CHAPTER 1

Introduction to Medical Laboratory and its Techniques

KEY TERMS

Biohazard: Any biological material that poses a risk to human health.
Centrifugation: The process of separating components of a mixture by spinning it at high speed.
Disinfection: The process of reducing or eliminating harmful microorganisms from surfaces or instruments using disinfectants.
External quality control (EQC): This involves participation in external programs where the laboratory's results are compared with those from other laboratories. This process ensures standardization and identifies any discrepancies, providing an objective assessment of the laboratory's performance.
Internal quality control (IQC): This refers to procedures conducted within the laboratory to ensure the accuracy and reliability of test results.
Laboratory technician: A trained professional who performs laboratory tests and procedures.
Microscopy: The use of microscopes to observe specimens that cannot be seen with the naked eye.
Pathology: The study of diseases, including their causes, processes, and effects.
Personal protective equipment (PPE): Protective clothing and equipment (e.g., gloves, masks, goggles) used to ensure safety.
Quality control (QC): Procedures to ensure accuracy and precision in laboratory results.
Reference range: The normal range of values for a laboratory test, used as a standard for comparison.
Sensitivity: The ability of a test to correctly identify individuals with a specific disease.
Specificity: The ability of a test to correctly identify individuals without the disease.

Case Scenarios

1. **Rejection due to improper sample container:** A urine sample was collected from a 45-year-old diabetic patient with suspected urinary tract infection (UTI) for culture and sensitivity testing. The sample was sent to the laboratory in a nonsterile container without a proper seal.

   Upon receiving the sample, the laboratory rejected it, citing contamination risk and noncompliance with standard protocols for microbiological testing.

   Unfortunately, the patient was unable to provide another sample due to dehydration and limited urine output, delaying the diagnostic process.
2. **Specimen did not reach the laboratory:** A critically ill 5-year-old child was undergoing evaluation for suspected meningitis. A lumbar puncture was performed, and cerebrospinal fluid (CSF) was collected for laboratory analysis. The laboratory report was eagerly awaited for 3 days to confirm the diagnosis and guide treatment.

   When the parents and physician inquired about the delay, the laboratory reported that it had never received the sample.

   Upon investigation, it was discovered that the nursing staff had mistakenly kept the CSF sample container in their pocket, inadvertently carried it out of the hospital, and lost it.

   Due to this mishap, the child had to undergo another lumbar puncture. However, since antibiotics had already been administered, the results of the second sample might not accurately reflect the initial infection, potentially delaying appropriate treatment.
3. **Misguided report due to inadequate information in test requisition form (TRF):** A urologist, concerned about the culture report of a pyelonephritis patient, contacted the microbiology laboratory to discuss the finding of 'insignificant bacteriuria'. The patient had undergone a percutaneous nephrostomy, and the urologist sought information on the organism and its antimicrobial sensitivity.

   The microbiologist reviewed the case and clarified that the TRF had labeled the specimen as a urine sample, not specifying it was from a percutaneous nephrostomy. Based on the provided information, the laboratory considered the gram-negative bacillus growth $<10^5$ CFU/mL as periurethral contamination and discarded the isolate.

   Since the organism was not preserved or subjected to further testing, accurate antimicrobial sensitivity results were not available, requiring a repeat collection for culture and sensitivity. This oversight delayed the patient's targeted treatment, emphasizing the critical need for accurate and detailed requisition forms.

VARIOUS BRANCHES OF MEDICAL LABORATORY SCIENCES

Medical laboratory sciences have different specialized branches that focus on different aspects of laboratory testing and analysis. Each branch

However, an employer must conduct a workplace assessment to ascertain whether the nature of certain tasks, workplace scenarios, or employee skill deficiencies creates a need for laboratory coats or other personal protective equipment to protect employees from exposure to blood.

It is an employer's responsibility to provide, clean, repair, replace, and/or dispose of personal protective equipment/clothing. As part of presenting a professional appearance, an institutional dress code may include wearing of a laboratory coat or smock.

If a blood sample is poorly collected, the results may be inaccurate and misleading to the clinician, and the patient may have to undergo the inconvenience of repeat testing.

Nursing Considerations

Accurate specimen collection, timely transport, and proper storage are essential for reliable laboratory results. Nurses must follow aseptic technique, use correct containers and labels, and ensure that the patient preparation requirements are met especially in inpatient department (IPD) settings. Specimens should be transported promptly at the recommended temperature and conditions to preserve integrity. Proper handling prevents contamination and ensures accurate diagnosis and effective patient care.

Best Practices in Phlebotomy

This involves the following factors:

- Proper planning
- Collection at appropriate location
- Keeping quality control
- Assuring standards for quality care for patients and health workers, which include availability of:
  - Appropriate supplies and protective equipment.
  - Postexposure prophylaxis (PEP).
  - Avoidance of contaminated phlebotomy equipment.
  - Training in phlebotomy.
  - Cooperation on the part of patients.
- Quality of laboratory sampling.

Elements of Quality Assurance in Phlebotomy

- Education and training are necessary for all staff carrying out phlebotomy.

TABLE 2.1: Effects of physiological factors on the composition of body fluids

Physical activity/posture	Tests	Effect on the test results (Increase/decrease)
Upright position (Levels of these parameters are higher in an upright position than in reclining position)		
	Alkaline phosphatase	7
	Amylase	6
	Calcium	3
	Cholesterol	7
	SGOT	5
	SGPT	7
	Thyroxine	11
	Albumin	9
	TG	6
	IgA, IgG, IgM	7
Prolonged bed rest (within 4 days):		
	HCT	Increases by 10%
	Plasma protein and albumin concentrations.	Decreases by an average of 0.5 and 0.3 g/dL.
Heavy exercise		
	Increased blood glucose	Increased
	Plasma lactate and pyruvate.	Increased
	Arterial PCO_2 and pH.	Reduced
Hospitalization and immobilization (within 4 days)		
	HCT	Increases by 10%
	Plasma protein and albumin concentrations.	Decreases by an average of 0.5 and 0.3 g/dL.
Oral contraceptives		
	Iron, TG, SGPT, GGPT and T4	**Increases**
Recent food ingestion (depends on the intake of food):		
	Glucose, total lipids, etc.	Increase in serum concentration.
	Alkaline phosphatase.	High level

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Physical activity/posture	Tests	Effect on the test results (Increase/decrease)
	Serum chlorides.	Decreases due to HCl synthesis, etc.
Malnutrition		
	Total serum protein, albumin and beta globulin.	Decreases
	Cholesterol	Decreases
	Triglycerides	Decreases
	LDL and VLDSL	Decreases
Caffeine		
	Plasma glucose concentration.	Slightly increase
	Plasma free fatty acids, glycerol, total lipids, lipoproteins and serum gastrin.	Increases
Smoking		
	Glucose	Concentration may be increased by 10 mg/dL, within 10 minutes of smoking a cigarette.
	Serum IgA, IgG, and IgM	Generally lowers
	IgE level	High
	Plasma total cholesterol, LDL and TGs.	High
	HDL-L	Lowers
	RBC count	Increases
	PO_2 of habitual smoker.	About 5 mm Hg less than of a nonsmoker.
Alcohol Ingestion (high volume)		
	Blood glucose	Increased by 20–50%, Increase is more in diabetics.
	Triglyceride	Marked hypertriglyceridemia (increased).

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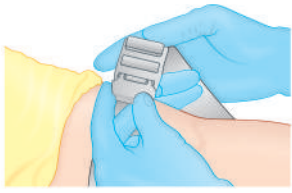
Step 1: Label the tube with the patient's particulars.

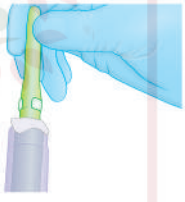


Step 2: Put tourniquet on the patient's arm about 3–4' above the venipuncture site.

Step 3: Ask the patient to form a fist so that the veins are more prominent.

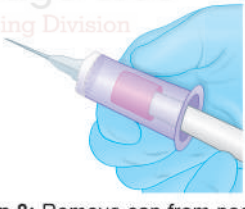
Step 4: Put on well-fitting, nonsterile gloves.

Step 5: Disinfect the site using 70% isopropyl alcohol for 30 seconds and allow to dry completely (30 seconds).

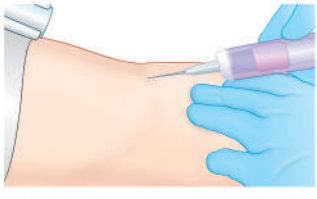


Step 6: Assemble needle and vacuum tube holder.

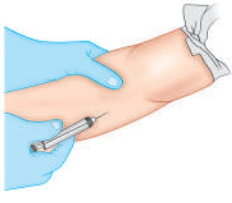
Step 7: Insert the collection tube into the holder until the tube reaches the needle.



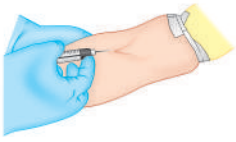
Step 8: Remove cap from needle.



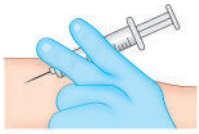
Step 9: Use thumb to draw skin tight about 1–2' below the venipuncture site.



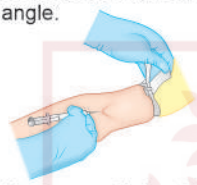
Step 10: Anchor the vein by holding the patient's arm and placing a thumb below the venipuncture site.



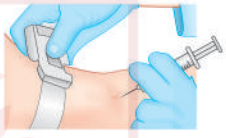
Step 11: Enter the vein swiftly at a 30° angle.



Step 12: Push the tube completely into the needle. Blood should begin to flow into the tube until vacuum tourniquet is exhausted.

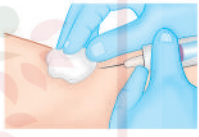


Step 13: Once sufficient blood has been collected, release the tourniquet before withdrawing the needle.



Step 14: Release the tourniquet.

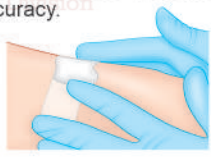
Step 15: Withdraw the needle gently and then give the patient a clean gauze or dry cotton-wool ball to apply to the site with gentle pressure.



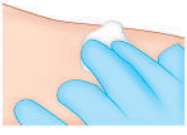
Step 16: After opening the patient's fist, place dry gauge over the venipuncture site and slowly remove the needle.

Step 18: Check the label and forms for accuracy.

Step 17: Discard the used needle and syringe or blood-sampling device into a puncture-resistant container.



Step 20: Apply bandage or continue applying mild pressure until bleeding has stopped.



Step 19: Apply mild pressure to the pad.

Step 22: Remove gloves and place them in the general waste. Perform hand hygiene. If using soap and water, dry hands with single-use towels.

Step 21: Discard sharps and broken glass into the sharps container. Place item that can drip blood or body fluids into the infectious waste.

Figure 2.2: Steps of venipuncture procedure

Did You Know?

Phlebotomists are generally limited to two attempts to obtain a blood sample. After two unsuccessful attempts, another phlebotomist or supervisor should be called for venipuncture.

Must Know

Summary of Phlebotomy/Venipuncture Procedure

- Patient identification
- Filling out the requisition
- Equipment
- Apply tourniquet and palpate for vein
- Sterilize the site
- Insert needle
- Drawing the specimen
- Releasing the tourniquet
- Applying pressure over the vein
- Applying bandage
- Disposing of the needle into sharps bin
- Labeling the specimens
- Transporting to lab

PATIENT'S AFTERCARE

Hematoma Formation

- Hematoma is a collection of blood under the skin. Hematoma formation can occur as a minor complication of venipuncture. It is the most common complication of venipuncture. This type of complication is caused by blood leaking into the tissues during or after venipuncture.
- A common sign of this complication is swelling at or near the venipuncture site. The hematoma forms under the skin adjacent to the punctured site. Hematoma occurs when the area around the puncture site begins to swell, indicating that blood is leaking into the tissues, which will result in a bruise due to partial insertion into the vein.
- When swelling occurs, release the tourniquet immediately, withdraw the needle, and apply firm pressure. If this happens, immediately remove the needle, apply pressure for 2 minutes and recheck to ensure that bleeding has stopped. If hematoma begins to form while blood is being withdrawn, the needle should be removed immediately and pressure maintained over the site.
- Some of the causes of hematoma formation after venipuncture are small fragile veins, needle too large, excessive probing to find vein,

Points to Remember

- Puncture no deeper than 2.4 mm (approximately 0.1 inches).
- Punctures to the posterior curvature of the heel can cause damage to the bones.
- Previous puncture sites should be avoided. Avoid bruising the infant's heel when obtaining blood.

Must Know

Don'ts of Blood Specimen Collection

- Do not prelabel specimen containers prior to collection.
- Do not leave the patient until all specimen containers are labeled.
- Do not use expired specimen containers.
- Do not affix specimen label to the biohazard transport bag or the container lid.
- Do not send unlabeled specimens with requisitions.
- Do not send specimens from more than one patient in the same bag.
- Never draw blood cultures from an intravenous catheter (line) unless specifically ordered, always draw by peripheral venipuncture.
- Do not cover the bar code label on the blood culture bottle with the patient label.

Complications During Blood Collection through Capillary Blood Specimen Collection

Complications that can arise in capillary sampling include:

- Collapse of veins if the tibial artery is lacerated from puncturing the medial aspect of the heel.
- Osteomyelitis of the heel bone (calcaneus).
- Nerve damage if the fingers of neonates are punctured.
- Hematoma and loss of access to the venous branch used.
- Scarring.
- Localized or generalized necrosis (a long-term effect).
- Skin breakdown from repeated use of adhesive strips (particularly in very young or very elderly patients)—this can be avoided if sufficient pressure is applied and the puncture site is observed after the procedure.

Advantages of Capillary Blood Collection

- Only a very small amount of blood is needed. ICU patients can lose up to 2% of their total blood volume every day when venous blood sampling is done.

Sample Collection Tubes

The colored caps of sample collection tubes are compiled in Table 2.5. Various types of swabs and sample containers for sample collection are given in Table 2.6.

TABLE 2.5: Blood collection tubes with cap colors and anticoagulants

Tube cap color or type		Additive/anticoagulant	Usage and comments
Blood culture tubes/ bottles: • Orange label- for anaerobes • Blue label—for aerobes (Adult patients) • Yellow label-for pediatrics		Sodium polyanethol sulfonate (anticoagulant) and growth media for microorganisms	• Usually draws first for minimal risk of microorganisms. • Two bottles are typically collected in one blood draw, one for aerobic organisms and another for anaerobic organisms.
Light blue		Sodium citrate	• Coagulase tests such as prothrombin time (PT), etc. • Example of tests: ▪ D-Dimer, Fibrinogen, Heparin Partial Thromboplastin Time (or APTT), Prothrombin Time (PT including INR), Protein S, Protein C, Antithrombin III, Factor Assays.
Plain red		No additive	Serum can be obtained for different biochemical and other tests.

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Tube cap color or type		Additive/anticoagulant	Usage and comments
Lavender (purple)		EDTA (chelating/ anticoagulant)	**Whole blood:** CBC, ESR, platelets, Coombs test, flow cytometry, etc.
Pink		EDTA (chelating/ anticoagulant)	Blood typing and crossmatching, direct Coombs test, HIV viral load, ABO type and RH antibody screen direct coombs, RHOGAM evaluation, transfusion reaction evaluation, crossmatch type and screen.
Royal blue		EDTA (chelating/ anticoagulant)	Trace elements, heavy metals and most drug levels, toxicology (Arsenic, Cadmium, Mercury).
Tan		EDTA (chelating/ anticoagulant)	Lead

Contd...

CBC can be done either through visualization of blood cells under a microscope or by using automatic electronic cell counter.

Tests: CBC, peripheral blood film (PBF)

Sample required: Whole blood

Description: 2–3 mL of whole blood is collected in a vacutainer tube containing anticoagulant EDTA (Lavender top) and sent to lab without any delay.

TABLE 3.1: Routine tests done in hematology (CBC)

Methodology: Microscopy or electronic cell counters				
Sl. no.	**Parameters**	**Biological reference range**		
		Male	**Female**	**Newborn**
	Total RBC count	4.6–5.9 x 10^{12}/L or 46,00,000–59,00,000 cells/mm^3	4.2–5.4 x 10^{12}/L or 42,00,000–54,00,000 cells/mm^3	4.1–6.2 x 10^{12}/L or 41,00,000–62,00,000 cells/mm^3
	Hemoglobin	14–18 g/dL	12–16 g/dL	15.4–25.5 g/dL or 9.6–15.3 mmol/L
	Hemoglobin A1 (major component)	96.1–99.0% of total hemoglobin		
	Hemoglobin A2 (major component)	0.8–3.4% of total hemoglobin		
	PCV/HCT	40–52%	37–47%	42–68% or 0.42–0.68 L/L
	MCV	77–99 fL		103–106 fL
	MCH	26–32 fL		36–38 pg
	MCHC	33–36 g/dL		34–36%
	RDW	12–15%		
Tests to check parameters of platelets **Methodology: Microscopy or electronic cell counters**				
Sl. no.	**Parameters**	**Biological reference range**		
		Male	**Female**	**Newborn**
	Total platelet count	1,40,000 – 4,00,000 lakh cells/µL		1,00,000–3,00,000 cells/µL

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TABLE 3.2: Various CBC parameters in children up to 1 year of age and in pregnancy

Up to 1 year of age	
Parameters	**Biological reference range**
Hemoglobin	9.0–14.5 g/dL or 5.6–9.1 mmol/L
Packed cell volume (PCV)/ Hematocrit (HCT)	29–41% or 0.29–0.42 L/L
Total RBC count	3.6–5.5 million/L or 3.6–5.5 x 1012/L
Mean corpuscular volume (MCV)	78 fL
Mean corpuscular hemoglobin (MCH)	25 pg
Mean corpuscular hemoglobin concentration (MCHC)	32%
Various CBC parameters in pregnancy	
PCV/HcT	
First trimester	35–46%
Second trimester	30–42%
Third trimester	34–44%
Total RBC count	
Trimester 1	4.0–5.5 million/µL or 4.0–5.0 x 10^{12}/L
Trimester 2	3.2–4.5 million/µL or 3.2–4.5 x 10^{12}/L
Trimester 3	3.0–4.9 million/µL or 3.0–4.9 x 10^{12}/L

Abbreviations: fL, femtoliter; L, liter; pg, picogram; µL, microliter

OTHER INVESTIGATIONS IN HEMATOLOGY

Erythrocyte Sedimentation Rate

The erythrocyte sedimentation rate (ESR), is a common hematology test used to assess inflammatory activity in the body (Tables 3.3 and 3.4).

- While it can indicate increased inflammation associated with conditions like autoimmune diseases, infections or tumors, it is not specific to a particular disease.
- The ESR is often used in conjunction with other tests to provide a comprehensive assessment of inflammatory activity.
- It is not highly sensitive or specific as a standalone screening test and may be elevated in various clinical situations.
- Interpretation of ESR results requires consideration of the overall clinical context and additional diagnostic information.

takes the final decision on whether blood should be collected from such a donor or not. In doubtful cases, the donor is deferred.

Blood donor's questionnaire:

- Are you at present in good health?
- When did you eat last?
- Are you taking any medication?
- Have you been vaccinated or immunized recently?
- Have you ever suffered from epileptic fits, convulsions or mental disorder?
- Have you ever had jaundice or hepatitis?
- Have you ever tested positive for HBV or HCV?
- Have you been in contact with a person suffering from jaundice (hepatitis) during the past 6 months?
- Do you know about AIDS?
- Have you ever had sexual contact with another man (for male donors)?
- Have you had unsafe sex with an individual at increased risk for AIDS?
- Have you lost significant weight in last 6 months?
- Have you ever tested positive for HIV?

MUST KNOW

Occupational hazards: Aircrews, drivers of long-distance heavy-duty vehicles and construction workers on high buildings are advised not to give blood within 12 hours of going on duty.

6. **Medical tests:** The medical tests which check the different medical issues, involve (Table 4.1):

TABLE 4.1: Medical issues that reject the blood donors

A. Respiratory infections	
Medical issues	**Decision taken**
Cold, flu, cough, sore throat or acute sinusitis	Defer until all symptoms subside and temperature is normal.
Chronic	No deferral unless using antibiotics.
Asthmatic attack	1 week after last attack if chest is clear.
Asthmatics on steroids	Defer

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B. Pregnancy and abortion	
Pregnant or recently delivered	Defer for 6 months after delivery.
Abortion	Defer 6 months after abortion.
Breastfeeding	After baby weaned (defer till baby is on breastfeeding).
C. Surgical procedures	
Major surgery	6 months after recovery
Minor surgery	3 months after recovery
Open-heart surgery-including by-pass surgery	Permanently defer
Cancer surgery	Permanently defer
Localized skin cancer that was removed	6 months after removal
Tooth extraction or dental manipulation.	Defer for 3 days
Dental surgery	1 month under anesthesia
D. Heart and cardiovascular diseases	
• High blood pressure controlled with medicine. • Has any active symptom such as (chest pain, shortness of breath, swelling of feet).	• Acceptable if BP is normal
• Restricted activity, cardiac medication (digitalis nitroglycerine) • Myocardial infraction • Coronary artery disease • Angina pectoris • Rheumatic heart	• Permanently defer
E. Seizures fainting and endocranial disorders	
Fainting, convulsions and epilepsy	• Defer ▪ If not taking medicine or free from seizures for >2 years can be accepted after evaluation.
Endocranial disorders	Permanently defer

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Did You Know?

- World Blood Donor Day (global) is observed on 14 June every year.
- National Voluntary Blood Donation Day (India) is observed on 1st October every year.

INDICATIONS OF USING VARIOUS BLOOD PRODUCTS

The decision to use blood components and products is governed by the principle of rational use of blood, meaning the patient receives only the specific component they lack (Table 4.7). Transfusion triggers (threshold values like hemoglobin or platelet counts) are generally used alongside the patient's clinical signs and symptoms.

TABLE 4.7: Various blood products and their uses/indications for transfusion

Blood/blood products	Indications for transfusion
Whole blood	Acute blood loss due to trauma, etc., where both red cells and volume are desired.
Red blood cells	To increase red cell mass (e.g., therapy of anemia); use with colloidal or crystalloids in active bleeding or massive transfusion.
• Red blood cells ▪ Additive solution added ▪ Leukocytes removed by centrifugation, washing or filtration	Packed red cells or whole blood used to increase red cell mass and avoid febrile allergic reactions caused by leukocytes or plasma proteins, and to prevent anaphylactic reaction.
Deglycerolized blood	To extent storage of red cells with rare blood types and autologous transfusions or to prevent HLA sensitization.
Platelet transfusions	This is indicated in treatment of bleeding due to thrombocytopenia or platelet function defects or in prevention of bleeding due to thrombocytopenia as in bone marrow failure, severe bleeding (massive transfusion), multiple traumas, traumatic brain injury or spontaneous intracerebral hemorrhage, bleeding that is not severe or life-threatening, disseminated intravascular coagulation (DIC) in presence of bleeding, etc.

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- Occupational exposure
- Needle-stick injuries, mucosal splash

Note: *HBV spreads via blood, sex, mother-to-baby.*

Modes of transmission of HCV:

- Blood-borne
- Sharing needles/syringes
- Unsafe medical injections
- Blood transfusion (before screening era)
- Needle-stick injuries (healthcare workers)
- Sexual transmission (rare)
- Mother-to-child transmission (rare)

Note: *HCV is mainly blood-borne, sexual/perinatal spread is less common than HBV.*

Tests for Influenza Virus A (H1N1) and B (HIB)

The H1N1 and HIB are enveloped, segmented, negative-sense RNA viruses. Both are transmitted through respiratory droplets, direct contact with infected individuals or contaminated surfaces. They cause seasonal flu outbreaks, with symptoms like fever, cough, and body aches (Tables 8.11 and 8.12).

TABLE 8.11: Tests for influenza viruses

Test's name	Sample required	Description
Rapid influenza diagnostic tests (RIDT) (antigen detection).	Nasopharyngeal (NP) swab, aspirate or wash, nasal swab, aspirate or wash, throat swab.	These tests are currently not preferred.
Rapid molecular assay (influenza viral RNA or nucleic acid detection).	NP swab, nasal swab	For point-of-care use.
Immunofluorescence, direct (DFA) or indirect (IFA) fluorescent antibody Staining (antigen detection).	NP swab or wash, bronchial wash, nasal or endotracheal aspirate.	—
Molecular testing: RT-PCR (singleplex and multiplex; real-time and other RNA-based)	NP swab, throat swab, NP or bronchial wash, nasal or endotracheal aspirate, sputum.	These are confirmatory tests.

STUDENT ASSIGNMENT

MULTIPLE CHOICE QUESTIONS

1. **What is the primary aim of a blood bank?**
   a. To store medical equipment
   b. To provide safe and adequate blood products
   c. To conduct medical research
   d. To diagnose diseases
2. **What type of donor recruitment is based on social pressure from friends or colleagues?**
   a. Voluntary-based
   b. Social persuasion-based
   c. Remunerated-based
   d. Compulsory-based
3. **Which type of donor's blood is considered unsafe and hazardous?**
   a. Voluntary donors
   b. Socially persuaded donors
   c. Paid professional donors
   d. Emergency donors
4. **What should be done to alleviate a donor's fear during blood donation?**
   a. Ignore their fears
   b. Convince them that it is painless and harmless
   c. Provide financial incentives
   d. Avoid communication with the donor
5. **Which group is considered at high-risk for HIV transmission?**
   a. Blood sellers
   b. Children under 12
   c. Vaccinated individuals
   d. Athletes
6. **What is the minimum age requirement for blood donation?**
   a. 16 years
   b. 18 years
   c. 21 years
   d. 25 years
7. **What should be the interval between two whole blood donations?**
   a. 4 weeks
   b. 8 weeks
   c. 12 weeks
   d. 16 weeks
8. **What is the acceptable minimum hemoglobin level for a donor?**
   a. 10 g/dL
   b. 11 g/dL
   c. 12.5 g/dL
   d. 13.5 g/dL
9. **What is the standard volume of blood collected from a donor weighing 45 kg?**
   a. 250 mL
   b. 350 mL
   c. 450 mL
   d. 500 mL