

**A-122 RAPID GROCOTT'S METHENAMINE-SILVER NITRATE  
METHOD FOR FUNGI (GMS) AND PNEUMOCYSTIS CARINII  
(MODIFIED FOR MICROWAVE)**

**FIXATION:** Formalin, 10% Buffered Neutral  
**SECTION:** Cut paraffin section at 4-6 microns

**STAINING PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. Oxidize in **Chromic Acid, 10%** (A-123-1), and microwave for 20 seconds at maximum power.
3. Let stand for 10 seconds in hot solution, then rinse in running tap water.
4. Place in **Sodium Bisulfite, 1% Aq.** (A-123-2), for 60 seconds.
5. Rinse in running tap water followed by 3 changes of distilled water.
6. Place slides in Methenamine Silver Nitrate Working Solution and microwave for 20 seconds at maximum power.

**\*To prepare working solution:**

**A. Methenamine – Silver Nitrate Stock Solution**

**Silver Nitrate, 5%, Aq.** (A-123-3) – 5ml

**Methenamine, 3%, Aq.** (A-123-4) – 100ml

**B. Methenamine Silver Nitrate Stock Working Solution**

Methenamine-Silver Nitrate Stock Sol'n Stock 25 ml

Distilled Water 25 ml

**Sodium Borate 5%, Aq.** (A-123-5) 2 ml

Prepare fresh prior to use. Do not use if cloudy.

7. Let slides stand in hot solution which has been placed in a beaker of water at 60°C for 30 or more seconds. The slides should remain until they are pale brown.
8. Rinse slides in 3 changes of distilled water.
9. Tone in **Gold Chloride 1%, aq.** (SO-227) for 10 seconds.
10. Rinse slides in distilled water.
11. Place in **Sodium Thiosulfate, 2% Aq.** (A-121-6) for 1 minute.
12. Wash in running tap water for 2 minutes.
13. Place in **Light Green Stock Solution, 0.2%** (A-121-7), for 30 seconds.
14. Rinse in distilled water.
15. Dehydrate, clear and mount in resinous medium.

**RESULTS:**

Fungi.....black, sharp detail  
Pneumocystis carinii black  
Mucin.....dark gray - taupe  
Inner areas Mycelia, Hyphae.....rose  
Background.....green, pale

**REFERENCES:**

Grocott, R.G.: Amer. J. Clin. Pathol., 25:975 (1955).

AFIP Manual of Histologic Staining Methods; ed. G. Luna, 3<sup>rd</sup>. ; New York:

McGraw-Hill Publications, c. 1968, p. 230

Reviewed and Approved

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