Department of biology

Department of biology

Microbiology Lab

((Gram Stain))

Lab/5

2 stage

By

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Gram Stain:

The Gram staining is one of the most crucial staining techniques in microbiology. It gets its name from the Danish bacteriologist Hans Christian Gram, who first introduced it in 1882, mainly to identify organisms causing pneumonia.

The term for organisms that retain the primary color and appear purple-brown under a microscope is Gram-positive organisms. The organisms that do not take up primary stain appear red or pink under a microscope and are Gram-negative organisms.

How Does Gram Staining Work?

Gram staining involves three processes: staining with a water-soluble dye called crystal violet, decolorization, and counterstaining, usually with safanin. Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram positive and Gram negative bacteria,Gram positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolorization process, while Gram negative bacteria lose the crystal violet stain and areinstead stained by the safranin in the final staining process.

Staining Protocol:

Crystal violet (primary stain)

Iodine solution/Gram's Iodine (mordant that fixes crystal violet to cell wall)

Decolorizer (e.g.ethanol)

Safranin (secondary stain)

Water (preferably in a squirt botle)

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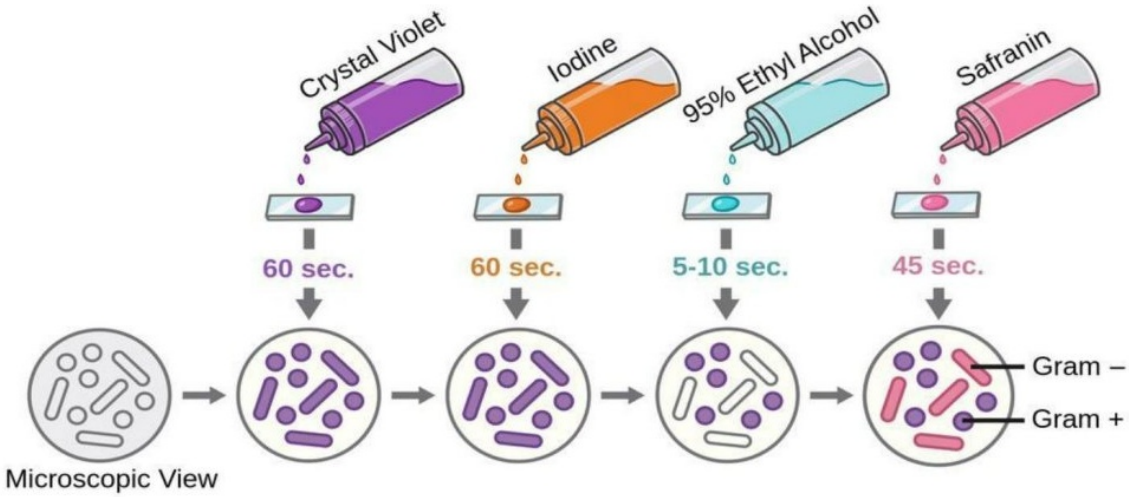
The process involves four steps:

1-Add the primary stain (crystal violet) to the sample/slide and incubate for 1 minute. Rinse slide with a gentle stream of water for a maximum of 5 seconds to remove unbound crystal violet.

2-Add iodine for 1 minute, or an agent that fixes the crystal violet to the bacterial cell wall.

3-Rinse sample/slide with acetone or alcohol for ~3 seconds and rinse with a gentle stream of water. The alcohol will decolorize the sample if it is Gram negative removing the crystal violet.

4-Add the secondary stain, safranin, to the slide and incubate for 1minute. Wash with a gentle stream of water for a maximum of 5 seconds.If the bacteria is Gram positive, it will retain the primary stain (crystal violet) and not take the secondary stain (safranin), causing it to look violet/purple under a microscope. If the bacteria is Gram negative, it will lose the primary stain and take the secondary stain(safranin), causing it to appear red(pink) when viewed under a microscope.



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