Gram Stain

Introduction

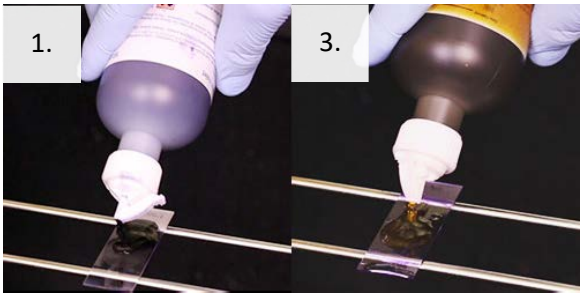
The Gram stain is a differential staining procedure used to categorize bacteria as Gram positive or Gram negative based on the chemical and physical properties of their cell's walls. The bacteria are differentiated through a series of staining and decolorization steps. Gram positive cells will stain purple and Gram negative cells will stain red to pink.

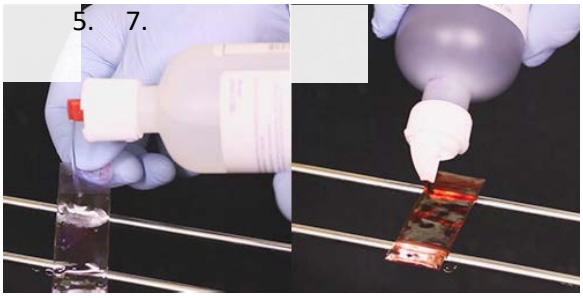
Supplies and Reagents

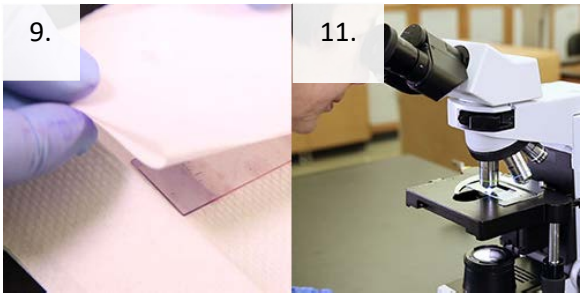
1. Personal protective equipment (PPE)
2. Slide rack
3. Timer
4. Absorbent paper, such as bibulous paper
5. Water (tap water or deionized)
6. Crystal violet
7. Gram's iodine
8. Decolorizer
9. Safranin (or carbol fuchsin)
10. Brightfield microscope with 100X objective
11. Immersion oil

Instructions

1. Use appropriate PPE (gloves, laboratory coat, and face and eye protection) according to your laboratory's procedures and safety manual.
2. Place the prepared fixed smear on a slide rack then flood the slide with crystal violet.
3. Wait at least 15 seconds* then rinse the slide with water.
4. Flood the slide with Gram's iodine.
5. After 15 seconds* rinse the slide with water.
6. Apply the decolorizer to the slide.
7. Rinse the slide immediately with water.
8. Flood the slide with counterstain.
9. Wait at least 15 seconds* then rinse the slide with water.
10. Blot the slide with absorbent paper. Be careful not to wipe the cells off the slide.
11. Allow the newly stained slide to air dry completely.
12. View the slide under oil using the oil immersion objective for a total magnification of 1000X.
13. Record results based on your laboratory's criteria.







* Be sure to check manufacturer's instructions for the timing of each step.