

Legionella sp. Control Slides – Technical Memo

CONTROL SLIDES:	Part 4400A	Part 4400B
	10 Slide/Set	98 Slide/Set

Legionella sp. Control Slides contain sections of positive staining lung and negative staining lung.

PRODUCT DESCRIPTION:

The enclosed positive control slides are intended to be used to verify histological techniques and reagent reactivity. These slides are to be used for the qualitative purpose of determining positive or negative results, and are not intended to be used for any quantitative purpose. The first serial section within the control box is stained and provided for your reference. **Before using the unstained slides, review the enclosed stained slide with your pathologist to ensure that this tissue source is acceptable. Newcomer Supply will not accept a return with missing slides in the series. Newcomer Supply guarantees reactivity of these control slides for one year from the date of receipt. Revalidate after one year to verify continued reactivity. Store at 15-30°C in a light deprived and humidity controlled environment.**

These positive control slides were produced from human surgical or autopsy tissues under carefully controlled conditions. Reasonable measures are used to deliver quality control slides that are as consistent as possible. However, characteristics of quality control slides may be dissimilar due to variations in the reagents, stains, techniques, laboratory conditions, and tissue sources used. Newcomer Supply Laboratory uses a manual method of performing quality control procedures, specifically avoiding automation, in order to provide reactive control slides for even less aggressive methods of staining that our customers may be using.

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

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

APPLICATION:

Newcomer Supply *Legionella sp.* Control Slides are for the positive histochemical staining of *Legionella sp.*, a gram negative bacteria readily visualized with silver staining techniques.

METHOD:

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

Technique: Paraffin sections cut at 5 microns on Superfrost® Plus

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

NEWCOMER SUPPLY VALIDATION 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
 - 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:

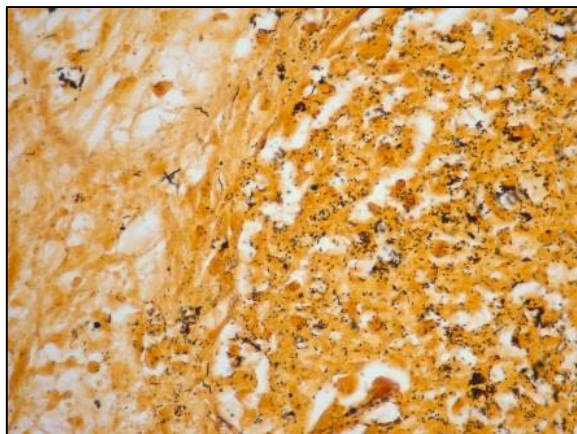
a. Solution C: Gum Mastic 2.5%, Alcoholic	15 ml
b. Hydroquinone Working Solution (Step #6)	25 ml
c. <u>Filter, then add and mix well:</u>	
d. Solution B: Silver Nitrate 1%, Aqueous	0.3 ml
13. Preheat fresh Reducing Solution in a 45°C water bath. Place slides in preheated solution and incubate in 45°C 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:

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



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*. Gaithersburg, 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.