

Name: _____ Seat # _____

Do not write in pencil (use pen).

Do not use Liquid Paper(draw one line through error).

Date: ____/____/____ Periods _____

Please staple all work.

Gram Stain Lab

10

Lab Number

Purpose: The purpose of this lab is to learn how to perform a gram stain correctly so that proper identification on unknown microorganisms can be made.

Materials:

- | | | |
|----------------------------------|--|----------------------------|
| 1. Glass slide | 10. Safranin | 19. Work Mat (paper towel) |
| 2. Wax pencil | 11. Beaker of water or running tap water | 20. Laboratory Coat |
| 3. Wire loop | 12. Paper towel (bibulous paper) | 21. Disinfecting wipes |
| 4. Bacti- Cinerator | 13. Forceps | 22. Biohazard containers |
| 5. Water (Dropper Bottle) | 14. Oil | |
| 6. Organism | 15. Microscope | |
| 7. Crystal violet | 16. Staining rack | |
| 8. Gram iodine | 17. Lens cleaner | |
| 9. Decolorizer (acetone-alcohol) | 18. Lens paper | |

Procedure: See next page for pictures of procedure. (Label Slide)

1. Take a clean glass slide and draw a small circle in the middle of the slide.
2. Turn the slide over so that the wax is under the slide, and it will not interfere with the gram stain.
3. Place a small drop of water inside the circle.
4. Using aseptic technique (flaming loop before and after use), transfer a loopful of bacterial culture to the inside of the circle next to the water.
5. Mix the bacteria and water together to cover the circle made with the wax pencil.
6. Allow the slide to air dry, and heat fix by putting the slide on top of the bacti-centerator with the organisms side facing up.
7. Place the slide on the rack of a staining tray.
8. Flood the slide from edge to edge with crystal violet. Let stand for one minute.
9. Using a squeeze bottle or beaker of water, indirectly wash off the dye. Do not squirt the water directly on the inoculum as it may completely wash off the slide.
10. Flood the slide from edge to edge with Gram's iodine. Let stand for one minute.
11. Rinse indirectly with water.
12. Decolorize with acetone – alcohol for about 10 seconds or until the alcohol drippings from the slide run clear.
13. Immediately rinse with water.
14. Counterstain with safranin for one minute.
15. Rinse with water and blot dry with bibulous paper.
16. Place the slide on the stage of the microscope and focus with the 10 X objective in place. Proceed to 40 X and, finally, add a drop of immersion oil directly onto the slide and rotate the 100 X objective into place.
17. Fine focus and examine the bacteria on 100 X. Observe for gram reaction, morphology, and arrangement of the bacteria. Record data in result section.
18. Replace and dispose of all supplies and equipment according to instructor (also see lab disposal book).
19. Disinfect work area, chair, test tube rack and any other item that may be contaminated with body fluids.

Raw Data/Calculations: N/A

Results (40 points):

| Team number and Unknown Letter (i.e. Team 1 and Unk A) | <u>Gram stain reaction</u> (positive or negative) | <u>Morphology (Shape)</u> (cocci or rods, or oval shaped yeast) | <u>Arrangement</u> (singles, pairs, chains, or clusters, tetrads etc.) |
|--|---|--|--|
| | | | |

Normal Range: N/A

Conclusion (20 points): Write a sentence stating if your gram stain was performed correctly according to the instructor.

Clinical Significance (20 points): Write a sentence stating why the gram stain test is necessary.

Questions (20 points):

1. What part of the organisms determines the gram stain reaction? _____
2. What is the purpose of heat fixing the slide prior to staining? _____
3. What is the name and purpose of each of the four solutions of the gram stain?
 - a. _____
 - b. _____
 - c. _____
 - d. _____
4. List two major bacterial morphologies. _____
5. Describe how the bacteria will appear at the end of the gram stain procedure if the decolorizer is left on too long? _____
6. List 4 different bacteria arrangements.
 - a. _____
 - b. _____
 - c. _____
 - d. _____