Lab-6 Bacterial Staining

Stain:- The process of adding a dye to a bacterial culture.

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

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

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

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

Staining allows for:

- Observing bacterial morphology and arrangements
- Other critical information such as cell wall structure

Types of Stains

- **Simple stain**

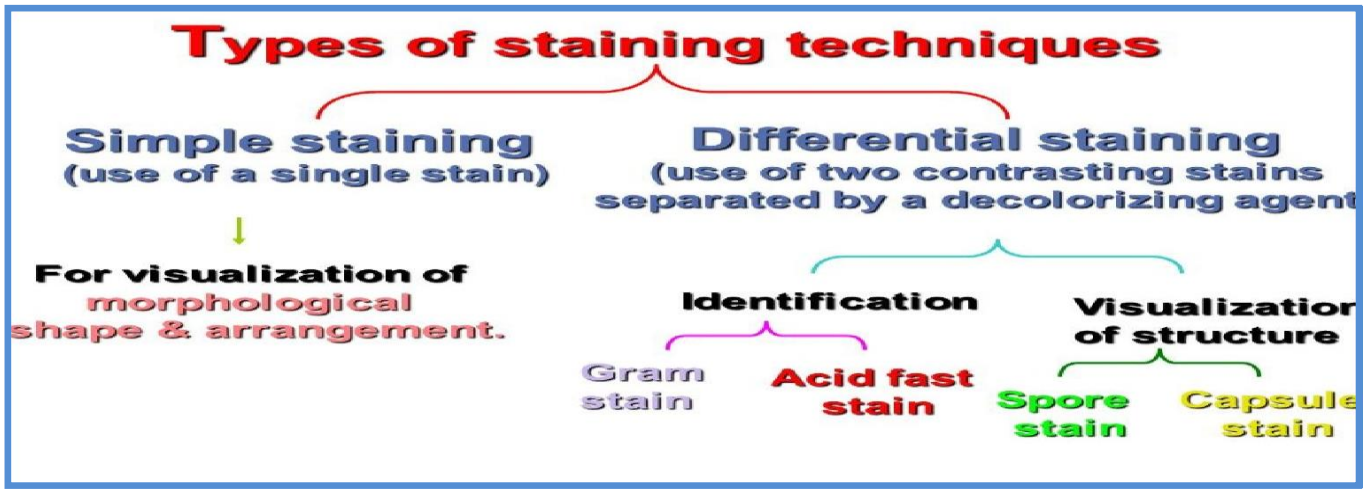
look at morphology and arrangement Good for observing morphology Result-all bacterial cells stain the same color Stains might include methylene blue, basic fuchsin, crystal violet

- **Differential stain**

Used to identification different types of organisms . Involves exposing cells to more than on stain Ex. Gram Stain, Acid-Fast stain.

- **Special-stain**

specific structures of bacteria Capsule stain, Endospore stain , flagella stain



Smear Preparation and simple stain

To prepare a smear m we need the following :

1- Broth or solid media .
2- Bacteriological loop.
3- Clean microscopic glass slide .
4- Bunsen Burner .

Smear:

A thin film of material containing Microorganisms which is spread over the surface of the slide .

Culture: Propagation of Microorganism in growth media.

Growth media :-An artificial media contains basic requirement needed for M.O.s growth .

1- Liquid (Broth) in test tube
2- Solid (agar) in Petri dish.

Specimens :

1- Urine 2- Stool 3- Blood 4- Sputum 5- CSF 6- Pus 7- discharge 8- pleural fluid , peritoneal fluid . 9- Broth or agar culture .

The Smear for preparation of two types:

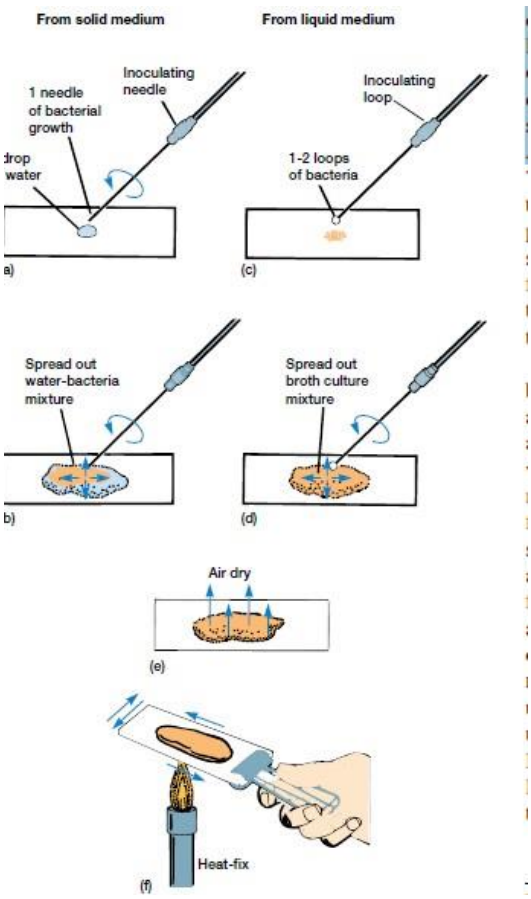
A) From fluid material :

(Broth culture ,urine ,sputum ,pus, purulent exudates etc----)

1) Sterilize the loop over Bunsen flame then let it cool.
2) A loopful of specimen and spread it over the surface of a clean slide to from a thin film of 1-2 cm in diameter (in the center of the slide).
3) Allow the film to dry by air
4) The film is fixed on the slide by passing it (3-4)time, through the Bunsen flame and allow the slide to cool before staining .

B) From Solid material (Colonies culture agar):

1) Sterilize the loop in Bunsen flame and let it cool.
2) Place a loopful of a clean water on the center of a clean slide
3) Resterilization the loop ,transfer a small portion of the colony and emulsify thoroughly with water and spread the mixture on the slide to form a thin film of 1-2 cm in diameter
4) Dry and fix .



Fixation of the slide:

1) To kill the M.Os
2) To stuck the M.Os on the surface of the slide.
3) To prevent the autolytic changes of M.Os

1-Simple staining

A staining method that uses only a single dye that uses only a single dye that which does not differentiate between type of organism .

Aim:- To observe the morphology of bacteria by using simple stain

Requirements :- Loeffler's methylene blue , Iodine, distilled water , slide and compound microscope .

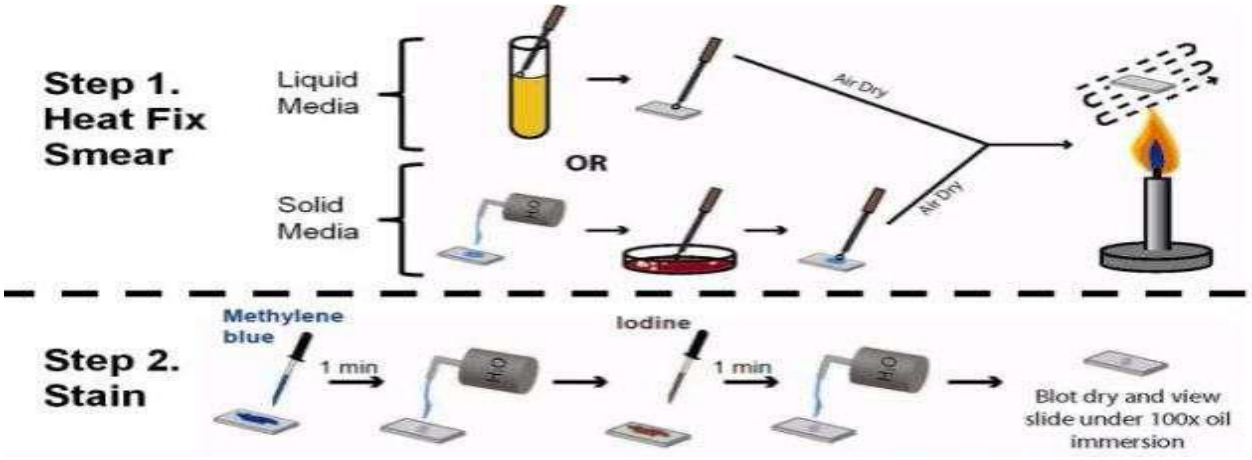
Procedure

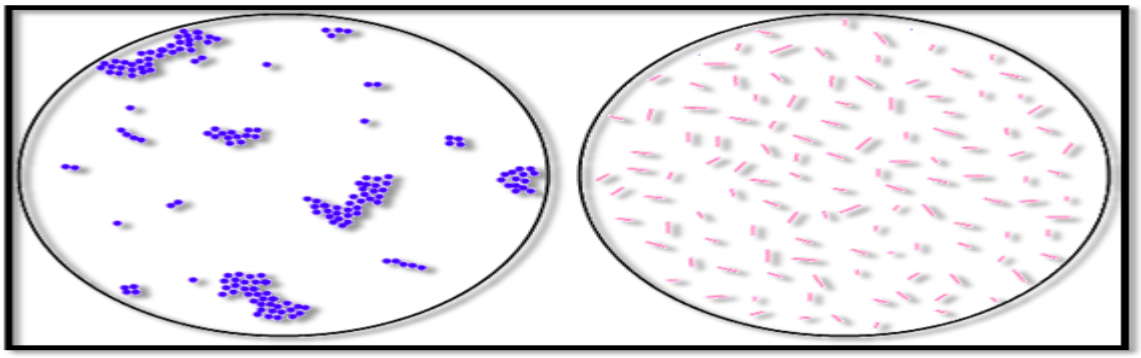
1- Cover the smear with methylene blue and allow the dye to remain in the smear for approximately one minute (Staining time is not critical here; somewhere between 30 seconds to 2 minutes should give you an acceptable stain, the longer you leave the dye in it, the darker will be the stain).

2- Using distilled water wash bottle, gently wash off the excess methylene blue from the slide by directing a gentle stream of water over the surface of the slide.

3- Wash off any stain that got on the bottom of the slide as well.

4- Saturate the smear again but this time with Iodine. Iodine will set the stain
5- Wash of any excess iodine with gently running tap water. Rinse thoroughly.
6- Wipe the back of the slide and blot the stained surface with bibulous paper or with a paper towel.
7- Place the stained smear on the microscope stage smear side up and focus the smear using the 10X objective.
8- Choose an area of the smear in which the cells are well spread in a monolayer. Center the area to be studied, apply immersion oil directly to the smear, and focus the smear under oil with the 100X objective.





Left: Cocci in Cluster; Right: Bacilli

Uses:

Diagnostic microbiology laboratory generally does not perform simple staining method. Differential staining such as Gram Staining and AFB Staining are commonly used to identify and differentiate the bacterial isolates. Simple staining can be useful in the following circumstances.

1- To differentiate bacteria from yeast cells: When endocervical swab culture is done in Blood agar both *Staphylococcus spp* and yeast cells may give similar looking colonies in Blood agar (a common error for newtechnologist or microbiologist with less experience). Performing wet mount technique or simple staining from the isolate can be helpful.

2- To presumptively identify the bacterial isolate Due to their ubiquitous presence, *Bacillus spp* may present as a contaminant in the culture plates. In some circumstances (e.g. growth in Blood Agar but no growth in MacConkey Agar), identifying the shape of the bacteria (rod or cocci) may help to eliminate the isolate as possible contaminants (*e.g., Bacillus spp*) or further process as potential pathogen (cocci).