

STEINER MODIFIED METHOD FOR SPIROCHETES

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of Spirochetes.
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 positive for 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 micrometers
SOLUTIONS:	<ol style="list-style-type: none"> 1. Uranyl Nitrate 1% Aqueous Item# s288B 2. Silver Nitrate 1% Aqueous Item# s1888 3. Gum Mastic 2.5% Alcoholic Item# s2195 4. Components for Reducing Solution (prepare just before use): Gum Mastic 2.5% Alcoholic..... 20.0 mL Hydroquinone 2% Aqueous 50.0 mL Absolute Alcohol 10.0 mL Silver Nitrate 0.04% 5.0 mL Combine the Gum Mastic Solution and the Hydroquinone Solution. Add the Absolute Alcohol and mix well. Filter through Whatman #4 filter paper. Immediately before placing slides in solution, add 5 mL of 0.04% Silver Nitrate. <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	
REFERENCE:	Garvey, W., Fathi, A. and Bigelow. F "Modified Steiner for the Demonstration of Spirochetes". <i>J. Histotechnology</i> 8:15-17, 1985.

STAINING PROCEDURE:

1. Deparaffinize and hydrate slides to distilled water.
2. Place in Uranyl Nitrate 1% Aqueous, preheated to 60°C, for 15 minutes.
3. Rinse thoroughly in distilled water.
4. Place in Silver Nitrate 1% Aqueous for 1 hour at 60°C.
5. Rinse in 2 changes of distilled water.
6. Dehydrate in 2 changes of 95% and 2 changes of 100% Alcohol.
7. Gum Mastic 2.5% Alcoholic for 5 minutes.
8. Allow sections to air dry for 1 minute.
9. Rinse in 2 changes of distilled water.
10. Place sections in Reducing Solution that has been preheated to 45°C. Allow sections to develop for 15–20 minutes, occasionally agitating sections.
11. Rinse in distilled water to stop reduction.
12. Dehydrate, clear, and mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Spirochetes, Fungi, and Most BacteriaDark Brown to Black

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