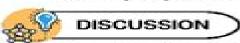# LABORATORY MODULE 4
GRAM STAIN

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:

- Explain the chemical and theoretical basis for differential staining procedures.
- Describe the chemical basis for the Gram stain.
- 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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Linda M. Marler,Jean A. Siders,Stephen D. Allen (MD.)

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The Gram Stain Gilda L. Jones,1986 *Direct Smear Atlas* Linda M. Marler,Jean A. Siders,Stephen D. Allen (MD.),2001 This atlas is a full color spiralbound manual of Gram stained direct smears for medical technologist and medical laboratory technician students and practitioners microbiologists pathologists infectious disease specialists and anyone who interprets Gram stained specimens Featuring over 250 clear photomicrographs taken from actual patient specimens the manual presents a simple easy to use format brief legends accompany each image Three introductory chapters cover Gram stain procedures quality control evaluation and more An invaluable reference and educational tool this manual is effective both as a standalone text and as an accompaniment to Marler Siders and Allen s Direct Smear Atlas CD ROM Lippincott Williams Wilkins 1998

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