

# Steiner-Chapman Modified Silver Stain Kit - Technical Memo

## KIT INCLUDES:

	Part 9172A	Part 9172B
Solution A: Zinc Formalin Sensitizer	250 ml	500 ml
Solution B: Silver Nitrate 1%, Aqueous	250 ml	500 ml
Solution C: Gum Mastic 2.5%, Alcoholic	175 ml x 2	350 ml x 2
Ingredient D: Hydroquinone, Powder	5 Grams	10 Grams
Mini Sampling Spoon		

**COMPLIMENTARY POSITIVE CONTROL SLIDES:** Enclosed are two complimentary unstained positive control slides 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](http://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
Acidulated Water pH 4.0-4.1	Part 10013
Coplin Jar, Plastic	Part 5184 (for microwave modification)

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

## APPLICATION:

Newcomer Supply Steiner-Chapman Modified Silver Stain Kit 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)

**Technique:** Paraffin sections cut at 4 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 provided staining procedure. Some solutions in the kit may contain extra volumes.

## PRESTAINING PREPARATION:

- If necessary, heat dry tissue sections/slides in oven.
- All glassware/plasticware must be acid cleaned prior to use.
  - See Procedure Notes #1 and #2 (page 2).
- Preheat Solution A: Zinc Formalin Sensitizer to 60°C.
- Preheat Solution B: Silver Nitrate 1%, Aqueous to 60°C.
- Prepare Hydroquinone Working Solution; combine and mix well.
  - Ingredient D: Hydroquinone, Powder 0.5 gm  
(or one rounded scoop with reusable mini sampling spoon)
  - Distilled water 25 ml
- Prepare fresh Reducing Solution by combining:
  - Solution C: Gum Mastic 2.5 %, Alcoholic 15 ml
  - Hydroquinone Working Solution (Step #5) 25 ml
  - Filter, then add and mix well;
  - Solution B: Silver Nitrate 1%, Aqueous 0.6 ml
  - Preheat solution in 45°C water bath. Save for Step #16.
- Do not preheat solutions if using Microwave Modification.**

## 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.
  - See Procedure Note #3 (page 2)
- Wash well in two changes of Acidulated Water pH 4.0-4.1 (10013).
  - See Procedure Note #4 (page 2).
- Sensitize slides in preheated Solution A: Zinc Formalin Sensitizer (Step #3) in a 60°C water bath or oven for 10 minutes.

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

- Place slides in a plastic Coplin jar with Solution A: Zinc Formalin Sensitizer. Microwave at 60°C for 1 minute.
  - Remove from microwave; Move to fume hood, cover and incubate for 1 minute with gentle agitation.
- Rinse in two changes of Acidulated Water pH 4.0-4.1 (10013).
  - Place slides in preheated Solution B: Silver Nitrate 1%, Aqueous (Step #4) and incubate in 60°C water bath or oven for 15 minutes.
- Microwave Modification:**
- Place slides in plastic Coplin jar with Solution B: Silver Nitrate 1%, Aqueous. Microwave at 70°C for 1 minute.
  - Remove from microwave and let stand an additional 90 seconds; agitate occasionally for even heat distribution.
- Rinse well in several changes of distilled water.
    - Excessive rinsing may cause nuclei to pick up silver.
  - Dip briefly in two changes each of 95% and 100% ethyl alcohols.
  - Place in Solution C: Gum Mastic 2.5%, Alcoholic for 3 minutes.
  - Place slides in preheated Reducing Solution (Step #6) in 45°C water bath for 15-30 minutes with frequent agitation. Examine microscopically after 15 minutes of incubation.
    - Check microscopically by dipping slide in 100% alcohol.
    - Review for desired staining results.
    - If necessary, return to warm solution; check every 2 minutes until results are achieved.

## Microwave Modification:

- Place slides in a plastic Coplin jar with Reducing Solution. Microwave at 70°C for 1 minute. Remove from microwave.
  - Pipette solution twice with plastic pipette to evenly distribute heated solution.
  - Cover and let sit for 2-4 minutes.
  - Check microscopically by dipping slide in 100% alcohol.
  - Review for desired staining results.
  - If necessary, return to warm solution, check every 2 minutes until desired results are achieved.
- Dehydrate in two changes of 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.
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 slides after each step to prevent solution carry over.
4. The use of Acidulated Water pH 4.0-4.1 rinses (Steps #9 and #11) is recommended for proper tissue pH and enhanced staining.
5. The suggested microwave procedure has been tested at Newcomer Supply. This procedure is a guideline and techniques should be developed for use in your laboratory.
6. If using a xylene substitute 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.