Deep Science Publishing
https://doi.org/10.70593/978-81-981367-7-0_5

Chapter 5

Staining techniques

Eswari Beeram
DBCS, Mohan Babu University, A.P, India.
b.eswari@mbu.asia

Abstract: Staining of bacteria is necessary to visualize the fine structure of bacteria under the microscope. Simple stains like methylene blue and crystal violet can be used to study the viability whereas differentiating stains like Gram's staining and acid-fast staining is used to differentiate bacteria in to gram positive and negative as well as acid fast and non-acid fast. Special staining techniques like flagella staining, capsule staining and endospore staining is used to study the specialised structures of bacteria.

Keywords: Acid Fast, Endospore, Crystal Violet, Methylene blue, Simple stain

Citation: Beeram E., (2024). Staining Techniques. In *Basic Concepts of Microbiology and Principles of Sterilization, Microscopy, and Virology* (pp. 27-31). Deep Science Publishing. https://doi.org/10.70593/978-81-981367-7-0_5

1. Classification of stains:

A stain is usually referred as agent used to colour the biological specimens where as a dye is referred as a coloring agent.

Based on origin stains are classified as :

1.1. **Natural stains:** These stains are prepared from natural products. For example Hematoxylin stain obtained from the hard wood of a tree (Haematoxylon campechianum). Natural stains are mostly used as Histological stains

1.2. **Synthetic stains:** Synthetic stains are prepared from the chemical substances like coal tar particles. Most of the synthetic stains are the derivatives of Aniline. For example Methylene blue, Saffranin, Crystal violet and Acid fuschin are the stains derived from Aniline.

On the basis of chemical behaviour stains are majorly classified in to :

- Acidic stains
- Basic stains
- Neutral stains.

Acidic stains are used to stain the cytoplasmic components and basic dyes are used to stain nuclear components. Acidic stains consists of chromophore as anion where as basic stains contains chromophore with cation.

1.3. Theories of staining:

Majorly theories of staining are:
1. Physical Theories
2. Chemical Theories

1.4. Physical Theories:

Physical theories are proposed based on Simple solubility and absorption

Solubility: Fat stains are efficient because these stains are more soluble in fat than in 70% alcohol.

Absorption: It is the ability of the larger molecule to attract the small molecules from a surrounding medium.

1.5. Chemical theories:

Chemical theories states that acid dyes stain basic components (Cytoplasm) and basic dyes stains acidic components (nuclear). But in few cases like Hematoxylin which is an acidic stain but in the presence of mordant it stains the nuclear components.

2. Gram staining:

Gram staining was devised by Danish physicist Christian Gram in 1883 later on he published his work in 1884. Gram staining method differentiates bacteria in to two major groups Gram positive or gram negative based on physico chemical properties of the bacterial cell walls.

Principle of Gram staining:

Gram positive cell walls possess thick peptidoglycan layer and teichoic acid where as gram positive cell wall contains thin peptidoglycan layer and high lipid content. Cells that take primary stain and resists the decolorisation with mordantand therefore appears violet and gram negative organisms with high lipid content looses primary stain with mordant and counter stained with saffranin and appears pink.

Procedure of staining:

- Prepare a thin smear and heat fix it

- The smear is flooded with crystal violet and allowed for incubation for 1 min
- Wash the slide under running tap water and flooded with iodine and subjected to incubation for 1 min
- Wash the slide under running tap water and flooded with ethanol for 15- 30 sec.
- Wash the slide under running tap water and counter stained with saffranin for 1 min.
- Wash the slide under running water and air dried and observed under 10X objective lens of the microscope.

3. Acid fast staining:

This staining technique is also referred as differential staining. The procedure for the staining is proposed by Paul Ehrlichand later on two German doctors Ziehl and Neelsen modified the procedure and hence the technique is also referred to as Ziehl- Neelsen technique. This staining technique is used to differentiate acid fast bacteria like Mycobacterium from non acid fast bacteria.

Principle:

Acid fast bacteria contain mycolic acids, waxes and complex lipids in their cell membrane hence almost impermeable to stains. Acid fast bacteria retain carbol fuschin even after treatment with acid solvents and hence appear red where as non acid fast bacteria loses the stain and appear green.

Procedure:

- Take a clean glass slide and make a thin smear
- Air dry the smear and heat fix it
- Flood the smear with carbol fuschin and exposed to steam over water bath for 5 min.
- Remove the slide and washed under running tap water and flooded with acid solvent for 10-15 sec.
- Wash the slide under running tap water and counter stained with methylene blue for 1min.
- Wash the slide under running tap water, air dried and observe under the microscope.

4. Capsule staining:

Capsules are non ionic and hence they would not stain with acid or basic stains and further capsules are water soluble and hence simple stains would not impart colour. Hence special stains make capsules to appear colorless against dark background.

Principle:

Negative staining methods impart translucent, dark background with non coloured capsules. The background staining can be imparted using stains like Nigrosin, congo red or Indian Ink. The cells are counter stained by methylene which makes the cell membrane appear coloured with unstained capsules. Capsules are usually dessicated, degraded or destroyed by heating. A drop of serum can be used to enhance the visibility of the capsule easily with the compound microscope.

Procedure:

- Place a drop of negative stain on the slide
- Add loopful of culture to the stain and mix it with the culture
- Spread the culture and make a thin smear using another slide and allow it to stand for 5- 7 minutes.
- Allow to air dry
- Spread the culture with crystal violet for 1 min and the stain is drained at an angle of 450 and allowed to air dry and observed under the microscope.

5. Endospore staining:

Endospore staining is a differential staining technique used to differentiate endospores from the vegetative cells. The technique was designed by Schaeffer and Fulton during the 1930's to stain the spores.

Principle:

Endospores are usually resistant to staining and primary stain Malachite green is forced to enter and stain spores by heating. Once stain enters the spore it is fixed. When washed with water the stain is removed hence water is used as decoloriser in the staining procedure.

Procedure:

- Prepare a thin smear
- Allow it to heat fix
- Stain the smear with malachite green and exposed to steam for 3-5 minutes until the smear dries.
- Remove the blotting paper and the slide is washed under running tap water.
- The slide is counter stained with saffranin for 2min and washed under running tap water.
- Allow it to air dry and observed under 100X oil immersion objective.

6. Flagella Staining:

Principle:

A wet mount technique for staining the flagella is simple and when arrangement and number of flagella is critical to identify the bacterial strain. The wet mount technique is also known as RYU - staining method as because it uses Ryu flagella stain for staining the flagella.

Procedure:

- Add a loopful of water on the clean glass slide
- Touch loopful of sterile water along the margins of the colony (It results in movement of bacteria towards margin).
- Spread the loopful of culture on to the water
- Place a cover slip over the drop and observed under 40X objective for the motile cells.
- If the motile cells are seen then the cells are stained by touching Ryu stain at the edges of the cover slip.
- Allow the stain to penetrate and stand for 5- 10 min and the cells are observed for the arrangement and number of flagella under the 100x objective.

References:

Atanasova, Kalina. (2010). Interactions between porcine respiratory coronavirus and bacterial cell wall toxins in the lungs of pigs.

Claude m. Fauquet (1999).Taxonomy, classification and nomenclature of viruses in encyclopedia of virology (second edition).

Demeter LM. JC, BK, and other polyomaviruses; progressive multifocal leukoencephalopathy. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Disease. London: Churchill Livingstone, 1995:1400-1406.

Human Genome Organization (http://www.hugo-international.org/) nomenclature page (http://www.genenames.org/).

Maximova, Natalia & Pizzol, Antonio & Sonzogni, Aurelio & Gregori, Massimo & Granzotto, Marilena & Tamaro, Paolo. (2015). Polyclonal gammopathy after BKV infection in HSCT recipient: A novel trigger for plasma cells replication?. Virology Journal. 12. 10.1186/s12985-015-0254-z.

Miller, Eric & Kutter, Elizabeth & Mosig, Gisela & Arisaka, Fumio & Kunisawa, Takashi & Rüger, Wolfgang. (2003). Bacteriophage T4 Genome. Microbiology and molecular biology reviews : MMBR. 67. 86-156, table of contents. 10.1128/MMBR.67.1.86-156.2003.

once, Adrian & Connon, Stephanie & Yung, Pun. (2008). Detection and Viability Assessment of Endospore-Forming Pathogens. 10.1007/978-0-387-75113-9_19.

Vander Elst, Niels & Meyer, Evelyne. (2018). Potential therapeutic application of bacteriophages and phage-derived endolysins as alternative treatment of bovine mastitis. Vlaams Diergeneeskundig Tijdschrift. 87. 181-186. 10.21825/ vdt.v87i4.16 065.