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

Title: Gram Stain SOP

Approvals:
Preparer: Melanie Lenahan_____ Date: 052907_____
Reviewer: Martha Salas_____ Date: 052907_____

1. **Purpose:**
   1.1. To perform a Gram Stain for identification of gram negative and gram positive bacteria.
2. **Scope:**
   2.1. This procedure is intended as a standard test for identification of gram negative and gram positive bacteria.
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 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. Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – cGMP (FDA publication, September 2004)
   4.2. United States Pharmacopeia
   4.3. Bergey's Manual of Systematic Bacteriology
5. **Definitions:**
   5.1. Gram stain (named after Christian Gram, 1853-1938) is the most useful and widely employed differential stain in bacteriology.
6. **Precautions**: Aseptic technique and standard precautions for handling microbial cultures. Gram crystal violet, safranin and iodine can cause irritation to the eyes, respiratory and skin. Wear suitable protective equipment.
7. **Materials:**
   7.1. Bacterial cultures including E. coli and S. aureus as controls
   7.2. Solutions of crystal violet, Gram's iodine, 95% ethanol and safranin
   7.3. Clean glass slides
   7.4. Inoculating loop
   7.5. Bunsen burner
   7.6. Bibulous paper
   7.7. Microscope
   7.8. Lens paper and lens cleaner
   7.9. Immersion oil
   7.10 Staining rack
   7.11 Squirt bottle filled with distilled water

8. **Procedure:**
   8.1. Prepare heat-fixed smears of the bacterial cultures provided by your instructor.
   8.1.1. With a wax pencil, mark the reference number of the bacterial culture in the far left corner on a clean glass slide.
   8.1.2. Place a loopful of water in the center of a clean glass slide.

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8.1.3. With the inoculating loop, aseptically pick up a very small amount of culture and mix into the drop of water.

8.1.4. Spread this out to a ½ - inch area.

8.1.5. Allow the slide to air dry or place on a slide warmer.

8.1.6. Pass the slide through a Bunsen burner flame three time to heat-fix the bacteria.

8.2. Staining.

8.2.1. See Figure 1 for graphical representation of staining procedure.

8.2.2. Place the slide on the staining rack.

8.2.3. Flood the slides smears with crystal violet and let stand for 30 seconds.

8.2.4. Rinse with water for 5 seconds.

8.2.5. Cover with Gram's iodine and let stand for 1 minute.

8.2.6. Rinse with water for 5 seconds.

8.2.7. Decolorize with 95% ethanol for 15 to 30 seconds.

8.2.8. Rinse with water for 5 seconds.

8.2.9. Counterstain with safranin for about 60 to 80 seconds.

8.2.10. Rinse with water for 5 seconds.

8.2.11. Blot dry with bibulous paper and examine under oil immersion. Gram positive organisms stain blue to purple; gram negative organisms stain pink to red. See Figure 2 for an example of gram-positive and gram-negative bacteria.

Figure 1: Graphical representation of staining procedure.

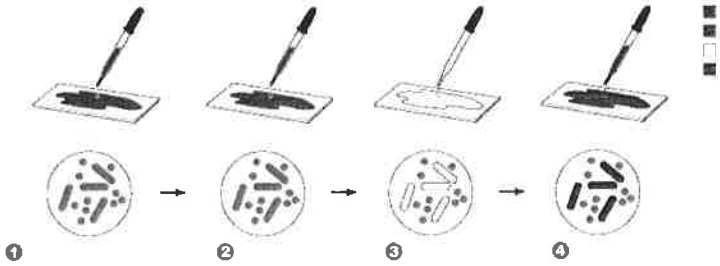

Figure 2: Example slide of gram-positive and gram-negative bacteria.

9. History:

9.1. Melanie Lenahan, 052907, initial release

9.2. Sheila Byrne, 063014, revision 1, SOP name change