A Video To Teach The Gram Stain Procedure In A Flipped Laboratory Or Lecture

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Video URL: https://www.youtube.com/watch?v=4QlimbyuK0M

Overview: The Gram stain is a common microbiological techniques used to differentiate between two major groups of bacteria, gram-positive and gram-negative, based on the composition of the bacterial cell wall. Cell walls with a thick peptidoglycan layer in the cell wall are termed gram-positive. They will appear purple due to the crystal violet stain held in the peptidoglycan layer. Gram-negative bacteria have a thin peptidoglycan layer and appear pink to red due to the safranin counterstain that masks the crystal violet. This is an important, fundamental, microbiological technique that is used throughout microbiology. This video can be used as a stand-alone resource to teach the Gram staining technique and the process of visualizing and identifying bacterial cells. It can also be used as a resource for a “flipped” laboratory exercise in which students first view the video on their own prior to attending a lab session when they will conduct a gram stain.

Learning Objectives: After viewing this video, students should:

- Understand the basic structural features of the bacterial cell wall.
- Understand the steps of the Gram stain procedure.
- Develop skills to identify and differentiate gram-positive and gram-negative bacteria.

Introduction: The Gram stain is an important technique in microbiology that is often a first step in characterizing and identifying bacteria. In this procedure, cells are fixed to a microscope slide and a series of stains, washes, and counterstains are applied to the cells. The Gram stain differentiates bacteria into two major groups based on the thickness of the peptidoglycan layer in the bacterial cell wall. Bacterial cell walls contain a peptidoglycan layer that differs in thickness among groups. Cells with a thick peptidoglycan layer in the cell wall are termed gram-positive. They appear purple due to the crystal violet stain held in the thick peptidoglycan layer. Gram-negative bacteria have a thin peptidoglycan layer that does not retain as much of the crystal violet. Instead, they will appear pink to red due to the safranin counterstain that masks the crystal violet in the thin peptidoglycan layer. These differences in the cell wall structure can be important determinants of bacterial sensitivity to antibiotics making the Gram stain an essential technique for students preparing for careers in the health professions to encounter early in their education. We developed this video to support teaching the Gram stain technique.

Although this video can be successfully used as a stand-alone resource, we typically use it as part of a flipped laboratory design in which background information that would typically be delivered in a lecture at the beginning of the laboratory meeting is provided to students in a video format. Students are expected to view prior to attending a class or laboratory session when they will conduct a Gram stain using information about the procedure presented in the video. Students can view the video prior to lab and engage with the material repeatedly to improve their preparation for lab and be ready to conduct the Gram stain upon arriving in lab giving them more time to engage in other activities.

Procedure: Students are assigned the video prior to attending lab. It is recommended that instructors should administer a quiz or other appropriate assessment at the beginning of lab to promote attentive viewing of the video and preparation for the laboratory sessions. Smith and Hussey (2005) provide details of the Gram stain procedure, reagents, and theoretical basis of the protocol.

Transcript with Timing

0:15 The Gram Stain is a simple microbiology technique used to differentiate between Gram-positive

0:20 and Gram-negative bacteria based on how cells stain.

0:25 For the Gram stain you will need the materials listed here

0:40 To begin, sanitize the bench

0:43 cleaning the workspace is essential when working with

0:46 microbes to retain sterile conditions.

0:55 Next pour a small amount of water into

0:58 the beaker.

1:05 Using a permanent marker, label the slide with the sample name or the organism

1:09 being tested.

1:24 Using a match, light the alcohol lamp.

1:33 Place the tip of the loop into the flame until it glows red.

1:45 Using the loop place a

1:51 small drop of water onto the slide, lightly tap to transfer

1:56 After transferring the water, re-flame the loop until it glows red.

2:01 Allow the loop to briefly cool

2:09 and pick up the glass tube, remove the lid and gently flame sterilize the opening.

2:13 Insert the loop into the tube without touching the sides and gather a small

2:17 amount of bacteria from the slant.

2:21 After removing the loop gently flame sterilize the opening of the glass

2:23 tube and return the cap.

2:28 Next, pick up the slide and add the bacteria to the water droplet.

2:32 Mix and smear into a very thin layer layer.

2:38 creating a thin layer promotes drying as well as reducing clumps of bacteria.

2:41 After creating the smear, re-flame the loop until it glows red.

2:47 Allow the slide to air dry, until it looks hazy with no water droplets.

2:51 Next the sample needs to be heat fixed.

2:55 This is done by passing the slide through the flame

2:56 two or three times.

3:00 In this way, it allows bacteria to stick to the side.

3:03 However, if the slide becomes too hot to

3:07 the touch you need to restart, as the bacteria have been cooked.

3:14 Working over a sink or beaker flood, the slide with Crystal Violet to cover the bacterial smear.

3:28 Allow to stain for one minute.

3:32 After one minute, rinse the Crystal Violet away with water

3:35 until the water running off the slide is clear.

3:39 Take care to avoid directly spraying the smear with the jet of water when rinsing

3:45 as the bacteria in the smear can be washed off.

3:52 Flood the slide with Gram's Iodine, to cover the bacterial smear, and allow to stain for one minute.

4:06 Rinse the Gram's Iodine away with water the water till the water running off the slide is clear.

4:11 Decolorize the sample with ethanol which dissolve the outer membrane and washes away the

4:14 crystal Violet from gram negative bacteria.

4:15 This must be done quickly to

4:17 not decolorize Gram-positive bacteria.

4:19 With a dropper full of ethanol hold the

4:22 slide at a 45 degree angle and apply to the top.

4:25 The sample is decolorized when the liquid

4:27 running off the slide is faintly purple.

4:30 This should take less than 30 seconds.

4:34 To stop decolorizing, quickly rinse the ethanol away

4:41 taking care when rinsing to not wash away the smear.

4:50 Flood the slide with Safranin to cover the bacterial smear and allow to stain for one minute.

4:59 The Safranin will stain the decolorized Gram-negative bacteria pink.

5:09 Rinse the Safranin away with water, until the water running off the slide is clear.

5:15 Now the slide needs to be dried.

5:20 Place within the Bibulous Paper and close the booklet.

5:23 Lightly press, but do not rub

5:27 Carefully remove the slide from the Bibulous paper

5:30 The bacteria on the slide can now be viewed using oil immersion

5:37 Both Gram-positive and Gram-negative bacteria, contain peptidoglycan in their cell walls.

5:39 which is stained with Crystal Violet

5:42 In this Gram-positive cross-section notice the thick layer of peptidoglycan

5:46 orange on the outside of the cell membrane.

5:48 Gram-negative bacteria have

5:52 an inner cell membrane, a THIN layer of peptidoglycan and an outer membrane.

5:55 This outer membrane is dissolved and

5:58 the thin layer of peptidoglycan looses it Crystal Violet when decolorized.

6:01 Safranin was used to counter-stain the Gram-negative cells

6:03 pink to make them visible.

6:08 Here is an example of Gram-negative bacteria stained, notice the pink color.

6:14 This is an example of a mixed culture showing both Gram-negative and Gram-positive bacteria.

6:20 Note the differences in cell shape, size and color.

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References

Ann C. Smith, Marise A. Hussey. 2005. Gram stain protocols. American Society of Microbiology. Available at: http://www.asmscience.org/content/education/protocol/protocol.2886