

Gram-staining Procedure

Gram-staining is a four part procedure which uses certain dyes to make a bacterial cell stand out against its background. The specimen should be mounted and fixed on a slide before you proceed to stain it. The reagents you will need to successfully perform this operation are:

- Crystal Violet (the Primary Stain)
- Iodine Solution (the Mordant)
- Decolorizer (ethanol is a good choice)
- Safranin (the Counterstain)
- Water (preferably in a squirt bottle)



Before starting, make sure that all reagents, as well as the squirt-bottle of water, are easily accessible because you won't have time to go get them during the staining procedure.

STEP 1: Place your slide on a slide holder or a rack. Flood (cover completely) the entire slide with crystal violet. Let the crystal violet stand for about 60 seconds. When the time has elapsed, wash your slide for 5 seconds with water. The specimen should appear blue-violet when observed with the naked eye.



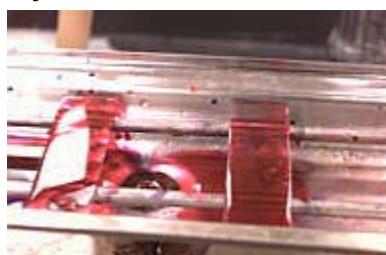
STEP 2: Now, flood your slide with the iodine solution. Let it stand about a minute as well. When time has expired, rinse the slide with water for 5 seconds and immediately proceed to step three. At this point, the specimen should still be blue-violet.



STEP 3: This step involves addition of the decolorizer, ethanol. Step 3 is somewhat subjective because using too much decolorizer could result in a false Gram (-) result. Likewise, not using enough decolorizer may yield a false Gram (+) results. To be safe, add the ethanol drop wise until the blue-violet color is no longer emitted from your specimen. As in the previous steps, rinse with the water for 5 seconds.



STEP 4: The final step involves applying the counterstain, safranin. Flood the slide with the dye as you did in steps 1 and 2. Let this stand for about a minute to allow the bacteria to incorporate the saffranin. Gram positive cells will incorporate little or no counterstain and will remain blue-violet in appearance. Gram negative bacteria, however, take on a pink color and are easily distinguishable from the Gram positives. Again, rinse with water for 5 seconds to remove any excess of dye.



After you have completed steps 1 through 4, you should blot the slide gently with bibulous paper or allow it to air dry before viewing it under the microscope. DO NOT RUB THE SMEAR!