

BROWN & BRENN STAIN FOR 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 Acetone.
CONTROL:	Tissue known positive for Gram positive and negative bacteria <i>Control Slides can be ordered 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. Sodium Bicarbonate 5% Aqueous Item# s275 3. Basic Fuchsin Working Solution Item# s2010 4. Picric Acid Acetone 0.1% Item# s1867 5. Gram's Iodine Item# s204 6. Ether Acetone 1:1 Item# s178P <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	
REFERENCE:	Carson, F. <u>Histotechnology: A Self-Instructional Text</u> . ASCP Press. Chicago, IL. 1990. pp. 126-127.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Place slides on staining rack. Place approximately 1mL (20 drops) of Crystal Violet 1% Aqueous and add 5 drops of Sodium Bicarbonate 5% Aqueous for 1 minute. Agitate slide slowly. If preferred, the two solutions may be mixed just before use.
3. Rinse in tap water.
4. Flood slides with Gram's Iodine for 1 minute.
5. Rinse in running water. Blot with bibulous paper.
6. Decolorize with Ether Acetone until no more color runs off.
7. Place directly into Basic Fuchsin Working Solution for 1 minute.
8. Wash in water, blot gently and dip in Acetone to start reaction.
9. Dip in Picric Acid Acetone until sections are yellowish pink.
10. Rinse quickly in Acetone, then in Acetone Xylene solution (50:50), 2 changes.
11. Clear in Xylene, 2 changes.
12. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Gram Negative Organisms Pink to Red
 Gram Positive Organisms Blue
 Nuclei Red
 Filaments of Nocardia and Actinomyces Blue
 Tissue Elements Yellow

Poly Scientific R&D Corp.

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