TUTORIAL 1

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SECTION/COURSE CODE	**: [SEBB 4173] [01] – [CELL & MOLECULAR BIOLOGY FOR BIOINFOMATICS]**
LECTURER	**: [DR. NORHANA]**
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Case Study Report submitted to School of Computing, Univerisiti Teknologi Malaysia in partial fulfilment to complete the SEBB 4173 Assignment for Semester 2 session 2020/2021

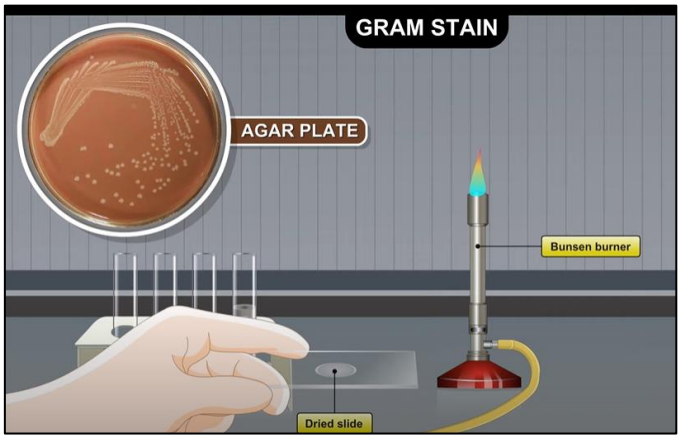
1. **What is the objective of Gram Stain?**

Gram Staining is a staining method to differentiate and distinguish Gram-positive bacteria and Gram-negative bacteria. Through the gram staining process, Gram-positive cells will be violet while Gram-negative cells will be red. This is due to the thickness of peptidoglycan wall of the Gram-positive cells and Gram-negative cells. Gram-positive bacteria have a very thick cell wall consists of multi-layered of peptidoglycan that prevent soluble crystal violet-iodine complex from escaping. However, thin peptidoglycan layer of Gram-negative bacteria causes the crystal violet-iodine complex removed easily.

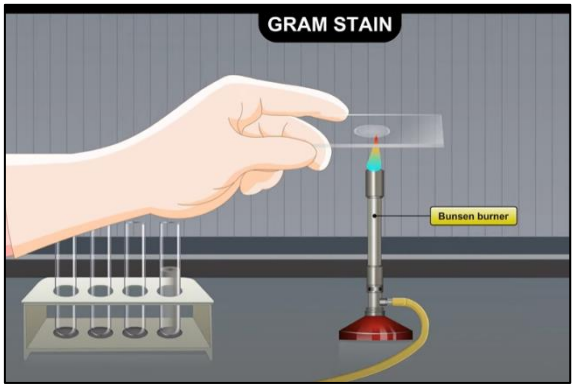
Hence, we can conclude that the objective of the Gram Stain is to identify the type of bacteria and based on the differences in the biochemistry and structure of the cell wall, understand how the Gram staining reaction affects Gram-positive and Gram-negative bacteria.

2. **Describe the principles involve in the gram staining procedure.**

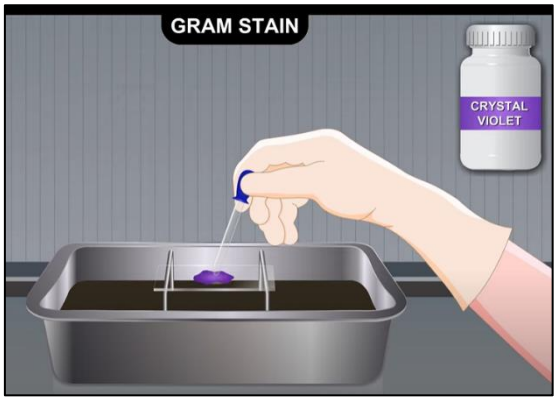
Step 1: Make a slide of cell sample to be stained.



Step 2: Heat fix the sample by passing the slide with a drop of sample on it through a Bunsen Burner.



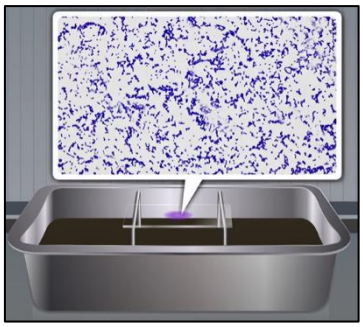
Step 3: Add the crystal violet to the sample and incubate for 1 minute.



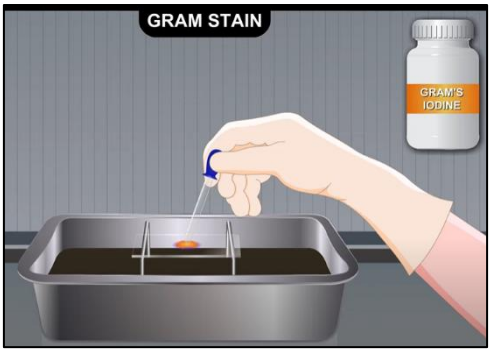
Principle: Crystal violet will dissociate CV+ and Cl- ions. Then, CV+ and Cl- ions penetrate through the cell wall and plasma membrane of Gram-positive cells and Gram-negative cells.

Step 4: Rinse the slide with the water for 5 seconds to remove excess stain.

Result: All cells are in purple colour.

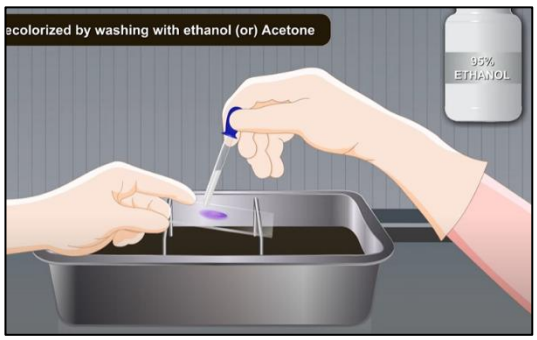


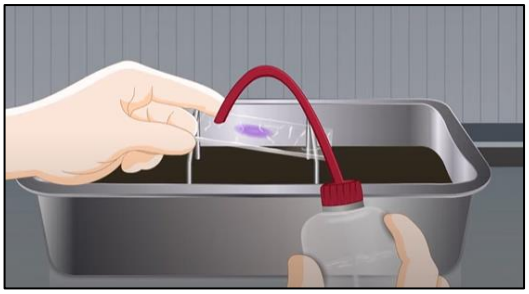
Step 5: Add iodine solution for 1 minute.



Principle: Iodide ions will interact with CV+ to form CV-I, a large crystal violet-iodine complex.

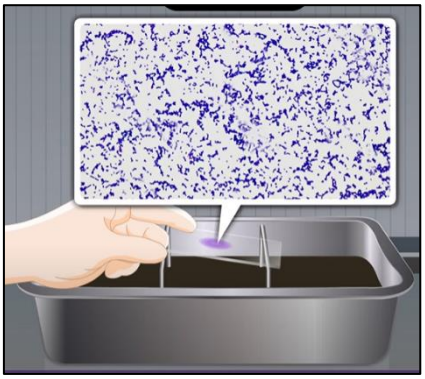
Step 6: Rinse the sample with ethanol for 3 seconds and rinse with the water.



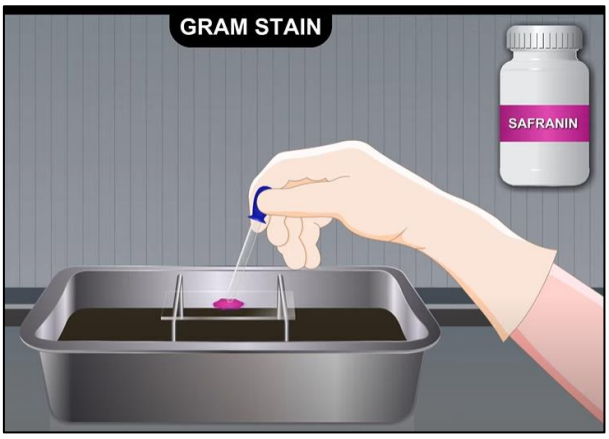


Principle: Ethanol will act as decolourized agent and interact with the lipid's membranes of both Gram-positive cells and Gram-negative cells. Gram-negative cells have thin layers of peptidoglycan which allow CV-I to be removed easily. Gram-positive cells have multi-layered peptidoglycan and link by peptide interbridge make the CV-I harder to escape.

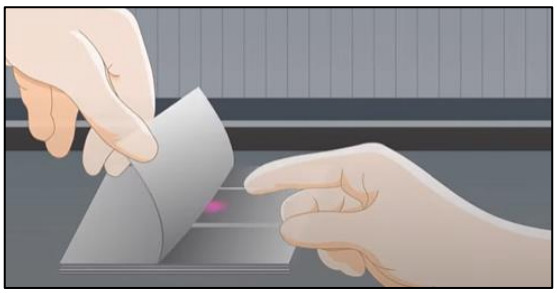
Result: Gram-negative cells will be decolourized since the crystal violet had been removed while Gram-positive cells remains in violet colour.



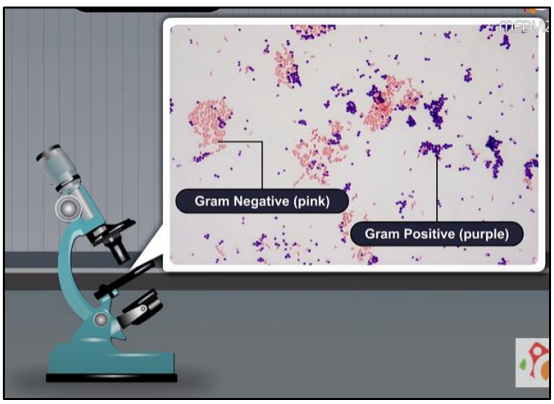
Step 7: Add the safranin to the slide and incubate for 1 minute.



Step 8: Wash with the water for 5 seconds and dry by filter paper.



Result: Gram-positive cells remains as violet while Gram-negative cells turn pink to red.



3. What is the limitation on Gram Stain?

- False result of Gram-negative may obtained if the decolourized is over while under decolourization will lead to false identification of Gram-positive.
- Smears cannot be too thick or too sticky because it may retain too many original stains, making it difficult to identify the correct Gram stain response.
- Cultures over 16 to 18 hours contain live and dead cells which will deteriorate the dead cells and the stain does not retain properly.
- Precipitate may form by the stain overtime. Excess crystal will be removed by filtering through gauze.

REFERENCE

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