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Part 4235
Revised October 2017

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

CONTROL SLIDES: **Part 4235A** 10 Slide/Set **Part 4235B** 98 Slide/Set

Fungus, GMS, Multi-Tissue, Artificial Control Slides contain sections of *Aspergillus sp.* positive staining rat lung, *Candida sp.* positive staining rat lung, *Histoplasma sp.* positive staining animal tissue and negative staining human 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 Fungus, GMS, Multi-Tissue, Artificial Control Slides were produced at the Newcomer Supply Laboratory under carefully controlled conditions. The positive control sections are not human tissue. The microorganisms for the *Aspergillus sp.* and *Candida sp.* positive sections were grown in pure culture, harvested, formalized and each introduced into individual freshly harvested rat lungs. No infective process occurred. *Aspergillus fumigatus* and *Candida albicans* were used to produce these control sections, and were purchased from Remel Microbiology Products (R4601018 and R4601503 ATCC® 10231™). 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 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

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

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.

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.

PRESTAINING PREPARATION:

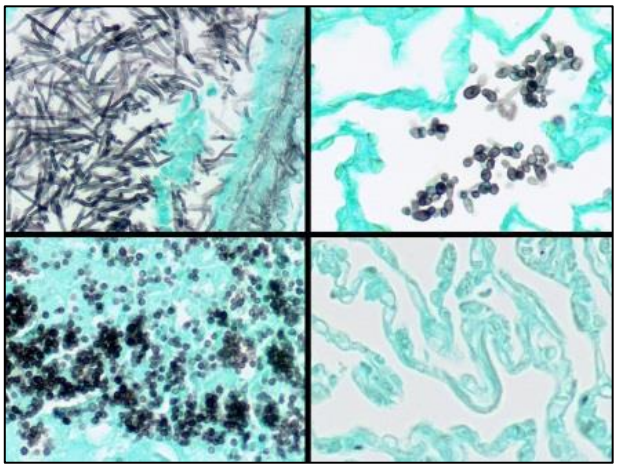
1. All glassware/plasticware must be acid cleaned prior to use.
   a. *See Procedure Notes #1 and #2 (page 2).*
2. Prepare Silver-Methenamine Working Solution and mix well.
   a. *Solution C: Silver Nitrate 20 ml*
   b. *Solution D: Methenamine Borate 20 ml*
3. Preheat Silver-Methenamine Working Solution to 45°C - 60°C approximately 20 to 30 minutes before use.
   a. *See Procedure Notes #3 and #4 (page 2).*
   b. ***Do not preheat if using Microwave Modification; Step 10.***

NEWCOMER SUPPLY VALIDATION PROCEDURE:

4. 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).*
5. 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.*
6. Wash well in running tap water; rinse in distilled water.
7. Place in Solution B: Sodium Bisulfite 1%, Aqueous for 1 minute.
8. Wash in running tap water for 5 minutes; rinse well in distilled water.
9. Incubate slides in preheated Silver-Methenamine Working Solution 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.*
10. ***Microwave Modification:*** *See Procedure Note #7 (page 2)*
    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.*
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; 20 seconds to 1 minute.
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 0.02%, 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:

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. 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. 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 staining rack/slides after each step to prevent solution carry over.
6. Do not allow sections to dry out at any point during staining procedure.
7. 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.
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.