

LABORATORY MODULE 4

GRAM STAIN



INTRODUCTION

The simple staining procedure makes it possible to see bacteria clearly, but it does not distinguish between organisms of similar morphology. Hence, **differential staining** was developed. It is a type staining technique that requires the use of at least four chemical reagents that are applied sequentially to a heat-fixed smear. The first reagent is called the **primary stain**. Its function is to impart its color to all cells. The second stain is a **mordant** used to intensify the color of the primary stain. In order to establish a color contrast, the third reagent used is the decolorizing agent. Based on the chemical composition of cellular components, the **decolorizing agent** may remove the primary stain from the entire cell or only from certain cell structures. The final reagent, the **counterstain**, has a contrasting color to that of the primary stain. Following decolorization, if the primary stain is not washed out, the counterstain cannot be absorbed, and the cell or its components will retain the color of the primary stain. If the primary stain is removed, the decolorized cellular components will accept and assume the contrasting color of the counterstain. In this way, cell types or their structures can be distinguished from each other on the basis of the stain that is retained.



OBJECTIVES

Once this module has been completed, the student should be able to:

- ➊ Explain the chemical and theoretical basis for differential staining procedures.
- ➋ Describe the chemical basis for the Gram stain.
- ➌ Perform the procedure to differentiate between two principal groups of bacteria: gram positive and gram negative.



DISCUSSION

The most important differential stain used in bacteriology is the **Gram stain**, named after Dr. Hans Christian Gram. It divides bacterial cells into two major groups, gram-positive and gram-negative, which makes it an essential tool for classification and differentiation of microorganisms.

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Gilda L. Jones



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Table of Contents Lab Module Gram Staining Introduction Clark College

1. Understanding the eBook Lab Module Gram Staining Introduction Clark College
 - The Rise of Digital Reading Lab Module Gram Staining Introduction Clark College
 - Advantages of eBooks Over Traditional Books
2. Identifying Lab Module Gram Staining Introduction Clark College
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Lab Module Gram Staining Introduction Clark College
 - User-Friendly Interface
4. Exploring eBook Recommendations from Lab Module Gram Staining Introduction Clark College
 - Personalized Recommendations
 - Lab Module Gram Staining Introduction Clark College User Reviews and Ratings
 - Lab Module Gram Staining Introduction Clark College and Bestseller Lists

5. Accessing Lab Module Gram Staining Introduction Clark College Free and Paid eBooks
 - Lab Module Gram Staining Introduction Clark College Public Domain eBooks
 - Lab Module Gram Staining Introduction Clark College eBook Subscription Services
 - Lab Module Gram Staining Introduction Clark College Budget-Friendly Options
6. Navigating Lab Module Gram Staining Introduction Clark College eBook Formats
 - ePub, PDF, MOBI, and More
 - Lab Module Gram Staining Introduction Clark College Compatibility with Devices
 - Lab Module Gram Staining Introduction Clark College Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Lab Module Gram Staining Introduction Clark College
 - Highlighting and Note-Taking Lab Module Gram Staining Introduction Clark College
 - Interactive Elements Lab Module Gram Staining Introduction Clark College
8. Staying Engaged with Lab Module Gram Staining Introduction Clark College
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Lab Module Gram Staining Introduction Clark College
9. Balancing eBooks and Physical Books Lab Module Gram Staining Introduction Clark College
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Lab Module Gram Staining Introduction Clark College
10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine Lab Module Gram Staining Introduction Clark College
 - Setting Reading Goals Lab Module Gram Staining Introduction Clark College
 - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Lab Module Gram Staining Introduction Clark College
 - Fact-Checking eBook Content of Lab Module Gram Staining Introduction Clark College
 - Distinguishing Credible Sources
13. Promoting Lifelong Learning

- Utilizing eBooks for Skill Development
- Exploring Educational eBooks

14. Embracing eBook Trends

- Integration of Multimedia Elements
- Interactive and Gamified eBooks

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