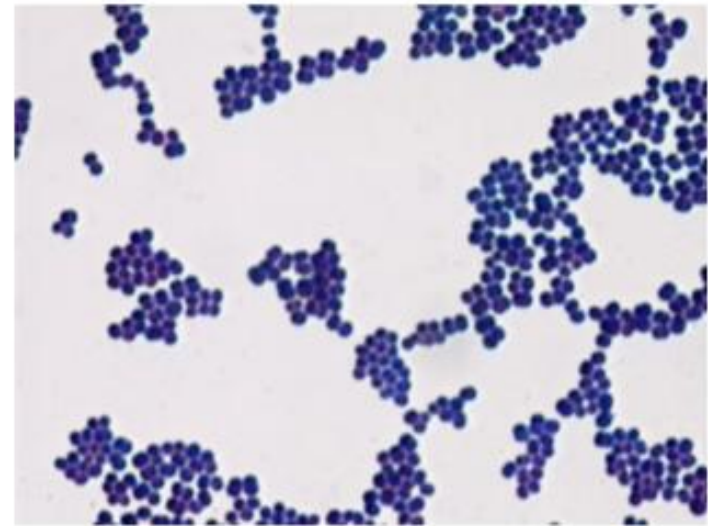
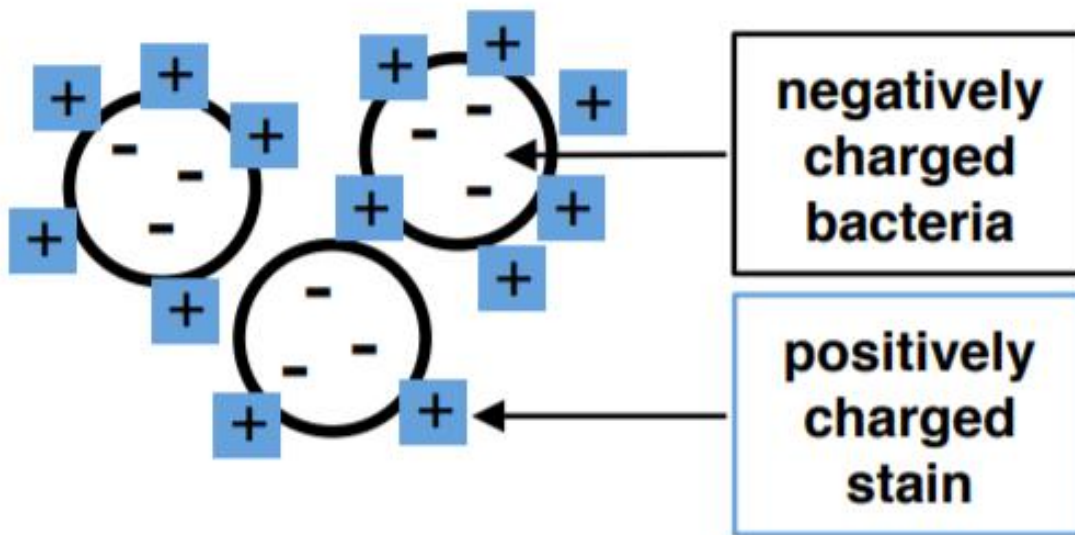


Simple staining



Gram stain process			
Gram staining steps	Cell effects	Gram-positive	Gram-negative
Step 1 Crystal violet <i>primary stain added to specimen smear.</i>	Stains cells purple or blue.		
Step 2 Iodine <i>mordant makes dye less soluble so it adheres to cell walls.</i>	Cells remain purple or blue.		
Step 3 Alcohol <i>decolorizer washes away stain from gram-negative cell walls.</i>	Gram-positive cells remain purple or blue. Gram-negative cells are colorless.		
Step 4 Safranin <i>counterstain allows dye adherence to gram-negative cells.</i>	Gram-positive cells remain purple or blue. Gram-negative cells appear pink or red.		

Gram Staining Procedure

Gram (+)

Gram (+) cell walls have single membrane enclosed by thick, cross linked peptidoglycan

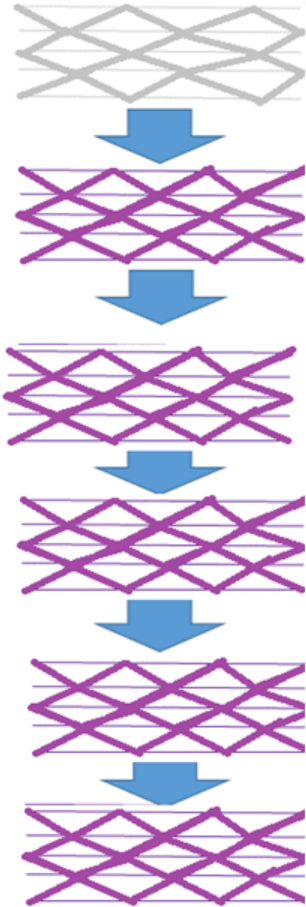
Thick peptidoglycan takes up dye. Appears purple.

Dye and mordant complex forms. Adheres firmly to thick peptidoglycan layer.

Alcohol cannot wash out the dye-mordant complex because it is firmly secured in the thick peptidoglycan layer.

Saturated with the crystal violet dye, the cell counter stain has little to no effect.

Cell wall ranges in color from mid to dark purple.



1. Heat fix cells to slide

2. Saturate with crystal violet dye for 60 seconds

3. Add iodine (mordant) for 60 seconds

4. Rinse slide with alcohol for 20 seconds

5. Stain slide with safranin (counter stain)

Gram (-)

Gram (-) cell walls have a thin layer of peptidoglycan in the periplasmic space within its inner and outer lipid membranes

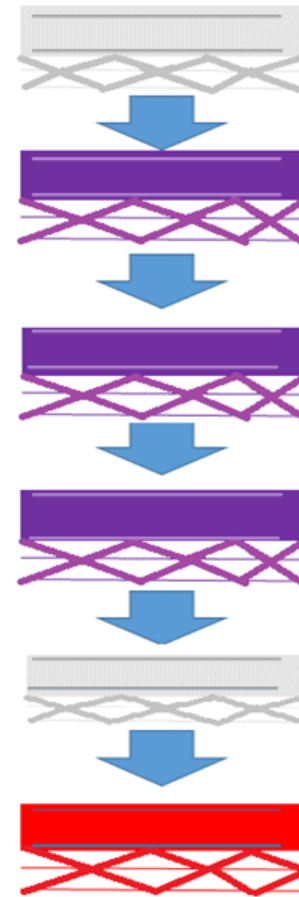
Cell wall takes up dye. Appears purple.

Dye and mordant form complex, but does not adhere to the thin layer of peptidoglycan.

Dye and mordant complex is easily removed from peptidoglycan layer with alcohol.

Colorless cell wall can easily take up counter stain.

Cell wall, counter stained with safranin, ranges in color from pink to red.




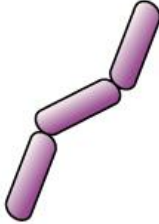

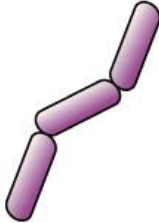

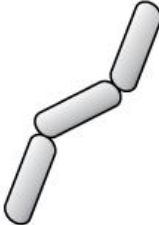

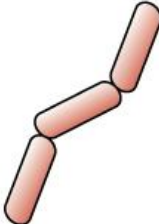
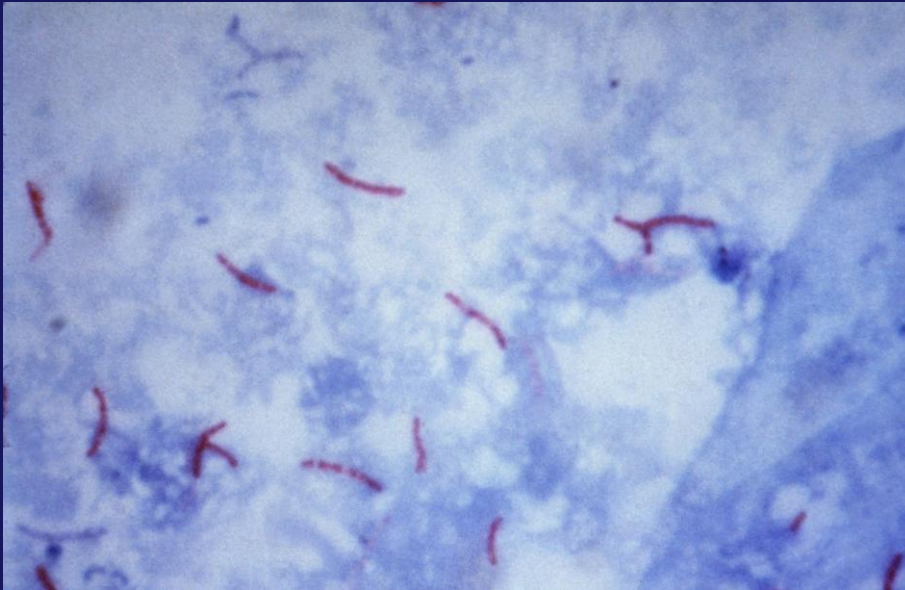
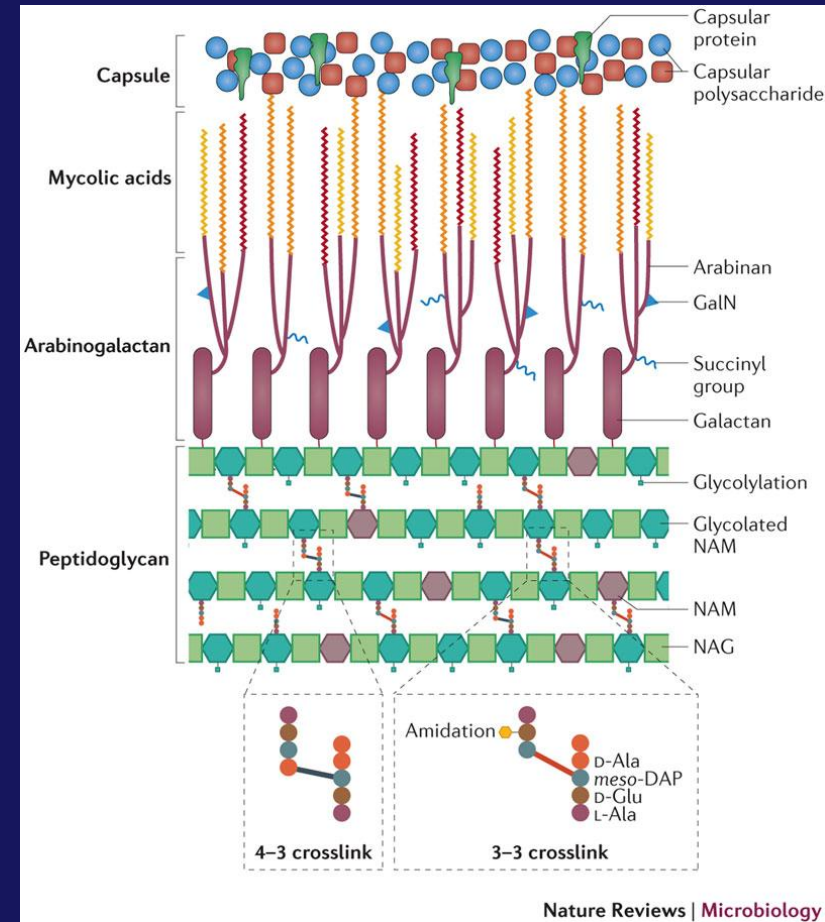
Gram stain process			
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Image: [Figure 2.33/OpenStax Microbiology/Creative Commons](#)

The acid-fast stain is used to stain species of *Mycobacterium*



Specimen: Mycobacterium tuberculosis Ziehl-Neelsen stain 02.jpg, Public Domain, CDC/Dr. George P. Kubica - phil.cdc.gov CDC-PHIL ID #5789



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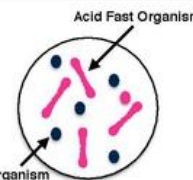
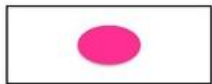
http://www.nature.com/nrmicro/journal/v12/n8/box/nrmicro3299_BX1.html

1. Apply primary stain of carbofuchsin for 30 seconds

2. Heat fix cells to the slide using flame

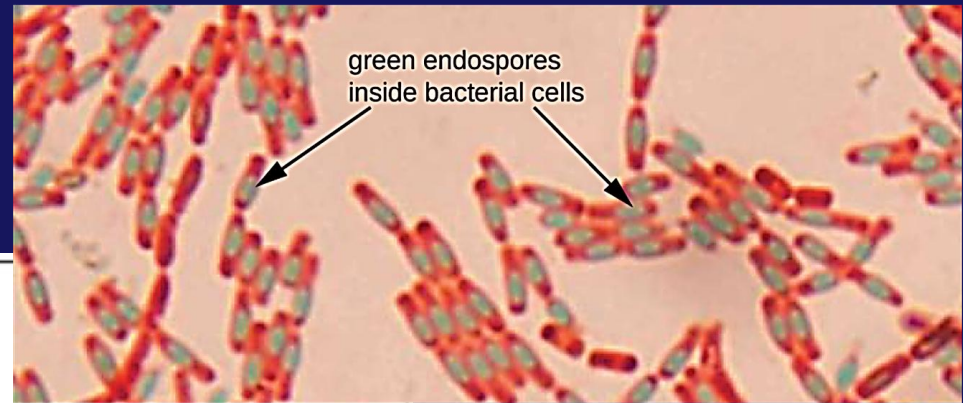
3. Decolorize with acid alcohol for 15-20 seconds

4. Apply counterstain of methylene blue for 30 seconds then rinse excess stain

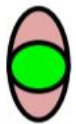
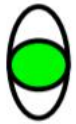
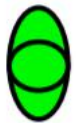


Non Acid Fast Organism

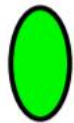
The spore stain is used to detect endospores in species of *Bacillus* and *Clostridium*



Endospore (+)
bacteria



Endospore (-)
bacteria



1. Bacteria and endospores are transparent prior to staining

2. Steam is used to force the MALACHITE GREEN into all the bacteria and endospores

3. Decolorization with water removes the malachite green from the bacteria but NOT the endospores

4. Safranin is the counterstain, which stains all bacteria but CAN NOT STAIN endospores

Image: Figure 2.38 OpenStax Microbiology/Creative Commons

[https://bio.libretexts.org/Learning_Objects/Laboratory_Experiments/Microbiology_Labs/Book%3A_General_Microbiology_Lab_Manual_\(Pakpour_and_Horgan\)/Lab_04%3A_Acid-Fast_Spores_and_Capsule_Stains](https://bio.libretexts.org/Learning_Objects/Laboratory_Experiments/Microbiology_Labs/Book%3A_General_Microbiology_Lab_Manual_(Pakpour_and_Horgan)/Lab_04%3A_Acid-Fast_Spores_and_Capsule_Stains)
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