Introduction to Medical Microbiology

Medical microbiology is the branch of microbiology that studies microorganisms responsible for human diseases

It focuses on understanding the biology of pathogenic microbes, the mechanisms of disease production, host responses, and methods for prevention and treatment

This field plays a key role in diagnosing infectious diseases, developing vaccines, and improving public health

.Microorganisms

Microorganisms (also called microbes) are tiny living organisms that can only be seen with a microscope

They include

1-Bacteria – single-celled prokaryotic organisms

2-Viruses – acellular particles requiring host cells for replication

3-Fungi – unicellular (yeasts) or multicellular (molds) eukaryotic organisms

4- Parasites – protozoa and helminths that can infect humans

5- Prions – infectious protein particles without nucleic acids

Some microbes are harmless or beneficial, but others are pathogenic and cause diseases

.**Interaction with the Host**

Microorganisms constantly interact with the human host. These interactions can be:

Commensalism – microbes live on or within the host without causing harm (e.g., normal flora).

Mutualism – both microbe and host benefit (e.g., gut bacteria producing vitamins).

Parasitism – microbes benefit at the expense of the host, potentially causing disease

The host's immune system (innate and adaptive immunity) acts as a defense against pathogenic microbes. The outcome of infection depends on the balance between microbial virulence and host immunity

Microbial Virulence

Virulence refers to the degree of pathogenicity of a microorganism

Factors that determine microbial virulence include

1-Adhesion factors – help microbes attach to host tissues

2-Invasion factors – allow penetration into host cells

3-Toxins – such as exotoxins and endotoxins that damage host tissues

4-Immune evasion mechanisms – such as capsules, antigenic variation, or secretion of enzymes to escape host defenses

Highly virulent microbes cause severe disease even in healthy hosts, whereas low-virulence microbes may cause disease mainly in immunocompromised individuals

.Historical Significance

The history of medical microbiology is marked by key discoveries

Antonie van Leeuwenhoek (1676): First to observe microorganisms using a microscope

Louis Pasteur (19th century): Developed the germ theory of disease, pasteurization, and vaccines for rabies and anthrax

Robert Koch (19th century): Identified specific microbes causing tuberculosis, cholera, and anthrax; established Koch's postulates

Joseph Lister: Introduced antiseptic surgery

Alexander Fleming (1928): Discovered penicillin, the first widely used antibiotic.

Microbiology laboratory

Microbiology laboratory is a specialized facility designed for the study, isolation, identification, and manipulation of microorganisms

Purpose

Diagnosis of infectious diseases

Research on microbial physiology, genetics, and resistance

Teaching and training students in laboratory techniques

Importance

Working with microorganisms requires strict safety measures to prevent contamination of the samples, environment, and personnel

Behavior Inside the Laboratory

General Rules

1-Personal Protective Equipment (PPE): Always wear lab coats, gloves, and protective eyewear

2-Hand Hygiene: Wash hands before and after each laboratory session

3-Food and Drink: Eating, drinking, and chewing gum are strictly prohibited in the laboratory

4-Labeling: Clearly label all tubes, plates, and bottles with the organism name, date, and your initials

5-Housekeeping: Keep the workspace clean, organized, and free from unnecessary items

Handling Microorganisms

Treat all microorganisms as potential pathogens

Use aseptic technique to prevent contamination

Never pipette by mouth; always use mechanical pipetting devices

Dispose of cultures and contaminated materials in biohazard containers

Emergency Procedures

Know the location of eyewash stations, safety showers, and fire extinguishers

Report all spills, injuries, or exposures immediately to the instructor or supervisor

Sterilization and Disinfection Methods

Definitions

Sterilization: The process of killing or removing all forms of microbial life, including spores.

Disinfection: The process of reducing or eliminating pathogenic microorganisms on inanimate objects, but not necessarily spores

Sterilization Methods

A. Physical Methods

Heat Sterilization

Moist Heat Autoclaving

Standard: 121 °C at 15 psi for 15–20 minutes

Kills bacteria, viruses, and spores

Dry Heat

Hot Air Oven

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Used for glassware, metal instruments

.**Filtration**

Used for heat-sensitive liquids e.g., serum, antibiotic solutions

Radiation

UV Radiation: Used for surface and air disinfection

Gamma Radiation: Used for disposable medical products

B. Chemical Sterilization

Ethylene oxide gas: Used for plastics, catheters, and other heat-sensitive items

Disinfection Methods

A. Liquid Disinfectants

Alcohols (70% ethanol or isopropanol): Skin antiseptic and surface disinfection

Phenolic compounds: Disinfect benches and equipment

B. Pasteurization

Mild heat treatment to reduce microbial load in liquids (e.g., milk) without destroying quality

Microscopic Examination of Infected Materials

Introduction

The microscopic examination of infected materials is one of the most important initial steps in the diagnosis of infectious diseases.

It allows direct visualization of microorganisms in clinical specimens and provides rapid presumptive information about the type of infection (bacterial, fungal, or parasitic.(

It also guides the selection of appropriate culture media and further laboratory tes

.Objectives of Microscopic Examination

• To detect microorganisms directly from clinical specimens before culture.

• To differentiate between bacterial, fungal, and parasitic infections.

• To help in choosing appropriate staining techniques and media.

• To provide early information to clinicians for timely therapy.

.٣Preparation of Specimens for Microscopy

A. Choice of Specimen

• The sample should represent the site of infection accurately.

• Collect before antibiotic treatment if possible.

B. Preparation of Smear or Imprint

• Fluids (e.g., CSF, pus, urine): a drop is placed on a clean glass slide.

Concentration by centrifugation may be used if the specimen is dilute.

• Tissues or solid materials: sections are made, or “touch imprints” are prepared.

• Mucus or viscous samples: spread gently to avoid clumping.

C. Fixation and Staining

• Smears are air-dried and heat-fixed, or fixed chemically.

• Then stained using appropriate stains depending on the suspected pathogen.

.٤Common Staining Techniques

Microscopic Observation

A. Low-power (4×–10×) Objective

• Used to locate the stained area and check overall distribution of material.

B. Oil Immersion (100×) Objective

• Used for detailed visualization of bacterial cells and structures.

C. What to Observe

• Shape and arrangement: cocci, bacilli, spirilla, clusters, or chains.

• Special structures: spores, capsules, flagella, fungal hyphae.

• Host elements: white blood cells, epithelial cells.

• Background: pus, mucus, red cells, debris.

• Quantity and distribution: heavy, moderate, or scanty organisms.

٦. Limitations of Microscopy

• Low sensitivity if organism numbers are small.

• Contaminating flora may obscure the true pathogen.

• Some microorganisms (e.g., viruses, small bacteria) cannot be visualized by light microscopy.

• Microscopy provides presumptive but not definitive identification.

٧. Applications and Examples

Example Pathogen

Sputum

Gram stain for bacterial pneumonia

Streptococcus pneumoniae

CSF

India ink or Gram stain

Cryptococcus neoformans, Neisseria meningitidis

Blood smear

Giemsa stain

Plasmodium (malaria(

Skin scraping / tissue

KOH or LPCB stain

Dermatophytes, Candida

Urine

Gram stain

Microscopic examination of infected materials is a rapid, inexpensive, and essential diagnostic procedure.

It allows early detection of pathogens, provides clues for culture selection, and supports the presumptive diagnosis of infectious diseases.

Specimen Collection and Processing

1. Introduction

Specimen collection and processing are critical steps in the clinical laboratory workflow. Accurate diagnosis and effective treatment depend on the proper collection, labeling, transportation, and handling of specimens. Errors at this stage can lead to false results and compromise patient care.

2. Definition

A specimen is a biological sample collected from a patient for laboratory testing to assist in diagnosis, treatment, and monitoring of diseases. Examples include: blood, urine, sputum, stool, cerebrospinal fluid (CSF), swabs, and tissue biopsies.

3. Phases of Laboratory Testing

There are three major phases in laboratory testing:

- Pre-analytical phase – specimen collection, identification, transportation, and preparation.

- Analytical phase – actual testing and analysis in the laboratory.

- Post-analytical phase – result interpretation, validation, and reporting.

The pre-analytical phase contributes to 60–70% of laboratory errors, so correct collection and handling are crucial.

4. General Principles of Specimen Collection

- Proper patient identification – verify name, ID number, and date of birth.

- Correct test request form – must include patient details, physician's name, date, and requested tests.

- Use of appropriate container – sterile and properly labeled.

- Aseptic technique – to prevent contamination.

- Correct timing – e.g., fasting blood glucose after 8 hours of fasting.

- Label immediately after collection to avoid mix-ups.

- Transport promptly to the laboratory, maintaining proper temperature if required.

5. Types of Specimens

a. Blood

Collected by venipuncture, finger prick, or heel prick (in infants). Use appropriate tubes (e.g., EDTA for hematology, citrate for coagulation, serum tubes for chemistry). Avoid hemolysis by gentle mixing and correct needle size.

b. Urine

Midstream clean-catch urine is preferred for microbiological and chemical analysis. First morning urine is ideal for detecting proteins or pregnancy hormones. Transport to the lab within 1 hour or refrigerate at 4°C.

c. Sputum

Collected early in the morning before eating or brushing teeth. Rinse mouth with water before collection to minimize contamination with saliva.

d. Stool

Used for parasitological and microbiological tests. Should be collected in a clean, dry container and transported immediately.

e. Cerebrospinal Fluid (CSF)

Collected by lumbar puncture under strict aseptic conditions. Three tubes: chemistry, microbiology, and cytology.

f. Swabs

Used for throat, wound, nasal, or genital specimens. Transport in appropriate transport media (e.g., Stuart or Amies medium).

6. Specimen Processing

- Receiving and accessioning – checking labeling, documentation, and condition of specimens.

- Aliquoting – dividing samples into portions for different tests.

- Centrifugation – separating serum or plasma from blood cells.

- Storage – depending on test requirements (room temp, 4°C, or -20°C).

- Rejection criteria – hemolyzed, clotted, improperly labeled, or insufficient samples must be rejected with documentation.

7. Biosafety and Precautions

- Always treat all specimens as potentially infectious.

- Use personal protective equipment (PPE): gloves, lab coat, mask, eye protection.

- No eating, drinking, or smoking in specimen collection areas.

Methods of Inoculation in the Microbiology Laboratory

Introduction

Inoculation is the process of transferring microorganisms from one medium to another under sterile conditions.

Goals of Inoculation

- To obtain pure cultures

- To isolate individual colonies

- To maintain microorganisms for further studies

- To test microbial characteristics

- To avoid contamination

Aseptic Technique

- Work near a flame or inside a biosafety cabinet

- Sterilize inoculating loop/needle until red hot

- Avoid touching non-sterile surfaces

- Keep tubes and plates open briefly

- Disinfect workspace

Types of Inoculation Methods

A. Streak Plate Method

Purpose: To isolate single colonies.

B. Spread Plate Method

Purpose: Counting bacteria or obtaining isolated colonies.

C. Pour Plate Method

Purpose: Counting bacteria in samples with high microbial load.

D. Inoculation of Broth Media

Purpose: Produce large numbers of microbes or for biochemical tests.

E. Stab Method

Purpose: Study oxygen requirements and motility.

Observations After Incubation

- Colony morphology

- Color/shape

- Hemolysis

- Growth patterns

Introduction

Biochemical identification of bacteria is an essential step in microbiology for determining the type of microorganism present in a sample

After isolation and observation of colony morphology and staining (such as Gram staining), biochemical tests are performed to identify bacterial species based on their metabolic and enzymatic activities

Principle of Biochemical Identification

Each bacterial species possesses a unique set of enzymes that allow it to metabolize certain substrates By testing for the presence or absence of these metabolic reactions, we can identify bacteria.

Biochemical identification is based on

(Enzyme production (e.g., catalase, oxidase, urease)

Fermentation or oxidation of sugars

Utilization of specific substrates

Production of metabolic end-products (acid, gas, H_2S, et)

Types of Biochemical Tests .

A. Enzyme Activity Tests

Catalase Test

Principle: Detects the enzyme catalase, which breaks down hydrogen .peroxide (H_2O_2) into water and oxygen

Reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$

Positive: Bubbling (e.g., Staphylococcus aureus)

Negative: No bubbles (e.g., Streptococcus spp)

Oxidase Test

Principle: Detects cytochrome c oxidase enzyme in the electron transport chain

Positive: Purple color (e.g., Pseudomonas aeruginosa)

Negative: No color change (e.g., Escherichia coli)

Urease Test

Principle: Detects urease enzyme that hydrolyzes urea into ammonia and CO_2

Medium: Urea agar or broth

.Positive: Pink color (alkaline) — Proteus spp

Negative: No color change — E. coli

Coagulase Test

Principle: Detects coagulase enzyme that converts fibrinogen to fibrin

Positive: Clot formation — Staphylococcus aureus

Negative: No clot — Staphylococcus epidermidis

B. Carbohydrate Fermentation Tests

Principle: Detects the ability of bacteria to ferment sugars producing acid (pH change) and/or gas

Medium: Phenol red broth base + specific sugar glucose,lactose,sucrose)

Indicator: Phenol red

Acid production → Yellow color •

Gas production → Bubble in Durham tube •

Example

E. coli: Ferments glucose and lactose → yellow with gas

Salmonella: Ferments glucose only → yellow without gas in lactose broth

C. Hydrogen Sulfide (H_2S) Production

Principle: Detects bacteria that produce H_2S from sulfur-containing compounds

Medium: Triple Sugar Iron (TSI) agar or SIM medium

Indicator: Iron salts → form black precipitate when H_2S is present

Example

Salmonella spp.: H_2S positive (black butt

Shigella spp.: H_2S negative

D. Indole Test

Principle: Detects the ability to produce indole from tryptophan by the enzyme tryptophanase

Medium: Tryptone broth

Reagent: Kovac’s reagent

(Positive: Red ring on top (E. coli)

(Negative: Yellow ring (Klebsiella pneumoniae)

E. Citrate Utilization Test

Principle: Tests whether bacteria can use citrate as the sole carbon source

Medium: Simmons citrate agar

Indicator: Bromothymol blue

Positive: Blue color (Enterobacter, Klebsiella)

Negative: Green (E. Coli)

F. Methyl Red (MR) and Voges-Proskauer (VP) Tests

Methyl Red Test (MR)

Detects strong acid production from glucose fermentation

Positive: Red after adding methyl red (E. Coli)

Negative: Yellow (Enterobacter aerogenes)

Voges-Proskauer Test (VP)

Detects acetoin production from glucose fermentation

Reagents: α-naphthol + KOH

Positive: Red color (Enterobacter aerogenes)

Negative: No color (E. Coli)

G. Triple Sugar Iron (TSI) Agar Test

Detects glucose, lactose, sucrose fermentation, gas, and H_2S production

Interpretation

Yellow butt / red slant: Glucose fermentation only (Shigella)

Yellow butt / yellow slant: Lactose and/or sucrose fermentation (E. coli)

Black butt: H_2S production (Salmonella)

Gas bubbles: Gas production

Automated and Commercial Identification Systems

Modern laboratories use automated systems for faster and more accurate identification

API (Analytical Profile Index)

VITEK system

Importance of Biochemical Identification

Confirms bacterial genus and species

Helps in selecting appropriate antimicrobial therapy

Useful in epidemiological investigations

Essential for diagnostic, clinical, and research microbiology