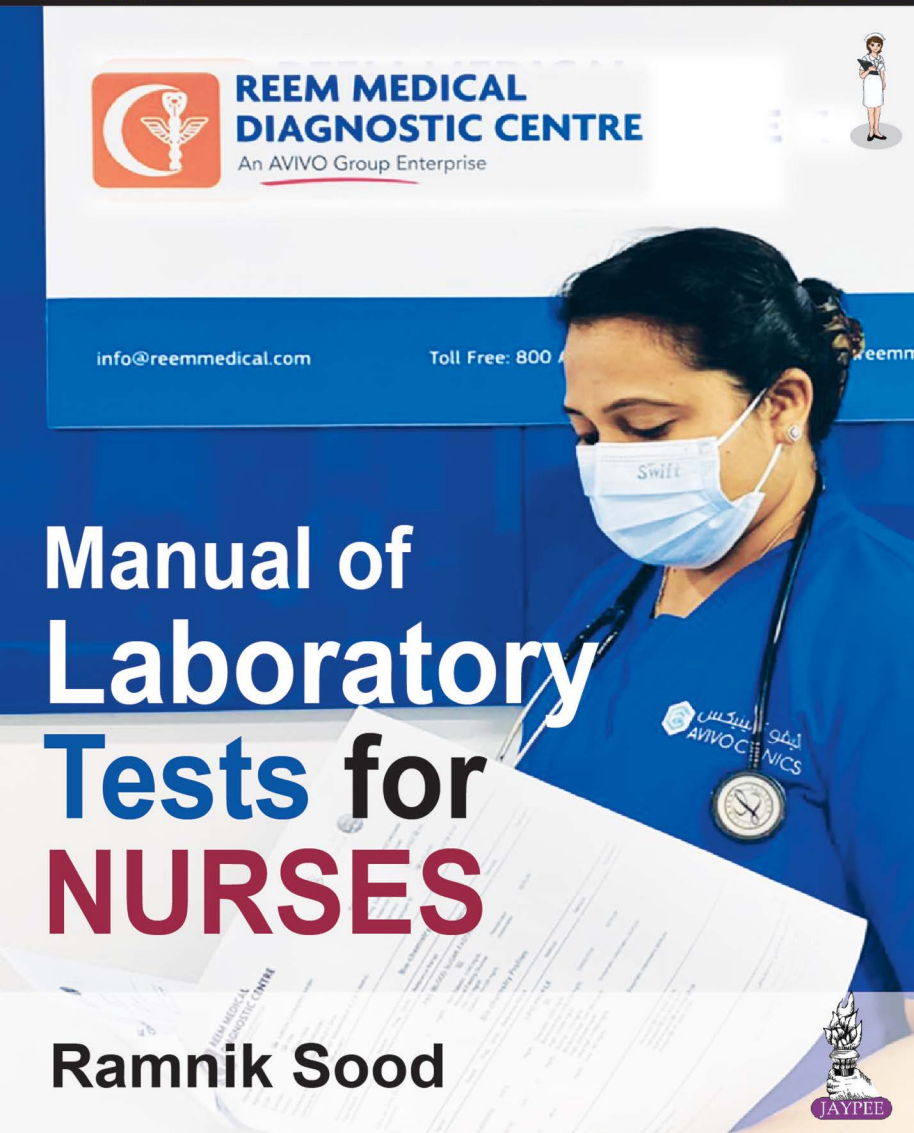


Manual of Laboratory Tests for NURSES

Ramnik Sood

JAYPEE

Contents

Chapter 3 Sample Collection by Technical Personnel and Ward Sisters

INTRODUCTION

Collections of sample of blood, urine, stool, swabs and a few other procedures are routinely performed in the Hospital settings as well as outside **(Table 3.1)**. The purpose of this chapter is to know about the procedure of sample collection, about the policies, standard operating procedure, proper safety, maintenance of time and understanding the nature of urgency.

Policy

This varies in every set up according to the decision of the management. However, a few are commonly thought about, discussed and finally implemented. The policies of sample collection in hospital set up are following:

Table 3.1: List of samples in hospital setup.

◆ Venous blood	◆ Urine collection directly or from urobags
◆ Capillary blood	◆ Stool
◆ Arterial puncture	◆ Swabs, etc.

- Proper collection of sample by trained personnel.
- Maintenance of time in all respects.
- Maintenance of safety measures in routine and regular fashion as well as in emergency situations.
- Counseling.
- Adequate communication with the information system.

PROCEDURE OF SAMPLE COLLECTION

Collection of peripheral venous blood is known as Phlebotomy and personnel who are trained for this are known as Phlebotomists. Ward sisters are usually trained enough for that.

The nature of sample, the specificity of the tests, time of collections are all done according to the directions of the attending medical personnel. Collection of samples other than blood are also performed in the same way.

Largely phlebotomies are done while the patient is lying on the bed, awake, properly introduced. A short while procedure of counseling of the patient in assertive manner to avoid panic and to reduce pain.

Gentleness, patience, knowledge and purpose of the activity are of prime importance to win the confidence of the ailing. For conscious inquisitive patients care must be taken to answer their queries.

Selection of the site venipuncture is the next matter to be thought. Antecubital veins are most preferred sites. Other sites are forearm, dorsum of wrist, neck, sides of the heel and femoral veins.

The limb should be free from infusion or transfusion set up. In cases when both upper limbs even all four limbs are occupied with fluid lines or channels, one can find veins in the neck. In small

babies skin puncture at inferolateral or inferomedial aspects of the heel can also be used with similar yield.

Situations where no favorable sites are available for venipuncture, one can stop on infusion set, wait for a few minutes and draw blood sample. Selection of vein is state of art. Subcutaneous veins are better seen than felt. Skin fairness facilitates this issue. However, experienced personnel with sensitive finger tips, focus and concentration can feel the veins easily in any kind of situations. A good eyesight is mandatory.

Sample collection trolley is to be made available before everything. A collection tray should have the following in order to have a smooth function of the procedure **(Table 3.2)**.

Table 3.2: Instruments for collection tray.

- Sterile and disposable nitrile gloves
- Large achromatic eye covering goggles
- Pared nails to facilitate palpation and to avoid injuries
- Adequate syringes with proper needles or evacuated vials with holders and special double ended needles
- Sterile swabs with 70% isopropanol in water
- Tube stand
- Tourniquet
- Stop watch
- Glass capillaries
- Round filter paper
- Thermometer and pulse oximeter, if necessary
- Receptacles for disposal of the waste
- Clean glass slide and a good spreader for smearing
- Pencil, glass marking pen and diamond marker
- Betadine
- Tissue paper

PROCEDURE OF VENIPUNCTURE

A rubber tourniquet or an elastic arm tourniquet is tied a few centimetres above the chosen site. Tourniquet can be tied for a period <1 minute duration. It is not wise to waste time for cleaning the puncture site after applying the tourniquet. A small straight segment (at least of > 2 cm long) of a palpable vein is fixed and made prominent by grabbing the elbow from behind and asking the patient to clench the fist. The needle should be introduced gently, in a straight or swing fashion at an angle of 15° to 30° from the skin surface within seconds into the lumen of the desired vein. Blood is immediately seen at the base of the needle when hypodermic syringe is used. However, it is totally blind in case of evacuated system of collection. In the former, a fixed volume of blood can be drawn by a single puncture. On the other hand, multiple vials can be filled in case of evacuated sampling. However, one can use butterfly needle for any volume of blood.

Once the vein is punctured tourniquet and the clenched fist are to be released immediately. In cases of collections of blood for calcium and lithium both tourniquet and clenching must not be done.

Glove hygiene is to be maintained in every individual patient. The personnel are to clean hands before and after use of single set of gloves. Needlestick should be avoided by all means.

ORDER OF DRAW

A definite order is maintained to avoid cross contamination of additives between the tubes **(Table 3.3)**. The order of sampling is as follows.

- **1st :** Blood culture in bottle or yellow/black topped plastic tube containing broth mixture. The latter keeps the viability of the microorganisms and make it suitable for cultures of aerobes, anaerobes and fungi.

Table 3.3: Vacutainer/sample tube types for venipuncture/phlebotomy.

Tube cap color or type	*Additive*	*Usage and comments*
Blood culture bottle	Sodium polyanethol sulfonate (anticoagulant) and growth media for microorganisms	Usually drawn first for minimal risk of contamination. Two bottles are typically collected in one blood draw; one for aerobic organisms and one for anaerobic organisms
Light blue	Sodium citrate (anticoagulant)	Coagulation tests such as prothrombin time (PT) and partial thromboplastin time (PTT) and thrombin time (TT). Tube must be filled 100%
Plain red	No additive	Serum: Total complement activity, cryoglobulins
Gold	Clot activator and serum separating gel	Serum-separating tube: Tube inversions promote clotting. Most chemistry, endocrine and serology tests, including hepatitis and HIV
Dark green	Sodium heparin (anticoagulant)	Chromosome testing, HLA typing, ammonia, lactate
Mint green	Lithium heparin (anticoagulant)	Plasma. Tube inversions prevent clotting
Lavender (purple)	EDTA (chelator/ anticoagulant)	Whole blood: CBC, ESR, Coombs test, platelet antibodies, flow cytometry, blood levels of tacrolimus and cyclosporin

Contd...

Contd...

Tube cap color or type	*Additive*	*Usage and comments*
Pink	EDTA (chelator/ anticoagulant)	Blood typing and cross-matching, direct Coombs test, HIV viral load
Royal blue	EDTA (chelator/ anticoagulant)	Trace elements, heavy metals, most drug levels, toxicology
Tan	EDTA (chelator/ anticoagulant)	Lead
Gray	◆ Sodium fluoride (glycolysis inhibitor) ◆ Potassium oxalate (anticoagulant)	Glucose, lactate
Yellow	Acid-citrate-dextrose A (anticoagulant)	Tissue typing, DNA studies, HIV cultures
Pearl (white)	Separating gel and (K_2) EDTA	PCR for adenovirus, toxoplasma and HHV-6

- **2nd:** Coagulation tube for routine coagulation assay if ordered only. If there is a concern about tissue thromboplastin, then one may draw a non-additive tube first and light blue topped coagulation tube containing Na-citrate. In this case blood is to be drawn upto the mark.
- **3rd:** Red topped non-additive tubes are used next for Chemistries, Immunology, Serology and Blood bank purposes.
- **4th:** Afterwards all tubes containing additives are used in the following order. The SST (Serum separation tube) containing a

gel separates serum (top) and blood (bottom) on centrifugation. These tubes are gold capped.

- **5th:** Orange topped tube containing thrombin which quickly clots blood and express serum quickly. This is used for urgent situations.
- **6th:** Light green lithium heparin tube with plasma gel separating tube (PST) for chemistries.
- **7th:** Dark green topped Na-heparin anticoagulant tube for chromosome testings, HLA typing, ammonia and lactate estimations.
- **8th:** Lavender or purple topped tube with EDTA as additive for complete blood count, ESR, Blood bank. This requires full draw and proper mixing. Other uses are: Direct Coombs test, Platelet antibodies, Flow cytometries, Blood level of tacrolimus, Cyclosporine.

Each sample container is to be labelled for proper identification immediately after the sample collection. The label should contain patient's name, test name, time. A bar code sticker can be pasted for better information.

SAMPLE COLLECTION ORDER FORM/REQUISITION

A requisition form must accompany each sample submitted to the laboratory. This order form must be in duplicate and must accompany individual's samples in zipped plastic bag to the lab. The following are the essential elements of an order form:

- Patient's name, age, sex.
- Patient's ID: Hospital ID and a personal ID.
- Date of birth.
- Doctor's name and telephone number.
- Date and time of the order.

- Date and time of collection.
- Collector's name and signature.
- Primary samples, e.g., blood, urine, etc.
- Tests required.

PROCEDURAL ISSUES

- The sample collector must apply a complete professional and courteous attitude to gain the confidence of the patient and be satisfied with the whole process.
- Greet the patient at the same time the collector clears his/her identification to the patient. Express in brief about the purpose and procedure confidently and gently. Both verbal and nonverbal communications are essential. If possible speak with the patient during the procedure to keep the patient's attention away. Always thank the patient and excuse yourself courteously when the procedure is completed.

Patient's Bill of Rights

The patient has the right to:

- Impartial access to treatment or accommodations that are available or medically indicated, regardless of race, creed, sex, national origin, or sources of payment for care.
- Considerate and respectful care.
- Confidentiality of all communications and other records pertaining to the patient's care.
- Expect that any discussion or consultation involving the patient's case will be conducted discretely and that individuals not directly involved in the case will not be present without patient permission.
- Expect reasonable safety congruent with the hospital practices and environment.

- Know the identity and professional status of individuals providing service and to know which physician or other practitioner is primarily responsible for his or her care.
- Obtain from the practitioner complete and current information about diagnosis, treatment, and any known prognosis, in terms the patient can reasonably be expected to understand.
- Reasonable informed participation in decisions involving the patient's health care. The patient shall be informed if the hospital proposes to engage in or perform human experimentation or other research/educational profits affecting his or her care or treatment. The patient has the right to refuse participation in such activity.
- Consult a specialist at the patient's own request and expense.
- Refuse treatment to the extent permitted by law.
- Regardless of the source of payment, request and receive an itemized and detailed explanation of the total bill for services rendered in the hospital.
- Be informed of the hospital rules and regulations regarding patient conduct.

Issues on Venipuncture Site Selection

Although the median cubital vein or cephalic vein of the arm are most commonly selected for venipuncture, other veins like basilic vein, veins of dorsum of arm, veins of dorsum of wrist may used. However, foot veins are least commonly used as chances of complications are more common.

Sites to be Avoided for Venipuncture

- Foot veins
- Scar over burns or surgery

- Upper extremities on the side of mastectomy as the results of the tests may be affected by lymphedema.
- Avoid hematoma to get rid of erroneous results.
- Intravenous line in one arm: One can use the arm or the same as distal to the IV line puncture site. In the case of the latter, it is advisable to turn of the flow of the IV line for at least 2 minutes before the sample collection. Apply the tourniquet below the IV site, avoid the same vein being used for the infusion line; draw and discard at least 5 ml of blood and finally draw for tests.
- Drawing from the IV line may be rarely needed but warrant problems. However, one has to flush the line first. Then using butterfly needle, one has to discard at 5 ml of blood before using for tests.
- Cannula: Drawing blood from cannula or fistula with heparin lock should not be done without consulting the attending physician.

Palpation of Vein is an Art

- Veins are soft compressible without any pulsation. Vein can rolled under the palpating fingertips.
- Verify the following details about the patient's condition before phlebotomy, such as state of fasting and its duration, dietary restrictions, medications, history of allergies to antiseptics or adhesives.
- Patient's position is important to get the most suitable site of venipuncture. Arm should be hyperextended.
- The tourniquet is to be placed at least 3–4 inches above the chosen site of venipuncture. The tourniquet is never to be kept tight for more than two minutes. If so, one has to release it immediately, wait for another two minutes and then reapply.

- Prepare the patient's arm using an alcohol prep. Cleanse in a circular fashion, beginning at the site and working outward. Allow to dry.
- Patient should be asked to clench the fist without pumping.
- The needle should angle 15° to 30° to the surface. Needle is to be inserted steadily without trauma and excessive probing.
- When the last tube to be drawn is filling, remove the tourniquet.
- While withdrawing the needle, press the gauze deeper and after complete withdrawal of the needle press the gauze tightly.
- Mix and label all the tubes and place within a zipped plastic bag.
- Dispose all the contaminated materials to the designated containers.

PERFORMANCE OF FINGERSTICK COLLECTION

The best locations for fingersticks are the 3rd (middle) and 4th (ring) fingers of the non-dominant hand. Do not use the tip of the finger or the center of the finger. Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, callused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash.

- Using a sterile lancet, make a skin puncture just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.
- Wipe away the first drop of blood, which tends to contain excess tissue fluid.
- Collect drops of blood into the collection device by gently massaging the finger. Avoid excessive pressure that may squeeze tissue fluid into the drop of blood.

- Cap, rotate and invert the collection device to mix the blood collected.
- Have the patient hold a small gauze pad over the puncture site for a couple of minutes to stop the bleeding.
- Dispose of contaminated materials/supplies in designated containers.
- Label all appropriate tubes at the patient bedside.
- Deliver specimens promptly to the laboratory.

OTHER COLLECTION ISSUES

Prevention of Hematoma

- Complete penetration of the superficial wall of the major superficial vein and continue penetration for a certain distain distance show that the needle tip remains within the venous lumen sufficiently and safely inside; never to penetrate through the deeper wall of the vein. Both partial penetration and penetration of both the walls lead to hematoma.
- To keep a continuous pressure on the puncture site by the other hand till the needle is removed.
- Always remove the tourniquet before removal of the needle.
- Put firm pressure at the puncture site after removal of the needle.

Prevention of Hemolysis

- Always mix blood with its anticoagulant gently, thoroughly, continuously and completely (5–10 times).
- In case of hypodermic syringe and needle, the needle should not be too thin, the plunger of the syringe should not be drawn out forcefully and suddenly, there should not be any creation of froth or bubbles within the syringe.

- Never to draw blood from a hematoma.
- The venipuncture site should be dry.
- Avoid too much probing or negotiation to get the venous wall or its lumen.
- Avoid prolonged application of tourniquet or clenching of patient's fist.

Indwelling Catheter

- Avoid it as it contains heparin to keep the catheter patent. This is a potential source of error.
- To avoid the error one has to discard at least a few ml of blood before actually filling the collection tubes.

Prevention of Hemoconcentration

In this situation there is an increase in concentration of larger molecules. To prevent that one has to take care of the following:

- Never to keep the tourniquet fastened and tight for more than 1 minute (vide infra).
- Never to ask the patient to keep the fist clenched once the drawing of blood is started.
- Never to ask the patient to pump by the fist during the time of drawing of blood.
- Not to massage or probe the puncture site.
- Not to use indwelling catheter, prolonged used for infusion, sclerosed or thrombosed veins.

Prolonged Tourniquet Application

- This leads to increase in local increase of hydrostatic pressure → passage of water and filterable elements out of the blood vessels → hemoconcentration.

- Hemoconcentration leads to increase in serum total protein, AST, total lipids, cholesterol, iron, etc. There may be increase in Hct, and other cellular parameters of blood.
- Prolonged tourniquet application leads to hemolysis and erroneous increase in serum potassium.

Patient Preparation Factors

- Maintain proper time of collecting samples
- Patient should be away from exhaustive activities before sampling. Patient should preferably remain restful for at 10–15 minnutes before sample collection procedures. Exercise leads to increase in creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and platelet count.

Therapeutic Drug Monitoring

Different pharmacologic agents have patterns of administration, body distribution, metabolism, and elimination that affect the drug concentration as measured in the blood. Many drugs will have "peak" and "trough" levels that vary according to dosage levels and intervals. Check for timing instructions for drawing the appropriate samples.

Diurnal Rhythm

Diurnal rhythm is an important factor in cases of serum cortisol which is highest in the morning and go to its nadir in the afternoon. Serum iron remains low during daytime than during the dusk.

Posture is an important factor in cases of large filterable materials in blood. Enzymes, proteins, lipids, iron, and calcium are significantly increased with changes in position.

Other Factors

Age, sex or pregnancy may affect the test results. The reference values are to be written as per age, sex or other state matched.

Reasons for Cancellation of Tests

- Nontechnical reasons:
  - Duplicate test request
  - Incorrect test ordered
  - Test no longer needed
- Technical reasons:
  - Hemolysis of the specimen
  - Clotted specimen
  - Quantity of specimen not sufficient
  - Collection of specimen in incorrect tube
  - Contaminated specimen
  - Identification of the specimen is suspected
  - Delay in transport – specimen too old.

SAFETY AND INFECTION CONTROL

Protect Yourself

- Because the ward sisters or hospital lab collectors are almost always with the patients, knowledge of protecting oneself about prevention from infection, accident or infections are to be kept in mind.
- Protections: Wearing gloves, lab coats, eye shield with regular and judicious changing of gloves, washing hands after each set of collection.
- Dispose of items in appropriate containers.

- Dispose of needles immediately upon removal from the patient's vein. Do not bend, break, recap, or re-sheath needles to avoid accidental needle puncture or splashing of contents.
- Clean up any blood spills with a disinfectant such as freshly made 2% bleach.

Measures in cases of accidental stick by contaminated needle:

- Remove the gloves and dispose of them properly.
- Squeeze puncture site to promote bleeding.
- Wash the area well with soap and water.
- Record the patient's name and ID number.
- Follow institution's guidelines regarding treatment and follow-up.

Protect the Patient

- Place the collection material and collected samples away from the patients especially suffering from psychiatric illness or children.
- Maintain cleanliness and prevent cross contamination.

TROUBLESHOOTING GUIDELINES

If there is an Incomplete Collection or No Blood is Obtained

The following are a few measures:

- Change the position of the needle. Move it forward (it may not be in the lumen) or move it backward (it may have penetrated too far).
- Adjust the angle (the bevel may be against the vein wall).
- Loosen the tourniquet. It may be obstructing blood flow.

- Try another tube. Use a smaller tube with less vacuum. There may be no vacuum in the tube being used.
- Re-anchor the vein. Veins sometimes roll away from the point of the needle and puncture site.
- Have the patient make a fist and flex the arm, which helps engorge muscles to fill veins.
- Pre-warm the region of the vein to reduce vasoconstriction and increase blood flow.
- Have the patient drink fluids if dehydrated.

If Blood Stops Flowing into the Tube or Syringe

- Re-secure the tourniquet to increase the venous pressure. If that is not successful then withdraw the needle, take care of the puncture site and choose another puncture site for a repeat puncture.
- Hold the collecting needle holder firmly against the skin to vein junction to keep the needle tip in position.

Other Troubleshootings

Inadvertent puncture of artery: Blood is usually bright red. The puncture site is to be secured very firmly for a prolonged period.

If hematoma is already formed, stop collection, withdraw the needle, secure the puncture site till there is no increase in the size of hematoma. Consult attending physician for further management of the hematoma.

Blood Collections on Babies

Inferolateral and inferomedial aspect of the pre-warmed (at 42°C) heel are sites of collection of newborn babies blood. Take meticulous

care about the pre-warming temperature. Hold the baby steadily and firmly. Clean the area with alcohol sponge and dry it with sterile cotton pad. Using sterile lancet cut the sides of the heel so that a clear drop of blood comes out immediately. Wipe away the first drop of blood with sterile dry cotton.

- Fill the capillary tube(s) or micro collection device(s) as needed.
- When finished, elevate the heel, place a piece of clean, dry cotton on the puncture site, and hold it in place until the bleeding has stopped.
- Be sure to dispose of the lancet in the appropriate sharps container.
- Dispose of contaminated materials in appropriate waste receptacles.
- Remove your gloves and wash your hands.

Pediatric Phlebotomy

Children, particularly under the age of 10, may experience pain and anxiety during the phlebotomy procedure. A variety of techniques can be employed to reduce pain and anxiety. Effective methods use distraction. These may include listening to music or a story, watching a video, playing with a toy, having a parent provide distraction with talk or touch, using flash cards, and squeezing a rubber ball.

Femoral puncture is an age old procedure for collection of blood. With appropriate hand this is done quite smoothly. However, the risk of femoral arterial puncture is high and the method is not very attractive now-a-days.

Collection Tubes for Phlebotomy

- Collection tubes can vary in size for volume of blood drawn, appropriate to the tests ordered with sample size required, and

vary in the kind of additive for anticoagulation, separation of plasma, or preservation of analyte **(Fig. 3.1)**.

- Larger tube sizes typically provide for collection of samples from 6 to 10 ml.
- Smaller collection tubes for sample sizes of 2 ml or less may be appropriate in situations where a smaller amount blood should be drawn, as in pediatric patients, or to minimize hemolysis during collection, or to avoid insufficient sample volume in the collection tube.

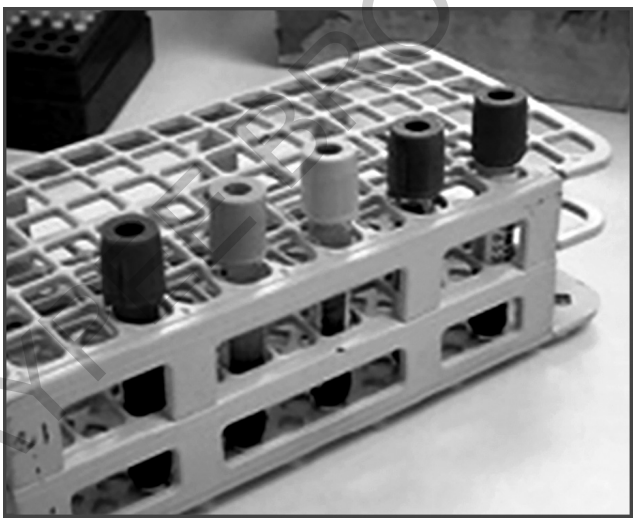
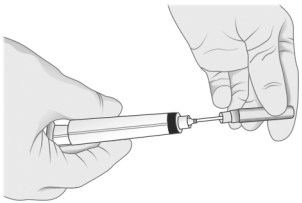
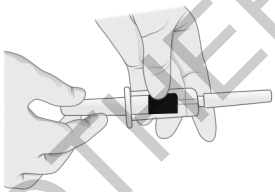


Fig. 3.1: A range of Vacutainer tubes containing blood.

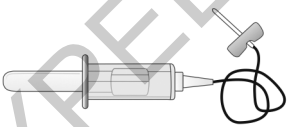
BLOOD SAMPLING SYSTEM



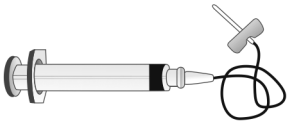
Needle and syringe system
Remove the syringe from the packaging and insert the nozzle of the syringe firmly into the exposed hub of the capped hypodermic needle



Vacuum extraction system
The barrel holds the sample collection tube in place and protects the phlebotomist from direct contact with blood. Do not push the laboratory tube onto the needle inside the barrel until the needle is in the blood vessel, or the vacuum will be lost



Winged butterfly system (vacuum extraction)
A vacuum system combined with a winged butterfly needle
Do not push the laboratory tube onto the needle inside the barrel until the winged needle is inside the blood vessel or the vacuum will be lost



Winged butterfly system (syringe)
A syringe combined with a winged butterfly needle

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