Bacterial Staining Methods

Staining is a technique used in microscopy to enhance contrast in a microscopic image. Stains and dyes are frequently used to highlight structures in microbes for viewing, often with the aid of microscopes. Bacteria cannot be seen under the microscope unless they are stained because 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.

A stain is a substance that adheres to a cell, giving the cell color.
Chemically, Stain an organic compound composed of a benzene ring, a chromophore (color) and auxochrome group (ionization properties).
Benzene is an organic colorless solvent. Chromophore is the molecule that gives color to benzene.
Benzene + Chromophore = Chromogen (Chromogen isn't a stain, just a colored compound)
Auxochrome ionizes the chromogen, giving it a charge. This helps the chromogen form salts and bind to substances.

Importance of staining

1. To reveal the morphological feature of bacteria including size, shape, and arrangement.
2. To visualize cell structures such as endospore, capsule, and flagella.
3. Have an important role in the identification and differentiation of different genera of bacteria.

Stains are classified as

Simple stain, it is classified into:

1. **Acidic stain:** has a negative charge so they bind to positively charged cell structures like some proteins. Acidic dyes are not very often used in Microbiology labs, except to provide background staining like Capsule staining. Examples: Nigrosine, Eosin, India ink, etc.
2. **Basic stain:** has a positive charge and binds to negatively charged molecules (nucleic acid, -COOH, -OH). Since, the surface of bacterial cells are negatively charged (in G+ due to Teichoic acid, while in G- is due to the outer membrane), basic dyes are most commonly used in bacteriology. Examples: Crystal Violet, Methylene Blue, Safranin, etc.
3. **Neutral stain:** This is produced when aqueous acidic and basic stains are combined. Neutral dyes stain nucleic acids & cytoplasm. Examples: Eosinate of Methylene blue, Giemsa stain.

I. Differential stain (compound)

a. Gram stain
b. Acid-fast stain

II. Structural or special stains

a. Capsule stain
b. Endospore stain
c. Flagella stain
d. Nuclear stain

Smear Preparation: is the first step in most bacterial staining. In a smear preparation, cells are spread in a thin film over a small area of a slide, dried, and then fixed to the slide by heating.

A good smear preparation should be...

1. A **thin layer** of cells so that individual cells can be observed.
2. Fixed appropriately to allow repeated washings during staining.
3. Heat fixing gently so as not to cause shrinking to the cells.

Procedure: Preparation of a bacterial smear

1. Clean microscope slides and draws a circle (about 2cm in diameter) on the bottom side of the slide.

A. From a culture on an agar plate

a. Place one drop of distilled water on the center of the slide.
b. Use the narrow end of a toothpick to take a small colony and mix with a water drop.
c. Disperse the bacterial cells in the water drop and spread them over a circular area in the center of the slide, and allow the slide to dry.

B. From a broth medium

a. Place 2-3 loops full of broth culture on the center of the slide (Note you must flame the loop between each transfer if you want to keep the culture for later use).
b. Spread the droplets over a circular area in the center of the slide, and allow the slide to dry.

2. Pass the slide slowly through the flame of a Bunsen burner 3-4 times to fix the bacteria to the slide.

Simple staining

The use of a single stain or dye to color a bacterium is called a **simple stain.**

Positive (Direct) Stains:

1. Prepare a bacterial smear and cover it with methylene blue
2. Allow the dye to remain on the smear for approximately 1 minute.
3. Gently wash off the excess stain from the slide by directing a gentle stream of distilled water over the surface of the slide.
4. Dry the slide at room temperature, and examine the slide under the 100x oil lens of the microscope.
5. Record the shape, arrangement, and approximate size of the organisms.

Negative (Indirect) Stains:

1. Clean microscope slides **thoroughly**.
2. Place a small drop (one-two loop-full) of the negative stain (Eosin) near one edge of the slide.
3. Mix a loop-full of bacterial culture into the drop of Eosin.
4. Place a second slide lengthwise in front of the drop of stain. Starting in the middle of the slide which contains the stain, move the other slide back at a 45^{o} angle until it touches the stain, then push forward to spread the stain.
5. Air dries (5 - 10 min.) **DO NOT heat fix!**
6. Examine under oil immersion and Record the shape, arrangement, and approximate size of the organisms.