# PW 02: STAINING of BACTERIAL SPECIMENS

## 1. Introduction

Bacterial specimens are often stained prior to microscopy studies, which allows better visualization of specimens using microscopy. Simply speaking, a stain is a substance that adheres to a cell, giving the cell color. Different stains have different affinities for different organisms, or different parts of organisms. They may be used to differentiate different types of organisms or to view specific parts of organisms.

There are several types of stains which are commonly used in microbiology. The first is a **simple stain**, which uses only one reagent which provides contrast between the background and the heat-fixed bacterium itself. The bacterium takes up stain and becomes colored, while the background remains unstained. Simple stains are typically used on bacterial smears which have been heat-fixed and thus contain non-living microbes.

A second type of stain is a **negative stain**, which uses a single reagent to provide contrast between the background and the living bacterium. Thus, the background is "stained", while bacterium does not take up any stain. Negative stains are typically used when observing live bacteria is desired.

A third type of stain is a **differential stain** which is a type of staining that allows you to distinguish between types of bacteria or between specific structures in a bacterium. A differential stain typically uses two or more reagents, a primary stain and a counter stain.

Chemically, there are two main types of stains: **basic stains**, which have a positive charge (cationic) and **acidic stains**, which have a negative charge (anionic). Basic stains have an affinity for negative components of cells, and include dyes such as methylene blue, crystal violet, and carbol-fuchsin. Acidic stains have an affinity for positive components of cells, and include dyes such as nigrosin, India ink, and picric acid. Since cell walls are negatively charged, a positive dye will be attracted to and stain the cell wall, whereas a negative dye will be repulsed by the cell wall and not directly stain the cell.

## 2. Preparation of bacterial specimens

In order to stain bacterial specimens, the bacteria must be placed on a glass slide and prepared for the specific staining process. This is typically done by preparing a **bacterial smear**. A smear is a small volume (a loopful) of specimen-containing medium that is spread (smeared) onto a microscope slide. In preparing smears, the "Goldilocks principle" applies, everything has to be done just right! If smears are too thick, you will have trouble seeing individual cells. If smears are too thin, you may not find the organism. If you stir the drop of medium too much as you spread it on the slide, you will disrupt cell arrangements such as chains or clusters.

Following preparation of a bacterial smear, most smears need to be heat fixed before they are stained. Heat fixation accomplishes three things: (1) it kills the organisms; (2) it causes the organisms to adhere to the slide; and (3) it alters the organisms so that they more readily accept stains (dyes). Again, the Goldilocks principle applies. Slides must be completely dry before heat-fixing, or the organism will be boiled and destroyed. If you heat-fix too little, the organism will wash off the slide. If you heat-fix too much, the organisms may be incinerated.

## 3. Procedure for preparing a bacterial smear

1. Obtain a glass slide and clean if necessary.

2. Using a **broth culture**:

    a. Gently agitate your culture broth tube to disperse the bacteria.

    b. Sterilize your inoculating loop using an incinerator or a Bunsen burner, and let cool for 20-30 seconds.

    c. Place loop in the bacterial broth and put the loopful of the broth onto the glass slide. Rub the drop into a nickel-sized smear. Sterilize the loop again to kill any remaining bacteria. Let the smear **air** dry completely. Do **not** use heat to dry your smear!

3. Using an **agar plate**:

    a. Place a **small** drop of water in the center of the slide.

    b. Sterilize your inoculating loop using an incinerator or a Bunsen burner, and let cool for 20-30 seconds.

    c. Use the sterile loop to pick up a small amount of bacterial growth from the surface of the plate. Do not dig into the agar. Put the loopful of bacteria into the drop of water on the glass

slide, and rub the drop into a nickel-sized smear. Sterilize the loop again to kill any remaining bacteria. Let the smear **air** dry completely. Do **not** use heat to dry your smear!

4. **Heat fix** the slide:

a. **Incinerator method:** Hold the slide with a wooden clothes pin approximately 1cm over the barrel of a hot incinerator for 20–30 seconds. Let the slide cool.

b. **Bunsen burner method:** Hold the slide with a wooden clothes pin, and pass 10-12 times through the flame. Let the slide cool.

5. **Optional:** After the slide has cooled us a marker or wax pencil to outline the area of the smear on the underside of the slide. This will help you locate your sample later.