

WARTHIN STARRY METHOD FOR SPIROCHETES & OTHER MICROORGANISMS (Microwave)

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of Spirochetes and other microorganisms.
PRINCIPLE:	The Spirochetes, and other tissue components to a lesser degree, absorb silver from the Silver Solution. This deposited silver acts as a catalyst, facilitating the reduction of silver ions in the Developer by Hydroquinone to the 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 (do not use dichromate fixatives), paraffin embedded sections cut at 4-6 micrometers
SOLUTIONS:	<ol style="list-style-type: none"> 1. Acidulated Water Item# s108D 2. Silver Nitrate 1% Item# s2165 3. Silver Nitrate 2% Item# s2166 4. Gelatin 5% Solution Item# s190B 5. Hydroquinone 0.15% Solution Acidified Item# s1834 <p><u>Developer Solution:</u> Must be prepared fresh. Use chemically clean glassware. Combine in the order given: Silver Nitrate 2% 7.5 mL Gelatin 5% Solution 17.5 mL Hydroquinone 0.15% Solution Acidified 10 mL</p> <p><i>Solutions can be purchased separately from Poly Scientific.</i></p>
NOTES:	Do not use any metal. If staining is too dark reduce the time in the Developer. <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>
REFERENCE:	Luna, Lee G. <u>Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts</u> . American Histolabs Inc. Gaithersburg, MD. 1992. pp. 238-240.

STAINING PROCEDURE:

Note: Use paraffin coated forceps for steps 2 and 3.

1. Deparaffinize and hydrate to Acidulated Water.
2. Place 40 mL of Silver Nitrate 1% in a plastic coplin jar (loosely apply cap). Microwave for 20 seconds at 80% power. Stir and re-microwave for 20 seconds at 80% power. Stir and place slides in solution. Microwave an additional 30 seconds at 80% power. The temperature of the solution should not exceed 56°C. Remove from microwave and place on counter for 5 minutes. Prepare Developer at this point.
3. To prepare Developer place 17.5 mL of Gelatin 5% Solution in a plastic coplin jar (loosely apply cap). Microwave for 10 seconds at 80% power. Combine Developer contents in the order given. Flood sections that have been laid across glass rods with the Developer Solution, which must be used as soon as it is mixed. Allow sections to develop until they are light brown or grayish in color. This color change usually begins in 30–60 seconds and reaction is complete in 3-5 minutes. Check known control under microscope. The Spirochetes should be black and the background light brown or yellow.
4. Wash quickly in hot water, approximately 56°C.
5. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
6. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Spirochetes, Helicobacter Pylori and Other Microorganisms Black
Background Tan to Yellow

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