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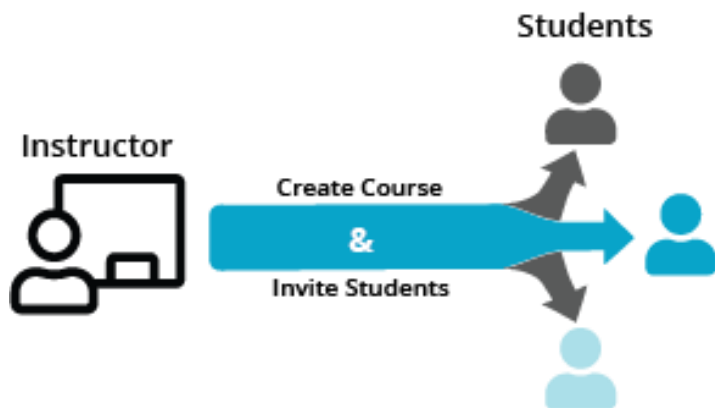
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Gram Stain Protocol

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Attribution

Smith, Molly and Selby, Sara, "[Microbiology for Allied Health Students: Lab Manual](#)" (2017). Biological Sciences Open Textbooks. 16. is licensed under [CC BY 4.0](#).

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Topic

- Gram stain
- Gram positive
- Gram negative
- Safranin
- Crystal violet
- Gram's iodine
- Aseptic technique

Timeline

- **Procedure:** < 1 hour

Safety

- Wear gloves and safety goggles!
- Please refer to the [biosafety guidelines](#) for handling microorganisms in teaching laboratories.

Protocol

Preparation of Microscope Slide:

1. Clean slide with a Kimwipe and alcohol to remove any fingerprints.
2. Draw two circles with your Sharpie on the **bottom** of the slide.
3. Using your inoculation loop, put two **small** drops of water in each circle.
4. Using aseptic technique, remove a **very small** amount of bacteria from the culture tube. Make sure you flame the tube before and after you enter.
5. Smear the bacteria in the drop of water on your slide. You may go out of the perimeter of your circles!
6. Let the slide **air dry completely**.
7. Heat-fix the slide by running it through the flame 3-4 times with the 'smear' side up. Do not flame the side with the bacteria!
8. Let the slide cool completely and you are ready to stain it.

Staining Procedure:

1. At a sink, place **crystal violet** on each smear for 1 minute.
2. Rinse the crystal violet off of the slide by swishing the slide gently in the large beakers labeled 'Crystal Violet'.
3. Tap slide on paper towel to remove most of the water.
4. Place **Gram's iodine** on each smear for 1 minute.
5. Rinse by running water from the tap very slowly over the surface of the slide while holding it at an angle.
6. Tap slide on paper towel to remove most of the water.
7. Place **ethanol** on each smear and for 15-30 seconds. This is the most variable step.
8. Rinse with water and tap dry.
9. Place **safranin** on each smear for 1 minute.
10. Rinse with water and tap dry.
11. Blot *gently* with bibulous paper.
12. Dry the bottom of the slide before placing it on the stage of the microscope and view with the oil immersion lens.

Protocol – Microbiology: Gram Stain

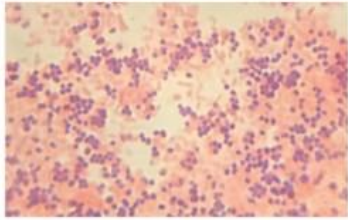
DIFFERENTIAL STAINS				
Stain Type	Specific Dyes	Purpose	Outcome	Sample Images
Gram stain	Uses crystal violet, Gram's iodine, ethanol (decolorizer), and safranin	Used to distinguish cells by cell-wall type (gram-positive, gram-negative)	Gram-positive cells stain purple/violet. Gram-negative cells stain pink.	

Figure 1: Information on Gram stain.


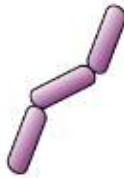

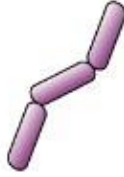

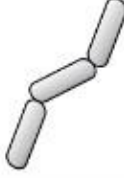

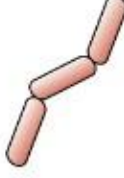
Gram stain process			
Gram staining steps	Cell effects	Gram-positive	Gram-negative
Step 1 Crystal violet <i>primary stain added to specimen smear.</i>	Stains cells purple or blue.		
Step 2 Iodine <i>mordant makes dye less soluble so it adheres to cell walls.</i>	Cells remain purple or blue.		
Step 3 Alcohol <i>decolorizer washes away stain from gram-negative cell walls.</i>	Gram-positive cells remain purple or blue. Gram-negative cells are colorless.		
Step 4 Safranin <i>counterstain allows dye adherence to gram-negative cells.</i>	Gram-positive cells remain purple or blue. Gram-negative cells appear pink or red.		

Figure 2: Gram-staining is a differential staining technique that uses a primary stain and a secondary counterstain to distinguish between gram-positive and gram-negative bacteria.