MODULE 3: Introduction to Staining

LEARNING OUTCOMES

1. Explain the purpose of staining in microbiology.
2. Discuss the action of basic and acidic dyes when applied to bacterial cells.
3. Name several examples of simple and differential dyes.
4. Compare and contrast simple and differential staining techniques.

Dyes Used in Staining

In their natural state, most of the cells and microorganisms that we observe under the microscope lack color and contrast. This makes it difficult, if not impossible, to detect important cellular structures and their distinguishing characteristics. Staining bacteria for viewing with a microscope provides valuable information about their size, shape, arrangement, and other cellular characteristics that assist in identification.

Dyes are selected for staining based on the chemical properties of the specimen being observed as well as the *chromophore,* which is the charged part of a dye that is responsible for color. Bacterial cells carry a net negative charge, so in most cases, it is preferable to use a basic (alkaline) dye that has a positively charged chromophore. Basic dyes are absorbed by cells to make them visible against a light background (Figure 3.1). Commonly used basic dyes include crystal violet, methylene blue, and safranin.

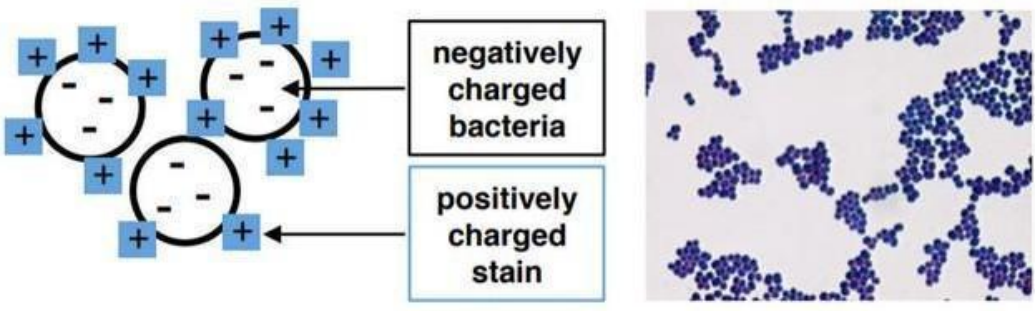


Figure 3.1: The positively charged chromophores of a basic dye such as methylene blue are absorbed by negatively charged bacterial cells.

However, there are times when it is advantageous to use an acidic dye, such as nigrosin or eosin, when staining a sample. These dyes have a negatively charged chromophore which is repelled by the cells, resulting in colorless cells against a dark background (Figure 3.2).

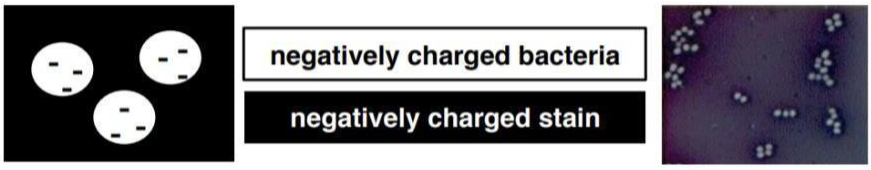
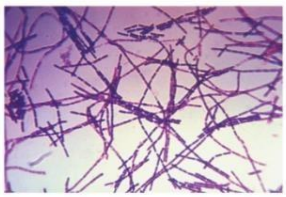


Figure 3.2: Negatively charged chromophores of an acidic dye such as nigrosin are repelled by negatively charged bacterial cells.

Simple vs. Differential Stains

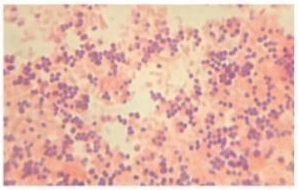
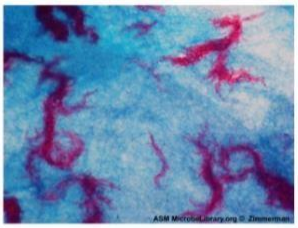
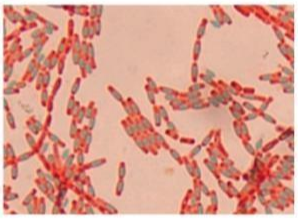
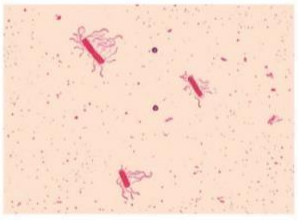
Simple staining techniques involve the application of only one dye to a sample to determine the size, shape, and arrangement of cells or to emphasize certain cellular structures. A simple stain makes all cells in a sample appear to be the same color, even if the sample contains more than one type of organism. Although it is a quick method to determine basic cellular morphology, simple staining often does not provide enough information to distinguish bacteria present in each sample. Table 3.1 shows common simple stains.

Table 3.1. Simple Stains

SIMPLE STAINS				
Stain Type	**Specific Dyes**	**Purpose**	**Outcome**	**Sample Images**
Basic stains	Methylene blue, crystal violet, malachite green, basic fuchsin, carbolfuchsin, safranin	Stain negatively charged molecules and structures, such as nucleic acids and proteins	Positive stain	
Acidic stains	Eosin, acid fuchsin, rose bengal, Congo red	Stain positively charged molecules and structures, such as proteins	Can be either a positive or negative stain, depending on the cell's chemistry.	
Negative stains	India ink, nigrosin	Stains background, not specimen	Dark background with light specimen	

In contrast to simple staining, *differential staining techniques* use multiple dyes and offer more information about cell types (Table 3.2). This may include cell wall thickness or the presence of mycolic acids, which are found in bacteria that cause tuberculosis. Since each dye of the procedure interacts with specific cellular components, this method distinguishes differences between bacteria and thus provides more details for identification. The most common differential stains performed in clinical microbiology laboratories are the Gram stain and acid-fast stains. Other differential stains include those for endospores, capsules, and flagella.

Table 3.2. Differential Stains

DIFFERENTIAL STAINS				
Stain Type	**Specific Dyes**	**Purpose**	**Outcome**	**Sample Images**
Gram stain	Uses crystal violet, Gram's iodine, ethanol (decolorizer), and safranin	Used to distinguish cells by cell-wall type (gram-positive, gram-negative)	Gram-positive cells stain purple/violet. Gram-negative cells stain pink.	
Acid-fast stain	After staining with basic fuchsin, acid-fast bacteria resist decolorization by acid-alcohol. Non acid-fast bacteria are counterstained with methylene blue.	Used to distinguish acid-fast bacteria such as *M. tuberculosis*, from non–acid-fast cells	Acid-fast bacteria are red; non–acid-fast cells are blue.	
Endospore stain	Uses heat to stain endospores with malachite green (Schaeffer-Fulton procedure), then cell is washed and counterstained with safranin.	Used to distinguish organisms with endospores from those without; used to study the endospore.	Endospores appear bluish-green; other structures appear pink to red.	
Flagella stain	Flagella are coated with a tannic acid or potassium alum mordant, then stained using either pararosaline or basic fuchsin.	Used to view and study flagella in bacteria that have them.	Flagella are visible if present.	
Capsule stain	Negative staining with India ink or nigrosin is used to stain the background, leaving a clear area of the cell and the capsule. Counterstaining can be used to stain the cell while leaving the capsule clear.	Used to distinguish cells with capsules from those without.	Capsules appear clear or as halos if present.