Bacterial Staining

Stain is a substance that adheres to a cell giving the cell color.

Staining is a technique used to enhance contrast in samples, generally at the microscopic level. **Staining** means coloring microorganism with a dye that emphasizes a certain structure. **Staining** makes the microscopic observation possible for the internal and external structures of microorganisms. The dyes used to stain bacteria are basic dyes. These dyes contain ionizable groups (positive charge) that are attracted to the negatively charge components of the bacterial cell.

Staining bacterial cell for microscopic examination makes it more visible for human eye and to study unique characteristic including cell size, shape, arrangement and other properties.

- There are three types of stains in microbiology lab:
  1. Simple stains.
  2. Differential stains (Gram stain & Acid-Fast stain)
  3. Structural stain (capsular, endospores, flagellar stains)

Stains can be classified based on the nature of chromogen into:

1. Acidic stain (Anionic stain) with –ve charge
   - Nigrosin
   - Malchite green
2. Basic stain (Cationic stain) with +ve charge
   - Crystal violet
   - Methylene blue
   - Safrinin
   - Basic fuchsin
3. neutral stain:
   - Eosinate of methylene blue
   - Giesma stain

- Steps of Bacteria Staining include:
  A. Smear Preparation
  B. Staining
  - Smear: is a thin film of material containing microorganism which spread over the slide.

Specimens: urine, stool, blood, CSF, Pus, pleural fluid, broth or agar media and others.

In a smear preparation, cells from a culture or a specimen spread in a thin film over a small area of a microscope slide, dried, and then fixed to the slide by heating or other chemical fixatives.

"A good smear preparation is the key to a good stain"

The purpose of making a smear is to fix the bacteria on the slide and to prevent the sample from being lost during a staining procedure.

- Procedure:
  1. Label a clean glass slide using wax marker. "Note that it is important to recognize the side of the glass slide that you put your bacterial sample on".
  2. Add a small drop of saline to the slide.
  3. With an inoculation loop or stick, pick up a small amount of bacteria. Mix it well with the saline and spread the mixture over a wider area of the slide.
  4. Air dry the bacterial specimen on the slide.
  5. Heat fix the bacterial specimen by passing the slide slowly over the flame twice.
     * "Heat fixing kills cells, and adheres them to the slide".
     * Cells will be rinsed off the slides if they are not heat fixed properly.
     * Be careful not to overheat the slides in this procedure.

After heat-fixing is complete, you are ready to simple or gram stain your slide.

B. Staining

"Simple Stain"

Generally, Simple staining include directly staining the bacterial cell with a positively charged dye in order to see bacterial detail such as size and morphology of cells, in contrast to negative staining where the bacteria remain unstained against a dark background.

- using **one stain only** such as safranin, crystal violate or methylene blue and appears in one color.
- **It is used to determine the:**
  1. **Size** of microorganism
  2. **Shape:** spherical (cocci), rod (bacilli), pleomorphic (coccobacilli)

and spiral (spirillum).

3. **Grouping or arrangement:** single, pairs, chain or cluster.

Simple Stain Procedure:

1. **Prepare** a fixed smear of bacteria.
2. **Flood** the slide with simple stain for (1 minute).
3. **Wash off** the stain with tap water.
4. **Dry** the slide (place the slide between the layers of filter papers).
5. **Examine** under oil immersion lens.

"Differential stain"

Using two or more dyes successively to differentiate between organisms according to their response to these dyes, include:

1. Gram stain
2. Acid fast stain

"Gram Stain"

The name comes from the Danish bacteriologist Hans Christian Gram, who developed this technique in 1884. The Gram stain is the most common differential stain used in microbiology. The unique cellular components of the bacteria will determine how they will react to the different dyes.

This method of staining is used to classify bacterial species into two large important groups, **Gram-positive** bacteria such as *Staphylococcus aureus* and **Gram-negative** bacteria Such as *E.coli*.

We have two stains used in this technique: (Primary and counter stain)

Bacteria that retain the primary dye **(crystal violet)** appear **dark purple** and are called **gram positive**. While bacteria that lose the primary dye after the application of the decolorizer agent, take up the counter stain **(safranin)** or **(basic fuchsin stain)** and appear red are termed **gram negative**.

"The ability to resist decolorization is related to the chemical composition and structure of cell wall".

"The decolorization step is the critical step that differentiate the bacteria"

"Grams iodine is a mordant that increase the affinity of the primary stain to the bacterial cells".

Gram staining includes four basic steps:

1. Applying a **primary stain** (crystal violet).
2. Adding a **mordant** (Gram's iodine).
3. Rapid **decolorization** with ethanol, acetone or a mixture of both.
4. **Counterstaining** with safranin.

Procedure:

1. Prepare a fixed smear.
2. **Flood** the smear with **crystal violet** for (1 minute), wash with water.
3. **Flood** the smear with **grams iodine** for (1 minute), wash with water.
4. **Decolorize** with ethyl alcohol by adding drops of solution until no more color appear (30 seconds), (The time may vary depending on the thickness of the smear), Wash with water.
5. **Counterstain** with dilute carbol fuchsin (1%) or safranin for (1 minute), wash with water.
6. **Observe** under oil immersion

"**Gram positive** bacteria are **dark purple** and the **Gram negative** are **red or pink** in color"

"Acid fast stain"

Acid-fast stain: is a laboratory test used mainly for diagnosis the causative microbe for **tuberculosis (TB)** in a sample of tissue, blood, or other body fluids.

It is a differential stain that is used in the identification of *Mycobacterium* and other acid fast bacteria. Acid – fast **means** the ability of a microorganism to resist depolarization by acid alcohol after primary staining. Acid alcohol resistant is due to the presence of a high lipid content (40-60%) in the cell envelope (gram negative bacteria have no more than (20%) while gram positive bacteria have (1-4%) lipid in their cell envelope).

Acid – fast staining procedure also called the ziehl-neelson technique, The lipoid capsule of an acid-fast organism stains with carbol-fuchsin and resists decolorization with dilute acid rinse. The acid-fast bacilli will stain **bright red**, and the background will stain **blue**.

- **Principle:** "The lipoid capsule of the acid-fast organism takes up **carbol fuchsin** and resists decolorization with a dilute acid rinse".

Procedure:

1. Prepare fixed smear of a mixture of two bacteria for example *E.coli* and *Mycobacterium* on the slide.
2. **Flood** the smear with concentrated **carbol fuchsin** with flaming until steaming for (5 minutes), wash with water.
3. Add the **decolorizer** acid alcohol (20% H_2SO_4 in ethanol or 3% HCL in ethanol), until no more color appears, Wash with water.
4. **Flood** with **methylene blue** for (1 minute), Wash with water.
5. **Dry** the slide and **observe** under oil immersion.

Acid fast bacteria appear **red** and non-acid fast bacteria appear **blue**.