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Anju Dhir

Textbook of

Applied Microbiology

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Second Edition

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3 CHAPTER

Structure and Classification of Microbes

CHAPTER OUTLINE

- Methods Used in Classification
- Tools of Identification
- Classification

INTRODUCTION

Classification is a way of arranging a wide variety of organisms in nature. It is not possible to study the characteristics of each and every microorganism, therefore, we study the characteristics of a population of different groups of microorganisms by classifying them. The requisites for classification are:

- **Stability:** Every attempt should be made to devise classifications that need only minor changes as new information becomes available.
- **Predictability:** By knowing the characteristics of one member of a taxonomic group, it is possible to assume that the *other members of the same group probably have similar characteristics*.

METHODS USED IN CLASSIFICATION

Generally, three methods are used for arranging bacteria into Texas, which are as follows:

1. **Intuitive method:** It is *based on the properties of the organisms*.
2. **Numerical taxonomy:** This method of classification has great practical usefulness and is unbiased also. Equal weightage is given to each characteristic of microorganism. By using the computational methods, one can calculate the similarity percentage of each strain.

   For any two strains,

   $$\%S = NS/(NS + ND) \text{ (For 2 strains)}$$

   Here,

   NS is the number of characteristics that are similar,
   ND is the number of characteristics that are different,
   %S is percentage of similar characters.
3. **Genetic relatedness:** It depends on the genetic relatedness between organisms. This method is stable and more predictable. It is most objective and is based on the hereditary material, i.e., DNA. Table 3.1 summarizes different criteria used in classification of bacteria.

TOOLS OF IDENTIFICATION

Biochemical testing: It is based on:

- The nutrient requirements and *'metabolic byproducts'* of a particular microorganism.
- The physiological behavior of the organism under observation.

Dichotomous keys series: The tests are done in a logical order and each test result indicates next test to be done. The collective results of multiple tests create a profile allowing identification of microorganisms (Fig. 3.1).

TABLE 3.1: Different criteria used in the classification of bacteria

Norms of classification	Examples	Benefits
Genetical Make up	% of G and C ratio along with DNA hybridization	It determines the relatedness within genera and families
Physiological behavior	pH, temperature, concentration of salts required to grow, osmotic pressure, light energy, sensitivity toward antibiotics, etc.	Helps in distinguishing species, genera or higher orders
Biochemistry	Nature of cellular components like RNA, inclusion bodies, different pigments, nuclear material and cell wall	Helps in distinguishing species, genera and higher groups of bacteria
Serology	Fluorescent antibody technique and slide agglutination	Distinguishes strains and species
Sequence of bases in tRNA	tRNA sequencing	Shows relatedness among all living things
Phage typing	Susceptibility of cells to a group of bacteriophages	Distinguishing and identification of strains
Protein profiles	Separation of proteins by electrophoresis	Distinguishes different strains
Morphology	Size and shape of cells, arrangement, presence or absence of flagella, pili, capsules and endospores	First level of separation into genera and species
Growth	Characteristics of growth in solid and liquid media	Distinguishes into species, genera and higher groups of organisms
Staining	Gram staining, acid-fast staining or other differential staining techniques	Separate bacteria into main divisions
Nutrition	Autotrophs or heterotrophs and the kind of source energy is used by them like carbon, nitrogen or sulphur, etc.	Distinguishes species, genera and higher groups of organisms

Abbreviations: C, cytosine; DNA, deoxyribonucleic acid; G, guanine; tRNA, transfer ribonucleic acid

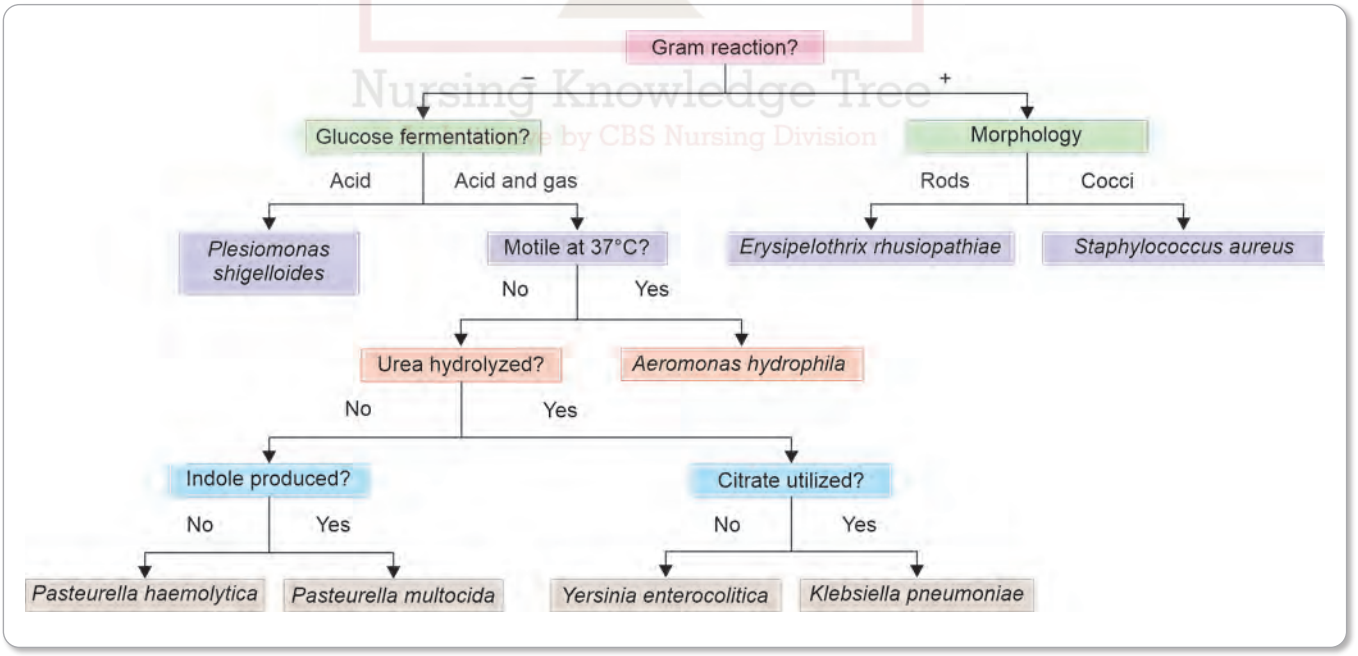


Fig. 3.1: Dichotomous key

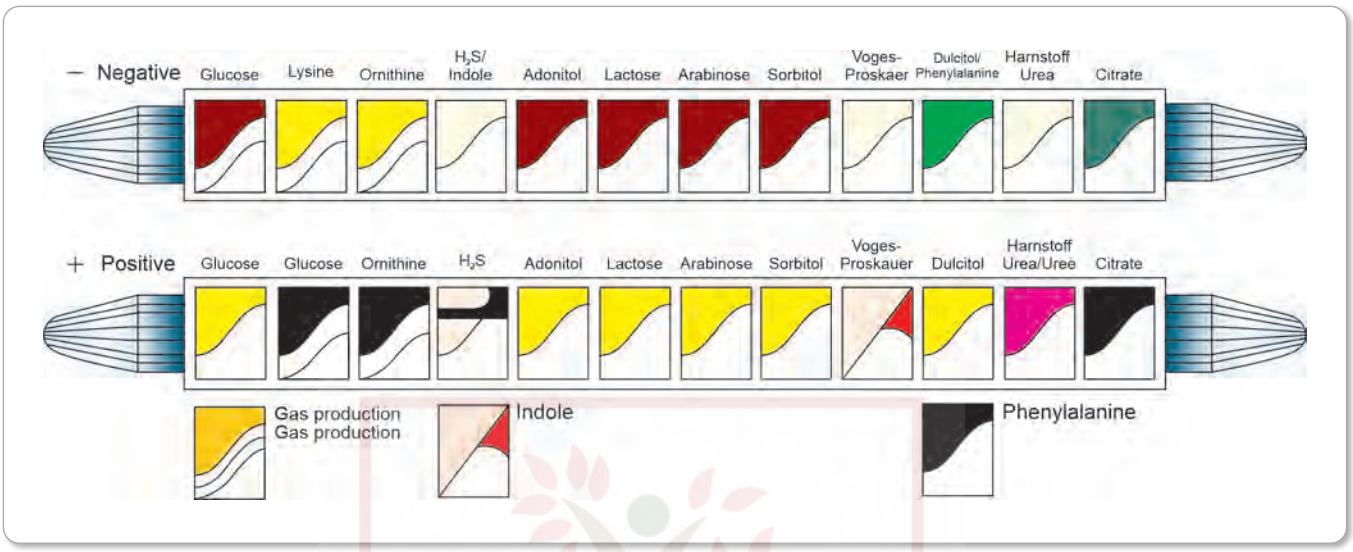


Fig. 3.2: Enterotube II

- ***Enterotube:*** In order to identify microorganisms, one of the *commercial devices* for rapid identification is Enterotube II (Fig. 3.2). It performs multiple tests simultaneously. Results are obtained within 24 hours and the identity of microorganism is revealed.
- **Serology:** Differences in antibody reactivity toward an antigen also reveal different bacterial strains.
- **Phage typing:** When different test phage samples are applied (in dots) to the surface of bacteria (to be tested) grown on agar plates, clear zones appear where bacteria have been infected and killed after 24 hours. Profile of phage sensitivity reveals the identification of bacteria.
- **DNA base composition:** Members of the same genera or species have nearly identical DNA sequences, and hence, the same proportions of G/C base pairs and A/T base pairs because the base pair, G = C and A = T.

  G/C + A/T = 100%

  (e.g., if G/C = 40%, then A/T = 60%)

  By determining the G/C content of the DNA from a test organism and comparing this to known values, is a quick way to identify microorganisms.
  - If %G/C is different, the microorganism cannot be a match.
  - If %G/C is same, there might be a match, but additional testing is necessary to confirm the identity.
- **DNA hybridization:** It is done on the basis of capability of separated DNA strand to make another complementary strand according to the base pairs. With enough heat, DNA strands are separated. It is cooled down and it allows complementary strands to make base pairs. This technique is used in a variety of ways to see if DNA from two different sources are similar or not. Usually, the DNA from one source is immobilized, the other is labeled to allow detection of complementary base pairs.
- **Ribosomal RNA comparison:** Prokaryotic ribosomes contain three different rRNA molecules—large subunit contains 23S and 5S rRNA, and small subunit contains 16S rRNA sequence which is typically used for ribotyping. The sequence on 16 rRNA is highly specific, therefore, a degree of difference reflects that the organism is evolutionary apart. This method is primarily used for classifying prokaryotic microorganisms.

CLASSIFICATION

So far 1.7 million organisms have been identified on the earth. All cellular organisms *evolved from a common ancestor* and it is suggested because of similar plasma membrane, using adenosine triphosphate (ATP) for energy and using DNA for genetic storage. They later evolved in different domains due to random mutation and natural selection (Fig. 3.3).

Gene sequencing now allows for more accurate and precise placement of organisms in the taxonomic pyramid of relatedness. The rRNA sequences show three distinct groups of life:

1. Bacteria
2. Archaea
3. Eukarya—protists, fungi, animals and plants

(Prior to sequencing, Bacteria and Archaea had been grouped together in the kingdom Monera).

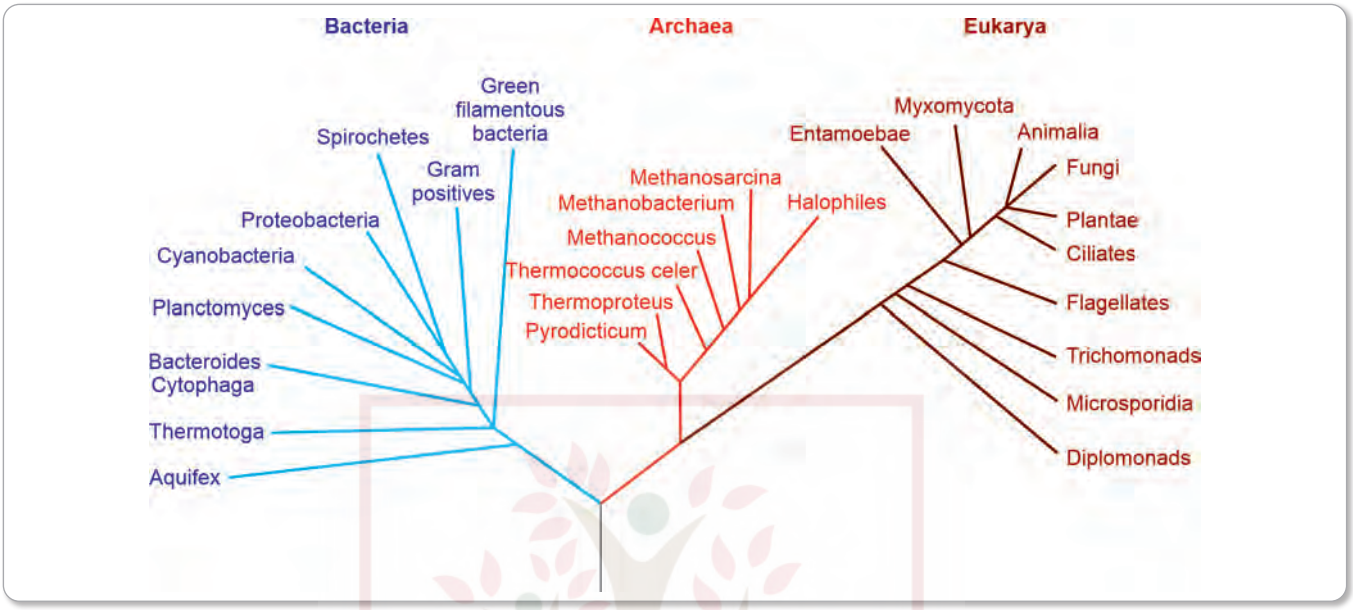


Fig. 3.3: Schematic representation of three domains of life: Bacteria, Archae and Eukarya

Bacteria

Bacteria are single-celled, microscopic living organisms. The bacterial classification not only helps in studying them in a systematic way but also helps in getting hold of treatment of a particular disease because mostly, the bacteria showing similar characteristics are sensitive to same type of chemotherapeutic drugs. Bacterial classification based on different factors is discussed here as follows.

Classification Based on Staining

This is based on the difference lying in the structure of bacterial cell wall (Table 3.2). The bacterial cell wall is composed of lipopolysaccharide (lipid), peptidoglycan (protein) and carbohydrates. Difference lies in the different ratio of lipopolysaccharide and peptidoglycan among different bacteria. Gram staining helps in categorizing bacteria in two broad classes as Gram-positive and Gram-negative.

1. **Gram-positive bacteria**, have a thick layer of peptidoglycan. The Gram-positive bacteria *do not let the primary stain to be removed* from their cell wall when decolorized and appear as violet colored organisms under the microscope.
2. **Gram-negative bacteria**, have less amount of peptidoglycan and more lipopolysaccharide in cell wall. They get decolorized and *primary stain is not retained* by their cell walls and appear as pink organisms due to the color of counter stain, Safranin.

TABLE 3.2: Different microorganisms and staining methods for microscopic study

Microorganisms	Methods of observation under microscope	Reasons
Coliform bacilli and cocci	Gram staining method	Difference in cell wall makes them fall into two main categories: Gram-positive and Gram-negative
Treponema pallidum	Fluorescent antibody technique or dark field microscopy	Too thin and delicate to make smears as is done in Gram staining
Mycobacteria: *M. leprae* and *M. tuberculosis*	Acid fast staining	Due to high content of fatty acids in cell wall simple dyes cannot penetrate
Mycoplasma pneumoniae	No staining technique	Cell wall is absent so staining cannot be done as it depends on presence of cell wall in an organism
Rickettsiae	Tissue stains like Giemsa staining	Very small intracellular microorganisms
Chlamydiae trachomatis	Inclusion bodies are observed under the microscope	Very small intracellular microorganisms
Legionella pneumophila	Difficult intake of safranin as counterstain	Prolonged period of time is required to counterstain

3. **Mycoplasma** are neither *Gram-positive* nor *Gram-negative* bacteria because they lack true cell wall. **Mycobacteria** have rigid cell wall and can be stained with acid fast staining. They can be stained by *acid fast staining* technique due to the presence of mycolic acid in their cell wall.
4. **Chlamydiae** and **Rickettsiae** are *Gram-negative* but due to their very small size and their existence as intracellular parasite, cannot be observed under microscope.
5. **Legionella** needs special induced application of *safranin dye* to show characteristics of Gram-negative bacteria.

Figure 3.4 shows the arrangements and shapes of bacteria.

Classification Based on Morphology

Cohn classified the bacteria in 1872, in following categories.

- **Cocci (In Greek 'kokkos' means berry):** Spheres or oval in shape.
  - **Monococci:** Existing as single cells, e.g., *Monococcus flavus.*
  - **Diplococci:** When the cell divides in such a way that after division the cells do not separate from each other, e.g., *Diplococcus pneumoniae.*
  - **Staphylococci:** Cell division occurs in three planes and cells do not separate from one another, instead they arrange themselves as bunches of grapes, e.g., *Staph. aureus.*
  - **Streptococci:** Cells do not separate from each other and form a chain, like *Streptococcus pyogenes.*
  - **Tetracocci:** When the cells are arranged in groups of four due to cell division taking place at right angles, e.g., *Gaffkya tetragena.*
  - **Sarcina:** Cell division is in three planes and it arranges the cells in the form of a cube, e.g., *Sarcina lutea.*
- **Bacilli (rod-shaped):** These are straight rod-shaped microorganisms.
  - **Diplobacilli:** When these exist as two cells.
  - **Palisades:** When arranged in groups of four or more.
  - **Streptobacilli:** In chains.
- **Vibrio:** Comma-shaped and curved bacteria, e.g., *Vibrio cholerae.*
- **Spirilla:** These are helically curved rigid rods, e.g., *Spirillum minus.*

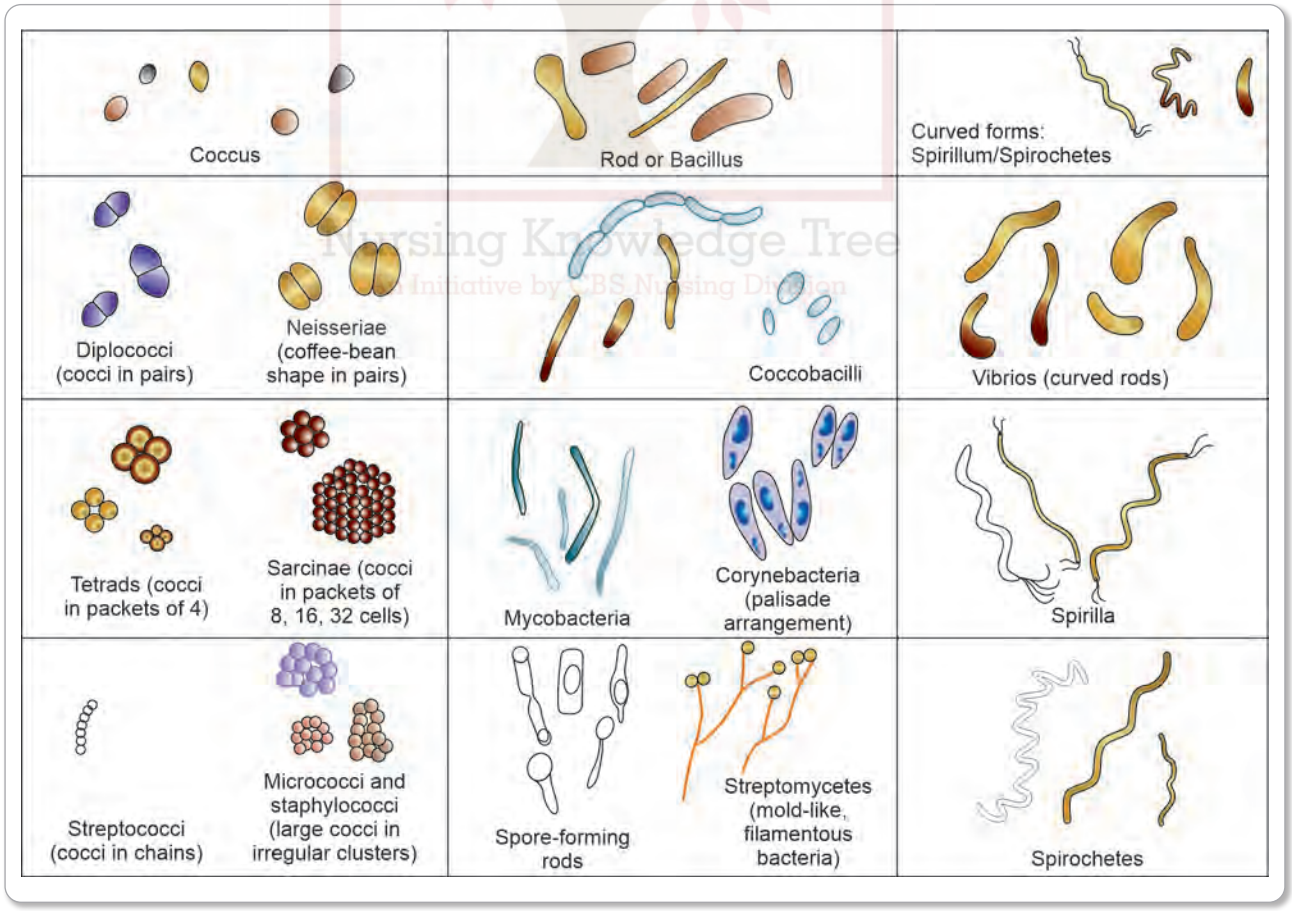


Fig. 3.4: Arrangements and shapes of bacteria

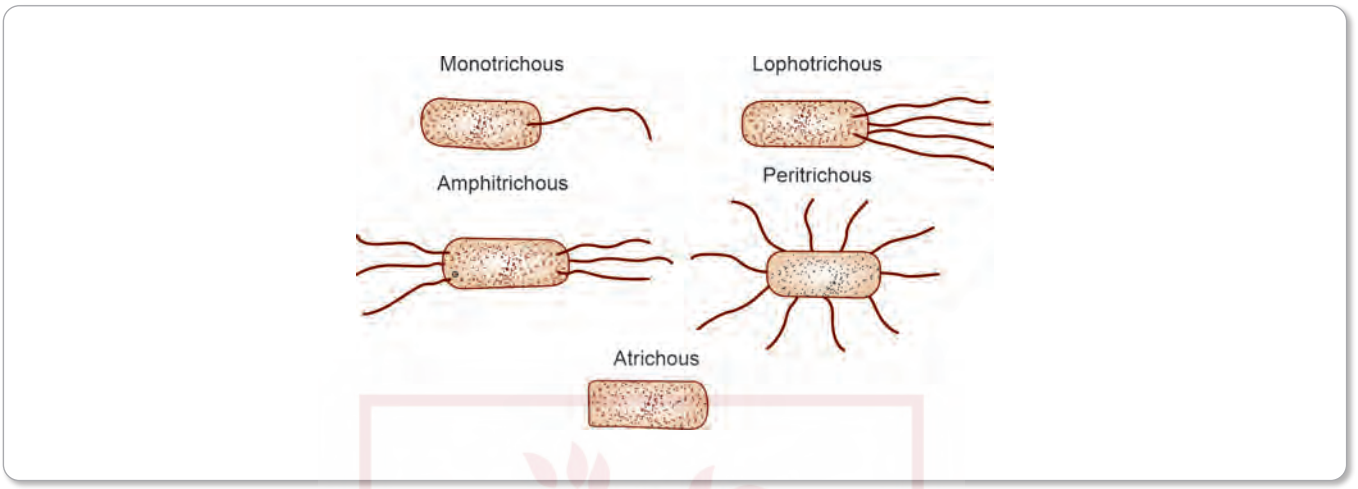


Fig. 3.5: Types of bacteria based on number and position of flagella

- **Spirochetes**: These are the rods with highly flexible forms, e.g., *Treponema pallidum*.
- **Coccobacilli:** These bacteria are intermediate between cocci and bacilli, e.g., *Brucella*.
- **Actinomycetes:** These are bacteria having branched filaments, e.g., *Actinomycetes meyeri*.
- **Mycoplasma:** These are wall-less organisms and have no fixed shape, e.g., *M. pneumoniae*.
- **Chlamydia:** These organisms resemble viruses in size and reproduce only inside the cells. They are obligate intracellular parasites, filterable and fail to grow in cell-free media. They possess both DNA and RNA, e.g., *Chlamydia trachomatis*.

Classification Based on Presence of Flagella

The flagella are used for movement by the bacteria and are rooted in the cell membrane. On the basis of position of flagella, the bacteria can be classified as: (Fig. 3.5)

- **Atrichous:** When the bacteria do not possess flagella and are, therefore, non-motile.
- **Monotrichous:** When the bacteria have a single flagellum, e.g., *V. cholerae*.
- **Amphitrichous:** Bacteria have flagella situated at two ends of the cell, e.g., *Alcaligenes faecalis*.
- **Peritrichous:** Which has the flagella arranged on all over the cell, e.g., *S. typhi*.
- **Lophotrichous:** When the flagella form a tuft at one particular point.
- **Polytrichous:** Bacteria have a number of flagella at different locations on the cell.

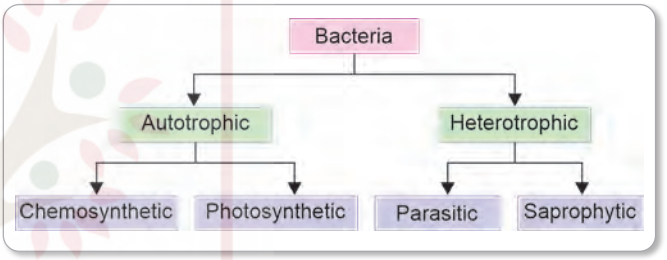


Fig. 3.6: Classification based on nutrient requirements

Classification Based on Nutrient Requirements (Fig. 3.6)

(*Given in detail in Chapter 6*)

Classification Based on Existence at Different Temperature

(*Given in detail in Chapter 6*)

Classification Based on Oxygen Requirement

(*Given in detail in Chapter 6*)

Classification Based on Hydrogen Ion Concentration

(*Given in detail in Chapter 6*)

Classification Based on Medical Importance

The bacteria can be classified as extracellular and facultative parasites or as obligate parasites (Fig. 3.7).

The **facultative pathogens** can be ***Gram-positive*** **such as** cocci like, Staphylococci and enterococci, or bacilli

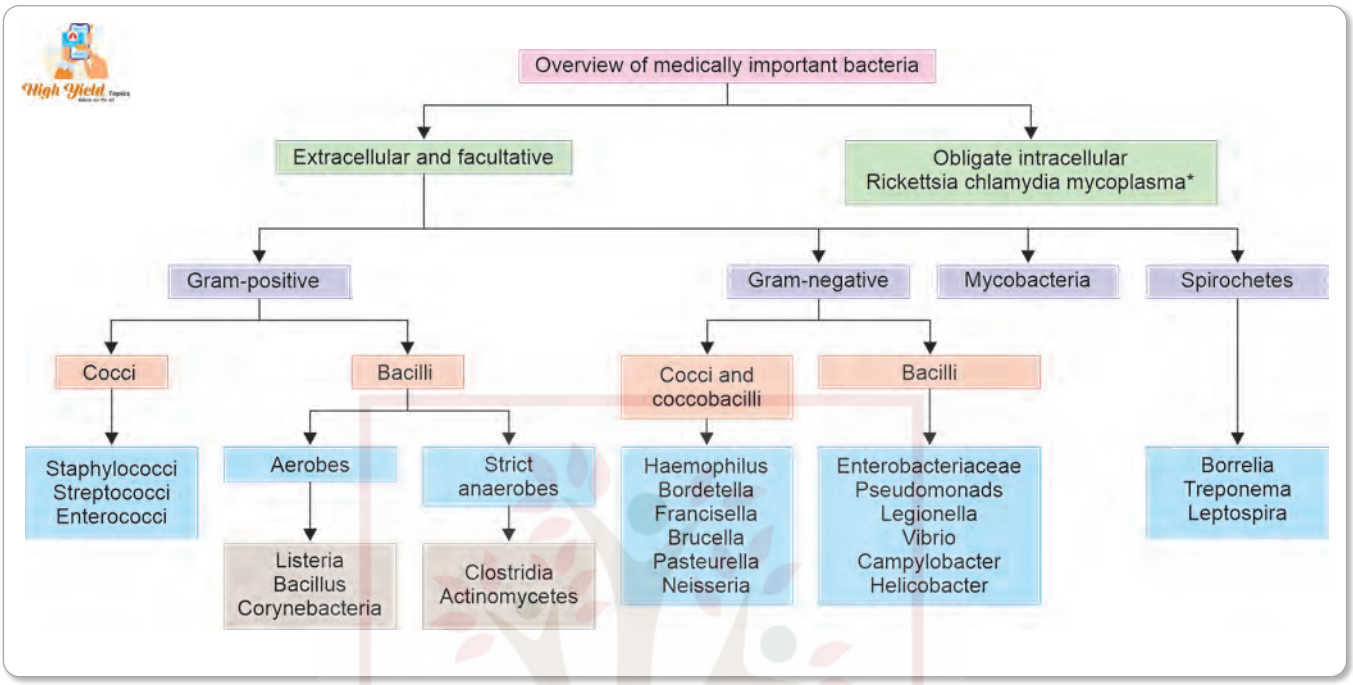


Fig. 3.7: Medically important bacteria

like, Corynebacterium. They can be ***Gram-negative*** such as cocci like Brucella and Bordetella, and bacilli like, Vibrio and Pseudomonas.

The spirochetes like, Borrelia, Treponema and *Mycobacterium tuberculosis* are **obligate human pathogens.**

- **Prokaryotic species:** It is defined as a population of cells with similar characteristics (no sexual reproduction).
- **Pure culture:** These are clones/populations derived from a single cell that are genetically identical.
- **Strains:** Each culture or group that is slightly different is called a strain. For example, *Escherichia coli* (*E. coli*)—normal intestinal flora *E. coli* 0157:H7 that produces a toxin which all other stains do not produce and is a deadly pathogen of humans.

Archaea

These include all prokaryotes with walls that do not have peptidoglycan. They often carry out unusual metabolism and live in extreme environmental conditions. There are no kingdoms to which it belongs.

Eukarya

Domain eukarya has four kingdoms:

1. **Kingdom Protista (unicellular eukaryotes):** These are simple eukaryotes which do not fit elsewhere and are nutritionally diverse: Autotrophs, heterotrophs, intracellular parasites. For example, algae and protozoa.
2. **Kingdom Fungi:** They absorb organic material through the plasma membrane and are mostly saprophytic, e.g., yeasts, molds and mushrooms.
3. **Kingdom Animalia:** They are multicellular animals and ingest organic food through the mouth. The cells are organized into tissues.
4. **Kingdom Plantae:** The multicellular plants are included in this kingdom. They undergo photosynthesis to convert $CO_2 + H_2O$ into organic molecules. The cells are organized into tissues.

Viruses

The viruses do not fit the domain system as they are acellular and usually classified by Family and Genus. They are usually referred by common name, e.g., human immunodeficiency virus (HIV), Genus—Lentivirus, Family—Retroviridae.

Viral species: It is defined as a population of viruses with similar characteristics (including morphology, genes and enzymes) that occupy a particular ecological niche.

Viruses have developed themselves to stay as intracellular parasites. They usually only infect one type of cells, which are the one that best supports the viral replication.

The viruses tend to be very specific about their preference site of infection, e.g., HIV infects only human T helper cells.

ASSESS YOURSELF

Long Answer Questions

1. What are the different criteria used for classification of bacteria? Discuss the different tools of classification.
2. What are the different ways of classifying bacteria?

Short Notes

Write notes on:

1. Classification based on pathogenicity
2. Classification based on morphology

Multiple Choice Questions

1. The organism's name is in:
   a. English b. Latin
   c. French d. None of these
2. Name the apparatus used to put test in one place to identify an unknown organism is:
   a. Enterotube II b. Enterotoxic tube
   c. Both (a) and (b) d. None of these
3. Bacilli are:
   a. Rods b. Cocci
   c. Coccobacilli d. Spirilla
4. The name of organism has a specific pattern:
   a. First genus and then species
   b. First species and then genus
   c. Both (a) and (b)
   d. None of the above
5. On the basis of rRNA, the three domains of life are:
   a. Bacilli, cocci and spirilla
   b. Bacteria, archaea and eukarya
   c. Bacteria, algae and fungi
   d. None of the above
6. % Similarity (%S) of each strain to every other strain is calculated by which method?
   a. Intuitive method
   b. Numerical taxonomy
   c. Genetic relatedness
   d. DNA homology experiments
7. Two organisms which are very closely related to each other have which of the following property?
   a. Similar mol% G+C values
   b. Different mol% G+C values
   c. Similar mol% G+C values and heteroduplexes are formed
   d. Different mol% G+C values and heteroduplexes are not formed
8. Which among the following kingdoms were proposed by Whittaker?
   a. Monera
   b. Protista, Fungi
   c. Plantae, Animalia
   d. Monera, Protista, Fungi, Plantae, Animalia

Answer Key

Multiple Choice Questions

1. b **2.** a **3.** a **4.** a **5.** b **6.** b **7.** c **8.** d

UNIT V

Review of Specimen Collection

The specimens to be analysed in a lab are collected and transported according to a guideline. The process followed to collect sample must be carefully so that false or incorrect lab results are avoided. The chapter given here discusses the principles of sample collection along with rules followed by a healthcare personnel during clinical sample collection.

LEARNING OBJECTIVE

After going through this unit, you will be able to:

- Discusses on what, when, how, why specimens are collected to optimize the diagnosis for treatment and management.

UNIT OUTLINE

The chapter included in this unit is as follows:

36
CHAPTER

Specimen Collection and Transportation

CHAPTER OUTLINE

- Principles of Specimen Collection
- General Rules for Specimen Collection
- Collection Techniques and Special Considerations
- Types of Clinical Specimen
- Staff Precautions

INTRODUCTION

Specimen collection is the *first step* of interaction between the patient and the laboratory. Before collecting specimen, appropriate counseling should be done and consent is taken. *Any error in specimen collection can lead to inaccurate results.* It is, therefore, an important step for a clinical laboratory (pre-analytical control). Specimen collection can be done at the *patient's bedside*, in the *laboratory* or in the *field* with the help of trained staff.

PRINCIPLES OF SPECIMEN COLLECTION

Collect specimen:

1. At the correct time (stage of infection)
2. In correct way (keep in mind the route)
3. From the correct site of infection
4. In sufficient volume to carry out all required tests
5. Correctly label with name, date, type, age, sex, etc.

GENERAL RULES FOR SPECIMEN COLLECTION

The specimen can be collected according to the following rules:

First step: Site selection and time of collection:

1. *Who* will take the sample and where? Representative and place.
2. *When*: In acute phase of the disease, before antimicrobial therapy and in start of the day.

Second step: Contamination should be avoided from normal and transient flora.

Third step: Type of specimen depends on the site from which sample is collected like tissue section, aspirate, etc. Two samples are taken one for culture and another for antigen detection or staining.

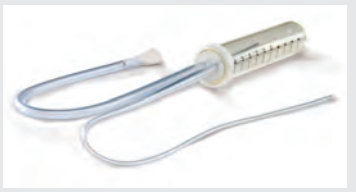
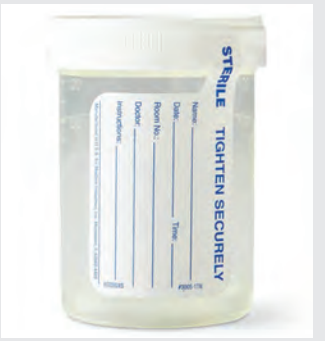
Fourth step: *Quantity* of specimen may vary according to requirement.

Fifth step: ***Specimen collection appropriate containers***—must be sterile and leak proof. Containers must be made of translucent and clear material. They should have screw cap with water tight seal to avoid spillage. The labeling could be done easily on its walls.

Sixth step: ***Labeling*** - It includes, name of patient, ID, site, date and time, initials of the collector, history, and diagnostic tests that are requested.

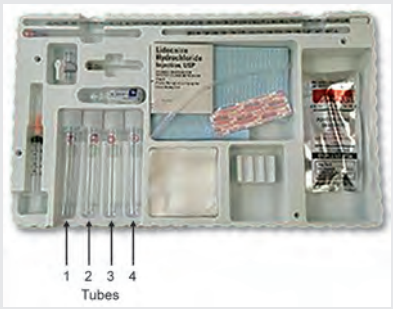
Containers for Sample Collection are shown in Table 36.1 and Commonly Used Blood Collection Tubes are enlisted in Table 36.2.

TABLE 36.1: Containers for sample collection

	Urine collection container
	Stool collection container
	Urine bottles
	Mucus specimen traps
	Suction catheter with mucus trap (non-vented, 20 mL graduated)
	Click seal sterile specimen container
	Pneumatic tube system specimen container
	Leak resistant sterile specimen container
	Commode specimen container
	Mucus specimen trap
	Pediatric urine specimen kit
	Swab collection kit

Contd...

Contd...

	CSF collection kit
	Clean catch collection kit
	Respiratory virus sample collection kit
	Fecal swab collection kit
	Anaerobic tissue transport kit

Contd...

	MRSA/MSSA PCR and Group B Strep testing Copan dual swab
	BD Vacutainer – no additive
	Anaerobic blood culture bottle
	Aerobic blood culture bottle

TABLE 36.2: Commonly used blood collection tubes

Stopper color	Used for	Additive used
Red (RT)	Collection of serum for chemical, serological and bacteriologic studies. May be used for any procedure requiring serum except HLA antibody tests.	Sterile, no anticoagulant or additives.
Special Red (RT) (7 mL)	Can be used for collection of serum for HLA antibody screen/PRA and platelet-specific antibody screen.	-
Gold (GT)	• Collection of serum for chemistry studies. • Not for use for blood bank procedures	-
Lavender (LT)	Primarily for collection of blood bank procedures, hematology studies and certain chemistries.	Sterile, contains EDTA (Ethylene Diamine Tetra Acetate) as the anticoagulant.
Blue (BLT)	Tube calibrated to draw only 4.5 mL of blood. Primarily for collection of samples for coagulation studies.	Sterile, contains sodium citrate (0.109M, 3.2%) solution as the anticoagulant.
Gray (GYT)	• Used for the collection of glucose and lactate samples. • Not suitable for enzymes or electrolytes.	Sterile, contains potassium oxalate and sodium fluoride as the anticoagulant.
Green (GRN)	• For collection of other miscellaneous studies. • Electrolytes, glucose, Blood Urea Nitrogen can be performed more quickly than from a red top • Especially useful for patients in Diabetic Ketoacidosis.	Sterile, contains lithium heparin as the anticoagulant.
Special Green (SGRN)	For collection of flow cytometry specimens.	Sterile, contains sodium heparin as the anticoagulant
Yellow (YT)	For determination of HLA-ABC antigens, HLA-B27, HLA Molecular Typing, G6PD levels and acid phosphatase levels.	Sterile, contains ACD (Acid Citrate Dextrose) as the anticoagulant.
Royal Blue (RBL)	For detection of trace metals (i.e., Arsenic, Zinc, etc.).	Sterile, contains no anticoagulant.
Pink (PT)	For detection of HLA antibodies in serum (CYTS).	Sterile, does not contain any anticoagulant, serum separator, or silicone coating.
Tan	For lead determinations. Tube inversions prevent clotting.	Sodium heparin (glass) or K_2EDTA (plastic). This tube contains less than 0.01µg/mL (ppm) lead.
Pearl Top (PPT, Plasma Preparation Tube) – (5 mL)	For viral load monitoring or viral detection	Contains 9 mg K_2EDTA.

COLLECTION TECHNIQUES AND SPECIAL CONSIDERATIONS

- Specimen must be collected under *aseptic conditions*.
- Collect specimen *before antibiotic therapy*. If this is not possible, specify antibiotic on requisition.
- *First morning specimens* are usually preferred, especially if urine or sputum specimens for TB is to be taken.
- Specimens collected on swabs are not acceptable for AFB or fungal cultures. The optimal specimen for anaerobic or aerobic culture is an aspirate or tissue.
- *Avoid contamination* of the sample with normal flora by decontaminating surface area prior to collecting the specimen. For urine sample, although midstream sample is taken but *single catheterized specimens are cleaner than midstream clean specimens. Deep wounds collected by aspiration are preferable to superficial swab collection.*
- Do not allow swabs to dry out. Follow instructions for proper use of specimen collection devices provided with each unit. Use *no fixatives or bacteriostatic agents* for cultures.
- To collect a specimen, *dry, sterile, leak proof container* that should be free from disinfectant, is required.
- Collection kits are available from the laboratory for ova and parasite examination. If preservatives are not used, specimens *must be sent to the laboratory within 1 hour* of collection.
- Whenever submitting body fluid for culture, send the fluid in a *sterile container* specifically for microbiology. *Do not inoculate blood culture media except for peritoneal fluid and also send un-inoculated fluid in a sterile container.*
- *Positive culture plates must be held for 7 days* in the event as non-routine antimicrobial susceptibility testing might be requested.

- Every laboratory test must be ordered *under the license of a physician* and must be *requested using the appropriate electronic ordering system*, e.g., EPIC, or on the appropriate requisition form.
- Mention the investigation required with clinical notes like signs, symptoms, abnormal test results, exposure to communicable diseases or other reasons.
- If the patient's information is missing, the specimen will be considered unacceptable.

Specimen Transportation

- **Time**
  - Immediately as soon as possible
  - Within 15 minutes in case of CSF and tissue sections
  - For hospital patients and on-site health care facilities, the specimen must be delivered to laboratory within 2 hours or earlier, if possible. *Deliver the specimen immediately if results are needed STAT* (Latin word—*statum, means immediate*).
  - Urine can be *refrigerated,* if delivery is delayed. Rapid delivery is especially important for CSF specimens, stools for ova and parasite examination (O&P examination), Neisseria cultures, and anaerobic specimens.
  - For off-site clinics or doctors' practices, various *transport systems* are available like, provision of transport tubes and transport media like media used for specimens suspected to have *Neisseria gonorrhoeae.*
  - In case of malaria, informing labs before collection of specimens is necessary, so that processing can begin immediately upon receipt of clinical sample.
- **Medium for transport**
  - It must keep organisms viable but must not promote their growth, e.g., Stuart's medium, Cary-Blair medium, anaerobic transport medium, etc.
- **Temperature**
  - Room temperature
  - If more than 2 hours' delay, then specimen can be stored by refrigeration
- **Mailing and shipping:** Before mailing the specimen is packed in:
  - Primary container
  - Secondary container
  - Mailing container
  - And a label of biohazard is put on it

Facteria

In countries like the U.S., **'ICD-10 code'** is also required in the request form. **ICD-10** is the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD). It has codes for diseases, signs and symptoms, abnormal findings, complaints, social circumstances and external causes of injury or diseases. The code set allows more than 14,400 different codes and permits the tracking of many new diagnoses. The WHO provides detailed online information about ICD along with ICD-10 training and study guide materials. For example, A00-B99 is code for certain infectious and parasitic diseases, C00-D48 is code for neoplasms, J00-J99 is for respiratory disease, etc.

TYPES OF CLINICAL SPECIMEN

Types of specimen collected for lab investigation are blood, urine, saliva, feces, spinal fluid, synovial fluid, amniotic fluid, pleural, pericardial and ascitic fluid, solid tissue, etc. Proper way of collecting different samples has been summarized in Table 36.3.

Blood

Blood sampling should be *done by a trained health care worker* who is competent in the procedures. There are many hematological, biochemical, immunological and microbiological blood tests to be performed, so appropriate laboratory containers are required for specific tests and the amount of blood required must be known to the health care worker. PPE must be used.

For *aerobic and anaerobic blood culture*, media set or vacutainer tube with *Sodium Polyanethol Sulfonate* (SPS) is used. *The blood is drawn from both arms so to have two different sites for collection of blood for aerobic and anaerobic blood culture.* The blood sample is transported to lab within 2 hours at room temperature. In case of delay, it is stored at 37°C in incubator.

Blood Sample for Culture

- Requires aseptic technique.
- Collected as early as possible after the onset of disease. In case of suspected endocarditis, three sets of blood cultures are done.
- The quantity of venous blood is collected in:
  a. Infants: 0.5–2 mL
  b. Children: 2–5 mL
  c. Adults: 5–10 mL

TABLE 36.3: Collection of samples/specimen

Specimen	Specimen transportation schedule	Patient's preparation	Storage instructions	Container	Volume
Blood	Within 2 hours at room temperature	Blood is aspirated in febrile period from both arms	Incubated at 37°C	Aerobic and anaerobic blood culture media sets In vacutainers	5–7 mL
Bone marrow	Within 2 hours at room temperature	Blood is aspirated in febrile period from both arms	Incubated at 37°C	Aerobic and anaerobic blood culture media sets In vacutainers	2–5 mL
Urine	Within 2 hours at 2°–8°C	Urine is collected after social hygiene as 'clean catch' sample	24 hours at 2°–8°C	Sterile screw capped wide mouth bottle or tube	5 mL
CSF	Immediately at room temperature	Collected after disinfecting the skin	6 hours at room temperature	Sterile screw capped wide mouth bottle or tube	1–2 mL
Sputum	Within 2 hours at 2°–8°C	Collected after deep coughing	24 hours at room temperature	Sterile screw capped wide mouth bottle or tube	1–2 mL and may vary
Swabs	Within 24 hours at 2°–8°C	Rubbing mucus membranes gently and quickly	72 hours at 2°–8°C	Placed in transport media in multiples of 4	Multiples of 4
Feces	Within 24 hours at 2°–8°C	Preferably morning sample	72 hours at 2°–8°C	In containers without preservative	5 mL
Tissue	Within 24 hours at room temperature	Infected portion of tissue is taken by biopsy	24 hours at room temperature	Anaerobic transport medium	Varies

Biopsy Material

Specimens like tissues from skin, muscles, kidney, liver, jejunum or brain are used for biopsy (Fig. 36.1). A sterile technique is required for all these procedures. The *most appropriate specimen from the selected site is taken* so as to undertake laboratory tests. The specimen should be in adequate amount. *In case of microbiological investigation, formalin is not used to preserve the specimen.*

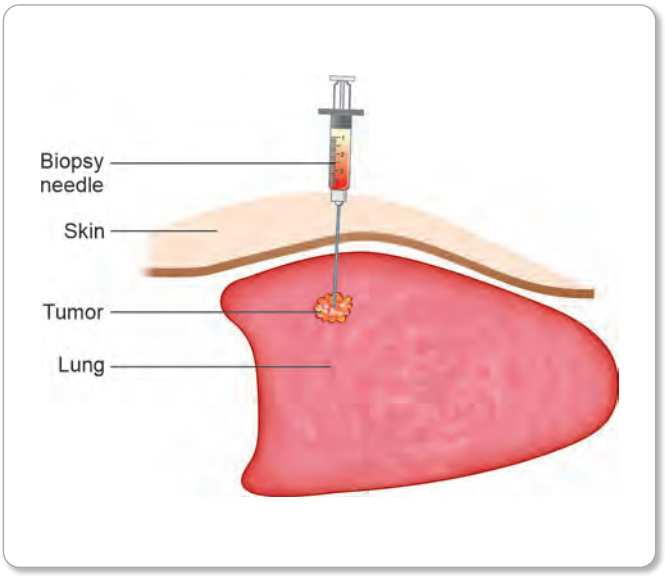


Fig. 36.1: Biopsy

Cerebrospinal Fluid

For accurate diagnosis of encephalitis and infective meningitis, sampling of cerebrospinal fluid (CSF) is necessary. CSF is obtained via a lumbar puncture performed by trained medical staff using a *sterile technique in a sterile screw capped tube or bottle.* Specimens of CSF should be dispatched to the laboratory immediately at room temperature. It can be stored for 6 hours at 37°C with 5% CO_2. *It is important not to store the specimen in a refrigerator as the cells may deteriorate or lyse giving rise to misleading results* (Fig. 36.2).

Ear Swabs

No antibiotics or other therapeutic agents should have been used in the aural region for about 3 hours prior to sampling the area as this may inhibit the growth of organisms. If there is purulent discharge, then place a sterile swab into the outer ear and gently rotate to collect the secretions (avoid damaging the eardrum). Now it can be transported in transport medium.

Nose Swabs

If the nose is dry, moisten the swab with sterile 0.9% saline solution and insert the swab into the anterior nares and direct it up into the tip of the nose and gently rotate. Both nares should be swabbed using the same swab to obtain adequate material. Place it in transport medium and send it to the lab within 24 hours.

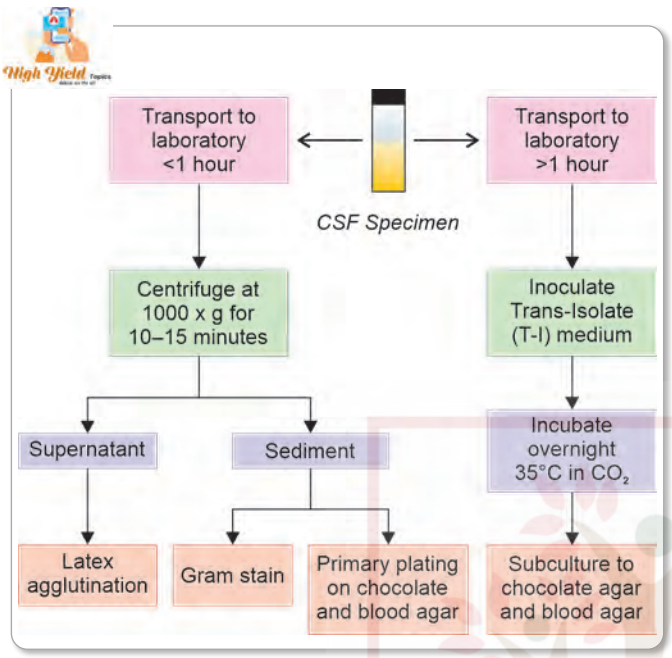


Fig. 36.2: Processing of cerebrospinal fluid specimen

Throat Swabs

By placing patient in a position with a good light source (to ensure maximum visibility of the tonsils), depress the tongue with a spatula. Quickly but gently, rub the swab over the tonsillar fossa or tonsillar bed and transport in transport medium. In case of delay, the specimen is *transported in Stuart's medium* to lab, otherwise it should be cultured on the same day to see β-hemolytic streptococci.

Throat/Oropharyngeal Swab Collection

- Gently tilt the patient's head back. Steady the chin.
- Ask the patient to open his/her mouth.
- Use a disposable tongue depressor to hold the tongue well. Insert a sterile swab.
- Swab both the tonsils and the posterior pharynx vigorously with a rotating motion, till the patient starts to gag.
- Remove the swab without touching the tongue. The swab is then placed in the labeled tube containing VTM
- The applicator stick is broken off at the indicated mark (if provided) or at below the level of the tube opening.
- Close and tightly screw cap the tube.

Specimen Collection Guidelines in COVID-19

(Indian Council of Medical Research—National Institute of Epidemiology)

Responsibilities of:

Clinician:

- To identify the appropriate patient for COVID-19 testing.
- To identify the appropriate specimen to be collected.

Lab technician:

- Should have received appropriate training for sample collection from suspected SARI/COVID-19 cases.
- To collect appropriate samples as decided by the treating doctor.
- To wear proper PPE as prescribed by NIV Pune.
- To strictly follow biosafety measures during collection and packing of samples and to follow the biomedical waste management guidelines for safe disposal of generated bio-waste.

Materials required: Materials needed for the sample collection: Nasal and Throat swab Specimen Collection Details – COVID-19 and Omicron and Equipment Required for Collecting COVID-19 and Omicron Specimen are given in Tables 36.4 and 36.5 respectively.

TABLE 36.4: Specimen collection details—COVID-19 and Omicron
(Adapted from the WHO Guidelines on 2019)

Specimen type	Collection materials	Transport to laboratory	Storage till testing	Comment
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs*	4°C	≤5 days: 4°C >5 days: –70°C	The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load.
Bronchoalveolar lavage	Sterile container*	4°C	≤48 hours: 4°C >48 hours: –70°C	There may be some dilution of pathogen, but still a worthwhile specimen
Tracheal aspirate, nasopharyngeal aspirate or nasal wash	Sterile container*	4°C	≤48 hours: 4°C >48 hours: –70°C	Not applicable
Sputum	Sterile container	4°C	≤48 hours: 4°C >48 hours: –70°C	Ensure the material is from the lower respiratory tract

Contd...

Specimen type	Collection materials	Transport to laboratory	Storage till testing	Comment
Tissue from biopsy or autopsy including from lung	Sterile container with saline	4°C	≤24 hours: 4°C >24 hours: –70°C	Autopsy sample collection preferably to be avoided
Serum (2 samples—acute and convalescent)	Serum separator tubes (adults: collect 3–5 mL whole blood)	4°C	≤5 days: 4°C >5 days: –70°C	Collect paired samples: • Acute–first week of Illness • Convalescent–2–3 weeks later
*For transport of sample for viral detection, use VTM (viral transport medium) containing antifungal and antibiotic supplements. Avoid repeated freezing and thawing of specimens.				

TABLE 36.5: Equipment required for collecting COVID-19 and Omicron specimen

Sl. No.	Item	Description/Details (if any)	Cat no. (If any)
1	Viral transport medium (VTM)#	Hi-Media	AL167
2	Polyester tipped plastic shaft swab	Fisher Brand	23-400-111
		Hi-Media	PW1180
3	Flocked Swab	Hi-Media	PW1172-1X500NO
		Copan	Ref 519CS01
4	Tongue depressor	Disposable	
5	Face mask	N95	
6	Apron	Disposable apron	
7	Gloves	Disposable nitrile gloves	
8	Hand sanitizer	Alcohol based rub	
9	Discard bag	Appropriate biohazard discard bag	
10	Cool box	Thermocol box	
11	Gel packs	To be frozen at –20°C prior use	
12	Head cap	Disposable	
13	Goggle	Reusable lab goggle	
14	Brown tape	Sealing the sample box	
15	Surgical spirit	Disinfect sample collection area	
16	Cryo label	Sample labeling	

Note: #VTM precautions: Ready to use VTM to be used within the expiry date. Always check for turbidity or any growth in VTM prior to use for sample collection or before dispatch of VTM to surveillance site. If any turbidity or growth is found, please discard the whole batch of VTM.
Note: Use of cotton-tipped or calcium alginate swabs or swabs with wooden shafts are **not recommended**.

Current Testing Modalities for Laboratory Diagnosis of COVID-19

- Real Time PCR testing is the recommended testing for COVID-19 diagnosis.
- Viral RNA is extracted from the nasal/throat swab specimen in VTM.
- Initial Screening RT PCR involves detection of 'E' gene (coding for SARSCoV-2 viral envelope).
- Confirmation of samples positive in screening PCR involves detection of one of the following two gene targets:
  - RdRp gene (coding for SARS-CoV-2 RNA dependent RNA polymerase).
  - ORF gene (coding for SARS-CoV-2 Open Reading Frame).

Summary of **Rapid antibody based blood test in** COVID-19 is given in Figure 36.3.

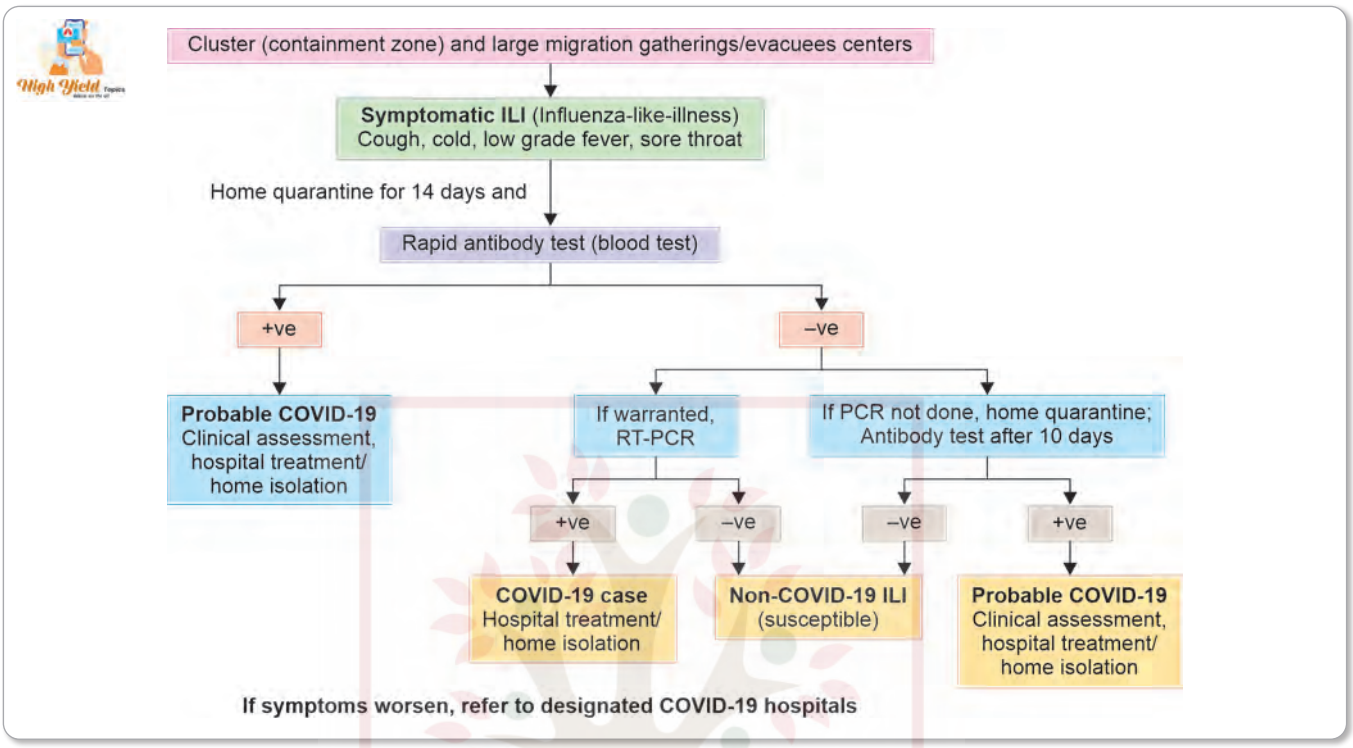


Fig. 36.3: Rapid antibody based blood test in COVID-19

Note *COVID-19 donning and doffing is given in chapter on Standard Safety Measures.*

Steps to be followed for Specimen Collection (using Aseptic Method): Nasal swab and/or throat swab specimens are to be collected as per GOI guidelines.

- Sample collection area should be kept clean and table or work surface should be properly disinfected with surgical spirit.
- Collect required details from the patient and fill the sample collection form.
- Ensure that patient is seated in a comfortable seating position.
- Clearly explain the sample collection procedure to ensure full cooperation from the patient.
- Wear appropriate personal protective equipment (PPE) such as gloves, apron, N95 mask, head cap, goggles before starting the procedure.
- Label the specimen collection vial containing VTM with the unique participant/sample ID.
- Specimen should be collected under good illumination.

Nasal Swab

- Take a fresh sterile swab.
- Gently tilt the patient's head backward and steady the chin.
- Insert the swab into the nostril parallel (1–2 cm) to the palate until the resistance is met at turbinate.
- Hold the swab in that position for a few seconds and then withdraw slowly in a firmly rotating motion. (five times clockwise and five times anticlockwise).
  - Appropriate precautions should be taken in collecting specimens since this may expose the lab technician/ sample collector to respiratory secretions from the patient.
  - Specimens from both nostrils are obtained with the same swab vigorously, irrespective of nasal congestion if any.
  - The nasal swab is placed in the same VTM tube containing throat swab. The applicator stick is broken off as done for throat swab and the tube is screw capped tightly.
  - The vial is then placed in the cool box containing tube rack in between the frozen gel packs.

Sample Handling at Collection Site

- A unique specimen ID is written/pasted on each VTM sample by the lab technician.
- VTM containing samples are to be kept in cool box immediately after collection.
- VTM tube IDs are cross checked with the details in the filled sample collection form.

- If the specimens cannot be sent to the laboratory within specified time frame, they should be stored at or below –70°C in ultra-low freezer. Repeated freezing and thawing must be avoided.

Transportation of Samples from Field Site to Laboratory

- Samples should be transported at 2°–8°C within specified time frame to the testing laboratory.
- Samples should be kept in proper standing position in appropriate test tube rack.
- The VTM containing part of the tube should be in direct contact with frozen gel packs.

Precautions

Always use PPE, e.g., laboratory apron/ gown, face mask, gloves and goggles. Wipe gloves thoroughly with a disinfectant (e.g., surgical spirit) before and after taking the sample.

Eye Swabs

Clean the eye first with sterile normal saline to obtain a clear view of the conjunctiva. With the help of sterile cotton wool swab, take the specimen by rolling the swab over the conjunctival sac. Place the swab in the transport medium—Stuart's medium. The eye swab is helpful in diagnosing infections such as *Chlamydia trachomatis.*

Rectal Swab

Swab is collected from the anal region by inserting 2.5 cm past anal sphincter and transported in a transport medium within 24 hours at 2°–8°C. For the identification of *Enterobius vermicularis*—threadworm or pinworm infection, specimen should be obtained in the morning by *using a clear adhesive tape* such as cello tape and a slide. Place the sticky side of a strip of tape over the anal region to obtain the material and stick the tape smoothly onto a glass slide. The worm can then be identified under the microscope. (Thread-worms lay their ova on the perianal skin at night and, therefore, are not seen in a fecal specimen).

Stool/Feces

A fecal specimen is more suitable than a rectal swab. The *morning sample* is preferred. A clean leak proof screw capped wide mouth container is chosen for sample collection. The specimen is transported to lab within 24 hours at 2°–8°C or can be stored at same temperature for 72 hours.

Vaginal Swabs

For investigation of simple vaginal discharge by using *charcoal swab*, gently insert the swab into the outer entrance of the vagina. Care must be taken not to tear the hymen. Place the swab into the transport medium and send to the lab.

Hair, Nail and Skin

Samples of infected hair, nails or deep scrapings should be removed by plucking the hair with forceps or gloves. The root of the hair is infected not the shaft. Samples of the whole thickness of the nail or deep scrapings or epidermal scales could be taken as a specimen after disinfecting the site. The specimen is *collected in anaerobic transport media* and transported within 24 hours at room temperature to the lab.

Sputum

Sputum is collected in sterile screw capped, wide-mouthed bottle after asking the patient to deep cough. The specimen is transported to lab within 2 hours at room temperature. If delay in transportation is inevitable, then sample could be stored at room temperature for 24 hours.

When it is difficult to obtain sputum in sufficient quantity as in *case of children who swallow sputum, gastric washings* are obtained for laboratory analysis to aid diagnosis of pulmonary *Mycobacterium tuberculosis*.

Urine

Bedside urine testing for the presence of blood, protein, etc., is usually undertaken with *reagent strips*. The results obtained from this indicate whether further laboratory investigation is required or not. The *normal social hygiene*, such as washing the genitalia with soap and water and drying thoroughly is considered sufficient to minimize contamination from the skin prior to collection of the specimen. Urine samples should be dispatched to the laboratory as soon as possible, i.e., within 2 hours. It can be stored for 24 hours at 2°–8°C.

Midstream or 'Clean Catch' Specimen

The "clean catch" method is the most popular noninvasive method used. Before collecting the specimen, ask the female to separate labia whilst passing urine and in the male, encourage retraction of the prepuce whilst passing urine. Ask the person to void a small amount of urine into the toilet first. Then collect 10–20 mL urine directly into the specimen container and the remaining urine can be passed into the toilet. The container used for collection is wide mouthed screw capped bottle.

Urine collection pads or urine collection bags are also used to obtain specimen in case of non-toilet trained children or patients unable to respond.

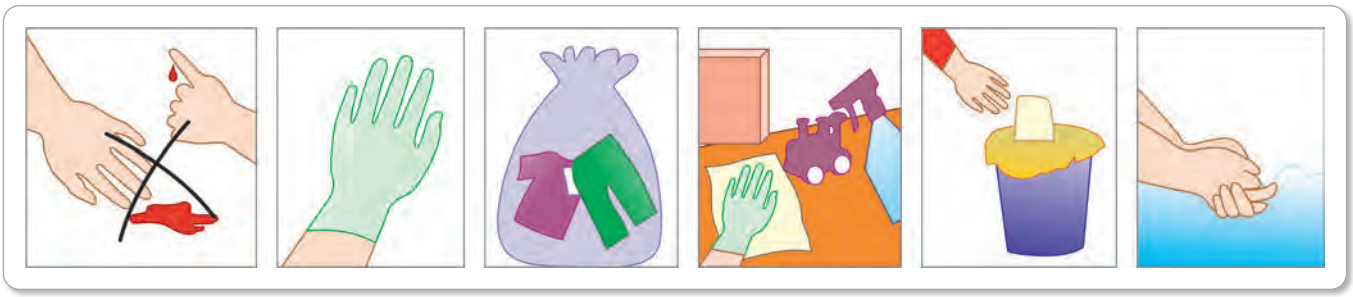


Fig. 36.4: Safety while handling blood and body fluids

STAFF PRECAUTIONS

Safety Precautions While Handling Clinical Samples of Blood and Body Fluids

The blood and body fluids serve as vehicles for transmission of various human diseases. Some of the diseases are very contagious. It is *necessary to follow cautions* while handling bodily fluids due to the risk of contracting infectious diseases (Fig. 36.4). The risk of contracting an infection from direct contact with blood, urine, vomit and feces is based on many factors like:

- The infectious agent involved.
- Duration and type of exposure.
- The amount of body fluids involved in the exposure.
- The amount of infectious agent in the body fluids or feces at the time of exposure.

The modern medical science, public health and personal hygienic practices consider body fluids as *potentially unclean*. This is because they *can be vectors for infectious diseases* like STD or other blood-borne diseases. Universal precautions help in avoiding exchange of body fluids (Fig. 36.4). *The safety precautions have been dealt separately in Chapter 42, Safety Protocols in this book.*

1. Do not directly touch the spilled sample
2. Use PPE
3. Dispose off the used PPE properly
4. Disinfect the work place
5. Throw the infected materials in yellow bin
6. Follow hand hygiene

- The exposed person *must report* any occupational exposures immediately to the medical support team.
- Removable soiled items *must be disposed gently and immediately*.
- *Treatment should be available* during all working hours in a lab or health care facility.
- On exposure to body fluids, a person *must inform* about this to a designated professional, while maintaining the confidentiality about the source of the sample.
- Sample of serum should be collected from the patient and *expert counseling* must be provided.
- *Documents of the incident must be prepared* which must include; the date, time and type of exposure along with how the incident occurred with name of source.

Post-exposure Management of the Source of Infection

The person whose body fluids are the source of an infection should be *evaluated for infection* with HIV, HBV, and HCV. If the source is known to have HIV infection, then detailed information must be collected like stage of infection, current and previous antiretroviral therapy, etc.

In a case when the status of the *source is not known,* then the person should be tested for the infectious source with his consent after counseling. The source person should be analyzed for HIV antibody, HCV antibody and antibody to HBV surface antigen, i.e., HBs Ag.

Post-exposure Management of the Exposed Person

The contaminated *clothing should be removed* and the injured area should be *washed* with soap and water. The *antiseptic* is applied on the exposed site. Affected mucus membranes should be *flushed* with large amounts of water. If eyes are contaminated, they are washed gently but thoroughly with water or normal saline, while kept open. The exposed person should have a *medical evaluation* including information about medications one is taking along with underlying medical conditions or circumstances (Table 36.6).

Post-exposure follow-up should be done by a *specialist* having knowledge of blood-borne infections. If it is confirmed that a person has been exposed to a blood-borne pathogen, he should *abstain from donating blood, semen, organs or tissue for 6 months* and should not share contaminated razors or toothbrushes, etc. The HIV and HBV, exposed person should be informed about the risk of transmission through sexual contact or injecting.

TABLE 36.6: Management of exposure to blood and body fluids

Post-exposure	Action to be taken
Immediately	First aid, risk assessment, post-exposure prophylaxis in case of significant injury
As soon as possible (same day)	Source assessment, documentation of exposure, prevention of transmission and exposure, pre-test counseling, referral to specialist, baseline serology
1–3 weeks	Post-test counseling with baseline serology, occupational health and safety review
3 months	Pre-HIV test counseling, follow-up serology
6 months	Follow-up serology

(All standard precautions are followed – *Standard Precautions are discussed separately in Chapter 33*)

ASSESS YOURSELF

Long Answer Questions

1. What are the guidelines followed while collecting a clinical specimen?
2. What is a STAT report? Why is it important to report in a proper pattern?

Short Answer Questions

1. What is the Principle of specimen collection?
2. What are the types of specimens collected for lab diagnosis?

Multiple Choice Questions

1. Urine sample is collected by:
   a. Clean catch method
   b. Midstream urine
   c. Both (a) and (b) are correct and similar
   d. None is correct
2. The code for nurses that tells the responsibilities is:
   a. The ICN Code of Ethics
   b. The IMN Code of conduct
   c. The ICN Code of Principles
   d. None of the above
3. Transport media used for transferring throat, nasal or vaginal swab is:
   a. Nutrient broth b. Blood agar
   c. Stuart's medium d. All of these
4. The specimen of CSF should not be refrigerated because:
   a. It clots b. It gets contaminated
   c. Cells are destroyed or lysed d. All of these
5. The emergency request for lab report from ICU is called:
   a. Emergency report b. STAT report
   c. Both (a) and (b) d. None of these

Answer Key

Multiple Choice Questions

1. c **2.** a **3.** c **4.** c **5.** b

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Apart from Microbiology, she is equally passionate about literature and has contributed chapters to reference books on English literature. Her online book on "Indian Common Krait - Bungarus caeruleus" has been published by Amazon on Kindle. She shares knowledge through presentations on slideshare.com by LinkedIn. Knowledge of microbiology is foundation stone in health industry. The present book is one more milestone in her career which certainly is going to help the students by transforming their knowledge into practicality.

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