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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 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.**
- **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.

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