Laboratory
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College of Pharmacy
Medical Microbiology Class : 2 nd

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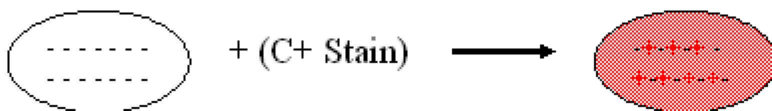
Staining Bacteria and smear preparation

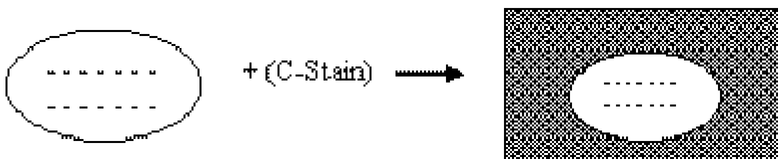
Staining Bacteria

Bacterial cells are usually colorless because cytoplasm, for the most part, is transparent. Since the bacteria are colorless, it is almost essential to add a stain to make the bacteria more visible. Once stained, cell morphology can be observed.

Stains are solutions that contain a solute called a chromophore dissolved in a solvent. A chromophore is the color possessing portion of the solution and is therefore responsible for the stains color. Bacterial cells usually have a negative surface charge, meaning that a positively charged stain is needed to stain the surface of the cell. Most stains are basic and have a positively charged chromophore. When the stain is applied, there is an attraction between the negatively charged cell surface and the positively charged chromophore, leading to the surface of the cell taking on the color of the stain.



A stain that stains the background of a slide is referred to as a negative stain because of its negative charge. Remember that the surface of bacterial cells are negative. When a negative stain is applied, the stain is repelled from the surface of the cell because of the like charges. This causes the background to stained and the cell to remain colorless. This technique is useful for determining cell shape and arrangement.



Procedure for preparing a bacterial smear

1.Obtain a glass slide and clean if necessary.

2. Using a broth culture:

a. Gently agitate your culture broth tube to disperse the bacteria.

b. Sterilize your inoculating loop using Bunsen burner, and let cool for 20-30 seconds.

c. Place loop in the bacterial broth and put the loopful of the broth onto the glass slide. Rub the drop into a slide. Sterilize the loop again to kill any remaining bacteria. Let the smear dry completely.

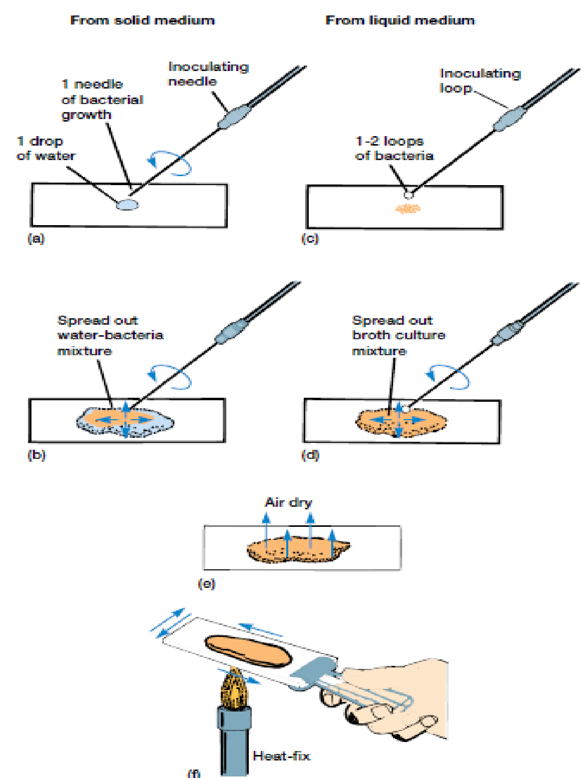
3. Using an agar plate:

a. Place a **small** drop of water in the center of the slide.

b. Sterilize your inoculating loop using a Bunsen burner, and let cool for 20-30 seconds.

c. Use the sterile loop to pick up a small amount of bacterial growth from the surface of the plate. Do not dig into the agar. Put the loopful of bacteria into the drop of water on the glass slide, and rub the drop into a slide.

Sterilize the loop again to kill any remaining bacteria. Let the smear dry completely.



4. Heat fix the slide.

Bunsen burner method: Hold the slide with a wooden clothes pin, and pass 10-12 times through the flame. Let the slide cool.

GRAM'S STAINING METHOD

1-fix smear by heat
2- Add **Crystal violet stain**
Allow to act for**10-30 sec**
3. Wash off stain with water.
4. Cover with Grams iodine **10-30 sec**.
5- Wash with water.
6- Decolorized with acetone for not more than **5 sec**. or decolorized for **10-30 sec**.with (acetone **30** ml with alcohol **70** ml)

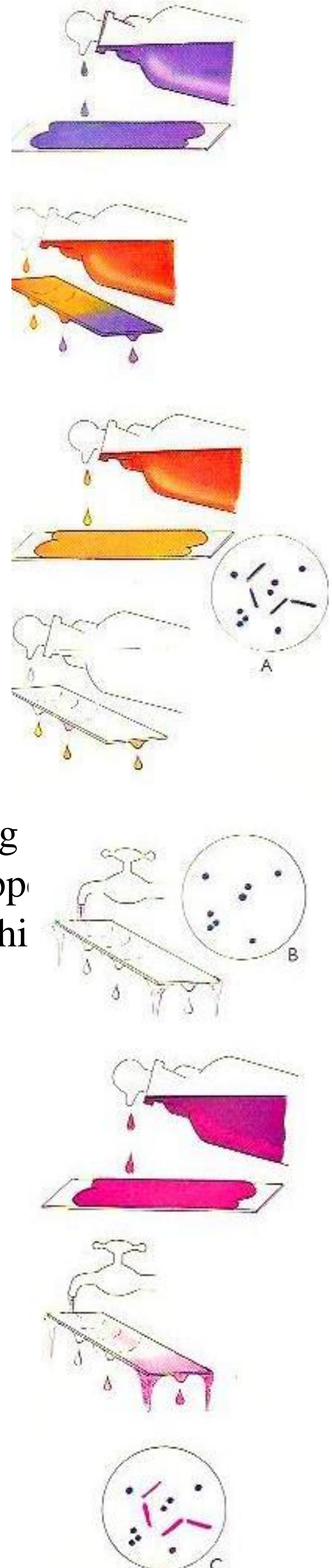
7- Wash slide immediately in water.
8- Cover for **10 30 sec**. with **safranin.**
9- Wash in water, blot and dry in air.
The inserts indicate the appearance of a mixed Gram +ve and Gram ve film at different stages during

- Before acetone decolorisation all organisims app
- After acetone decolrisation those organisims whi

are no longer visible.

- These Grams -ve organisims are visualized

After the application of the counterstain.



Gram Positive Bacteria vs. Gram Negative Bacteria