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

KIT INCLUDES:

- Solution A: Chromic Acid 5%, Aqueous
- Solution B: Sodium Bisulfite 1%, Aqueous
- Solution C: Silver Nitrate
- Solution D: Methenamine Borate
- Solution E: Gold Chloride 0.1%, Aqueous
- Solution F: Sodium Thiosulfate 2%, Aqueous
- Solution G: Light Green SF Yellowish Stain 0.02%, Aqueous

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.

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

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.

PRESTAINING PREPARATION:

1. All glassware/plasticware must be acid cleaned prior to use.
   - a. See Procedure Notes #1 and #2 (page 2).
   - 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.

STAINING PROCEDURE:

4. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohol, 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.
   - b. Do not preheat if using Microwave Modification; Step 10.

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. Pneumocystis may take longer to stain than other fungus.
   - d. 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.
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:

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

SUPPORT/WARRANTY: For assistance regarding this product contact Newcomer Supply at 800-383-7799 or info@newcomersupply.com. The information presented in this technical memo is the best of our knowledge accurate. No warranty is expressed or implied. The user is responsible for determining the suitability of this product for their use and upon receipt assumes all liability for its use and responsibility for compliance with any laws or regulations. Please refer to www.newcomersupply.com for complete warranty information. © Newcomer Supply, Inc., 2017
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:


5. Modifications developed by Newcomer Supply Laboratory.