Microbial Culture Sensitivity Testing

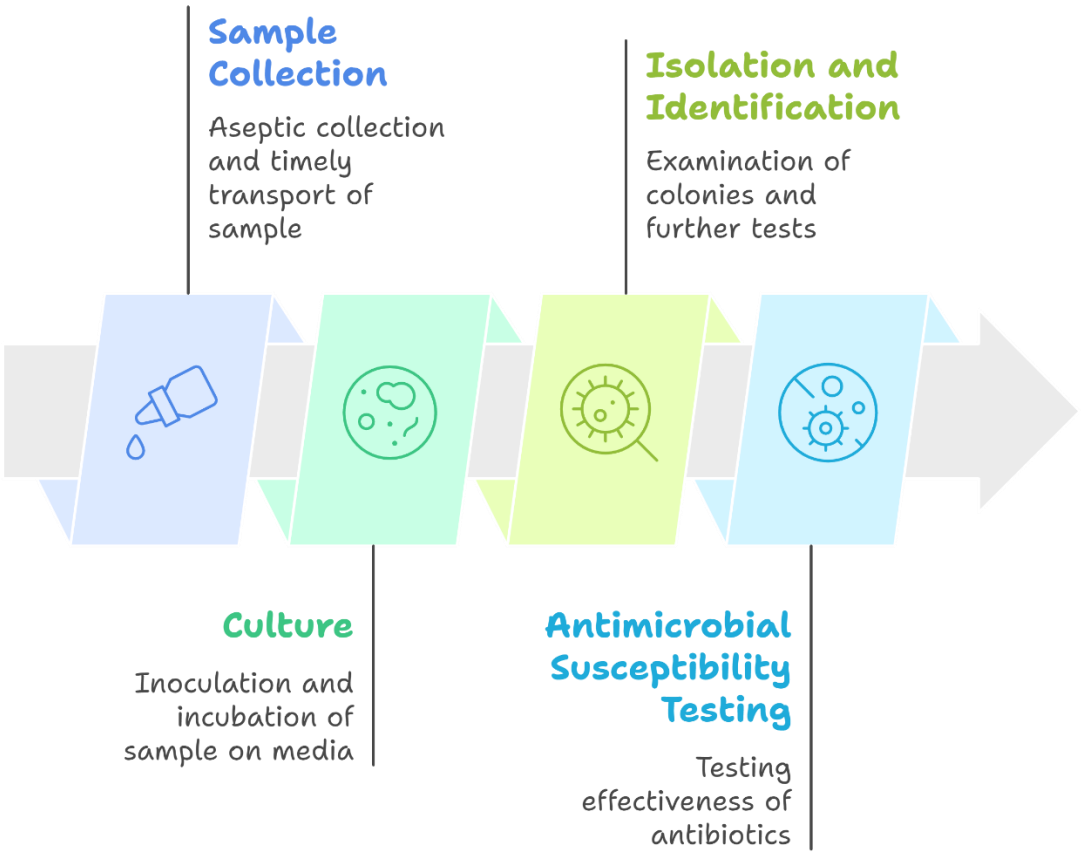
Introduction:

- Microbial culture and sensitivity testing (CST) is a diagnostic laboratory procedure used primarily in microbiology to detect and identify pathogens (like bacteria or fungi) in a biological sample (e.g., urine, blood, sputum, pus, etc.)
- and determine the susceptibility or resistance of those microorganisms to various antimicrobial agents.

Purpose:

- To identify the causative organism of an infection.
- To choose the most effective antimicrobial drug for treatment.
- To avoid the use of broad-spectrum antibiotics unnecessarily and reduce the risk of developing antimicrobial resistance.

Basic Steps Involved:



1. Collection of Sample:

- The sample must be collected aseptically from the infection site.
- Transport to lab must be timely to preserve organism viability.

2. Culture:

- The sample is inoculated onto or into specific media (agar plates or broth) to grow the microorganisms.

- Common media include blood agar, MacConkey agar, and Sabouraud agar.
- Plates are incubated under suitable conditions (temperature, oxygen requirement).

3. Isolation and Identification:

- After incubation, colonies are examined for morphology.
- Further biochemical or molecular tests may be done to identify the microorganism.

4. Antimicrobial Susceptibility Testing (AST):

- Once the microorganism is identified, susceptibility testing is done to determine which antibiotics are effective.
- Common methods: Disk diffusion (Kirby-Bauer), broth dilution, E-test.

Methods of Sensitivity Testing



1. Kirby-Bauer Disk Diffusion

- Antibiotic discs are placed on agar.
- Measure diameter of the **zone of inhibition** (no bacterial growth).
- Compare with **CLSI/EUCAST standards** for susceptibility.

2. Broth Dilution (MIC Testing)

- Antibiotics are diluted in broth; bacteria are added.
- The **Minimum Inhibitory Concentration (MIC)** is the lowest concentration preventing visible growth.

3. E-Test (Epsilometer Test)

- A plastic strip with a gradient of antibiotic concentrations is used.
- MIC is read where the zone edge intersects the strip.

Interpretation of Results

When interpreting microbial culture and sensitivity results, the report usually includes:

A. Culture Result:

- **Positive Culture**: Indicates growth of a specific microorganism.
- **Negative Culture**: No organism was isolated (may mean no infection or false negative due to sampling/transport issues).

B. Organism Identified:

- Specifies which bacteria or fungus was grown (e.g., *Escherichia coli*, *Staphylococcus aureus*).

C. Antibiotic Sensitivity Pattern:

- This section usually shows results such as:

Antibiotic	Result	Zone of Inhibition / MIC Value
Amoxicillin	Resistant	Zone < 13 mm / MIC > 32 µg/mL
Ciprofloxacin	Sensitive	Zone > 21 mm / MIC < 1 µg/mL
Tetracycline	Intermediate	Zone 14–18 mm

- This section usually shows results such as:
  - **Sensitive (S)**: The microorganism is inhibited by the drug at standard dosage.
  - **Resistant (R)**: The microorganism is not inhibited and drug will likely be ineffective.
  - **Intermediate (I)**: The drug may be effective at higher doses or in body sites where it is concentrated.

D. Clinical Correlation:

- Lab results must be correlated with clinical symptoms.
- Colonizers or contaminants may not indicate infection (e.g., *Staphylococcus epidermidis* on skin).

Important Considerations:

- Multi-drug resistant organisms (MDROs) like MRSA, ESBL-producing *E. coli*, and carbapenem-resistant *Klebsiella* must be highlighted.
- Breakpoints for sensitivity are set by organizations like CLSI (Clinical and Laboratory Standards Institute) or EUCAST.

Principles and Methods of Different Microbiological Assays

Definition

- Microbiological assays are analytical methods that use microorganisms to estimate the concentration or potency of a substance, usually an antibiotic, vitamin, or other bioactive compound.

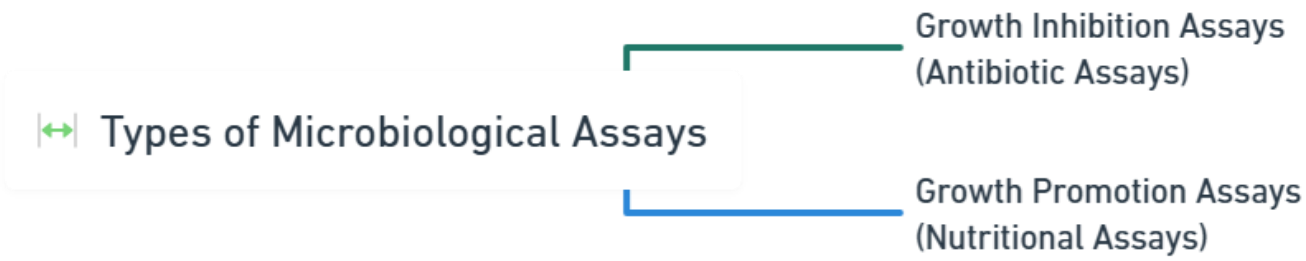
Principle

- The fundamental principle is that certain microorganisms have a specific requirement for a substance, or are inhibited by it.

- By measuring:
  - **Growth promotion** (for vitamins, amino acids, etc.) or
  - **Growth inhibition** (for antibiotics),
    we can quantify the **amount or activity** of the test substance by comparing it with a standard of known concentration.

Types of Microbiological Assays

- There are two major categories:



1. Assays based on growth inhibition (Antibiotic assays)

- The test substance **inhibits the growth** of a sensitive microorganism.
- The amount of inhibition is proportional to the concentration.
- Examples: Assay of Penicillin, Streptomycin.

2. Assays based on growth promotion (Nutritional assays)

- Microorganisms that require a specific nutrient for growth are used.
- The **growth response** is proportional to the nutrient's concentration.
- Examples: Assay of Vitamin B2 (Riboflavin), Vitamin B12 (Cobalamin).

Methods of Microbiological Assays



1. Agar Diffusion Method (Cup/Disc method)

- Test and standard solutions are applied to wells or discs on an agar plate seeded with test organism.
- After incubation, zones of inhibition are measured.
- The size of the zone correlates with the concentration.

2. Turbidimetric Method (Tube assay)

- Based on measuring **turbidity (optical density)** caused by microbial growth in a liquid medium.
- Suitable for both inhibition and growth-promotion assays.
- Growth is measured spectrophotometrically at a specific wavelength.

3. End-point (Titration) Assay

- Based on determining the **lowest concentration** of the test substance that shows a detectable effect (e.g., no visible growth).
- Used in MIC determination and bioassay standardization.

General Requirements

- **Test microorganism** must be sensitive and specific to the compound.
- **Standard preparation** with known potency.
- **Controlled conditions**: pH, temperature, nutrient media, incubation time.
- **Statistical analysis** to validate the results (e.g., parallel line assay method).

Applications

- Used for potency determination of:
  - **Antibiotics:** Penicillin, Streptomycin, etc.
  - **Vitamins:** B1, B2, B6, B12
  - **Amino acids**
- Commonly used in pharmaceutical quality control.

Advantages

- Measures biological activity directly.
- Useful when no reliable chemical method exists.
- High sensitivity.

Limitations

- Time-consuming (requires incubation).
- Sensitive to growth conditions.
- Requires strict control and standardization.

Microbiological Assay of Penicillin

Introduction

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