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Part 4290
Revised February 2024

Histoplasma, Animal Control Slides – Technical Memo

CONTROL SLIDES:

Part 4290A	**Part 4290B**
10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:
Tissue: Positive staining animal organ.
Fixation: Formalin 10%, Phosphate Buffered (Part 1090).
Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.
Quality Control Stain: Grocott Methenamine Silver 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 Fungus, Grocott Methenamine Silver (GMS) Stain Kit:	**Part 9121A/B**	**Individual Stain Solution**
Solution A: Chromic Acid 5%, Aqueous	250/500 ml	Part 10341
Solution B: Sodium Bisulfite 1%, Aqueous	250/500 ml	Part 13821
Solution C: Silver Nitrate	125/250 ml	Part 1142
Solution D: Methenamine Borate	125/250 ml	Part 1142
Solution E: Gold Chloride 0.1%, Aqueous	250/500 ml	Part 11285
Solution F: Sodium Thiosulfate 2%, Aqueous	250/500 ml	Part 13888
Solution G: Light Green SF Yellowish Stain 0.2%, Aqueous	250/500 ml	Part 12202

APPLICATION:

Newcomer Supply *Histoplasma*, Animal Control Slides are for the positive histochemical staining of fungal organisms in tissue sections. The morphology of the organisms is consistent with *Histoplasma sp*.

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. Prepare Silver-Methenamine Working Solution and mix well.
   a. *Solution C: Silver Nitrate* *20 ml*
   b. *Solution D: Methenamine Borate* *20 ml*
4. Preheat Silver-Methenamine Working Solution to 45°C-60°C in a water bath approximately 20 to 30 minutes before use.
   a. *See Procedure Note #3 (page 2).*
   b. **Do not preheat if using Microwave Modification; Step 11.**

STAINING PROCEDURE:

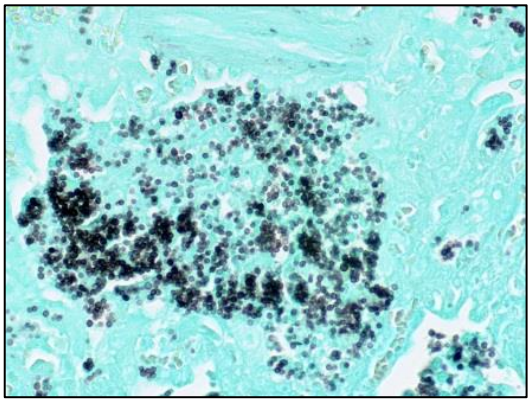
5. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
   a. *See Procedure Notes #4 and #5 (page 2).*
6. Oxidize in Solution A: Chromic Acid 5%, Aqueous for 1 hour.
   Microwave Modification: *See Procedure Note #6 (page 2)*
   a. *Microwave Solution A: Chromic Acid 5%, Aqueous* ***without slides*** *in a plastic Coplin jar (5184) for 1 minute at 60°C. Add slides to heated Solution A and oxidize for 10 minutes.*
7. Wash well in running tap water; rinse in distilled water.
8. Place in Solution B: Sodium Bisulfite 1%, Aqueous for 1 minute.
9. Wash in running tap water for 5 minutes; rinse well in distilled water.
10. Incubate slides in preheated Silver-Methenamine Working Solution (Step #4) at 45°C-60°C or at room temperature, for 12-18 minutes until sections appear paper-bag brown.
    a. *Periodically remove control, rinse in warm distilled water, check microscopically for adequate silver impregnation. Fungi should be dark brown.*
    b. *If organisms are not sufficiently dark, return slides to warm silver solution. Recheck at 2-3 minute intervals until desired intensity is achieved.*
    c. *Staining at room temperature will require longer incubation.*

Microwave Modification:

    a. *Incubate slides in a plastic Coplin jar containing Silver-Methenamine Working Solution and microwave for 5 minutes at 45°C.*
    b. *Check microscopically for adequate development.*
    c. *If additional incubation is required, return slides to warm silver solution. Recheck at 3-5 minute intervals.*
11. Rinse in three to four changes of distilled water.
    a. *Do not use tap water at this step.*
12. Tone in Solution E: Gold Chloride 0.1%, Aqueous until sections turn gray; 10-30 seconds.
13. Rinse well in distilled water.
14. Remove unreduced silver in Solution F: Sodium Thiosulfate 2%, Aqueous for 2 minutes.
15. Wash in running tap water for 5 minutes; rinse in distilled water.
16. Counterstain in Solution G: Light Green SF Yellowish Stain 0.2%, Aqueous for 2 minutes.
17. Dehydrate quickly 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:

Histoplasma	Sharply outlined in black
Background	Green



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. Maintain solution between 45°C-60°C to minimize precipitate.
4. Drain slides after each step to prevent solution carry over.
5. Do not allow sections to dry out at any point during procedure.
6. 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.
7. If using a xylene substitute, follow manufacturer's recommendation for deparaffinization and clearing steps.

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REFERENCES:

1. Carson, Freida L., and Christa Cappellano. *Histotechnology: A Self-Instructional Text*. 5th ed. Chicago: ASCP Press, 2020. 221-226.
2. Grocott, R G, "A Stain for Fungi in Tissue Sections and Smears using Gomori Methenamine Silver Nitrate Technic". *American Journal of Clinical Pathology* 25 (1955): 975-979.
3. Koski, John. "Silver Methenamine Borate (SMB): Cost Reduction with Technical Improvement in Silver Nitrate-Gold Chloride Impregnations." *The Journal of Histotechnology* 4.3 (1981): 115-119.
4. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 245-246.
5. Modifications developed by Newcomer Supply Laboratory.