**Gram Stain Procedure**

**Reagents**

1. Hucker’s Crystal Violet (primary stain)
2. Gram’s Iodine (mordant)
3. 95% Ethyl Alcohol (decolorizer)
4. Safranin (counter or secondary stain)

1. Make bacterial smear, air dry, heat fix.
2. Gently flood smears with Hucker’s Crystal Violet and let stand for 1 minute.
3. Gently wash with tap water.
4. Gently flood smears with the Gram’s Iodine mordant and let stand for 1 minute.
5. Gently wash with tap water.
6. Decolorize with 95% Ethyl Alcohol. Note: Do not over-decolorize. Add reagent drop by drop until alcohol runs almost clear, showing only a blue tinge.
7. Gently wash with tap water.
8. Counterstain with Safranin for 1 minute.
9. Gently wash with tap water.
10. Blot dry with bibulous paper and examine under low power, high dry and then oil immersion.