

Standard Laboratory Operating Procedure #2019

Bacteria Staining

Laboratory: Science and Technology of Foods

Location: CS203

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General: Gram staining is generally the first step in identifying an unknown bacterial species, the resulting color of the Gram stain will classify bacteria as either Gram-positive (purple) or Gram-negative (red). The Gram staining technique requires relatively few reagents, is well defined, and can be successfully practiced with a few simple steps.

Safety: gloves, safety glasses, Gram stain material can be disposed of according to *Flinn Suggested Disposal Method #26b* in the *Flinn Scientific Catalog/Reference Manual*

Materials

crystal violet	distilled water	wooden clothespins
ethyl alcohol, 95%	disposable pipettes	
gram iodine	bunsen burner	
gram safranin	compound microscope	
microscope slides	inoculating loop	

Procedure

Heat-Fixing Bacterial Slides:

1. Using an inoculating loop, select a colony of soil bacteria from a plate and smear a thin layer of the colony on to microscope slide.
2. Allow 5 minutes to air dry smear and then heat-fix slide by passing the slide, smear side up over alcohol or Bunsen burner five or six times.
3. Repeat steps 1-2 for each soil plate.

Staining of Bacterial Slides:

1. Apply 1mL of Gram crystal violet stain to flood the slide with stain and allow to stand for 1 minute. Gently rinse slide with water or dip into beaker of clean water to remove excess stain.
2. Next apply 1mL of Gram iodine (retains the dye) to flood slide and allow to stand for 1 minute. Gently rinse slide with water or dip into beaker of clean water to remove excess stain.
3. Decolorize slides with 95% ethyl alcohol by applying 1 drop at a time until no more color runs off (approx. 30 seconds). Gently rinse slide with water or dip into beaker of clean water to stop the action of the alcohol de-colorizer.
4. Apply 1mL of Gram safranin counterstain and allow to stand for 30 seconds to stain the bacteria that did not stain with crystal violet. Rinse with water, blot slide dry with paper towel and view under microscope.

**Note: Gram-positive bacteria will appear to be stained purple color, whereas Gram-negative bacteria will appear red in color.

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