GRAM STAINING in BACTERIAL CULTIVATION (Automated with PREVI™ Color)

1. *Introduction*

Gram staining technique differentiates bacteria into two large groups (Gram Positive & Gram Negative) based on composition of cell wall. Hence, it is one of the primary methods used for identification of bacteria.

2. *Requirement for Gram Staining*

- Bacterial colony on agar plate
- Glass slide
- Inoculating loop
- Burner
- Previ color Stainer and its reagent bottles
- Bibulous paper
- Immersion oil
- Microscope

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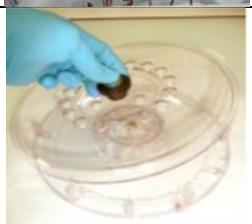
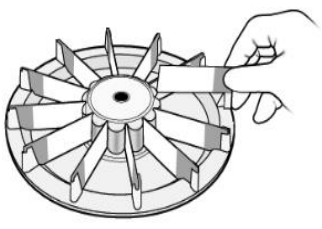
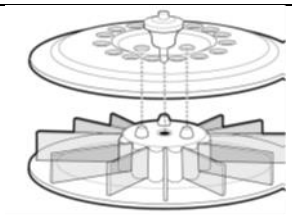
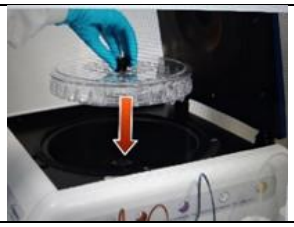
Table 1 Requirement for Gram Staining procedure

3. *Gram staining procedure (Automated)*

PREVI™ Color Gram is an automated Gram staining system that provides rapid, standardized results for all types of specimens and streamlines the workflow of microbiology labs. Please read the user manual sheet carefully before using the PREVI™ Color Gram. In all cases of maintenance and repair, work should only be undertaken by a skilled personal.

Step 1 - Loading the Slide in Previ Machine

	• Remove the carousel from the bowl.
	• Remove the carousel lid by pressing the button and lifting the lid away.
	• Insert the slides into the carousel. The slides must be inserted diametrically opposite one to the other to balance the carousel. • Be sure that slides are loaded in balanced pairs. If an odd number of slides need to be stained, use a blank slide. ***A signal tone sounds during the run if the position of the slides is unbalanced.***

	• Replace the lid by pressing the button and lowering the lid over the indexing posts. • Release the button and press the lid handle until it clicks into place.
	• Place the carousel in the bowl and close the lid.

Step 2 - Starting Staining Cycle

- If the carousel is full, press "**run**".
- If not, enter the number of slides that have been loaded into the carousel (even number). Then press "**run**".
- Staining procedure in machine
  - Spray Crystal violet to slide for 30 sec.
  - Wash with distilled water for 30 sec.
  - Spray Iodine solution to slide for 30 sec.
  - Wash with distilled water for 30 sec.
  - Spray Acetone fuchsin to slide for 30 sec.
  - Wash with distilled water for 30 sec and dry the slides.

Step 3 - Unloading slides from machine

	• Remove the carousel from the bowl.
	• Remove the carousel lid by pressing the button and lifting the lid away.
	• Carefully remove each slide already dried and read the Gram results with a microscope.
	• Discard slides into a biohazard container.

4. Result Interpretation

Violet color Bacteria indicates that the given bacteria are gram positive and pink color indicates that the given bacteria are gram negative.

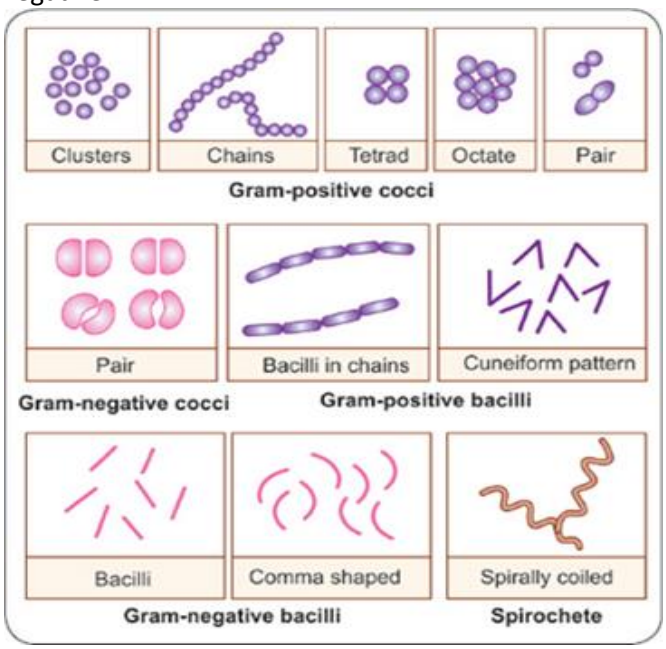


Figure 1 : Result Interpretation of Gram Staining Slide

References

- https://serc.carleton.edu/microbelife/research_methods/microscopy/gramstain.html
- http://www.frankshospitalworkshop.com/equipment/documents/automated_analyzer/user_manuals/Biomerieux%20Previ%20Color%20Gram%20-%20User%20Manual.pdf
- University of Medical Technology University: Microbiology General Practical Guidance