Gram Stain Set Instructions

Introduction

One of the most frequently cited criteria by which bacteria are classified involves the reaction of individual species to the Gram staining procedure. As a result, bacteria are classified as either Gram-positive or Gram-negative. Gram staining is generally the first step in identifying an unknown bacterial species and is routinely used in even the most sophisticated microbiological laboratories. The Gram staining technique requires relatively few reagents, is well defined, and can be successfully practiced with a few simple steps.

Materials

Crystal violet solution, 5–10 mL*
Ethyl alcohol, 95%,5 mL*
Gram iodine, 5–10 mL*
Gram safranin, 5–10 mL*
**Materials included in kit.*
Water, distilled or deionized
Beral pipet, thin-stem*
Bunsen burner
Compound microscope
Slide

Safety Precautions

Gram stain materials will stain skin and other materials. Care should be taken when flame-fixing the slides. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Safety Data Sheets for additional safety, handling, and disposal information. Wash hands thoroughly with soap and water before leaving the laboratory.

Procedure

1. Thin smears of bacterial cultures to be tested should be air-dried and flame-fixed on clean glass slides. Fix by passing the slide, smear side up, over an alcohol or Bunsen burner five or six times. 18- to 24-hour cultures will give the best results.
2. Apply Gram crystal violet stain (Hucker formula) from an eye-dropper or dropper bottle. Slide should be flooded with stain and let stand for one minute. Gently rinse slide with water from a dropper or by dipping in a beaker or cup of clean water. Rinsing should be sufficient to remove excess stain.
3. Flood smears with Gram iodine (serves as a mordant, or dye retainer) and let stand for one minute. Rinse with clean water as above.
4. Decolorize smears with 95 percent ethyl alcohol by applying one drop at a time—just until no more color runs off—for approximately 30 seconds. Decolorization time is determined primarily by the thickness of the smear—shorter for thin smears, longer for thick smears. It is very important not to over-decolorize in this step. Rinse with water as above. This rinsing step is necessary to stop the action of the alcohol decolorizer.
5. Apply Gram safranin counterstain and let stand for 30–45 seconds. This step serves to stain those bacteria (Gram-negative) not holding the crystal violet after step 4. Rinse very briefly with water as above. Air or blot dry the slides and examine under oil-immersion.

Note: Upon examination, Gram-positive bacteria will appear to be stained a bluish black color while Gram-negative species will be red. During the various steps it is important not to allow any of the stains to evaporate to dryness on the slides. Simply add more stain until the recommended staining period is over and the slides are ready to be rinsed. Common spring-type wooden clothespins make excellent slide holders during flaming, staining and rinsing.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. Gram stain material can be disposed of according to Flinn Suggested Disposal Method #26b.

Publication No. 10065
061616