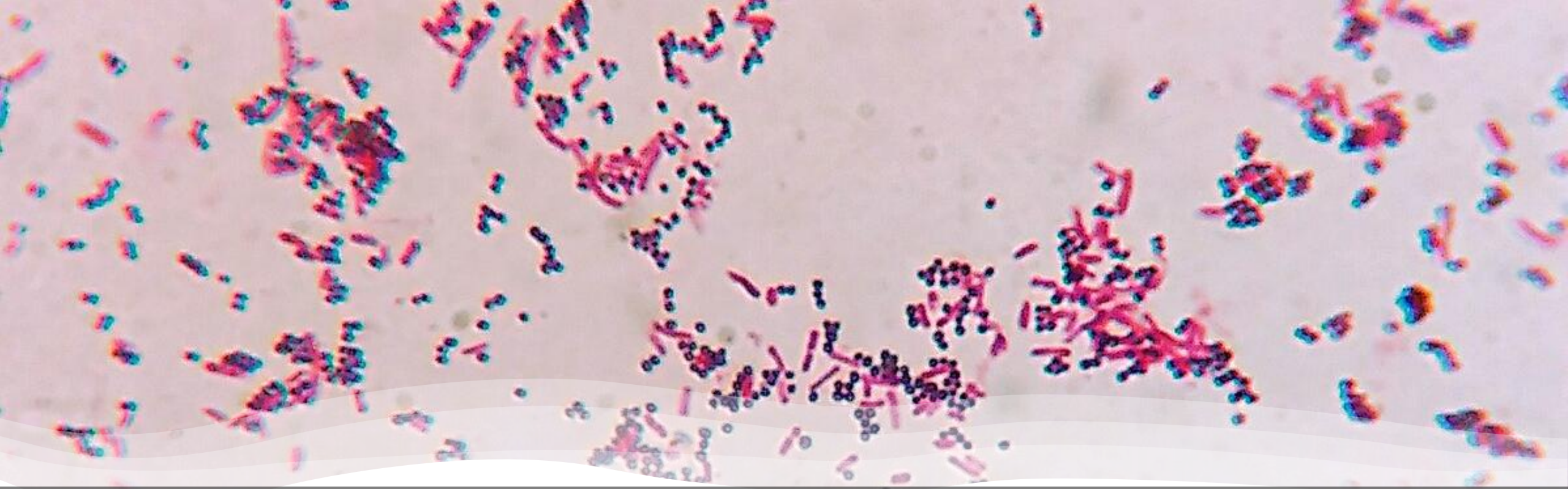

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Gram Staining

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Learning Objectives

By the end of this lab, nursing students will be able to:

1. **Explain the purpose** of Gram staining and its importance in clinical microbiology.
2. **Describe the differences** between Gram-positive and Gram-negative bacteria based on cell wall structure and stain uptake.
3. **Perform the Gram stain procedure** step-by-step using proper laboratory technique.
4. **Identify bacteria** under the microscope as Gram-positive or Gram-negative based on color and morphology.
5. **Demonstrate correct microscope handling** and slide preparation skills.
6. **Interpret Gram stain results** to aid in preliminary diagnosis and treatment decisions.

Overview

What is Gram Staining?

Gram stain is a key diagnostic tool that guides clinical decisions in infection management.

It is a differential staining technique used to classify bacteria into two groups : Gram-positive and Gram-negative.

Why gram staining matter in nursing?

- Provide quick identification of bacteria type
- Helps guide antibiotic therapy
- Aids in understanding infection control protocols

Clinical Relevance:

- Gram-negative infections may be more resistant to antibiotics
- Certain infections require rapid identification for isolation (e.g., MRSA)
- Knowing the Gram status helps predict severity and course of treatment

Bacteria Type	**Color After Staining**	**Cell Wall Structure**
Gram Positive	Purple / Violet	Thick peptidoglycan
Gram Negative	Pink / Red	Thin peptidoglycan wall and have an outer membrane

Examples of Gram Negative and Positive Bacteria

Gram-positive:

- Staphylococcus aureus (Cocci in clusters)
- Streptococcus pneumoniae (Cocci in chain)
- Bacillus subtilis (Rod-shaped)

Gram-negative:

- Escherichia coli (Rod-shaped)
- Pseudomonas aeruginosa (Rod-shaped)

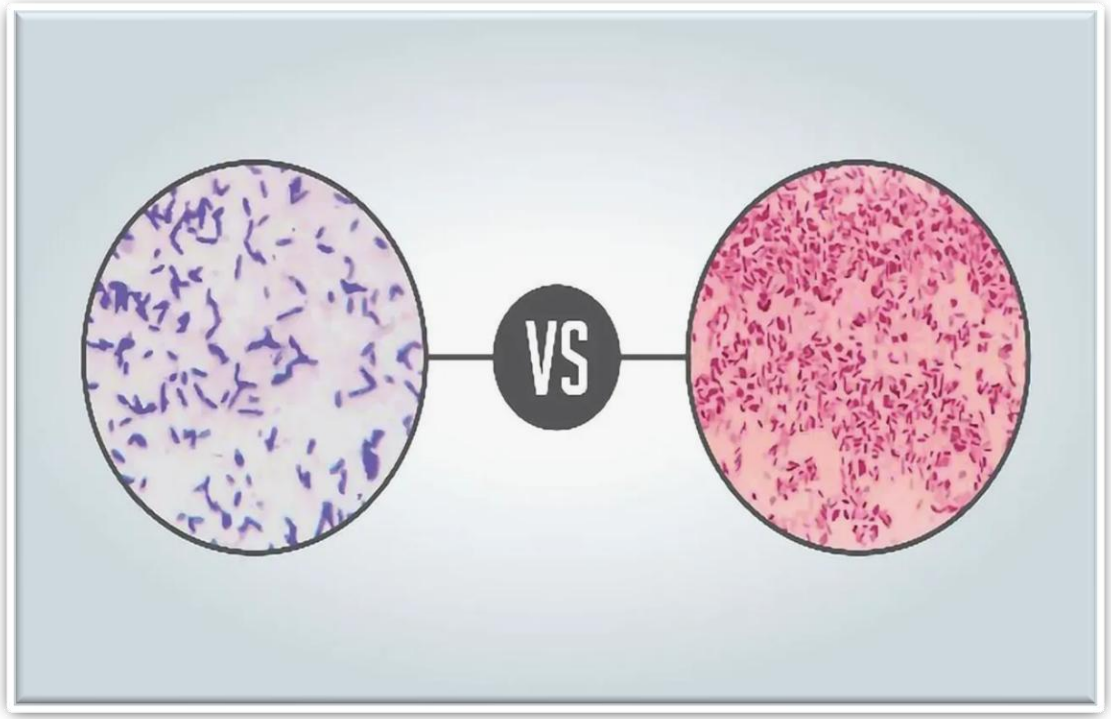

Photo credit to https://assets.technologynetworks.com/production/dynamic/images/content/323007/gram-positive-vs-gram-negative-323007-1280x720.webp?cb=13323480

Step-by-Step Gram Staining Procedure:

1. Prepare a Bacterial Smear

1. Place a small drop of water on a clean slide (if using solid culture).
2. Using a sterile loop, transfer a small amount of bacteria to the drop and spread thinly.
3. Air dry the smear completely.

2. Heat Fix the Slide

Pass the slide through a flame 2–3 times (smear side up) to fix the bacteria.

3. Stain with Crystal Violet (Primary Stain)

1. Cover the smear with **crystal violet** for **1 minute**.
2. Rinse gently with **distilled water**.

4. Apply Gram's Iodine (Mordant)

1. Flood the slide with **iodine solution** for **1 minute** (acts as a mordant).
2. Rinse with **distilled water**.

4. Decolorize with Alcohol or Acetone

1. Gently apply **alcohol or acetone** for **10–20 seconds** (until runoff is clear).
2. Immediately rinse with **distilled water** to stop decolorization.

5. Counterstain with Safranin

1. Stain with **safranin** for **1 minute**.
2. Rinse with **distilled water** and blot dry with bibulous paper.

6. Examine Under Microscope

Use **oil immersion (100x objective)** to observe bacteria.

- **Gram-positive** bacteria appear **purple/violet**.
- **Gram-negative** bacteria appear **pink/red**.