



High School Science Virtual Learning

**Applied Biological Sciences**

**Gram Staining**

April 8, 2020



Applied Biological Sciences  
Lesson: April 8, 2020

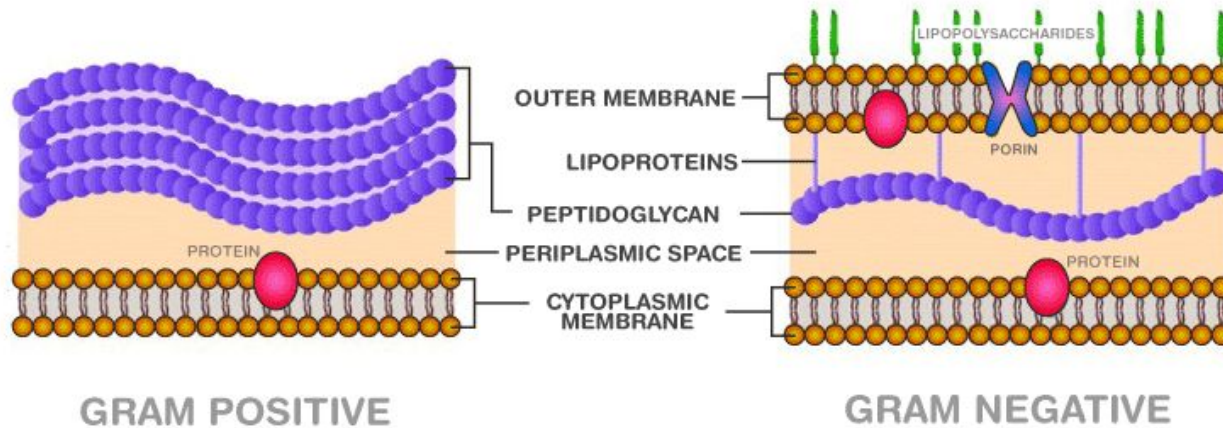
**Objective/Learning Target:**

Understand the importance and process for conducting a Gram Stain procedure. (review)

# Let's Get Started:

1. Using the image below, what are the physical differences between a gram negative and gram positive bacteria?
2. How does this relate to the gram stain reaction?

## GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA



## Let's Get Started: Answer

1. Using the image below, what are the physical differences between a gram negative and gram positive bacteria?
  - a. Gram-positive bacteria have a cell wall composed of a single macromolecule of peptidoglycan. Gram-negative bacteria have a thin peptidoglycan layer surrounded by a lipopolysaccharide-containing bilayer.
2. How does this relate to the gram stain reaction?
  - a. These differences in cell wall structure cause the difference in the Gram-stain reaction.

# Activity

View the [Gram Stain Virtual lab](#) and answer the following in your notebooks or on a sheet of paper:

1. What color do gram negative bacteria stain? Gram positive?
2. Why is it important to sterilize equipment when doing gram stains?
3. What 4 chemicals are needed to do a gram stain? What does each do?
4. Summarize the steps of how to do a gram stain with the sample.

Practice performing a gram stain

# Activity - Answers

1. What color do gram negative bacteria stain? Gram positive?
  - a. Gram positive stain purple, gram negative stains pink
2. Why is it important to sterilize equipment when doing gram stains?
  - a. Reduce contamination of the sample
3. What 4 chemicals are needed to do a gram stain? What does each do?
  - a. Crystal violet - stains purple to indicate gram positive bacteria
  - b. Gram's iodine - acts to fix crystal violet to cell wall
  - c. 95% alcohol - washes all excess dye off the slide
  - d. Safranin - stains pink to indicate gram positive bacteria
4. Summarize the basic steps of how to do a gram stain with the sample.
  - a. Heat fix bacteria to slide, add crystal violet and wash, flood with iodine and wash, add alcohol and wash, add safranin and wash, pat dry slide

# Why is the Gram-stain reaction so important?

Use the evidence in the table to the right to develop your CER response to the question above:

- Claim
- Evidence
- Reasoning

Empirically prescribed antibiotics	Gram negative bacteria		Gram positive bacteria	
	Sensitive	Resistant	Sensitive	Resistant
Ceftriaxone	5	4	1	1
Nitrofurantoin	1	1	0	0
Ofloxacin	3	3	1	2
Norfloxacin	4	5	1	1
Amoxicillin-clavulanate	1	0	1	0
Ciprofloxacin	4	3	1	0
Cefixime	0	5	0	1

# Why is the Gram-stain reaction so important? **Answer**

Use the evidence in the table to the right to develop your CER:

- **Claim**
  - Gram staining is important for determining which antibiotic to choose.
- **Evidence**
  - Gram negative bacteria are resistant to 6/7 antibiotics.
- **Reasoning**
  - Some antibiotics are not effective against gram negative bacteria.

Empirically prescribed antibiotics	Gram negative bacteria		Gram positive bacteria	
	Sensitive	Resistant	Sensitive	Resistant
Ceftriaxone	5	4	1	1
Nitrofurantoin	1	1	0	0
Ofloxacin	3	3	1	2
Norfloxacin	4	5	1	1
Amoxicillin-clavulanate	1	0	1	0
Ciprofloxacin	4	3	1	0
Cefixime	0	5	0	1



# Practice

Answer the following multiple choice questions.

1. During staining, the smear is heat-fixed in order to.
  - a. kill the organism so that dyes will penetrate
  - b. attach the organism firmly to the slide
  - c. kill the organism and attach the organism firmly to the slide
  - d. neither kill the organism nor attach the organism firmly to the slide
2. If the iodine step were omitted in the Gram-staining procedure, what color would you expect Gram-positive bacteria to stain and what color would you expect Gram-negative bacteria to stain?
  - a. Gram-positive = purple; Gram-negative = pink
  - b. Gram-positive = purple; Gram-negative = purple
  - c. Gram-positive = purple; Gram-negative = colorless
  - d. Gram-positive = pink; Gram-negative = purple
  - e. Gram-positive = pink; Gram-negative = pink

# Practice

3. The substances listed below are used in various differential staining techniques. Which of the following lists the correct order for the solutions used in the Gram stain?

1. Alcohol
2. Gram's iodine
3. Carbol fuchsin
4. Crystal violet
5. Methyl red
6. Methylene blue
7. Safranin (or basic fuchsin)

- a. 1 - 3 - 4 - 2
- b. 1 - 7 - 2 - 4
- c. 4 - 2 - 1 - 7
- d. 1 - 4 - 3 - 6
- e. 4 - 7 - 1 - 5

# Practice

4. Let's assume that you were teaching the Gram stain to 5 students, and each student did one thing wrong during the Gram-staining procedure, as shown in the following list. All five students were staining slides containing a mixed culture of a Gram-positive rod and a Gram-negative coccus. Of the 5 students listed below which one will probably get a CORRECT Gram reactions (assuming everything else was done correctly)?
- One put the alcohol on first instead of when it should have been applied.
  - One used safranin (a red dye) instead of basic fuchsin as a counterstain.
  - One forgot the alcohol step completely.
  - One forgot to use a counter stain.
  - One left the alcohol on for 20 minutes instead of 20 seconds.

# Practice - Answers

1. C
2. E
3. C
4. B

# Additional Practice

1. View this [instructional video](#) to see how this procedure is done in a lab.
2. Complete this [online worksheet](#) to check your understanding.