

Steiner-Chapman Modified Silver Stain - Technical Memo

SOLUTIONS:	250 ml	500 ml	1 Liter
Zinc Formalin Fixative			Part 1482A
Silver Nitrate 1%, Aqueous	Part 13804A	Part 13804B	
Acidulated Water pH 4.0-4.1		Part 10013A	Part 10013B
Gum Mastic 2.5%, Alcoholic	Part 1145A	Part 1145B	

Additionally Needed:

Helicobacter sp., Animal Control Slides	Part 4274	or	Helicobacter sp., Artificial Control Slides	Part 4275
Xylene, ACS	Part 1445			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Alcohol, Ethyl Denatured, 95%	Part 10842			
Hydroquinone, Powder	Part 12089			
Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)			
Coplin Jar, Plastic	Part 5184 (for microwave modification)			

For storage requirements and expiration date refer to individual product labels.

APPLICATION:

Newcomer Supply Steiner-Chapman Modified Silver Stain procedure, with included microwave modification, is used for staining spirochetes, *Helicobacter pylori*, *Legionella pneumophila*, other nonfilamentous bacteria and fungus. This modified method eliminates the use of Uranyl Nitrate and its regulatory and disposal requirements.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)

- Mercury fixatives will inhibit silver staining

Technique: Paraffin sections cut at 5 microns

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

PRESTAINING PREPARATION:

1. All glassware/plasticware must be acid cleaned prior to use.
 - a. See Procedure Notes #1 and #2 (page 2).
2. Preheat Zinc Formalin Fixative (1482) to 60°C.
 - a. **Do not preheat if using Microwave Modification.**
3. Preheat Silver Nitrate 1%, Aqueous (13804) to 60°C.
 - a. **Do not preheat if using Microwave Modification.**
4. Prepare Hydroquinone Working Solution; combine and mix well.
 - a. Hydroquinone, Powder (12089) 0.5 gm
 - b. Distilled water 25 ml
 - c. Save for use in Step #13.

STAINING PROCEDURE:

5. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with Acidulated Water pH 4.0-4.1 (10013); two changes, 5 minutes each.
 - a. See Procedure Notes #3 and #4 (page 2).
6. Sensitize slides in preheated Zinc Formalin Fixative (Step #2) in a 60°C water bath or oven for 15 minutes.

Microwave Modification: See Procedure Note #5 (page 2).

 - a. Place slides in a plastic Coplin jar containing Zinc Formalin Fixative and microwave to 60°C.
 - b. Remove from microwave; incubate and agitate in warm solution under fume hood for an additional 5 minutes.
 - c. See Procedure Note #6 (page 2).
7. Rinse in several changes of Acidulated Water pH 4.0-4.1 (10013).

8. Place slides in preheated Silver Nitrate 1%, Aqueous (Step #3) and incubate in a 60°C water bath or oven for 1 hour.

Microwave Modification:

- a. Place slides in a plastic Coplin jar containing Silver Nitrate 1%, Aqueous and microwave to 60°C.
 - b. Remove from microwave and let stand for an additional 1 minute 30 seconds; agitate occasionally for even heat distribution.
9. Rinse well in several changes of distilled water.
 - a. Excessive rinsing may cause nuclei to pick up silver.
 10. Dip briefly in two changes each of 95% and 100% ethyl alcohols.
 11. Place slides in Gum Mastic 2.5%, Alcoholic (1145) for 5 minutes.
 12. Air-dry slides for 1-5 minutes until slides are milky white.
 13. Prepare fresh Reducing Solution by combining:

a. Gum Mastic 2.5 %, Alcoholic	15 ml
b. Hydroquinone Working Solution (Step #4)	25 ml
c. <u>Filter, then add and mix well:</u>	
d. Silver Nitrate 1%, Aqueous	0.3 ml
 14. Rinse air-dried slides quickly in distilled water.
 15. Place slides in fresh Reducing Solution. Incubate in a 45°C water bath for 10-30 minutes with frequent agitation; examine microscopically at 10 minutes.
 - a. Check staining progress at timed intervals.
 - b. Tissue will turn tan in color; bacteria will be black when the tissue reaches a golden brown color.
 - c. Dip in warm distilled water before and after examination.

Microwave Modification: See Procedure Note #7 (page 2).

 - a. Heat slides in a plastic Coplin jar containing fresh Reducing Solution and microwave at 45°C for 30 seconds.
 - b. Remove from microwave; continue to incubate in warm solution for an additional 2 minutes.
 16. Wash for 3 minutes in running tap water; rinse in distilled water.
 17. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Spirochetes	Dark brown to black
<i>Helicobacter pylori</i>	Dark brown to black
<i>Legionella pneumophila</i>	Dark brown to black
Nonfilamentous bacteria and fungus	Dark brown to black
Background	Golden brown

PROCEDURE NOTES:

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water. Cleaning glassware with bleach is not equivalent to acid washing.
2. Plastic (5500), plastic tipped or paraffin coated metal forceps must be used with any silver solution to prevent precipitation of silver salts. No metals of any kind should be in contact with any silver solution. Only glass thermometers should be used.
3. Drain staining rack/slides after each step to prevent solution carry over.
4. The use of Acidulated Water pH 4.0-4.1 rinses (Steps #5 and #7) is recommended to ensure proper tissue pH, enhanced staining and reduced background staining.
5. The suggested microwave procedure has been tested at Newcomer Supply using an "EB Sciences", 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer's instructions, to prevent exposure to chemical vapors.
6. Heating Zinc Formalin Fixative will result in liberation of toxic fumes. Use appropriate protective actions.
7. The Reducing Solution contains alcohol and will reduce its boiling point. Do not boil solution. Adjust microwave times and power levels accordingly.
8. The use of some xylene substitutes may result in diminished spirochete staining. If using a xylene substitute exercise caution and closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 238-239.
2. Chapman, Clifford, and Lorelei Margeson. "Use of Zinc Formalin as a Sensitizer in Silver Stains for Spirochetes." *The Journal of Histotechnology* 19.2 (1996): 135-138.
3. Steiner, Gabriel, and Grete Steiner. "New Simple Silver Stain for Demonstration of Bacteria, Spirochetes and Fungi in Sections of Paraffin Embedded Tissue Blocks." *Journal of Laboratory Clinical Medicine* 29 (1944). 868-871.
4. Modifications developed by Newcomer Supply Laboratory.