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Biochemistry Laboratory Practical Manual

for Phase-I MBBS Students



As per the Competency-Based Medical Education Curriculum (NMC)

Name of the Candidate: _____

Name of the Institution: _____

_____

Roll No.: _____

Academic Year: _____ **Batch:** _____

Pankaja Naik

2nd Edition

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DOAP: Demonstrate, Observe, Assess, and Perform; SGD: Small Group Discussion

EXPERIMENT

4

Sample Collection

COMPETENCY		LEARNING OBJECTIVES
BI11.1	Describe commonly used laboratory apparatus and equipment, good safe laboratory practice and waste disposal.	1. Describe different types of biological samples. 2. Describe methods of collection of blood sample. 3. Describe color-coded vacuum evacuated tubes, their additives and uses in laboratory. 4. Describe types of urine samples and their collection.

INTRODUCTION

Proper specimen collection and handling is an integral part of obtaining a valid and timely laboratory test result. Specimens must be obtained in the proper tubes or containers, correctly labeled, and then promptly transported to the laboratory.

TYPES OF BIOLOGICAL SAMPLES

Types of biological specimens that are analysed in clinical laboratories include:

- Whole blood
- Serum
- Plasma
- Urine
- Feces
- Various fluids, such as: pleural fluid, ascitic fluid, cerebrospinal fluid (CSF), synovial fluid, pericardial fluid, and amniotic fluid.
  - *Plasma:* Obtained by centrifugation of anticoagulant added blood and it contain all coagulation factors except calcium ions.
  - *Serum:* Obtained when blood undergoes clotting and it does not contain clotting factors.

Among the above, blood and urine are the most common samples analyzed in the laboratory.

METHODS OF BLOOD SAMPLE COLLECTION

Blood samples intended for laboratory analysis are usually collected in glass or plastic tubes that have been partially evacuated so that their internal air pressure is lower than atmospheric pressure. These tubes have color-coded polymer stoppers that indicate their contents.

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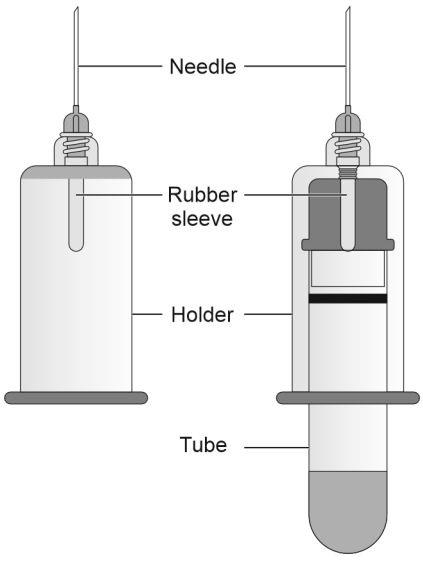

Figure 4.1: Vacuum evacuated tube system.



Figure 4.2: Different types of vacuum evacuated tube.

Blood for analysis is collected from **veins, arteries** or **capillaries**. Most of the tests are performed on venous blood, the process of collection of venous blood is called **venipuncture** or **phlebotomy** (from Greek words; **phleb**, which means vein, and **tome**, to cut or incise). Venipuncture can be performed by three basic methods.

1. **Vacuum evacuated tube system:** This system contains a needle, holder, and vacuum tube **(Figure 4.1).** Vacuum evacuated tubes are plastic tubes with color-coded tops indicating tube contents **(Figure 4.2).** Most tubes contain an additive that either accelerates clotting of the blood (clot activator) or prevents the blood from clotting (anticoagulant). They are used to collect blood for various investigation.
2. **Needle and syringe**: Blood collected in a syringe can be transferred to an evacuated tube.
3. **Winged infusion set**: Winged infusion sets are used when blood is collected from a very small vein, e.g., pediatric patient.

ETS is the preferred method. In this method, blood is collected directly from the vein into a tube, minimizing the risk of specimen contamination.

Color-coded Vacuum Evacuated Tubes, their Additives and Uses in Laboratory

Commonly used vacuum evacuated tubes in clinical chemistry lab are **(Table 4.1):**

- **Green top tube:** This less commonly used tube is for biochemistry tests which require heparinized plasma or whole blood for analysis.
  - *Additive:* Sodium or lithium heparin. Inhibits thrombin formation to prevent clotting
  - *Laboratory uses:* ABG analysis and osmotic fragility test.
- **Gray top tube:**
  - *Additive:* Potassium oxalate and sodium fluoride. Potassium oxalate acts as an anticoagulant and sodium fluoride acts as an antiglycolytic agent that inhibits enolase enzyme of glycolysis to ensure that no further glucose breakdown occurs within the sample after it is taken. Potassium oxalate removes calcium and acts as an anticoagulant.
  - *Laboratory uses:* Estimation of blood glucose
- **Light blue top tube**
  - *Additive:* Anticoagulant; sodium citrate. Binds and remove calcium to prevent blood from clotting
  - *Laboratory uses:* Hematology tests involving coagulation studies which include PT (Prothrombin time), TT (thrombin time) and factor assays.

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- **Purple top tube:**
  - *Additive:* Anticoagulant; EDTA (Ethylenediaminetetraacetic acid). It removes calcium preventing clotting of blood
  - *Laboratory uses:* Generally used for hematology tests where whole blood is required for analysis like complete blood count, blood film for abnormal cells or malaria parasites, total leucocyte count, differential leucocyte count, platelets, Hb electrophoresis, erythrocyte sedimentation rate, HbA1C, blood bank testing (cross match, blood grouping).
- **Red top tube:**
  - *Additive:* None or contains silica particles which act as clot activators. Clot activator promotes blood clotting.
  - *Laboratory uses:* Used for biochemistry tests requiring serum, blood bank, and serology/Immunology (Hepatitis A, B, C, rheumatoid factor, HIV, viral antibodies)
- **Royal blue top tube:**
  - *Additive:* Sodium heparin and sodium EDTA. Inhibits thrombin formation to prevent clotting.
  - *Laboratory uses:* Trace elements, such as zinc, copper, lead and mercury.
- **Yellow top tube:**
  - *Additive:* anticoagulant SPS (Sodium polyanethol sulfonate) and ACD (acid citrate dextrose). Prevents the blood from clotting.
  - *Laboratory uses:* Blood and body fluid cultures (HLA, DNA, paternity).

Order of Draw

The order of draw is a special sequence of tube collection that reduces the risk of specimen contamination by microorganisms (e.g., blood cultures) and additive carry over, which affects the test results. Recommended order of draw is as given below:

- Sterile tube (e.g., blood culture)
- Coagulation tube (Blue top)
- Serum tube with/without clot activator (Red top, yellow top)
- Heparin tube (Green top)
- EDTA tube (Purple top)
- Glycolytic inhibitor tube (Gray top)

Table 4.1: Commonly used color-coded vacuum tubes, their additives and uses in laboratory.

Tube cap color	*Additive*	*Use*
Green	Anticoagulant (Lithium heparin/sodium heparin)	ABG analysis Osmotic fragility test
Grey	Potassium oxalate and sodium fluoride	Blood glucose
Light blue	Sodium citrate	**Hematology tests** involving coagulation studies which include PT (Prothrombin time), TT (Thrombin time) and factor assays
Purple	Anticoagulant; EDTA (Ethylenediaminetetra-acetic acid)	**Biochemistry**: HbA1C **Hematology**: Complete blood count, blood film for abnormal cells or malaria parasites, total leukocyte count, differential leucocyte count, platelets, Hb electrophoresis, erythrocyte sedimentation rate (ESR) **Blood bank**: Cross match, blood grouping
Red	None/ or clot activator (silica particles). Clot activator promotes blood clotting	**Biochemistry** tests requiring serum**, blood bank**, and **serology**
Royal blue	Anticoagulant; Sodium heparin and sodium EDTA	Trace elements such as zinc, copper, lead and mercury
Yellow	Anticoagulant (SPS, sodium polyanethole sulfonate ACD: acid citrate dextrose)	Blood and body fluid cultures (HLA, DNA and paternity testing)

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URINE SAMPLE COLLECTION

Urine is the second most common sample received in the biochemistry laboratory after the blood samples.

Types of Urine Samples and their Collection

Random Sample

- This is collected at any time of during the day. Usually used only for routine screening.
- For sample collection, the patient is given a non-sterile collection container **(Figure 4.3)** and instructed to collect a mid-stream specimen in the container. This type of specimen is routinely used for urinalysis and may not be used for a culture and sensitivity.



Figure 4.3: Universal urine container.

First Morning Sample/ First Voided Sample

- This is the first sample when the patient gets up in the morning. A first voided specimen is the most concentrated and is the preferred specimen for **microscopic examinations**. This is also the sample choice for the urine constituents to detect protein, HCG, and other substances.
- For sample collection, the patient is given a urine container to take home. Because urine is not stable, the specimen should be returned to the laboratory within one hour of collection. If that is not possible, the specimen should be refrigerated until it can be tested.

Timed Specimens

- These samples are collected at specific intervals during the day, such as 1, 4 or 24 hours. Twenty-four hour urine specimens are required for creatinine clearance tests and many other hormone studies.
- For sample collection, the patient is given a large container (1000–5000 mL capacity) containing preservative **(Figure 4.4)** that is labeled with the patient's name and date. Patient is advised to discard first morning sample on the day of collection and to collect all subsequent urine voided till next morning, including first voided sample. The 24-hour urine specimen is brought to the laboratory as soon as possible as the 24-hour period is over.



Figure 4.4: 24-hour urine collection container.

Clean-Catch Midstream Specimen

- In this case, the patient is instructed to clean the genitalia with water and soap. Then the midstream sample is collected in a universal sterile container.
- These are used for the bacteriological culture. Caution should be taken that the container should not touch the genitalia. Bring the specimen to the lab within 1 hour of collection or store refrigerated for up to 24 hours.

Catheterized Specimen

- These are usually collected from seriously-ill patients who are already catheterized or can insert the catheter into the urinary bladder to collect the sample who are unable to void urine. This procedure is done only by especially trained staffs.
- These are used for the microbiological examination of critically-ill patients.

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Urine Storage and Preservation

The stability of some of the components excreted in the urine is affected by the change in pH that occurs in the collected sample. Urine pH often becomes more alkaline upon collection due to the presence and ongoing metabolism of bacteria and viable cells that are excreted.

- Use of preservatives reduce bacterial growth; decrease the decomposition of the chemicals associated with variable handling and storage conditions.
- Unpreserved urine specimens are subjected both to microbiologic decomposition and to inherent chemical changes.

Various urine preservatives are:

- Refrigeration of samples till analysis
- 6N HCl
- Boric acid
- Formalin (formaldehyde) is the best for urine sediments.
- Sodium fluoride
- Phenol
- Toluene
- Thymol
- Commercial preservative tablets

QUESTIONS

1. Enumerate different types of biological samples.
2. Give methods of collection of blood sample.
3. Describe color-coded vacuum evacuated tubes, their additives and uses in laboratory.
4. Describe types of urine samples.
5. Describe collection of different types of urine samples.
6. Write additives used in grey evacuated tube and its importance.
7. Enumerate different types of urine preservatives.
8. What are vacutainer tubes?
9. Mention the different vacutainer tubes and their uses.
10. What is the difference between plasma and serum?

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Biochemistry
Laboratory Practical Manual
for Phase-I MBBS Students

Salient Features

- The 2nd edition of *Biochemistry Laboratory Practical Manual for Phase-I MBBS Students* has been updated in a user-friendly manner according to the requirements of medical students. Practical syllabus is divided into four sections: A, B, C, and D with the learning domain involved as per the Competency-Based Medical Education Curriculum (NMC) for 1st MBBS students.

1. **Section A**: SGD/Demo
2. **Section B**: Qualitative Experiments (DOAP/SGD)
3. **Section C**: Quantitative Experiments (Practical/DOAP)
4. **Section D**: Basis and Rationale and Biochemical Tests Done in Various Disorders (SGD)

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1. Biochemistry (6th Edition): Approved for the course of MBBS, as textbook by Maharashtra University of Health Sciences, Rajeev Gandhi University of Health Sciences & various deemed universities.
2. Essentials of Biochemistry (3rd Edition): Approved for the course of BDS, BTh and BOT as textbook by Maharashtra University of Health Sciences & various deemed universities.
3. Textbook of Biochemistry for BSc Nursing (1st Edition).

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