# Staining Techniques

# Types of staining techniques

## Simple staining
(use of a single stain)

For visualization of morphological shape & arrangement.

## Differential staining
(use of two contrasting stains separated by a decolorizing agent)

- Identification
  - Gram stain
  - Acid fast stain
- Visualization of structure
  - Spore stain
  - Capsule stain

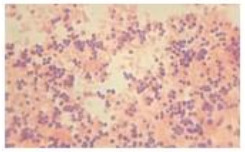
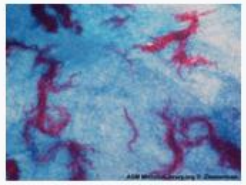
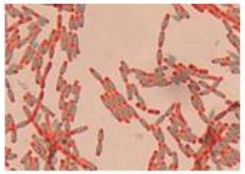
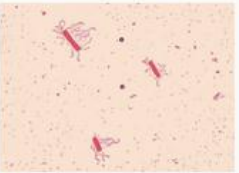
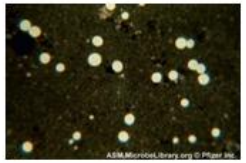
# Simple stain

**Table 2. Simple Stains**[1]

| Stain Type | Specific Dyes | Purpose | Outcome | Sample Images |
|---|---|---|---|---|
| *Basic stains* | Methylene blue, crystal violet, malachite green, basic fuschsin, carbolfuschsin, safranin | Stain negatively charged molecules and structures, such as nucleic acids and proteins | Positive stain |  |
| *Acidic stains* | Eosine, acid fuchsin, rose bengal, Congo red | Stain positively charged molecules and structures, such as proteins | Can either be positive or negative stain, depending on the cell's chemistry |  |
| *Negative stains* | India ink, nigrosine | Stains background, not specimen | Dark background with a light specimen |  |

# Differential staining

**Table 3. Differential Stains**[2]

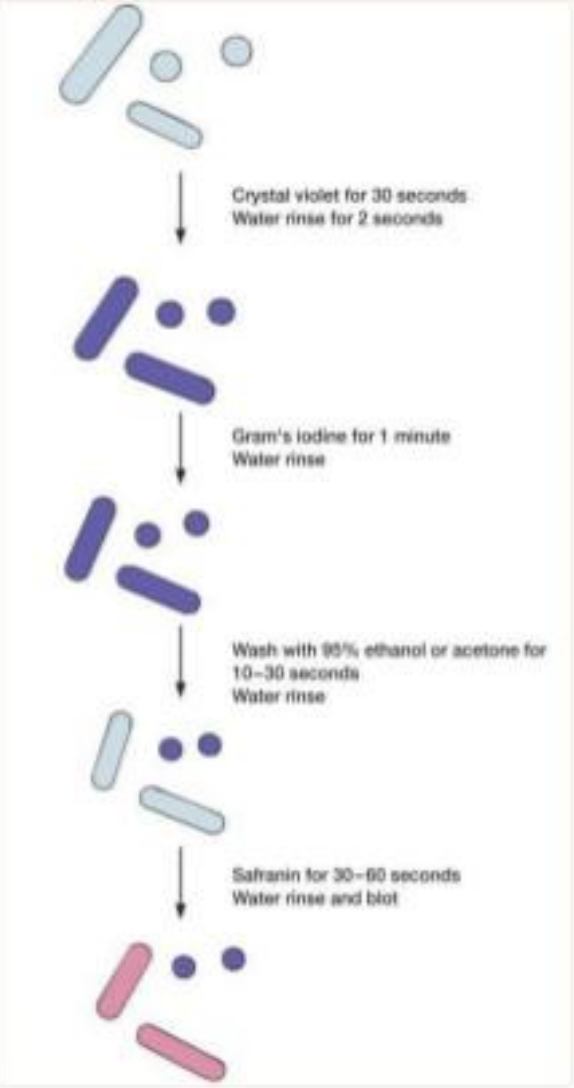
| Stain Type | Specific Dyes | Purpose | Outcome | Sample Images |
|---|---|---|---|---|
| *Gram stain* | Uses crystal violet, Gram's iodine, ethanol (decolorizer), and safranin | Used to distinguish cells by cell-wall type (gram-positive, gram-negative) | Gram-positive cells stain purple/violet. Gram-negative cells stain pink |  |
| *Acid-fast stain* | After staining with basic fuchsin, acid-fast bacteria resist decolonization by acid-alcohol. Non-acid-fast bacteria are counterstained with methylene blue. | Used to distinguish acid-fast bacteria such as *M. tuberculosis*, from non-acid-fast cells. | Acid-fast bacteria are red; non-acid-fast cells are blue. |  |
| *Endospore stain* | Uses heat to stain endospores with malachite green (Schaeffer-Fulton procedure), then cell is washed and counterstained with safranin. | Used to distinguish organisms with endospores from those without; used to study the endospore | Endospores appear bluish-green; other structures appear pink to red. |  |
| *Flagella stain* | Flagella are coated with a tannic acid or potassium alum mordant, then stained using either pararosaline or basic fuchsin | Used to view and study flagella in bacteria that have them. | Flagella are visible if present |  |
| *Capsule stain* | Negative staining with India ink or nigrosine is used to stain the background, leaving a clear area of the cell and the capsule Counterstaining can be used to stain the cell while leaving the capsule clear | Used to distinguish cells with capsules from those without. | Capsules appear clear or as halos if present. |  |

# Gram stain procedure

- The **Gram stain procedure** is a differential staining procedure that involves multiple steps. It was developed by Danish microbiologist Hans Christian **Gram** in 1884 as an effective method to distinguish between bacteria with different types of cell walls, and even today it remains one of the most frequently used staining techniques. The steps of the Gram stain procedure are listed below and illustrated in Table 1.
- First, **crystal violet**, a **primary stain**, is applied to a heat-fixed smear, giving all of the cells a purple color.
- Next, **Gram's iodine**, a **mordant**, is added. A mordant is a substance used to set or stabilize stains or dyes; in this case, Gram's iodine acts like a trapping agent that complexes with the crystal violet, making the crystal violet–iodine complex clump and stay contained in thick layers of peptidoglycan in the cell walls.
- Next, a **decolorizing agent** is added, usually ethanol or an acetone/ethanol solution. Cells that have thick peptidoglycan layers in their cell walls are much less affected by the decolorizing agent; they generally retain the crystal violet dye and remain purple. However, the decolorizing agent more easily washes the dye out of cells with thinner peptidoglycan layers, making them again colorless.
- Finally, a secondary **counterstain**, usually **safranin**, is added. This stains the decolorized cells pink and is less noticeable in the cells that still contain the crystal violet dye.
- Gram-staining is a differential staining technique that uses a primary stain and a secondary counterstain to distinguish between gram-positive and gram-negative bacteria.

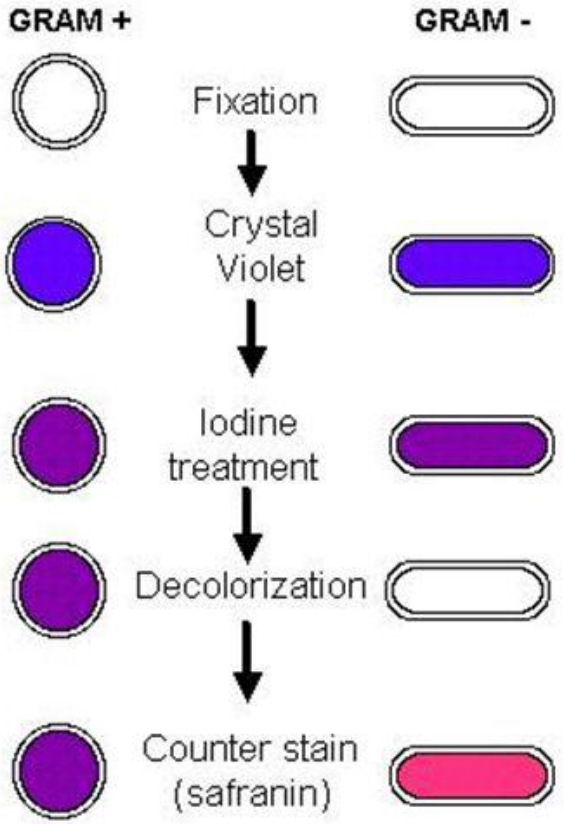
# GRAM STAIN TECHNIQUES

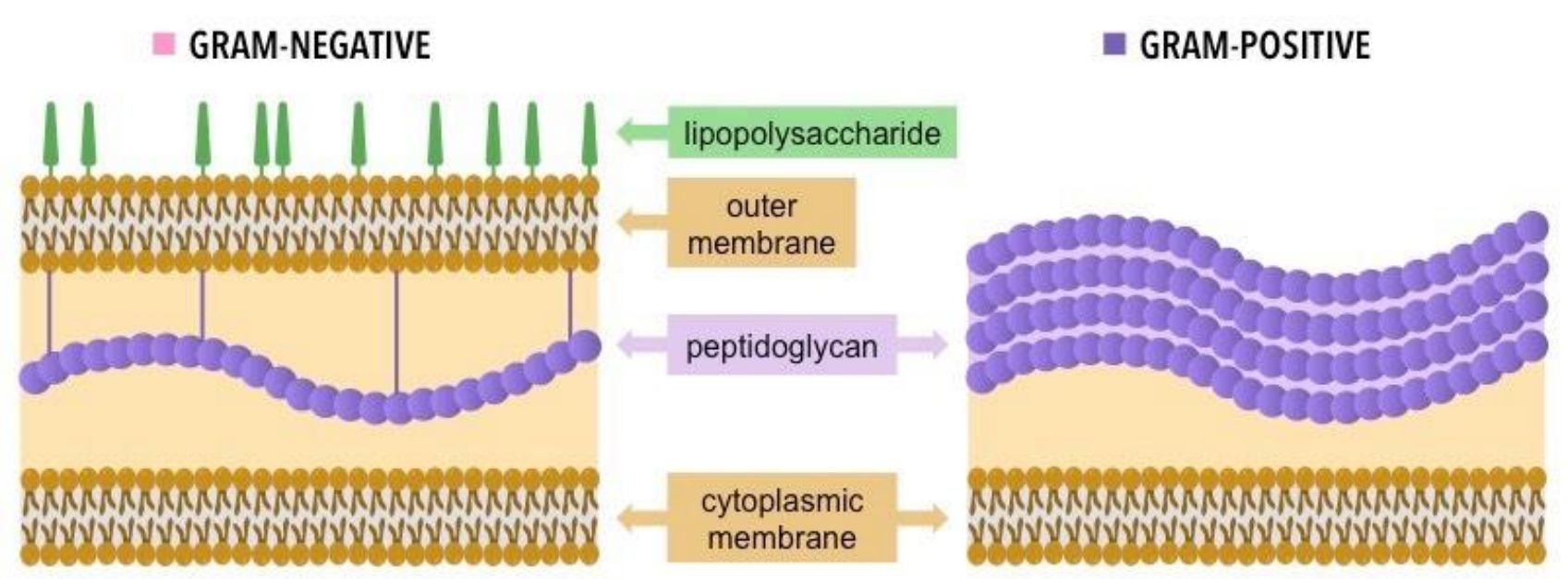
- Prepare bacterial smear on the clean slide.
- Pass the slide through over the flame 2–3 times. (**Heat fixing**)
- Apply Crystal Violet (**Primary stain**) on smear for 1 minutes & rinse with water.
- Apply Gram's iodine (**Mordant**) for 1 minute & wash with water.
- Then wash with 95% alcohol (**Decolouriser**) for 10-20 seconds & rinse with water.
- Apply Safranin (**Seconday stain**) for 1 minute & wash with water.
- Air dry, Blot dry & Observe under Microscope.

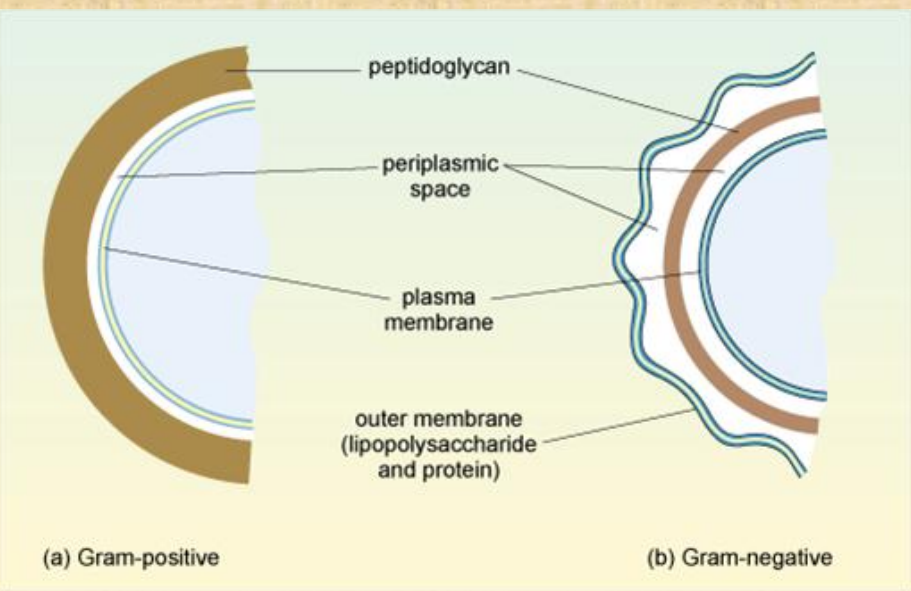


# The gram stain consists of these steps:

- **Crystal violet** - stains both gram negative and positive bacteria
- Gram's iodine - fixes the stain in gram positive bacteria
- Ethanol or acetone - washes the stain from gram negative bacteria
- **Safranin** - counterstain, will re-stain gram negative bacteria while not interfering with the previous stain in gram positive bacteria

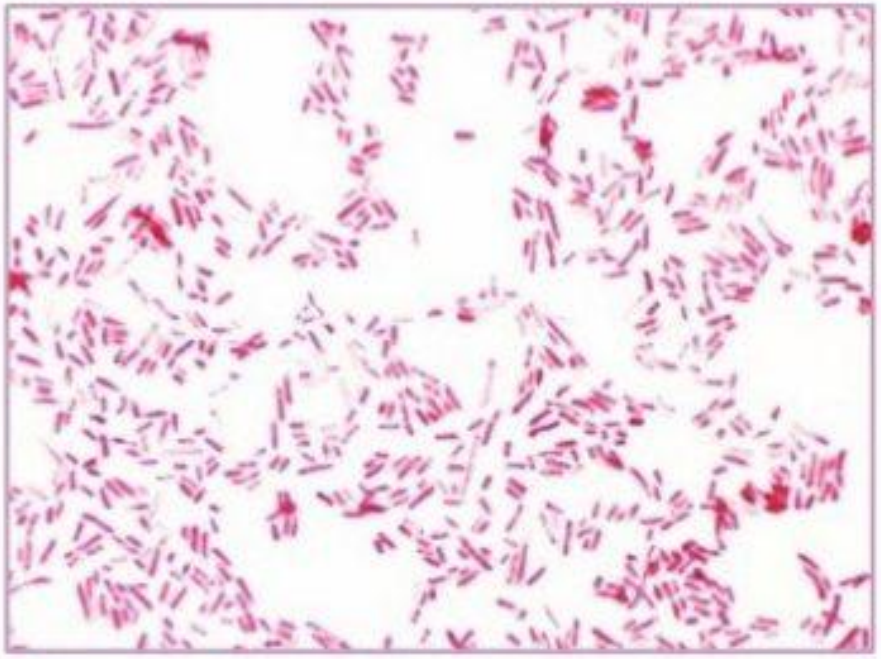




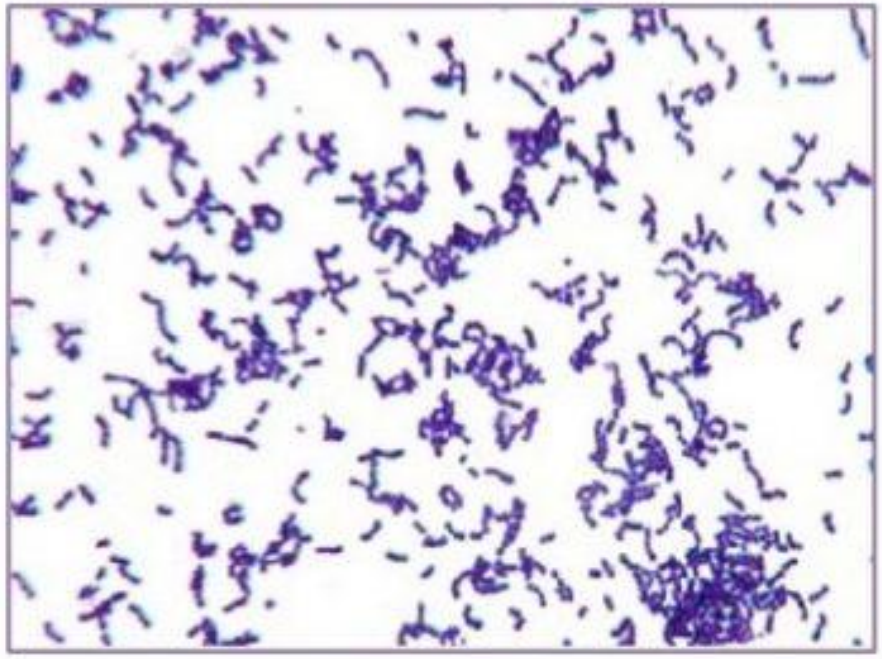




In the Gram-positive Bacteria, the cell wall is thick (15-80 nanometers), consisting of several layers of peptidoglycan. In the Gram-negative Bacteria the cell wall is relatively thin (10 nanometers) and is composed of a single layer of peptidoglycan surrounded by an outer membrane

# Gram staining of Bacteria



Gram-Negative Bacteria



Gram-Positive Bacteria