

Slide Preparation:

1. After obtaining a fecal sample, take a small amount using the wooden end of a cotton tipped swab and make a thin smear of the feces along the slide using a rolling motion (similar to preparing ear cytology)
2. Air-dry the smear or heat fix it over a gentle flame.
 - Moving the slide in a circular motion will help to prevent over heating a particular area.

Gram Staining:

1. Add crystal violet stain over the fixed fecal smear.
 - Let stand for 10 to 60 seconds.
 - Pour off the stain and gently rinse the excess stain with a stream of water from a faucet or a plastic water bottle.
 - Note that the objective of this step is to wash off the stain, not the fixed culture.
2. Add the iodine solution on the slide, enough to cover the fecal smear.
 - Let stand for 10 to 60 seconds.
 - Pour off the iodine solution and rinse the slide with running water.
 - Shake off the excess water from the surface.
3. Add a few drops of decolorizer so the solution trickles down the slide.
 - Rinse it off with water after 5 seconds.
 - The exact time to stop is when the solvent is no longer colored as it flows over the slide.
 - Further delay will cause excess decolorization in the gram-positive cells, and the purpose of staining will be defeated.
4. Counterstain with Safranin solution for 40 to 60 seconds.
 - Wash off the solution with water.
 - Blot with bibulous paper to remove the excess water.

- Alternatively, the slide may be shaken to remove most of the water and air-dried.