



Routine Microscopy Gram Stain Procedure

There are variations of the gram stain procedure regarding the timing and reagents, but the basic steps are the same. You will need to follow your institution's procedures. For this gram stain demonstration, we are going to follow the American Society of Microbiology's manual of Clinical Microbiology Procedures 10th Edition.

Position your slide rack over the sink and place the fixed slide on the slide rack. Flood the slide with crystal violet. Wait at least 15 seconds, then gently rinse the slide with water. Tilt the slide to remove the excess water.

Next, flood the slide with gram's iodine. After 15 seconds, gently rinse the slide with water. Tilt the slide to remove the excess water. Add the decolorizer to the slide and rinse immediately with water to help prevent over-decolorizing.

Tilt the slide again to remove the excess water. Last, flood the slide with safranin. Wait at least 15 seconds and then gently rinse the slide with water, tilting the slide to remove any excess water. You can gently blot the slide with the bibulous paper but be careful not to wipe the smear off the slide, then allow the slide to air dry. Once the stained slide is dry, it is ready to be viewed under a bright-field microscope.

First, locate the cells under the 40X objective, then add a drop of immersion oil to the center of the smear and view under the 100X oil objective. Grampositive cells will appear purple and gram-negative cells will appear pink.

Link to video job aid [Routine Microscopy – Gram Stain Procedure | OneLab REACH \(cdc.gov\)](#)