

Gram Staining

Supplies

- ▶ Microscope Slide
- ▶ Inoculating Loop or Wood stick
- ▶ Paper Towels



Material

- ▶ Crystal Violet Stain
- ▶ Safranin Red Stain
- ▶ Gram's Iodine
- ▶ Ethyl Alcohol (denatured)
- ▶ Burner
- ▶ Wax Pencil
- ▶ Microscope



Procedure

- ▶ Obtain a clean microscope slide
- ▶ Draw a circle on your slide with wax pencil
- ▶ Warming the slide will make the wax pencil work better when drawing your circle



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- ▶ Place 1-2 drops of water inside the circle on your microscope slide
 - ▶ Use the inoculating loop to obtain a tiny sample of bacteria from your bacteria culture
 - ▶ Add the bacteria sample to the water on your microscope slide, mixing to create a suspension of bacteria



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- ▶ **Heat Fixing Bacteria to the Microscope Slide**
 - ▶ Pass your microscope slide through the flame until all of the water evaporates
 - ▶ This will leave your bacteria stuck or “fixed” to the microscope slide



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- ▶ **Primary Staining of Gram Positive Bacteria**
 - ▶ Place 2-3 drops of Crystal Violet Primary Stain inside your circle (on your bacteria)
 - ▶ Leave the stain on the bacteria sample for one minute
 - ▶ Rinse stain off with water (gently)



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- ▶ Add the Gram's Iodine to your bacteria sample inside the circle
 - ▶ Leave Gram's Iodine on your sample for one minute
 - ▶ Rinse off with water



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- ▶ Add ethyl alcohol to the bacteria
 - ▶ Let the alcohol set, then rinse with water
 - ▶ Continue de-colorizing until the crystal violet stain is no longer released from your sample.
 - ▶ You should just be able to see a hint of purple at this point



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- ▶ Add 2-3 drops of Safranin Red Stain to your bacteria sample inside the circle on your slide
 - ▶ Leave safranin red on your bacteria sample for one minute
 - ▶ Rinse with water, blot or air dry

