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Review of Medical Laboratory Techniques

MCQs Based on Theoretical Knowledge and Clinical Practice

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7 Bacteriology

CHAPTER

GENERAL MICROBIOLOGY

Louis Pasteur is also known as the father of microbiology. He described fermentation, pasteurization, germ theory of diseases, anthrax vaccine and rabies vaccine.

Robert Koch discovered the anthrax disease and the bacteria responsible for tuberculosis and cholera.

Koch's postulates: (1) The microorganism should be constantly associated with the lesions of the disease; (2) It should be possible to isolate the organism in pure culture from the lesions of the disease; (3) Inoculation of a laboratory animal with the cultured microorganism must recapitulate the disease; (4) It should be possible to re-isolate the organism in pure culture from the lesions in the experimental animal.

Molecular Koch' postulate: A gene found in a pathogenic microorganism encodes a product that contributes to the disease (virulence factor) caused by the pathogen.

Paul Ehrlich discovered the method of staining the tubercle bacillus. With his side-chain theory, Paul Ehrlich explained the basic principle of immunity. He is called the father of chemotherapy for formulating the arsenic compound, Salvarsan, which was used in the treatment of syphilis during the first half of this century until penicillin was discovered by Alexander Fleming.

Joseph Lister is called the father of antiseptic surgery.

Antoni van Leeuwenhoek: Father of microscopy.

Microscope: The parts of a microscope is shown in **Figure 7.1**.

Types of Microscopes

- **Bright field:** Forms a dark image against a brighter background, used for stained samples.
- **Dark field:** Used to illuminate and identify living, unstained cells bacteria causing them to appear brightly lit against a dark background, e.g., for *Spirochaetes*.
- **Phase contrast:** It is possible to visualize certain cell organelles and structures that are invisible with bright-field. It is useful for studying motility, endospores and inclusion bodies.
- **Fluorescent microscope:** It uses fluorescent molecules, fluorophores for the labelling of defined cellular structures which absorb light at a specific wavelength (excitation) and emit it at a specific higher wavelength (emission), e.g., acridine orange for malaria parasite and Auramine for *M. tuberculosis*.

Staining Techniques

- **Simple stain:** Involves directly staining the bacterial cell with a positively charged dye (basic dye),e.g., basic fuchsin.
- **Negative stain:** Where the bacteria remain unstained against a dark background, e.g., India Ink.

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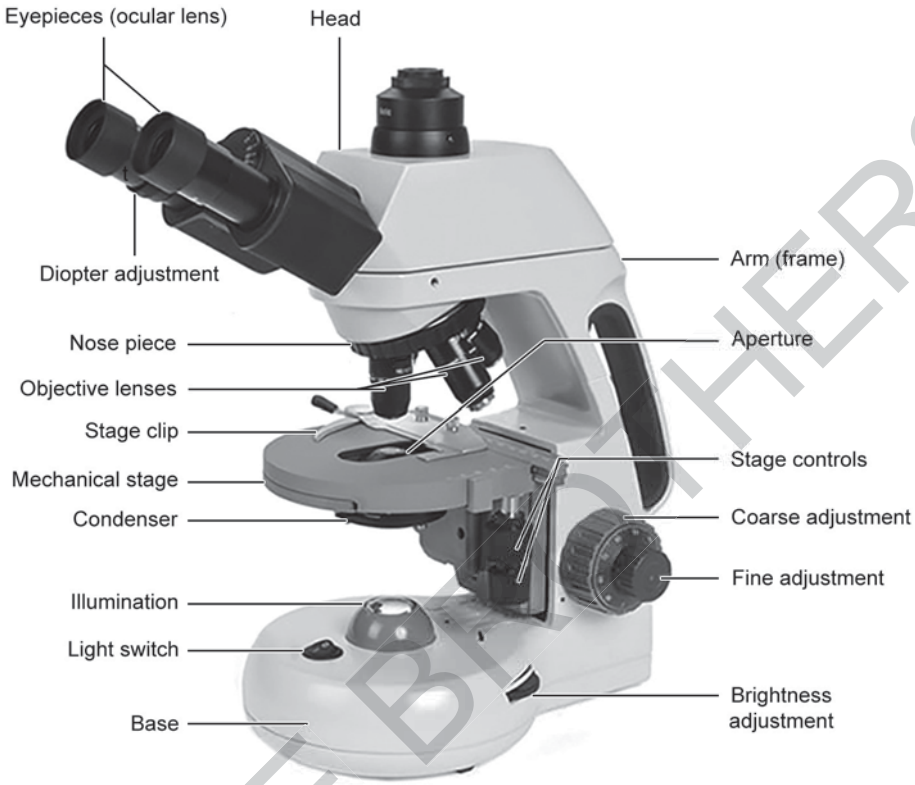

Fig. 7.1: Parts of microscope.

- **Impregnation method:** Silver impregnation is the traditional method for detection of *T. pallidum* in formalin-fixed tissues. *Borrelia spp., Bartonella spp., Leptospira spp.,* and *Calymmatobacterium*. Weakly staining gram-negative bacteria, including *Legionella spp., Burkholderia spp., Francisella spp., and Helicobacter*, are also best demonstrated by silver impregnation.
- **Differential stain:** It use more than one stain, which impart different appearance based on their structural properties. Some examples of differential stains are the Gram stain, acid-fast stain and the Kinyoun method of staining does not require heating.
- **Endospore stain:** Schaeffer fulton method with malachite green 5% and 0.5% safranin, which results in the spore appear green and vegetative cells red. Acid-fast with 0.25% sulphuric acid.
- **Flagellar stain:** The Leifson flagella stain method, all flagella stains use mordants, like tannic acid and potassium alum, to coat and thus thicken the flagellum in order to be observable by light microscopy.
- **Capsular stain:** Wet-mount method using india ink where the capsule is visualized as a refractile zone surrounding a cell. Dry-mount method that precipitates copper sulfate and leaves the capsule as a pale blue zone. The polychrome methylene

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blue staining procedure for blood or tissue smears found from dead animals **(M'Fadyean's reaction)** rapid diagnostic test for anthrax bacilli bearing polypeptide capsule.

Acid-Fast Organisms

- Mycobacteria
- Nocardia
- Bacterial endospores
- Head of sperm
- *Cryptosporidium parvum*
- *Cryptoisospora belli*
- *Cyclospora cayetanensis*
- *Taenia saginata eggs*
- Hydatid hooklets

Demonstration of Bacterial Motility

- Hanging drop
- Semisolid agar
- Cragie tube method

Characteristic Motility of Bacteria

- **Darting motility:** *Vibrio cholerae* and *Campylobacter jejuni*
- **Tumbling motility:** *Listeria monocytogenes*
- **Corkscrew motility:** *Spirochetes*
- **Swarming:** *Proteus* and *Clostridium tetani*

Morphology of Bacteria

- **Cell wall:** Gram-negative bacteria are surrounded by a thin peptidoglycan cell wall, which itself is surrounded by an outer membrane containing lipopolysaccharide. A peptidoglycan monomer consists of two joined amino sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). Gram-positive bacteria lack an outer membrane but are surrounded by layers of peptidoglycan many times thicker. Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram positive and Gram negative bacteria, Gram positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolorization process, while gram negative bacteria lose the crystal violet stain. Teichoic acids acidic polymers found in the cell walls, capsules, and membranes of all gram-positive bacteria give them an overall negative charge due to the presence of phosphodiester bonds between teichoic acid monomers.
- **Capsule:** The bacterial capsule is usually a hydrated polysaccharide structure that covers the outer layer of the cell wall. The capsule of *Bacillus anthracis* is an exception its capsule is composed of polypeptide. Capsule resists phagocytosis from ingesting and destroying the bacterial cell.
- **Flagella:** They are the organelles for bacterial locomotion. It extends from the cytoplasm to the cell exterior and are composed of three major structural elements, the basal body, the hook and the filament.
- **Fimbriae or pili:** Fimbriae and pili are hair-like appendages present on the bacterial cell wall similar to flagella. They are shorter than flagella and more in number. They are involved in the bacterial conjugation, attachment to the surface and motility
- **Spores:** They are the most dormant form of bacteria since they exhibit minimal metabolism. Gram-positive bacteria produce intracellular spores called endospores as a survival mechanism. Spore forming bacteria include *Bacillus* (aerobic) and *Clostridium* (anaerobic) species.

Physiology of Bacteria

Bacterial Growth Curve (Fig. 7.2)

The bacterial growth curve represents the number of live cells in a bacterial population over a period of time. There are four distinct phases of the growth curve: lag, exponential

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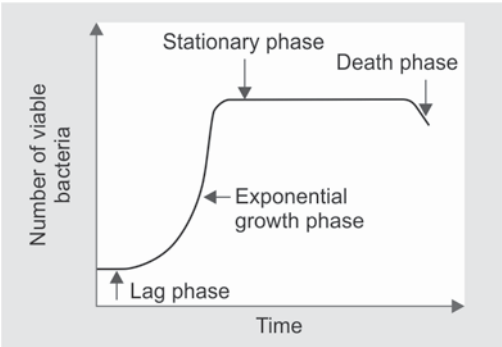

Fig. 7.2: Bacterial growth curve.

(log), stationary, and death. The initial phase is the lag phase where bacteria are metabolically active but not dividing.

Obligate Intracellular Organism

Obligate intracellular bacteria, which include *Chlamydia spp., Anaplasma spp., Ehrlichia spp., Rickettsia spp., Orientia spp,* and *Coxiella spp.*, replicate exclusively inside of eukaryotic host cells.

Endotoxin

Endotoxins or lipopolysaccharide (LPS)are the main component of the outer membrane of the cell wall of gram-negative bacteria.

Exotoxin

They are secreted proteins which act locally and at distance of the bacterial colonization site.

The pathogenic bacteria that produce exotoxins mainly include *Clostridium tetani, Clostridium botulinum, Clostridium perfringens, Corynebacterium diphtheriae, Group A streptococcus and Staphylococcus aureus.*

STERILIZATION AND DISINFECTION

Definition

- **Sterilization:** A process that destroys or eliminates all forms of microbial life including viable spores with reduction of at least 10^6 log colony forming units.
- **Disinfection:** Describes a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects (10^3 log colony forming units).
- **Cleaning:** Is the removal of visible soil (e.g., organic and inorganic material) from objects and surfaces.
- **Asepsis:** It is a process where the chemical agent (antiseptic) applied to body surfaces will kill or inhibit the pathogenic microorganisms (and also commensals) present on skin.
- **Decontamination:** It is reduction of pathogenic microbes to a level at which items are considered safe to handle and with reduction of atleast 1 log CFU of microorganism but not spores.

Factors Influencing Efficacy of Sterilant

- Organism Load
- **Nature of Organism:** Decreasing order of resistance of microorganisms: Prions > bacterial spores > coccidian cyst > mycobacteria > nonenveloped viruses > fungi > vegetative bacteria > enveloped viruses.
- Concentration of the sterilant/disinfectant and temperature of the physical agent.
- Nature of the sterilant/disinfectant
- Duration of exposure
- pH
- Biofilm formation

The Spaulding Classification

This classification places reusable medical instruments or devices into three categories of ascending risk for infection **(Table 7.1)**.

Sterilization is carried out by physical or chemical methods (Table 7.2)

Physical:

Moist heat can be used:

- At temperatures below 100°C.
- At a temperature at 100°C.
- At a temperature above 100°C (in saturated steam under increased pressure).

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Table 7.1: Spaulding classification.

Spaulding classification	*Medical device contacts*	*Disinfection level*
Critical	Sterile tissue or the bloodstream, e.g., catheters	Sterilization
Semi-critical	Mucous membranes or nonintact skin, e.g., endoscopes	High level disinfection (HLD)
Noncritical	Intact skin only, e.g., thermometer	Intermediate level (ILD) or low level disinfection (LLD)

Table 7.2: Applications/limitations of different sterlization methods.

Process	*Conditions*	*Applications/limitations*
Heat sterilization		
Dry Heat		
Flaming	High temperature and short time processing	To sterilize loops and points of forceps in the flame of a bunsen burner until it is red
Hot air oven	160°C for 120 minutes	Powders and petroleum products
Infra-red rays	Infrared technology include short cycle time, low energy consumption, no cycle residuals, and no toxicologic or environmental effects.	For sterilization of selected heat-resistant instruments (not FDA approved) has longer wavelength and lower energy it cannot penetrate substances and can only be used in sterilizing surfaces
Radiation		
Nonionizing rays: Rays of wavelength longer than the visible light are nonionizing microbicidal wavelength of UV rays lie in the range of 200–280 nm, with 260 nm being most effective	UV rays are generated using a high-pressure mercury vapor lamp	UV light in biosafety cabinets can cause skin erythema and keratoconjunctivitis
Ionizing rays: Electron beams and gamma rays	Gamma rays have more penetrative power than electron beam but require longer time of exposure	To sterilize disposable petri dishes, plastic syringes, antibiotics, vitamins, hormones, glasswares and fabrics
Moist heat (below 100°C)		
Pasteurization	63°C for 30 minutes (the holder method) or 73°C for 20 seconds (the flash method)	Can destroy all nonspore forming pathogens in milk except *Coxiella burnetii* in holder method
Serum bath	Serum can be inactivated by heating in a water bath at 56 °C for 1 h on several successive days	Only vegetative bacteria are killed and spores survive. Proteins in the serum will coagulate at higher temperature
Vaccine bath	Vaccine preparation can be inactivated by heating in a water bath at 60 °C for 1 h	Only vegetative bacteria are killed and spores survive

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Process	*Conditions*	*Applications/limitations*
Inspissation	The medium containing serum or egg are placed in the slopes of an inspissator and heated at 80–85°C for 30 minutes on three successive days. On the 1st day, the vegetative bacteria would die and those spores that germinate by next day are then killed the following day	LJ media/Loeffler's serum slope. If the spores fail to germinate then this technique cannot be considered sterilization
Moist heat (at 100°C)		
Boiling	Placing items like glassware in boiling water for 10–20 minutes	Certain bacterial toxins such as Staphylococcal enterotoxin are also heat resistant. Some bacterial spores are resistant to boiling and survive
***Steam at 100°C* (Arnold's and Koch's steamers) 100 °C**	The articles are subjected to free steam at 100°C for 90 minutes	Media such as TCBS, DCA and selenite broth are sterilized by steaming. An autoclave (with discharge tap open) can also serve the same purpose
Tyndallization or fractional sterilization or intermittent sterilization	Free steaming for 20 minutes for three successive days. The vegetative bacteria are killed in the first exposure and the spores that germinate by next day are killed in subsequent days. The success of process depends on the germination of spores	Media containing sugar and gelatin
***Moist heat* (above 100°C)**		
Steam sterilization (autoclave) types—gravity displacement and pre vacuum	121°C for 15 minutes at 15 psi or 134°C for 3 minutes (pre vacuum autoclave)	For sterilization of most culture media, glassware and other laboratory materials. Decontamination of microbiological waste may require at least 45 minutes at 121°C because the entrapped air remaining in a load of waste retards steam permeation and heating efficiency
Microwave	30-minute cycle with to 110°C. The microwave unit transmits energy as microwaves and this energy turns into heat inside the wet waste	Laboratory/hospital waste treatment
Chemical sterilization		
Glutaraldehyde 2%	For sterilization of medical instruments (exposure >10 hours is required)	Meticulous cleaning to remove organic matter. instruments should be rinsed with filtered water before use
Per acetic acid (0.2%)	0.2% for 12 minutes sterilize medical, (e.g., GI endoscopes) and surgical (e.g., flexible endoscopes) instruments	The instrument should be rinsed four times with filtered water before use
Gaseous sterilization		
Ethylene oxide (EtO) gas sterilization	Concentration of 450–1200 mg/L, at temperatures of 37–63°C and RH of 40–80% for 1–6 h	Heat-sensitive and moisture sensitive equipment and instruments

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Process	*Conditions*	*Applications/limitations*
Hydrogen peroxide vapor (HPV) and hydrogen peroxide gas plasma (HPGP) sterilization	Concentration of 6 mg/L, temperature range of 37 time of 75 minutes	Heat-sensitive and moisture sensitive equipment and instruments
Ozone	The duration of the sterilization cycle is about 30–35°C; 4 h and 15 m	Processing reusable medical devices

- **Principle of an autoclave:** There are four parameters of steam sterilization: steam, pressure, temperature, and time. Steam is admitted at the top or the sides of the autoclave chamber and, because the steam is lighter than air, forces air out the bottom of the chamber through the drain vent. By pushing the air out, the steam is able to make direct contact with the the load and begin to sterilize it.
- **Autoclave tape:** To indicate whether a specific temperature has been reached.
- **Bowie-dick indicator:** A commercially available bowie-dick indicator **(Fig. 7.3)** is placed in the center of the autoclave in an empty cycle and run at 134°C for 3.5 minutes. Autoclave performance is acceptable if the sheet inside the test pack shows a uniform color change. Used in Central Sterile Supply Department (CSSD) department.
- **Chemical indicators class 5 and 6 (Fig. 7.4):** They are devices used to monitor the presence or attainment of one or more of the parameters required for a satisfactory sterilization process. This change in color of the indicator is observed and interpreted as a pass or fail. Used in CSSD department.

Fig. 7.3: Bowie-dick indicator.

Fig. 7.4: Chemical indicators class 5 and 6.

Sterilization monitoring/Process control

See **Table 7.3**.

Disinfection in Healthcare

High-level Disinfection

It destroys all microorganisms but not bacterial spores, e.g., glutaraldehyde, hydrogen peroxide, etc.

Table 7.3: Different sterlization process and their biological indicator.

Process	*Biological indicator (Figs. 7.5A and B)*
Hot air oven	*B. atrophaeus*
Autoclave	*Geobacillus stearothermophilus* spores
ETO	*Bacillus atrophaeus*
Plasma sterilization	*Bacillus atrophaeus spores*
Ionizing radiation	*Bacillus pumilus*
Filtration	*Serratia marcescens Brevundimonas diminuta*

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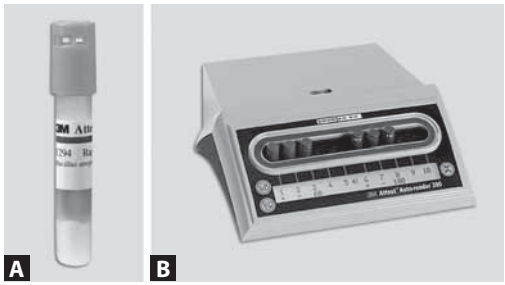

Figs. 7.5A and B: (A) Biological indicator; (B) Incubator.

Intermediate-level Disinfection

It destroys all microorganisms, but not is bacterial pores and some small nonenveloped viruses, e.g., alcohol, QACs. Intermediate-level disinfection is used for noncritical items.

Low-level Disinfection

It destroys most microorganisms and some viruses but has no action on *Mycobacterium tuberculosis* and spores, e.g., alcohol or QACs, etc., at lower exposures.

Commonly used Chemical Disinfectants in Health Care/Laboratory

See **Table 7.4**.

Testing of Disinfectants

Disinfectants are known to lose their activity on standing as well as in the presence of organic matter, their activity can be tested by the following methods:

- **Riedel Walker method (phenol coefficient):** For phenolic agents
- Chick Martin test

Table 7.4: Chemical disinfectants with their uses and limitations.

Chemical	*Uses*	*Limitation*
Alcohols ethyl alcohol (ethanol, alcohol) and isopropyl alcohol, 60–90%	Environmental surface cleaning	No sporicidal activity, concentrations less than 50% have poor antimicrobial activity and skin irritant
Hypochlorite solutions: 0.1% (1,000 parts per million/ppm) for surface cleaning; (1.0 %) (10,000 ppm) for large (>10 mL) spills of blood and body fluids and *C. auris* and *C. difficile*	Environmental surface cleaning	Irritant of eyes, skin, and mucous membrane can cause asthma, corrosion of metal Fresh dilution should be prepared daily Solution should not be exposed to direct sunlight or kept open for long time
Hydrogen peroxide >0.5%	Environmental surface cleaning	Irritant for eyes organisms with high cellular catalase activity such as *Staphylococcus aureus*, *Serratia marcescens*, and *Proteus mirabilis* are relatively resistant and require nearly an hour of exposure. Solution should not be exposed to direct sunlight or kept open for long time
Phenols (5% phenol, 1–5% cresol, 5% lysol)	Can be used in discarding jars in TB laboratories	Inactive against spores and most viruses
Halogen-releasing agents—iodine and iodophors	Skin preparation	
Quaternary ammonium compounds (QACs)	Environmental sanitation of noncritical surfaces, such as floors, furniture, and walls	Follow manufacturer's recommendation
Chlorhexidine	Antimicrobial dressings, gargles or mouthwash	Maximum bactericidal effect occurring within 20 seconds

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Chemical	*Uses*	*Limitation*
Heavy metals	1% silver nitrate solution; copper salts ; merthiolate	As treatment for ophthalmia neonatorum; copper salts are used as a fungicide Merthiolate at a concentration of 1:10,000 is used in preservation of serum
Cetrimide and benzalkonium chloride (cationic detergents)	They are widely used as disinfectants at dilution of 1–2% for domestic use and in hospitals	Pseudomonas can grow in cetrimide
Aniline dyes such as crystal violet	Bacteriostatic against gram positive bacteria	The dyes are used as selective agents in certain selective media. Malachite-green dye is added to LJ media to inhibit microorganisms other than mycobacteria and as a pH indicator

- Capacity use dilution test (Kelsey-Sykes test)
- In-use test

Filters (Table 7.5)

- **Sporicidal agents:** Include gluteraldehyde, sodium hypochlorite, iodine iodophors, hydrogen peroxide and peracetic acid.
- **Virucidal agents:** Ethyl alcohol 60%–80%, 3% hydrogen peroxide, povidone-iodine (PVP-I), sodium hypochlorite, glutaraldehyde and quaternary ammonium compounds (QACs)
- **Mycobacteriocidal agents** 5% phenols, chlorine and alcohol.
- **Bactericidal agents:** Alcohol, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, hydrogen peroxide, iodophors, ortho-phthalaldehyde (OPA), peracetic acid.

Calculation of Sodium Hypochlorite Concentrations

[% chlorine in liquid sodium hypochlorite/% chlorine desired] – 1 = Total parts of water for each part sodium hypochlorite

Example: [4% in liquid sodium hypochlorite/1% chlorine desired]–1 = 3 parts of water for each part sodium

Table 7.5: Filters with their characteristics and uses and limitations.

Type	*Characteristic*	*Use*	*Limitation*
Earthenware filters Pasteur-Chamberland filter/Berkefeld filter	Are made up of porcelain (sand and kaolin) or Kieselguhr	Used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions and urea solution	The disadvantages of depth filters are migration of filter material into the filtrate, absorption or retention of certain volume of liquid by the filters, pore sizes are not definite and viruses and mycoplasma could pass through
Asbestos filters	Magnesium silicate sterilized by autoclaving		Not reusable
Sintered glass filters	Pore diameter of 1–1.5 μm cleaned with warm concentrated H_2SO_4 and sterilized by autoclaving		

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Type	*Characteristic*	*Use*	*Limitation*
Membrane filters	Cellulose diacetate; a pore diameter ranging from 0.015 μm to 12 μm		Little loading capacity and are fragile
HEPA (High Efficiency Particle Air) filters	99.97% efficient for removing particles >0.3 μm in diameter	Operating rooms and biosafety cabinets	

hypochlorite i.e., if 1 liter of 1% sodium hypochlorite is needed dilute 25 mL with 75 mL of water.

CULTURE MEDIA AND METHODS

- **Liquid media:** It offers a uniform culture condition for the growth of bacteria producing general turbidity, e.g., nutrient broth and brain heart infusion broth.
- **Semi-solid media:** They are prepared with lower agar concentrations of 0.2 to 0.5%. They have a soft and are used to cultivate microaerophilic bacteria or determine bacterial motility by cultivation in stab tubes, e.g., Hugh and Leifson's oxidation fermentation medium, Stuart's and Amies media, and Mannitol motility media.
- **Solid media:** They are prepared by adding 1 to 2% agar. They make it possible to obtain isolated colonies of different bacterial species, which can be identified, e.g., MacConkey agar and nutrient agar.

Media are Classified into Six Types

1. Basal media
2. Enriched media
3. Selective
4. Indicator media/differential media
5. Transport media
6. Storage media

1. **Basal media:** Media do not require enrichment sources and are suitable for growing nonfastidious bacteria like *Staphylococcus* and Enterobacteriaceae. They are generally used to isolate microorganisms in labs or in sub-culturing processes, e.g., are nutrient broth, nutrient agar, and peptone water.
2. **Enriched media:** Addition of extra nutrients in the form of blood, serum, egg yolk, etc., to basal medium makes them enriched media for the growth of fastidious bacteria, e.g., are blood agar, chocolate agar, loeffler's serum, MacConkey agar and Lowenstein-jensen media.
3. **Selective:** A selective medium is a medium that allows the growth of one or more types of microorganisms while inhibiting the growth of other flora with the help of additives such as antibiotics. Cetrimide agar base is a culture medium used to selectively isolate and identify *Pseudomonas aeruginosa*.
4. **Indicator media/differential media:** These media thus allow to differentiate various kinds of microorganisms on the same agar plate, e.g., is blood agar.

   MacConkey agar is a selective and differential culture medium as it is designed to selectively isolate gram-negative and enteric bacteria and differentiate them based on lactose fermentation. MacConkey medium include crystal violet dye, bile salts, lactose, and neutral red (pH indicator). Crystal violet dye and bile salts halt the growth of gram-positive bacteria.

   TCBS agar (thiosulfate-citrate-bile-sucrose agar) is a selective differential medium for isolating and cultivating *Vibrio cholerae*.

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Cystine-lactose-electrolyte-deficient agar (**CLED agar**) supports the growth of urinary pathogens but prevents undue swarming of *Proteus species* due to its lack of electrolytes.

5. **Transport media:** They are essentially buffer solutions containing carbohydrates, peptones and other nutrients (excluding growth factors) designed to preserve the viability of bacteria during transport without allowing them to multiply.
   Cary-Blair medium and Venkatraman Ramakrishnan (VR) medium for fecal and rectal samples, is ideal for transport *V. cholerae*;amies medium (*Neisseria gonorrheae)*, stuart medium (*Neisseria gonorrheae),* pike's medium *(Streptococci)*
6. **Storage media:** Media used for storing the bacteria for a long period of time, e.g., egg saline medium and chalk cooked meat broth.
   Enrichment Broth: Liquid medium that only permits a specific species (pathogen) of a microbe to grow in it while inhibiting others (normal flora), e.g., selenite F. broth and tetrathionate broth for *Salmonella* and *Shigella,* alkaline peptone water (APW) for *V. cholerae.*
   Agar which is extracted from species of Gelidium and Gracilaria is a commonly used solidifying agent (1–2%) in microbiological media. Agar is an ideal solidifying agent for microbiological media because of its melting properties and because it has no nutritive value for the vast majority of bacteria. Solid agar melts at about 100°C; liquid agar solidifies at about 42°C. Smaller amounts (0.05–0.5%) are used in media for motility studies and for growth of anaerobes (0.1%) and microaerophiles.

Anaerobic Culture Media

1. Robertson's cooked meat broth (RCM)
2. Thioglycollate broth
3. Neomycin blood agar
4. PRAS (Prereduced anaerobically sterilized media)

RCM is suitable for growing anaerobic bacteria in air and also for the transport and preservation of their stock cultures.

Aerobic Culture Methods: Streak, Lawn, Stoke and Stab Method

Microaerophilic culture: Candle jars are used to grow bacteria requiring an increased (5–7%) CO_2 concentration (capnophilic bacteria), e.g., *Haemophilus influenzae, neisseria meningitidis and streptococcus pneumoniae.*

Anaerobic culture methods:

1. **McIntosh and Fildes anaerobic jar (Fig. 7.6)** works on the concept of replacement and evacuation, in which the air within the chamber is removed and replaced with a mixture made of gas (consisting of 5% CO_2, 10% H_2 and 85% N_2).
2. **GasPak system (Fig. 7.7)** generates an anaerobic environment by means of a gas generating pouch, the hydrogen thus produced reacts with oxygen present inside the jar producing water (which forms as condensation on the inside of the jar).
   $2H_2 + O_2 + \text{catalyst} = 2H_2O$

   Anaerobic indicator strips:
   - **Impregnated with methylene blue:** Remains colorless in anaerobic conditions, but turns blue on exposure to oxygen.
   - **Impregnated with resazurin, a redox indicator:** Colorless (white) in anaerobic conditions but turns to pink in the presence of oxygen.
   - **Biological indicator:** A plate inoculated with *Pseudomonas* is incubated along with other inoculated plates for anaerobic cultures.
3. **Anoxomat anaerobic culture system:**

Bacterial identification

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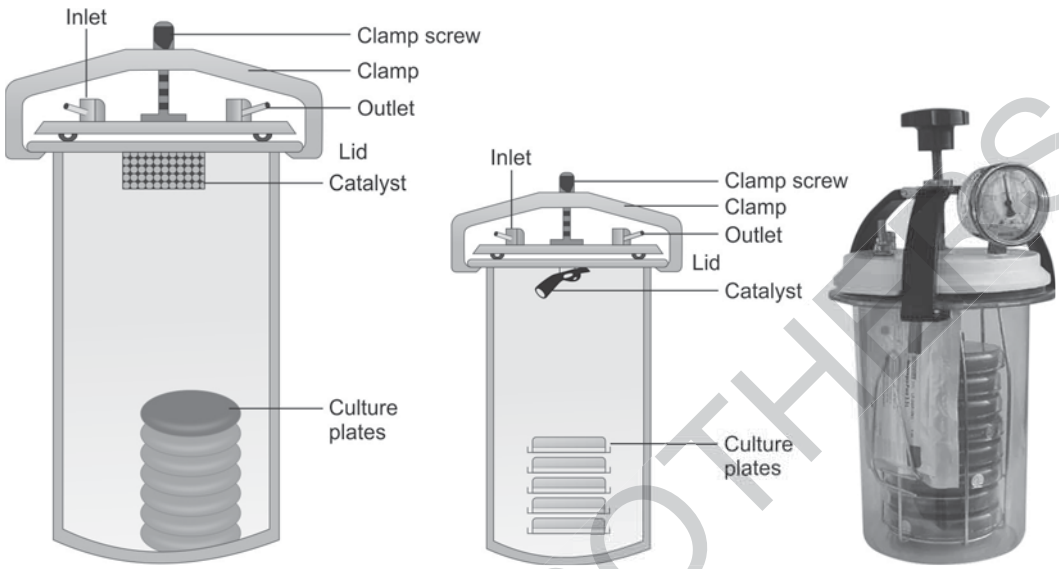

Fig. 7.6: McIntosh and Fildes anaerobic jar.

1. Conventional (identification by biochemical reactions).
2. Automated (VITEK 2, Phoenix, MicroScan WalkAway, MALDI-TOF)).
3. Molecular (PCR)

Biochemical Reactions

Gram Positive Cocci

Fig. 7.7: GasPak system.

- **Catalase (3% H_2O_2):** *Streptococcus species* (catalase-negative), *Staphylococcus species* (catalase-positive), and *Listeria species* (catalase-positive) can be differentiated. Positive organisms will evolve gas bubbles in the presence of H_2O_2.
- **Coagulase (slide and tube):** Coagulase test is used to differentiate *Staphylococcus aureus* (positive) which produce the enzyme coagulase, from *S. epidermis* and *S. saprophyticus* (negative) which do not produce coagulase. Both tests utilize EDTA-treated rabbit plasma. Positive test will produce clumps of cells.
- **CAMP test:** The Christie–atkins–munch-peterson(CAMP test) is a test to identify group B β-hemolytic *Streptococci* (*Streptococcus agalactiae*) based on their formation of a substance (CAMP factor) that enlarges the area of hemolysis formed by the β-hemolysin elaborated from *Staphylococcus aureus*.

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Review of Medical Laboratory Techniques

Salient Features

Review of Medical Laboratory Techniques stands out as a comprehensive and indispensable resource for students and professionals in the field, covering a wide range of topics encompassing medical laboratory techniques, spanning Human Anatomy, Physiology, Biochemistry, Hematology, Cytology, Histopathology, Microbiology, Immunology, and Molecular Diagnostics.

- The content is presented in a reader-friendly manner, ensuring accessibility for learners at various stages of their educational or professional journey.
- Practical application is a key focus, with real-world scenarios integrated into the learning process, allowing readers to bridge the gap between theory and practical implementation.
- The inclusion of detailed step-by-step procedures and illustrations enhances clarity, making complex techniques more understandable.
- The updated content is to reflect the latest advancements in medical laboratory technology.
- The book offers a diverse array of multiple-choice questions (MCQs) that challenge learners at various levels, from basic recall to critical thinking scenarios.
- The book serves as a valuable self-assessment tool, enabling readers to gauge their understanding and enhancing confidence and proficiency in applying knowledge to diverse scenarios.

Overall, the book is designed to be a comprehensive, up-to-date, and user-friendly guide for anyone seeking proficiency in medical laboratory techniques.

Reetika Menia DNB (Pathology) is Assistant Professor, Department of Pathology, All India Institute of Medical Sciences (AIIMS), Vijaypur, Jammu (J&K), India. Armed with advanced degrees in pathology and a rich background in medical research, she has emerged as a prominent figure in the realm of laboratory science and diagnostics. Her academic journey is marked by a dedication to academic excellence, evident through publications in renowned journals and active participation in scientific communities. Her contribution to this book stands as a testament to her unwavering commitment to advancing knowledge, fostering excellence, and shaping the future of medical laboratory science.

Shivani Gandhi MD (Pathology) is Assistant Professor, Department of Pathology, All India Institute of Medical Sciences (AIIMS), Vijaypur, Jammu (J&K), India. Her research contributions and publications in reputable journals reflect a deep involvement in advancing the knowledge frontier within pathology and laboratory science. Also, her practical experience in clinical settings and a passion for teaching have influenced the book's content, ensuring its relevance and applicability to the needs of students and professionals alike. She is actively engaged in professional societies and collaborative initiatives within the field of healthcare. Her wealth of knowledge, practical experience, and passion have helped in guiding her journey in the field of medical sciences.

Ishani Gupta MD (Pathology) is Assistant Professor, Department of Pathology, All India Institute of Medical Sciences (AIIMS), Vijaypur, Jammu (J&K), India, stands as a distinguished figure in the pathology field. Being both a seasoned practitioner and a dedicated educator, she brings a unique blend of theoretical expertise and practical insights to her work. Her academic journey is marked by a series of notable achievements, including significant contributions to research and publications in esteemed journals. Her participation in this book reflects her commitment to excellence and precision in shaping the minds of future laboratory professionals, instilling in them a deep understanding of the intricacies of medical laboratory techniques.

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Shelving Recommendation
PATHOLOGY

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