STAINING A SPECIMEN

Not all specimens can be clearly seen under a microscope. Sometimes the specimen blends with other objects in the background because they absorb and reflect approximately the same light waves. You can enhance the appearance of a specimen by using a stain. A stain is used to contrast the specimen from the background.

A stain is a chemical that adheres to structures of the microorganisms and in effect dyes the microorganisms so t can be easily seen under a microscope. Stains used in microbiology are either basic or acidic.

Basic stains are cationic and have positive charge. Common basic stains are methylene blue, crystal violet, safranin, and malachite green. These are ideal for staining chromosomes and the cell membranes of many bacteria.

Acid stains are used to identify bacteria that have a waxy material in their cell walls. This form of staining differentiates bacteria.

Quick Guide for Staining Techniques

Type	Number of Dyes Used	Observations	Examples
Simple stains	Use a single dye	Size, shape and arrangement of cells	Methylene blue Safranin Crystal violet
Differential stains	Use two or more dyes to distinguish different types or different structures of bacteria	Distinguishes gram-positive or gram-negative. Distinguishes the member of mycobacteria anmd nocardia from other bacteria	Gram stain Zehl-Nielsen acid-faast stain
Special stains	These stains identify specialized structures	Exhibits the presence of flagella. Exhibits endospores	Shaefter-Fultion spore staining

Types of Stains

There are two types of Stains: simple and differential.

Simple Stain

A simple stain has a simple basic dye that is used to show shapes of cells and the structures within a cell. Methylene blue, safranin, carbolfluchsin and crystal violet are common simple stains that are found in most microbiology laboratories.

Differential Stain

A differential stain consists of two or more dyes and is used in the procedure to identify bacteria. Two of the most commonly used differential stains are the Gram stain and the Zehl-Nelson acid-fast stain.

In 1884 Hans Christian Gram, a Danish physician, developed the Gram stain. Gram-stain is a method for the differential staining of bacteria. Gram-positive microorganisms stain purple. *Gram-negative* microorganisms stain pink *Staphylococcus ureus,* a common bacterium that causes food poisoning, is gram-positive, Escherichia coli is gram-negative.

The *Ziehl-Neelsen* acid-fast stain, developed by Franz Ziehl and *Friedrick Neelsen,* a red dye that *attraches* to the waxy material in the cell walls of bacteria such as *Mycobacterim tuberculosis,* which is the bacterim that causes tuberculosis, and *Mycobacterim leprae,* which is the bacterium that causes leprosy. Microorganisms that retain the Zichl-Neelsen acid-fast strain are called acid-fast. Those that do not retain it turn blue because the microorganism doesn't absorb the Ziehl-Neelsen acid-fast stain.

How to Gram-stain a specimen.

Observing Microorganisms

1. Prepare the specimen using the heat fixation process (see "Smear" above).
2. Place a drop of crystal violet stain on the specimen.
3. Apply iodine on the specimen using an eyedropper. The iodine helps the crystal violet stain adhere to the specimen. Iodine is a mordant which is a chemical that fixes the stain to the specimen.
4. Wash the specimen with ethanol or alcohol-acetone solution, then wash with water.
5. Wash the specimen to remove excess iodine. The specimen appears purple in colour.
6. Apply the safranin stain to the specimen using an eyedropper.
7. Wash the specimen.
8. Use a paper towel and blot the specimen until the specimen is dry.
9. the specimen is ready to be viewed under the microscope. Gram-positive bacteria appear purple and gram-negative bacteria appear pink.

Here is how to apply the Zichi-Neelsen acid-fast stain to a specimen.

1. Prepare the specimen (see "Smear" earlier in this chapter).
2. Apply the red dye carbon-fuchsin stain generously using an eyedropper.
3. Let the specimen sit for a few minutes.
4. Warm the specimen over steaming water. The heat will cause the stain to penetrate the cell wall.
5. Wash the specimen with an alcohol-acid or acid-alcohol decolorizing solution consisting of 3 percent hydrochloric acid and 93 percent ethanol. The hydrochloric acid will remove

the color from non-acid-fast cells and the background. Acid-fast cells will stay red because the acid cannot penetrate the cell wall.

6. Apply methylene blue stain on the specimen using an eyedropper.

Special Stains

Special stains are paired to dye specific structures of microorganisms such as endospores, flagella and gelatinous capsules. One stain in the pair is used as a negative stain. A negative stain is used to stain the background of the microorganism

Table 3-5. Scientists and Their Contribution

Year	Scientists	Contribution
1854	Hans Christian Gram	Developed the Gram stain used to stain and identify bacteria.
1882	Franz Ziehl and Friedrick Neelsen	Developed the Ziehl-Neelsen acid-fast stain used to stain bacteria

Causing the microorganisms to appear clear. A second stain is used to colorize specific structures within the microorganism. For example, nigrosin and India ink are used as a negative stain and methylene blue is used as a positive stain.

The Schaeffer-Fulton endoscope stain is a special stain that is used to colorize the endospore. The endospore is a dormant part of the bacteria cell that protects the bacteria from the environment outside the cell.

Here is how to apply the Schaeffer-Fulton endospore stain.

1. Prepare the specimen (See "Senear" earlier in the chapter)
2. Heat the malchite gree stain over a Bunsen burner until it becomes fluid.
3. Apply the malachite green to the specimen using an eyedropper.
4. Wash the specimen for 30 seconds.
5. Apply the safranin stain using an eyedropper to the specimen to stain parts of the cell other than the endospore.
6. Observe the specimen under the microscope.

Prokaryotic Cells and Eukaryotic Cells

The life processes of a living thing include

* Metabolism. Breakdown nutrients for energy or extract from the environment.
* Responsiveness. React to internal and external environmental changes.
* Movement. Whether it is the entire organism relocating within its environment, cells within that organism or the organelles inside those cells.

* Growing. Increase the size or number of cells.
* Differentiation. The process whereby cells that are unspecialized become specialized. (An example would be a single fertilized human egg, developing into an individual) Prokaryotic cells do not differentiate.
* Reproduction. Form new cells to create a new individual.

Prokaryotic Cells

A prokaryotic cells is a cell that does not have a true nucleus. The nuclearstructure is called a nuclenid. The nuclenid contains most of the cell's genetic material and is usually a single circular molecule of DNA. Karyo-is Grek for "kernet". A prokaryotic organism, such as a bacterium, is a cell that lacks a membrance-bound nucleus or membrane-bound organetles. The exterior of the cell usually has glycocalyx, flagellum, fimbriae, and pili.

Differences between Prokaryotic and Eukaryotic Cells

Characteristics	**Prokaryotic Cells**	**Eukaryotic Cells**
Cells wall	Include peptidoglycan Chemically complex	Chemically simple
Plasma membrane	No carbohydrates No sterols	Contain carbohydrates Contain sterols
Glycocalyx	Contain a capsule or a slime layer	Contained in cells that lack a cell wall
Flagella	Protein building blacks	Multiple micronutrient
Cytoplasm	No cytoplasmic streaming	Contain cytoskeleton Contain cytoplasmic streaming
Membrane-bound Organelles	None	Endoplasmic reticulum Golgi complex Lysosomes Mitochrondria Chloroplasts
Ribosomes	70S	SOS Ribosomes located in Organelles are 70S
Nucleus	No nuclear membranes No nucleui 0.2-2.0 mm un diameter	Have a nucleus Have a nuclear membrane Have a nucleoli 10-100 m in diameter
Chromosomes	Single circular chromosome No Bistones	Multiple linear chromosomes Have histones
Cell division	Binary fussion	Mitosis
Sexual reproduction	No melosis DNA transferred in fragments	Metosis

Parts of Prokaryotic Cells

Glycocalyx

Glycocalyx is a strictly, sugary envelope composed of polysaccharides and/or polypeptides that surround the cell. Glycocalyx is found in one or two states. It can be firmly attached to the cell's surface, called capsule, or loosely attaché, called slime layer. A slime layer is water-soluble and is used by the prokaryotic to adhere to surfaces external to the cell.
Glycicalyx is used by a prokaryotic cell to protect it against attack from the body's immune system.

Flagella

Flagella made of protein and appear "whip-like." They are used by the prokaryotic cell for mobility. Flagella propel the microorganism away from harm and towards food in a movement known as taxis. Movement caused by a light stimulus is referred to as phototaxis and a chemical stimulus causes a chemotaxis movement to occur.

Flagella can exist in the following form

Monotrichous: One Flagellum
Lophotrichus: Two or more Flagella that are at one end of the cell
Amphitrichous: Flagella at two ends of the cell.
Endoflagellum: A type of amphitrichous flagellum that is tightly wrapped around spirochetes. A spirochete is a spiral-shaped bacterium that moves in a corkscrew motion. *Borrelia burgdorferi*, which is the bacterium that causes lyme disease, exhibits an endoflagellum.

Fimbriae

Fimbrae are proteinaceous, sticky, bristle-like projections used by cells to attach to each other and to objects around them. *Neiseseria gonorrhoeae*, the bacterium that causes gonorrhea uses fimbriae to adhere to the body and to cluster cells of bacteria.

Pili

Pili are tubules that are used to transfer DNA from one cell to another cell similar to tubes used to fuels aircraft in flight. Some are also used to attach one cell to another cell. The tubules are made of protein and are shorter in length than flagella and longer than fimbriae

Cell Wall

The prokaryotic cell's wall is located outside the plasma membrane and gives the cell its shape, providing rigid structural support for the cell. The cell wall also protects the cell from its environment.
Pressure within the cells builds as fluid containing nutrients enters the cells. It is the job of the cell wall to resist the pressure the same way that the walls of a balloon resist the build-up pressure whan it is inflated. If pressure inside the cell becomes too great, the cell wall bursts, which is referred to as lysis.

The cell wall of many bacteria is composed of *peptidoglycan*, which covers the entire surface of the cell. *Peptidoglycan* is made up of a combination of peptide bonds and carbohydrates, either N-acetylmuramic acid, commonly referred to as NAM, or N-acetylglycosamine, which is known as NAG
The wall of a bacterium is classified in two ways:

Gram-positive: A gram-positive cell wall has many layers of *Peptidoglycan* that retain crystal violet dye when the cell is stained. This gives the cell a purple color when seen under a microscope

Gram-negative: A gram negative cell wall is thin. The inside is made of *Peptidoglycan.* The outer membrane is composed of phospholipids and lipopolysaccarides. The cell wall does not retain the crystal violet dye when the cell is stained. The cell appears pink when view with a microscope.