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Bacterial staining

Simple Stains: The simple stain can be used to determine cell shape, size, and arrangement. The simple stain is a very simple staining procedure involving only one stain e.g. crystal violet.

Differential Stains: is a staining process which uses more than one chemical stain. Using multiple stains can better differentiate between different microorganisms. One commonly recognizable use of differential staining is the Gram stain.

Special Stains - These are stains that include the acid-fast, endospore, capsule ,flagellar stains.

Acid-Fast Stain - is used for staining cells of ***Mycobacterium*** and ***Nocardia.***

Albert stain: a stain for diphtheria bacilli and their metachromatic granules.

Gram stain

Gram stain is a common technique used to differentiate two large bacterial species into two large groups (Gram-positive and Gram-negative). Based on their different cell wall constituents. The **Gram stain** procedure distinguishes between **Gram** positive and **Gram** negative groups by coloring these cells red or violet.

Gram's method (Procedure):

Steps

1-Sterilze the loop by Bunsen burner.

2- Taking bacterial colony from a petri dish (or culture plate), and added a drop of distal water on the slide and mixed with bacterial colony .

3- Heat and fix the bacteria to the slide, by quickly pass the slide two to three times through a Bunsen burner flame.

4-Added several drops of crystal violet dye, sometimes called gentian violet. Wait (3-5 minute).

5-Wash the slide by serial distal water.

6- Added drops of iodine and wait (2 minute). Also wash the slide by sterile distal water.

7-Then add the decolorize by (Alcohol 90%) for 10 seconds and wash the slide by sterile distal water.

8-Added counters stain typically is safranin for 45 seconds and wash the slide by sterile distal water.

9- The dry the slide by using dry filter paper.

10-Prepare the light microscope**:** Place the slide under the light microscope. Bacteria vary greatly in size, so the total magnification required will vary from 400x to 1000x. Place a drop of immersion oil on the slide.

11-Identify gram-positive bacteria by shape and bacterial color appear as purple color under the microscope e.g. *Staphylococci ,Streptococci* .

12-Identify gram-negative bacteria under the microscope appear as red in color e.g. *Neisseria* spp , *Bordetella*, *Brucella*, *Haemophilus*, and *Pasteurella*.

Staining mechanism

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in a thick layer in Gram-positive bacteria. In a Gram stain test, Gram-positive bacteria retain the crystal violet dye, while a counterstain (commonly safranin or fuchsine) added after the crystal violet gives all Gram-negative bacteria a red or pink coloring.

1-Gram-positive bacteria have a thick cell wall made of peptidoglycan, and as a result are stained cell wall, so do not retain crystal violet stain and stained red in violet color by crystal violet, whereas gram-negative bacteria have thin color by the Safranin.

2-When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. A gram-negative cell loses lipids outer lipopolysaccharide membrane, and the inner peptidoglycan layer .

3-After decolorization, the gram-positive cell remains purple or violet color and the gram-negative cell loses its purple color or violet color. Counterstain, which is usually positively charged safranin or basic fuchsine, is applied last to give decolorized gram-negative bacteria a pink or red color.

Added **to** bacterial sample several drops of crystal violet dye, sometimes called gentian violet. Wait thirty to3-5 minute . Crystal violet (CV) dissociates in aqueous solutions into CV+ and chloride (Cl–) ions. These ions penetrate through the cell wall and cell membrane of both gram-positive and gram-negative cells. The CV+ ion interacts with negatively charged components of bacterial cells to stain the cells purple.
Then added iodine. Let it sit for at least 2 minute . Iodine, in the form of negatively charged ions, interacts with CV+ to form large complexes of crystal violet and iodine (CV–I complexes) within the inner and outer layers of the cell. This will trap the purple crystal violet color in the cell, wherever it has stained.
Hold the slide at an angle and added the decoloriser (Alcohol 90%). This typically takes under 10 seconds, or even less time if the decoloriser. The decolorize will remove the crystal violet stain from both gram-positive and negative cells, and the stain will have to be repeated.
A counterstain, typically safranin added for 45 second to differentiate between gram-negative and gram-positive bacteria, by staining decolorised (gram-negative) bacteria pink or red.

Why are gram-positive bacteria purple and gram-negative bacteria pink?

Gram stain involves staining a group of bacteria with four different liquids. First, crystal violet is added. Then stained with iodine, and finally with safranin. Then it goes through an alcohol wash.

Cristal violett is added to the sample and penetrates the cell walls and stains the cells, iodine is then added to fix the stain, then the cells are washed with Alcohol to remove the stain .

Gram positive bacteria : The bacteria that retain the purple stain from the crystal violet are gram-positive .

Gram positive bacteria have a thick layer of peptidoglycan, which absorbs the gram stain.

Gram positive bacteria are purple because they have cell walls. While staining you use crystal violet and then iodine to set the stain. If the bacteria are gram positive (or have cell walls), they will retain the stain.

Gram negative bacteria: Take the pink stain from the safranin are gram-negative.

Gram-negative bacteria have a thick lipid bilayer on the outside.

Gram negative bacteria cannot hold the stain because they lack the cell walls.