

**STEINER MODIFIED METHOD FOR SPIROCHETES (Microwave)**

<b>PURPOSE:</b>	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of fungi and Pneumocystis Carinii in tissue.
<b>PRINCIPLE:</b>	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.
<b>CONTROL:</b>	Any tissue known positive for Spirochetes <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs012.</i>
<b>SPECIMEN PREPARATION:</b>	Formalin fixed, paraffin embedded sections cut at 6 micrometers
<b>SOLUTIONS:</b>	<p>1. Uranyl Nitrate 1% Aqueous Item# s288B                  2. Silver Nitrate 1% Aqueous Item# s1888                  3. Gum Mastic 2.5% Alcoholic Item# s2195</p> <p><i>Reducing Solution: Prepare just before use.</i>                  Gum Mastic 2.5% Alcoholic..... 20 mL                  Hydroquinone 2% Aqueous ..... 50 mL                  Absolute Alcohol ..... 10 mL                  Silver Nitrate 0.04% Aqueous ..... 5 mL</p> <p>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 Silver Nitrate 0.04% Aqueous.</p> <p><i>Solutions can be purchased separately from Poly Scientific.</i></p>
<b>NOTES:</b>	<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>
<b>REFERENCE:</b>	Luna, Lee G. <u>Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts</u> . American Histolabs Inc. Gaithersburg, MD. 1992. p. 218.

**STAINING PROCEDURE:**

1. Deparaffinize and hydrate slides to distilled water.
2. Using a plastic coplin jar, place slides in 45 mL of Uranyl Nitrate 1% Aqueous and microwave on high for 1 minute.
3. Rinse thoroughly in distilled water.
4. Using a plastic coplin jar, place slides in 45 mL of Silver Nitrate 1% Aqueous and microwave on high for one minute. Remove from oven and allow slides to stand in hot Silver Nitrate for 2-3 minutes.
5. Rinse in distilled water, 3 changes.
6. Dehydrate in 2 changes of 95% Alcohol and 2 changes of Absolute Alcohol.
7. Treat with 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. Preheat Reducing Solution in microwave on high for 20 seconds. Place slides in Reducing Solution, place in water bath at 45°C for 10–20 minutes or until sections have developed satisfactorily with Spirochetes at desired intensity and background is light yellow.
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 Bacteria .....Dark Brown to Black  
 Background.....Light Yellow to Brown

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