2505 Parview Road • Middleton, WI 53562-2579 • 800-383-7799 • www.newcomersupply.com • newly@newcomersupply.com

Part 9121
Revised June 2015

Fungus, Grocott Methenamine Silver (GMS) Stain Kit - Technical Memo

KIT INCLUDES:	Part 9121A	Part 9121B
Solution A: Chromic Acid 5%, Aqueous	250 ml	500 ml
Solution B: Sodium Bisulfite 1%, Aqueous	250 ml	500 ml
Solution C: Silver Nitrate	125 ml	250 ml
Solution D: Methenamine Borate	125 ml	250 ml
Solution E: Gold Chloride 0.1%, Aqueous	250 ml	500 ml
Solution F: Sodium Thiosulfate 2%, Aqueous	250 ml	500 ml
Solution G: Light Green SF Yellowish Stain 0.02%, Aqueous	250 ml	500 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:	
Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Coplin Jar, Plastic	Part 5184 (for microwave modification)

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

APPLICATION:

Newcomer Supply Fungus, Grocott Methenamine Silver (GMS) Stain Kit procedure, with included microwave modification, is one of the best staining methods for demonstrating a variety of fungal organisms including: *Pneumocystis carinii, Aspergillus, Blastomyces, Candida* and *Histoplasma.*

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns
- a. *See Procedure Note #1 (page 2).*

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

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

STAINING PROCEDURE:

1. All glassware/plasticware must be acid cleaned prior to use.
   a. *See Procedure Notes #2 and #3 (page 2).*
2. 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).*
3. Oxidize in Solution A: Chromic Acid 5%, Aqueous for 1 hour.
   Microwave Modification: *See Procedure Note #6 (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.*
4. Wash well in running tap water; rinse in distilled water.
5. Place slides in Solution B: Sodium Bisulfite 1%, Aqueous for 1 minute.
6. Wash for 5 minutes in running tap water; followed by three to four changes of distilled water.
7. Prepare Silver-Methenamine Working Solution and mix well:
   a. *Solution C: Silver Nitrate 20 ml*
   b. *Solution D: Methenamine Borate 20 ml*
   c. ***Proceed to Step 10 for Microwave Modification.***
8. Preheat the Silver-Methenamine Working Solution to 45°C-60°C.
   a. *See Procedure Notes #7 and #8 (page 2).*
9. Place slides in preheated Silver-Methenamine Working Solution and incubate in 45°C-60°C oven or water bath, or bench top/room temperature, for 12-18 minutes until sections appear paper-bag brown. Periodically remove control, rinse in warm distilled water, check microscopically for adequate silver impregnation. Fungi should be dark brown. If organisms are not sufficiently dark, return slides to the warm silver solution. Recheck at 2-3 minute intervals until desired intensity is achieved.
   a. *Pneumocystis generally takes longer to stain than other fungal organisms. Staining at room temperature will require an overall longer incubation time*.
10. ***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. If additional incubation is required, return slides to the warm Silver-Methenamine Working Solution. Recheck at 2-3 minute intervals.*
11. Rinse in three to four changes of distilled water.
    a. *Never 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 by placing slides 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 0.02%, Aqueous for 2 minutes.
    a. *Over counterstaining could mask organisms.*
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:

Fungi	Crisp black cell walls with visible internal structures
Background	Green
Mucin	Taupe to dark gray

PROCEDURE NOTES:

1. When staining for pneumocystis with another organism, run a separate control slide that is specific for pneumocystis.
2. 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.
3. Plastic (5500), plastic-tipped (5502, 5503), 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.
4. Drain staining rack/slides after each step to prevent solution carry over.
5. Do not allow sections to dry out at any point during staining procedure.
6. 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.
7. 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 if a microwave is unavailable. Begin preheating the silver solution approximately 20-30 minutes before use.
8. 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.
9. 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.