

Title: Four Step Gram Stain SOP

Approvals:

Preparer: Bob O'Brien Date 04Apr08
Reviewer: Deb Audino Date 04Apr08

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

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- 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 10 μ L 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 10 μ L 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

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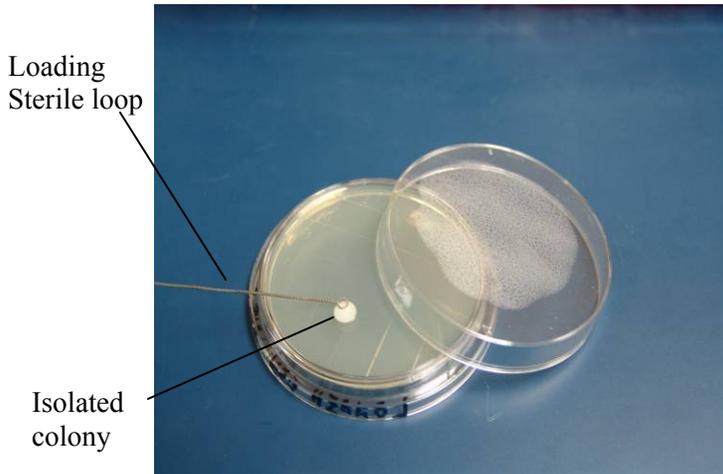


Figure 1: Taking sample colony

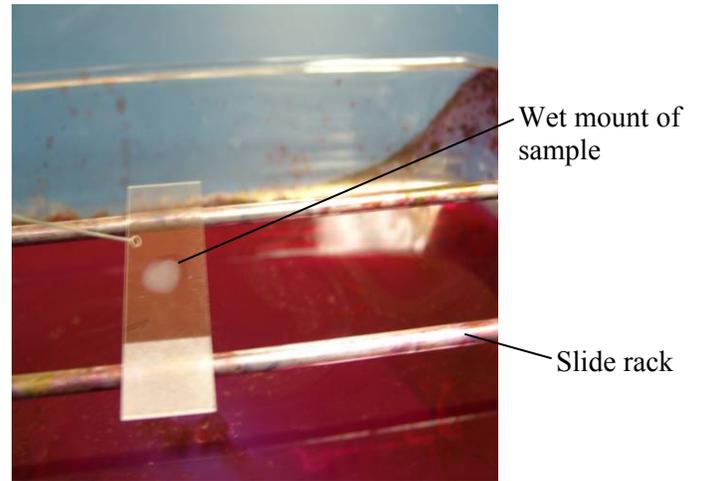


Figure 2: Spreading sample colony thin film

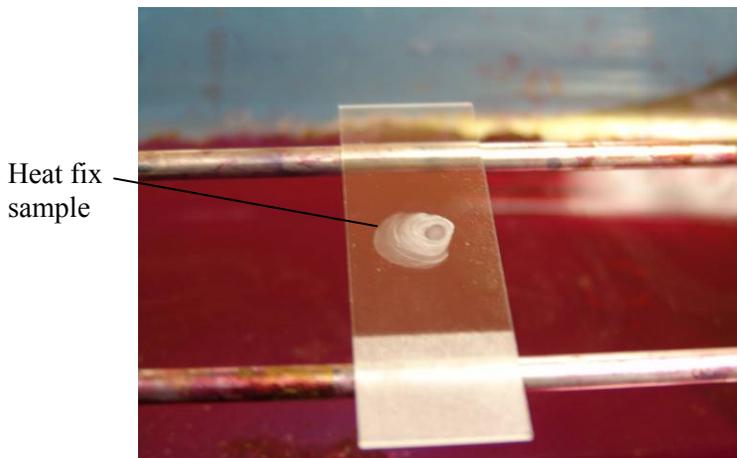


Figure 3: Heat fix sample

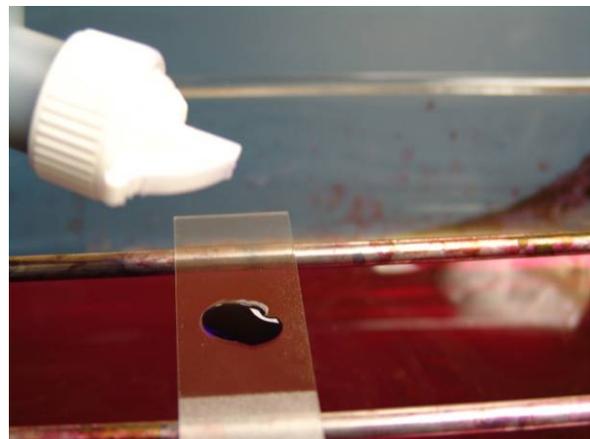


Figure 4: Sample covered with crystal violet

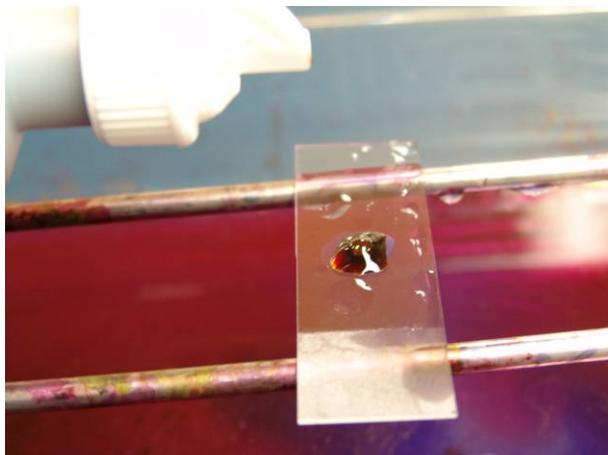


Figure 5: Sample covered with iodine mordant

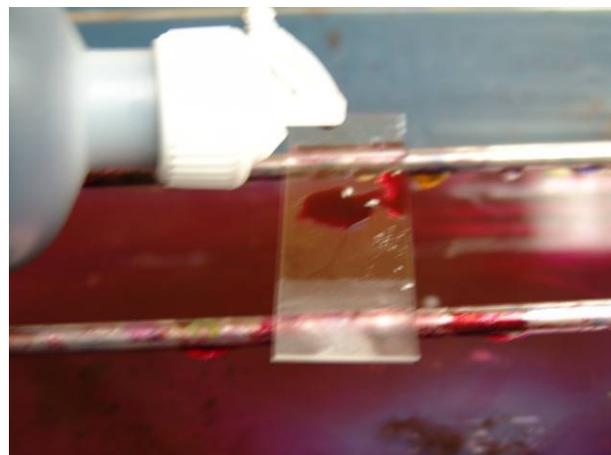


Figure 6: Sample Covered with safranin