

Lab #3: Making Bacteria Visible

Purpose:

To examine bacteria under a compound microscope and see their different shapes.

Procedure:

1. Create a slide smear of your bacteria:
 - a) Sterilize the slide and the wire loop by passing them through the alcohol burner flame once or twice. Let the slide and loop cool in your hand.
 - b) Place a drop of water on the slide. Using the wire loop, mix bacteria from one colony into the water drop.
 - c) Spread the water drop **thinly** (smear) on the slide. If it is too thick you won't see individual bacteria.
 - d) Fix the bacteria to the slide by passing it over the flame. You should still be able to touch the bottom of the slide – otherwise your bacteria will be MUSH!

2. Gram Staining Bacteria: (**Stains will stain fingers & clothes**)
 - i. Put 5 drops of **crystal violet or methylene blue** stain over your smear. Let it stand for **one minute**. **Gently** rinse with water to remove the excess stain.
 - ii. Next, put 5 drops of **iodine solution** on the smear. Let it stand for **one minute**. **Gently** rinse with water to remove excess stain.
 - iii. Briefly wash the slide with **acetone or ethanol** for **2-3 seconds** (until colour dissipates). Immediately after, **gently** wash it with water to remove the alcohol.
 - iv. Put about 5 drops of **safranin or congo red** solution on the culture. Let it stand for **two minutes**. **Gently** rinse with water.
 - v. Shake off excess water.

3. Examine the slides under the compound microscope on **oil immersion**.

4. Identify the bacteria as **Gram positive (+) (blue-violet / purple brown)** or **Gram negative (-) (red / pink)**.

Observations:

Make a biological drawing of the bacteria that you see in the microscope. Pay special attention to the shape of the cells. Label the drawing by stating if the bacteria are Gram + or Gram – and what shapes the bacteria have. The name of the specimen can be the place where the bacteria were found. Include actual size and drawing magnification.

Wash the slides, wipe off the microscope objective lens well and **WASH YOUR HANDS**.
Wipe down lab bench.

Conclusions:

Discuss the purpose. Did you find more Gram + or Gram – bacteria?