

Microbiology lab essay sample



**ASSIGN
BUSTER**

The primary focus of this lab was on microscopy and simple stains. Crystal violet and Carbol fuchsine, simple staining components, were used to stain the slide in order to see the different microbes in order to determine their cellular shape and identify unknown ones by comparing. Introduction

Bacterial cells are usually colorless because cytoplasm, for the most part, is transparent. Since the bacteria are colorless, it is almost essential to add a stain to make the bacteria more visible. Once stained, cell morphology can be observed. Stains are solutions that contain a solute called a chromophore dissolved in a solvent. A chromophore is the color possessing portion of the solution and is therefore responsible for the stains color. The ability of a stain to bind to macromolecular cellular components such as protein or nucleic acids depends on the electrical charge found on the chromogen portion, as well as on the cellular component to be stained.

There are 2 types of stain: Acidic stains - a chromogen portion exhibits a negative charge, therefore has a strong affinity for the positive constituents of the cell Basic stains - a chromogen portion exhibits a positive charge, therefore has a strong affinity for the negative constituents of the cell

Bacterial cells usually have a negative surface charge, meaning that a positively charged stain is needed to stain the surface of the cell. When the stain is applied, there is an attraction between the negatively charged cell surface and the positively charged chromophore, leading to the surface of the cell taking on the color of the stain. Numerous staining techniques are available for visualization, differentiation and separation of bacteria in terms of morphological characteristics and cellular structures. Simple staining used

for visualization of morphological shape and arrangement Differential staining used for:

- 1) Separation into groups (Gram stain & Acid-fast stain)
- 2) Visualization of structures (Flagella, Capsule, Spore & Nuclear stains)

Methods & Materials

Bacterial loop

Glass slides

Staining tray with slide holders

Crystal violet and Carbol fuchsine dyes

Cultures of bacteria: *Escherichia coli*, *Staphylococcus aureus* & *Serratia marcescens* Bunsen burner

Beaker with tap water

Disposable pipettes

Microscope, dispenser with immersion oil

Part I: Preparation of bacterial smears

1. Glass slides were labelled by the species name of microorganism
2. One drop of distilled water was placed on the slide. A small of the bacterial inoculum from the culture was transferred into the drop of the distilled water and spread with a circular motions
3. Spot of culture was allowed to dry completely
4. Same procedure was repeated for each culture twice: *Escherichia coli*, *Staphylococcus aureus* & *Serratia marcescens*
5. All smears were hot-fixed: slides were passed several times over the flame
6. Slides were placed in a washing tray. By using a pipettes 1-5 drops of the

Crystal violet were placed on smears of *Escherichia coli*, *Staphylococcus aureus* & *Serratia marcescens* and left for 1-2 min

7. Smears were washed with water until no more stain is released from the stained smear. Slides were air-dried and examined under the microscope

8. Steps 6-7 were repeated using another stain - Carbol fuchsine Part II:

Identification of unknowns

1. Glass slides were labelled as 1, 2, 3

2. Smears were produced from liquid cultures from unnamed tubes in the same way as in Part I

3. Smears were hot-fixed

4. Slides were placed in a washing tray.

5. By using a pipette 1-5 drops of Crystal violet were placed on samples 1 and 2; and on sample 3 drops of Carbol fuchsine were placed

6. Smears were washed with water until no more stain is released from the stained smear

7. Glass slides were air-dried completely and examined under the microscope

Results

Table 1. Observation of the slides stained with 2 types of stain under the microscope Bacteria

Crystal violet

Carbol Fuchsine

Escherichia coli

Staphylococcus aureus

Serratia marcescens

Table 2. Identification of unknown samples

Tube " 1"

Tube " 2"

Tube " 3"

Visualization under the microscope

Observations

Staining was not satisfactory.

Hard to define, but possibly it is *E. coli*.

Hair-like microorganisms.

Color absorption is poor.

Possibly it is *Serratia marcescens*.

Connection of bacteria is quite well seen.

Cocci shaped.

Staining is better than in previous two unknowns.

Possibly it is *Staphylococcus aureus*

Questions

1. Why very thick smear or smears with much excess of bacteria (dense smear) will unlikely result in good microscopic evaluation? It will diminish the amount of light that can pass through, making it difficult to see under the microscope

2. Why it is essential that smears are air-dried to complete dryness before hot-fixing? Overheat will denature and rupture the cell wall. Excess water on the slides will boil during fixation

3. Why it is important not to overheat slide during the hot-fixing?
Overheating will distort the morphology through plasmolysis of the cell wall

4. Why presence of grease or dirt on a glass slide will result I a poor smear preparation? 1. Bacteria will attach to the grease or dirt and wash away when staining. 2. It can also diminish the microscopic light making it hard to see the cells.

5. Why are basic dyes more effective for bacterial staining than acidic dyes?
Basic stains with a positively charge chromogen are preferred because bacterial nucleic acid and certain cell wall components carry a negative charge that strongly attract and binds to the cationic chromogen.

6. Can simple staining technique be used to identify more than the morphology of microorganisms? Explain. Yes, the purpose of simple staining is to elucidate the morphology and also arrangement of bacteria cells.