Staining techniques of bacteria

Most of bacteria have no color, so they generate little contrast in the microscope field. Therefore, to see bacteria obviously for recognition and identification, it is necessary to apply color by using a staining reagent. Once stained, the bacteria may be observed and studied with respect to their shape, size, and arrangement.

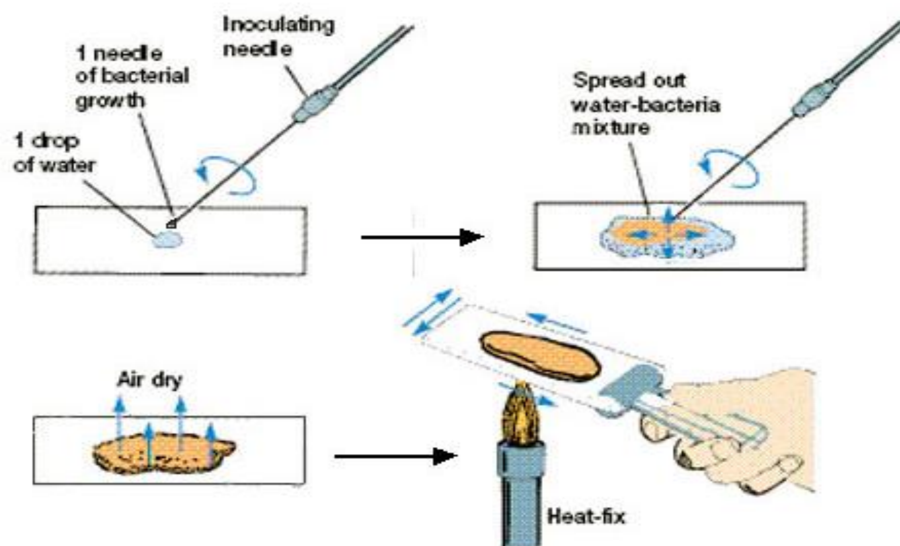
Preparation of bacterial smear

Bacterial smear is a thin layer of bacteria placed on a slide for staining.

The preparation of bacterial smear involves several steps:

First, bacteria are placed on a glass slide and ˝fixed˝ with heat, by passing 2-3 times on flame to ensure that they remain attached to the slide.

The making and fixing a film performed either by a saline suspension of a colony or a sample from a fluid culture.



Bacterial smear preparation

Simple staining

This technique is a rapid and effective way for general bacterial viewing (size, shape and arrangement), performed by using a single basic stain that chemically unites with the bacterial cytoplasm. The remaining stain is then washed away.

Basic stains like: crystal violet, safranin, methylene blue, carbol fuchsin.

Differential staining

Differential stains, such as Gram stain and acid-fast stain, differentiate bacteria based on the chemical composition of their cell wall.

These stains use two stains (dyes) instead of one. The first stain is called primary stain, and the second is called the counter stain.

Gram stain

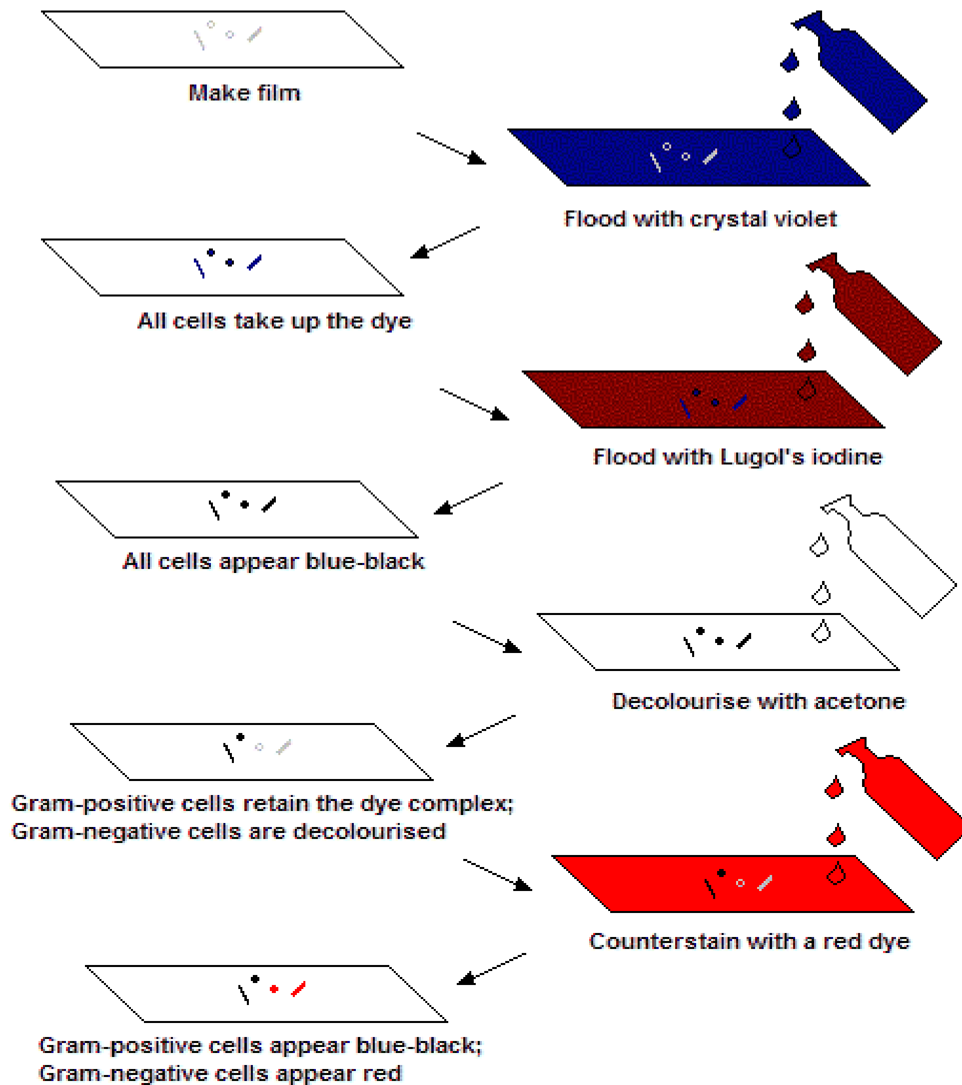
This is the most commonly employed and important of all diagnostic staining techniques, that performed as the following steps:

1- Make bacterial smear on clean dry slide, and left to air dry at room temperature and fixed by pass it through flame 2-3 times.
2- Place the slide on the rack, and put a few drops of crystal violet (basic stain) for 3 min.
3- Briefly rinse the slide with water. The heat-fixed cells should look purple at this stage.
4- Add drops of iodine solution and wait for 1 min. This acts as a mordant and fixes the dye.
5- Briefly rinse with water.
6- Decolorize the sample by applying 95% ethanol. This step washes away unbound crystal violet, leaving Gram-positive organisms stained purple and Gram-negative organisms colorless.
7- Rinse with water to stop decolorization.
8- Rinse the slide with a counterstain (safranin or carbol fuchsin) which stains all cells red. The counterstain stains both gram-negative and gram-positive cells. However, the purple gram-positive color is not altered by the presence of the counter-stain,

it's effect is only seen in the previously colorless gram-negative cells which now appear pink/red.

9- Blot gently and allow the slide to dry.

10- Then examined by oil immersion lens 100X.



Most bacteria posses a cell wall that contains either **a thick peptidoglycan layer** or **a thin peptidoglycan layer** with an additional lipopolysaccharide layer. This chemical difference is distinguished with Gram stain.

By this method bacteria can be recognized as **Gram-positive** bacteria **(purple to blue)** if they retain the primary-dye complex in the face of

attempted decolorisation. Otherwise if decolorisation occurs, allowing to accept the counter stain, bacteria appear as **Gram-negative (red to pink)**