

DIETERLE'S METHOD FOR SPIROCHETES & LEGIONNAIRES' DISEASE (Microwave)

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative 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 visible 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 4-6 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 5x0.75 grams, Sodium Sulfite 5x0.15 grams, Distilled Water 5x30 mL, Acetone 5x5 mL, Formaldehyde 5x5 mL, Pyridine 5x5 mL, Gum Mastic 10% Alcoholic 5x5 mL.</p> <p>16oz kit contains: Hydroquinone 10x0.75 grams, Sodium Sulfite 10x0.15 grams, Distilled Water 10x30 mL, Acetone 10x5 mL, Formaldehyde 10x5 mL, Pyridine 10x5 mL, Gum Mastic 10% Alcoholic 10x5 mL.</p> <p><i>Solutions can be purchased separately from Poly Scientific.</i></p>
NOTES:	<p>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.</p> <p><i>Variations in timing may occur due to the power wattage of the microwave oven. Provided times and power levels are based on 1000 watt microwave oven.</i></p>
REFERENCE:	Rhatigan, K. Personal interview. McClain Laboratories, LLC. Smithtown, NY. 2004.

STAINING PROCEDURE:

1. Deparaffinize and hydrate sections to distilled water.
2. Microwave Uranyl Nitrate for 25 seconds at 70% power twice, stirring in-between. Place sections in solution for 2 minutes.
3. Distilled Water, 1 dip.
4. 95% Alcohol, 10 dips.
5. Gum Mastic 10% Alcoholic, 15 seconds.
6. 95% Alcohol, 10 dips.
7. Rinse in distilled water.
8. Place sections in Silver Nitrate 1% Aqueous and microwave for 60 seconds at 70% power. Let slides sit for 5 minutes.
9. Distilled water, 2 dips.
10. Microwave Developer 30-45 seconds. Leave slides in hot Developer for 4 minutes. Sections should turn light brown to pale yellow. Hold sections up to a strong incandescent light bulb for 30 seconds to increase stain intensity.
11. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
12. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Spirochetes, Bacteria Dark Brown to Black
Background Pale Yellow to Tan

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

Poly Scientific R&D Corp.

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