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Part 1142
Revised September 2021

Grocott Methenamine Silver Set, GMS - Technical Memo

SET INCLUDES:	**Part 1142A**	**Part 1142B**
Solution A: Silver Nitrate	500 ml	1000 ml
Solution B: Methenamine Borate	500 ml	1000 ml

Additionally Needed For Fungus Stain, Grocott Methenamine Silver, GMS:

Fungus, GMS, Multi-Tissue, Artificial Control Slides	Part 4235
Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)
Chromic Acid 5%, Aqueous	Part 10341
Sodium Bisulfite 1%, Aqueous	Part 13821
Gold Chloride 0.1%, Aqueous	Part 11285
Sodium Thiosulfate 2%, Aqueous	Part 13888
Light Green SF Yellowish Stain 0.02%, Aqueous	Part 12204
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 product labels.

APPLICATION:

Newcomer Supply Grocott Methenamine Silver Set, GMS with included microwave modifications, provides the silver solutions for the Fungus GMS stain procedure. This is one of the best staining methods to demonstrate a variety of fungal organisms including: *Pneumocystis, Aspergillus, Blastomyces, Candida* and *Histoplasma.*

When staining for *Pneumocystis* with other fungal organisms, running a separate control specific for *Pneumocystis* (4556) is recommended.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 4 microns
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

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

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
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 A: Silver Nitrate* *20 ml*
   b. *Solution B: Methenamine Borate* *20 ml*
4. Preheat Silver-Methenamine Working Solution to 45°C-60°C in a water bath 20 minutes before use.
   a. *Maintain solution between 45°C-60°C to minimize precipitate.*
   b. *Save for use in Step #10.*
   c. ***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 #3 and #4 (page 2).*
6. Oxidize in Chromic Acid 5%, Aqueous (10341) for 1 hour.
   Microwave Modification: *See Procedure Note #5 (page 2).*
   a. *Oxidize slides in a plastic Coplin jar with Chromic Acid 5%, Aqueous. Microwave for 1 minute and 20 seconds at 60°C.*
7. Wash well in running tap water; rinse in distilled water.
8. Place in Sodium Bisulfite 1%, Aqueous (13821) for 1 minute.
9. Wash for 5 minutes in running tap water; 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. *Pneumocystis may take longer to stain than other fungus.*
    d. *Staining at room temperature will require longer incubation.*
11. ***Microwave Modification:***
    a. *Incubate slides in a plastic Coplin jar with Silver-Methenamine Working Solution (Step #3). Microwave for 1 minute at 70°C.*
    b. *Check microscopically for adequate development.*
    c. *If additional incubation is required, return slides to warm silver solution. Recheck at 2-3 minute intervals.*
12. Rinse in three to four changes of distilled water.
    a. *Do not use tap water at this step.*
13. Tone in Gold Chloride 0.1%, Aqueous (11285) until sections turn gray; 20 seconds to 1 minute.
14. Rinse well in distilled water.
15. Remove unreduced silver in Sodium Thiosulfate 2%, Aqueous (13888) for 2 minutes.
16. Wash in running tap water for 5 minutes; rinse in distilled water.
17. Counterstain in Light Green SF Yellowish Stain 0.02%, Aqueous (12204) for 2 minutes.
18. 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. 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. Do not allow sections to dry out at any point during procedure.
5. 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.
6. If using a xylene substitute, 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.