

Leptospira sp., Animal Control Slides – Technical Memo

CONTROL SLIDES:	Part 4402A	Part 4402B
	10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:

Tissue: Positive staining hamster liver and negative staining human lung.

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

Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.

Quality Control Stain: Steiner-Steiner Modified quality control stained slide(s) included.

Reactivity: Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year to verify continued reactivity.

Storage: 15-30°C in a light deprived and humidity controlled environment.

Intended Use: To verify histological techniques and reagent reactivity.

Before using unstained control slides, review the enclosed stained slide(s) to ensure that this tissue source is acceptable for testing needs.

CONTROL SLIDE VALIDATION:

With Steiner-Steiner Modified Silver Stain Kit:	Part 9171A	Individual Stain Solution
Solution A: Uranyl Nitrate 1%, Aqueous	250 ml	Part 14036
Solution B: Silver Nitrate 1%, Aqueous	250 ml	Part 13804
Solution C: Gum Mastic 2.5%, Alcoholic	350 ml	Part 1145
Ingredient D: Hydroquinone, Powder	5 grams	Part 12089

APPLICATION:

Newcomer Supply *Leptospira sp.*, Animal Control Slides are for the positive histochemical staining of *Leptospira sp.*, a corkscrew shaped bacteria readily visualized with silver staining techniques.

PRESTAINING PREPARATION:

1. Heat dry sections in oven according to your laboratory protocol.
2. All glassware/plasticware must be acid cleaned prior to use.
 - a. See Procedure Notes #1 and #2 (page 2).
3. Preheat Solution A: Uranyl Nitrate 1%, Aqueous to 60°C in a water bath. Save for Step #9.
4. Preheat Solution B: Silver Nitrate 1%, Aqueous to 60°C in a water bath. Save for Step #11.
5. Prepare Hydroquinone 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
6. Prepare fresh Reducing Solution by combining in order listed.
 - a. Hydroquinone Solution (Step #5) 25 ml
 - b. Solution C: Gum Mastic 2.5%, Alcoholic 15 ml
 - c. Solution B: Silver Nitrate 1%, Aqueous 0.6 ml
 - d. Solution will turn milky white after addition of Gum Mastic.
 - e. Preheat solution in 45°C water bath. Save for Step #15.
7. **Do not preheat solutions if using Microwave Modifications.**

STAINING PROCEDURE:

8. 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)
9. Sensitize in preheated Solution A: Uranyl Nitrate 1%, Aqueous (Step #3) for 10 minutes in a 60°C water bath.

Microwave Modification: See Procedure Note #4 (page 2).

 - a. Place slides in a plastic Coplin jar with Solution A: Uranyl Nitrate 1%, Aqueous. Microwave at 70°C for 1 minute.
10. Rinse well in several changes of distilled water.
11. Place slides in preheated Solution B: Silver Nitrate 1%, Aqueous (Step #4) and incubate in a 60°C water bath for 15 minutes.

Microwave Modification:

 - a. Place slides in a plastic Coplin jar with Solution B: Silver Nitrate 1%, Aqueous. Microwave at 70°C for 1 minute.
 - b. Remove from microwave, cover and let sit for 1 minute.
12. Rinse well in several changes of distilled water.
 - a. Excessive rinsing may cause nucleus to pick up silver.

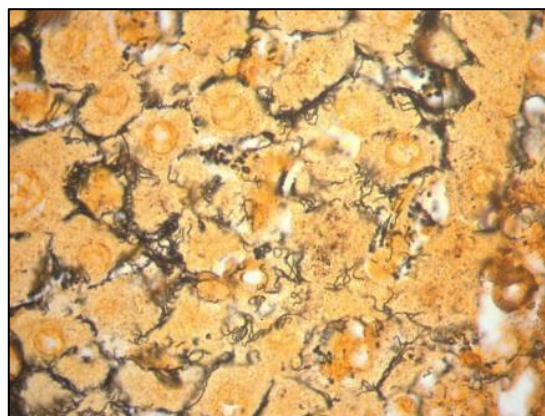
13. Dip 5 times in two changes each of 95% and 100% ethyl alcohols.
14. Place in Solution C: Gum Mastic 2.5%, Alcoholic for 3 minutes.
15. Place slides in preheated Reducing Solution (Step #6) in 45°C water bath for 10-30 minutes with frequent agitation. Examine microscopically after 10 minutes of incubation.
 - a. Check microscopically by dipping slide in 100% alcohol.
 - b. Review for desired staining results.
 - c. If necessary, return to warm solution; check every 2-5 minutes until desired results are achieved.

Microwave Modification:

- a. Place slides in a plastic Coplin jar with Reducing Solution. Microwave at 70°C for 1 minute. Remove from microwave.
 - b. Pipette solution twice with plastic pipette to evenly distribute heated solution.
 - c. Cover and let sit for 1 minute.
 - d. Check microscopically by dipping slide in 100% alcohol.
 - e. Review for desired staining results.
 - f. If necessary, return to warm solution, check every 1 minute until desired results are achieved.
16. Directly dehydrate in two changes of 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

<i>Leptospira sp.</i>	Dark brown to black
Background	Golden brown
Negative lung	Negative for <i>Leptospira sp.</i>



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 suggested microwave procedure has been tested at Newcomer Supply. This procedure is a guideline and techniques should be developed for your laboratory.
5. If using a xylene substitute, follow manufacturer's recommendation for deparaffinization and clearing steps.

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.