

Steiner-Steiner Modified Silver Stain - Technical Memo

SOLUTIONS:

	250 ml	500 ml	1 Liter
Uranyl Nitrate 1%, Aqueous	Part 14036A	Part 14036B	Part 14036C
Silver Nitrate 1%, Aqueous	Part 13804A	Part 13804B	
Gum Mastic 2.5%, Alcoholic	Part 1145A	Part 1145B	

Additionally Needed:

Helicobacter sp., Animal Control Slides	Part 4274	or Helicobacter sp., Control Slides	Part 4276
Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)		
Xylene, ACS	Part 1445		
Alcohol, Ethyl Denatured, 100%	Part 10841		
Alcohol, Ethyl Denatured, 95%	Part 10842		
Hydroquinone, Powder	Part 12089		
Coplin Jar, Plastic	Part 5184 (for microwave modification)		

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

APPLICATION:

Newcomer Supply Steiner-Steiner Modified Silver procedure, with included microwave modification, is a silver technique effective for the demonstration of spirochetes, *Helicobacter pylori*, *Legionella pneumophila*, other nonfilamentous bacteria and fungus.

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.

STAINING PROCEDURE:

- All glassware/plasticware must be acid cleaned prior to use.
 - See Procedure Notes #1 and #2 (page 2).
- Preheat Uranyl Nitrate 1%, Aqueous and Silver Nitrate 1%, Aqueous to 60°C in a water bath.
 - Skip Step #2 if using Microwave Modification.**
- 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 distilled water.
 - See Procedure Note #3 (page 2)
- Sensitize slides in preheated Uranyl Nitrate 1%, Aqueous for 15 minutes in a 60°C water bath. Agitate solution to evenly distribute heat.
 - See Procedure Note #4 (page 2).

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

 - Place slides in a plastic Coplin jar containing Uranyl Nitrate 1%, Aqueous and microwave at 60°C for 5 minutes.
- Rinse well in several changes of distilled water.
- Prepare Hydroquinone Working Solution; combine and mix well.
 - Hydroquinone, Powder (12089) 0.5 gm
 - Distilled Water 25 ml
 - Save for use in Step #12.
- Place slides in preheated Silver Nitrate 1%, Aqueous and incubate in a 60°C water bath for 30 minutes.

Microwave Modification:

 - Place slides in a plastic Coplin jar containing Silver Nitrate 1%, Aqueous and microwave at 70°C for 5 minutes.

- Rinse well in several changes of distilled water.
 - Excessive rinsing may cause nucleus to pick up silver.
- Dip briefly in two changes each of 95% and 100% ethyl alcohols.
- Place slides in Gum Mastic 2.5%, Alcoholic for 5 minutes.
- Air-dry for 1-5 minutes until slides are milky white.
- Prepare fresh Reducing Solution by combining:
 - Gum Mastic 2.5%, Alcoholic 15 ml
 - Hydroquinone Working Solution (Step #6) 25 ml
 - Filter, then add and mix well:
 - Silver Nitrate 1%, Aqueous 0.3 ml
- Preheat fresh Reducing Solution in a 45°C water bath. Place slides in preheated solution and incubate in a 45°C water bath for 10-30 minutes with frequent agitation; examine microscopically at 10 minutes.
 - Check staining progress at timed intervals. Tissue will turn tan in color; continue to check staining progress at timed intervals. Bacteria will be black when the tissue reaches a golden brown color.
 - Dip in warm distilled water before and after examination.

Microwave Modification: See Procedure Note #6 (page 2)

 - Heat slides in a plastic Coplin jar containing fresh Reducing Solution at 45°C for 30 seconds.
 - Remove from microwave. Continue to incubate slides in the warm solution for an additional 2 minutes.
- Wash for 3 minutes in running tap water; rinse in distilled water.
- 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 (5502), 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. Dispose of Uranyl Nitrate as hazardous waste and/or according to local and state environmental regulations. Refer to SDS for personal protective measures and handling information.
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. The Reducing Solution contains alcohol and will reduce its boiling point. Do not boil solution. Adjust microwave times and power levels accordingly.
7. The use of some xylene substitutes have resulted in diminished spirochete staining. If using a xylene substitute exercise caution and closely follow the manufacturer's recommendation for deparaffinization and clearing steps.

REFERENCES:

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8. Swisher, Billie. "Modified Steiner Procedure for Microwave Staining of Spirochetes and Nonfilamentous Bacteria." *The Journal of Histotechnology* 10.4 (1987): 241-243.
9. Modifications developed by Newcomer Supply Laboratory.