

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:

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|---------------------------------------|--|----|---|-----------|
| Spirochete, Artificial Control Slides | Part 4655 | 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 modifications) | | | |

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

APPLICATION:

Newcomer Supply Steiner-Steiner Modified Silver procedure, with included microwave modifications, 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.

PRESTAINING PREPARATION:

- All glassware/plasticware must be acid cleaned prior to use.
 - See Procedure Notes #1 and #2 (page 2).
- Preheat Uranyl Nitrate 1%, Aqueous to 60°C in a water bath. Save for Step #8.
- Preheat Silver Nitrate 1%, Aqueous (13804) to 60°C in a water bath. Save for Step #10.
- Prepare Hydroquinone Solution; combine and mix well.
 - Hydroquinone, Powder (12089) 0.5 gm
 - Distilled Water 25 ml
- Prepare fresh Reducing Solution by combining in order listed.
 - Hydroquinone Solution (Step #4) 25 ml
 - Gum Mastic 2.5%, Alcoholic (1145) 15 ml
 - Silver Nitrate 1%, Aqueous (13804) 0.3 ml
 - Solution will turn milky white after addition of Gum Mastic.
 - Preheat solution in 45°C water bath. Save for Step #16.
- Do not preheat solutions if using Microwave Modifications.

STAINING PROCEDURE:

- 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.

Microwave Modification: See Procedure Note #4 (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.
- Place slides in preheated Silver Nitrate 1%, Aqueous (Step #3) and incubate in a 60°C water bath for 30 minutes.
 - Agitate solution to evenly distribute heat.

Microwave Modification:

 - Place slides in a plastic Coplin jar containing Silver Nitrate 1%, Aqueous (13804) 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 5 times in each of two changes of fresh 95% ethyl alcohol.
- Dip 5 times in each of two changes of fresh 100% ethyl alcohol.
- Place in Gum Mastic 2.5%, Alcoholic (1145) for 5 minutes.
- Air-dry for 5 minutes. Slides/sections will be milky white.
- Place slides in preheated Reducing Solution (Step #5) and incubate in 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/after each examination.

Microwave Modification: See Procedure Note #5 (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 warm solution for an additional 2 minutes.
- Rinse well in several changes of 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 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 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.
5. The Reducing Solution contains alcohol and will reduce its boiling point. To avoid boiling solution, adjust microwave times and power levels accordingly.
6. 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.
7. 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.

REFERENCES:

1. Garvey, Winsome. "Some Favorite Silver Stains." *The Journal of Histotechnology* 19.3 (1996): 269-278.
2. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolabs, 1992. 218-219.
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. Swisher, Billie. "Modified Steiner Procedure for Microwave Staining of Spirochetes and Nonfilamentous Bacteria." *The Journal of Histotechnology* 10.4 (1987): 241-243.
5. Modifications developed by Newcomer Supply Laboratory.