

TML/MSH Microbiology Department Policy & Procedure Manual	Policy #MI\TECH\42\04\v01	Page 1 of 2
Section: Technical Manual	Subject Title: Bacto 3-Step Gram Stain Procedure	
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BACTO 3-STEP GRAM STAIN PROCEDURE

Principle

To be used for problem smears to determine the Gram reaction of organisms.

Materials

3-Step Stabilized Iodine Technique

Bacto Gram Crystal Violet
Bacto Stabilized Gram Iodine
Bacto 3-Step Gram Safranin-S

3-Step Technical Iodine Technique

Bacto Gram Crystal Violet
Bacto Gram Iodine
Bacto 3-Step Gram Safranin-T

Microscope slides
Bunsen burner or methanol
Bacteriological loop
Swabs
Blotting paper
Microscope with oil immersion lens
Bactrol™ Gram Slide
Bactrol™ Disks

Procedure

1. Flood the fixed smear with primary stain (Bacto Gram Crystal Violet) and stain for 1 minute.
2. Remove the primary stain by gently washing with cold tap water.
3. Flood the slide with mordant (Bacto Stabilized Gram Iodine or Bacto Gram Iodine (traditional formulation) and retain on the slide for 1 minute. (Refer to LIMITATIONS OF THE PROCEDURE, #5)
4. Wash off the mordant with decolourizer / counterstain (Bacto 3-Step Gram Safranin-S or Bacto 3-Step Gram Safranin-T). (**NOTE:** Do not wash off iodine with water). Add more decolourizer / counterstain solution to the slide and stain 20-50 seconds.
5. Remove the decolourizer / counterstain solution by gently washing the slide with cold tap water.
6. Blot with blotting paper or paper towel or allow to air dry.
7. Examine the smear under an oil immersion lens.

Interpretation

REACTION	3-STEP TECHNIQUE using either Bacto Gram Safranin-S or Bacto Gram Safranin-T
Gram-positive	Purple-black to purple cells
Gram-negative	Red-pink to fuchsia cells

Quality Control

Run controls daily using 18-24 hour cultures of known gram-positive and gram-negative microorganisms.