Bio I

Lab: Gram Staining

In 1884 the Danish bacteriologist Christian Gram developed a staining technique that separated bacteria into two groups: those that are gram-positive and those that are gram-negative. The procedure is based on the ability of microorganisms to retain the purple color of crystal violet during decolorization with alcohol. Gram-negative bacteria are decolorized by the alcohol, losing the purple color of crystal violet. Gram-positive bacteria are not decolorized and remain purple. After decolorization, safranin, a red counterstain, is used to impart a pink color to the decolorized gram-negative organisms.

Materials

Bacillus sp.	wash bottle
slides	microscope
Gram staining kit	oil of immersion
paper towel	

Procedure

1. Cover the smear with crystal violet. Let stand for **20 minutes**.

2. Briefly wash off the stain, using a wash bottle of distilled water. Drain off excess water.

3. Cover the smear with Gram's iodine solution and let stand for **one minute.**

4. Pour off the Gram's iodine and flood the smear with 95% ethyl alcohol for **10 to 20 seconds**. This step is critical. Thick smears will require more time than thin ones. Decolorization has occurred when the solvent flows colorlessly from the slide.

5. Stop the action of the alcohol by rinsing the slide with the wash bottle for a **few seconds**.

6. Cover the smear with safranin for **20 seconds**.

7. Wash gently for a few seconds, blot with a paper towel and let dry at room temperature.The slide may be examined under oil immersion immediately.