College of pharmacy

Clinical laboratory training

Fifth stage

Dr. Maytham Ahmed

Lecture: 1

Diagnostic test basics, collecting &transporting specimens, venipuncture, urine specimen, stool specimen.

Lecture: 1 **Dr. Maytham Ahmed**

Introduction

The results of the study or tests affected by sample collection such as sample taking and handling, labeling, processing (additives, centrifugation), storage, contamination and transportation.

Information Linked to Sample

Time and date of collection, recent diet and supplement use, reproductive information (menstrual cycle), recent smoking, current medication use, recent medical illness, storage conditions.

1: Specimen Collection

The specimens used for biochemical analyses are various such as:

venous blood (serum or plasma), arterial blood, capillary blood, blood spot on a filter paper (Guthrie Card), urine, faeces, cerebrospinal fluid (CSF), sputum and saliva, tissue and cells, aspirates, e.g. pleural fluid, ascites, joint (synovial) fluid, intestinal (duodenal), pancreatic pseudocysts, calculi (stones).

Blood Sample Collection

Venipuncture is the process of puncturing a vein with a needle for any medical purpose. **Phlebotomy** is venipuncture performed specifically to obtain blood samples for study. **Venipuncture** is one of the most routinely performed invasive procedures and is carried out for any of five reasons:

1. To obtain blood for diagnostic purposes
2. To monitor levels of blood components
3. To administer therapeutic treatments
4. To remove blood due to excess levels of iron or erythrocytes (red blood cells)
5. To collect blood for later uses, mainly transfusion either in the donor or in another person.

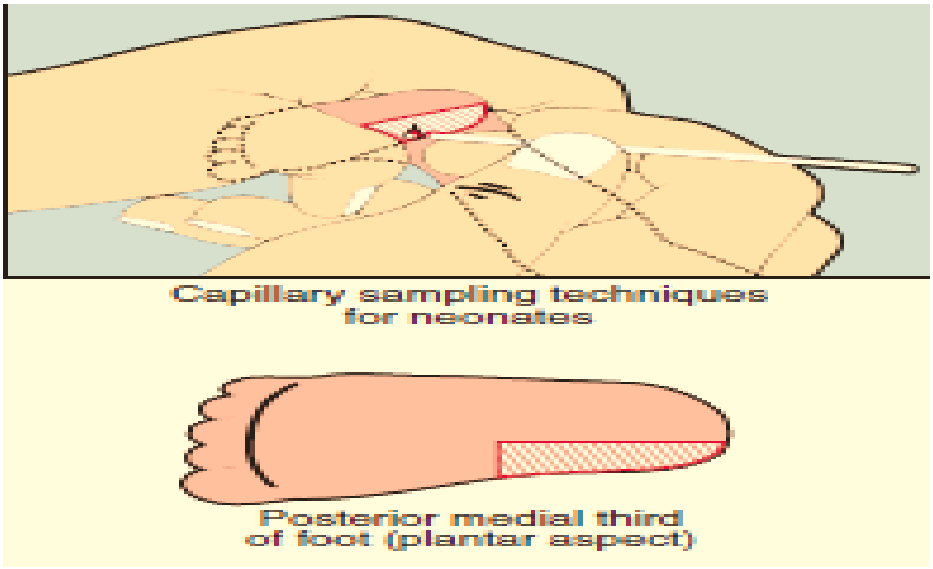
Blood is most commonly obtained from the superficial veins of the upper limb.

Commonly used sites in the arm for phlebotomy

• When a large amount of blood sample needed, an evacuated tube system with interchangeable glass tubes can be used to avoid multiple venipunctures.

• Evacuated tubes are commercially prepared with or without additives and with sufficient vacuum to draw a predetermined blood volume per tube.

Minute quantities of blood may be taken by fingerstick sampling and collected from infants by means of a heel prick or from scalp veins with a winged infusion needle.



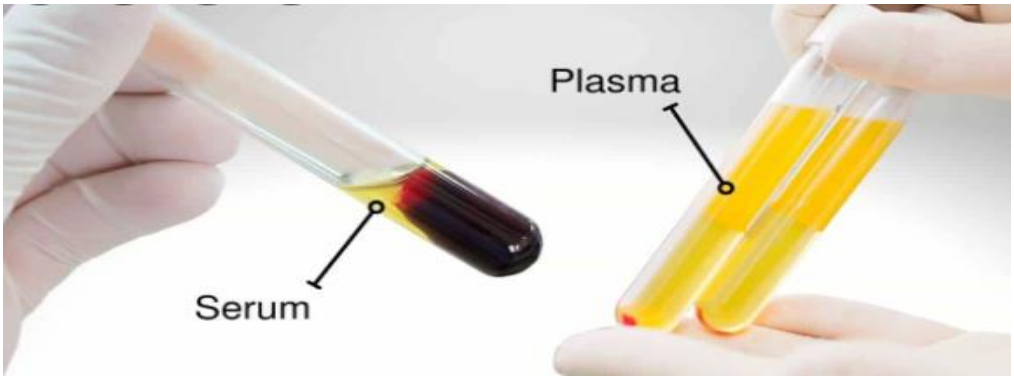
Blood Specimens

If blood is collected into a plain tube and allowed to clot, after centrifugation a serum specimen is obtained**.** If the blood is collected into a tube containing an anticoagulant such as heparin, when centrifuged, the supernatant is called plasma.

Blood specimen tubes for specific biochemical tests are illustrated as follows:

Serum = Plasma – Clotting Factors

Characteristics	Serum	Plasma
Definition	Serum is the liquid part of the blood after the coagulation.	Plasma is a clear and yellowish fluid part of the blood.
Composition	Serum is the water fluid from blood without the clotting factors.	Plasma is the blood fluid that contains blood clotting agents.
Volume	Volume of serum is less in comparison to plasma.	Plasma is a clear yellow liquid which is 55% of total volume of blood.
Isolation	Serum is acquired from the process of spinning after clotting.	Plasma is acquired from the process of spinning before clotting.



Blood Collection: Color-code Tubes

Green-top tubes contain heparin.

Blue-top tubes contain sodium citrate and citric acid.

Black-top tubes contain sodium oxalate.

Yellow-top tubes contain acid-citrate-dextrose (ACD) solution.

Grey-top tubes contain a glycolytic inhibitor.

VACUTAINER TUBES

Color	Anticoagulant	Uses
	No anticoagulant	Serological examination in biochemistry
	**Sodium Fluoride**	Glucose estimation
	**EDTA**	Hematological examination like complete hemogram, ESR
	**3.2% sodium citrate**	Coagulation studies like PT, APTT
	**Heparin**	Bone marrow studies
	**Citrate**	Blood culture
	**(K2)EDTA**	Blood Bank tests. Blood typing. ABO grouping etc

Sampling Errors

There are a number of potential **errors** that may contribute to the **failure** of the laboratory **in providing the correct answers** to the clinician's questions. Some of these problems arise when a clinician first obtains specimens from the patient.

•Blood Sampling Technique:

1. **Difficulty in obtaining a blood specimen** may lead to hemolysis with consequent release of potassium and other red cell constituents.

2. **Prolonged stasis during venipuncture**: Plasma water diffuses into the interstitial space and the serum or plasma sample obtained will be concentrated. Proteins and protein bound components of plasma, such as calcium or thyroxin, will be falsely elevated.

3. **Insufficient specimen**: It may prove to be impossible for the laboratory to measure everything requested on a small volume.

4. **Errors in timing:** The biggest source of error in the measurement of any analyte in a specimen is in the errors in timing.

5. **Incorrect specimen container:** For many analyses the blood must be collected into a container with anticoagulant and/or preservative. For example, samples for glucose should be collected into a special container containing **fluoride**, which inhibits glycolysis; otherwise the time taken to deliver the sample to the laboratory can affect the result. If a sample is collected into the wrong container, it should never be decanted into another type of tube. For example, blood that has been exposed, even briefly, to **potassium EDTA containing tube** will have a markedly reduced calcium concentration, approaching zero, along with an artefactually high potassium concentration. This is because **EDTA** (an anticoagulant) is a **chelator** of calcium and is **present as its potassium salt**.

6. **Inappropriate sampling site:** Blood samples should not be taken 'downstream' from an intravenous drip. It is not unheard of for the laboratory to receive a blood glucose request on a specimen taken from the same arm into which 5% glucose is being infused. Usually the results are biochemically incredible but it is just possible that they may be acted upon with disastrous consequences for the patient.

7. **Incorrect specimen storage:** A blood sample stored overnight before being sent to the laboratory will show falsely high potassium, phosphate and red cell enzymes, such as lactate dehydrogenase, because of leakage into the extracellular fluid from the cells.

•Biological Factors Affecting the Interpretation of Results:

The discrimination between normal and abnormal results is affected by various physiological factors that must be considered when interpreting any given result. These include:

1. **Sex:** Reference intervals for some analytes such as serum creatinine are different for men and women.

2. **Age:** There may be different reference intervals for neonates, children, adults and the elderly.

3. **Diet:** The sample may be inappropriate if taken when the patient is fasting or after a meal.

4. **Timing:** There may be variations during the day and night.

5. **Stress and anxiety:** These may affect the analyte of interest.

6. **Posture of the patient:** Redistribution of fluid may affect the result.

7. **Effects of exercise:** Strenuous exercise can release enzymes from tissues.

8. **Medical history:** Infection and/or tissue injury can affect biochemical values independently of the disease process being investigated.

9. **Pregnancy:** This alters some reference intervals.

Urine Sample Collection

• **Urine** is an ultrafiltrate of the plasma. It can be used to evaluate and monitor body metabolic disease process, exposure to xenobiotic agents, mutagenicity, exfoliated cells, DNA adducts, etc.

• **Urine collection** is not invasive and readily obtainable. Clean and dry plastic or glass containers (50-3000 ml capacity) are used. Errors in timing in urine collection should be avoided because the biggest source of error in the measurement of any analyte in a 24-hour urine specimen is in the collection of an inaccurately timed volume of urine. Total volume must be recorded.

• **The** type of urine selected and the collection procedure used depend on the tests to be performed. The specimen should be well mixed to ensure homogeneity and aliquots for specific assays.

• If you can't hand your urine sample in within an hour you should keep it in the fridge at around 4 °C for no longer than 24 hours to avoid multiplying of bacteria. Specimen can be stored in refrigerator at 2 to 8 °C for up to 2 days or frozen at 0 °C before testing. However it is strongly recommended testing urine sample as soon as possible after collection. A urine containers with preservatives may be needed depending on the proposed assay as in 24 hours urine collection.

Types of Urine Specimens

Over the course of a 24-hour period, the **composition** and **concentration** of urine **changes continuously**. For this reason, various types of specimens may be collected, including:

1- First morning urine specimen (also known as an eight-hour specimen). The urine is generally more concentrated in this type of specimen because it has been in the bladder for an extended period of time. **A first morning** specimen is collected, the subject voids before going to sleep and **immediately upon rising** collects a urine specimen.

2- Random urine specimen can be collected **at any time**. These specimens are usually satisfactory for routine screening and for cytology studies.

3- Fractional urine collection. A collection of urine taken during a **few specified hours** or from a **specified quantity** rather than from the entire amount voided during a day.

4- Timed urine collection. **Timed short-term** specimens for 2 hour collections. **Timed long term** specimens usually done over 12-24 hour period, this method allow day-to-day comparison.

5- Midstream clean-catch specimens. **A mid-stream** urine sample means you don't collect the first or last part of urine that comes out. This reduces the risk of the sample being contaminated with bacteria from: your hands, the skin around the urethra, the tube that carries urine out of the body. Used for urine culture and cytological analyses.

6- Double-voided specimen. This refers to a urine specimen which is collected after first emptying the bladder and then **waiting until another specimen** can be collected. These specimens are more accurate for purposes of glucose testing.

7- Urinary catheter specimen. A urinary catheter is a flexible tube used to empty the bladder and collect urine in a drainage bag.

8- Suprapubic transabdominal needle aspiration of the bladder urine. Suprapubic aspiration is a procedure to take a urine sample. It involves **putting a needle** through the skin just above the pubic bone **into the bladder**. It is typically used as a method to collect urine in child less than 2 years of age who is not yet toilet trained in an effort to diagnose a urinary tract infection.

Stool Samples Collection

The importance of stool tests

Stool tests can be a valuable diagnostic tool to **assess digestive tract** if there are signs and symptoms of gut infections as severe abdominal pain, bloating, frequent bowel movements, diarrhea, bloody diarrhea, or mucous in stool.The test includes a search for certain cells of interest, infectious markers and stool DNA.

Stool Sample Collection

It should be collected in a sterile or clean, dry screw-top container. **Freshly** passed stool samples are taken and avoid specimens from a bedpan. Stool samples **must be fresh** – if they aren't, the bacteria in them can multiply.
Stool samples should be handed as soon as possible. If you can't hand the stool sample in immediately, you should store it in a fridge, but for **no longer than 24 hours**. Stool is stable for 1 hour at room temperature or 24 hours in the refrigerator. Specimen's greater than 96 hours **(4 days) will be rejected**. Stool

collection for culture and sensitivity instructions leave at room temperature **do not refrigerate**.

What is the Best Time of Day to Collect a Stool Sample?

Specimens are best obtained a few hours after the person has rested, between 9 p.m. and midnight, or in the morning immediately upon arising before bathing or bowel movement.

What is Normal Stool Report?

Normal: **The stool appears brown, soft, and well-formed in consistency**. The stool does not contain blood, mucus, pus, undigested meat fibers, harmful bacteria, viruses, fungi, or parasites.

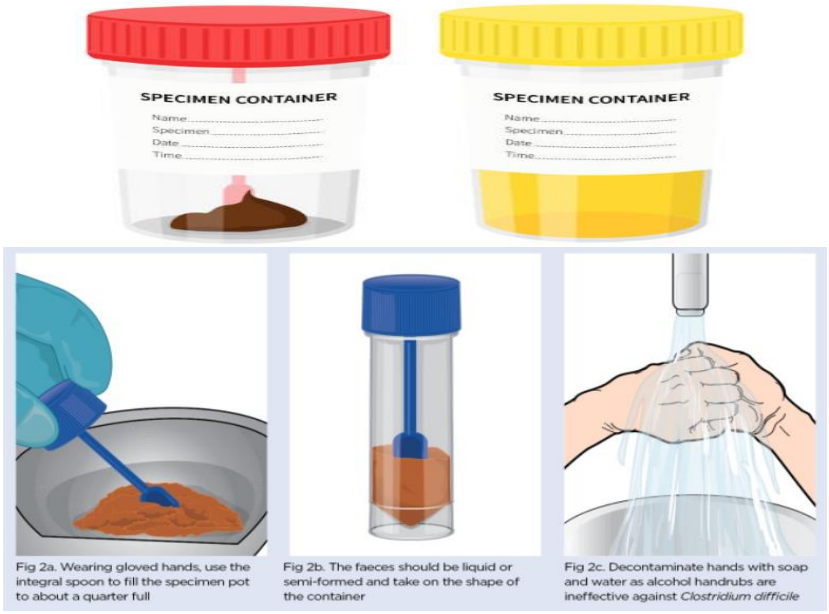


Fig 2a. Wearing gloved hands, use the integral spoon to fill the specimen pot to about a quarter full

Fig 2b. The faeces should be liquid or semi-formed and take on the shape of the container

Fig 2c. Decontaminate hands with soap and water as alcohol handrubs are ineffective against *Clostridium difficile*

2- Transportation

• Sample transportation requirements depends on the time, distance, climate, season, method, applicable regulations, type of specimen and markers to be assayed.

• **Polyurethane boxes containing ice** are used to ship and transport samples that require **low temperature**. For samples require **very** low temperature, liquid nitrogen container can be used. Liquid nitrogen containers are used for long term storage and transportation of biological specimens in the liquid nitrogen temperature zone (-196 ℃), such as blood samples, tissues, vaccines and viruses, etc. of animal, plants or humans.

Polyurethane ice box

Liquid nitrogen containers

3-Storage temperature

Specimen collection requires storage system that capable of maintaining the optimal temperature for the diverse type of specimens. For example DNA storage at -20 °C and -80 °C can prevent DNA degradation for months or years. Laboratory kits are stored according to manufacturer instructions.

Criteria for Rejecting Samples

1- Mismatch of information on the label and the request.

2- Inappropriate transport temperature.

3- Excessive delay in transportation.

4- Insufficient quantity.