Function of Bacterial Cell Wall

Bacterial Staining

Staining principle

Stains, or dyes, contain salts made up of a positive ion and a negative ion. Depending on the type of dye, the positive or the negative ion may be the chromophore (the colored ion); the other, uncolored ion is called the counter ion. If the chromophore is the positively charged ion, the stain is classified as a **basic dye**; if the negative ion is the chromophore, the stain is considered an **acidic dye**.

For example, the dye Methylene blue is composed of positively charged ion so it binds to the negatively charged parts of bacteria such as DNA and proteins with ionic bonds called cationic chromophore while the negative dyes have anionic chromophore, such as the Eosin dye, is associated with the positively charged parts of the bacterial cell, such as some amino acids.

Preparation and Fixation of Bacterial Smear for Staining

There are two methods of fixing bacterial smear for staining:-

- **Heat Fixing:** A thin layer of the specimen is spread on the slide (called a smear), and the slide is then briefly heated over a heat source to attaching the specimen to the slide, fixation also kills microorganisms in the specimen, stopping their movement and metabolism while preserving the integrity of their cellular components for observation.
- **Chemical fixatives:**Apply methyl alcohol 95% for one minute and then tilt the slide to remove the excess alcohol.

Materials:

- Prepared young cultures between 18-24 hours of age.
- slide, inoculating loop, Bunsen.

The method of work:

- We pass a clean slide surface into the Bunsen flame several times to partially sterilize and remove fat particles stuck to it, then leave it on its holder to cool.
- By inoculating loop, we place two drops of liquid culture on the surface of the slide

*In the case of solid culture, we put a drop of normal saline or a sterile water in the center of the slide, then take a colony of the bacterial growth by the needle with the inoculating loop and mix them well.

- By using the loop, we spread the culture mixture on an area of about 1 cm in the middle of the slide, to form a regular thin film.
- Leave the film to dry completely in the air dry at room temperature.
- After that, we fix the formed thin layer by passing the slides in the flame several times (three times).

Types of staining

First: Simple staining

-Simple staining refers to the use of only one stain in staining bacteria, and several drops of the dye are placed on Smear fixed for a period of minutes, depending on the type of dye used, then the dye is washed with water and the slide is dried. Then check it by using the of microscope.

- The simple dye depends on the fact that the bacteria cells are chemically different from the surrounding environment, so they are stained to show the contrast between them and the medium.
- Simple staining is useful to clarify differences in cell size, morphology and arrangement.
- Among the most popular dyes used in it are methylene blue, safranin, and crystal violet.

-The simple stain that targets the bacterial cells themselves is called the direct stain

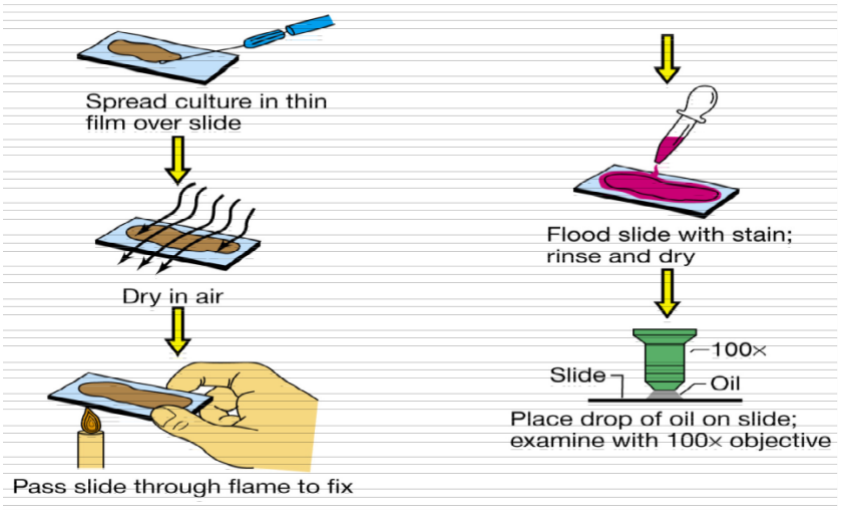
-A simple dye that stains the background and leaves bacterial cells unpainted is called a negative stain

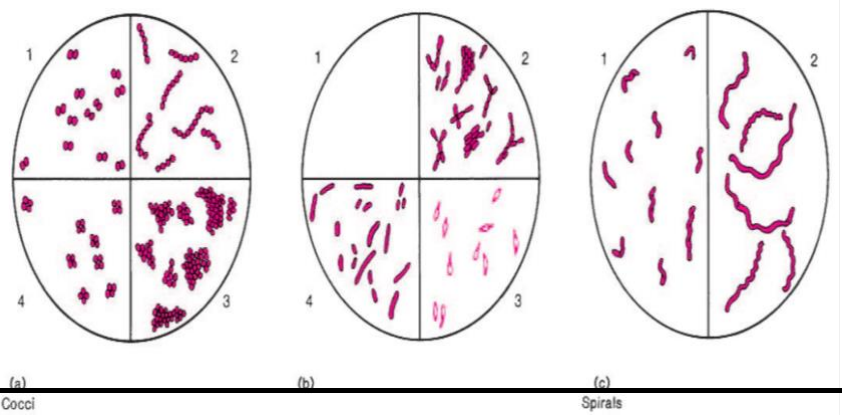
Materials:

- A young bacterial culture, 18-24 hours old.
- Methylene blue or Safranine.
- Glass Slides.

The method of work:

- We place the slide on which the bacterial smear is fixed on a dye wire.
- Soak the smear with the required dye, and let it have sufficient time for reaction: 30 to 60 seconds for methylene blue or safranin and crystal violet.
- We get rid of the dye solution and then wash the dyed smear with water, to remove the excess dye.
- We dry the slide by placing it between two clean blotting papers and then passing it over the flame several times.
- Put a drop of cedar oil on the dyed smear, then examine it with the oil lens





Second: differential or compound staining: It is intended to use more than one dye, in order to distinguish between different bacterial groups.

Gram Staining

The **Gram stain procedure** is a differential staining procedure that involves multiple steps. It was developed by Danish microbiologist Hans Christian **Gram** in 1884 as an effective method to distinguish between bacteria with different types of cell walls, and even today it remains one of the most frequently used staining techniques.

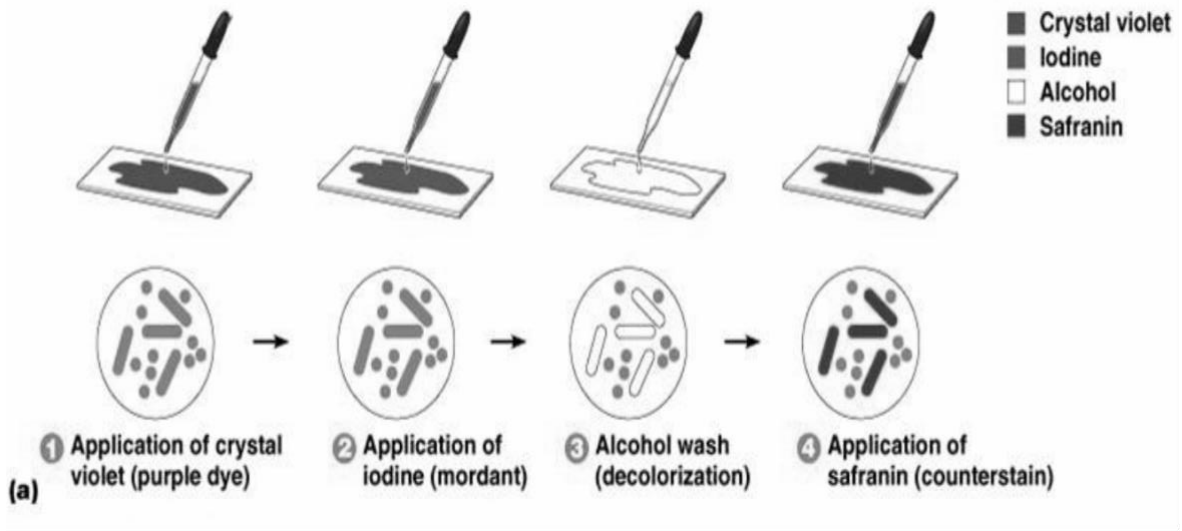
1. First, **crystal violet**, a **primary stain**, is applied to a heat-fixed smear, giving all of the cells a purple color.
2. Next, **Gram's iodine**, a **mordant**, is added. A mordant is a substance used to set or stabilize stains or dyes; in this case, Gram's iodine acts like a trapping agent that complexes with the crystal violet, making the crystal violet–iodine complex clump and stay contained in thick layers of peptidoglycan in the cell walls.
3. Next, a **decolorizing agent** is added, usually ethanol or an acetone/ethanol solution. Cells that have thick peptidoglycan layers in their cell walls are much less affected by the decolorizing agent; they generally retain the crystal violet dye and remain purple. However, the decolorizing agent more easily washes the dye out of cells with thinner peptidoglycan layers, making them again colorless.
4. Finally, a secondary **counterstain**, usually **Safranine**, is added. This stains the decolorized cells pink and is less noticeable in the cells that still contain the crystal violet dye.

Gram-staining is a differential staining technique that uses a primary stain and a secondary counterstain to distinguish between gram-positive and gram-negative bacteria.

Mechanism of action of Gram stain: Cram-negative bacteria contain a higher proportion of lipids in the composition of their cell wall (outer membrane) and a thin layer of peptidoglycan, which allows the penetration of this complex compound from the dye to the outside of the cell when

adding ethanol alcohol, where the fat layer melts, leading to an increase in volume Holes in the wall allowing the dye to escape from these bacteria.

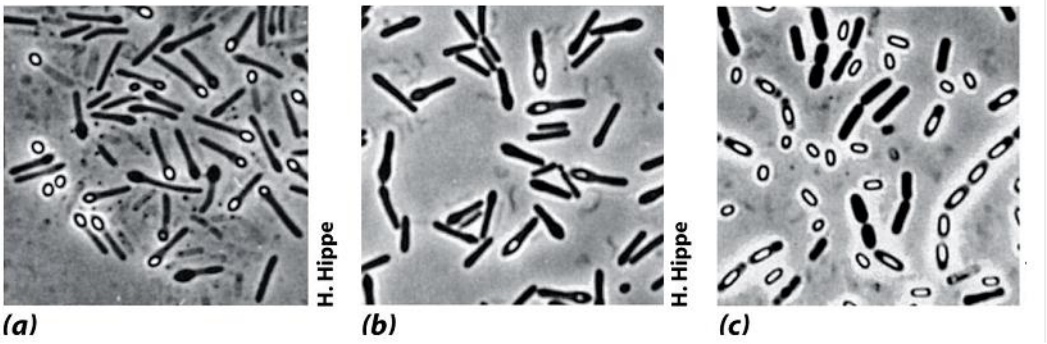
As for the positive cells of the Cram stain, they contain a very small percentage of fat and a thick layer of peptidoclycan that narrows the peptidoclycan holes when treated with alcohol, as it shrinks the cell wall of bacteria, so when washing with alcohol, the complex compound is not allowed to exit from these narrow holes, and thus the rinsing with alcohol does not lead to Removal of the violet-colored complex**.**



Third: the special stain: It is used to stain special parts or components of bacteria such as the endospore and the capsule.

Endospore Staining

- Bring the slides and fix bacterial smear with the flame
- Cover the slide with Malachite green dye 5% and heat the slide for 5 minutes
- Wash with water
- We put **Safranine** for half a minute, and this dye in this case is called Counterstain.
- The end result appear inner part endospore are staining green color while other parts of bacteria appear red.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H. Hippe
(a)
(b)
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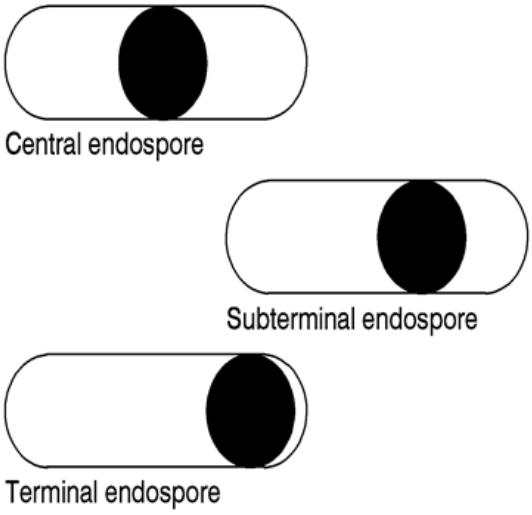
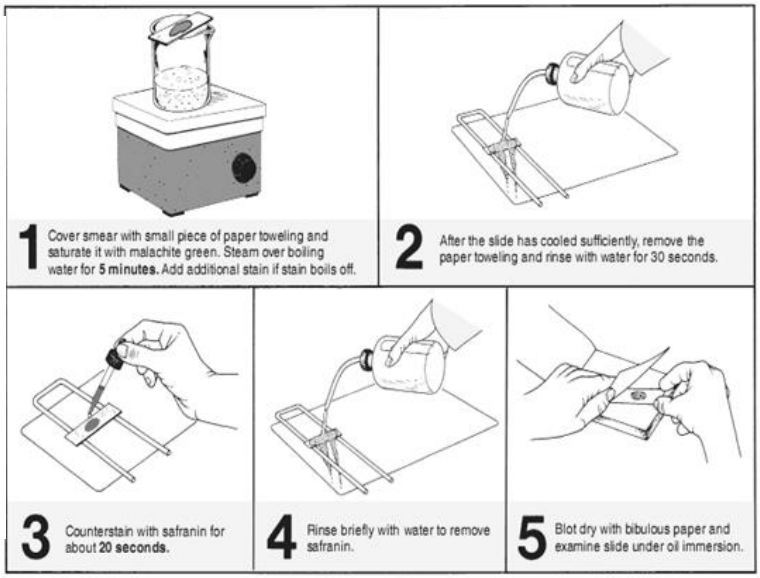

Central endospore
Subterminal endospore
Terminal endospore


1 Cover smear with small piece of paper toweling and saturate it with malachite green. Steam over boiling water for 5 minutes. Add additional stain if stain boils off.
2 After the slide has cooled sufficiently, remove the paper toweling and rinse with water for 30 seconds.
3 Counterstain with safranin for about 20 seconds.
4 Rinse briefly with water to remove safranin.
5 Blot dry with bibulous paper and examine slide under oil immersion.