

**A-115 MODIFIED STEINER PROCEDURE FOR
MICROWAVE STAINING OF SPIROCHETES AND
NONFILAMENTOUS BACTERIA**

FIXATION: Formalin, 10% Buffered Neutral

STAINING PROCEDURE: *ACID WASH ALL GLASS AND PLASTIC WARE*

1. Deparaffinize and rehydrate tissue in distilled water.
2. Sensitize sections by placing in room temperature **Zinc Formalin W/Zinc Sulfate, (A-115-1A)** and microwaving sections just until boiling point is reached in 25-30 seconds. Do not boil. Immediately remove slide to distilled water.
3. Rinse in distilled water until possibility of cross contamination is eliminated.
4. Place in room temperature **Silver Nitrate, 1%**, (A-115-2) and microwave just until boiling point is reached in 30-45 seconds. Do not boil. Remove from oven, loosely cover jar, and allow to stand in hot silver nitrate for 5-10 minutes. Alternatively, preheat **Silver Nitrate, 1%**, (A-115-2) for 20-30 minutes in a 60°C water bath, add slides, and allow them to be impregnated at 60°C for 1-1 1/2 hours.
5. Rinse in 3 changes of distilled water.
6. Dehydrate in 2 changes of 95% alcohol.
7. Dehydrate in 2 changes of absolute alcohol.
8. Treat with **Gum Mastic, 2.5%**, (A-115-3) for 5 minutes.
9. Allow to air dry for 1 minute.
10. Rinse in 2 changes of distilled water. Slides may stand in distilled water while reducing solution is being prepared, or longer if necessary.
 - *To prepare reducing solution mix:
 - 10ml **Gum Mastic, 2.5%** (A-115-3)
 - 25ml **Hydroquinone, 2%** (A-115-4)
 - 5ml Absolute Alcohol
 - Prepare just before use-filter with #4 filter-paper and add 2.5ml of **Silver Nitrate, 0.04%** (A-115-5). DO NOT FILTER this solution. This reducing solution should have a milky appearance when the Gum Mastic is added.
11. Reduce in preheated reducing solution in a 45° water bath for 10-25 minutes or until sections have developed satisfactorily, with black spirochetes and background a light yellow. Avoid an intensely stained background that will interfere with spirochete identification. Coplin jars should be loosely covered.
12. Rinse in distilled water to stop reduction.
13. Dehydrate through graded alcohols, clear in xylene or other solvent that has no limonene, and mount with synthetic mountant.

RESULTS:

Spirochetes, Calymmatobacterium granulomatis, Legionella pneumophila, leptospira, and other nonfilamentous bacteria stain dark brown to black

Backgrounds stain bright yellow to light brown.

REFERENCES:

Billie L. Swisher, Modified Steiner Procedure for Microwave Staining of Spirochetes and Nonfilamentous Bacteria, The Journal of Histotechnology, Vol. 10., No.4, (Dec. 1987) 241-243