

TWORT'S METHOD FOR GRAM POSITIVE & NEGATIVE BACTERIA

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of Gram positive and 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. Neutral Red is taken up by the colorless bacteria and the excess is removed by Acetone.
CONTROL:	Any 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 4 micrometers
SOLUTIONS:	1. Crystal Violet Hucker's Item# s169 2. Lugol's Iodine Working Solution Item# s234A 3. Neutral Red Saturated Alcoholic Item# s2128 4. Twort's Green Item# s2587 <u>Twort's Working Solution:</u> Neutral Red 4.5 mL Twort's Green 0.5 mL Distilled Water 15 mL 5. Acetone Alcohol 1:1 Item# s103 <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	
REFERENCE:	Bancroft, J. D. & Stevens, A. <u>Theory and Practice of Histological Techniques</u> . 4th Ed. Churchill Livingstone. New York. 1996. pp 294-295.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to water.
2. Stain in filtered Crystal Violet Hucker's for 3 minutes on staining rack.
3. Mordant with Lugol's Iodine for 3 minutes.
4. Wash with water for 5 seconds.
5. Decolorize with Acetone Alcohol until the blue color ceases to run off section (about 4 dips in coplin jar).
6. Quickly rinse in water.
7. Stain in Twort's Working Solution for 5 minutes.
8. Decolorize in Acetone Alcohol 1:1.
9. Dehydrate in Absolute Alcohol, clear in Xylene and mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Gram Positive Bacteria..... Blue
 Gram Negative Organisms and Nuclei Red
 Background Blue to Green

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