Gram Stain

Gram stain is a test that checks for bacteria at the site of a suspected infection such as the throat, lungs, genitals, or in skin wounds. Gram stains may also be used to check for bacteria in certain body fluids, such as blood or urine.

There are two main categories of bacterial infections: Gram-positive and Gram-negative. The categories are diagnosed based on the how the bacteria react to the Gram stain. A Gram stain is colored purple. When the stain combines with bacteria in a sample, the bacteria will either stay purple or turn pink or red.

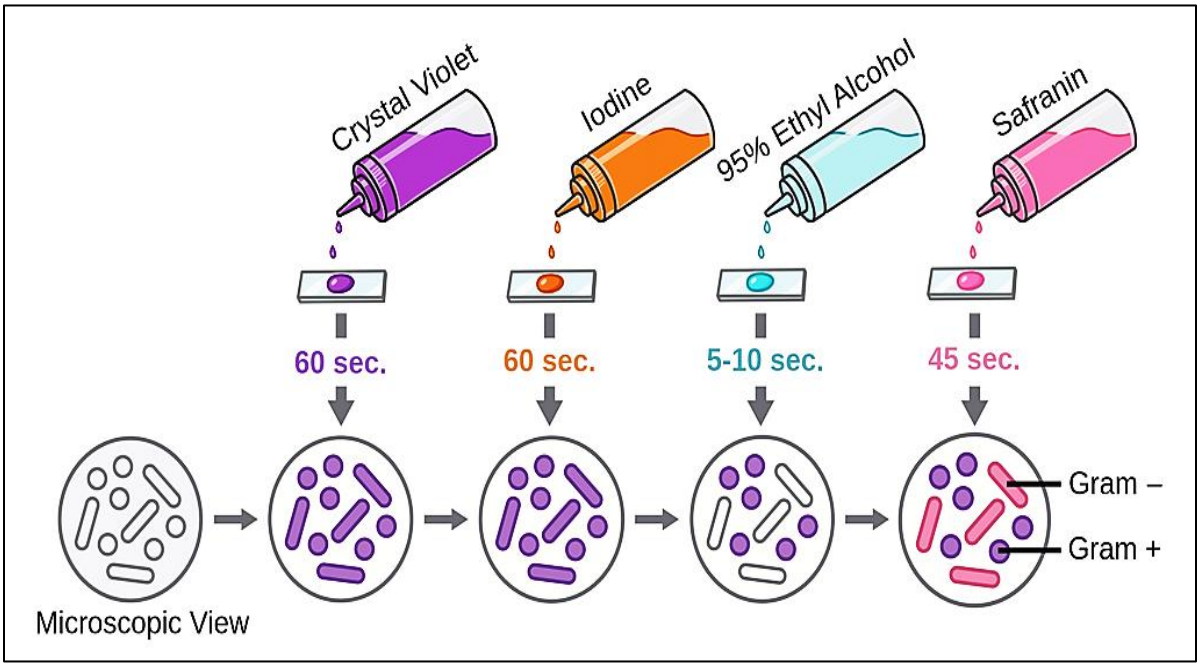
- If the bacteria stay purple, they are Gram-positive.
- If the bacteria turn pink or red, they are Gram-negative.

Gram stain is most often used to find out if you have a bacterial infection. If you do, the test will show if your infection is Gram-positive or Gram-negative.

A Gram stain may also be used to diagnose fungal infections(yeast).

❖ **Steps to perform Gram-Staining:**

1) Apply crystal violet on the slide and keep it for 60 seconds. Wash the slide with water.
2) Apply iodine on the slide and keep it for 60 seconds. Wash the slide with water.
3) Apply 95% ethyl alcohol on the slide and keep it for 5 to 10 seconds. Wash the slide with water.
4) Finally, apply safranin on the slide and keep it for 45 seconds. Wash the slide with water.



Gram-positive organisms (+)

Gram-positive bacteria have cell walls that contain thick layers of peptidoglycan, a substance that forms the cell walls of many bacteria. The peptidoglycan forms about 90% of the cell wall in gram-positive bacteria. This causes them to appear blue to purple under a Gram stain.

Gram-positive organisms include:

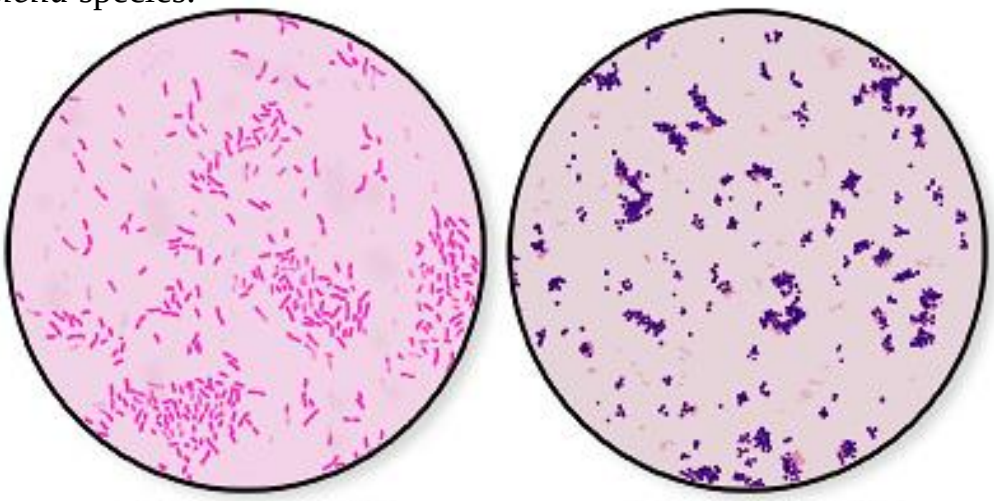
- *Staphylococcus* species.
- *Streptococcus* species.
- *Corynebacterium* species.
- *Clostridium* species.
- *Listeria* species.

Gram-negative organisms (-)

Gram-negative bacteria have cell walls with thin layers of peptidoglycan (10% of the cell wall) and high lipid (fatty acid) content. This causes them to appear red to pink under a Gram stain.

Gram-negative organisms include:

- *Neisseria gonorrheae* and *Neisseria meningitides*.
- *Moraxella* species.
- *Escherichia coli* (*E. coli*).
- *Pseudomonas* species.
- *Proteus* species.
- *Klebsiella* species.



Gram-negative Gram-positive