Bacterial Staining

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Outline

- Introduction
- Principles of Staining
- Gram Stain
- Acid-Fast Stain

Why do we stain microorganisms?

- Bacteria are colourless and transparent - invisible under light microscopy without staining
- Staining allows visualization, identification, and classification (Shape, size, arrangement)
- **Different staining properties** reflect **fundamental structural differences**
    - Classify bacteria into groups

How Do Stains Work?

Dyes have two important parts:

- **Chromophore** (coloured part) - gives the colour
  - But Colour Alone Isn't Enough!
- **Auxochrome** (charged part) - helps it bind to bacteria

How Do Stains Work?

Types of Dyes Based on Charge:

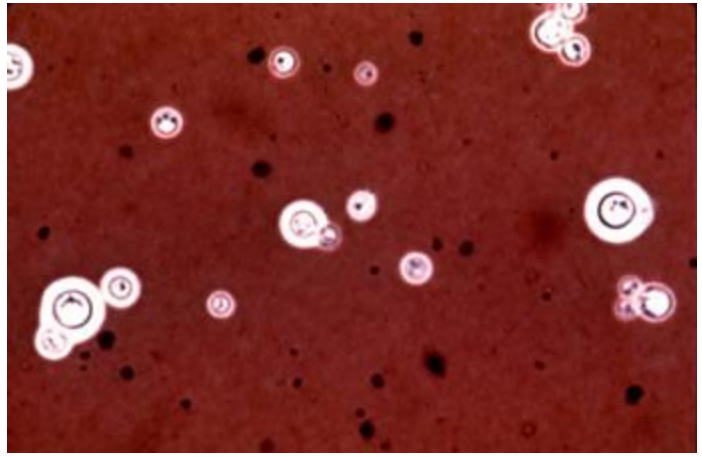
A. Basic (cationic) dyes - *positive charge*

- **Examples:** Crystal violet, Methylene blue, Safranin
- **Mechanism:** Positively charged chromophore (cation)
- **Binds to:** Negatively charged bacterial components
  - Nucleic acids (DNA/RNA), Acidic proteins, Cell wall components (peptidoglycan, teichoic acids)
- **Most commonly used** because bacteria are naturally negative

How Do Stains Work?

B. Acidic (anionic) dyes - *negative charge*

- **Examples:** Eosin, India ink
- **Mechanism:** Negatively charged chromophore (anion)
- **Repelled by:** Bacterial cells (negative charge)
- **Used for:** Background staining (negative staining)



Differential staining

Differential staining: Uses **multiple dyes** to distinguish between **different types** of bacteria

Purpose:

Classify bacteria into groups based on cell wall structure or properties

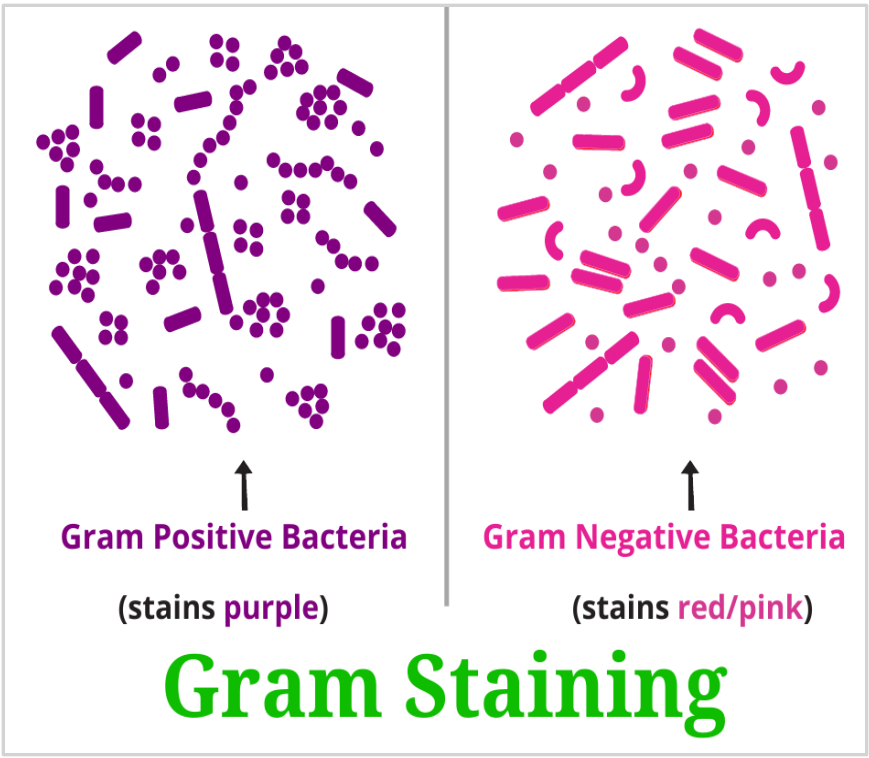
Key Examples:

A. Gram Stain ⭐ *MOST IMPORTANT*

B. Acid-Fast Stain (Ziehl-Neelsen, Kinyoun)

Differential staining: Gram Stain

- The Gram Stain developed by **Hans Gram** (in 1884)
- The most important staining technique in clinical microbiology
- **Differential stain** - divides bacteria into two major groups based on their reaction to the staining procedure



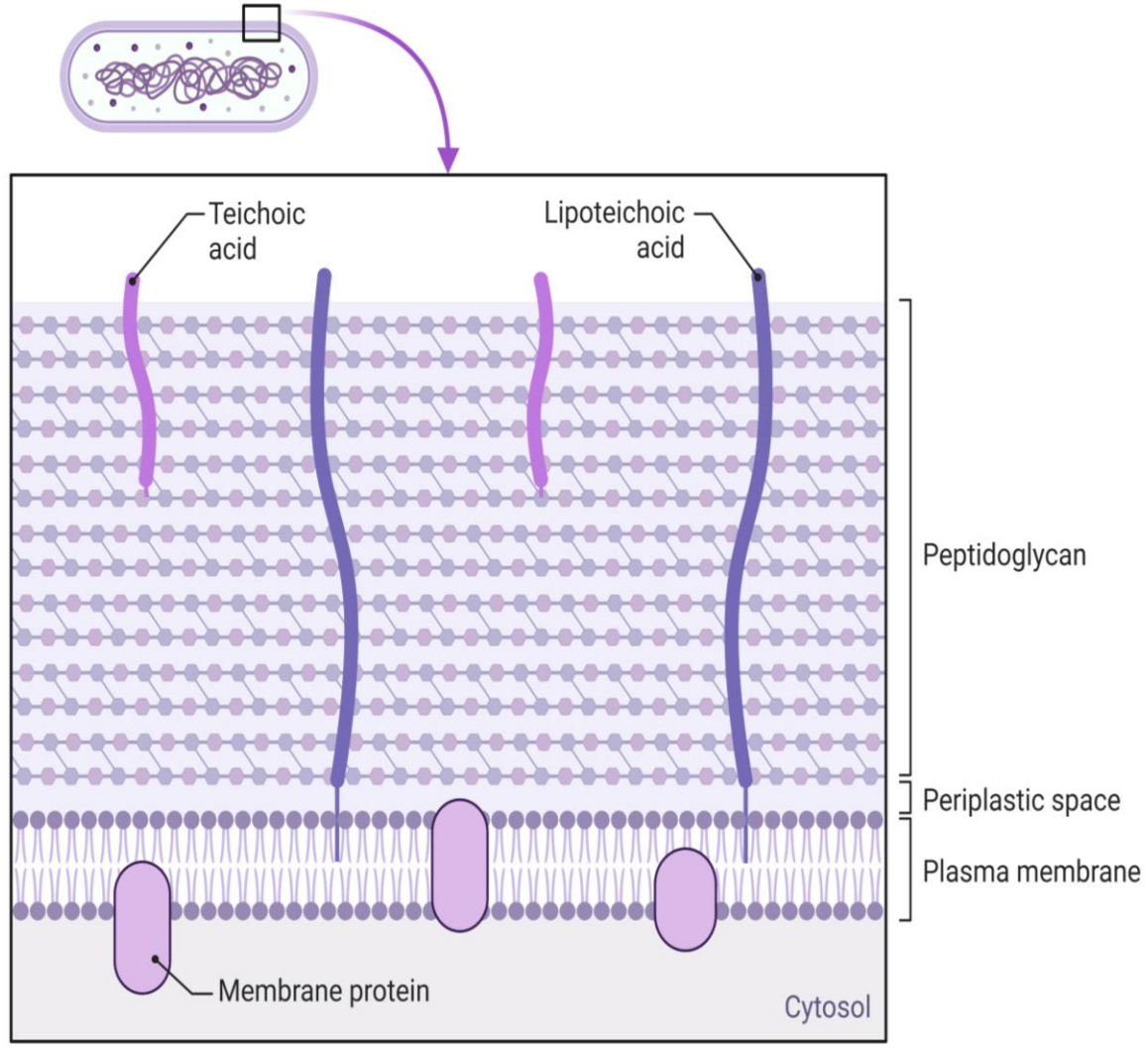
- **Two groups:**

  Gram-positive bacteria → retain primary stain → appear **Purple/Blue**

  Gram-negative bacteria → lose primary stain → appear **Pink/Red**

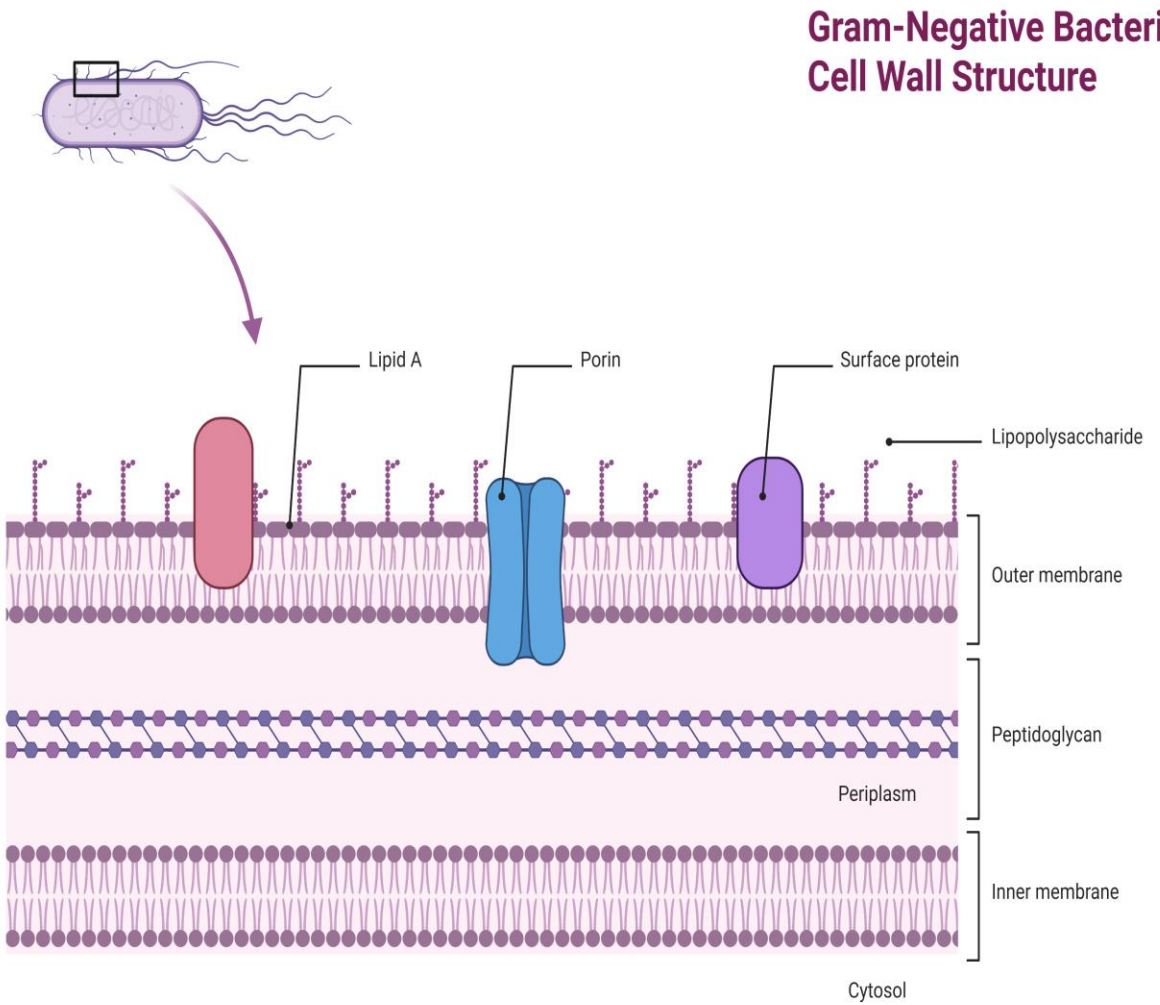
Differential staining: Gram Stain- Gram-Positive Bacteria

- **Thick peptidoglycan layer** (20-80 nm, multiple layers)
  - Makes up 90% of the cell wall
  - Like a thick protective wall
- **No outer membrane**
- Contains **teichoic acids** and **lipoteichoic acids**



Differential staining: Gram Stain- Gram-Negative Bacteria

- **Thin peptidoglycan layer** (2-7 nm, single layer)
  - Only 10% of cell wall
  - Like a thin framework
- **Has outer membrane** (unique feature!)
  - Contains **lipopolysaccharide (LPS/endotoxin)**
  - Acts as permeability barrier



Gram Stain: How Does Gram Staining Work?

STEP 1: Primary stain (crystal violet)

- **All bacteria** are stained **PURPLE**
- Crystal violet (basic dye; positive charge) enters all cells → Binds to negatively charged cell components

STEP 2: Mordant (trapping agent)

- Iodine forms complex with crystal violet; Crystal Violet-Iodine complex (CV-I)
- Large complex gets **trapped** inside cells → Makes the stain harder to remove

Gram Stain: How Does Gram Staining Work?

STEP 3: Decolorization (alcohol or acetone-alcohol)

⭐ **Most critical step!**

- **Gram-Positive bacteria:**
  - Thick peptidoglycan acts like a **mesh/sponge**
  - Alcohol dehydrates peptidoglycan → pores **shrink/close**
  - CV-I complex **TRAPPED** inside → **RETAIN purple colour** ✅
- **Gram-Negative bacteria:**
  - Alcohol dissolves outer lipid membrane
  - Thin peptidoglycan cannot trap CV-I complex
  - CV-I complex **WASHES OUT → LOSE purple colour** → become colourless

Gram Stain: How Does Gram Staining Work?

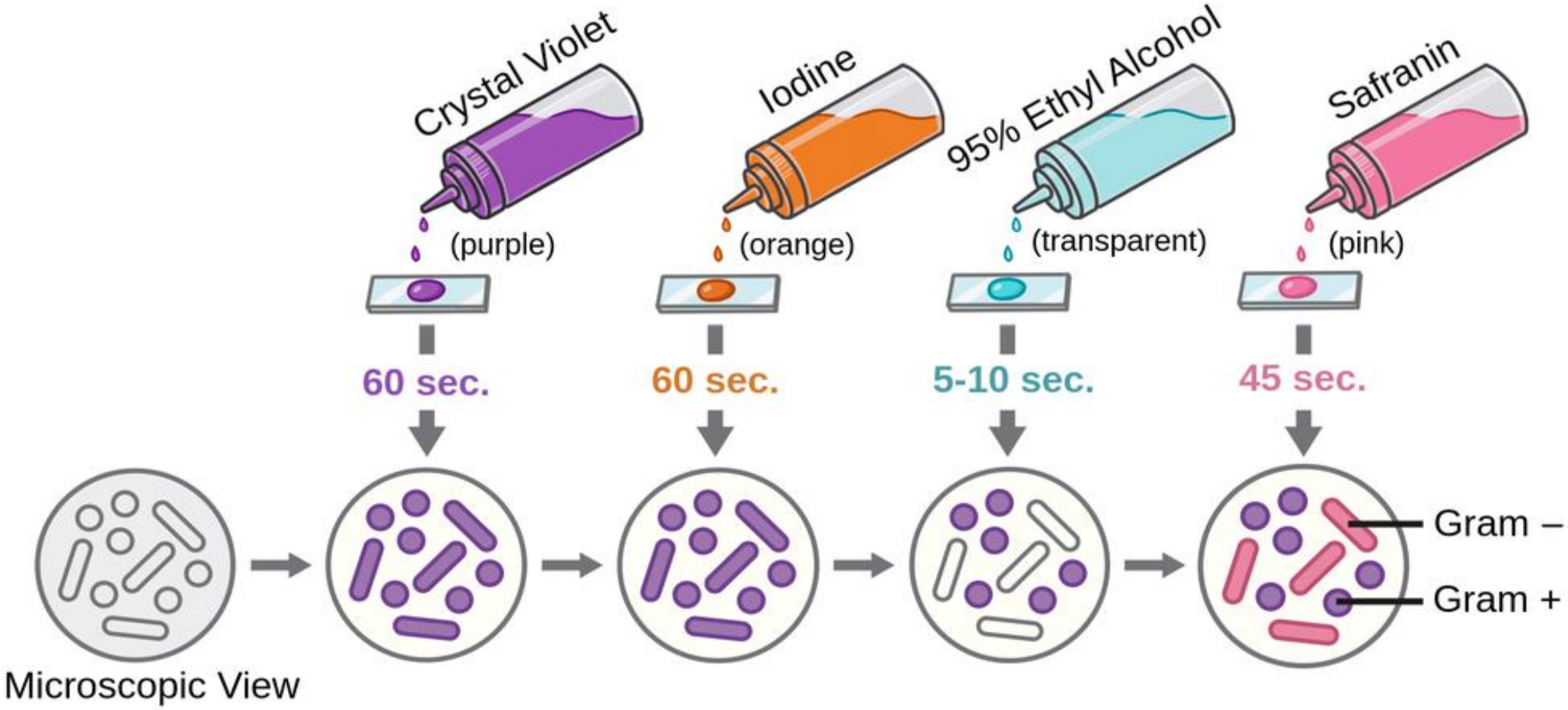
STEP 4: Counterstain (Safranin)

- Safranin (red/pink dye) stains ALL cells
- Gram-positive bacteria already full of **purple** → remain purple
- Gram-negative bacteria (colourless) → take up **pink** stain

Final result:

- **Gram-positive** = purple/blue
- **Gram-negative** = pink/red

Gram Stain: How Does Gram Staining Work?



Differential staining: Acid-Fast Stain

It's a **differential stain** used to identify bacteria with **waxy, lipid-rich cell walls** (mycolic acids) that resist decolorization by acid-alcohol.

- These bacteria **CANNOT be stained by Gram stain**

Which Bacteria are Acid-Fast?

- **Main Group: Mycobacteria,** such as *Mycobacterium tuberculosis* and *Mycobacterium leprae*
- **Non-Acid-Fast Bacteria:** All other bacteria (Gram-positive and Gram-negative)

Differential staining: Acid-Fast Stain-Principles

STEP 1: PRIMARY STAIN (Carbolfuchsin)

Challenge: Mycolic acid barrier prevents dye penetration

Solutions:

- **Heat** (Ziehl-Neelsen method) OR
- **Detergent** (Kinyoun method - cold)

What happens:

- Heat/detergent disrupts waxy layer
- Carbolfuchsin (BASIC dye, RED/PINK) penetrates cell wall
- Dye binds to mycolic acids
- **ALL bacteria become RED/PINK**

Differential staining: Acid-Fast Stain-Principles

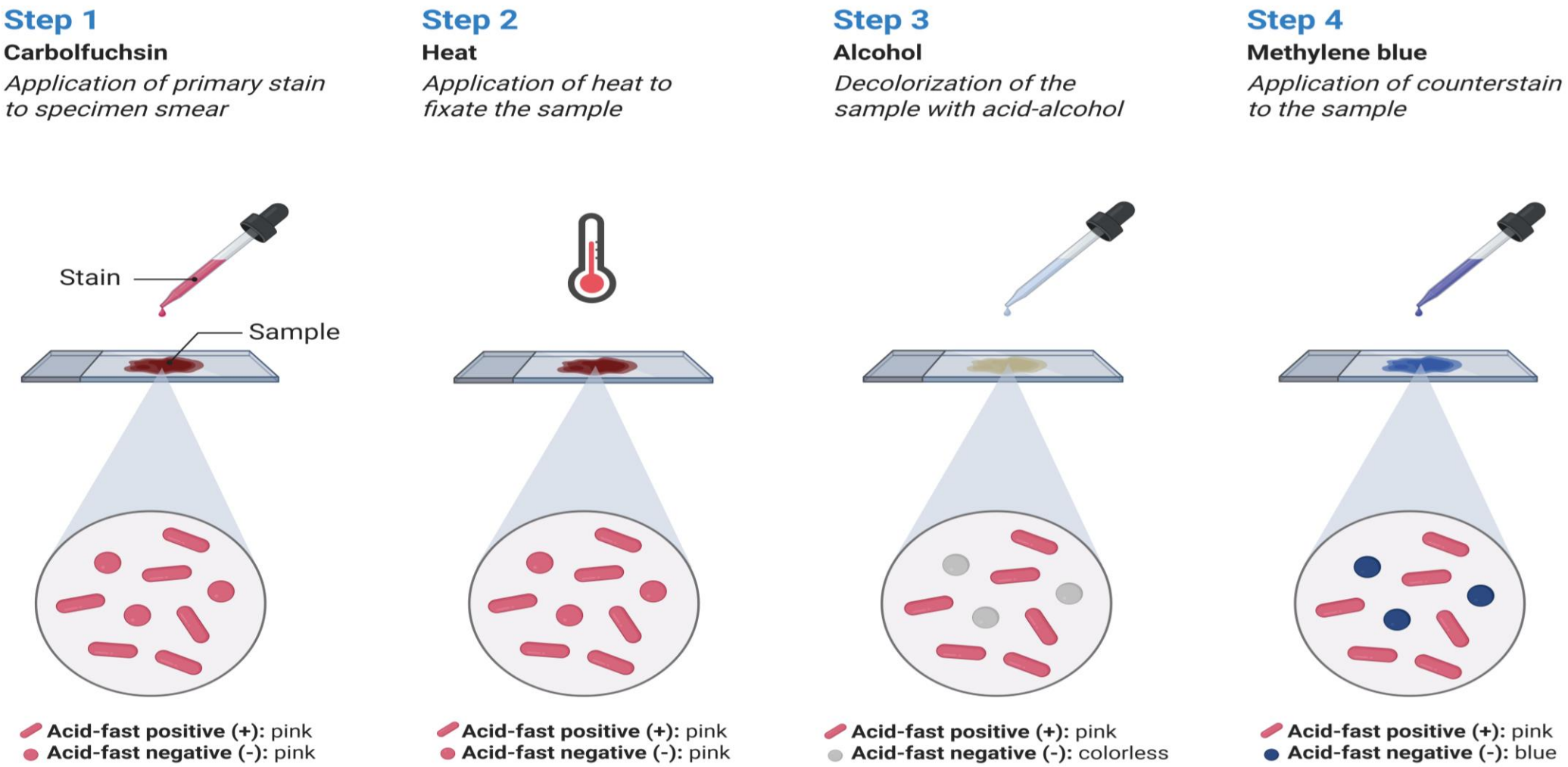
STEP 2: Decolorization (Acid-Alcohol)

- **Acid-fast bacteria (Mycobacteria):** Waxy mycolic acid layer acts as **barrier** → Carbolfuchsin gets **TRAPPED** by lipids → **RETAIN RED/PINK colour**
- **Non-acid-fast bacteria:** No mycolic acid barrier → Carbolfuchsin is removed by acid-alcohol → **LOSE colour** → **become colourless**

STEP 3: Counterstain

- Counterstain (Methylene Blue) stains all decolorized bacteria
- **Acid-fast bacteria** (already red) → remain **RED/PINK** 🔴
- **Non-acid-fast bacteria** → become **BLUE** 🔵

Differential staining: Acid-Fast Stain



Thank you