

Lec (3-4)

Bacterial Staining

Stains are:

1-Simple stains:

a-Basic dye. b-Acidic dye.

2-Differential stains:

a-Gram stain . b-Acid-fast stain.

3-Structural stains:

a-Feulgen stain. b-Endospore stain. c-Cell wall stain. d-Capsule stain. e-Flagella stain

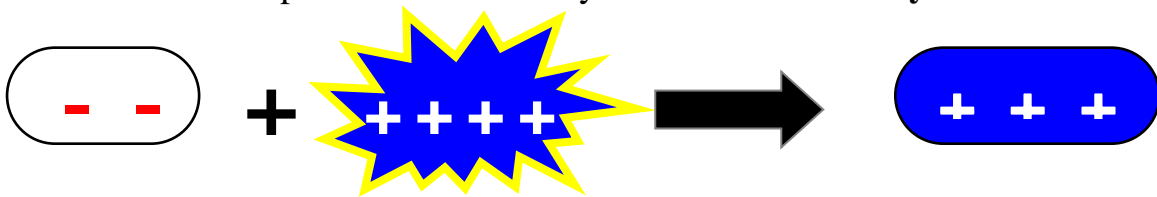
Dyes:- are generally salts in which one of the ions are colored. A salt is a compound composed of a positive and negative ions.

1-Simple stains: single stain to color the organism .

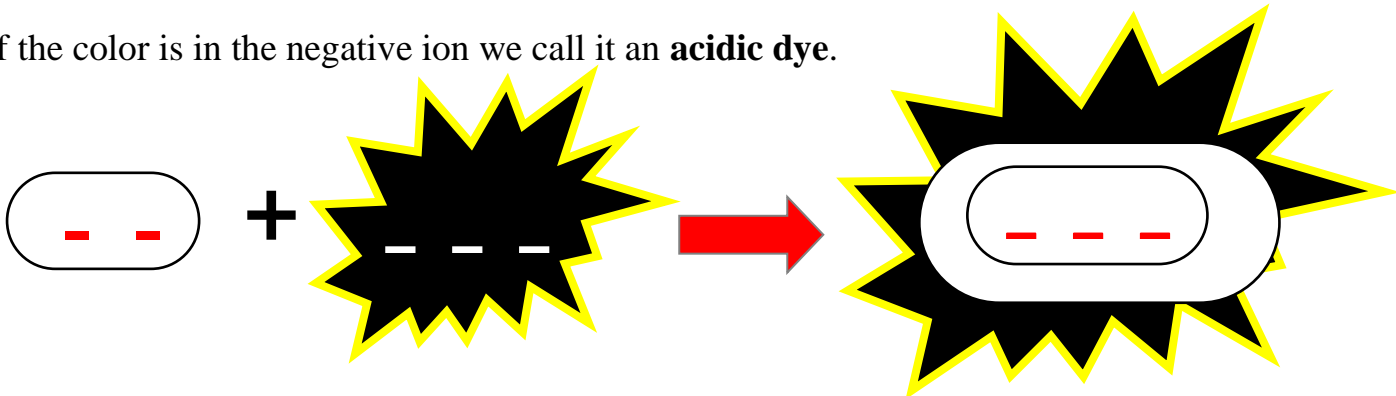
The simple dye methylene blue is the salt blue chloride:



If the color is in the positive ion of the dye we call it a **basic dye** .



If the color is in the negative ion we call it an **acidic dye**.



Advantages: study the morphology of bacterial cell , size , arrangement of bacteria and principle diagnosis of *Pasteurella* (Bipolar staining).

Smear Preparation:

1- Clean the slide with 50% of ethyl alcohol.

2- If the bacteria are growing in a liquid media, one starts by placing two loopful of liquid media directly on the slide. From solid media, one starts by placing one or two loopful of water on the slide and mix with one loopful of organism.

3- Spread the drop on the slide to form thin film.

4- Allow the slide to dry in the air.

5- When the film dry, pass the slide, film side up three times through the Bunsen flame.

6-Staining.

Crystal violet

Safranin

Methylene blue

Carbol fuchsin

The purpose of fixation:

is to kill the micro organisms cause it to adhere to the slide.

2-Differential stains:

a-Gram stain

It is divide bacteria in to two groups gram positive , gram negative. The difference in staining is due to the variance in surface layer of the two types of cells. Gram positive consist of thick layer of peptidoglycan which resist decolorize with alcohol so gram positive bacteria retain a crystal violet through decolonization and appear purple, but gram negative consist of vary thin layer of peptidoglycan and lipopolysaccharide which decolorize with alcohol and take the counter stain with red dye of safranin.

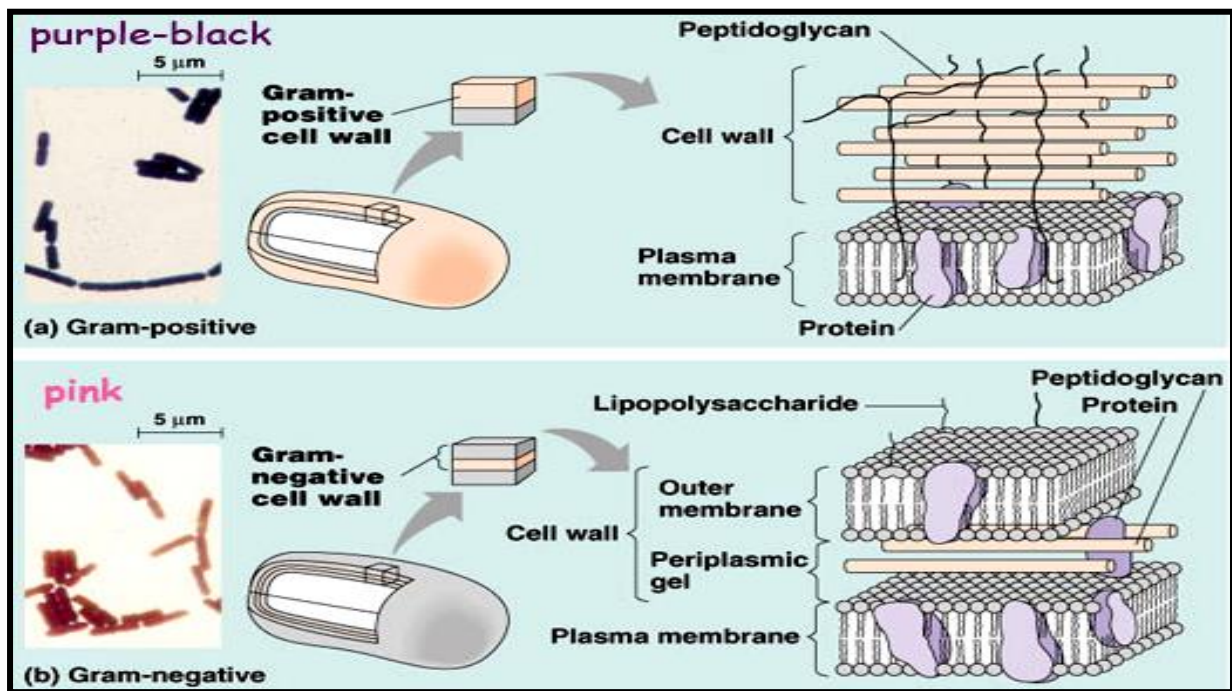


Figure (G-) & (G+)

Gram's stain steps

- 1-Prepare smear and fixed.
- 2-Crystal violet for 30 second and wash with water.
- 3-Gram s iodine for 30 second and wash with water.
- 4-Decolorized with alcohol 95% for 10 – 20 second and wash with water.
- 5-Counter stain (Safranin) for 30 second and wash with water and examine with oil immersion lens