

# THE GRAM STAIN

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## OBJECTIVE/RATIONALE

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One of the first procedures performed by the medical microbiologist for the identification of bacteria is the Gram Stain. The student will learn the procedure for performing the gram stain.

TEKS 121.14 (c) 1A, 1B, 4A, 4B, 4C

TAKS ELA 1, 4

Mathematics 8

Science 1, 2, 4

National Science Education Standards A9-12; C9-12

National Health Care Skills Standards .01, .04, .05, .06, .07, .08

National Curriculum Standards for School Mathematics S1; S3; S10; S11

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## KEY POINTS

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### **Gram Stain Power Point Presentation**

- I. Bacteria (Microorganisms)
  - A. A pathogen is microorganism that causes disease.
  - B. Nonpathogenic – microorganisms that do not cause disease
    - Microorganisms that live within the body and do not cause disease are called normal flora.
  - C. Infection is caused by microorganisms entering the host and multiplying.
  - D. Toxins – poisonous substance produced by a cell.
    - 1 Exotoxins
    - 2 Endotoxins
  - E. Bacteria damage the host with exotoxins: intracellular toxin released into the environment (the human body) by living gram-positive bacteria. Endotoxins are phospholipopolysaccharide macromolecules that comprise the gram-negative bacterial cell wall and are released only when the cells is lysed as it dies.
  - F. Types of bacteria
    1. Bacilli (rod-shaped)
    2. Cocci (round shaped)
    3. Spirilli (spiral shaped)
  - G. Koch's postulates - Robert Koch, a German microbiologist who identified four conditions that must be satisfied to prove that an agent causes disease:
    1. The organism should be found in all cases of the disease, and in the locations affected by the disease.
    2. The organism should be grown in pure culture.
    3. The organism should reproduce the disease when introduced into another susceptible host.

4. The same organism should be re-isolated from the intentionally infected individual.
- II. Differentiation
- A. Gram Stain
1. Discovered by Hans Christian Gram in the 1800's.
  2. Differentiates Gram-positive from Gram-negative bacteria
  3. Procedure: See handout
  4. Reagents:
    - a. Crystal violet-Primary stain; stains all bacteria blue to purple
    - b. Gram's iodine-A mordant that enhances reaction between the cell wall and the primary stain.
    - c. Ethyl alcohol or acetone (Gram decolorizer)-Gram-positive bacteria retain the primary stain (crystal violet) because of the makeup of their cell wall. Gram-negative bacteria do not retain the primary stain during the decolorizing process because of the large amount of lipopolysaccharide in the cell wall.
    - d. Safranin-This is the counterstain. It will have no effect on the Gram-positive bacteria, but stains the Gram-negative bacteria pink to red.
- B. Results
- a. Gram-positive bacteria: Stain dark blue to purple.
  - b. Gram-negative bacteria: Stain pink to red.
  - c. Note: Do not use cultures older than 48 hours because Gram-positive may stain as Gram-negative bacteria.
- III. Pathogens
- a. Gram-positive pathogens
    1. Staphylococcus aureus (cocci in clusters, skin infections)
    2. Streptococcus pyogenes (cocci in chains, strep-throat)
    3. Clostridium tetani (rods, tetanus)
  - b. Gram-negative pathogens
    1. Neisseria gonorrhoea (cocci in pairs called diplococci, gonorrhoea)
    2. Pseudomonas aeruginosa (rod, opportunistic infection microorganism)
    3. Vibrio cholera (rod, cholera)

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## ACTIVITIES

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- I. Complete the Gram Stain Laboratory Investigation.

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## MATERIAL/RESOURCES

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### Gram Stain Procedure Handout

Prepared slides of Gram-negative and Gram-positive bacteria

Gram stain supplies: Crystal Violet

Gram's Iodine

Acetone or Ethyl Alcohol (Gram decolorizer)

Safranin

(Hint: Number the bottles 1 through 4 for student use)

Bacterial cultures of Gram-positive and Gram-negative bacilli and cocci

Microscope slides

Microscopes

Sinks

Gloves

Inoculating Loops

Bunsen Burners

Oil for oil immersion lens

Timer

Biohazard container

Staining racks

Goggles

Bilbous paper

Disinfecting solution

Paper towels

Internet: [www.cells.com](http://www.cells.com)

[www.courses.ahc.umn.edu/pharmacy/5825/GSPage02.html](http://www.courses.ahc.umn.edu/pharmacy/5825/GSPage02.html)

<http://newmedia.kent.edu/microbiology/default.htm>

<http://www.carolinabiologicals.com>

Hunt, Madelyn D. and Lyons, Lisa C. (1996). Exploring the Unseen World of Microbes. New York: McGraw-Hill Publishing Co.

Burton, Gwendolyn R. W. and Engelkirk, Paul G. (2000). Microbiology for the Health Sciences, 6<sup>th</sup> ed. Baltimore: Lippincott Williams & Wilkins.

Delost, Maria Dannessa, Introduction to Diagnostic Microbiology, 1997, ISBN 0-8016-7853-6

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## ASSESSMENT

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Laboratory Investigation Rubric

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## ACCOMMODATIONS

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For reinforcement, the student will review the steps of the Gram stain procedure and repeat the laboratory investigation.

For enrichment, the student will choose a microorganism and research the characteristics of growth and the diseases it may cause and the consequences of an infection. Design a multimedia presentation.

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## REFLECTIONS

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## **GRAM STAIN PROCEDURE**

1. Prepare a thin smear of the culture or specimen to be examined. Allow the slide to air dry and heat fix. Place smear on the staining rack.
2. Flood the slide with crystal violet for 30 seconds to one minute.
3. Rinse with distilled water. Tilt the slide to remove excess water.
4. Flood the slide with Gram's iodine for one minute.
5. Rinse with distilled water. Tilt the slide to remove excess water.
6. Hold the slide by the short edge using forceps or clothespin. Flood the slide with ethyl alcohol or acetone (Gram decolorizer) for about 15 seconds.
7. Rinse with distilled water. Tilt the slide to remove excess water.
8. Counterstain the smear by flooding the slide with Safranin for 30 seconds.
9. Rinse with distilled water.
10. Blot dry with bibulous paper or air dry.
11. Examine the slide under oil immersion objective for Gram-positive or Gram-negative bacteria.
12. Record findings.

# THE GRAM STAIN LABORATORY INVESTIGATION

**NAME:**

**DATE:**

## **PURPOSE:**

In this laboratory investigation, the student will learn the procedure for performing the gram stain.

## **BACKGROUND INFORMATION:**

## **MATERIALS:**

Prepared slides of Gram-negative and Gram-positive bacteria

Gram stain supplies: Crystal Violet

Gram's Iodine

Acetone or Ethyl Alcohol (Gram decolorizer)

Safranin

(Hint: Number the bottles 1 through 4 for student use)

Bacterial cultures of Gram-positive and Gram-negative bacilli and cocci

Microscope slides

Microscopes

Sinks

Gloves

Inoculating Loops

Bunsen Burners

Oil for oil immersion lens

Timer

Biohazard container

Staining racks

Goggles

Bilbous paper

Disinfecting solution

Paper towels



## **CONCLUSION:**

Please answer the following questions.

1. What is the color seen of Gram-positive bacteria?
2. What is the color seen of Gram-negative bacteria?
3. Describe the three types of bacteria. Draw pictures of their shapes.
4. What is the purpose of Gram's iodine?
5. The oil immersion objective lens provides a **total** magnification of \_\_\_\_\_.
6. A 16-year old high school student presents with sores on his legs that keep spreading. He states he works out every day in the gym and shares towels with other students. The physician cultures the sores and sends two swabs to the laboratory for identification and antibiotic sensitivity. The laboratory technologist performs a Gram stain on one of the swabs. Results of the Gram stain are: Gram-positive cocci in clusters and many white blood cells. What is the suspected bacteria causing the sores (infection of the skin)?