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# Thermo Scientific Richard-Allan Scientific Chromaview – Advanced Testing Gram Stain – Film Instructions for Use

## Technical Discussion

**Preparation**
Monolayer smear on a clean glass slide.

**Fixation**
Slide flooding with absolute methanol or heat fixing by flame.

**Quality Control**
The use of a control slide with known Gram positive and known Gram negative organisms is recommended.

## Technical Procedure

**Standard Staining Protocol**

1. Flood smear with Crystal Violet Solution for 1 minute.
   **Optional Step:** Add 5 drops of Sodium Bicarbonate Solution to smear to enhance Crystal Violet retention.
2. Rinse off excess stain in deionized water for 30 seconds.
3. Flood smear with Gram's Iodine Solution for 1 minute.
4. Rinse off excess stain in deionized water for 30 seconds.
5. Differentiate smear with Decolorizing Solution.
6. Rinse in deionized water for 30 seconds.
7. Flood smear with Safranin O Stain Solution for 1 minute.
8. Rinse off excess stain in deionized water for 30 seconds.
9. Blot slide with absorbent paper.
10. Examine under microscope.

## Results

Gram Positive Organisms – Blue

Gram Negative Organisms – Red

## Discussion

All staining reagents should be stored at room temperature. The Gram Stain reagents are for "In Vitro" use only. Refer to the Material Safety Data Sheet for health and safety information. All reagents are stable and should not form precipitants under ordinary storage parameters. These stains should not be diluted and are ready for use. All dyes used in these formulations are certified by the Biological Stain Commission.

## Technical Comments

If precipitation occurs in Crystal Violet Solution, place in warm oven (approximately 35°C) and stir. Gram's Iodine Solution may show a decrease in potency over time. The finished slide is stable indefinitely. When performing Gram stains from culture media, one should consider the age of the culture. This can affect the degree of Gram positive staining. Antibacterial treatments can also affect Gram positive staining – one should be aware that over-decolorizing is more probable.

## Probable Mode of Action

Bacteria can be classified into one of two families based upon the thickness of the peptidoglycan-containing cell wall. Gram positive bacteria have a thick peptidoglycan-containing cell wall. Gram negative bacteria have a thin peptidoglycan-containing cell wall. Both Gram positive and Gram negative bacteria stain with the dye lake created by the crystal violet stain and iodine mordant. However, during rinsing with Decolorizing Solution, the dye-lake is completely washed away from the thin, Gram negative bacteria. The cell wall will become counter-stained with the Safranin O Stain Solution. The short duration of the Decolorizing Solution rinse enables the dye lake to remain within the thicker, Gram positive cell wall. Take care when rinsing the slide with the Decolorizing Solution. Extended rinses with Decolorizing Solution can cause the crystal violet dye lake to wash out of the thick cell wall layer of Gram positive bacteria as well as the Gram negative bacteria.

## References


1. Bancroft, J.D. and Stevens, A. Theory and Practice of Histological Techniques. Churchill Livingstone, New York, NY, 1977.
2. Volk, W.A. Essentials of Medical Microbiology. J.B. Lippincott Company, Philadelphia, PA, 1982.
3. Manual of Clinical Microbiology of the American Society for Microbiology, 30-45: 1970.
4. Hucker G.J. and Conn, H.J. Further Studies on the Methods of Gram Staining. NY Agric Exp Stain Technology Bulletin. No. 128, 1927.
5. Cutlin, B.W. 1975. Cellular Elongation Under the Influence of Antibacterial Agents: Way to Differentiate Coccobacilli from Cocci. J. Clin, Micro 1:102-105.


## Order Information

| Product | Size | Qty. | REF |
|---|---|---|---|
| Gram Stain Kit (Film) | 1 Kit | 1 | 87101 |
| Crystal Violet Solution | 500ml | 1 | 88101 |
| Gram's Iodine Solution | 500ml | 1 | 88102 |
| Safranin O Stain Solution | 500ml | 1 | 88103 |
| Decolorizing Solution | 500ml | 1 | 88104 |
| Sodium Bicarbonate Solution | 8ml | 2/cs. | N/A |



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