



GRAM STAINING in BACTERIAL CULTIVATION (Manual Method)

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

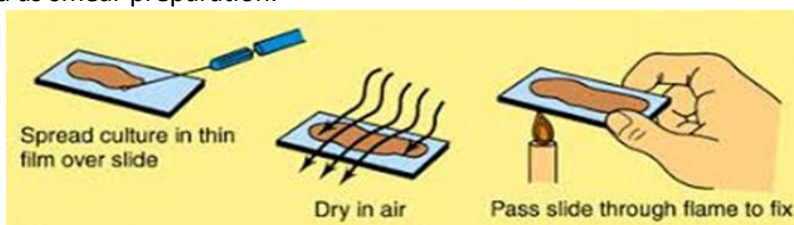
- Bacterial colony on agar plate
- Glass slide
- Inoculating loop
- Burner
- Manual Gram staining kit
- Bibulous paper
- Immersion oil
- Microscope



Table 1 Requirement for Gram Staining procedure


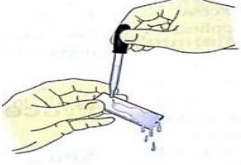

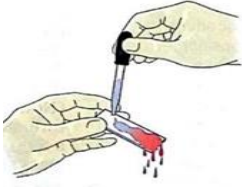
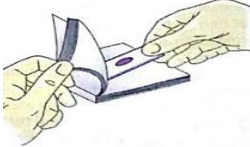
3. Gram Staining Procedure (Manual)

1. Wash and clean glass slide (make it dust and grease free).
2. Dry the glass slide.
3. With the help of inoculating loop, take a loopful of culture and spread it uniformly on the slide, this step is called as smear preparation.
4. Allow the smear to dry in air and then heat fixed it. Heat fixing kills bacteria and adheres them to the slide.



<p>Gently stain with crystal violet for 1 minute.</p>	<p>5. Flood the crystal violet (primary stain) on the smear for 1 minute.</p>
<p>Gently wash off the stain with tap water.</p>	<p>6. Run off the crystal violet by using distilled water or tap water.</p>



 <p>Gently apply Gram's iodine for 1 minute.</p>	<p>7. Gently flood gram's iodine over smear for 1 minute. At this moment the smear would appear violet or blue in color.</p>
 <p>Add 95% alcohol drop by drop until the alcohol runs almost clear.</p>	<p>8. Decolorize the smear by flooding the slide with 95% alcohol for 30 seconds.</p>
 <p>Counterstain with safranin for 45 seconds.</p>	<p>9. Flood the safranin (counter stain) over the slide and keep it for 45 seconds.</p>
 <p>Gently wash off the safranin with tap water.</p>	<p>10. Run off the safranin stain with distilled water.</p>
 <p>Blot dry with bibulous paper.</p>	<p>11. Allow the slide to blot dry with bibulous paper and observe under microscope.</p>

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.

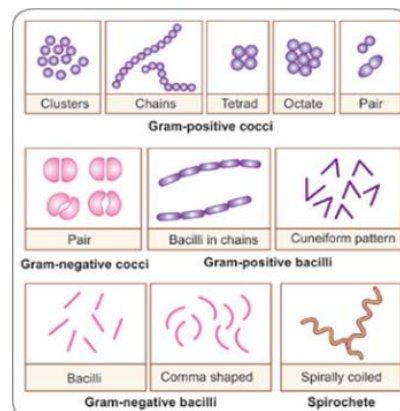


Figure 1 : Result Interpretation of Gram Staining Slide

References

- https://serc.carleton.edu/microbelife/research_methods/microscopy/gramstain.html
- University of Medical Technology University: Microbiology General Practical Guidance