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Part 4655
Revised January 2020

Spirochete, Artificial Control Slides – Technical Memo

CONTROL SLIDES:

	Part 4655A	**Part 4655B**
	10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:
Tissue: Positive staining rat 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 Spirochete, Artificial Control Slides are for the positive histochemical staining of spirochetes, the causative agent of a variety of diseases such as; syphilis, bejel, pinta, yaws and lyme. *Brachyspira hyodysenteriae,* purchased from American Type Culture Collection, is used to produce the positive control tissue.

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 0.3 ml*
   d. *Solution will turn milky white after addition of Gum Mastic.*
   e. *Preheat solution in 45°C water bath. Save for Step #17.*
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 slides in preheated Solution A: Uranyl Nitrate 1%, Aqueous (Step #3) for 15 minutes in a 60°C water bath.
   a. *Agitate solution to evenly distribute heat.*

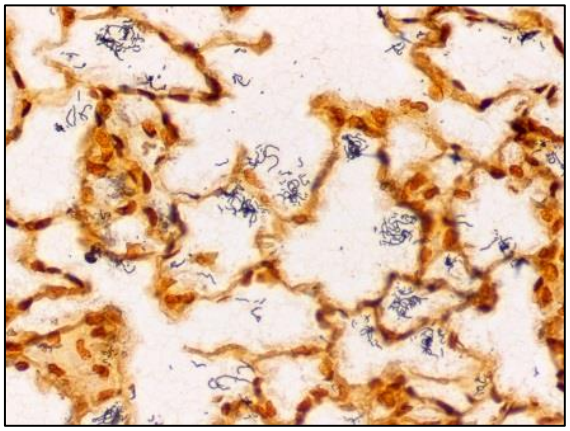
   Microwave Modification: *See Procedure Note #4 (page 2).*
   a. *Place slides in a plastic Coplin jar containing Solution A: Uranyl Nitrate 1%, Aqueous and microwave at 60°C for 5 minutes.*
10. Rinse well in several changes of distilled water.
11. Place slides in preheated Solution B: Silver Nitrate 1%, Aqueous (Step #4) and incubate in a 60°C water bath for 30 minutes.
    a. *Agitate solution to evenly distribute heat.*

    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.*
12. Rinse well in several changes of distilled water.
    a. *Excessive rinsing may cause nucleus to pick up silver.*
13. Dip 5 times in each of two changes of fresh 95% ethyl alcohol.
14. Dip 5 times in each of two changes of fresh 100% ethyl alcohol.
15. Place in Solution C: Gum Mastic 2.5%, Alcoholic for 5 minutes.
16. Air-dry for 5 minutes. Slides/sections will be milky white.
17. Place slides in preheated Reducing Solution (Step #6) 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.*
    b. *Tissue will turn tan in color; continue to check staining progress at timed intervals.*
    c. *Bacteria will be black when the tissue reaches a golden brown color.*
    d. *Dip in warm distilled water before/after each examination.*

    Microwave Modification: *See Procedure Note #5 (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 warm solution for an additional 2 minutes.*
18. Rinse well in several changes of distilled water.
19. 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
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 suggested microwave procedure has been tested at Newcomer Supply. This procedure is a guideline and techniques should be developed for use in your laboratory.
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 additional 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*. Gaitheresburg, 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.