Reading Material for
Medical Lab. Technician
(Bacteriology & Virology)

Compiled By:

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MICROBIOLOGY I

(BACTERIOLOGY AND VIROLOGY)

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BACTERIOLOGY AND VIROLOGY

1.Introduction to Clinical Microbiology

Clinical microbiology is the branch of medical science concerned with the prevention, diagnosis and treatment of infectious diseases.

Scope of Clinical Microbiology

Clinical microbiology involves the use of laboratory techniques to indentify microorganism and diagnose infectiousdiseases.

Relationship with other branches of Medical Sciences

Clinical microbiologists work with clinicians to assist in the diagnosis, management, and treatment of infectious diseases.

In addition to providing diagnostic services, Clinical Microbiology also contributes to facilities infection prevention and control, antimicrobial stewardship programs, and play an important role in laboratory public health surveillance programs.

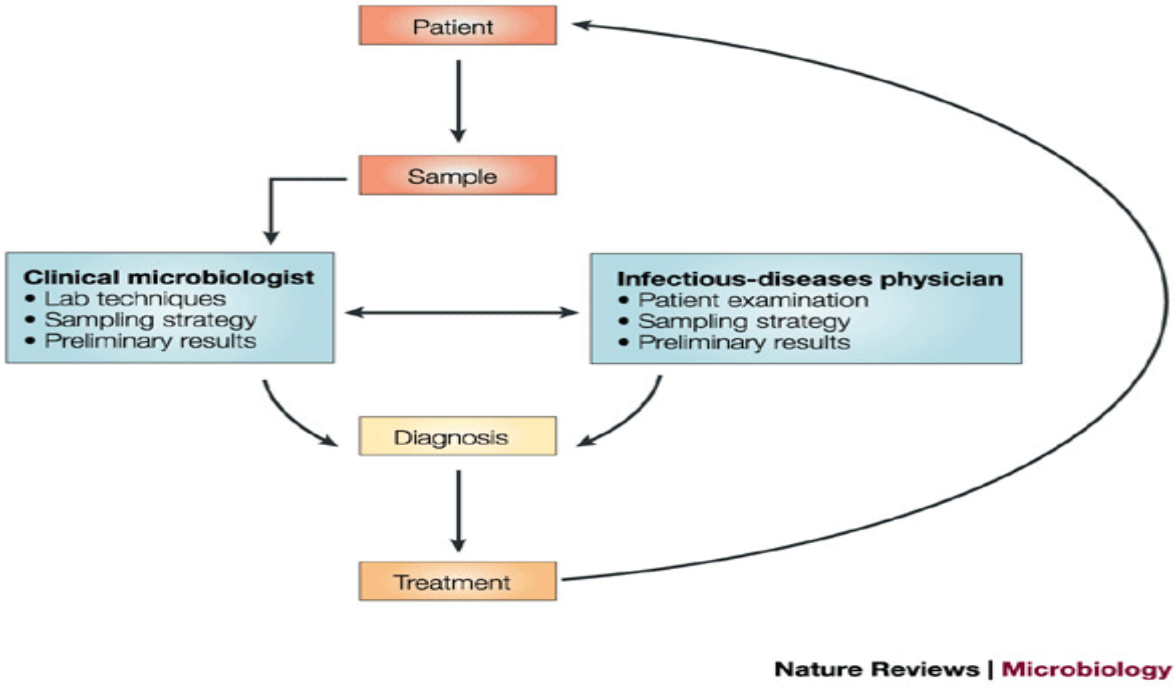


Figure 1: Role of Clinical Microbiology in medical practice

2. Microscope

History of microscope

Microscope is evolved from a Greek word mikron (small) and scopeos (to look) was invented by Antony Van Leuwenhoek (1632-1723). He was a Dutch lens maker and was the first person to observe Bacteria.

Theory of light microscope

The light microscope is one of the most basic and essential equipment used in any laboratory. It is used for visualizing very small objects like cells, bacteria, parasites, their ova/cysts and crystals etc., that are otherwise not visible to the naked eye. It comprises a series of lenses, which magnify an illuminated small object several times to make it recognizable with the naked eye and to study its details. Such a microscope is called compound light microscope. e. It has three basic components: • Foot piece • Body • Eye piece.

Nature of light, concept of amplitude and wave length

The light constitutes the raw material of the light microscopy. The light is a form of energy that travels in waves. **Wavelength** is the distance between two corresponding points on adjacent waves and determines the colour of light. Whereas the **amplitude**, i.e., vertical displacement of the wave from the optical axis determines the intensity or brightness.

Perception of colour and brightness

The visible light is a mixture of seven different colours with wavelength (λ) in the range of 400- 750 nm. The frequency (the number of variations per second) of these waves is responsible for differences in colour.

Formation of images in light microscope

The magnified image of the object (specimen) is first produced by a lens close to the object called the objective. This collects light from the specimen and forms the primary image. A second lens near the eye called the eyepiece enlarges the primary image, converting it into one that can enter the pupil of the eye.

2.1Types of light Microscope

There are 2 types of light microscope i.e. simple and compound microscope.

2.1.1 Simple microscope

A simple microscope is used at a basic level and a single lens is used in a simple microscope.

2.1.2Compound microscope

A compound microscope is used at an advance level as it has higher magnification and two lenses are used in a compound microscope.

Objectives of microscope

The magnified image of the object (specimen) is first produced by a lens close to the object called the objective. This collects light from the specimen and forms the primary image. A second lens near the eye called the eyepiece enlarges the primary image, converting it into one that can enter the pupil of the eye. The magnification of the objective multiplied by that of the eyepiece, gives the total magnification of the image seen in microscopes having a mechanical tube length (MTL) of 160 mm. The MTL is the distance between the shoulder of an objective and the rim of the eyepiece.

Examples

Objective magnification	Eyepiece magnification	Total magnification
10×	10×	100 diameters
40×	10×	400 diameters
100×	10×	1 000 diameters

Eyepieces and useful magnification

The objective provides all the detail available in the image. The eyepiece makes the detail large enough to be seen but provides no information not already present in the primary image formed by the objective. The magnification of eyepiece used should therefore be adequate to enable the relevant detail in the primary image to be seen clearly.

Eyepieces

The microscope should be supplied with 10X wide field eyepieces.

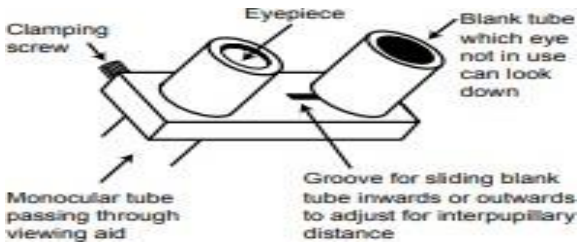


Figure:2.1 Eye-piece

Compensating eyepieces

These eyepieces are required for use with fluorite and apochromatic objectives.

High-eyepoint eyepieces

Some manufacturers equip their microscopes with high-eyepoint eyepieces which help spectacle wearers to use a microscope without removing their spectacles e.g. a person wearing spectacles to correct for astigmatism. High-eyepoint eyepieces also avoid sweating onto the eye lenses and are therefore useful in tropical climates.

Resolving and defining power of an objective

An objective accepts light leaving the specimen over a wide angle and recombines the diverging rays to form a point-for-point image of the specimen. Objectives of varying magnifications allow a specimen to be examined in broad detail over a wide area, and in increasing detail over a smaller area. This increase in magnifying power is always linked to an increase in resolving power. The higher the resolving power of an objective, the closer can be the fine lines or small dots in the specimen. The resolving power of an objective is therefore of great importance.

Figure 2.2: Objectives of Varying

Magnifications

Numerical aperture

The resolving power of an objective is of great importance. It is dependent on the numerical aperture (NA) of the objective. The NA is an exact figure that has been worked out mathematically from its equivalent focal length and lens diameter. Both the NA and magnification of an objective are usually engraved on it.

The following are the usual NAs of commonly used objectives:

10X objective NA 0.25

20X objective 0.45

40X objective 0.65

100X (oil) objective 1.25

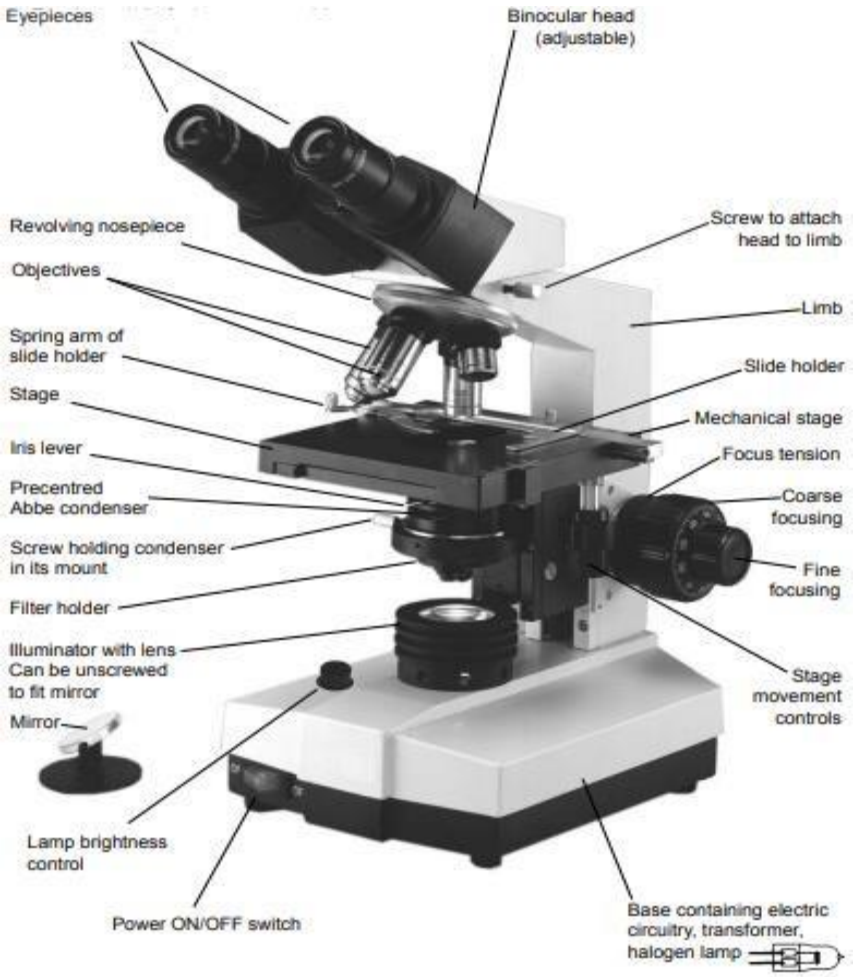


Figure2.3: Complete diagram of a Compound Microscope

3. Sterilization and Disinfection

Sterilization

Sterilization is the killing or removal of all microorganisms, including bacterial spores. It can be achieved by different means e.g physical and chemical methods.

Antisepsis

Chemicals used to kill microorganisms on the living surfaces like skin and mucous membranesis called antisepsis for example by:

- 70% alcohol
- 2% tincture of iodine

Disinfection

Disinfection is the elimination of pathogens, except spores from inanimate objects.

Figure3.1: Differences between Sterilization and Disinfection

3.1Methods of Sterilization

There are 2 methods for sterilization i.e; Physical and chemical.

3.1.1Physical Methods

a. Heat,

b. radiation,

c. filtration,

d. low temperature,

e. Desiccation

a. Heat

1. Dry heat
2. Moist heat

1. DRY HEAT

Red heat

When objects to be sterilized are held vertically in the flame, until red hot for example inoculating wires, loops, tips of needles and forceps.

Hot air oven

A hot air oven is an equipment that uses dry heat (hot air) to sterilize laboratory objects and samples. Temperature range- 160 to 180 celcius for two hours. Glass ware (tubes, flasks, cylinders, glass pipettes).

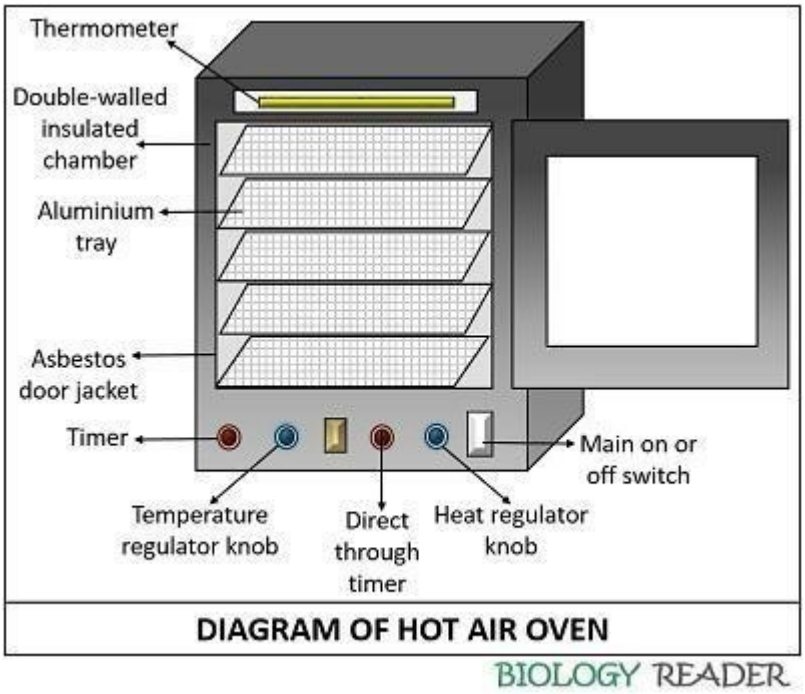


Figure3.2: Hot air Oven

Incinerator

It is a furnace or a container which burns waste materials.

Its working temperature is 800- 1000 degree Celsius. It reduces the volume of waste upto95%.

Figure3.3: Incinerator

2. Moist

heat

Autoclaving

An autoclave is a machine that uses steam under pressure to kill bacteria,viruses, fungi, and spores on items that are placed inside a pressure vessel.
Sterilization is usually carried out by autoclaving, which consists of exposure to steam at 121°C under a pressure of 15 lb/in for 15-20 minutes.

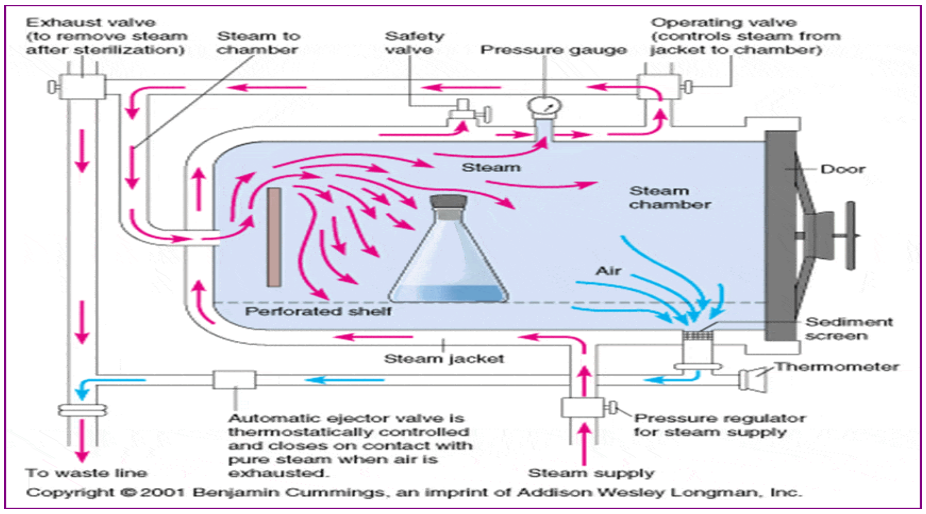


Figure3.4: Autoclave and its principle

There are 2 types of controls to test for the efficiency of autoclave, physical and biological.

Physical control

Thermocouple

Thermocouples are insulated cables can withstand the high pressure and temperature environment of the sterilization process in autoclaves under validation. They measure temperatures up to 150°C.

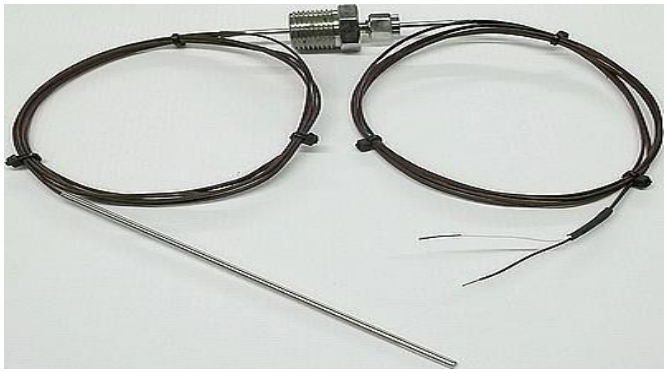


Figure3.5: Thermocouple

Chemical control

Browne's Control Tube

Browne's tube is a small glass tube containing a red heat-sensitive dye used as a chemical indicator for sterilization. The dye changes colour from red to green if sterilization is achieved successfully.

Bowie Dick Tape

It's a printed tape and printed lines on tape turn black in color when the required temperature is achieved.

3.1.2 CHEMICAL METHODS

Gases

Ethylene oxide gas

Formaldehyde gas

Liquids

Phenolic compounds (Phenol, Lysol, and Cresol)

Halogen compounds (Chlorine etc)

Aldehyde compounds (Formaldehyde)

TABLE 13-1 Clinical Use of Disinfection and Sterilization

Clinical Use	Commonly Used Disinfectant or Method of Sterilization
Disinfect surgeon's hands prior to surgery	Chlorhexidine
Disinfect surgical site prior to surgery	Iodophor
Disinfect skin prior to venipuncture or immunization	70% ethanol
Disinfect skin prior to blood culture or inserting vascular catheter	Tincture of iodine followed by 70% ethanol, iodophor, or chlorhexidine
Cleanse wounds	Thimerosal, chlorhexidine, hydrogen peroxide
Cleanse burn wounds	Silver sulfadiazine
Cleanup of blood spill from a patient with hepatitis B or C (disinfect area)	Hypochlorite (bleach, Clorox)
Sterilize surgical instruments and heat-sensitive materials (e.g., endoscopes, respiratory therapy equipment)	Ethylene oxide or glutaraldehyde
Sterilize non–heat-sensitive materials (e.g., surgical gowns, drapes)	Autoclave
Sterilize intravenous solutions	Filtration
Disinfect air in operating room (when not in use)	Ultraviolet light
Disinfect floor of operating room	Benzalkonium chloride (Lysol)
Disinfect stethoscope	70% ethanol
Preservative in vaccines	Thimerosal

SEITZ FILTER

These filters are asbestos pads which are used to retain bacteria and viruses from the fluids.

Figure3.6: Asbestos Filter

Inspissation

It is a process used for heating high-protein containing media for example to enable recovery of bacteria for testing.

Figure 3.7: Insipissator

4. Stains

What are stains?

Stain is a chemical or a physical union between the dye and like component of a cell. Stains or dyes are used to highlight the specimen at the microscopic level to study it at higher magnification for diagnostic purposes.

Principle

The principle of staining is to provide significant contrast between the microbial cell and its environment to study a cell's morphology. Direct staining allows for the observation of the shape, size, and arrangement of bacterial cells.

Classification of stains

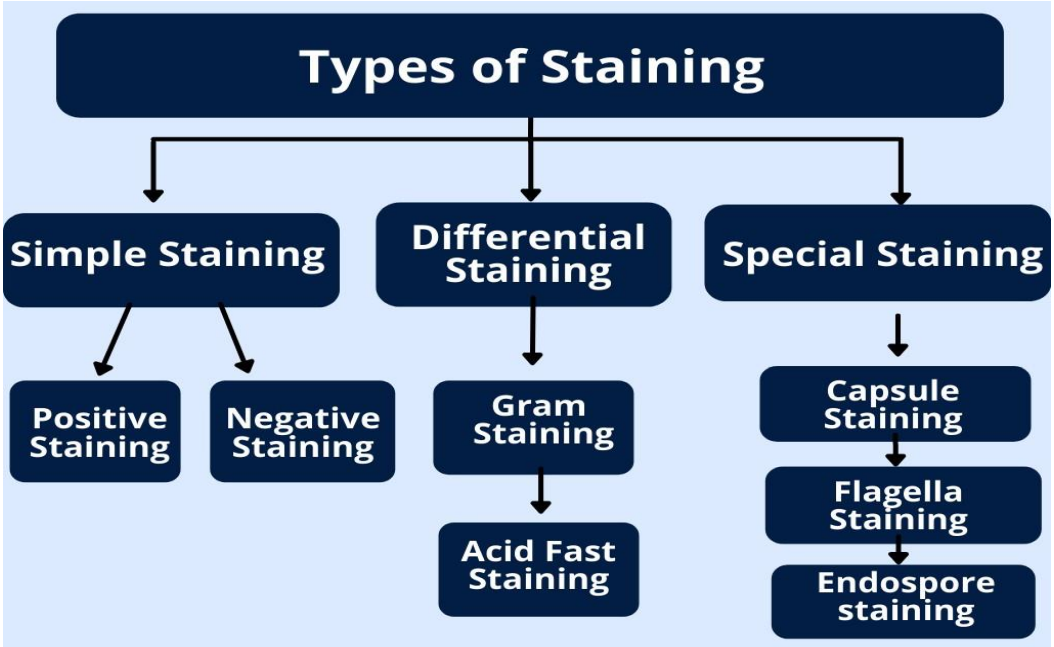


Figure4.1: Classification of stains

4.1Gram's Stain

In 1884 Hans Christian Gram described this method of staining, which is the most important stain in routine bacteriology.

Procedure

Make a thin smear, dry in air and fix in flame. Cover with crystal violet for 30 seconds. Wash and apply iodine solution for 30 seconds. Wash and decolourise with acetone iodine until no further violet washes off. Wash and counterstain with dilute carbol fuchsin for 30 seconds. Wash with water, blot and dry.

Figure 4.2 Gram Staining

4.2. ZIEHL-NEELSEN STAINING

The technique is used to stain Mycobacterium and Nocardia species.

Procedure

Fix smear by rapidly passing over flame. Cover with filtered carbol fuchsin and heat until steam rises. Allow staining for 5 min, heat being applied at intervals to keep the stain hot.

Do not boil or allow to dry. Wash with water. Decolourise with acid alcohol for 2 min. The red colour of the preparation changes to yellowish brown. Wash with distilled water and counter-stain with Loeffler's methylene blue for 15-20 seconds. Wash in distilled water, dry and examine.

Results:

Acid Fast Bacilli stain red against blue background.

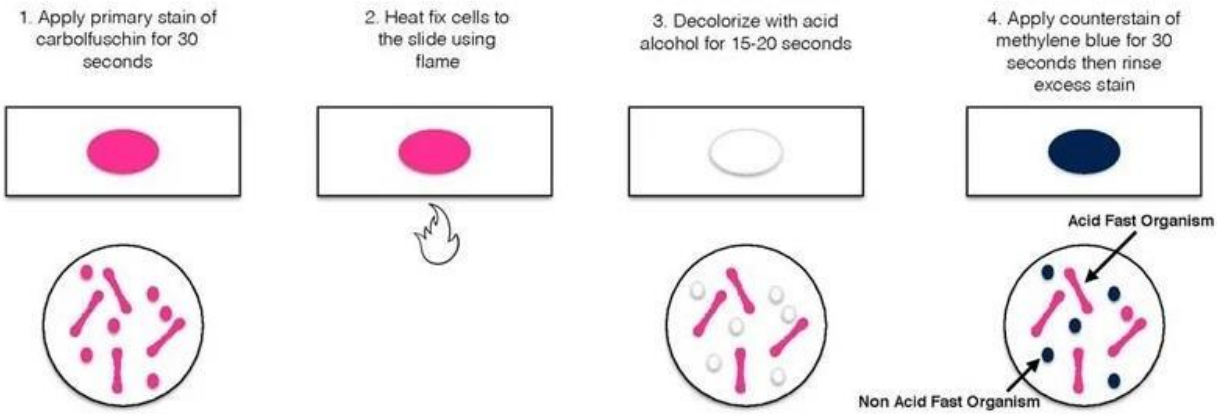


Figure 4. 3 ZN Staining

5. Culture Medium

Nutritional requirement for bacterial growth

Bacteria require proteins, carbohydrates, water and iron for their growth.

Atmospheric requirement for bacterial growth

The response to oxygen is an important criterion for classifying bacteria and has great practical significance because specimens must be incubated in a proper atmosphere for the bacteria to grow.

Obligate aerobes

They require oxygen to grow.

Obligate anaerobes

They cannot grow in the presence of oxygen, they grow in the presence of CO_2 for example *Clostridium tetani*.

Facultative anaerobes

They can grow in the presence as well as in absence of Oxygen. They utilize oxygen, if it is present, but they can use the fermentation pathway to survivein the absence of oxygen.

Temperature requirement for bacterial growthThe temperature required for routine culturing of bacteria is 35–37 C.

Culture medium

The purpose of using cultural techniques in microbiology is to demonstrate the presence of organisms which may be causing disease. For a culture medium to be successful in growing the bacteria it must provide all essential nutrients, ions, and moisture, maintain the correct pH and osmotic pressure, and neutralize any toxic materials produced.

5.1Classification of culture media

Culture media can be classified by consistency as:

- Solid
- Semi-solid
- Liquid

5.1.1 Solid media

Solid media are used mainly in petri dishes as culture Plates. Also in bottles or tubes as stab (deeps) or slope cultures. The purpose of culturing on solid medium is principally to isolate colonies of each organism present in the specimen. This will enable pure cultures to be produced for identification and sensitivity testing.

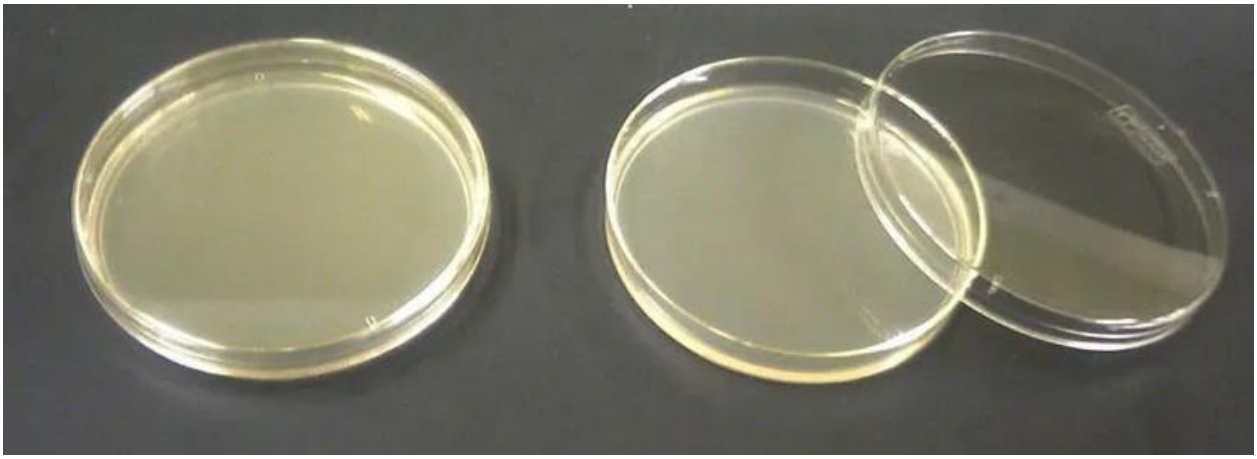


Figure 5.1: Solid culture media (Nutrient agar plates)

5.1.2 Semi-solid culture media

This form of culture medium is prepared by adding a small amount of agar (0.4–0.5% w/v) to a liquid medium. Semi-solid media are used mainly as transport media, and formotility and biochemical tests.

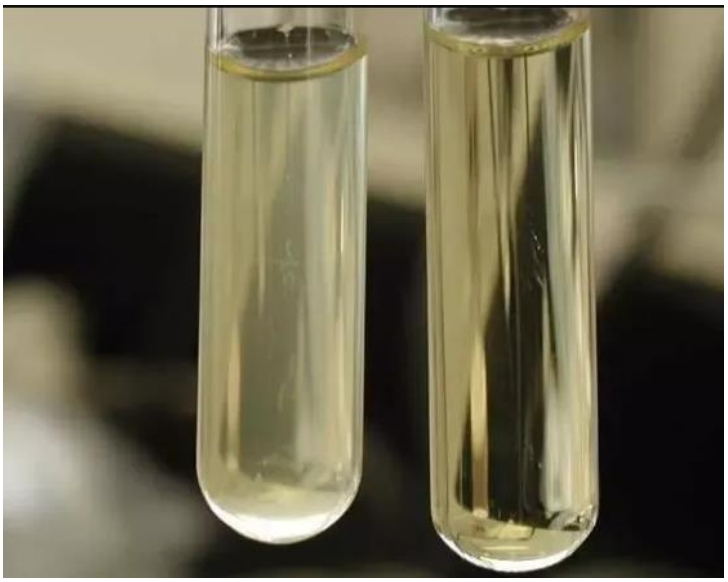


Figure 5.2: Semi-solid and fluid culture media

5.1.3 Liquid culture media

Liquid media are most commonly used as enrichment where organisms are likely to be few e.g. blood culture. Some organisms produce a surface growth on the medium in which they are growing e.g. Vibrio cholerae when growing in alkaline peptone water. Fluid media may also be used for biochemical testing.

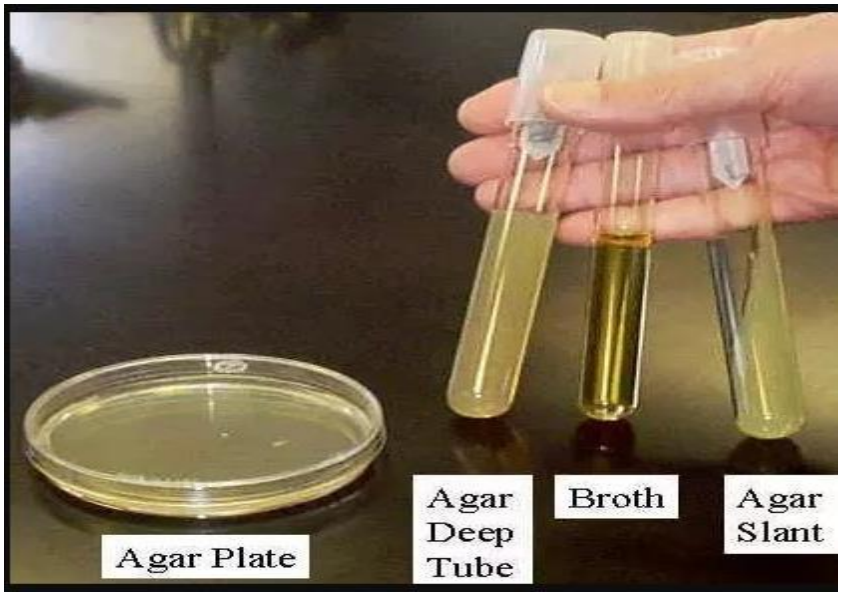


Figure 5.3: Examples of Solid, semi-solid and fluid culture media

The composition and use of culture media

5.2.1Basic media

These are simple media such as nutrient agar and nutrient broth that will support the growth of microorganisms that do not have special nutritional requirement.

5.2.2 Enriched media

Enriched media are required for the growth of organisms with exacting growth requirements. Basic media may be enriched with whole or lyzed blood, serum, peptones, yeast extract, vitamins and other growth factors e.g Blood agar.

5.2.3 Selective media

These are solid media which contain substances which inhibit the growth of one organism to allow the growth of another e.g Mannitol Salt agar.

5.2.4 Indicator media

These are media to which dyes are added to differentiate microorganisms MacConkey agar.

5.2.5 Transport media

These are mostly semisolid media and their use is particularly important when transporting microbiological specimens from one laboratory to another e.g Carry-Blair medium.

5.2.6 Identification media

These include media to which substrates or chemicals are added to help identify bacteria isolated on primary cultures e.g Krigler iron agar.

6. Identification of bacteria

Morphological classification

On the basis of morphology bacteria are divided into the following groups:

a. Cocci: round or oval in shape
b. Bacilli: rod shaped
c. Cocco-baccilli : short rods
d. Vibrios: coma shaped
e. Spirochaetes: spiral like

6.1 Classification on the basis of Gram staining

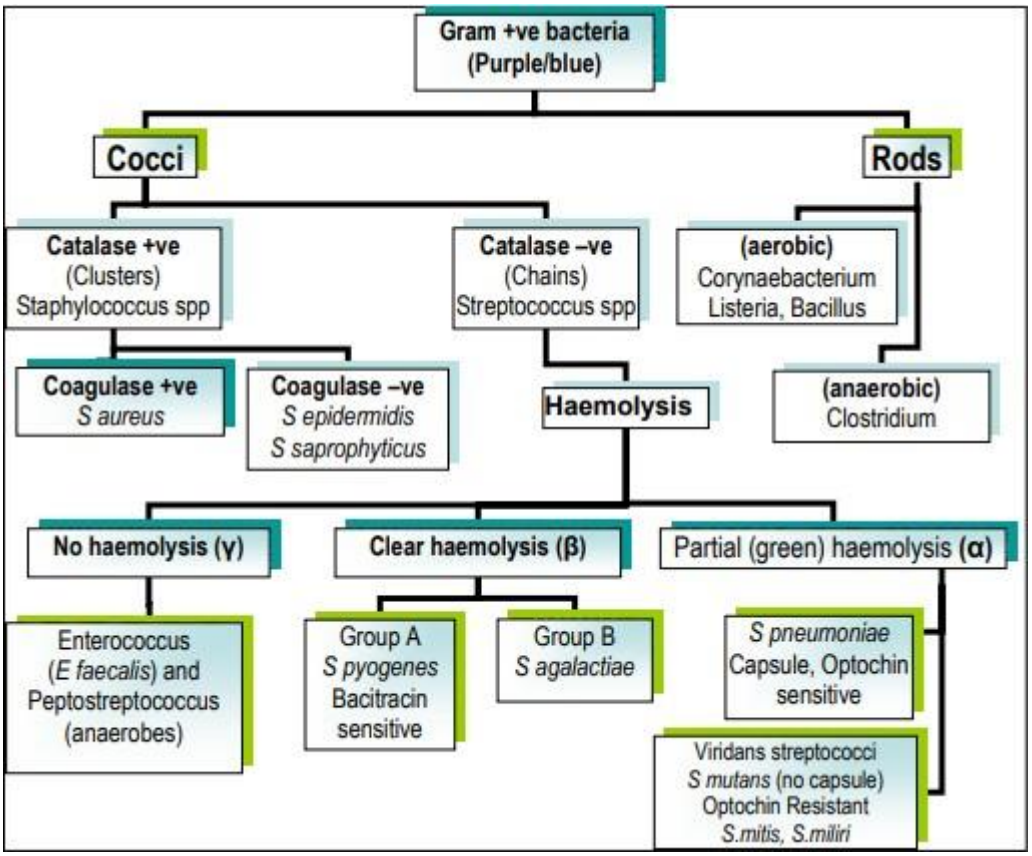


Figure6.1: Gram positive bacteria

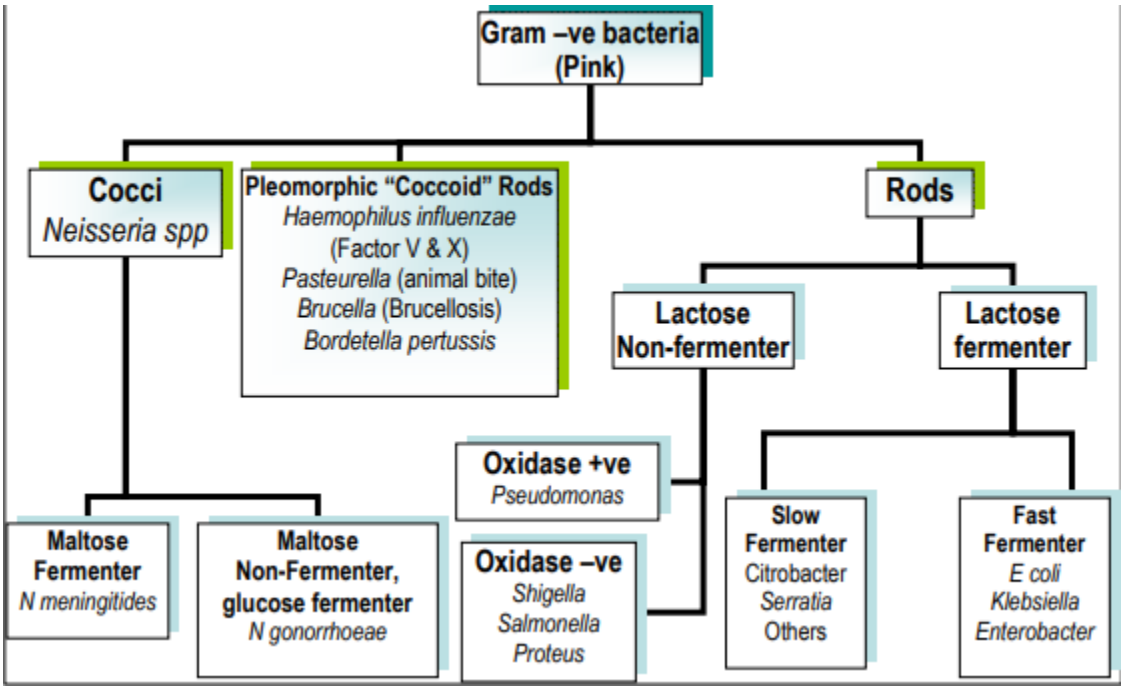


Figure6.2: Gram negative bacteria

7. Study of Common Pathogens

7.1 Possible pathogens isolated from Blood cultures

Gram positive bacteria	Gram negative bacteria
Staphylococcus aureus	Salmonella typhi
Viridans streptococci	Salmonella serovars
Streptococcus pneumoniae	Brucella species
Streptococcus pyogenes	Pseudomonas aeruginosa
Enterococcus faecalis	Haemophilus influenzae
	Klebsiella strains
	Escherichia coli

7.2 Possible pathogens isolated from urine.

Gram positive bacteria	Gram negative bacteria
Staphylococcus saprophyticus	Escherichia coli
Haemolytic streptococci	Proteus species
	Pseudomonas aeruginosa
	Klebsiella strains
	Neisseria gonorrhoeae

7.3Possible pathogens isolated from stool.

Gram positive bacteria	Gram negative bacteria
Clostridium perfringens types	Shigella species
Bacillus cereus	Salmonella serovars
Clostridium difficile	Yersinia enterocolitica

7.4 Possible pathogens isolated from throat

Gram positive bacteria	Gram negative bacteria
Streptococcus pyogenes	Neisseria species
Staphylococcus aureus	Moraxella catarrhalis
	Haemophilus influenzae

7.5 Possible pathogens isolated from C.S.F

Gram positive bacteria	Gram negative bacteria
Streptococcus pneumoniae	Neisseria meningitidis
Streptococcus agalactiae	Haemophilus influenzae type b
Listeria monocytogenes	Escherichia coli

7. 6 Possible pathogens isolated from eye

Gram positive bacteria	Gram negative bacteria
Staphylococcus aureus	*Pseudomonas aeruginosa*
Streptococcus pneumoniae	Escherichia coli

7.7 Possible pathogens isolated from ear

Gram positive bacteria	Gram negative bacteria
Staphylococcus aureus	*Proteus species*
Streptococcus pneumoniae	*Pseudomonas aeruginosa*

8. Study of antibiotics

Principles of action of antibiotics on microorganisms

There are four major sites (cell wall, ribosomes, nucleic acids, and cell membrane) in the bacterial cell that serve as the basis for the action of antibiotics.

Mechanism of action

8.1

Inhibition of cell wall synthesis

Penicillins

Penicillins act by inhibiting transpeptidases, the enzymes that catalyze the final cross-linking step in the synthesis of peptidoglycan.

Cephalosporins

Cephalosporins are β-lactam drugs that act in the same manner as penicillins (i.e., they are bactericidal agents that inhibit the cross-linking of peptidoglycan).

Carbapenems

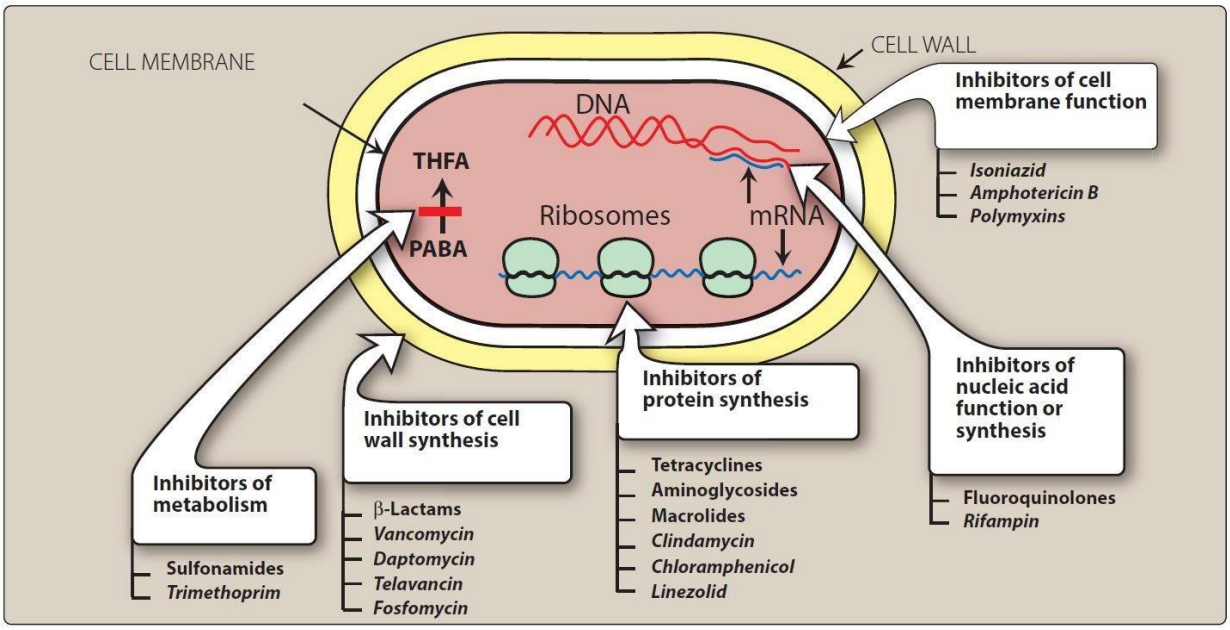
Carbapenems are β-lactam drugs that are structurally different from penicillins and cephalosporins but having same mechanism of action.

Monobactams

Monobactams are also β-lactam drugs that are structurally different from penicillins and cephalosporins but having same mechanism of action.

Vancomycin

Vancomycin is a glycopeptide that inhibits cell wall peptidoglycan synthesis by blocking transpeptidation but by a mechanism different from that of the β-lactam drugs.



Classification of some antimicrobial agents by their sites of action. (THFA = tetrahydrofolic acid; PABA = *p*-aminobenzoic acid.)

Source : Lippincott Illustrated Reviews, Pharmacology - Whalen, Karen

Figure 8.1: Mechanism of action of all antibiotics

8.2 Protein synthesis

inhibitors

Aminoglycosides

Aminoglycosides act by inhibition of the initiation complex and misreading of messenger RNA (mRNA).

Tetracycline

They inhibit protein synthesis by binding to the 30S ribosomal subunit and by blocking the aminoacyl transfer RNA (tRNA) from entering the acceptor site on the ribosome.

Chloramphenicol

Chloramphenicol inhibits protein synthesis by binding to the 50S ribosomal subunit and blocking the action of peptidyltransferase

Macrolides

Macrolides inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit and blocking translocation.

Clindamycin

Clindamycin binds to the 50S subunit and blocks peptide bond formation.

Linezolid

Linezolid binds to the 23S ribosomal RNA in the 50S subunit and inhibits protein synthesis.

8.3 Inhibition of nucleic acid Synthesis

Fluoroquinolones

Fluoroquinolones are bactericidal drugs that block bacterial DNA synthesis by inhibiting DNA gyrase (topoisomerase).

8.4 Mechanisms of antibiotic resistance

There are mainly four methods by which bacteria develop resistance against antibiotics,

8.5 Bacteria produce enzymes that inactivate drug
8.6 Bacteria synthesize modified targets sites against which the drug has a reduced effect.
8.7 Bacteria reduce permeability to the drug
8.8 Bacteria actively export drugs using a “multidrug-resistance pump”.

8.5 Methods of determination of sensitivity of antibiotics

Pathogenic bacteria show great variation in susceptibility to antibiotics. It is therefore essential to determine the susceptibility.

There are mainly 2 methods to determine the susceptibility

Diffusion tests

8.8.1 Strokes disc diffusion method
8.8.2 Kirby-Bauer disc diffusion method

Dilution tests

1. Broth dilution method
2. Agar dilution method

Kirby-Bauer disc diffusion method

This is used in routine in clinical laboratory. In disc diffusion methods the discs of filter paper are soaked in known quantity of antibiotics and are placed on plates of appropriate mediuminoculated with pure culture of organisms.

Antibiotics diffuse in the surrounding medium thus preventing the growth of organisms in an area where the antibiotic concentration remains sufficient for killing the organisms or preventing their division. A visible clear zone appears the diameter of which, is measured and compared with control organisms.

9 Virology

9.1 Broad classification of viruses

Viruses are classified on the basis of type of genetic material i.e., DNA or RNA viruses, presence or absence of envelope, shape and characteristics of their genome and the enzymes present in the viruses. They are mainly classified as:

Structure	Viruses
DNA enveloped viruses	Herpesviruses (herpes simples virus 1 and 2,varicella zoster virus, cytomegalo virus, Ebstein Barr virus) hepatitis B virus, small pox.
DNA Non-Enveloped viruses	Adenoviruses, papillomaviruses, B19 virus.
RNA enveloped viruses	Influenza, parainfluenza, respiratory syncytial virus, measles virus, mumps virus, rubella virus, rabies virus, HIV, HCV.
RNA Non-Enveloped viruses	Enteroviruses (polio virus, coxsackie virus, echovirus, HAV), rhino virus, rota virus, HEV, norovirus.

9.2 Identification of viruses

There are four approaches to the diagnosis of viral diseases by the use of clinicalspecimens:

(1) Identification of the virus in cell culture,

(2) Serologic procedures to detect a rise in antibody titer or the presence of IgM antibody,

(3) Detection of viral antigens in blood or body fluids,

(4) Detection of viral nucleic acids in blood or the patient's cells.

9.3 MICROSCOPIC IDENTIFICATION

Viruses can not be identified by direct microscopic examination of clinical specimens suchas biopsy material.

Three different procedures can be used.

(1) Light microscopy can reveal characteristic inclusion bodies or multinucleated giant cells in vesicular skin lesions.

(2) UV 592 microscopy is used for fluorescent antibody staining of the virus in infected cells.

(3) Electron microscopy detects virus particles, which can be characterized by their size and morphology.

Electron Microscopy:

Electron microscopy (EM) has long been used in the discovery and description of viruses. Organisms smaller than bacteria have been known to exist since the late 19th century, but the first EM visualization of a virus came only after the electron microscope was developed.

9.4 Routine procedure for isolation of virus:

Collection

For viral isolation, the specimen must be obtained as early as possible after the onset of the clinical disease. The specimens must be obtained from multiple sites i.e. throat swab, urine, faeces, CSF etc depending upon the organ system involved

Transport

The specimens are to be transported in a Virus transport Medium (VTM). It is basically a buffer with balanced salt composition and bovine albumin stabilise the viruses. VTM is obtained from the virus laboratory according to the need.

Storage

Such specimens must not be frozen and must be kept around 4°C. However, in case of delay beyond 72 hours these may be snap frozen at -70°C or transported in a container of liquid nitrogenor in dry ice.

MICROBIOLOGY-III

PRACTICAL ACTIVITIES

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22. **Stool culture-sampling media used-procedure-bacteria commonly isolated**
23. **Vaginal swab culture-sampling media used-procedure-bacteria commonly isolated**

24. **CSF culture-sampling media used-procedure-bacteria commonly isolated**
25. **Method of determination of sensitivity**

VIROLOGY

26. **Precautions to be observed in virology laboratory**
27. **Collection, transportation and storage of specimen for virological examination**
28. **Examination of virus under microscope**
29. **Staining procedures for viruses**
30. **Procedures for isolation of viruses**
31. **Diagnostic tests for viral diseases**

MICROBIOLOGY.II

1. Introduction of Microbiology Laboratory

A microbiology laboratory is a place for working with a variety of microorganisms. The Microbiology Laboratory receives samples from patients to identify organisms that are responsible for infection including bacteria, fungi and parasites.

General requirements for Microbiology laboratory

1. Wash your hands with disinfectant soap when you arrive at the lab and again before you leave.

2. Absolutely no food, drinks, chewing gum, or smoking is allowed in the laboratory.

3. Wear a lab coat and safety glasses, bring them to class, and use them.

4. Disinfect work areas before and after use with 70% ethanol or fresh 10% bleach.

5. Replace caps on reagents, solution bottles, and bacterial cultures. Do not open Petri dishes in the lab unless absolutely necessary.

6. Inoculating loops and needles should be flame sterilized in a Bunsen burner before laying them down.

7. Turn off Bunsen burners when not in use.

8. Treat all microorganisms as potential pathogens. Use appropriate care and do not take cultures out of the laboratory.

9. Sterilize equipment and materials.

10. Never pipette by mouth. Use a pipetting aid or adjustable volume pipettors.

11. Consider everything a biohazard. Do not pour anything down the sink. Autoclave liquids and broth cultures to sterilize them before discarding.

12. Dispose of all solid waste material in a biohazard bag and autoclave it before discarding in the regular trash.

13. Familiarize yourself with the location of safety equipment in the lab.

14. Dispose of broken glass in the broken glass container.

15. Dispose of razor blades, syringe needles, and sharp metal objects in the “sharps” container.

16. Report spills and accidents immediately to your instructor.

17. Report all injuries or accidents immediately to the instructor, no matter how small they seem.

2. Cleaning and washing of new and infected glass wares used in microbiology laboratory

All glassware for the laboratory must be washed and cleaned thoroughly. In most cases it must be cleaned chemically and in some cases it must be cleaned from microorganisms i.e., needs to be sterile.

Most glassware can be cleaned in the following way:

1. Put the specified amount of detergent into a dish-pan containing moderately warm water.

2. Rinse glassware in tap water and then put it in detergent solution for at least one hour.

3. Using a cleaning brush, thoroughly scrub the glassware. Avoid use of abrasive cleaners.

4. Rinse the glassware under running tap water. Allow the water to run into each piece of glassware, pour it out and repeat several times (7-10).

5. Rinse with distilled water.

6. Glassware may be dried in hot air oven at 50-100°C or at room temperature. Always dry glassware or other equipment in an inverted position.

7. Check the glassware for cleanliness by observing the water drainage.

3. Handling and disposal of Infected material

Handling and disposal of infectious laboratory waste requires the development of site-specific plans. Procedures developed by personnel within a facility will be appropriate for the specific needs of that facility and may gain a higher level of acceptance than will procedures imposed from outside sources.

Basic principles of handling

Persons who generate infectious laboratory waste are responsible for preparing the waste so that potential occupational exposures and environmental contamination are minimized.

Infectious waste needs to be segregated by the generator from other waste streams.

The waste can then be treated on-site to reduce the concentration of the pathogen to an acceptable level (decontamination), prior to terminal treatment.

Packages of infectious waste need to be identified so that the potential hazard clearly can be recognized and understood by others.

Disposal of infected material

Chemical Decontamination

Liquid and gaseous chemicals are used routinely for decontaminating infectious waste.

Chlorine bleach, iodophors, or phenolic disinfectants are used to decontaminat work stations.

Gaseous decontamination of HEPA filters is usually carried out with formaldehyde sublimed by heat from paraformaldehyde flakes in the presence of high humidity.

Steam Autoclaving

Steam autoclaving usually is considered to be the method of choice for decontaminating cultures, laboratory glassware, pipettes, syringes, or other small items known to be contaminated with infectious agents.

Incineration

Incineration is the method of choice for treating large volumes of infectious waste, animal carcasses, and contaminated bedding materials.

4. Sterilization and Disinfection: Different methods for sterilization

4.1 Sterilization

Sterilization is the killing or removal of all microorganisms, including bacterial spores. It can be achieved by different means e.g physical and chemical methods.

4.2 Antisepsis

Chemicals used to kill microorganisms on the living surfaces and mucous membranesis called antisepsis for example by:

- 70% alcohol
- 2% tincture of iodine

4.3 Disinfection

Disinfection is the elimination of pathogens, except spores from inanimate objects.

4.4 Sterilization methods

There are 2 methods for sterilization Physical and chemical.

4.4.1Physical Methods

Heat, radiation, filtration, low temperature, Desiccation

Heat

1. Dry heat
2. Moist heat

1.DRY HEAT

Red heat

When objects to be sterilized are held vertically in the flame, until red hot for example inoculating wires, loops, tips of needles and forceps.

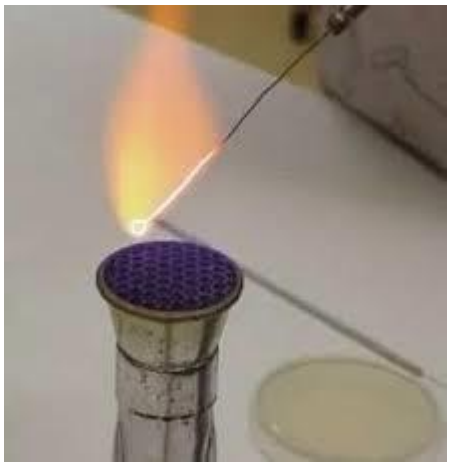


Figure4.1: wire loop sterilization in dry heat

Hot air oven

A hot air oven is an equipment that uses dry heat (hot air) to sterilize laboratory objects and samples. Temperature range- 160 to 180 celcius for two hours. Glass ware (tubes, flasks, cylinders, glass pipettes).

Incinerator

It is a furnace or a container which burns waste materials.

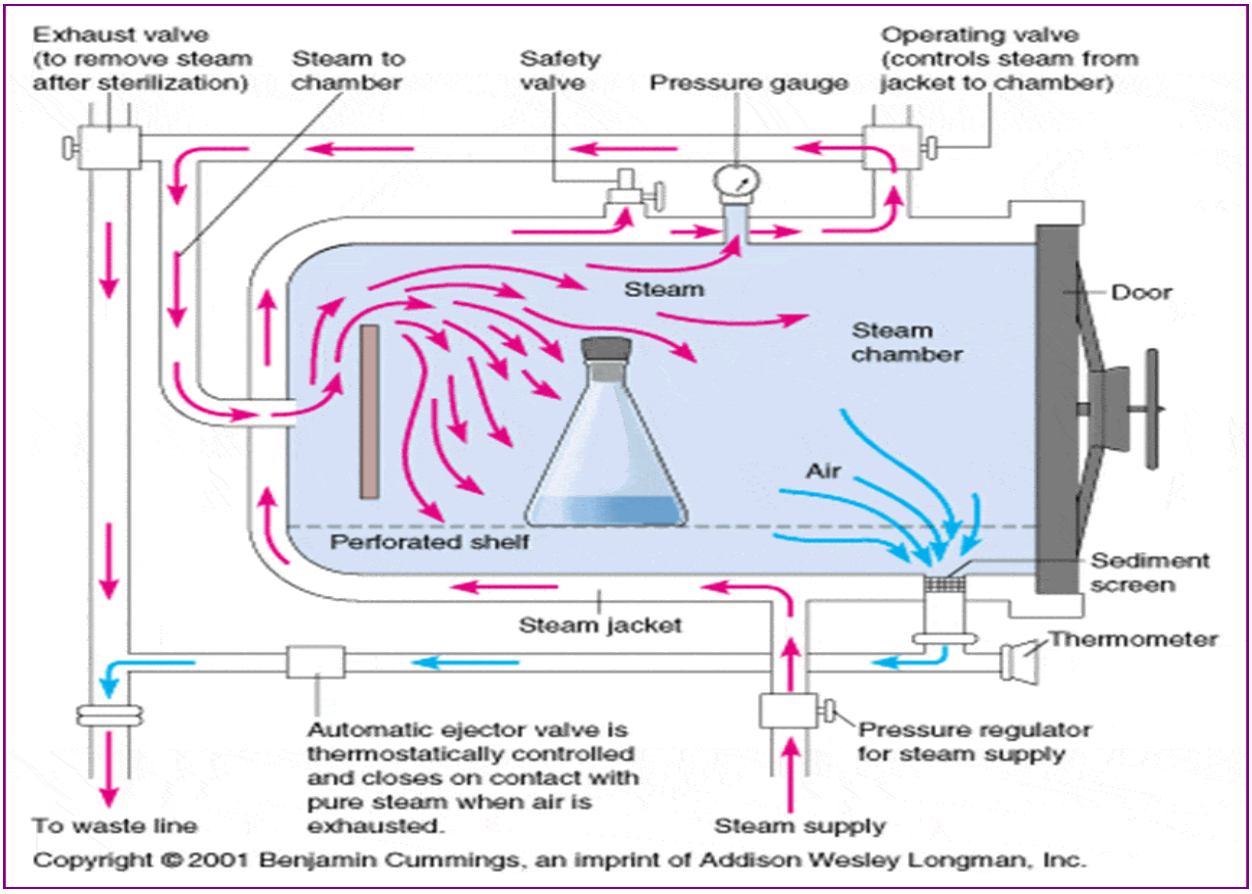
Its working temperature is 800- 1000 degree Celsius. It reduces the volume of waste upto95%.

2.Moist

heat

Autoclaving

An autoclave is a machine that uses steam under pressure to kill harmful bacteria, viruses, fungi, and spores on items that are placed inside a pressure vessel. Sterilization is usually carried out by autoclaving, which consists of exposure to steam at 121°C under a pressure of 15 lb/in 2 for 15-20 minutes.

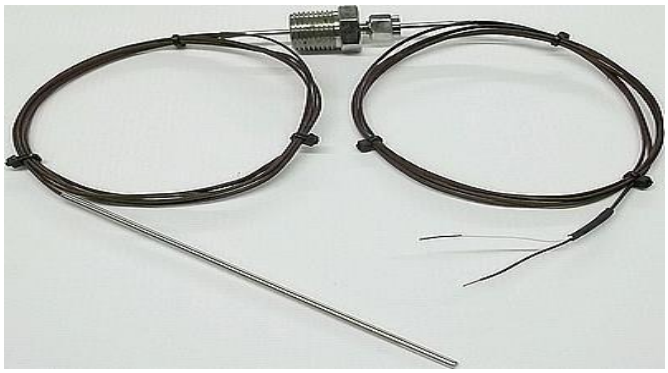


There are 2 methods to test for the efficiency of autoclave sterilization, physical and biological.

Physical control

Thermocouple

Thermocouples are insulated cables can withstand the high pressure and temperature environment of the sterilization process in autoclaves under validation. They measure temperatures up to 150°C.



Chemical control

Browne's Control Tube

Browne's tube is a small glass tube containing a red heat-sensitive dye used as a chemical indicator for sterilization. The dye changes colour from red to green if sterilization is achieved successfully.

Bowie Dick Tape

It's a printed tape and printed lines on tape turn black in color when the required temperature is achieved.

II.CHEMICAL METHODS

Gases

Ethylene oxide gas

Formaldehyde gas

Liquids

Phenolic compounds (Phenol, Lysol, and Cresol)

Halogen compounds (Chlorine etc)

Aldehyde compounds (Formaldehyde)

TABLE 13-1 Clinical Use of Disinfection and Sterilization

Clinical Use	Commonly Used Disinfectant or Method of Sterilization
Disinfect surgeon's hands prior to surgery	Chlorhexidine
Disinfect surgical site prior to surgery	Iodophor
Disinfect skin prior to venipuncture or immunization	70% ethanol
Disinfect skin prior to blood culture or inserting vascular catheter	Tincture of iodine followed by 70% ethanol, iodophor, or chlorhexidine
Cleanse wounds	Thimerosal, chlorhexidine, hydrogen peroxide
Cleanse burn wounds	Silver sulfadiazine
Cleanup of blood spill from a patient with hepatitis B or C (disinfect area)	Hypochlorite (bleach, Clorox)
Sterilize surgical instruments and heat-sensitive materials (e.g., endoscopes, respiratory therapy equipment)	Ethylene oxide or glutaraldehyde
Sterilize non–heat-sensitive materials (e.g., surgical gowns, drapes)	Autoclave
Sterilize intravenous solutions	Filtration
Disinfect air in operating room (when not in use)	Ultraviolet light
Disinfect floor of operating room	Benzalkonium chloride (Lysol)
Disinfect stethoscope	70% ethanol
Preservative in vaccines	Thimerosal

SEITZ FILTER

These filters are asbestos pads which are used to retain bacteria and viruses from the fluids.

Inspissation

It is a process used for heating high-protein containing media for example to enable recovery of bacteria for testing.

5.Microscope:Introduction different parts and their functions

Microscope

The light microscope is one of the most basic and essential equipment used in any laboratory. It is used for visualising very small objects like cells, bacteria, parasites, their ova/cysts and crystals etc., that are otherwise not visible to the naked eye. It comprises a series of lenses, which magnify an illuminated small object several times to make it recognisable with the naked eye. Such a microscope is called compound light microscope.

5.1Introduction to the microscope's parts

It has three basic components:

- Foot piece
- Body
- Eye piece

5.1.1Foot piece

It forms the base of the microscope and provides stability to the body and eyepieces.

5.1.2Body

The body of the microscope is mounted on the foot piece. It holds a sub-stage condenser, a stage and a nosepiece.

Sub-stage condenser is composed of a system of lenses and diaphragm. The intensity of light and the size of field illuminated by it are controlled by moving the condenser up or down and adjusting the aperture of the diaphragm.

The stage is a device for holding the objects for examination. It has a hole in the middle over which the object is placed. Exactly underneath the hole is the sub-stage condenser.

Nosepiece is the part of the body, which holds the objectives.

An objective comprises a system of lenses, which magnify the image several times. Each objective is marked with a coloured line, which indicates its magnification.

Following are the common objectives installed in an ordinary light microscope:

• Scanner: Red line, x4 magnification

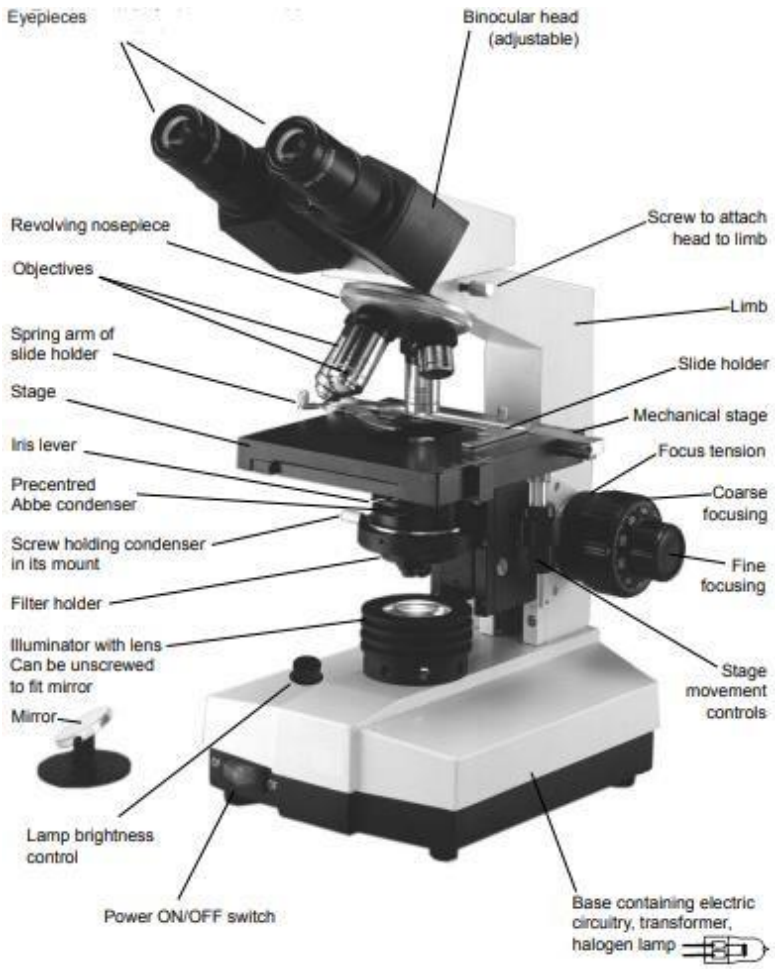
• Low power: Yellow line, x10 magnification

• Dry high power: Blue line, x 40 magnification

• Oil immersion: White line, x100 magnification

5.1.3Eyepiece

The observer, to look at the object under examination uses this part of the microscope.



6.Correct Use Of Microscope

1. The microscope should be placed on a level bench, which should be free of vibrations.

2. The power socket, to which the microscope is plugged, should not be loose and sparking.

3. The height of the microscope or chair should be adjusted in such a way that the eyes of the user are right on the eyepieces while maintaining the normal curvatures of the backbone.

4. The microscope should then be adjusted for the optimum resolution and contrast to ensure maximum definition of specimen details. It can be done by using **Köehler technique**.

6.1 Köehler technique

Turn on the microscope at very low illumination and give 1-2 min to the filament of the bulb to warm. Then adjust the light intensity.

• Place the specimen on the stage, switch to x10 objective and focus.

• Close the iris diaphragm of the sub-stage condenser and raise the sub-stage condenser to the top “stop”.

• Close the field iris diaphragm of the light assembly in the body.

• Move the sub-stage condenser down until the image of the field iris diaphragm is in sharp focus.

• Now re-focus the specimen.

• Centre field diaphragm image by using adjustment screws in the condenser.

• Enlarge field diaphragm image until it is just out of the field of view and the entire area under observation is illuminated.

• Remove one eyepiece and look down the tube.

• Adjust the aperture of diaphragm while observing the circular beam of the light so the light beam fills 75% of the field.

• Replace the eyepiece. Adjust the diopter setting and inter-pupillary distance.

Place your forearms flat on the surface of the table while using microscope.

7.Care Of Microscope

Microscope is very delicate equipment. Proper care not only enhances precision but also increases its life. Following points are helpful in the care of microscope:

1. Protect from heat.

2. Clean it daily. When not in use, keep it covered with a plastic cover or a piece of cloth but not with mesh gauze.

3. Clean the objectives with soft tissue paper soaked in xylol and then with lint free cloth. Be careful as excess of xylol may dissolve the cement with which lens is fixed in the objective and may trickle into it. Do not clean with alcohol.

4. Remove the dust from the eyepieces with the help of soft tissue paper.

5. Always use soft tissue paper or lint free cloth for cleaning lenses and never rub but wipe gently. This protects lenses from scratches.

6. Switch off the power at the end of microscopy session.

8. Common stains used in microbiology-preparation of stains-preparation of film-fixing and staining-Gram's staining-Ziehl Neelson staining. Sensitivity to antibiotics.

Common stains used in microbiology include Gram’s stain and Ziehl Neelson stain.

8.1 .Preparation of film-fixing and

stainingPreparation of smear

Using a sterile wire loop, make a thin preparation covering an area of about 15–20 mm diameter on a slide.

After making a smear, leave the slide in a safe place for the smear to air-dry, protected from dust, flies, cockroaches, ants, and direct sunlight.

Fixing of a smear

The purpose of fixation is to preserve micro-organisms and to prevent smears being washed from slides during staining. Smears are fixed by heat, alcohol, or occasionally by other chemicals.

1. Heat fixation

1 Allow the smear to air-dry completely.

2 Rapidly pass the slide, smear uppermost, three times through the flame of a spirit lamp or pilot flame of a Bunsen burner.

Note: After passing the slide through the flame three times, it should be possible to lay the slide on the back of the hand without the hand feeling uncomfortably hot. When this cannot be done, too much heat has been used.

3 Allow the smear to cool before staining it.

Figure 8.1: heat fixing of a smear

2. Alcohol fixation

1 Allow the smear to air-dry completely.

2 Depending on the type of smear, alcohol-fix as follows: -

For the detection of intracellular Gram negative diplococci, fix with one or two drops of absolute methanol or ethanol.

For the detection of other organisms including M. tuberculosis, fix with one or two drops of 70% v/v methanol or ethanol.

3 Leave the alcohol on the smear for a minimum of 2 minutes or until the alcohol evaporates.

8.2 Gram's Stain

In 1884 Hans Christian Gram described this method of staining, which is the most important stain in routine bacteriology.

Procedure

Make a thin smear, dry in air and fix in flame. Cover with crystal violet for 30 seconds. Wash and apply iodine solution for 30 seconds. Wash and decolourise with acetone

iodine until no further violet washes off. Wash and counterstain with dilute carbol fuchsin for 30 seconds. Wash with water, blot and dry.

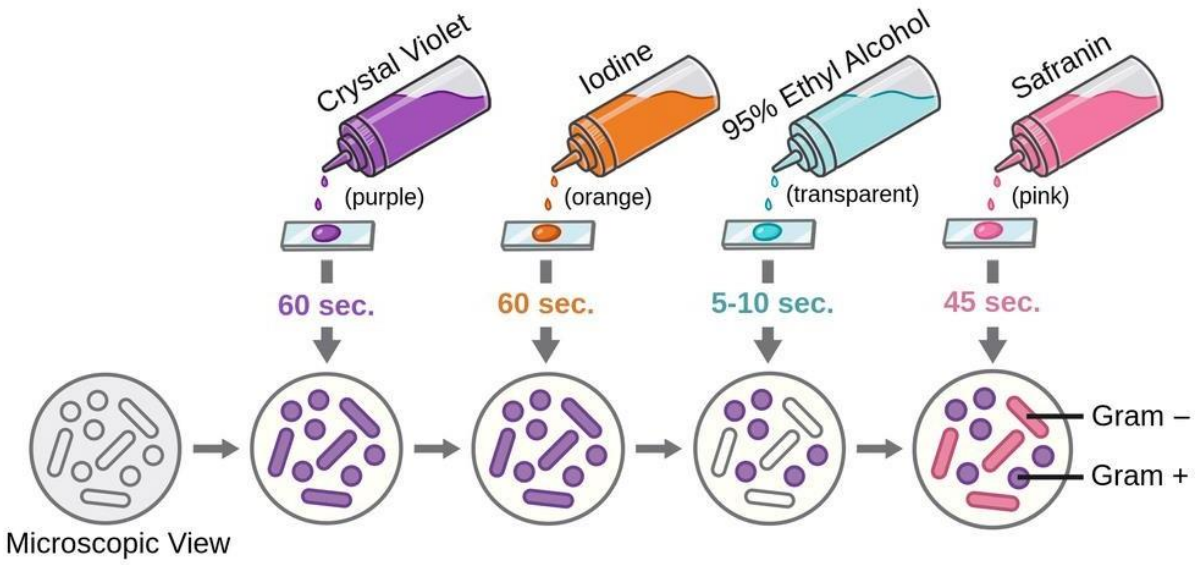


Figure 8.2 : Gram Staining

8.3 ZIEHL-NEELSEN STAINING

The technique is used to stain Mycobacterium and Nocardia species.

Procedure

Fix smear by rapidly passing over flame. Cover with filtered carbol fuchsin and heat until steam rises. Allow staining for 5 min, heat being applied at intervals to keep the stain hot.

Do not boil or allow to dry. Wash with water. Decolourise with acid alcohol for 2 min. The red colour of the preparation changes to yellowish brown. Wash with distilled water and counter-stain with Loeffler's methylene blue for 15-20 seconds. Wash in distilled water, dry and examine.

Results:

Acid Fast Bacilli stain red against blue background.

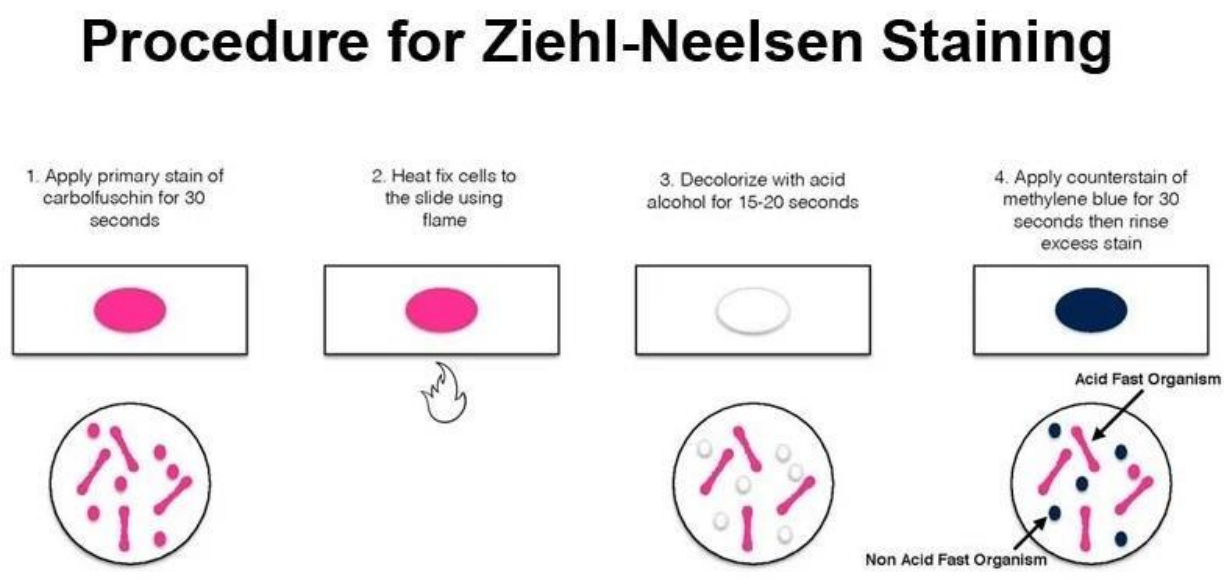


Figure 8.3: ZN Staining Procedure

9. Culture media-classification-composition and uses of important media-preparation of common media

Culture media

For a culture medium to be successful in growing the pathogen, it must provide all essential nutrients, ions, and moisture, maintain the correct pH and osmotic pressure, and neutralize any toxic materials produced. It is also essential to incubate the inoculated medium in the correct atmosphere, at the optimum temperature and for an adequate period.

9.1 Culture media classification

The main types of culture media are:

- Basic
- Enriched
- Selective
- Indicator
- Transport
- Identification

9.1.1Basic media

These are simple media such as nutrient agar and nutrient broth that will support the growth of micro-organisms that do not have special nutritional requirements e.g; Nutrient agar

Figure9.1: Basic media (nutrient agar)

9.1.2 Enriched Media

Enriched media are required for the growth of organisms with special growth requirements such as H. influenzae, Neisseria species, and Streptococcus species. Basic media may be enriched with whole or lyzed blood, serum, peptones, yeast extract, vitamins and other growth factors. E.g; Blood agar, Choclate agar

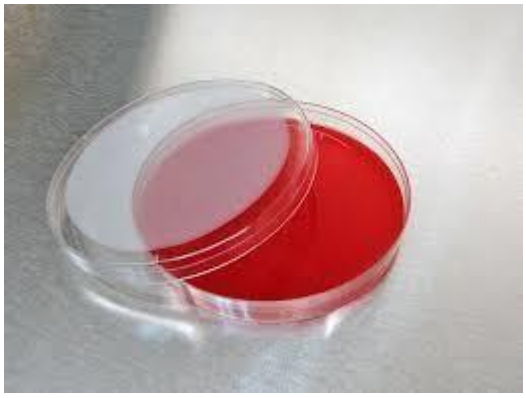


Figure9.2: Enriched medium (Blood agar)

9.1.3 Selective media

These are solid media which contain substances (e.g. bile salts or other chemicals, dyes, antibiotics) which inhibit the growth of one organism to allow the growth of another.

e.g; Mannitol Salt agar , TCBS agar

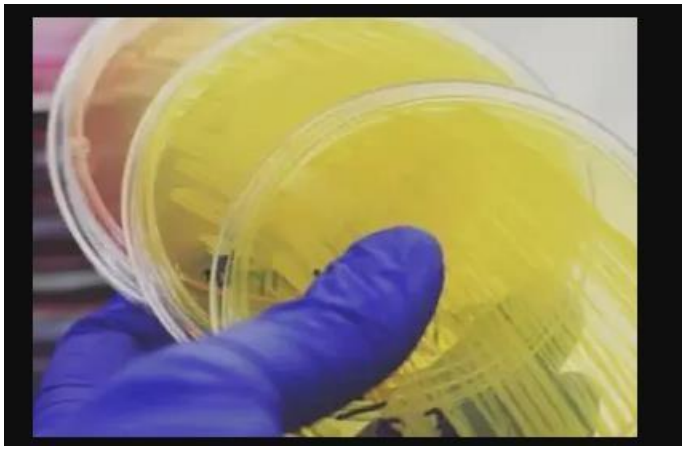


Figure 9.3: Selective media (Mannitol salt agar)

9.1.4 Indicator (differential) media

These are media to which dyes or other substances are added to differentiate micro-organisms. Many differential media distinguish between bacteria by incorporating an indicator which changes colour when acid is produced following fermentation of a specific carbohydrate e.g. MacConkey agar.

Figure9.4: Indicator media (MacConkey agar)

9.1.5Transport media

These are mostly semisolid media that contain ingredients to prevent the overgrowth of commensals and ensure the survival of aerobic and anaerobic pathogens when

specimens cannot be cultured immediately after collection. Their use is particularly important during transportation. E.g: Alkaline Peptone Water

10: Identification media

These include media to which substrates or chemicals are added to help identify bacteria isolated on primary cultures. Examples include peptone water sugars, urea broth, and Kligler iron agar. Organisms are mainly identified by a change in the colour of medium and or the production of gas.

10. Adjustment of PH of media

The pH of most culture media is near neutral. The pH of media should be adjusted as directed in the method of preparation. Minor adjustments should be carried out using 0.1 mol/l (N/10) sodium hydroxide when the medium is too acid, and 0.1 mol/l (N/10) hydrochloric acid when too alkaline.

11. Culture techniques-preparation and uses of wire loop, inoculation on plates and slopes-stab streaking-pour plate-shake culture-incubation

Culture techniques

The purpose of using cultural techniques in microbiology is to demonstrate the presence of organisms which may be causing disease, and when indicated, to test the susceptibility of pathogens to antimicrobial agents.

Preparation and uses of wire loop

Cut a piece of wire about 125 mm in length. Wind it around a loop holder.

Using a pair of scissors, cut off one arm of the wire leaving the loop and about 50 mm of wire. Bend the loop back to make it central using a pair of forceps.

Insert the wire in a loop holder and make sure the loop is completely closed.

Wire loop is used to inoculate and streak the culture media.

Figure11.1: Wire loop

Calibarated Wire Loops

commercially made calibrated disposable wire loops are also available. they usually available in 1 ul , 2ul, 10ul volumes

Inoculation on plates

1. Using a sterile loop or swab of the specimen, apply the inoculum to a small area of the plate (the 'well').
2. Flame sterilize the loop. When cool, spread the inoculum. This will ensure single isolated colony growth.

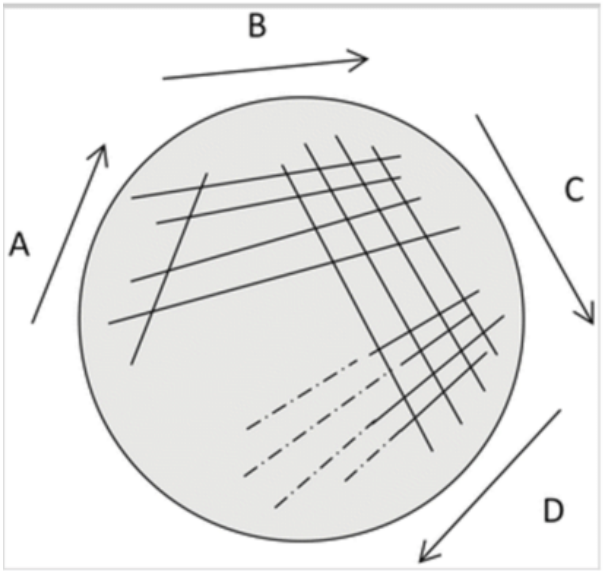


Figure11.2: Inoculation technique

Inoculation on slopes

To inoculate a slope and butt medium, such as Kligler iron agar, use a sterile straight wire to stab into the butt first and then use the same wire to streak the slope in a zig-zag pattern.

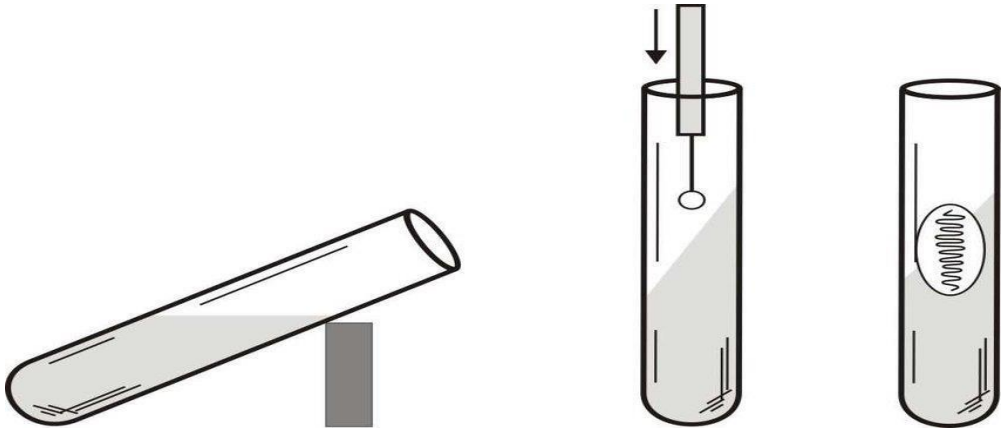


Figure 11.3: Inoculation on slopes

Stab inoculation

Use a sterile straight wire to inoculate a stab medium. Stab through the centre of the medium, taking care to withdraw the wire along the line of inoculum without making further stab lines.

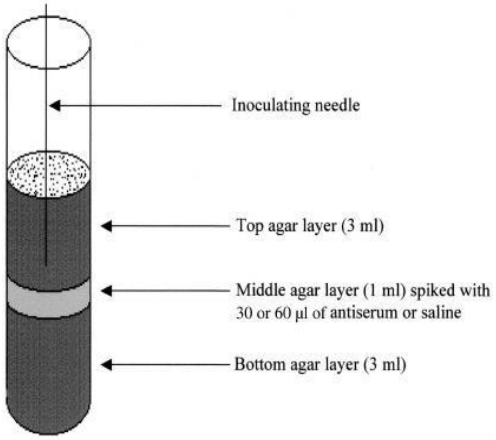


Figure11.4 : Stab inoculation

Pour plate

The pour plate method allows the plating of higher volumes of sample (1 mL) by dispensing a liquid inoculum onto an empty petri dish, which is then flooded with a molten medium.

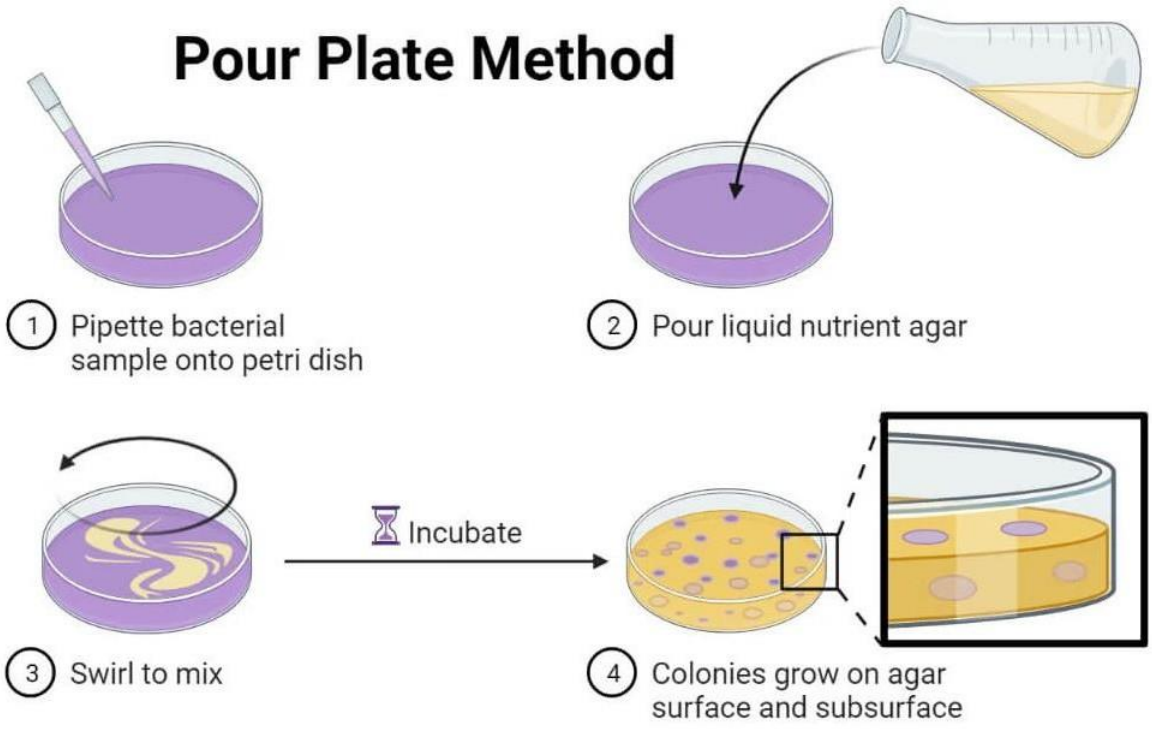


Figure 11.5: Pour plate method

Shake culture

In shake-flask cultures, the cells are exposed to low oxygen concentrations since the microorganisms are suspended in the culture broth.

Incubation

The temperature selected for routine culturing is 35-37C with most microbiologists recommending 35C in preference to 36C or 37C.

12. Aaerobic and anaerobic culture techniques

Aerobic techniques

In aerobic method, the organisms are incubated in a standard incubator under normal atmospheric conditions at 37°C.

Figure 12.1: Incubation of

culturesAnaerobic techniques

The usual method is to use McIntosh jar and a packet filled with powder is placed in the jar and is made airtight. The powder in the kit uses all the free oxygen in the jar in a chemical reaction and thus creates anaerobic atmosphere.

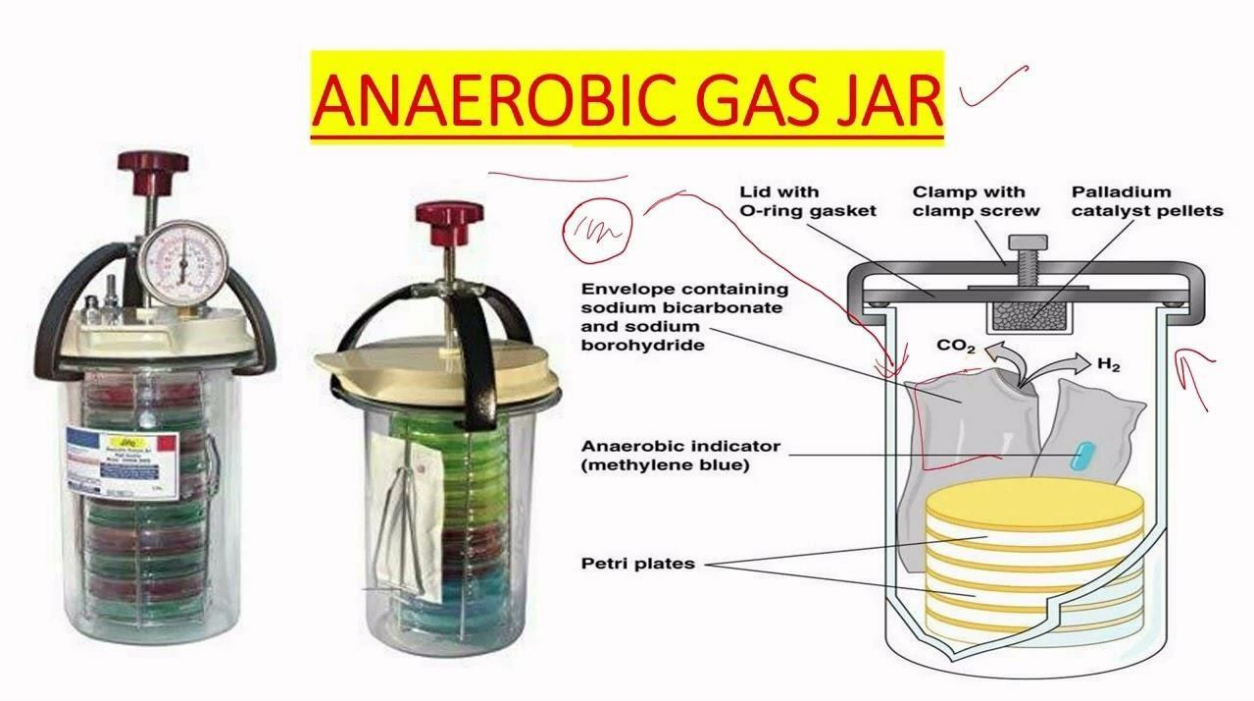


Figure 12.2: Anaerobic jar used for anaerobic culture

13. Examination of culture for growth

Bacteria grow on solid media as colonies. A colony is defined as a visible mass of microorganisms all originating from a single mother cell, therefore a colony constitutes a clone of bacteria all genetically alike.

In the identification of bacteria and fungi much weight is placed on how the organism grows in or on media. This exercise will help you identify the cultural characteristics of a bacterium on an agar plate - called **colony morphology**.

Procedure

Use a plate which has well-isolated colonies. Look at the largest colonies with the naked eye to determine general shape.

Use a colony counter since it has a magnifying glass, and a light behind the plate stage.

In order to determine CONSISTENCY, use an inoculating loop to pick up the colony and determine the consistency of the inoculum material as the loop leaves the agar medium.

Figure 13.1: Quebec colony counter

Shapes of colonies with characteristics

Whole shape of colony

Varies from round to irregular to filamentous and rhizoid (root-like)

Size of colony

Can vary from large colonies to tiny colonies less than 1mm.

Margins of colony

Magnified edge shape (use a dissecting microscope to see the margin edge well)

Chromogenesis

Color of colonies, pigmentation: white, buff, red, purple, etc.

Opacity of colony

Is the colony transparent (clear), opaque (not transparent or clear), translucent (almost clear.

Elevation of colony

How much does the colony rise above the agar?

Surface of colony

Smooth, glistening, rough, dull (opposite of glistening), rugose (wrinkled)

Consistency of colony

Butyrous (buttery), viscid (sticks to loop, hard to get off), brittle/friable (dry, breaks apart), mucoid (sticky, mucus-like).

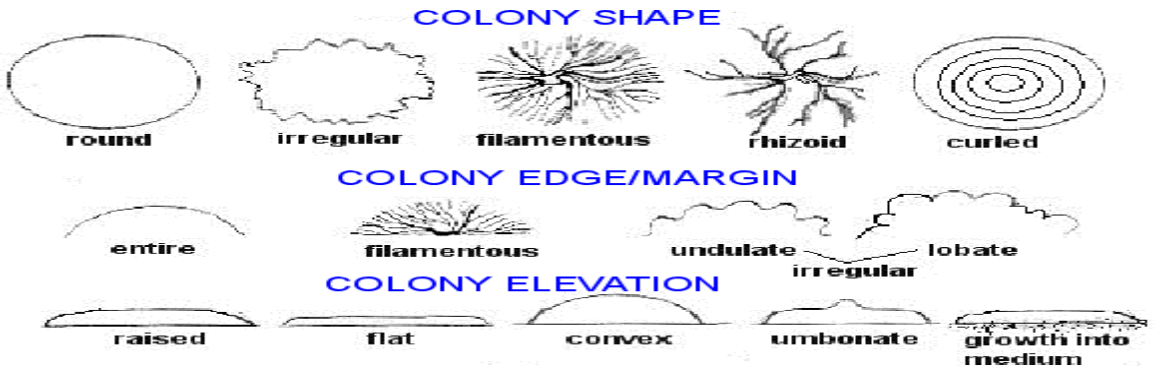


Figure13.2: Colony morphological features

14. Microscopic Examination of Bacterial Smear

Bacteria are colorless so they would be invisible to naked eye if observed under microscope thus bacteria should be stained with certain dyes in order to visualize bacterial cell or their internal structures using the light microscope.

1. If no microorganisms are seen in a smear of a clinical specimen, report “No microorganisms seen.
2. If microorganisms are seen describe morphology.

Identification of bacteria under microscope

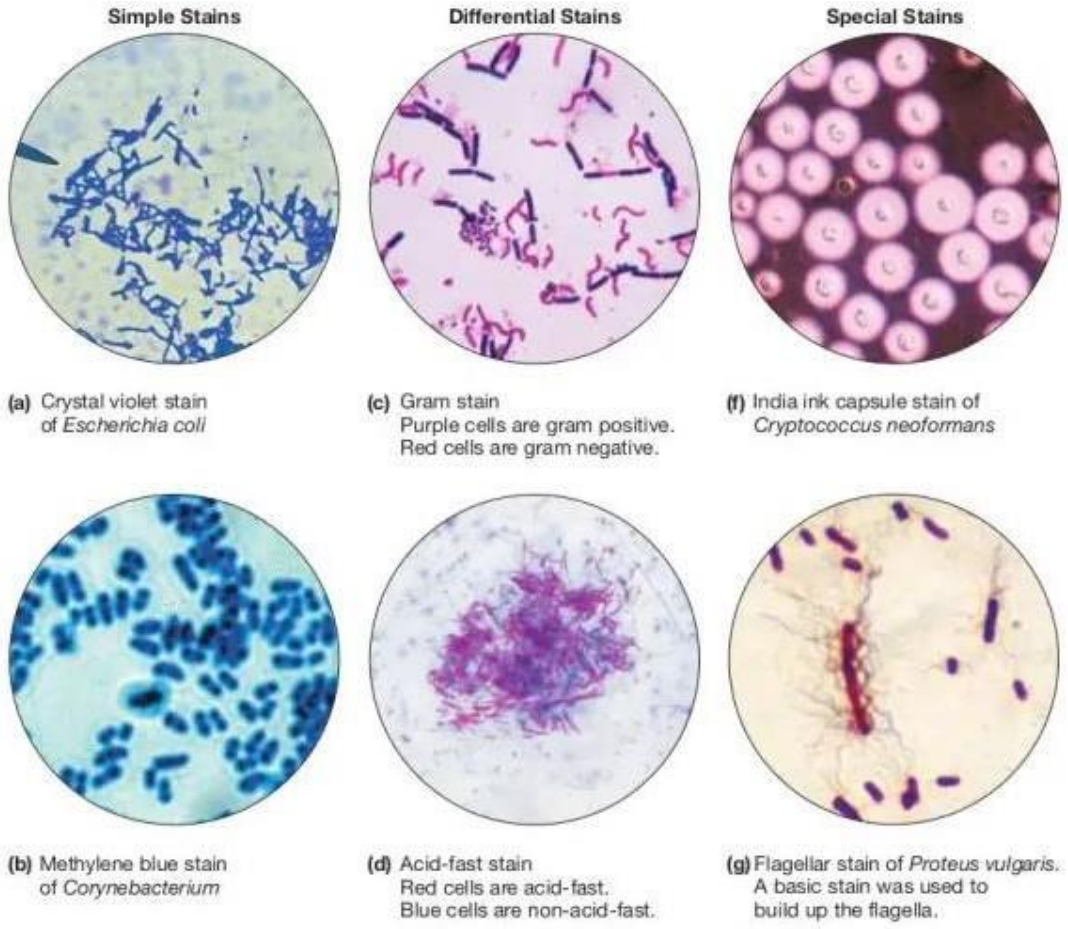


Figure14.1: Different bacteria under microscope

15. Morphological characters of important and common organisms

On the basis of shape bacteria are classified as

1. Cocci

Cocci are small, spherical or oval cells. In Greek 'Kokkos' means berry e.g. Staphylococcus.

2. Bacilli

They are rod shaped cells e.g Bacillus anthracis. It is derived from Greek word "Bacillus" meaning stick.

3. Vibrios

They are comma shaped curved rods e.g. Vibrio cholera.

4. Spirilla

They are longer rigid rods with several curves or coils.

5. Spirochetes

They are slender and flexuous spiral forms.

6. Actinomycetes

They are branching filamentous bacteria e.g. Streptomyces.

7. Mycoplasma

They are cell wall deficient bacteria and hence do not possess stable morphology. They occur as round or oval bodies with interlacing filaments.

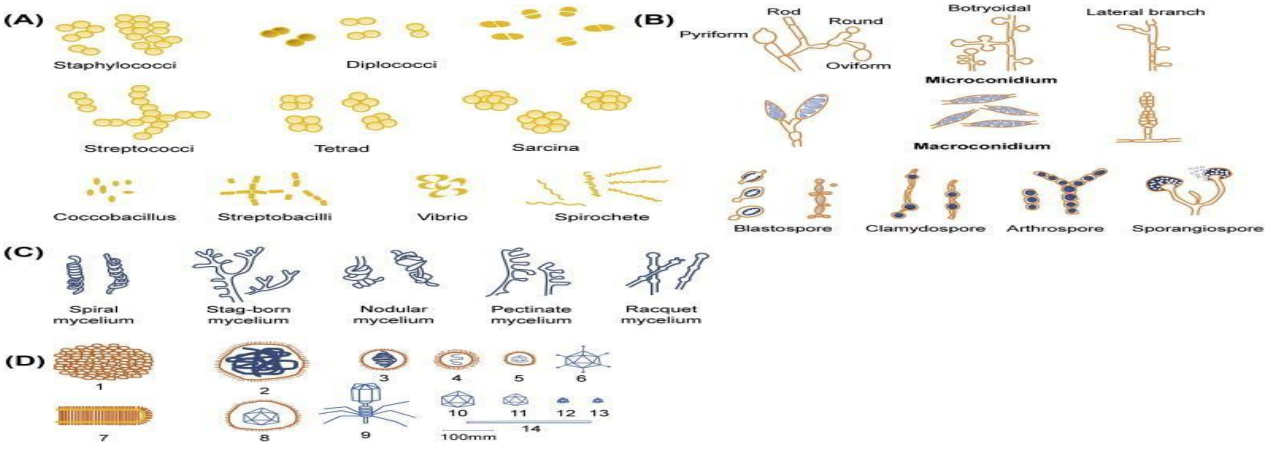


Figure15.1: Morphology of different bacteria

16. Study of common organisms-important tests cogulase test, catalase test, oxidase test, motility test

Coagulase test

This test is used to identify Staphylococcus aureus which produces the enzyme coagulase.

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Procedure

1 Place a drop of distilled water on each end of a slide or on two separate slides.

2 Emulsify a colony of the test organism in each of the drops to make two thick suspensions.

3 Add a loopful of plasma to one of the suspensions, and mix gently.

Look for clumping of the organisms within 10 seconds.

No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Results Clumping within 10 secs..... Staphylococcus aureus

No clumping within 10 secs..... No bound coagulase

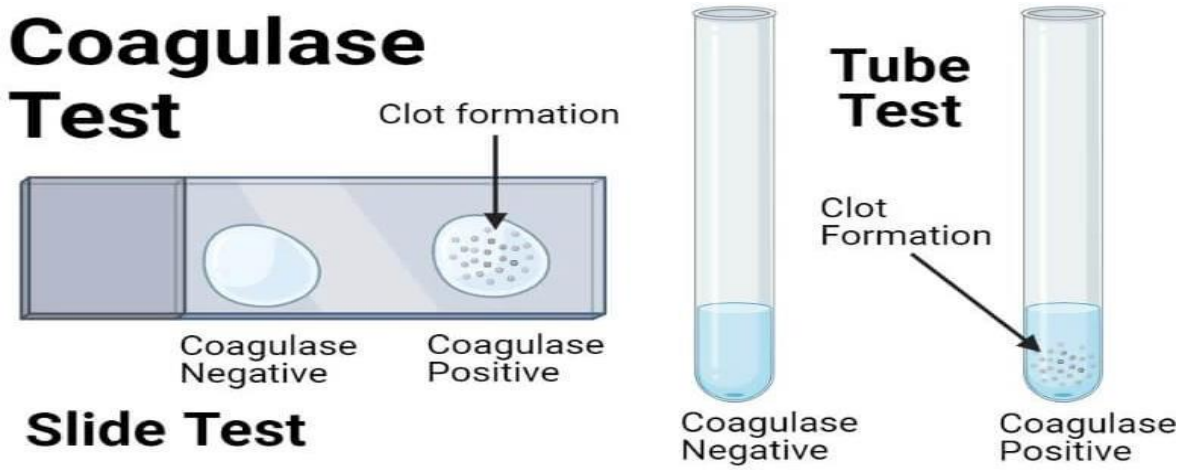


Figure 16.1: Coagulase test demonstration

Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci.

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

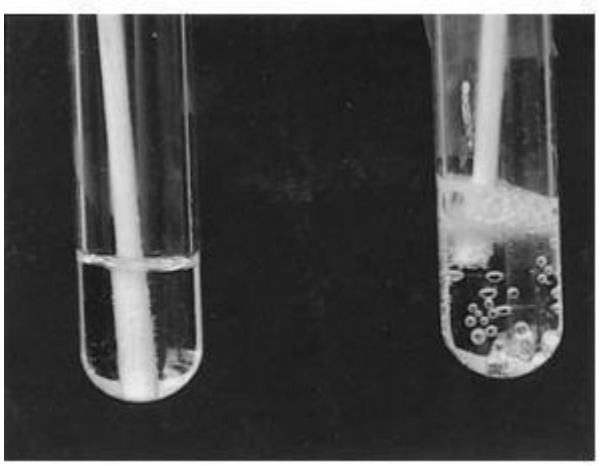


Figure 16.2: Left shows a negative catalase test while Right shows positive catalase test

Required

Hydrogen peroxide, 3% H_2O_2 (10 volume solution)

Method

1 Pour 2–3 ml of the hydrogen peroxide solution into a test tube.

2 Using a sterile wooden stick or a glass rod (not a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution. Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.

3 Look for immediate bubbling as shown in the figure.

Results

Active bubbling.....Positive catalase test

No bubbles.....Negative catalase test

Oxidase test (Cytochrome oxidase test)

The oxidase test is used to assist in the identification of Pseudomonas, Neisseria, Vibrio, Brucella, and Pasteurella species, all of which produce the enzyme cytochrome oxidase.

Principle

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour.

Required

Oxidase reagent freshly prepared. Note: Fresh oxidase reagent is easily oxidized. When oxidized it appears blue and must not be used. Stable oxidase reagent strips

Method

1 Place a piece of filter paper in a clean Petri dish and add 2 or 3 drops of freshly prepared oxidase reagent.

2 Using a piece of stick or glass rod, remove a colony of the test organism and smear it on the filter paper.

3 Look for the development of a blue-purple colour within a few seconds.

Results

Blue-purple colour .....Positive oxidase test (within 10 seconds)

No blue-purple colour..... Negative oxidase test (within 10 seconds)

Figure 16.3: Positive and negative oxidase test

17. Blood culture-sampling media used-procedure identification of organisms-bacteria commonly, isolated

Blood culture-sampling

1 Using a pressure cuff, locate a suitable vein in the arm. Deflate the cuff while disinfecting the venipuncture site.

2 Wearing gloves, thoroughly disinfect the venipuncture site as follows: – Using 70% ethanol, cleanse an area about 50 mm in diameter. Allow to air-dry. – Using 2% tincture of iodine, swab the area in circular manner. Allow the iodine to dry on the skin for at least 1 minute.

3 Remove the protective cover from the top of the culture bottle. Wipe the top of the bottle using an ethanol-ether swab.

4 Using a sterile syringe and needle, withdraw about 10 ml of blood from an adult or about 2 ml from a young child.

5 Insert the needle through the rubber liner of the bottle cap and dispense blood into the bottle.

6 Clearly label each bottle with the name and number of the patient, and the date and time of collection.

7 As soon as possible, incubate the inoculated media. Protect the cultures from direct sunlight until they are incubated.

Blood culture media

Columbia agar and Columbia broth diphasic medium for aerobes and facultative anaerobes and Thioglycollate broth medium to isolate strict anaerobes.

Identification of organisms-bacteria commonly, isolated

Colonial appearances

Colonies of staphylococci, S. typhi, Brucellae, and most coliforms can usually be seen easily, whereas colonies of S. pneumoniae, Neisseria species, S. pyogenes, and Y. pestis are less easily seen.

Pseudomonas and Proteus species produce a film of growth on the agar.

When growth is present:

Subculture on blood agar, chocolate agar, and MacConkey agar.

Incubate the blood agar and MacConkey agar plates.

Examine a Gram stained smear of the colonies.

Depending on the bacteria seen, test the colonies further (e.g. for coagulase, catalase, oxidase, urease, and motility).

When large Gram positive rods resembling C. perfringens are seen: Subculture also on lactose egg yolk milk agar and incubate the plate anaerobically.

When motile, urease and oxidase negative Gram negative rods are isolated: Subculture the colonies on Kligler iron agar.

When catalase positive Gram negative coccobacilli are isolated, suspect Brucella species and send the culture to a microbiology specialist laboratory for identification. Mark the culture 'High Risk'.

18. Urine culture-sampling-media used-procedure-bacteria commonly isolated

Sampling

Whenever possible, the first urine passed (midstream urine) by the patient at the beginning of the day should be sent for examination.

1 Give the patient a sterile, dry, wide-necked, leak-proof container and request a 10–20 ml specimen. Important: Explain to the patient the need to collect the urine with as little contamination as possible, i.e. a 'clean-catch' specimen.

Female patients: Wash the hands. Cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine with the labia held apart.

Male patients: Wash the hands before collecting a specimen (middle of the urine flow).

2 Label the container with the date, the name and number of the patient, and the time of collection.

Media used for urine culture

Cystine lactose electrolyte-deficient (CLED) agar is used to isolate urinary pathogens.

Bacteria commonly isolated

Escherichia coli, Proteus species, Pseudomonas aeruginosa, Klebsiella strains

Figure18.1: Bacterial growth on CLED agar and urine sample

19. Throat swab culture-sampling-media used procedure bacteria commonly isolated

Throat swab culture-sampling

In a good light and using the handle of a spoon to depress the tongue, examine the inside of the mouth. Look for inflammation, and the presence of any membrane, exudate, or pus.

Swab the affected area using a sterile cotton-wool swab. Taking care not to contaminate the swab with saliva, return it to its sterile container.

Figure 19.1: Throat swab sampling

Media used

Blood agar, Chocolate agar.

Procedure

Inoculate the swab on a plate of blood agar. Incubate the plate preferably anaerobically or, in a carbon dioxide enriched atmosphere overnight at 35–37 C.

Bacteria commonly isolated

Streptococcus pyogenes

20. Eye swab culture-sampling-media used-procedure-bacteria commonly isolated pathogen.

Sampling

1. Take swab for culture before application of topical anesthetics.
2. Cleanse skin around eye with mild antiseptic.
3. Collect specimen by swabbing.
4. Moisten sterile swab with sterile saline.
5. Pass moistened swab 2 times over lower conjunctiva.
6. Avoid eyelid border and lashes.
7. Insert swab into moist sponge in swab container to preserve specimen.
8. Label container with patient's legal name, date and actual time of collection, type of specimen, and as right or left eye.

Figure 20.1: Eye swab sampling

Media used

Blood agar and MacConkey agar

Procedure

Inoculate the swab on a plate of blood agar. Incubate the plate preferably anaerobically or, in a carbon dioxide enriched atmosphere overnight at 35-37 C.

Bacteria commonly isolated

Staphylococci, Streptococcus pyogenes and Pseudomonas aeruginosa.

21. Nasal and ear swab culture-sampling media used-procedure-bacteria commonly isolated

Nasal Swab Collection

Nasal swab is collected from the anterior turbinate.

Insert dry swab into nostril up to 1 inch.

Slowly rotate the swab, gently pressing against the inside of nostril at least 4 times for a total of 15 seconds. Get as much nasal discharge as possible on the soft end of the swab.

Slowly remove swab while slightly rotating it.

Use a same swab for another nostril.

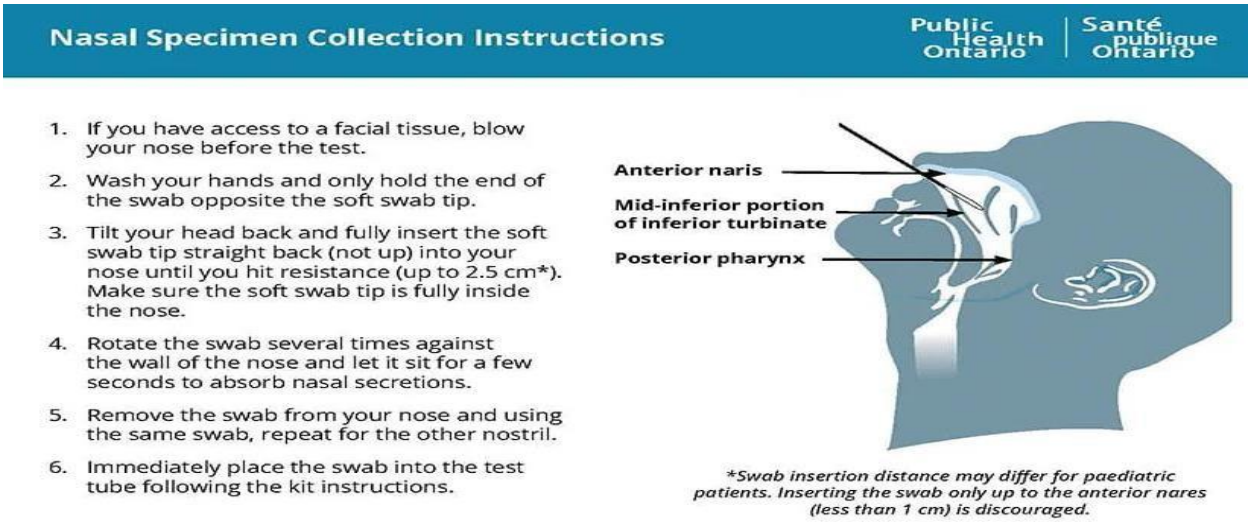


Figure 21.1: Nasal swab specimen collection

Ear swab collection

An ear drainage culture is collected by placing a cotton swab gently in the ear canal.

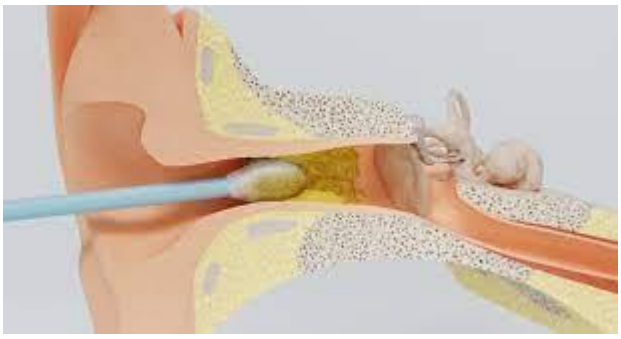


Figure21.2: Ear swab specimen collection

Media used

Blood agar, Choclate agar and MacConkey agar

Procedure

Inoculate the swab on a plate of blood agar. Incubate the plate preferably anaerobically or, in a carbon dioxide enriched atmosphere overnight at 35–37 C.

Bacteria commonly isolated

Streptococcus pneumoniae and Haemophilus influenzae

22. Stool culture-sampling media used-procedure-bacteria commonly isolated

Sampling

1 Give the patient a suitable wide-necked sterilized container in which to pass a specimen.

2 Transfer a portion (about a spoonful) of the specimen, especially that which contains mucus, pus, or blood, into a clean, dry, leakproof container.

3 Label the specimen and send it with a request form to reach the laboratory within 1 hour.

Figure 22.1: Stool specimen

collection Media used for culture

Xylose lysine deoxycholate (XLD) agar, MacConkey agar, Salmonella-Shigella agar

Procedure

Inoculate the swab on the selected agar. Incubate the plate preferably anaerobically or, in a carbon dioxide enriched atmosphere overnight at 35–37 C.

Commonly isolated pathogens

Salmonella and Shigella species

23. <u>Vaginal swab culture sampling media used procedure bacteria commonly isolated</u>

Vaginal swab sampling

1 Moisten the speculum with sterile warm water, and insert it into the vagina.

2 Cleanse the cervix using a swab moistened with sterile physiological saline.

3 Pass a sterile cotton-wool swab 20–30 mm into the endocervical canal and gently rotate the swab against the endocervical wall to obtain a specimen.

4 Label the specimens and deliver to the laboratory as soon as possible. Inoculated culture plates must be incubated within 30 minutes.

Media used

Thayer Martin medium, Blood agar, MacConkey agar

Procedure

Inoculate the swab on the selected agar. Incubate the plate overnight at 35–37 C for 24 hours.

Commonly isolated pathogens

Streptococcus pyogenes, Staphylococcus aureus, Clostridium perfringens.

24. CSF culture-sampling media used-procedure-bacteria commonly isolated

Sampling

Cerebrospinal fluid must be collected aseptically from the arachnoid space by an experienced medical officer. A sterile wide-bore needle is inserted between the fourth and fifth lumbar vertebrae and the c.s.f. is allowed to drip into a dry sterile container.

Media used

Chocolate agar, blood agar and MacConkey agar

Procedure

Inoculate the swab on the selected agar. Incubate the plate overnight at 35-37 C for 24 hours.

Commonly isolated pathogens

Streptococcus pneumoniae Streptococcus agalactiae, Neisseria meningitides, Haemophilus influenzae, Escherichia coli

25. Method of determination of sensitivity of antibiotics

Pathogenic bacteria show great variation in susceptibility to antibiotics. It is therefore essential to determine the susceptibility.

There are mainly 2 methods to determine the susceptibility

Diffusion tests

3. Strokes disc diffusion method
4. Kirby-Bauer disc diffusion method

Disc diffusion methods

Dilution tests

5. Broth dilution method
6. Agar dilution method

Broth dilution methods

For the clinical and surveillance purposes WHO recommends **modified Kirby-Bauer disc diffusion method.**

Kirby-Bauer disc diffusion method

This is used in routine in clinical laboratory. In disc diffusion methods the discs of filter paper soaked in known quantity of antibiotic are placed on plates of appropriate medium inoculated with pure culture of organisms.

Antibiotics diffuse in the surrounding medium thus preventing the growth of organisms in an area where the antibiotic concentration remains sufficient for killing the organisms. A visible clear zone appears the diameter of which, is measured and compared with control organisms.

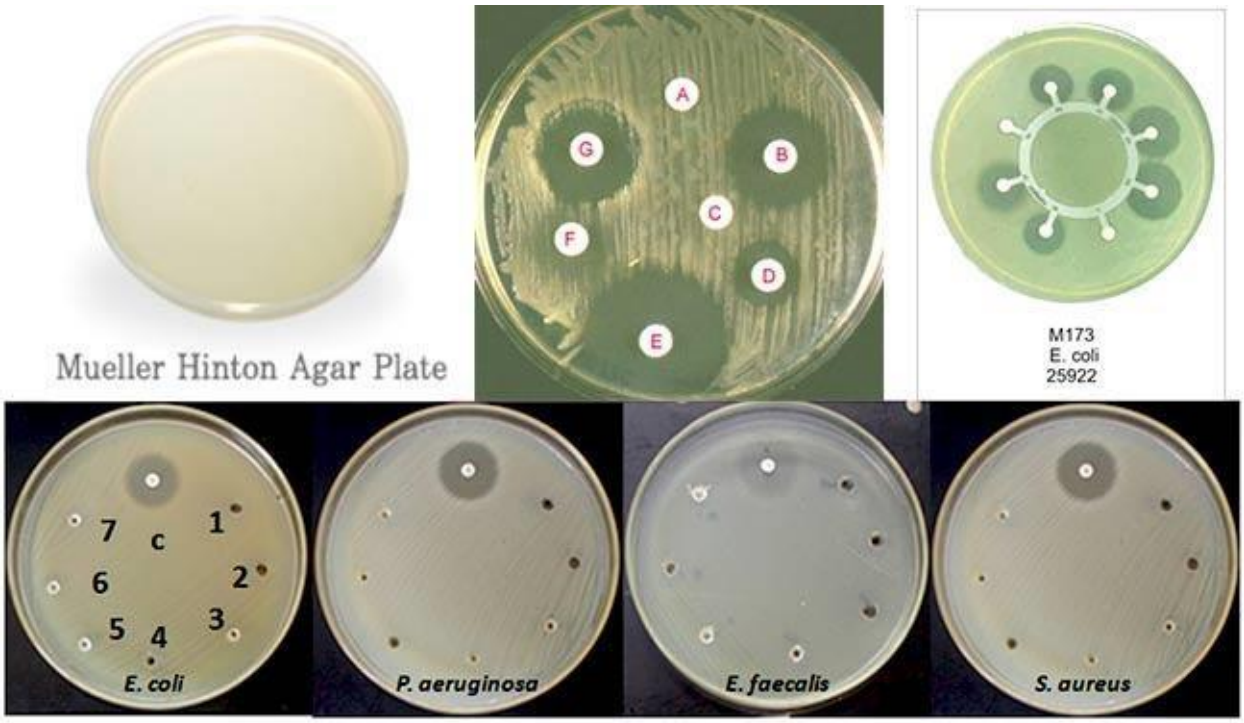


Figure 25.1: Mueller Hinton agar showing growth of bacteria and zones of inhibition

VIROLOGY

26. Precautions to be observed in virology laboratory

Enter the lab through the staircase/elevators/ on any given route.

Remove the street footwear and keep them in the designated place/ shoe rack and wear dedicated lab foot-wears.

Press the locking button to open the door and enter inside of the corridor area.

Sanitize hands with 70% IPA.

Open the garment cubicle, pick and wear the dedicated full sleeves white apron. Ü Wear the head cap, shoe cover before entering inside.

Enter the virology section through the corridor. For different areas such as lysis, extraction, Pre-PCR, analysis room enter through the general corridor and ensure that the personnel is wearing appropriate Personal Protective Equipment (PPE)

Before leaving the lab, remove the apron and hang them in the designated place of the garment cubicle or in the racks available in the changing room.

In the case of shoe covers, masks, gloves, remove them and discard in the dedicated dust bin.

Press interlocking button to open the air lock door. Open the exit door of main entry and keep the dedicated virology lab footwears in the designated place.

Open the door leading to exit from the corridor.

Figure 26.1: Personal protective equipments for a virology lab

27. Collection, transportation and storage of specimen for virological examination

Collection

Specimen collection

Use personal protective equipment

Wear PPE, before collecting patient sample such as SAR-CoV-2 or any other infectious agents.

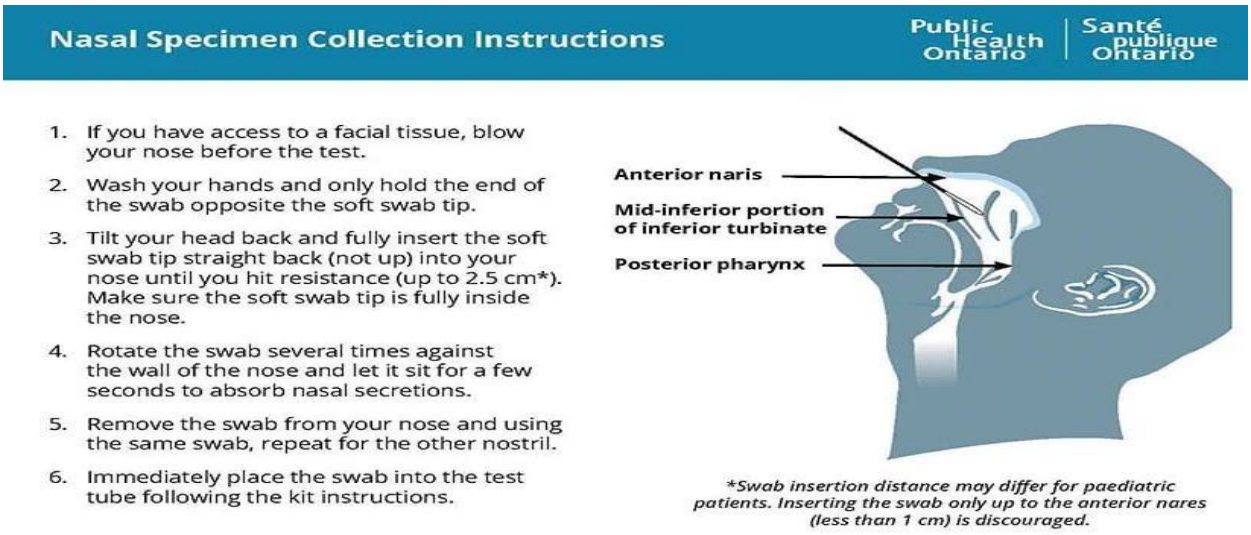


Figure 27.1: nasal specimen collection

Throat Swab Collection:

Have the patient open his/her mouth wide open

Use tongue depressor.

Sweep the swab over the back of throat including tonsils.

Send both nasal and throat swab of one patient in a single VTM tube.

Send filed requisition form also.

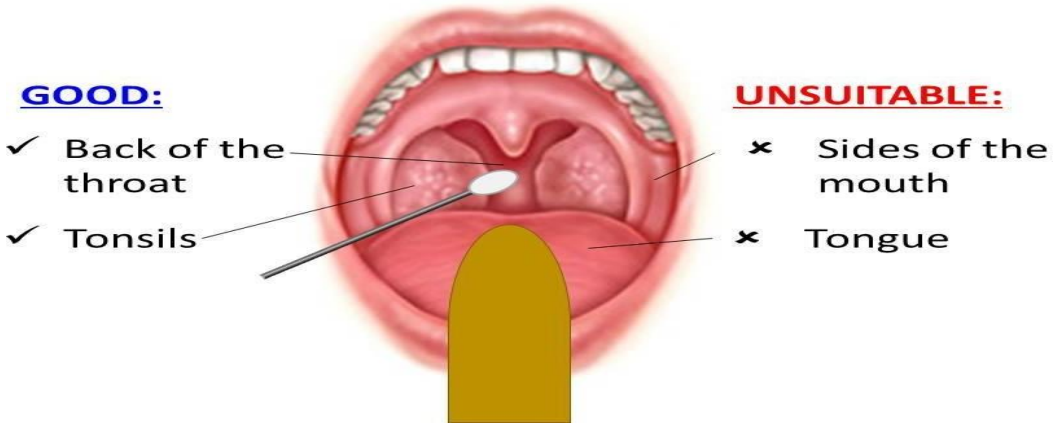


Figure27.2: Throat swab collection

Specimen packaging and transport

Triple layer sample packaging:

Sample should be safely packed in a triple layer container

The following are the key features of the sample packaging:

Protects the environment and the carrier

Protects the sample

Arrival in good condition for analysis

The packaging consists of three layers as follows:

Primary receptacle:

A labelled primary watertight, leak-proof receptacle containing the specimen.

The receptacle is wrapped in enough absorbent material so as to absorb all the fluid in case of a breakage

Secondary receptacle:

A second durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle.

Several wrapped primary receptacles may be placed in one secondary receptacle.

Sufficient additional absorbent material must be used to cushion multiple primary receptacles.

Outer shipping package:

The secondary receptacle is placed in an outer shipping package which protects it and its contents from outside influences such as physical damage and water while in transit.

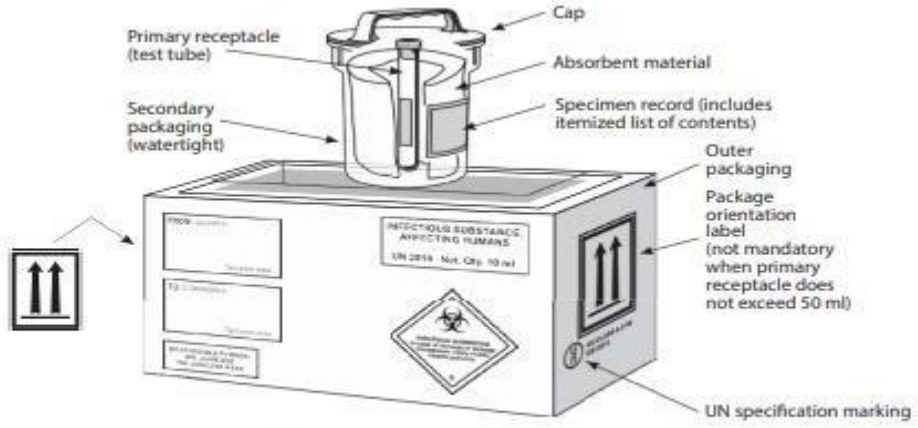


Figure 5: Transportation of specimen

Specimen data forms, letters, and other types of information that describe the specimen for “testing” and also identify the shipper should be taped to the outside of the second receptacle.

Sample should be transported in the cold chain to the laboratory with prior intimation.

All specimens should be delivered to the laboratory soon after collection owing to the loss of infectivity which occurs over time.

If immediate delivery is not possible, refrigerate specimens (2 °C to 8 °C), or place them on wet ice or a cold pack.

Do not freeze samples.

If the sample has to be frozen, freeze them rapidly at –20 °C or –70 °C.

Specimen collection, Storage and Transport details:

Specimen type	Collection material	Transport to laboratories. (48-72 hours.)	Storage till testing
Nasopharyngeal and oropharyngeal swab. (Both swabs should be placed in the same tube to increase the viral load.)	VTM	4° C	≤72 hours. 4 ° C. >72 hours. - 70 ° C.
Bronchoalveolar lavage.	Sterile container.	4 ° C	≤ 48 hours.4 ° C. > 48 hours. - 70 ° C
Tracheal aspirate Nasopharyngeal aspirate or nasal wash.	Sterile container.	4 ° C	≤ 48 hours.4 ° C. > 48 hours. - 70 ° C
Sputum. (Ensure the material is from the lower respiratory track.)	Sterile container	4 ° C	≤ 48 hours.4 ° C. > 48 hours. - 70 ° C
Cerebrospinal fluid (CSF).	Sterile round bottom tube	4 ° C	≤72 hours. 4 ° C.
Amniotic fluid	VTM or Sterile container	4 ° C	>72 hours. - 70 ° C.
Vitreous fluid.		4 ° C	≤72 hours. 4 ° C.
Urine.	Sterile tube/container	4 ° C	>72 hours. - 70 ° C.
Blood	In yellow ACD tube	4 ° C	≤72 hours. 4 ° C.
Bone marrow aspirate	In yellow ACD tube	4 ° C	>72 hours. - 70 ° C.
Faces.	In container without preservative.	4 ° C	≤72 hours. 4 ° C.
Tissue.	In sterile saline or viral transport media.	4 ° C	>72 hours. - 70 ° C.

28. Examination of virus under microscope

MICROSCOPIC IDENTIFICATION

Viruses cannot be detected and identified by direct microscopic examination of clinicalspecimens such as biopsy material or skin lesions. But Tissue Reaction or injury by virus can be idnetiifed microscopy

Three different procedures can be used.

Light microscopy

Light microscopy can reveal characteristic inclusion bodies or multinucleated giant cells.

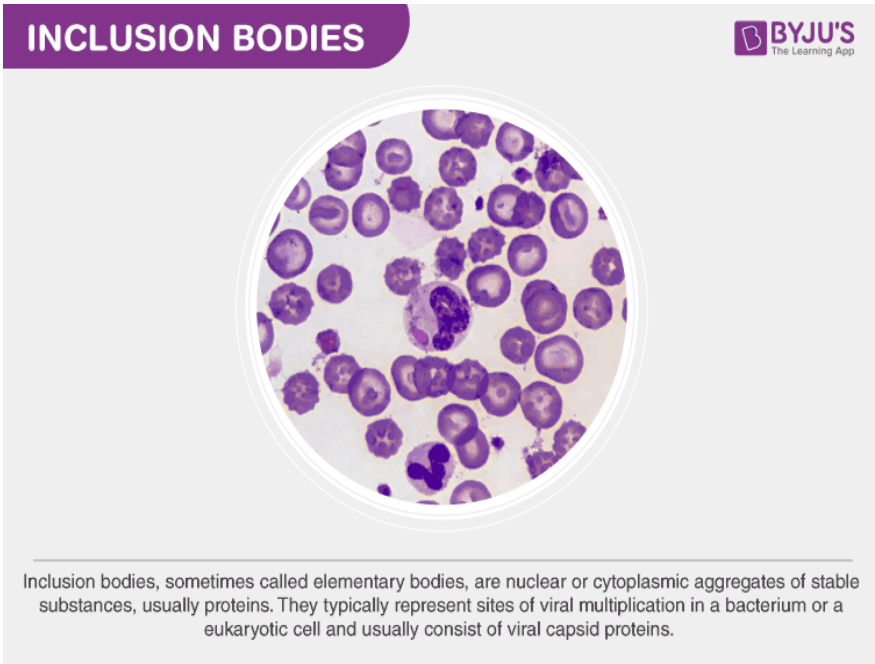


Figure 28.1: Inclusion bodies

The **Tzanck smear**, which shows herpesvirus-induced multinucleated giant cells in vesicular skin lesions, is a good example.

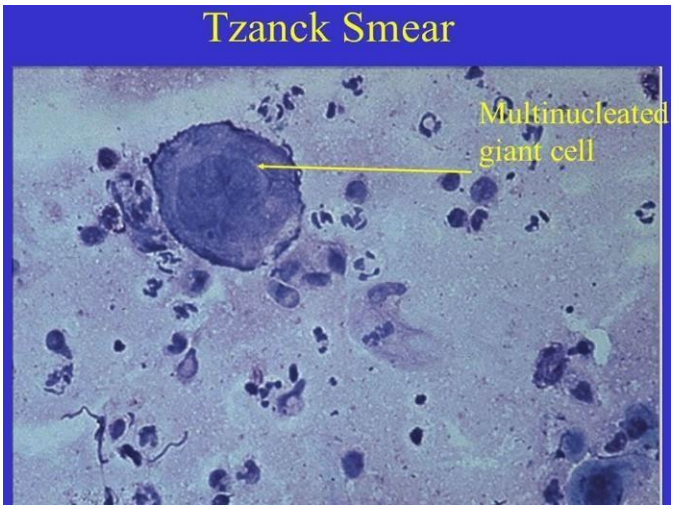
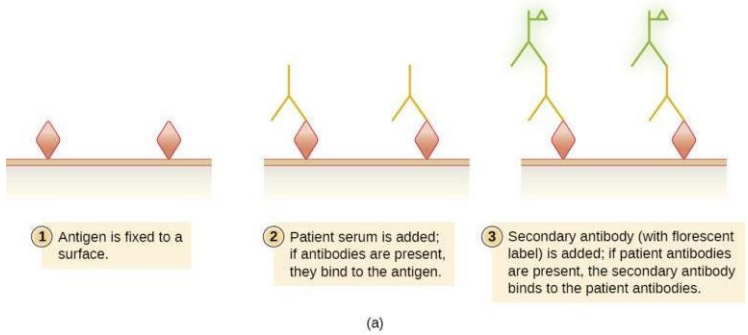


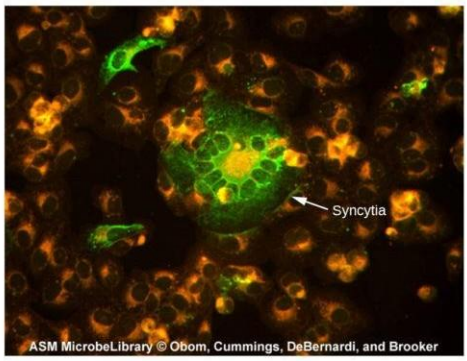
Figure 28.2: Tzank smear showing multinucleated giant cells

UV 592 microscopy

UV 592 microscopy is used for fluorescent antibody staining of the virus in infected cells.



(a)



(b)

Electron microscopy

Electron microscopy detects virus particles, which can be characterized by their size and morphology.

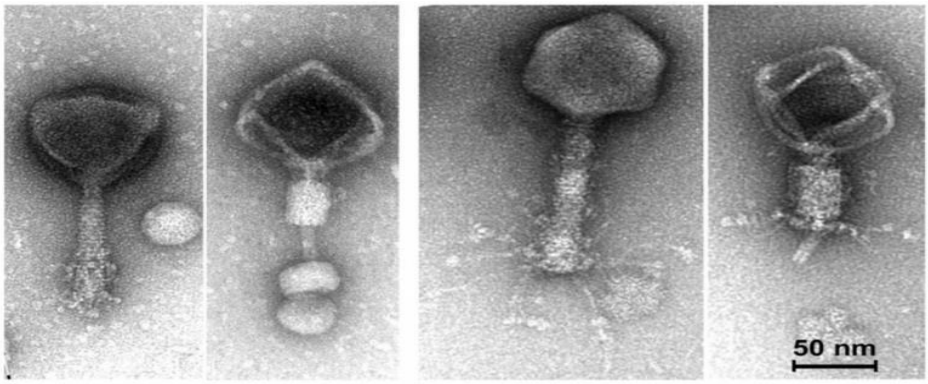


Figure28.3: Electron microscope showing different viral particles

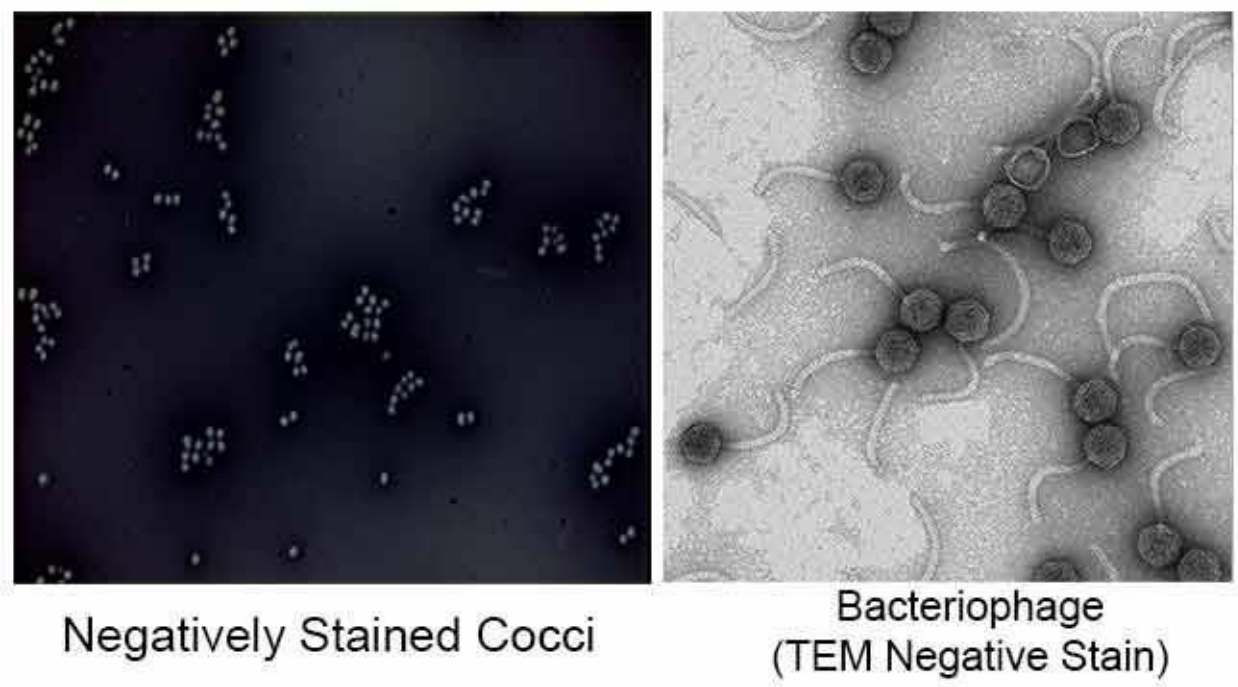
29. Staining procedures for viruses

Viral staining is a technique used in virology to enhance the visibility of viruses when viewed under a microscope. Different staining methods can be employed depending on the type of virus and the specific goals.

Here are some common viral staining methods:

Negative staining

In negative staining, the background around the virus particles is stained, leaving the viruses unstained and visible as clear areas against a dark background. This method is commonly used in electron microscopy. Common negative stains include India ink.



Negatively Stained Cocci

Bacteriophage
(TEM Negative Stain)

Immunohistochemical staining

Immunohistochemistry involves using antibodies that specifically bind to viral antigens. These antibodies can be visualized using fluorescent dyes or enzyme markers, allowing for the identification of viral particles in tissue samples.

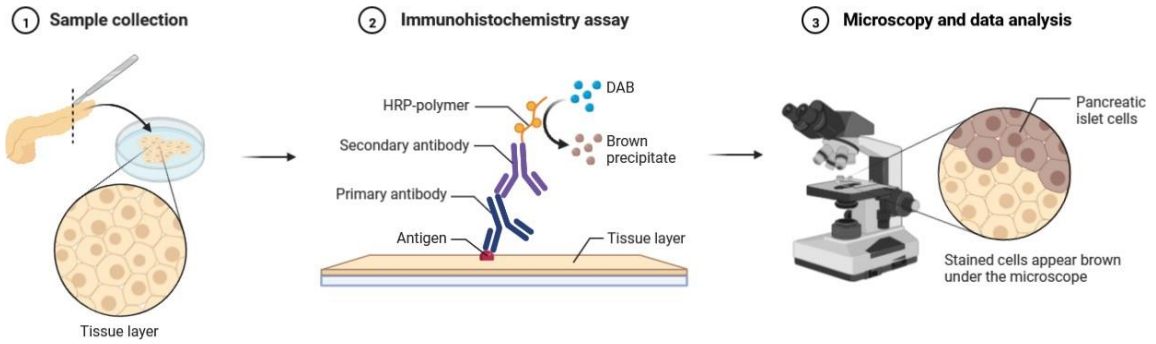
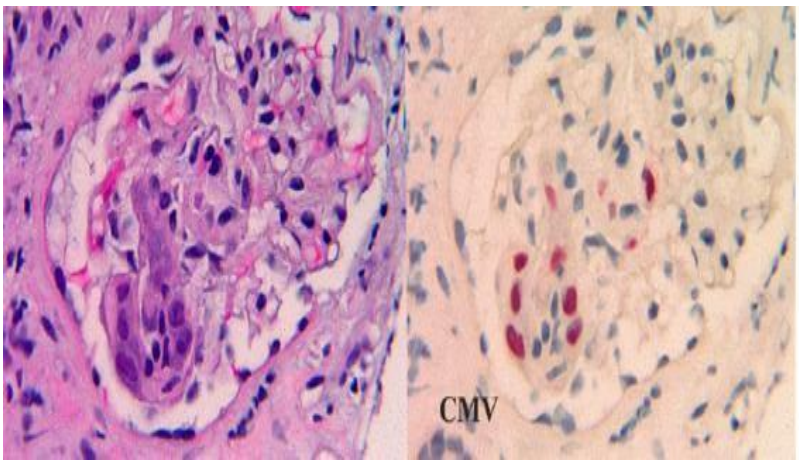


Figure29.1: Immunohistochemical staining

Hematoxylin and eosin staining

H&E staining is more commonly used for histological samples, it can be employed to visualize some viral infections. Hematoxylin stains cell nuclei blue, while eosin stains cytoplasm and extracellular structures pink.



glomerulitis. Hematoxylin and eosin (H&E) and CMV immunostain depicting a glomerulus with a cluster of capillaries occluded by enlarged and hyperchromatic endothelial cells. On the H&E stain (left), the combination of nuclear enlargement and basophilic granular cytoplasm in the abnormal cells is strongly suggestive of CMV cytopathic changes. The CMV immunostain (right) is confirmatory, marking the nuclei of multiple infected cells.

Figure 29.2: Hematoxylin and eosin staining showing cells infected by virus

Giemsa stain

Giemsa stain is commonly used in virology for light microscopy. It is also useful for visualizing certain viral particles in tissues.

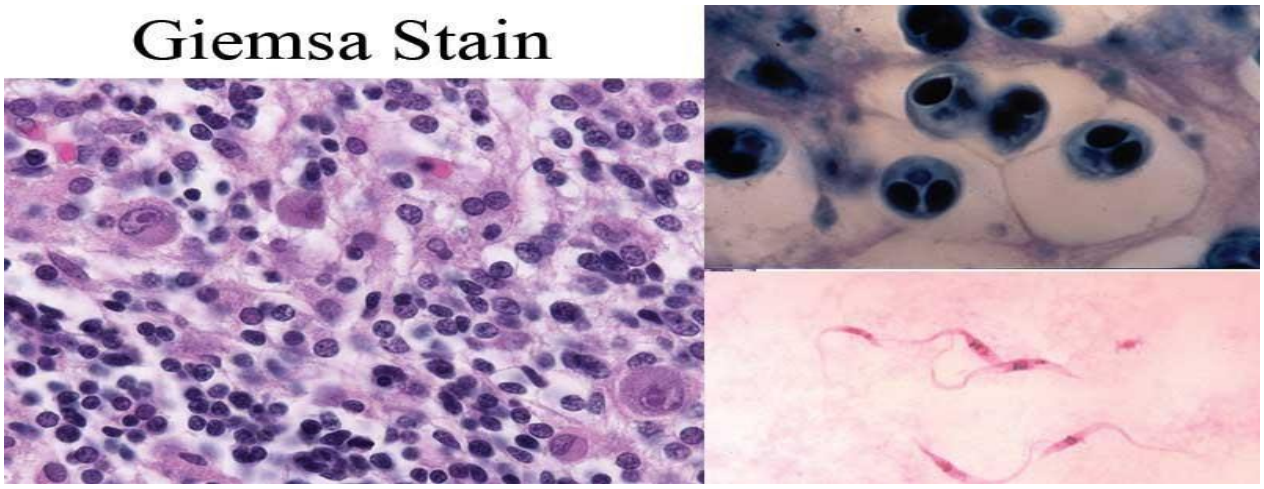


Figure 29.3: Giemsa staining showing owl's eye appearance in CMV infection

30. Procedures for isolation of viruses

There are four approaches to the diagnosis of viral diseases by the use of clinicalspecimens:

(1) Identification of the virus in cell culture.

(2) Serologic procedures to detect a rise in antibody titer or the presence of IgM antibody.

(3) Detection of viral antigens in blood or body fluids.

(4) Detection of viral nucleic acids in blood or the patient's cells.

31. Diagnostic tests for viral diseases

1. IDENTIFICATION IN CELL CULTURE

Sample of the antibody linked to an enzyme is added, which will attach to the bound virus. Then an enzyme is added, and the amount of the bound enzyme is determined.

2. MICROSCOPIC IDENTIFICATION

Viruses can be detected by direct microscopic examination of clinical specimens such as biopsy material or skin lesions. Three different procedures can be used.

(1) Light microscopy

(2) UV 592 microscopy is used for fluorescent antibody staining of the virus in infected cells.

(3) Electron microscopy detects virus particles, which can be characterized by their size and morphology.

3. SEROLOGIC PROCEDURES

A serum sample is obtained as soon as a viral etiology is suspected (acutephase), and a second sample is obtained 10 to 14 days later (convalescent-phase). If the antibody titer in the convalescent-phase serum sample is at least fourfold higher than the titer in the acute-phase serum sample, the patient is considered to be infected.

4. DETECTION OF VIRAL ANTIGENS

Viral antigens can be detected in the patient's blood or body fluids by various tests, but most often by an ELISA.

5. DETECTION OF VIRAL NUCLEIC ACIDS

Viral nucleic acids (i.e., either the viral genome or viral mRNA) can be detected in the patient's blood or tissues with complementary DNA or RNA (cDNA or cRNA) as a probe.

REFERENCES

1) Review of Medical microbiology and immunology, 11th Edition b Warren Levinson
2) Jawetz, Melenicks and Adelberg, Medical Microbiology 25th Edition
3) District Laboratory Practice in Tropical Countries, Monica Cheesbrough 2ndedition-Part 1 & 2
4) Manual of Laboratory Medicine, AFIP, 4th Edition