Montgomery County Community College
340 DeKalb Pike
Blue Bell, PA 19422

SOP: Gram Stain

Approvals:

Preparer: Dr. Maggie Bryans Date: 18FEB14
Reviewer: Jason McMillan Date: 19FEB14

1. Purpose:

1.1. To Gram stain samples.

2. Scope:

2.1. Applies to Gram staining samples using the 3-step method to detect the presence of Gram positive and Gram negative microorganisms.

3. Responsibilities:

3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:

4.1 Gram stain pack insert

4.2. microscope SOP

5. Definitions:

5.1. Gram positive microorganism: a microorganism that stains dark purple when treated with Gram staining solutions.

5.2. Gram negative microorganism: a microorganism that stains pink when treated with Gram staining solutions.

6. Precautions:

6.1. Gram Stain reagents are harmful. Wear gloves while performing this SOP.

7. Materials:

7.1. 4-step Gram stain kit
7.2. microscope slide
7.3. P20 pipet and tips
7.4. Bunsen burner
7.5. safety gas lighter with flint
7.6. tongs
7.7. inoculation loop
7.8. isopropanol
7.9. slide staining rack
7.10. timer
7.11. water
7.12. immersion oil
7.13. microscope with 1000X magnification
7.14. lab tissues
7.15. lab towels

8. Process:

Note: Refer to Figures 1-6 as needed before performing this SOP and throughout the procedure as needed.

8.1. **Sample preparation**

SOP: Gram Stain

8.1.1. Label a glass microscope slide with pertinent information.
8.1.2. Prepare slide following directions for the appropriate sample source:
8.1.2.1. If sample is from a liquid culture, pipet 10µL of the culture onto the microscope slide.
8.1.2.1.1. Spread into a thin film with the pipet tip.
8.1.2.2. If sample is from a colony, pipet 10µL of water onto the slide.
8.1.2.2.1. Take a sample of the colony using a sterile loop.
8.1.2.2.2. Place the loop full of sample on the glass microscope slide, mix with water and spread into a thin film.
8.1.3. Gently heat fix the microbes to the slide.
Note: Do not overheat the slide. Excessive heating will cause atypical staining.

8.2. **Gram stain**
8.2.1. Place the slide on a slide rack to cool to room temperature before staining.
8.2.2. Cover the fixed sample on the slide with crystal violet stain and leave for approximately 1 minute.
8.2.3. Wash with a stream of water until the water runs clear.
8.2.4. Cover the fixed sample on the slide with iodine mordant and leave for approximately 1 minute.
8.2.5. Wash with a stream of water until the water runs clear.
8.2.6. Rinse with decolorizer.
8.2.7. Wash with a stream of water until the water runs clear.
8.2.8. Cover the fixed sample with safranin and leave for 30-60 seconds.
8.2.9. Wash with a stream of cold water until the water runs clear.
8.2.10. Air-dry or blot with lab tissue.
Note: Do not rub glass slide with the lab tissue.
8.2.11. View with the light microscope at 100x magnification (using oil).
8.2.12. Record whether cells are Gram positive (dark purple) or Gram negative (pink).
8.2.13. Discard the slide in the biohazard sharps container.

9. Attachments: N/A
9.1. Figure 1: Taking sample colony
9.2. Figure 2: Spreading sample colony thin film
9.3. Figure 3: Heat fix sample
9.4. Figure 4: Sample covered with crystal violet
9.5. Figure 5: Sample covered with iodine mordant
9.6. Figure 6: Sample covered with safranin

10. History:

Name	Date	Amendment
Bob O'Brien	12Jun07	Initial release
Deb Audino	04Apr08	College name change
Jason McMillan	19FEB14	College name change

SOP: Gram Stain

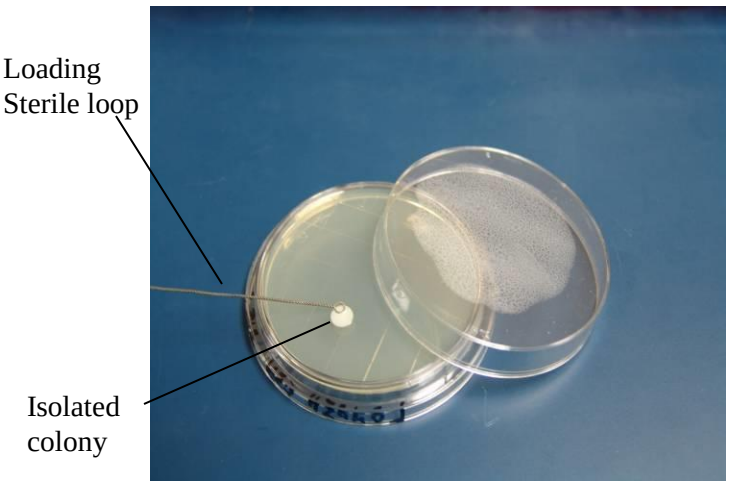

Figure 1: Taking sample colony

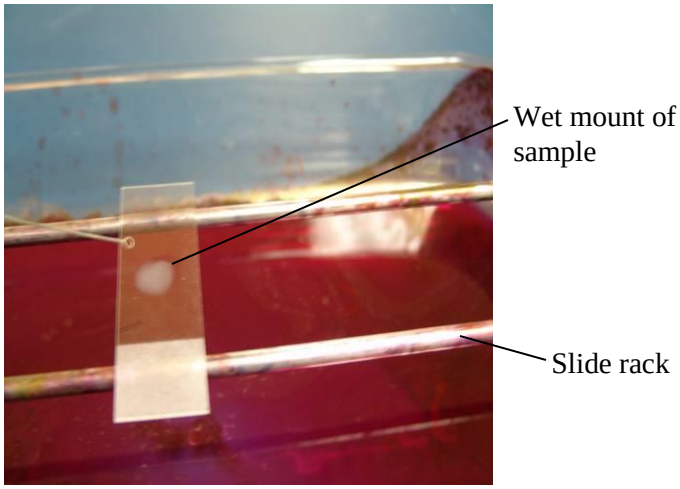

Figure 2: Spreading sample colony thin film

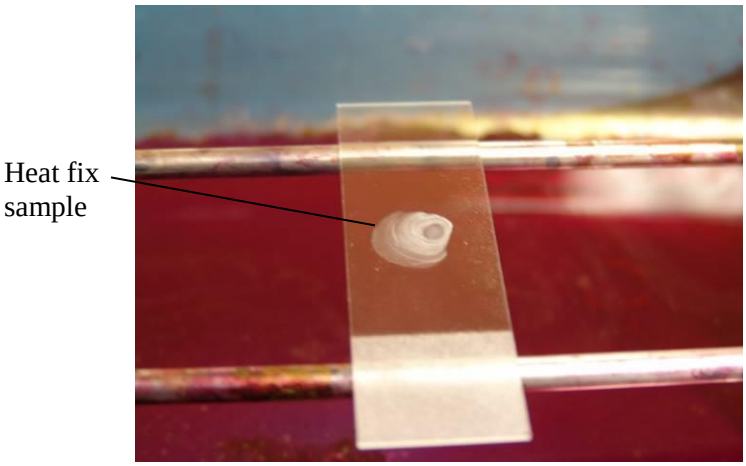

Figure 3: Heat fix sample

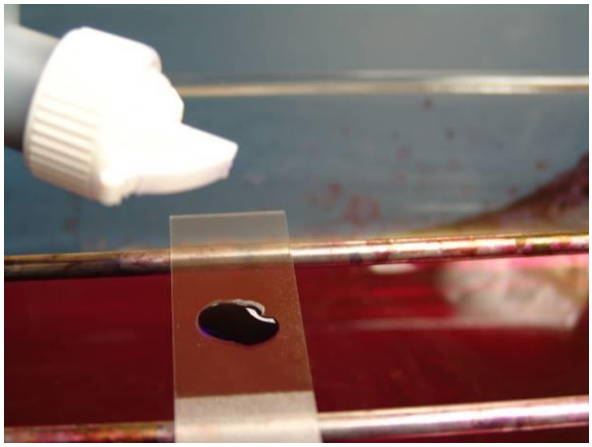

Figure 4: Sample covered with crystal violet

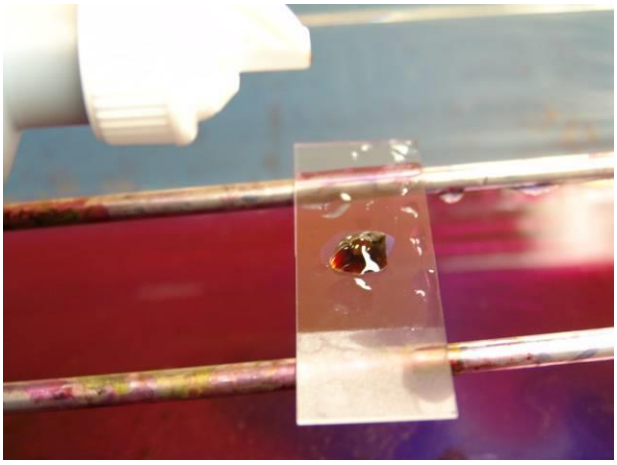

Figure 5: Sample covered with iodine mordant

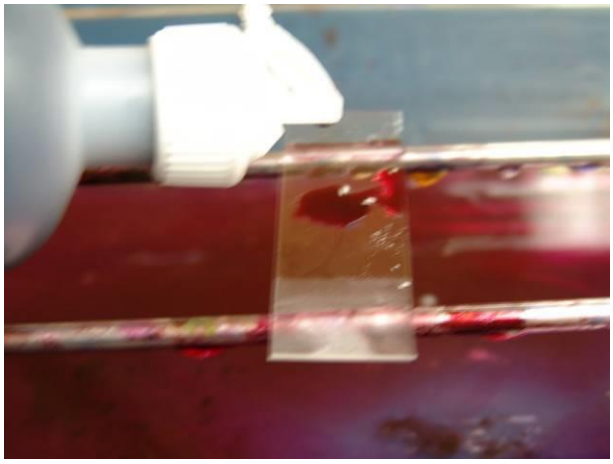

Figure 6: Sample Covered with safranin