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Part 4235
Revised January 2020

Fungus, GMS, Multi-Tissue, Artificial Control Slides – Technical Memo

CONTROL SLIDES:

	Part 4235A	**Part 4235B**
	10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:

Tissue: Positive staining *Aspergillus sp.* rat lung, positive staining *Candida sp.* rat lung, positive staining *Histoplasma sp.* animal tissue and negative staining lung.
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 0.02%, Aqueous	250/500 ml	Part 12204

APPLICATION:

Newcomer Supply Fungus, GMS, Multi-Tissue, Artificial Control Slides, use a variety of lung tissue sources for the positive histochemical staining of *Aspergillus sp*., *Candida sp*., and organisms exhibiting morphology consistent with *Histoplasma sp*. in separate tissue sections.

Aspergillus fumigatus and *Candida albicans* purchased from Remel Microbiology Products is used to produce the artificial positive control tissue.

PRESTAINING PREPARATION:

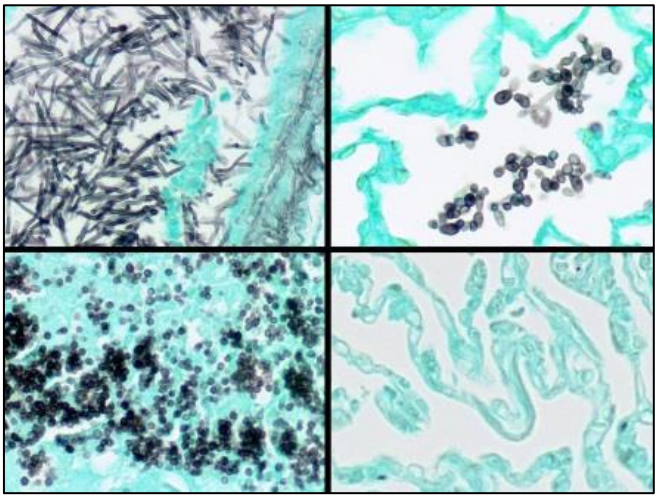
1. Heat dry sections in oven according to your laboratory protocol.
2. All glassware/plasticware must be acid cleaned prior to use.
   a. *See Procedure Notes #1 and #2 (page 2).*
3. Prepare Silver-Methenamine Working Solution and mix well:
   a. *Solution C: Silver Nitrate* *20 ml*
   b. *Solution D: Methenamine Borate* *20 ml*
4. Preheat Silver-Methenamine Working Solution to 45°C - 60°C.
   a. *See Procedure Notes #3 and #4 (page 2).*
5. **Do not preheat Silver-Methenamine Working Solution if using Microwave Modification in Step #12.**

NEWCOMER SUPPLY VALIDATION PROCEDURE:

6. 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 #5 and #6 (page 2).*
7. Oxidize in Solution A: Chromic Acid 5%, Aqueous for 1 hour.
   Microwave Modification: *See Procedure Note #7 (page 2).*
   a. *Oxidize slides in a plastic Coplin jar containing Solution A: Chromic Acid 5%, Aqueous and microwave for 1 minute and 20 seconds at 60°C.*
8. Wash well in running tap water; rinse in distilled water.
9. Place in Solution B: Sodium Bisulfite 1%, Aqueous for 1 minute.
10. Wash for 5 minutes in running tap water; rinse well in distilled water.
11. 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 an overall longer incubation time.*
12. **Microwave Modification:**
    a. *Incubate slides in a plastic Coplin jar containing Silver-Methenamine Working Solution and microwave for 1 minute at 70°C.*
    b. *Check microscopically for adequate development.*
    c. *If additional incubation is required, return slides to warm Silver-Methenamine Working Solution. Recheck at 2-3 minute intervals.*
13. Rinse in three to four changes of distilled water.
    a. *Never use tap water at this step.*
14. Tone in Solution E: Gold Chloride 0.1%, Aqueous until sections turn gray; 20 seconds to 1 minute.
15. Rinse well in distilled water.
16. Remove unreduced silver in Solution F: Sodium Thiosulfate 2%, Aqueous for 2 minutes.
17. Wash in running tap water for 5 minutes; rinse in distilled water.
18. Counterstain in Solution G: Light Green SF Yellowish 0.02%, Aqueous for 2 minutes.
    a. *Over counterstaining could mask organisms.*
19. 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:

Aspergillus sp.	Sharply outlined in black
Candida sp.	Sharply outlined in black
Histoplasma sp.	Sharply outlined in black
Background	Green
Negative lung	Negative for fungus



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. Preheating Silver-Methenamine Working Solution to 45°C-60°C prior to incubation is suggested for timely silver development. A water bath can be used for preheating. Begin preheating silver solution approximately 20-30 minutes before use.
4. Staining slides at higher temperatures will cause the development reaction to happen faster, but may also cause precipitate to form in the working silver solution and deposit on the slides. Maintaining the silver solution between 45°C-60°C will help to minimize precipitate.
5. Drain slides after each step to prevent solution carry over.
6. Do not allow sections to dry out at any point during procedure.
7. 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.
8. If using a xylene substitute, closely follow the manufacturer's recommendations 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. 239-243.
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.