Grocott Methenamine Silver Set, GMS - Technical Memo

**SET INCLUDES:**

<table>
<thead>
<tr>
<th>Part 1142A</th>
<th>Part 1142B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution A: Silver Nitrate</td>
<td>500 ml</td>
</tr>
<tr>
<td>Solution B: Methenamine Borate</td>
<td>500 ml</td>
</tr>
</tbody>
</table>

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

- **Fungus, GMS, Multi-Tissue, Artificial Control Slides**
- **Hydrochloric Acid 5%, Aqueous**
- **Chromic Acid 5%, Aqueous**
- **Sodium Bisulfite 1%, Aqueous**
- **Gold Chloride 0.1%, Aqueous**
- **Sodium Thiourea 2%, Aqueous**
- **Light Green SF Yellowish Stain 0.02%, Aqueous**
- **Xylene, ACS**
- **Ethyl Denatured Alcohol, 100%**
- **Ethyl Denatured Alcohol, 95%**
- **Coplin Jar, Plastic**
- **Sodium Thiosulfate**
- **Gold Chloride**
- **Sodium Bisulfite**
- **Chromic Acid**
- **Hydrochloric Acid 5%**
- **Mucin**
- **Formalin**
- **Paraffin sections cut at 5 microns**
- **Microwave Modifications**
- **Part 4235**
- **Part 1142A**
- **Part 1142B**
- **Part 1445**
- **Part 10841**
- **Part 10842**
- **Part 5184 (for microwave modification)**

**APPLICATION:**

Newcomer Supply Grocott Methenamine Silver Set, GMS with included microwave modifications, provides the silver staining solutions for the Fungus Stain, Grocott Methenamine Silver (GMS) procedure. This is one of the best staining methods for demonstrating a variety of fungal organisms including: *Pneumocystis carinii*, 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 5 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 staining procedure provided below. Some solutions in the set 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).
2. Prepare Silver-Methenamine Working Solution and mix well:
   a. Solution A: Silver Nitrate 20 ml
   b. Solution B: Methenamine Borate 20 ml
3. Preheat Silver-Methenamine Working Solution to 45°C - 60°C approximately 20-30 minutes before use.
   a. See Procedure Notes #3 and #4 (page 2)
   c. 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 alcohols, 10 dips each. Wash well with distilled water.
   a. See Procedure Notes #5 and #6 (page 2).
5. Oxidize in Chromic Acid 5%, Aqueous (10341) for 1 hour.
   **Microwave Modification:** See Procedure Note #7 (page 2).
   a. Oxidize slides in a plastic Coplin jar containing Chromic Acid 5%, Aqueous (10341) and microwave for 1 minute and 20 seconds at 60°C.
   b. Periodically remove control, rinse in warm distilled water, check microscopically for adequate silver impregnation. Fungi should be dark brown.
6. Wash well in running tap water; rinse in distilled water.
7. Place in Sodium Bisulfite 1%, Aqueous (13821) for 1 minute.
8. Wash for 5 minutes in running tap water; rinse well in distilled water.
9. Incubate slides in preheated Silver-Methenamine Working Solution (Step #3) at 45-50°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 (Step #2) 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 Gold Chloride 0.1%, Aqueous (11285) until sections turn gray; 20 seconds to 1 minute.
13. Rinse well in distilled water.
14. Remove unreacted silver in Sodium Thiourea 2%, Aqueous (13888) for 2 minutes.
15. Wash in running tap water for 5 minutes; rinse in distilled water.
16. Counterstain in Light Green SF Yellowish Stain 0.02%, Aqueous (12204) 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., 2018
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 the 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.