

Lab 1 – Gram Staining Bacteria

Kingdom Bacteria:

- Read background information from exercise 23 in the biology lab manual and write down key points (shapes and characteristics of bacteria), as well as gram staining information
- View prepared slides and make a chart of what it is and what it looks like in your data section
- Gram Stain teeth or tongue and supplied bacterial sample for a total of 2 samples and classify using your table. Work with group around you to stain different bacteria so all samples can be compared and classified.
- Make new data table with samples describing shape, gram +/-, and picture

Gram Staining

One of the most important techniques to classify bacteria is the Gram Stain, based on the different structural and chemical compositions of bacterial cell walls. Gram staining is important because it often correlates with the sensitivity of bacterium to antibiotics. Gram-positive bacteria have a thick cell wall that retains a purple dye, whereas Gram-negative bacteria have a much thinner cell wall that does not retain the dye.

1. Obtain *SMALL* drop of water on slide. Sterilize inoculating loop and cool
2. Streak a small amount of bacteria from the stock plate to a spot in the middle of a microscope slide
3. Heat the slide gently by holding it with a clothespin and passing it over the top of a flame three to four times (do not put too close to base of flame, and wait until water is gone). Drying is critical to success. This heat will adhere the bacteria to the slide.
4. When the slide has cooled, gently cover the spot of bacteria completely with several drops of crystal violet stain and incubate for 20 seconds.
5. Rinse the slide for 2 seconds with a gentle but steady stream of water from a water bottle.
6. Gently cover the spot of bacteria with several drops of iodine and incubate for 1 minute.
7. Drop decolorizer on the smear until no purple shows in the alcohol coming off the slide. Quickly rinse the slide with water to remove the alcohol.
8. Gently cover the bacterial spot with a few drops of safranin and incubate (hold) for 20 seconds.
9. Gently rinse the slide with water, air dry the slide and place a cover slip over the bacterial spot.
10. Observe the bacteria using a high power objective to determine shape and if the bacteria are gram positive or gram negative
11. Place slide and cover slip in bleach for sterilization
12. Clean up work area and wipe down station with ethanol before checking out with teacher

In your analysis section, compare and contrast a gram positive and gram negative bacteria, explain why the staining technique can differentiate the two, and summarize sterile technique