

DIETERLE'S METHOD FOR SPIROCHETES AND LEGIONNAIRES' DISEASE

PURPOSE:	For In Vitro Diagnostic Use: Intended for the demonstration of Spirochetes in tissue.
PRINCIPLE:	Uranyl Nitrate acts as a sensitizer for the organisms. The organisms are able to bind silver which is then reduced by the Hydroquinone to the metallic form.
CONTROL:	Any tissue known to contain Spirochetes <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs012.</i>
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	<p>1. Uranyl Nitrate Alcoholic Solution Item# s2028 2. Gum Mastic 10% Alcoholic Item# s1818 3. Silver Nitrate 1% Aqueous Item# s1888</p> <p><u>Developer Solution:</u> Add chemicals and reagents to prepare developer solution (mix in order). This must be made fresh.</p> <p>8oz kit contains: Hydroquinone 3x5 grams, Sodium Sulfite 3x0.83 grams, Distilled Water 3x200 mL, Acetone 3x33 mL, Formaldehyde 3x33 mL, Pyridine 3x33 mL, Gum Mastic 10% Alcoholic 3x33 mL.</p> <p>16oz kit contains: Hydroquinone 5x5 grams, Sodium Sulfite 5x0.83 grams, Distilled Water 5x200 mL, Acetone 5x33 mL, Formaldehyde 5x33 mL, Pyridine 5x33 mL, Gum Mastic 10% Alcoholic 5x33 mL.</p> <p><i>Solutions can be purchased separately from Poly Scientific.</i></p>
NOTES:	Developer Solution turns milky as the Gum Mastic is added and medium brown when standing in a well-lighted area. As the brown streaks appear, swirl flask gently. Developer is ready to use in about 6 hours and may be used until color becomes dark brown.
REFERENCE:	Luna, Lee G. <u>Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts</u> . American Histolabs Inc. Gaithersburg, MD. 1992. pp. 223-224.

STAINING PROCEDURE:

1. Preheat the Alcoholic Uranyl Nitrate Solution and the 1% Silver Nitrate Solution in a 55-58°C oven for at least 30 minutes.
2. Deparaffinize and hydrate sections to distilled water.
3. Place sections in preheated Alcoholic Uranyl Nitrate in a 55-58°C oven for 30 minutes to 1 hour.
4. Distilled water, 1 dip.
5. 95% Alcohol, 1 dip.
6. Gum Mastic 10% Alcoholic, 3 minutes.
7. 95% Alcohol, 1 quick dip.
8. Distilled water, 1 minute then allow slides to drain for 15-20 minutes until almost dry (may be left overnight).
9. Place sections in preheated 1% Silver Nitrate Solution in a 55-58°C oven in the dark for 4 hours.
10. Distilled water, 2 dips.
11. Developer, Dip until sections are light tan.
12. Distilled water, 2 dips.
13. 95% Alcohol, 2 dips.
14. Acetone, 2 dips.
15. Xylene, 2 dips.
16. Xylene, 2 dips.
17. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Spirochetes, Bacteria.....Dark Brown to Black

BackgroundPale Yellow to Tan

Other structures that stain are melanin granules, chromatin, formalin pigment, and some foreign material in macrophages.

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