

Simple Stain

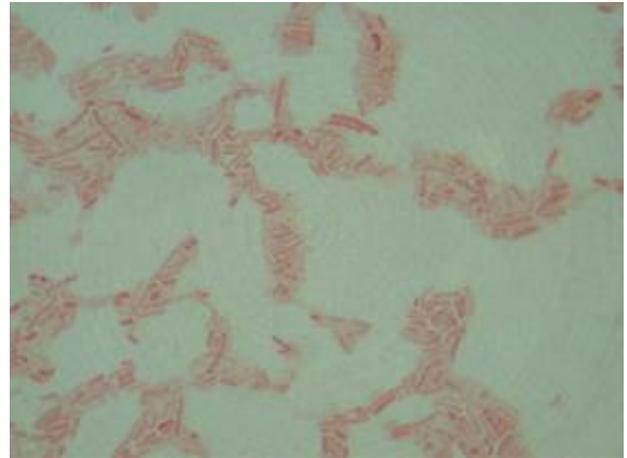
Simple stains are just that - add one stain to a fixed smear slide, let it sit, rinse it off, let it dry, and view. It is a quick procedure for determining the presence and morphology of bacteria in clinical samples such as stool and discharges. Methylene blue is used to determine the morphology of fusiform and spirochetes in oral infections. It is also the stain of choice for identifying the metachromatic granules in *Corynebacterium diphtheriae*. The granules will stain a distinctly deeper blue than the surrounding blue bacteria. Other species of *Corynebacterium* do not have the metachromatic granules. Any basic dyes, such as methylene blue, crystal violet, malachite green, or safranin work well. Basic (cationic or positively charged) dyes bind to negatively charged components in the cell membrane and cytoplasm.

Materials

- Methylene blue
- Safranin
- Crystal violet
- Malachite Green
- Staining racks
- Micro tool boxes
- Prepared smear slides

General Considerations

Staining is part art and part science. There are no hard and fast rules for staining and rinsing times. The times listed are suggestions that usually work well. You will need to experiment with what works for the bacteria you have and the techniques you use. It is essential that you record exactly what you do and the results you observe in your lab book. You will be repeating these stains later in the semester and you don't want to waste your time re-inventing your successful staining procedure. It would be useful for each lab bench member to pick a different stain so you can see what they all look like.



Enterobacter aerogenes

Simple Stain Procedure

1. Place your carefully prepared fixed smear slides on the stain rack over the sink.
 - Do one slide at a time.
 - Cover the smear with any of the basic dyes available to you.
 - You only need enough dye to cover the smear. The stain should not drip off the slide.
2. Let the stain sit for 1-5 minutes.
3. Using the clothespin, grab the long end of the slide, tilt the slide over the sink and rinse the stain off with a stream of water from the wash bottle.
 - Be sure to spray above the smear and let it dribble down.
 - If you spray directly on the smear you are liable to wash the smear off the slide.
 - Rinse till the water runs clear or is only slightly colored.
4. Touch the edge of the slide to a paper towel to remove excess water. You can now let it air dry. Alternately you can dry it with blotting paper by placing it in the blotting paper book and pressing lightly. While this method is quicker, you can also blot off a poorly adhered smear.
5. View your slide under oil immersion and record your observations in your lab book.
6. Discard your used stained slides in the disinfectant bucket in the sink.