

**SOP: SP036**

**Gram's Staining**

**Materials and Reagents:**

1. One dried and heat fixed glass microscope slide per sample
2. Staining tray
3. Crystal violet stain
4. Gram's iodine
5. Decolorizer (4:1 mixture of isopropyl alcohol/acetone)
6. Safranin stain
7. Bibulous paper
8. Clothespin
9. Paper towels
10. Sharps glass disposal bin

**Protocol:**

1. \_\_\_\_ Cover your thoroughly dried, heat fixed, and cooled slides with crystal violet. Leave on for one minute.
2. \_\_\_\_ Gently wash off the stain with a stream of running water until the water runs clear. Be careful to spray around the smear, not directly on it, or the smear may rinse off.
3. \_\_\_\_ Completely cover the slides with Gram's iodine and leave on for one minute.
4. \_\_\_\_ Gently wash off the iodine with a stream of running water until the water runs clear.
5. \_\_\_\_ Using a clothespin, hold the slide at an angle over the staining pan. Slowly dispense a few drops of decolorizer at the upper end of the slide above the smear (not on the smear itself) so that the decolorizer runs down over the smear. Blot the decolorizer that accumulates in the lower corner of the slide on a paper towel. The blotted decolorizer will be purple/blue in color at first. Apply more decolorizer, as above, just until the blotted decolorizer is colorless.
6. \_\_\_\_ Gently wash off the decolorizer with a stream of running water.
7. \_\_\_\_ Shake off any excess water, cover the smears with safranin, and leave on for one minute.
8. \_\_\_\_ Gently wash off the stain with a stream of running water until the water runs clear.
9. \_\_\_\_ Gently blot the slides dry, using bibulous paper. Do not rub or push down on the slide which will wipe off the smear. Wipe the back of the slide with a small piece of paper towel dampened with 2-3 drops of decolorizer.
10. \_\_\_\_ Examine the slide under a microscope, then discard the glass slides in the proper sharps disposal bin.