

BROWN HOPPS METHOD FOR GRAM POSITIVE & GRAM NEGATIVE BACTERIA

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of Gram positive and Gram negative 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. Basic Fuchsin is taken up by the colorless bacteria and the excess is removed by Gallego's Solution.
CONTROL:	Tissue known positive for Gram positive and negative bacteria <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs002.</i>
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	1. Crystal Violet 1% Aqueous Item# s167D 2. Gram's Iodine Item# s204 3. Basic Fuchsin 0.5% Aqueous Item# s122 4. Gallego's Differentiating Solution Item# s189 5. Tartrazine 1.5% Aqueous Item# s284 6. Cellosolve Item# s163D <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	If too red or pink, increase time in step 10 to 8 minutes.
REFERENCE:	Luna, Lee G. <u>Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology</u> . 3rd Ed. McGraw-Hill Book Co. New York. 1968. pp. 224-225.

STAINING PROCEDURE:

Note: Steps 6, 7, 13 and 14 are critical in regard to timing.

1. Deparaffinize and hydrate to water.
2. Place slides on staining rack and pour on filtered Crystal Violet 1% Aqueous for 2 minutes.
3. Wash under running water.
4. Mordant in filtered Grams Iodine for 5 minutes.
5. Rinse in distilled water in a coplin jar.
6. Differentiate in Cellosolve until blue color no longer streams away from the section, approximately 5-10 seconds.
7. Quickly rinse in distilled water. Check to make sure slides are blue.
8. Filtered Basic Fuchsin 0.5% Aqueous for 5 minutes.
9. Rinse in distilled water.
10. Gallego's Differentiating Solution for 5 minutes (differentiates and "fixes" the Basic Fuchsin).
11. Rinse thoroughly in distilled water.
12. Blot lightly only to remove excess water, not to dryness.
13. Tartrazine 1.5% Aqueous for 3 seconds. Immediately blot away excess, but not to dryness.
14. Cellosolve, 3 changes, 6 quick dips each.
15. Xylene, 3 changes, 10 dips each.
16. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Gram Positive Bacteria..... Blue
 Gram Negative Bacteria..... Red
 Background Yellow

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