Bacterial Staining

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image .Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes.

A dye is a colored substance that has an affinity to the substrate to which it is being applied. The dye is generally applied in an aqueous solution.

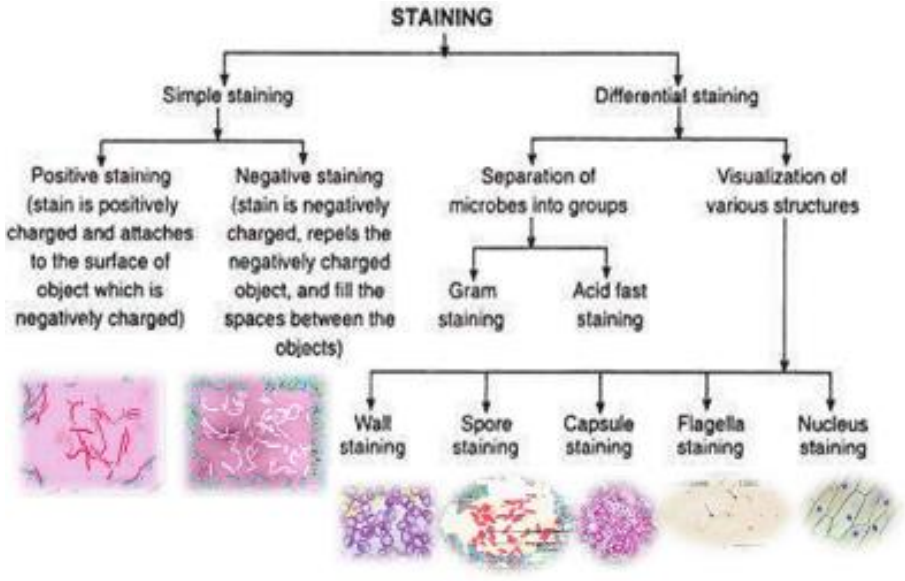
<u>The bacteria are stained for the following reasons:</u>

1) To study their shape.
2) To differentiate the bacterial species by using differential stain.
3) To study the internal components of the bacterial cell.

The stains divide into 2 groups (according to their affinity to cell components):

1. **Indirect stain (Negative stain):** It is can penetrate the cell envelope therefore the cell become very obvious by making counter dark (stain the slide but not the cell): India ink, Congo red

2. **Direct stain(Positive stain):** It has strong affinity to one or more of cell components:-
    - Simple stain: Acidic (Safranine) ,Basic (Methylene blue, Crystal violet)
    - Differential stain: Gram stain , Acid fast stain(Ziehl Neelson stain)
    - Selective stain:Spore stain , Capsule stain , Flagella stain , Cell wall stain , Nucleic acid stain

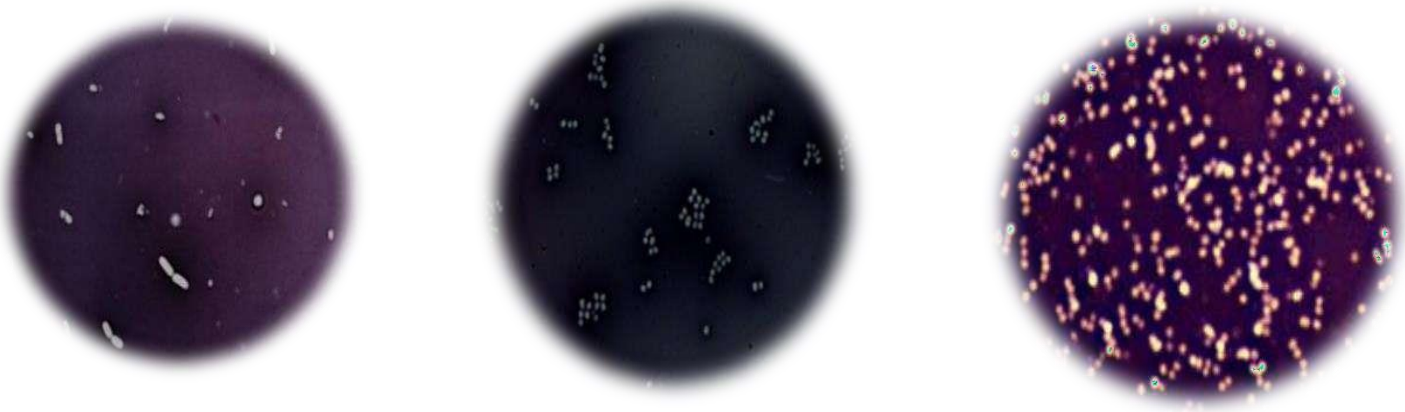


Indirect stain (negative stain)

The main purpose of Negative staining is to study the morphological shape, size and arrangement of the bacteria cells that is difficult to stain. Negative staining requires an acidic dye such as India ink or Nigrosine. India ink or Nigrosine is an acidic stain. Since the surface of most bacterial cells is negatively charged, the cell surface repels the stain.

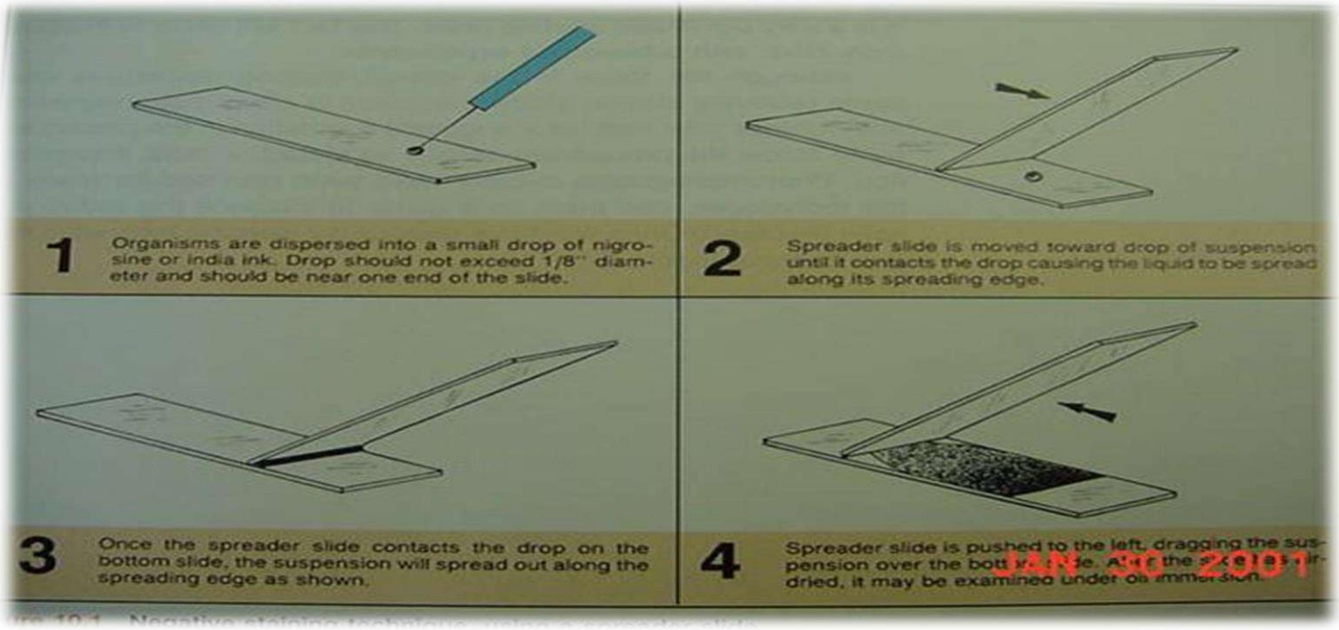
The glass of the slide will stain, but the bacterial cells will not.

The bacteria will show up as clear spots against a dark background.



Procedure:

1. Mix one drop of India ink with culture by sterile loop on slide.
2. Used another slide for dispensing the mixture.
3. Air dry the smear(do not use heat fixation).



Simple Stain (Using one dye –one step for staining)

There are two groups of simple stain:-

1- **Acidic stain**: has negative charge it is stain the basic compounds protein)of the cell with a positive charge(safranine , acid fuchsin)
2- **Basic or Alkaline stain**: has positive charge it is stain the acidic compounds
3- (RNA,DNA))of the cell with a negative charge(Methylene blue , Crystal violet)

Steps of work in general

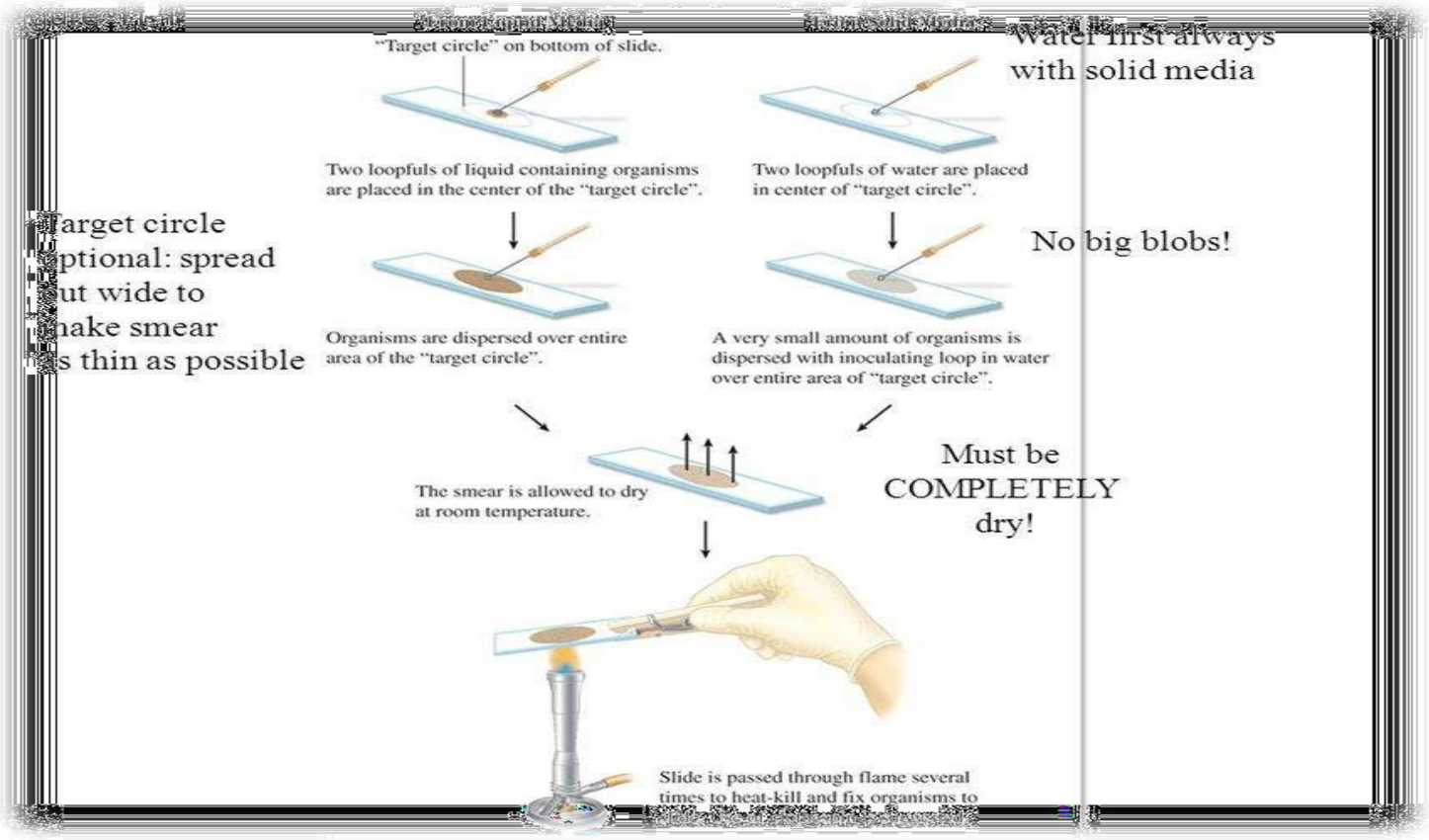
1- Smear preparation
2- Fixation
3- Staining with stain

Smear preparation

From broth: one loopful directly from broth on the slideFrom solid culture:put one drop of water in the center of the slide the take one touch from colony and mix well with the drop.

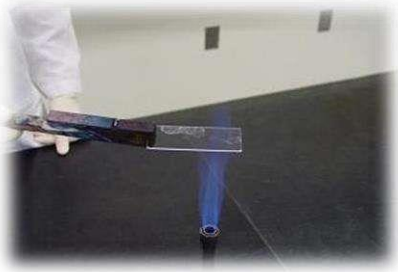
After air drying

The fixation will done by Passing the slide rapidly across the flame three times the slide should be warm not hot, why? Hot will change the shape of bacterial cell.



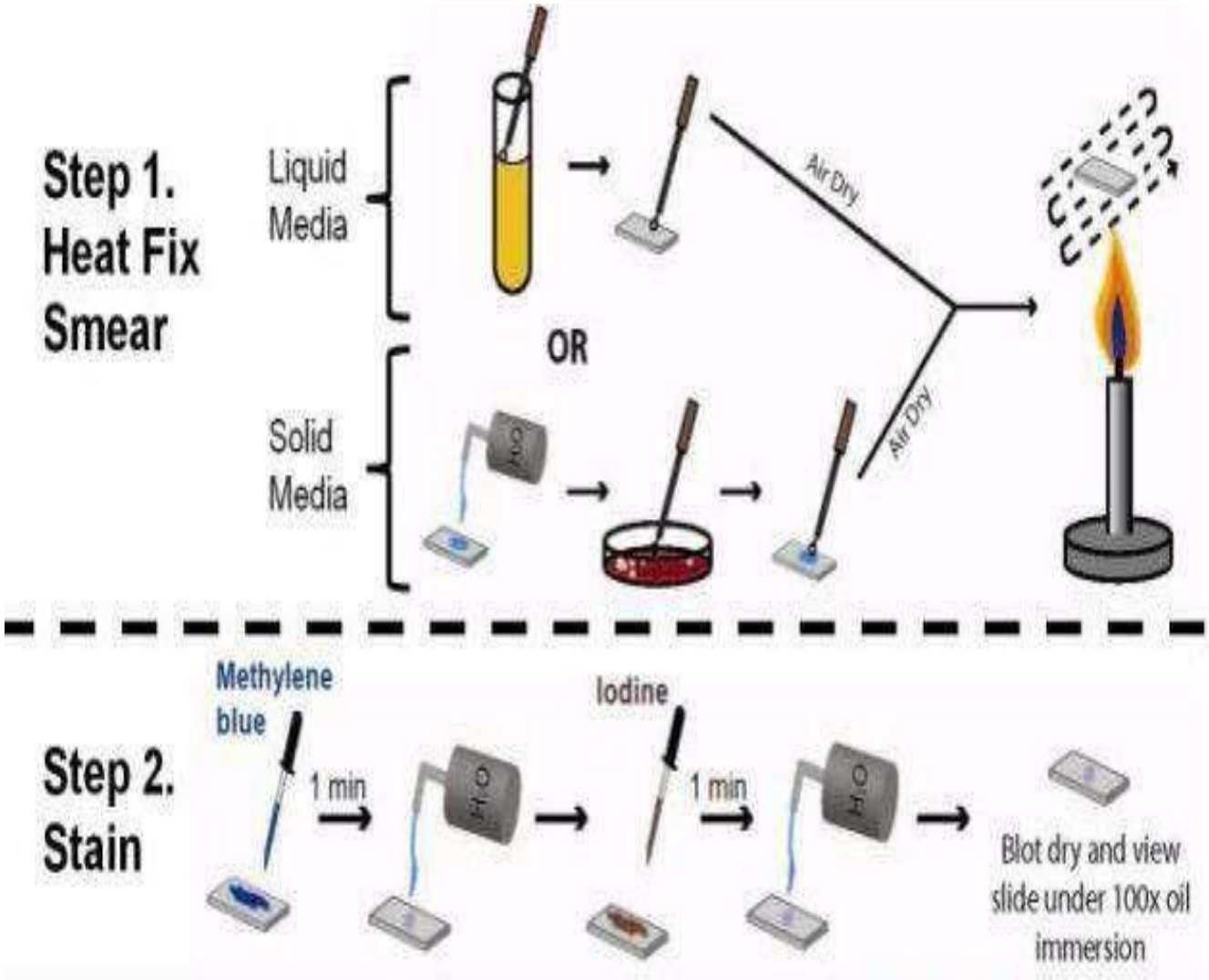
1- Kill the bacteria so the stain will penetrate without serious destruction of cell structure.

2- Fix the cell on the slid
(by coagulation of their protein) So they are not



The fixation will

1- Kill the bacteria so the stain will penetrate without serious destruction of cell structure.
2- Fix the cell on the slid (By coagulation of their protein) So they are not removed during staining.



Staining with dye:

- Put the slide on the staining rack and flood the smear with simple stain (Crystal violet or Safranine)for 1 min.
- Wash the slide with tap water gently and drain off excess water Air dry
- Examine with microscope under oil –immersion lens