Lesson 2: Basic Techniques in Microbiology Practical II

Introduction

Gram staining is a differential staining technique that divides all bacteria into two major groups – Gram positive and Gram negative. Gram positive bacteria have a thick layer of peptidoglycan in their cell walls that allow them to take up and retain the primary stain, Crystal Violet. On the other hand, Gram negative bacteria have a thin layer of peptidoglycan that is not enough to hold on to the primary stain. Thus, the primary stain gets washed out during the subsequent rinsing steps. Gram negative bacteria are stained with the counterstain, Safranin and Carbol Fuschin. Through this technique, Gram positive bacteria appear violet in color and Gram negative bacteria appear red or pink in color.

Question 1

What is the purpose of Gram-staining of control microorganisms?

Gram staining is a type of differential staining technique that divides all bacteria into two major groups – Gram positive and Gram negative. This division is based on the physical and checmical properties of the cell walls of bacteria. Apart from providing information about the cell wall content, this technique also allows us to view the morphology, arrangement, and shape of the bacteria. Gram staining of control microorganisms allows us to have a comparison standard for assessing the gram staining results of the given sample.

Methodology

- A clean dry slide was taken and labelled on the underside. Using an inoculating loop, a loopful of the given sample was placed on the centre of the slide and spread using a circular motion.
- The smear was completely air-dried and passed through the burner 2 to 3 times for heat fixation.
- Once the smear was ready, the slide was flooded with Crystal Violet stain and left for 1 minute. It was then rinsed with distilled water.
- Gram's iodine was added to the slide and left for 1 minute. It was then rinsed with distilled water.
- The smear was then decolorized using 95% ethanol for 5 to 10 seconds. It was then rinsed with distilled water.
- The slide was flooded with Safranin and left for 45 seconds. It was then rinsed with distilled water.
- The smear was blot dried using blotting paper and viewed under the microscope.

Question 2

Why is it necessary to air-dry the smear before carrying out heat fixation of the smear?

Before heat fixing the organisms on the slide, it is necessary to completely air-dry the smear or else they will be washed off during the staining process. If the smear is not sufficiently air-dried, heat fixing may lead to coagulation of proteins of the organisms, affecting the results of gram staining.

Question 3

Why is it necessary to heat fix the smear?

Heat fixation of the smear helps adherence of the bacterial cells to the slide so that they are not rinsed off during the staining process. Also, application of heat leads to denaturation of bacterial enzymes that may digest the parts of the cell via autolysis. If this happens, the structural integrity of the cells may be affected resulting in incorrect staining results.

Results and Discussions

Gram staining was performed on two individual bacterial cultures, one of *Staphylococcus epidermidis* and the other of *Escherichia coli*. Gram staining results of *Staphylococcus epidermidis* under the microscope

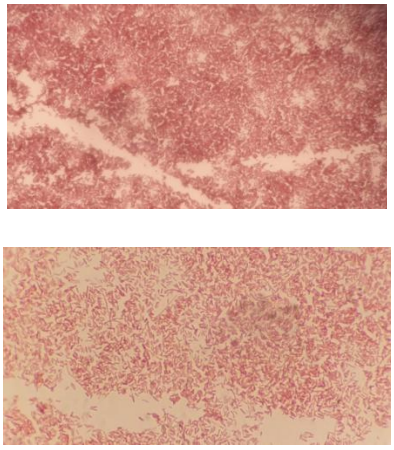
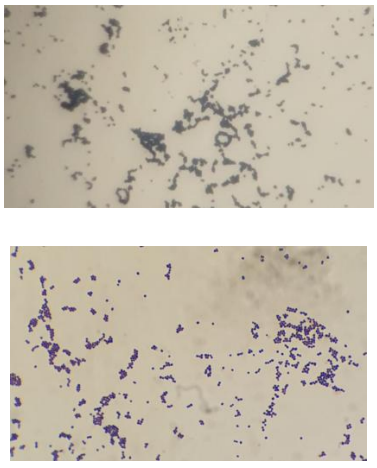
showed several clusters of violet colored cocci. This indicates that the organism is a Gram-positive circular bacterium that appears in clusters. On the other hand, Gram staining results of *Escherichia coli* under the microscope showed several individual pink-colored rods. This indicates that the organism is a Gram-negative rod-shaped bacterium that is present individually. Hence, Gram staining of two bacterial cultures successfully helped in the classification of the bacterial sample as Gram positive or Gram negative. Additionally, it also provided information about the shape and arrangement of the bacterial cells.

Following this, a mixed culture sample was used for Gram staining. The resulting slide was observed under the microscope and two types of organisms were found to be present. One was violet-colored cocci in clusters and the other was pink-colored rods present individually. These organisms were identified as *Staphylococcus epidermidis* and *Escherichia coli* respectively. Hence, Gram staining of a mixed culture sample successfully helped us differentiate between Gram positive and Gram negative organisms on a single slide.

Question 4

Simple staining and Gram-staining of *Escherichia coli* and *Staphylococcus epidermidis*

Record your observations (cell shape and cell arrangement, as well as colour and Gram-status) of each bacterial cell morphology as viewed under microscope in the table below.

Microorganism	Draw your observations	Description of observations
Escherichia coli		Cell shape: *rods* Cell arrangement: *Single* Stain Colour: *Red/ Pink* Gram Status: *Gram-negative*
Staphylococcus epidermidis		Cell shape: *Cocci* Cell arrangement: *Clusters* Stain Colour: *Violet/ Blue* Gram Status: *Gram-positive*

Question 5

Record the magnification of eye pieces and objective used and calculate the total magnification of microorganism observed above.

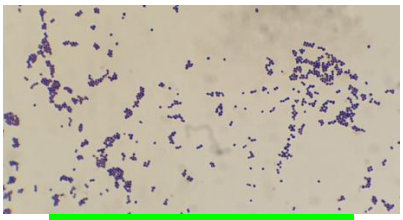
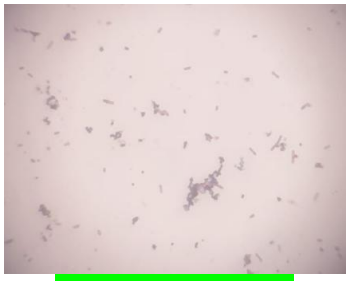
Magnification of eye piece: _____*10*___; Magnification of objective used: ___*100*_____

Total magnification of microorganisms = *1000*

Question 6

Simple staining and Gram-staining of mixed culture

Record your observations (cell shape and cell arrangement, as well as colour and Gram-status) of each bacterial cell morphology as viewed under microscope in the table below.

Method of staining	Draw your observations	Description of observations
Simple stain	 *Staphylococcus epidermidis*	Cell shape: *Cocci* Cell arrangement: *Clusters* Stain Colour: *Violet/ Blue* Gram Status: *Gram-positive*
Gram-stain	 *Mixed culture sample*	Cell shape: *Cocci & rods* Cell arrangement: *Clusters& Single* Stain Colour: *'Violet/ Blue' & 'Red/ Pink'* Gram Status: *Gram-positive & Gram-negative*

Question 7

Comment on the **differences** between **simple staining** and **Gram-staining techniques**.

- Simple staining is a technique used to merely observe bacterial cells under the microscope whereas Gram staining allows us to classify bacteria as Gram positive or Gram negative.
- Gram staining provides important information about the cell wall content of bacteria which is not possible through simple staining.
- Simple staining uses a single dye, usually methylene blue, whereas Gram staining uses two dyes, Crystal Violet and Safranin.

Question 8

Comparing the bacterial cell morphology and Gram-status of the **mixed culture sample**, comment if *E. coli* might be present in the culture.

The Gram staining results of the mixed culture sample showed two types of bacterial cells under the microscope. One type represented Gram positive cocci clusters and the other type represented Gram negative individual rods. We know that *E. coli* is a Gram negative rod, and so from the results, it is likely that *E. coli* is present in the culture.

Conclusion

Gram staining is a differential staining technique that is used to distinguish between bacteria based on their cell wall composition. Apart from helping us view the morphology of the bacterial cells, it can also help us identify the number of different organisms present in a mixed culture sample. We were successfully able to Gram stain the given samples, classify organisms as Gram positive or Gram negative, and view their shape and arrangement under the microscope.

References

Faurie, B. (2019). GRAM staining procedure. 10.13140/RG.2.2.29711.84644.

Smith, A. C.& Hussey, M. A. (2016). Gram Stain Protocols. American Society for Microbiology.