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Part 9171
Revised December 2015

# Steiner-Steiner Modified Silver Stain Kit - Technical Memo

| **KIT INCLUDES:** | **Part 9171A** |
|---|---|
| Solution A: Uranyl Nitrate 1%, Aqueous | 250 ml |
| Solution B: Silver Nitrate 1%, Aqueous | 250 ml |
| Solution C: Gum Mastic 2.5%, Alcoholic | 175 ml x 2 |
| Ingredient D: Hydroquinone, Powder | 5 grams |
| Mini Sampling Spoon | |

**COMPLIMENTARY POSITIVE CONTROL SLIDES**: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

*Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.*

| Additionally Needed: | |
|---|---|
| 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 |
| Coplin Jar, Plastic | Part 5184 (for microwave modification) |

***For storage requirements and expiration date refer to individual bottle labels.***

## APPLICATION:

Newcomer Supply Steiner-Steiner Modified Silver Stain Kit 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 Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

## STAINING PROCEDURE:

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

   ***Microwave Modification:*** *See Procedure Note #5 (page 2).*
   a. *Place slides in a plastic Coplin jar containing Solution A: Uranyl Nitrate 1%, Aqueous and microwave at 60°C for 5 minutes.*
5. Rinse well in several changes of distilled water.
6. Prepare Hydroquinone Working Solution; combine and mix well.
   a. *Ingredient D: Hydroquinone, Powder 0.5 gm*
      *(or one rounded scoop with reusable mini sampling spoon)*
   b. *Distilled Water 25 ml*
   c. *Save for use in Step #12.*
7. Place slides in preheated Solution B: Silver Nitrate 1%, Aqueous and incubate in a 60°C water bath for 30 minutes.

   ***Microwave Modification:***
   a. *Place slides in a plastic Coplin jar containing Solution B: Silver Nitrate 1%, Aqueous and microwave at 70°C for 5 minutes.*
8. Rinse well in several changes of distilled water.
   a. *Excessive rinsing may cause nucleus to pick up silver.*
9. Dip briefly in 2 changes each of 95% and 100% ethyl alcohols.
10. Place slides in Solution C: Gum Mastic 2.5%, Alcoholic for 5 minutes.
11. Air-dry for 1-5 minutes until slides are milky white.
12. Prepare fresh Reducing Solution by combining:
    a. *Solution C: Gum Mastic 2.5%, Alcoholic 15 ml*
    b. *Hydroquinone Working Solution (Step #6) 25 ml*
    c. *Filter, then add and mix well:*
    d. *Solution B: Silver Nitrate 1%, Aqueous 0.3 ml*
13. 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.
    a. *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.*
    b. *Dip in warm distilled water before and after examination.*

    ***Microwave Modification:*** *See Procedure Note #6 (page 2)*
    a. *Heat slides in a plastic Coplin jar containing fresh Reducing Solution at 45°C for 30 seconds.*
    b. *Remove from microwave. Continue to incubate slides in the warm solution for an additional 2 minutes.*
14. Wash for 3 minutes in running tap water; rinse in distilled water.
15. 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 |
| *Helicobacter pylori* | Dark brown to black |
| *Legionella pneumophila* | 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. 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. To avoid boiling 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:**

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 249-250.
2. Churukian, Charles, and Winsome Garvey. "Microwave Steiner Method for Spirochetes and Bacteria." *The Journal of Histotechnology* 13.1 (1990): 45-47.
3. Garvey, Winsome. "Some Favorite Silver Stains." *The Journal of Histotechnology* 19.3 (1996): 269-278.
4. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaitheresburg, MD: American Histolabs, 1992. 218-219.
5. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 241-242.
6. 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.
7. Swisher, Billie. "Modified Steiner Procedure for Microwave Staining of Spirochetes and Nonfilamentous Bacteria." *The Journal of Histotechnology* 10.4 (1987): 241-243.
8. Modifications developed by Newcomer Supply Laboratory.