Microbiology Laboratory

Lab 4 : Staining

Simple Staining

Simple staining is one of the conventional staining techniques. From the name, it is quite clear that it is a very simple or **direct staining method** that uses a **single stain** only. The microorganisms are invisible to the naked eye, and to make them visible, staining is performed that gives **divergence** to a microscopic image. Direct staining makes the use of basic dyes like methylene blue, safranin, crystal violet, malachite green etc. called "**simple** or **direct stains**".

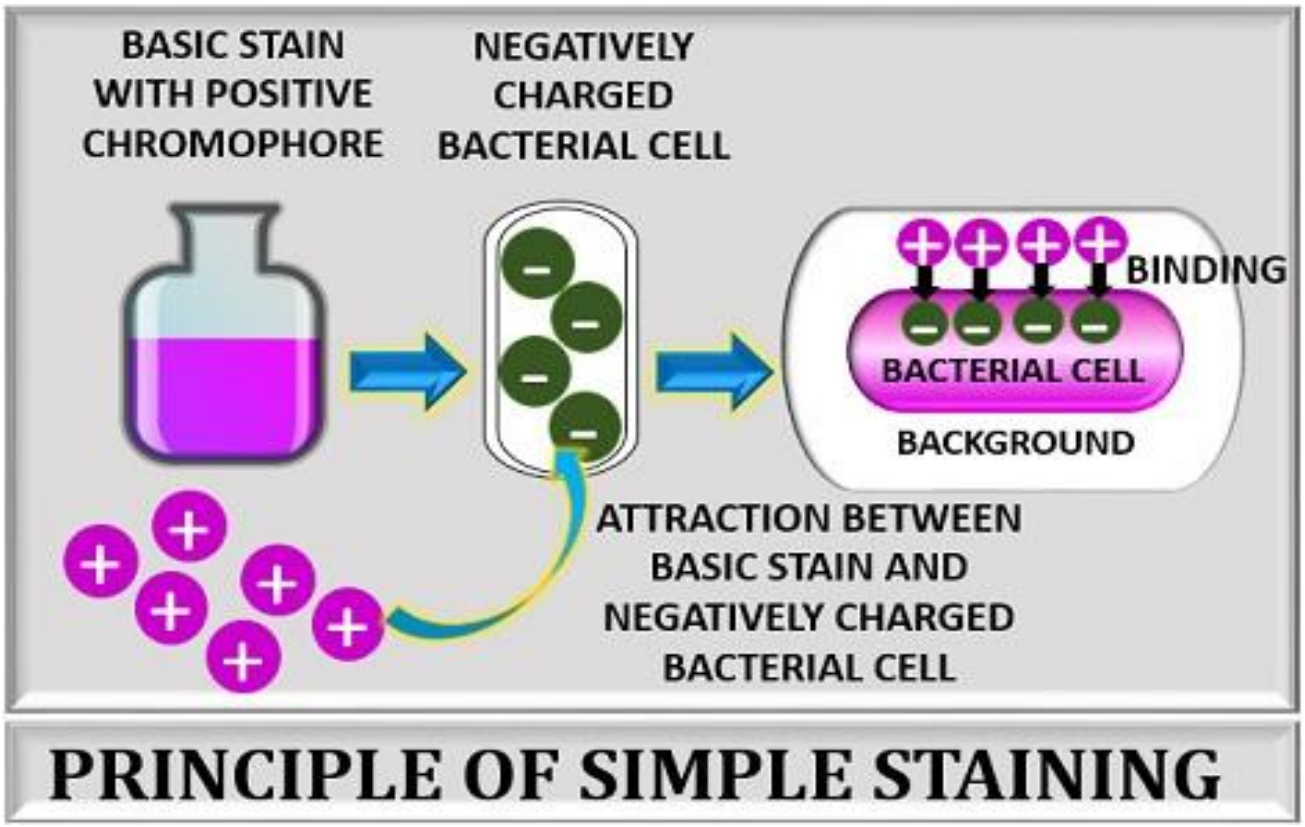
The simple stain can be used as a quick and easy way to determine the cell shape, size, and arrangement of bacteria. True to its name, the simple stain is a very simple staining procedure involving a single stain solution. Any basic dye, such as methylene blue, safranin, or crystal violet, can be used to color the bacterial cells. These stains will readily give up a hydroxide ion or accept a hydrogen ion, which leaves the stain positively charged. Since most bacterial cells and cytoplasm surface is negatively charged, these positively charged stains adhere readily to the cell surface. After staining, **bacterial cell morphology** (shape and arrangement) can be appreciated. **Simple staining is defined as one of the ordinaries yet the popular method used to elucidate the bacterial size, shape and arrangement to differentiate the various bacteria groups. It stains the bacterial cell** uniformly **and thus increases the visibility of an organism. The term simple staining sometimes interchangeable with the terms like direct, positive or monochrome staining. Also,** Simple stains can be defined as the **basic dyes**, which are the alcoholic or aqueous solutions (diluted up to 1-2%). These can easily release $\mathbf{OH^-}$ and accepts $\mathbf{H^+}$ ion, due to which the simple stains are positively charged. As the simple stains are **positively charged,** they usually termed as positive or cationic dyes. It is commonly used to color most bacteria. As the simple stain carry a positive charge, it firmly adheres to a negative bacterial cell and makes the organism colored by leaving a background colorless. **Examples** of simple stains include safranin, methylene blue, crystal violet etc.

The basic stains have different **exposure times** to penetrate and stain the bacterial cell.

Basic stains	**Exposure time to stain the bacteria**
Methylene blue	1-2 minutes
Crystal violet	20-60 seconds
Carbol fuschin	15-30 seconds
Safranin	30-60 seconds

Principle of Simple staining

In simple staining, the bacterial smear is stained with a single reagent, which produces a distinctive contrast between the organism and its background. Its principle is based on producing a **marked contrast** between the **organism** and its surroundings by using basic stain. A basic dye consists of a positive chromophore, which strongly attracts the negative cell components and charged molecules like nucleic acids and proteins, Basic stains with a positively charged chromogen are preferred because bacterial nucleic acids and certain cell wall components carry a negative charge that strongly attracts and binds to the cationic chromogen Thus, a simple staining technique result in a colored bacterial cell against a colorless background**.** The purpose of simple staining is to elucidate the morphology and arrangement of bacterial cells. The most commonly used basic stains are methylene blue, crystal violet, and carbol fuchsin



Reagents and Equipment's for Simple Staining

Methylene blue, crystal violet, and carbon fuchsin, Micro incinerator or Bunsen burner, inoculating loop, staining tray, microscope, lens paper, bibulous (highly absorbent) paper, and glass slides.

Procedure of Simple Staining

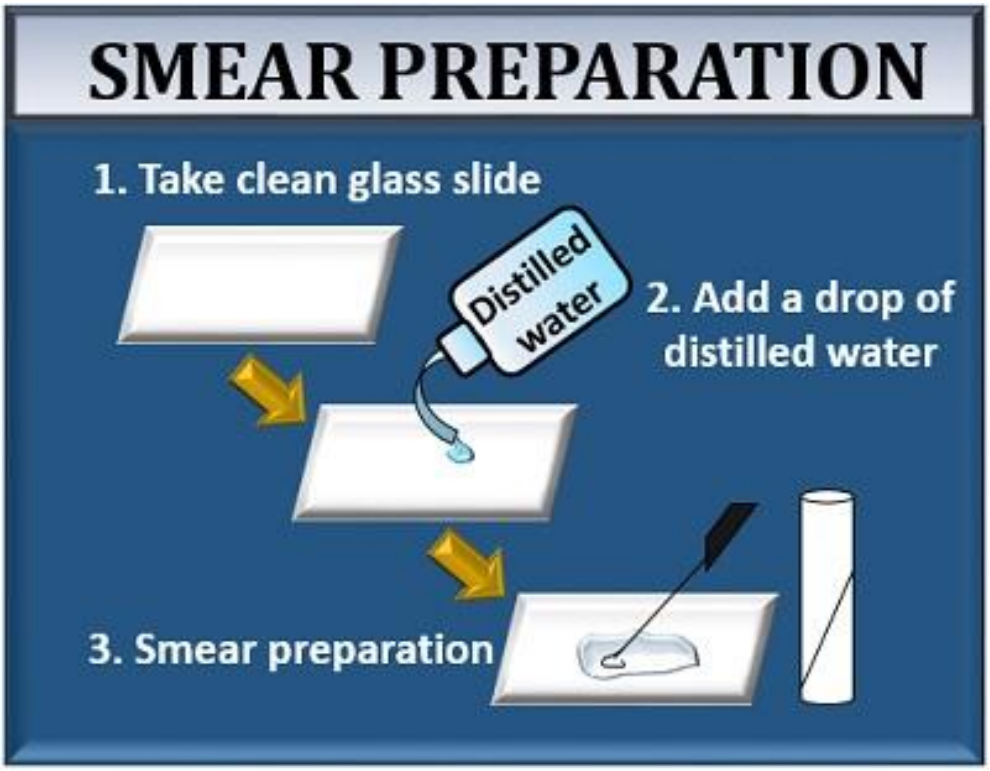
It involves the following three steps:

1. Smear preparation
2. Heat fixing
3. Staining

Smear Preparation

A bacterial smear appears as a thin film of bacterial culture. For the smear preparation, we need to perform the following steps:

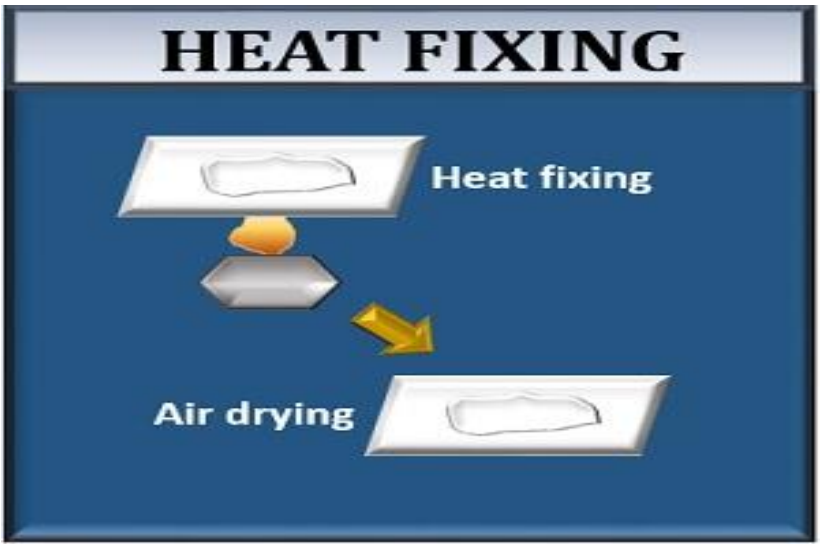
1. Take a clean, grease-free glass slide.
2. Add a drop of distilled water to the center of the glass slide.
3. Then, add inoculum from the bacterial culture with a sterilized inoculating loop on the glass slide.
4. After that, mix the inoculum with a drop of distilled water to make a thin film by uniformly rotating the inoculating loop until a thin bacterial film is formed.



Heat Fixing

After smear preparation, move the prepared slides over the Bunsen burners flame at least three times. Then, allow the slide to air dry. There are many reasons to perform heat fixing, and it cannot be skipped because:

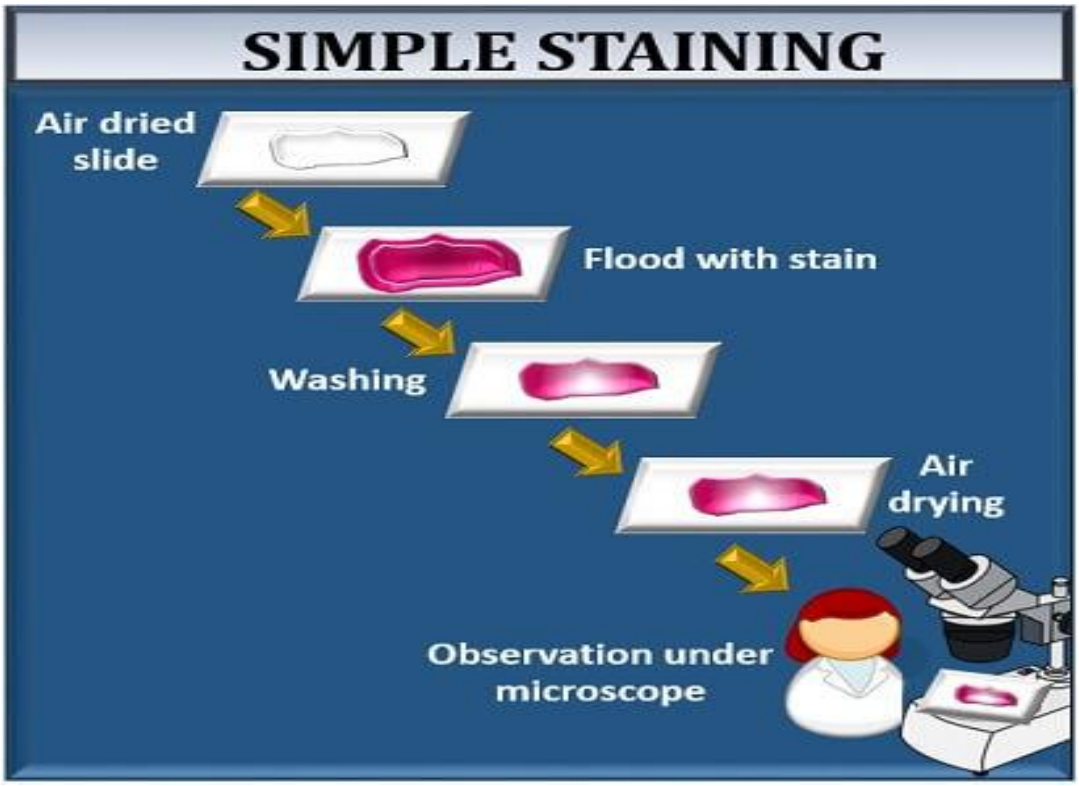
- Heat fixing helps in the fixation of a specimen to the glass slide.
- Heat fixing helps the stain to penetrate the smear.



Staining of Bacteria

It is the last and the most crucial step, in which one can identify the morphological characteristics of the bacteria through microscopic examination, once the cells get stained. This stage involves the following steps, which are as follows:

1. Add stain to the heat fixed smear.
2. Allow the stain to stand for at least 1 minute so that it can penetrate between the cells.
3. Wash off the glass slide carefully.
4. Blot dry the slide with absorbent paper (do not wipe the slide).
5. Examine the glass slide under the microscope from low to high power to get a magnified view of the specimen. One can also add a drop of oil



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.

