

GRAM STAIN METHOD

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of Gram negative and Gram positive bacteria
PRINCIPLE:	Crystal violet stains all bacteria. Iodine complexes with crystal violet and forms a large complex that is more easily removed by the decolorizer from the lipid rich bacteria leaving the gram negative bacteria colorless. Safranin O is taken up by the colorless bacteria and the excess is removed by water.
CONTROL:	Any sample known positive for gram + - bacteria.
SPECIMEN PREPARATION:	Fixed thin film.
SOLUTIONS:	<ol style="list-style-type: none"> 1. Crystal Violet Grams Item# s168 2. Gram's Iodine Item# s204 3. Safranin O Counterstain (Gram's)Item# s267 4. Gram's Decolorizer (Acetone Alcohol 1:1) Item# s103 <p>Solutions can be purchased separately from Poly Scientific.</p>
NOTES:	
REFERENCE:	Sydney M. Finegold and William J. Martin, Diagnostic Microbiology, Chapt. 3, Mosby Co., St. Louis, 1982.

STAINING PROCEDURE:

1. Prepare a thin film of material to be examined, dry and fix.
2. Stain with Crystal Violet-Gram's for 30-60 seconds.
3. Wash with water.
4. Stain with Gram's Iodine for 30 seconds.
5. Wash with water.
6. Decolorize with Gram's Decolorizer until solvent flows colorless from the slide. This usually takes 10-20 seconds depending on thickness of slide. Care should be taken no to over decolorize the film which may result in an incorrect reading.
7. Wash thoroughly with water.
8. Counterstain with Safranin O counterstain for 2 minutes.
9. Wash with water.
10. Air dry and examine.

RESULTS:

Gram Positive Organisms..... Purple
 Gram Negative Organisms..... Red

Poly Scientific R&D Corp.

Revision: B-18

