

**A-119      MODIFIED STEINER USING CHAPMAN'S  
MODIFICATION**

**FIXATION:**    **10% Buffered Neutral Formalin**

**SECTION:**    4-6 microns

**STAINING PROCEDURE:**

1. Deparaffinize and re-hydrate tissues to distilled water.
2. Sensitize sections in **Zinc Formalin** (A-119-1), at 60°C in a water bath for 15 minutes (cover jar loosely). Preheat this solution.
3. Rinse in distilled water, 3 times for 2 minutes each.
4. Place in **Silver Nitrate, 1%** (A-119-2), in a Coplin Jar. Place in microwave (800 watts); start and run for 80 seconds. Remove from microwave and allow to stand in hot silver solution for 20 minutes (cover jar loosely).
5. Rinse in distilled water 3 times for 2 minutes each.
6. Dehydrate in 2 changes of 95% ethanol followed by 2 changes of 100% ethanol.
7. Treat with **Gum Mastic, 2.5%** (A-119-3), for 5 minutes.
8. Allow to air dry for 1 minute.
9. Rinse in distilled water (2 changes). Slides may stand in distilled water while reducing solution is being made, or longer if necessary.
10. To prepare reducing solution, mix just before use:

a. Gum <b><u>Mastic, 2.5%</u></b> (A-119-3)	10.0ml
b. Hydroquinone, <b><u>2%</u></b> (A-119-4)	25.0ml
c. Ethanol, 100%	5.0ml

Filter and then add:

d. Silver <b><u>Nitrate, 0.04%</u></b> (A-119-5)	2.5ml
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**DO NOT filter this silver!**
11. Reducing solution at 45°C in water bath for 5-30 minutes or until sections have developed satisfactorily with the spirochetes at desired intensity and the background light yellow. Avoid an intensely stained background by checking microscopically. **DO NOT PREHEAT THIS SOLUTION.**
12. Rinse in distilled water to stop reaction.
13. Dehydrate through graded alcohols clear in, Xylene (not Xylene substitute).  
And mount with Permount (M-18).

**RESULTS:**

Spirochetes, Donovan Bodies, General Bacteria,  
Legionnaires' Disease Bacteria.....dark brown or black  
Background.....bright yellow to light brown

**\*NOTE: Acid wash all glassware used with silver solutions!**

**REFERENCES:**

Margeson, L.S. Chapman CM: Use of Zinc Formalin as a sensitizer in Silver Stains for Spirochetes. Journal of Histotechnology, 19:2 p. 135-138, 6-96.