**Microbiology Techniques**

**Staining Bacteria**

 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.

**Simple Staining**

Step 1. Bacteria from colonies. Clean a glass slide and place a small mark slightly off center using a grease pencil. Using your loop, transfer one small drop of water to the center the slide, being careful to be close to but not overlapping the grease pencil mark. Do not transfer too much water because these drops will have to air dry. Sterilize your loop and touch a single colony and transfer the bacteria to the water droplet on the slide and mix well.

Step 1. Bacteria from broth. Clean a glass slide and place a small mark slightly off center using a grease pencil. Mix the broth containing the bacteria well because the bacteria may sediment to the bottom of the container. Use a sterile loop and transfer one or two droplets of bacteria to the center of a cleaned glass slide, close to but not overlapping the grease pencil mark

Step 2. Drying. Allow the bacterial slurry (called the smear) to air dry. You CAN NOT heat the sample nor blow on it to hasten drying time because that could force bacteria into the air leading to contamination and possible infection

Step 3. Heat fixation. Holding the slide by one edge, pass it slowly through a bunsen burner flame. Do not move so slowly that the edge of the slide you're holding heats up to uncomfortable levels. This heat fixation step denatures bacterial proteins causing the cells to stick to the slide while also killing the bacteria making them safe for the following steps

Step 4. Staining. Place the stain in a staining rack and cover the smear with the stain of choice. Allow the stain to work for 30 seconds (some stains may have different staining times but this time will work well for simple stains). Remove the stain by rinsing with water from the squeeze bottle and gently blot (do not rub) the stain dry using bibulous paper. The slide is now ready to look at under the microscope. Because the bacteria were heat fixed, it will not be necessary to use a cover slip

**The Gram Stain**

The Gram stain is classifed as a differential stain because it allows us to distinguish between different types of bacteria. Bacteria can be quickly divided into two distinct morphological and functional groups on the basis of the Gram stain. By this technique, Gram positive bacteria stain purple and gram negative stain red. The bacteria are first stained with crystal violet followed by a brief treament with Gram's iodine. The iodine functions as a mordant to help the crystal violet bind more firmly. The bacteria are then rinsed with ethanol. Gram positive bacteria, which have multiple layers of peptidoglycan, retain the crystal violet while it is quickly rinsed out of Gram negative bacteria because their peptidoglycan is a single layer thick. The bacteria are stained a second time (counter stained) with the dye safranin which will not show up on the already purple Gram positive but will stain the decolorized Gram negative bacteria red

Step 1. Preparation. Smear and heat fix the bacteria as described above (steps 1 through 3) for the simple stain .

Step 2. Primary stain. Cover the smear with crystal violet and incubate for 30 seconds. Rinse the dye off with distilled water (dH2O) from the squeeze bottle

Step 3. Mordant. Cover the smear with grams iodine. After 20 seconds, rinse the slide with dH2O

Step 4. Decolorization Rinse the stain with 95% ethanol. This step must be done very carefully. Hold the slide at a 45o angle over the staining rack and rinse with ethanol one drop at a time. Watch the ethanol as it runs off the slide looking for blue color. Stop dropping ethanol as soon as no more color is releases and rinse the slide immediately with water. A few drops of ethanol too many and the gram positive bacteria will also lose their crystal violet

Step 5. Counterstain. Cover the bacteria with safrinin for 30 seconds. Rinse with dH2O and and blot the slide dry with bibulous paper

**Endospore staining**

[Endospore staining](https://en.wikipedia.org/wiki/Endospore_staining) is used to identify the presence or absence of [endospores](https://en.wikipedia.org/wiki/Endospore), which make bacteria very difficult to kill. This is particularly useful for identifying endospore-forming bacterial [pathogens](https://en.wikipedia.org/wiki/Pathogen) like [*Clostridium difficile*](https://en.wikipedia.org/wiki/Clostridium_difficile).

**Ziehl-Neelsen stain**

[Ziehl-Neelsen staining](https://en.wikipedia.org/wiki/Ziehl-Neelsen_stain) is used to stain species of [*Mycobacterium tuberculosis*](https://en.wikipedia.org/wiki/Mycobacterium_tuberculosis) that do not stain with the standard laboratory staining procedures like Gram staining.

The stains used are the red coloured [Carbol fuchsin](https://en.wikipedia.org/wiki/Carbol_fuchsin%22%20%5Co%20%22Carbol%20fuchsin) that stains the bacteria and a counter stain like [Methylene blue](https://en.wikipedia.org/wiki/Methylene_blue)



Microscopic view of a histologic specimen of human [lung](https://en.wikipedia.org/wiki/Lung) tissue