

Project No. _____

TITLE Gram Staining of Bacteria

Book No. _____

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Purpose: Make a statement of the purpose of gram staining and why it is important. This is also where you state your hypothesis. For this lab your hypothesis is that gram staining will allow you to determine the type of cell membrane a bacteria has.

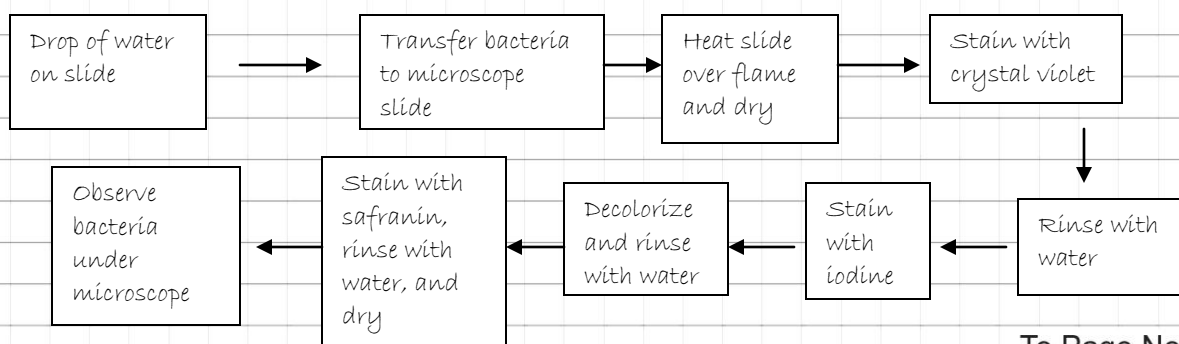
Materials and Methods: (See SOP page 3)

Microscope slide	inoculating loop	Safranin	Bunsen burner
Clothes pin	Kim wipes	Bleach	iodine
Crystal violet stain	ethyl alcohol squirt bottle		

Methods:

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

Flow Chart:



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Data:

Sample	shape	gram +/-	picture
<u>(This table is drawn in by hand and filled out as you do the lab. The picture is where you draw a picture of what you are seeing in the microscope for each sample)</u>			

Calculations:

There are no calculations for this lab so just state not "No calculations for this lab". If you did make any calculations such as figuring out how much of a stock solution to use or how many grams of a substance to weigh out you would write those calculations here.

Conclusion: Here you make a conclusion based on the data collected. Conclusions such as "it was good" or "I don't think it worked" will not earn points. If you make statements like that you will need to support them with specific pieces of data that you have collected. A conclusion should answer whether or not your data supports your hypothesis and why it does that.

Reflection: Write what you learned from the experiment. Also be specific about things that you didn't understand. This section is not data driven, but rather a personal reflection on your learning.

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Date

For the teacher to sign and date during grading.
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Today's date