Lab.8. Bacterial Staining

Staining : Process in which bacteria are stained to give color to them.

- Because microbes are colorless and highly transparent structures.
- Bacteria have nearly the same refractive index as water, therefore, when they are observed under a microscope they are opaque or nearly invisible to the naked eye.
- So different types of staining methods are used to make the cells and their internal structures more visible under the light microscope.

STAINS OR DYES

Stains or dyes : are organic compounds which carries either positive charges or negative charges or both.

- They adheres to a cell, giving the cell color as different stains have different affinities for different organisms, or different parts of organisms so they are used to differentiate different types of organisms.

❖ **Based on the charges**: the commonly used stains are salts.

➢ **Basic stains (+) :** react with acidic (-) parts of the cell

ex. crystal violet, safranin, methylene blue , stains that get inside the cell

➢ **Acidic stains (-)** : are repelled by the negatively charged cell surface

Ex. India ink , Stains the background, not the cells

Smear preparation

Microbial smear : It is a very small amount of microbial growth (broth or solid)spread on a clean slide and drying by air.

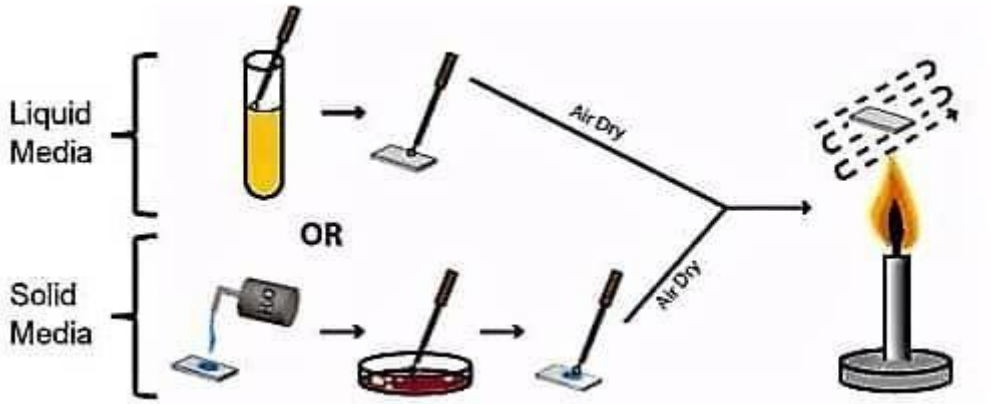
Fixation : The process of passing the smear after drying several times over benzene burner to fix the microbes on slide and prepare it for staining.

Note :The reason of fixation process is to kill the microbes, fix the microbe cells to the slide and prevent their removal during washing steps .

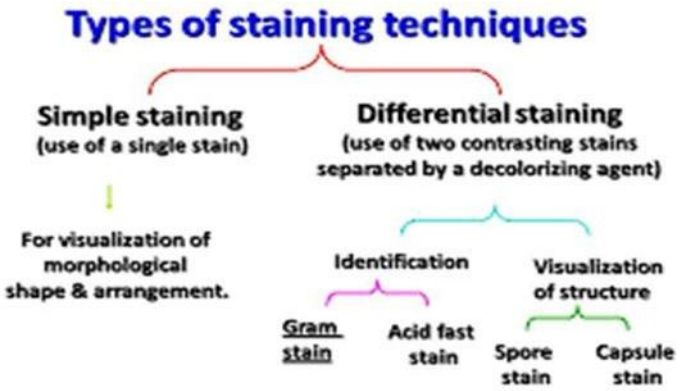
Steps of microbial smear preparation:

1. Handle a clean slide by its edge, label the target place at the bottom side o the slide by drawing a circle with a diameter about 2 cm using a marker.

2. Sterile the loop until reaching the red heat.
3. If the bacterial culture was broth, shake the culture and transfer loopful of broth to the center of the slide and spread over the target circle.
   While if the bacteria were grown on solid medium , place loopful of water on the slide then transfer inoculums to the water and homogenize the smear .
4. Sterile the loop.
5. Leave the smear to dry at room temperature (by air).
6. After drying , Pass the slide over the flame to fix the smear (avoid prolonged heating of the slide) .



Types of stains



Simple stain

In this process only one stain is used for staining . The most commonly used stains for simple staining are crystal violet , methylene blue , carbol fuchsin.

- This method of staining is **useful** determining basic morphology and the presence or absence of certain kinds of granules.

Steps of simple staining:

1. Prepare a fixed smear.
2. Stain the smear with crystal violet by putting a couple of the stain drops and let for 1 min.
3. Wash off by tap water gently, leave it to dry at room temperature (by air(
4. Examination .