



GRAM STAIN PROTOCOL

This technique is used to stain a slide such as a fecal smear to observe the bacterial microflora present based on their gram stain reaction.

- **“Heat-fix” the slide with the specimen by passing it over a heat source, such as a flame, several times using a clothes pin or forceps. The slide should be passed very quickly through the flame and not be heated excessively. Place slide on the staining tray.**
- **Flood the fixed smear with crystal violet solution (#1) and allow to remain for 1 minute.**
- **Rinse off the crystal violet with distilled or tap water.**
- **Flood the slide with iodine solution (#2). Allow to remain for one minute.**
- **Rinse off the iodine solution with distilled or tap water.**
- **Holding slide on a tilt with a clothes pin, flood the slide with decolorizer (#3) for one to five seconds.**
- **Rinse off the decolorizer with distilled or tap water.**
- **Flood the slide with safranin (#4). Allow to remain for 30 seconds.**
- **Rinse off the safranin with distilled or tap water.**
- **Dry the slide on bibulous paper or absorbent paper and place in an upright position.**

Microscopically examine the slide for bacterial organisms under a 100X objective. Observe several fields on the slide for bacterial organisms. Describe the gram reaction of any organisms seen. Gram-positive bacteria stain deep violet to blue and gram-negative bacteria

stain pink to red. If your slide is all one color (either pink or blue), then the slide may either have been over or under decolorized. Unless it is a gram stain of a pure culture, there normally should be material staining both colors somewhere on the slide.