JHARKHAND Rai University
RANCHI

Bachelor of Science in Medical Laboratory Technology (BMLT)

HAEMATOLOGY - II

Semester: Third (IIIrd)

PRACTICAL LABORATORY MANUAL

INDEX

EXPERIMENT- 1

Title of the Experiment: Blood Collection Techniques – Venipuncture and Capillary Collection.

Aim: To learn and perform proper techniques of blood collection through venipuncture and capillary methods using aseptic precautions for laboratory testing.

Principle

Accurate blood collection is essential for reliable laboratory results. Blood can be collected from veins (venipuncture) or capillaries (finger or heel prick). Proper technique ensures patient safety, prevents contamination, and avoids haemolysis or clotting of the sample.

Apparatus and Materials Required

- Tourniquet
- Sterile disposable syringe (2 ml, 5 ml) or vacutainer system
- Needles (18–23 gauge)
- Alcohol swabs (70% isopropyl alcohol)
- Sterile cotton/gauze
- Test tubes/ Vail (plain, EDTA, fluoride, citrate, etc.)
- Gloves
- Lancets (for capillary collection)
- Micropipettes (for capillary blood)
- Blood collection tray
- Labels and marker pens
- Biohazard waste container

Types of Blood Collection

1. Venous Blood Collection (Venipuncture)

- Most commonly used for routine lab tests.

2. Capillary Blood Collection

- Used in infants or when venipuncture is difficult (e.g., for glucose, bleeding time).

Procedure

A. Venipuncture

1. Greet and identify the patient.
2. Explain the procedure and get consent.
3. Wash hands and wear gloves.
4. Apply tourniquet 3–4 inches above the puncture site.
5. Palpate and select a vein (usually median cubital).
6. Clean the site with an alcohol swab in a circular motion.
7. Insert the needle at a 15–30° angle with bevel up.
8. Collect required blood in the appropriate tube.
9. Release the tourniquet **before** withdrawing the needle.
10. Withdraw the needle and press the site with sterile gauze.
11. Dispose of needle in sharps container.
12. Label the sample and record details.

B. Capillary Blood Collection

1. Warm the site if needed (fingertip or heel for infants).
2. Clean with alcohol swab and allow to dry.
3. Prick the site with a sterile lancet.
4. Wipe off the first drop; collect the subsequent drops.
5. Collect blood with capillary tube or micropipette.
6. Apply pressure with sterile cotton after collection.
7. Dispose of lancet in sharps container.

Anticoagulants and Tubes Used:

Tube Colour	**Additive**	**Use**
Purple/ Violet	EDTA	CBC, ESR
Blue	Sodium citrate	Coagulation tests
Grey	Sodium fluoride	Blood sugar
Green	Heparin	Blood gas analysis
Red/ Plain	No additive	Serology, Biochemistry

Precautions

- Always use sterile and disposable materials.
- Never reuse needles or lancets.
- Label samples immediately after collection.
- Avoid prolonged tourniquet application (>1 minute).

Normal Volumes Collected

- Venous blood: 2–5 ml (based on test requirements)
- Capillary blood: 20–100 µl

Clinical Significance

Proper blood collection is essential for:

- Accurate diagnosis
- Monitoring of therapy
- Health screening
- Research and transfusion purposes

EXPERIMENT- 2

Title of the Experiment: Preparation of Whole Blood Smear.

Aim: To prepare a peripheral (whole) blood smear from a fresh blood sample for morphological examination of blood cells under the microscope.

Principle: Peripheral blood smear is a vital haematological procedure used to study the morphology of red blood cells (RBCs), white blood cells (WBCs), and platelets. A properly prepared smear reveals diagnostic clues for various haematological disorders such as anaemia, leukaemia, malaria, and infections.

Materials Required:

Glassware & Consumables:

- Clean, grease-free glass slides
- Spreader slide (edge smoothened)
- Micropipette or dropper
- Staining rack

Reagents:

- Leishman stain
- Buffered distilled water
- Methanol (fixative, if using dried smear)

Equipment:

- Compound light microscope
- Timer

Sample: Fresh capillary blood from finger prick or EDTA anticoagulated venous blood.

Procedure:

A. Smear Preparation:

1. Clean and label the slide on the frosted end.
2. Place a small drop of blood near one end of the slide (not more than 1 cm from the edge).
3. Hold the spreader slide at a 30–45° angle to the slide.
4. Pull the spreader back to touch the drop and let the blood spread along its edge.
5. Push the spreader forward smoothly to spread the blood across the slide.
6. Allow the smear to air dry completely.

B. Staining Procedure (Leishman's Stain):

1. Place the air-dried smear on a staining rack.
2. Cover the smear with Leishman stain (without dilution) and allow to stand for 2 minutes to fix the smear.
3. Add double the volume of buffered distilled water and mix gently.
4. Allow the smear to stain for 7–10 minutes.
5. Wash gently with running distilled water.
6. Stand the slide vertically to air dry.

Microscopy:

- Start with 10x to assess smear quality.
- Use 100x oil immersion for cellular morphology:
  - RBC: shape, size, colour
  - WBC: type, count, nucleus
  - Platelets: number and clumping

Precautions:

- Use fresh blood for best results.
- Avoid dirty or oily slides.
- Do not blow or heat-dry smears.
- Standardize staining time and pH of water.
- Dispose of all sharps and blood-contaminated

Clinical Significance:

- Anaemia screening (common in Indian females and children)
- Malaria diagnosis (Plasmodium spp.)
- Leukaemia and blood cancers (increasing incidence in India)
- Public Health Programs: Used in NRHM and RBSK for disease screening

References:

1. National Institute of Biologicals (NIB), Noida – *Guidelines on Blood Smear Examination for Malaria & Blood Cell Morphology*
2. ICMR Manual of Laboratory Techniques (2021 Edition)
3. Ochei, J. and Kolhatkar, A. (2000). *Medical Laboratory Science Theory and Practice*. Tata McGraw-Hill Publishing.

EXPERIMENT- 3

Title of the Experiment: Morphology of Abnormal Red Blood Cells

Aim: To identify and interpret various abnormal red blood cell morphologies in peripheral blood smears under a microscope and correlate them with pathological conditions common in the Indian population.

Principle: Examination of RBC morphology on stained peripheral blood smears is an essential diagnostic tool in haematology. Abnormal RBC shapes, sizes, and staining patterns help detect anaemia, hemoglobinopathies, nutritional deficiencies, and systemic diseases, many of which are prevalent in India (e.g., iron deficiency anaemia, thalassemia, malaria).

Materials Required:
Equipment:
- Compound microscope (with 100x oil immersion objective)
- Slide staining rack
- Glass slides and cover slips

Reagents:
- Leishman stain or Giemsa stain
- Buffered distilled water (pH 6.8)
- Immersion oil

Samples:
- Previously prepared and stained peripheral blood smears.

Procedure:
1. Select a well-prepared and well-stained blood smear.
2. Focus using the 10x objective to locate the feathered edge.
3. Switch to 100x oil immersion for detailed examination of red cell morphology.

Observe for changes in:
- Size (anisocytosis)
- Shape (poikilocytosis)
- Colour/staining (hypochromia, polychromasia)
- Inclusions (basophilic stippling, Howell-Jolly bodies)

Types of Abnormal RBC Morphologies:

Morphology	Description	Associated Conditions
Microcytes	Small-sized RBCs	Iron deficiency anaemia
Macrocytes	Large-sized RBCs	Vitamin B12/Folic acid deficiency
Hypochromic cells	Increased central pallor	Iron deficiency anaemia
Spherocytes	Spherical, no central pallor	Hereditary spherocytosis, autoimmune haemolysis
Elliptocytes	Oval/elongated cells	Hereditary elliptocytosis
Target cells	Bull's eye appearance	Thalassemia, liver disease
Schistocytes	Fragmented RBCs	DIC, mechanical haemolysis

Tear drop cells	Teardrop shaped	Myelofibrosis, marrow infiltration
Sickle cells	Crescent shaped	Sickle cell anemia (rare in India but present in tribal areas)
Basophilic stippling	Fine blue granules in RBC cytoplasm	Lead poisoning, thalassemia
Howell-Jolly bodies	Round DNA remnants	Post-splenectomy, megaloblastic anaemia
Rouleaux formation	Stacked coin appearance	Multiple myeloma, infections
Polychromasia	Greyish-blue immature RBCs	Reticulocytosis

Clinical Significance:

- Iron Deficiency Anaemia: Most common anaemia in India, especially among women and children.
- Megaloblastic Anaemia: Due to poor diet and malabsorption in rural areas.
- Thalassemia & Hemoglobinopathies: Highly prevalent in Gujarat, Maharashtra, Bengal, Punjab, and tribal belts.
- Malaria: Common in endemic zones; RBC morphology may show haemolysis.

Precautions:

- Use freshly prepared and properly stained blood smears.
- Avoid using dirty or scratched slides.
- Examine only in monolayer area of the smear.
- Use oil immersion for accurate RBC morphology.

References:

1. Indian Council of Medical Research (ICMR) – *Guidelines on Haematology Techniques*, 2021.
2. Ochei and Kolhatkar (2000). *Medical Laboratory Science: Theory and Practice*. Tata McGraw-Hill.
3. Dacie, J.V. and Lewis, S.M. (2016). *Practical Haematology*.

EXPERIMENT- 4

Title of the Experiment: Determination of MCH, MCV, and MCHC (Red Cell Indices)

Aim: To determine the red cell indices:
- **MCV** – Mean Corpuscular Volume
- **MCH** – Mean Corpuscular Hemoglobin
- **MCHC** – Mean Corpuscular Hemoglobin Concentration

These indices are used to classify anaemia and assess red blood cell (RBC) characteristics.

Principle: Red cell indices are calculated parameters derived from:
- Hemoglobin concentration (Hb)
- Packed cell volume (PCV or haematocrit)
- RBC count

These indices help classify anaemia as microcytic, macrocytic, hypochromic, or normocytic-normochromic, which is vital in diagnosing common haematological conditions in India.

Materials Required:
- Haematology analyzer or manual method tools (Wintrobe tube, haemocytometer)
- EDTA-anticoagulated whole blood sample
- Calculator or software (if using manual method)
- Personal Protective Equipment (gloves, lab coat, mask)

Normal Reference Values:

Parameter	Normal Range (Adult Male)	Normal Range (Adult Female)
MCV	80 – 100 fL	80 – 100 fL
MCH	27 – 33 pg	27 – 32 pg
MCHC	32 – 36 g/dL	32 – 36 g/dL

Formulae:
1. **MCV (fL)** = (PCV × 10) / RBC count
2. **MCH (pg)** = (Hb × 10) / RBC count
3. **MCHC (g/dL)** = (Hb × 100) / PCV

Procedure (Manual Calculation Method):
A. Perform the following tests:
1. Estimate **Hemoglobin** using Sahli's method or Hemoglobinometer.
2. Determine **PCV** using Wintrobe's or Microhematocrit method.
3. Perform **Total RBC count** using Haemocytometer.

B. Insert values in the formulas:

- Example:
  - Hb = 12 g/dL
  - PCV = 36%
  - RBC = 4.5 million/cmm

- **MCV** = (36 × 10) / 4.5 = 80 fL
- **MCH** = (12 × 10) / 4.5 = 26.7 pg
- **MCHC** = (12 × 100) / 36 = 33.3 g/dL

Interpretation:

Index	Low Value Suggests	High Value Suggests
MCV ↓	Microcytic anaemia (e.g., Iron deficiency – common in India)	Macrocytic anaemia (e.g., B12/Folate deficiency)
MCH ↓	Hypochromic RBCs (Iron deficiency anaemia)	-
MCHC ↓	Hypochromia	Hereditary spherocytosis (↑ MCHC)

Precautions:

- Use fresh, properly mixed EDTA blood.
- Avoid clotted or haemolyzed samples.

EXPERIMENT- 5

Title of the Experiment: Determination of Absolute Eosinophil Count (AEC)

Aim: To determine the **Absolute Eosinophil Count** in a patient's blood sample using a manual or automated method. AEC is important in diagnosing allergic reactions, parasitic infestations, and certain haematological disorders.

Principle: Eosinophils are a type of granulocytic white blood cell involved in allergic and parasitic responses.
Absolute eosinophil count = **Total WBC count × % of eosinophils / 100**

In the **manual method**, a **diluting fluid** stains and lyses other cells, allowing eosinophils to be counted in a **Neubauer chamber**.

In the **automated method**, modern haematology analyzers directly report AEC based on flow cytometry or impedance.

Clinical Significance:

Condition	**AEC Findings**
Parasitic infections (e.g., filariasis, ascariasis)	Elevated AEC (>500 cells/µL)
Bronchial asthma, allergies	Moderate increase
Hyper eosinophilic syndrome	Markedly elevated (>1500/µL)
Drug-induced reactions	Mild to moderate increase
Autoimmune diseases	Variable

Materials Required (Manual Method):
Equipment:
- Microscope
- Neubauer counting chamber
- RBC pipette (or WBC pipette)
- Test tubes, pipettes

Reagents:
- **Dunger's solution** or **Pilot's solution**
- **EDTA blood sample**

Procedure (Manual Method):
1. Take **0.5 mL of Dunger's fluid** in a test tube.
2. Add **20 µL of well-mixed EDTA blood** to the fluid.
3. Mix gently and allow to stand for **5–10 minutes** for staining.
4. Charge a **Neubauer chamber** with the stained diluted blood.
5. Allow cells to settle for **2–3 minutes**.
6. Count eosinophils in **all 9 large squares** under **10x or 40x** magnification.

Calculation:
AEC (cells/µL) =
Number of eosinophils counted × dilution factor / volume of chamber

- Neubauer chamber volume = 0.1 µL
- If dilution = 1:20
- AEC = (Number of cells × 20) / 0.1 = Number of cells × 200

Example:
If you count 15 eosinophils → AEC = 15 × 200 = **300 cells/µL**

Normal Reference Range:

Age Group	AEC Normal Range (cells/µL)
Adults	40 – 440 /µL
Children	50 – 500 /µL

Precautions:

- Use **fresh, anticoagulated blood** (within 2–3 hours of collection)
- Avoid air bubbles while loading the chamber
- Let cells settle before counting
- Count eosinophils only (distinguished by **bi-lobed nucleus and red-orange granules**)
- Clean and dry Neubauer chamber after use

Interpretation Table:

AEC Level (cells/µL)	Interpretation
<40	Eosinopenia (rare; not usually clinically significant)
40–440	Normal
500–1500	Mild to moderate eosinophilia
>1500	Hypereosinophilic syndrome

References:

1. **ICMR Manual of Laboratory Techniques**, 2021
2. Ochei & Kolhatkar (2000). *Medical Laboratory Science: Theory and Practice*. Tata McGraw-Hill

EXPERIMENT- 6

Title of the Experiment: Reticulocyte Count (Manual Method)

Aim: To determine the **percentage of reticulocytes** (immature red blood cells) in a peripheral blood smear using supravital staining.

Principle: Reticulocytes are young, a nucleated red blood cells that contain residual ribonucleic acid (RNA). When stained with a **supravital stain** such as **New Methylene Blue (NMB)**, this RNA precipitates into a **reticular (mesh-like) network**, distinguishing reticulocytes from mature red cells. The **reticulocyte percentage** indicates **bone marrow activity** and **erythropoietic response** to anaemia.

Clinical Significance (India Context):

Condition	Reticulocyte Response
Iron deficiency anaemia	↓ or normal
Vitamin B12/Folic acid deficiency	↓
Haemolytic anaemia (e.g., malaria, thalassemia)	↑↑
Blood loss (surgery, trauma)	↑ after 3–5 days
Response to iron/B12 therapy	↑ within 7 days

Materials Required:
Reagents:
- 0.5% **New Methylene Blue (NMB)** (freshly prepared)
- EDTA anticoagulated blood (fresh, <4 hrs old)
- Distilled water (if needed)

Glassware and Equipment:
- Microscope (100x oil immersion)
- Glass slides and cover slips
- Capillary/micropipette
- Pasteur pipettes/test tubes
- Timer or stopwatch
- Personal protective equipment (PPE)

Procedure:
A. Sample Preparation:
1. **Mix equal volumes** of:
   - EDTA blood (e.g., 3 drops)
   - 0.5% New Methylene Blue stain (e.g., 3 drops)

2. Incubate the mixture at **room temperature for 15–20 minutes**.
3. Remix gently and prepare **two thin blood smears**.
4. **Air-dry** the smears completely (do not fix or counterstain).
5. Observe under **oil immersion (100x)** microscope.

Microscopic Examination:

- **Reticulocytes** appear as slightly larger RBCs with **bluish filaments or granules** (reticular network).
- **Mature RBCs** lack any internal network.

Counting and Calculation:

Relative Reticulocyte Count (%)
Count **at least 1000 RBCs** and identify how many are reticulocytes.

Formula:
Reticulocyte % = (Number of reticulocytes / Total RBCs counted) × 100

Example:
If 20 reticulocytes are seen in 1000 RBCs: Retic % = (20 / 1000) × 100 = **2.0%**

Absolute Reticulocyte Count (ARC)
Formula:
ARC = Retic % × RBC count (millions/μL) × 10
If Retic % = 2.0%, and RBC count = 4.5 million/μL: ARC = 2 × 4.5 × 10 = **90,000 cells/μL**

Normal Reference Values:

Group	Reticulocyte % (Normal)
Adults	0.5 – 2.5%
Newborns	2.5 – 6.0%

Interpretation:

Retic %	Clinical Interpretation
<0.5%	Aplastic anaemia, marrow suppression
0.5–2.5%	Normal range
>2.5%	Haemolytic anaemia, active erythropoiesis

Precautions:

- Use **freshly prepared stain** and **fresh blood (<4 hours)**.
- Avoid thick smears; use **monolayer** for counting.
- Do not heat-fix the smear.
- Identify true reticulocytes.

References:

1. **ICMR Manual of Laboratory Techniques** (2021), Indian Council of Medical Research
2. **AIIMS Laboratory Services Manual**, New Delhi
3. Ochei, J. & Kolhatkar, A. (2000). *Medical Laboratory Science: Theory and Practice*, Tata McGraw-Hill.

EXPERIMENT- 7

Title of the Experiment: Determination of Sickle Cell Smear

Aim: To prepare and examine a peripheral blood smear to identify sickle-shaped red blood cells indicative of **sickle cell anaemia or sickle cell trait**.

Principle: Sickle cell disease is a genetic disorder caused by the presence of **Hemoglobin S (Hb-S)**. Under hypoxic conditions, red blood cells become distorted into a sickle or crescent shape. A stained blood smear can reveal these characteristic sickle cells under a microscope. The sickling test may be supplemented with a sodium metabisulfite solution to induce deoxygenation and enhance detection of sickled cells.

Requirements:
- Sterile lancet or needle
- Clean glass slides
- Micropipette or capillary tube
- 70% isopropyl alcohol or spirit
- Giemsa or Leishman's stain
- Sodium metabisulfite solution (2%) *(optional for sickling test)*
- Microscope (light microscope, 100 x oil immersion)
- Immersion oil
- Cotton swabs
- Gloves and lab coat
- Slide box and marker

Specimen: Fresh capillary or EDTA-anticoagulated venous blood.

Procedure:

A. Preparation of Blood Smear:
1. **Clean the fingertip** of the subject with spirit and allow it to dry.
2. **Prick** the fingertip using a sterile lancet.
3. Wipe off the first drop of blood.
4. Place a **small drop** of blood near one end of a clean glass slide.
5. Using another slide as a spreader, hold it at a 30–45° angle and swiftly spread the blood to form a thin smear.
6. Allow the smear to **air dry**.

B. Fixation and Staining (Giemsa/Leishman):
1. Fix the dried smear by covering it with **methanol** for 2 minutes (if using Giemsa).
2. Air-dry again.
3. Flood the smear with **Leishman's stain** (undiluted) for 2 minutes, then add **double volume of buffered water** and allow to stand for 10 minutes.
4. Rinse gently with water and let it dry completely.

C. Sickling Test:
1. Mix a drop of blood with a drop of **2% sodium metabisulfite** on a slide.
2. Cover with a cover slip and seal the edges with petroleum jelly or wax.
3. Leave the slide in a **hypoxic condition** (room air, covered with a petri dish) for 30–60 minutes.
4. Examine under microscope for **sickled cells**.

Observation:
Examine the stained smear under **100x oil immersion** objective:

- **Normal RBCs:** Round, biconcave, with central pallor.
- **Sickled RBCs:** Elongated, crescent or "sickle" shaped cells with pointed ends.
- May also observe target cells, anisopoikilocytosis in severe cases.

Precautions:

- Use **fresh blood**; avoid haemolyzed or clotted samples.
- Prepare smear immediately to avoid artifacts.
- Handle all blood samples as **potentially infectious**.
- Ensure slides are **properly cleaned** for accurate results.
- Use **adequate staining time** for clear morphology.
- Avoid overheating during drying or staining.

Clinical Relevance:
This test is used as a preliminary diagnostic method for:

- **Sickle Cell Anaemia (HbS-S)**
- **Sickle Cell Trait (HbA-S)**
  It is particularly important in areas with a **high prevalence**, such as parts of Central and Eastern India.

References (Indian Sources):

1. **DC Dey.** *Textbook of Haematology*. New Central Book Agency.
2. **Ramnik Sood.** *Medical Laboratory Technology – Methods and Interpretations*. Jaypee Brothers.
3. National Health Mission – Screening Guidelines for Sickle Cell Disease in India.
4. ICMR Guidelines for Hemoglobinopathies (2021).

EXPERIMENT- 8

Title of the Experiment: Determination and Examination of Bone Marrow Smear

Aim: To prepare and stain a bone marrow smear for cytological examination in order to assess haematopoiesis and identify abnormal cells.

Principle: Bone marrow examination is a critical diagnostic tool used to study the proliferation and maturation of blood cell lines. The aspirated marrow is smeared on a slide and stained (typically with Leishman or Giemsa stain) for morphological evaluation. The procedure helps in diagnosing haematological disorders such as anaemia, leukaemia, multiple myeloma, and bone marrow infiltration.

Requirements:
- Sterile bone marrow aspiration needle (e.g., Salah or Klima needle)
- Syringe (10–20 mL)
- Antiseptic solution (povidone-iodine/spirit)
- Sterile gauze and cotton
- Local anaesthetic (e.g., 2% lignocaine)
- Clean glass slides (pre-labelled)
- Leishman or Giemsa stain
- Methanol (for fixation)
- Microscope (100x oil immersion)
- Immersion oil
- Gloves, mask, and sterile drapes
- Bone marrow aspiration tray
- Slide box and marker

Specimen: Fresh bone marrow aspirate (commonly from **posterior superior iliac spine** in adults or **sternum** in some cases).

Site of Aspiration:
- Adults: Posterior iliac crest (most common), sternum, anterior iliac crest
- Infants: Tibia (anteromedial surface)

Procedure:
A. Bone Marrow Aspiration:
(Performed by a trained clinician or pathologist under aseptic conditions)
1. Explain the procedure to the patient and obtain informed consent.
2. Position the patient (lateral or prone position for iliac crest aspiration).
3. Clean the skin with antiseptic and drape the area.
4. Administer local anaesthesia at the aspiration site.
5. Insert the sterile aspiration needle into the bone with a screwing motion until it enters the marrow cavity.
6. Attach a syringe and aspirate 0.2 to 0.5 mL of marrow.
7. Transfer aspirate immediately onto clean slides.

B. Preparation of Smears:

1. Place a drop of marrow aspirate near one end of the slide.
2. Use another slide as a spreader to make a **thin smear** using the wedge method.
3. Prepare **at least 5–6 slides** to ensure good cellularity.
4. Air-dry the smears quickly.

C. Staining (Leishman or Giemsa):

1. Fix the smear with **methanol** for 2 minutes.
2. Flood with **Leishman's stain** (undiluted) for 2 minutes.
3. Add **twice the volume of buffered water** and leave for 8–10 minutes.
4. Wash gently with running water.
5. Air-dry and examine under a microscope.

Observation:

Examine the stained smear under **oil immersion (100x)**:

- **Erythroid series**: proerythroblasts to normoblasts
- **Myeloid series**: myeloblasts to neutrophils
- **Megakaryocytes**: large cells with multilobulated nuclei
- **Lymphocytes, plasma cells, macrophages**
- **Abnormal cells** in leukaemia or metastatic disease

Precautions:

- Use **sterile techniques** throughout the procedure.
- Avoid air bubbles or blood dilution in smear.
- Smears must be **made immediately** to preserve morphology.
- Handle aspirate gently to avoid **cell lysis**.
- Use **proper fixation and staining times**.
- Dispose of sharps and biohazard waste safely.

Clinical Applications:

- Diagnosis and classification of **anaemia**, **leukaemia**, **lymphoma**
- Assessment of **bone marrow metastasis**
- Evaluation of **unexplained cytopenia**
- Monitoring **chemotherapy response**

References (Indian Context):

1. **Ramnik Sood.** *Medical Laboratory Technology – Methods and Interpretations*, Jaypee Brothers.
2. **DC Dey.** *Textbook of Haematology*, New Central Book Agency.
3. Indian Council of Medical Research (ICMR) – Haematology Guidelines
4. **Saxena R.** *Practical Haematology*, CBS Publishers & Distributors.

EXPERIMENT- 9

Title of the Experiment: Determination of Bleeding Time (BT)

Aim: To determine the **bleeding time**, which is the duration taken for bleeding to stop from a standardized puncture wound, indicating **platelet function and capillary integrity**.

Principle: Bleeding time measures **the time interval between skin puncture and cessation of bleeding**. It reflects the **functionality of platelets**, vascular integrity, and primary haemostasis. It is prolonged in conditions like **thrombocytopenia**, **platelet dysfunction**, and **vascular disorders**.

Two common methods:

- **Duke's Method** *(earlobe or fingertip puncture)*
- **Ivy's Method** *(more standardized, forearm incision with BP cuff)*

Requirements:

- Sterile lancet or disposable BT lancet
- Filter paper or blotting paper
- Stopwatch or timer
- 70% isopropyl alcohol
- Spirit swabs
- Cotton and adhesive bandage
- Gloves
- Blood pressure apparatus *(for Ivy's method)*
- Ruler *(for Ivy's method)*

Specimen:
Capillary blood from the fingertip or earlobe (Duke) or forearm (Ivy).

Procedure:

A. Duke's Method (Simpler, bedside test):

1. Clean the fingertip or earlobe with alcohol and allow it to dry.
2. Puncture the site with a sterile lancet to a depth of 3–4 mm.
3. Start the stopwatch immediately.
4. Blot the drop of blood every 30 seconds using filter paper, **without touching the wound**.
5. Note the time when **bleeding completely stops**.
6. Record the bleeding time in minutes and seconds.

B. Ivy's Method (More standardized and accurate):

1. Apply a **blood pressure cuff** to the upper arm and inflate it to **40 mm Hg**.
2. Clean the **volar aspect of the forearm** with spirit.
3. Make 2–3 small standardized incisions (5 mm long, 1 mm deep) using a BT device or lancet.
4. Start the stopwatch.
5. Every 30 seconds, blot the blood with filter paper near the wound (not directly on it).
6. Note the time when **bleeding stops completely**.
7. Remove the cuff, clean, and apply an adhesive dressing.

Normal Values:

Method	Normal Range
Duke's Method	1–3 minutes
Ivy's Method	2–7 minutes

Clinical Significance:

- **Prolonged Bleeding Time** is seen in:
  - Thrombocytopenia
  - Platelet function disorders (e.g., Glanzmann thrombasthenia)
  - Uraemia
  - Certain medications (e.g., aspirin)

- **Normal Bleeding Time**:
  - Rules out primary haemostatic defects (if platelet count is normal).

Precautions:

- Do not puncture too deeply; avoid profuse bleeding.
- Do not squeeze or press the site.
- Maintain **aseptic conditions**.
- Always **blot gently** at intervals of 30 seconds.
- Dispose of lancet and used paper properly (biohazard protocol).

Limitations:

- Subjective and operator-dependent.
- Affected by skin thickness, room temperature, puncture depth.
- Less commonly used now, replaced by **PFA-100** and **platelet aggregation tests** in modern labs.

References:

1. **Ramnik Sood.** *Medical Laboratory Technology – Methods and Interpretations*, Jaypee Brothers Medical Publishers.
2. **DC Dey.** *Textbook of Haematology*, New Central Book Agency.
3. **Satyanarayan & Chakrapani.** *Essentials of Biochemistry for Medical Students*, Elsevier.

EXPERIMENT- 10

Title of the Experiment: Determination of Clotting Time (CT)

Aim: To determine the **clotting time of blood**, i.e., the time taken for **capillary blood to clot** outside the body under controlled conditions. It reflects the efficiency of the **intrinsic coagulation pathway**.

Principle: Clotting time is the time required for **whole blood to form a clot** when placed in a capillary or test tube at body temperature. It assesses the functioning of coagulation factors, especially **factors I, II, V, VIII, IX, X, XI, and XII**.

Two common methods:
- **Capillary Tube Method (Wright's method)**
- **Test Tube Method (Lee and White method)**

Requirements:

A. Capillary Tube Method:
- Sterile lancet
- Capillary glass tubes (75 mm length)
- Stopwatch or timer
- Spirit swabs (70% isopropyl alcohol)
- Gloves and disposal container
- Water bath (optional for 37°C maintenance)
- Glass marking pencil

B. Test Tube Method:
- Sterile test tubes
- 10 mL syringe and needle
- Spirit swabs
- Stopwatch
- Water bath at 37°C
- Gloves

Specimen:
- **Capillary blood** (for capillary tube method)
- **Venous blood** (for test tube method)

Procedure:

A. Capillary Tube Method (Wright's Method):
1. Clean the fingertip with spirit and allow it to dry.
2. Prick with a sterile lancet and wipe away the first drop of blood.
3. Fill 2–3 clean capillary tubes with blood by capillary action.
4. Start the stopwatch immediately.
5. Break small segments of the capillary tube every **30 seconds**.
6. Observe for the **formation of a fibrin thread** between the broken ends.
7. Note the time when the **first thread appears** – this is the clotting time.

B. Test Tube Method (Lee and White Method):

1. Collect 1–2 mL of **venous blood** using sterile precautions.
2. Immediately transfer into **two clean, dry test tubes**.
3. Place the tubes in a **37°C water bath**.
4. Start the stopwatch.
5. Tilt the tubes gently every **30 seconds** until a **clot forms**.
6. Note the time at which **clotting occurs** – when blood stops flowing as you tilt.

Normal Values:

Method	**Normal Range**
Capillary Tube Method	3–6 minutes
Test Tube Method	5–11 minutes

Clinical Significance:

- **Prolonged Clotting Time** may indicate:

➢ Haemophilia A (Factor VIII deficiency)
➢ Haemophilia B (Factor IX deficiency)
➢ Severe liver disease (coagulation factors synthesized in liver)
➢ Vitamin K deficiency
➢ Use of anticoagulants (e.g., heparin)

Precautions:

- Capillary tubes and test tubes must be **dry and clean**, as moisture inhibits clotting.
- Avoid contamination with tissue fluid or antiseptics.
- **Do not squeeze the finger excessively** to fill capillary tubes.
- Perform the test at or near **body temperature (37°C)** for accuracy.
- **Start timing immediately** after collecting blood.

References:

1. **Ramnik Sood.** *Medical Laboratory Technology – Methods and Interpretations*, Jaypee Brothers.
2. **DC Dey.** *Textbook of Hematology*, New Central Book Agency.
3. **Satyanarayan & Chakrapani.** *Essentials of Biochemistry for Medical Students*, Elsevier India.