (Abs T/Abs S) ×200

Reference Range:

- **Desirable:** < 200 mg/dL
- **Borderline High:** 200–239 mg/dL
- **High:** ≥ 240 mg/dL