**Gram Staining Lab Name:**

**Block:**

**Pre-lab:**

Bacteria are extremely small and hard to see even under the microscope. They are so small that the light of the microscope will make them transparent to the point of invisibility. Microbiologists use the **Gram Staining** technique to dye the cells to have better visibility. This technique was developed by a Danish Bacteriologist, Hans Christian Gram in 1884. It is still used to this day as a quick way to identify bacteria.

Bacteria can be **Gram Positive** if they have a single membrane (monoderm) and are purple. They can also be **Gram Negative** if they have a double membrane (diderm) and end up being pink. In addition to the shape, type of growth, and mode of eating, Gram staining is an additional way to differentiate bacteria.

**Procedure:**

**READ** the procedure and then follow it to stain your bacteria.

1. Obtain a slide and smear a drop or two of yogurt water on it.
2. Allow it to air dry by moving it gently through the air.
3. On an alcohol lamp fix  the bacteria by gently moving the slide through the flame making sure not to burn the bacteria. All you want to do is warm the slide up.

**STAINING**

1. Wear a glove on the hand that you use to hold the slide.
2. Make sure ALL dying happens in the sink as the dyes are quite potent and will stain you and your surroundings if splashed.
3. Put a drop or two of CRYSTAL VIOLET (PURPLE) on the slide. GENTLY rinse with tap water.
4. Put a drop or two of IODINE (YELLOW) on the slide. GENTLY rinse with tap water.
5. Rinse with a squirt of 95% alcohol and EMMIDIATELY rinse with tap water.
6. Put a drop or two of SAFRANIN (RED) on the slide and rinse with tap water.

Your slide should be stained with purple / red colour. Allow for it to air dry and then view it under the microscope.

After viewing your bacteria and drawing it, place the slide in the designated beaker.

Observations and Questions:

1. Draw the yogurt bacteria on the back of this sheet making sure to follow all the drawing rules that we went over in the microscope notes last day.
2. Find the actual size of the bacteria using the calculations we learned about (on the back of this sheet).
3. Determine if the bacteria is gram positive or gram negative. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

What does that say about its cell wall? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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1. Use online resources to find what happens at each stage of gram staining and write it in point form below:

|  |  |
| --- | --- |
| Adding Crystal violet |  |
| Adding Iodine |  |
| Adding Alcohol |  |
| Adding Safranin |  |