Staining Techniques for Differentiation of Microorganisms

Prepared by : Dr O.H. AJIGBEWU

Department of Microbiology

Introduction

- Imagine trying to identify people in a crowded room where everyone is wearing the same colour of clothing.
- That’s exactly what happens when microbiologists look at microorganisms under the microscope
  - without staining—they all look nearly transparent and similar.
- Staining is like giving each microorganism a “unique outfit”
- It helps us see their shapes, structures, and even differences in their cell walls.
-

What is Staining

- Staining is a laboratory technique that applies chemical dyes (stains) to microbial cells, tissues, or cellular components so they take up color and become more visible or distinguishable under light microscopy.
- Stains are usually organic dyes that interact with cellular components by ionic or affinity-based binding
- The result is increased contrast between the specimen and the background or between different structures within the specimen.

Purposes of staining

1) **Visualization:** makes tiny, often transparent microbes visible under a light microscope.
2) **Differentiation:** separates organisms into groups (for example, Gram-positive vs Gram-negative) based on how they retain or lose particular dyes.
3) **Structural identification:** highlights specific structures such as capsules, spores, flagella, or cell walls so their presence, size, and shape can be studied.

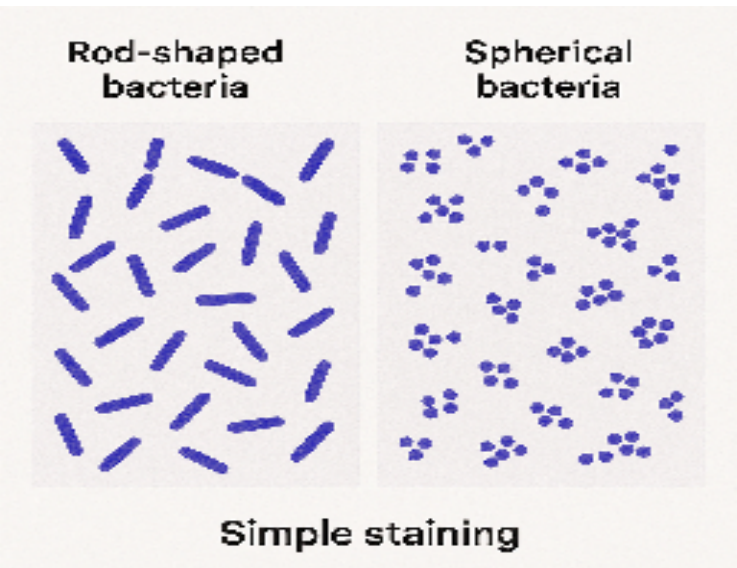
These purposes guide which staining method is chosen for a given diagnostic or research question.

Why Staining Matters in Microbiology

1) Helps identify pathogens in clinical samples.
2) Guides doctors in choosing treatments.
3) Reveals microbial structures important for survival and virulence.
4) Aids in teaching and research by making invisible organisms visible.

Types of Staining

1) Simple Staining
2) Differential Staining
3) Negative Staining
4) Special Staining



1. Simple Staining

Simple staining uses a single dye to colour microorganisms.

Purpose: It highlights the overall shape, size, and arrangement of cells.

Common dyes: Methylene blue, crystal violet, safranin

Procedure:

1. Prepare a smear of the microorganism on a slide.
2. Fix the smear by passing it over a flame.
3. Flood the slide with dye for 30–60 seconds.
4. Rinse gently with water and observe under the

Differential Staining

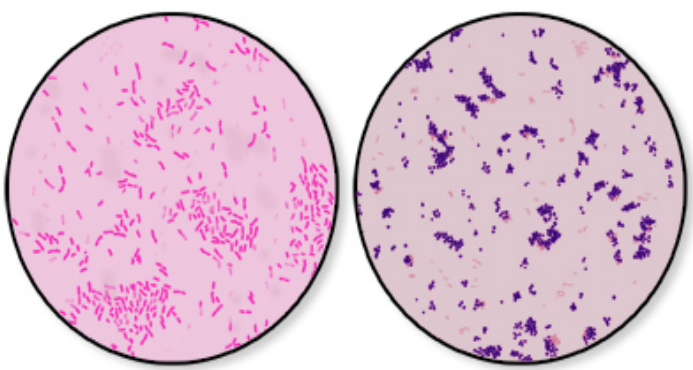
- Differential staining uses more than one dye to distinguish between different types of microorganisms or structures.
- It provides **diagnostic and taxonomic value**.
- It aids in the identification of pathogens and understanding of microbial physiology

- **Examples**
- Gram Staining
- Acid-Fast Staining
- Endospore Staining
- Capsule Staining
- Flagella Staining

Gram Staining

1) Gram Staining

- Invented by: Hans Christian Gram in 1884.
- Purpose: Differentiates bacteria into
  - Gram-positive
  -



- **Procedure:**

1) Apply crystal violet (primary stain).
2) Add iodine (mordant) to fix the dye.
3) Wash with alcohol (decolourizer).
4) Counterstain with safranin.

Result

- Gram-positive bacteria retain crystal violet (purple).
- Gram-negative bacteria lose crystal violet and take up safranin (pink).

Comparison table highlighting the differences between Gram-positive and Gram-negative bacteria

Feature	Gram-Positive Bacteria	Gram-Negative Bacteria
Colour After Staining	Purple or violet	Pink or red
Cell Wall Structure	Thick peptidoglycan layer	Thin peptidoglycan layer + outer membrane
Teichoic Acids	Present	Absent
Outer Membrane	Absent	Present (contains lipopolysaccharides)
Examples	*Staphylococcus aureus*, *Bacillus subtilis*	*Escherichia coli*, *Salmonella typhi*

2) Acid-Fast Staining (Ziehl-Neelsen)

Purpose

- Identifies bacteria with waxy cell walls (rich in mycolic acid),
- e.g. *Mycobacterium tuberculosis* and *M. ulcerans*

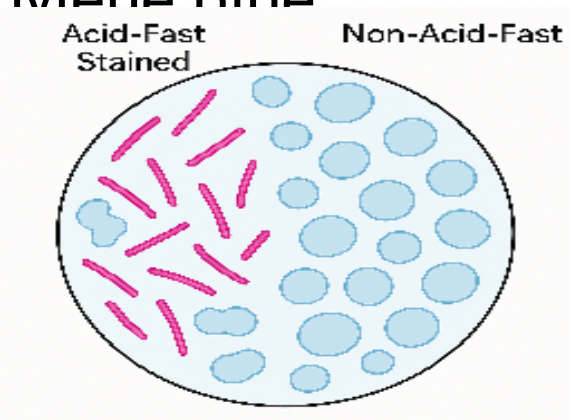
Result:

- Acid-fast bacteria: Red.

- Non-acid-fast bacteria: Blue.

Method

1. Stain with carbol fuchsin (red dye).
2. Heat gently to allow dye penetration.
3. Decolourize with acid-alcohol.
4. Counterstain with methylene blue



3) Endospore Staining

Purpose

- Detects bacterial endospores (survival structures).

Method (Schaeffer-Fulton)

1. Stain with malachite green and apply heat.
2. Wash with water.
3. Counterstain with safranin

Result:

- Endospores: Green.
- Vegetative cells: Red.

Example: *Bacillus* and *Clostridium* species.

4) Capsule Staining

- P**urpose**: Reveals capsules (protective layers around bacteria).
- **Method**: Negative staining with India ink or nigrosin.
- **Result:** Capsules appear as clear halos around stained cells.

e.g. *Klebsiella pneumoniae*

5) Flagella Staining

Purpose: Makes bacterial flagella visible.

Method: Uses special stains that coat flagella, making them thick enough to see under a microscope.

Result: Long, hair-like structures extending from cells.

Example: Salmonella* species with peritrichous flagella.

3) Negative Staining

Definition: Uses acidic dyes (e.g., nigrosin, India ink) that do not penetrate cells but stain the background.

Purpose: Shows cell shape and size without distortion.

Result: Cells appear clear against a dark background.

Example: Useful for observing delicate structures like capsules.

4) Special Stains

These highlight specific structures:

Spore stain– Endospores.

Capsule stain – Capsules.

Flagella stain – Flagella.

Nucleic acid stains – DNA/RNA.