

## GRAM STAIN METHOD-HUCKER MODIFICATION

### STAINING PROCEDURE:

1. Prepare a thin film of material to be examined, dry and fix.
2. Flood the slide with Crystal Violet-Hucker's and allow to remain for 10 seconds.
3. Pour off stain and wash off remaining stain with Gram's Iodine.
4. Mordant with additional Gram's Iodine for 10 seconds.
5. Rinse off with running water. Shake off excess 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 not to over decolorize the film which may result in an incorrect reading.
7. Counterstain with Safranin O (Hucker's) for 10 seconds.
8. Wash with water.
9. Blot between clean sheets of bibulous paper and examine under Immersion Oil.

<b>PURPOSE:</b>	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of Gram positive and Gram negative bacteria
<b>PRINCIPLE:</b>	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.
<b>CONTROL:</b>	Any sample known positive for Gram + - bacteria
<b>SPECIMEN PREPARATION:</b>	Fixed thin film.
<b>SOLUTIONS:</b>	1. Crystal Violet Huckers Item# s169 2. Gram's Iodine Item# s204 3. Safranin O Counterstain (Hucker's) Item# s1885 4. Gram's Decolorizer (Acetone Alcohol 1:1) Item# s103  Solutions can be purchased separately from Poly Scientific.
<b>NOTES:</b>	
<b>REFERENCE:</b>	C.R.C Manual of Clinical Lab. Proc., 2nd Ed., p.269 & 270 (1970).

### RESULTS:

Gram Negative Organisms..... Red  
Gram Positive Organisms ..... Blue

*Poly Scientific R&D Corp.*

