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**A-121      GROCOTT'S METHOD FOR FUNGI**  
(GMS)

**FIXATION:** **Formalin, 10% Buffered Neutral**

**SECTION:** Cut Paraffin sections at 6 microns

**STAINING PROCEDURE:**

Deparaffinize and hydrate to distilled water.

1. Oxidize in **Chromic Acid, 4%** (A-121-1), 1 hour; wash in tap water, 5-10 seconds.
2. Dip in Sodium **Bisulfite, 1%, Aq.** (A-121-4), for one minute; this should remove all Chromic Acid traces. Wash in running water, 5-10 minutes, and then rinse in 3-4 changes of distilled water.
3. Incubate at 60° C for 1 hour in the Working Methenamine – Silver Nitrate Reagent. First, prepare Methenamine – Silver Nitrate Stock.

**Methenamine – Silver Nitrate Stock:**

- a. **Silver Nitrate, 5%, Aq.** (A-121-3A) – 5ml
- b. **Methenamine, 3%, Aq.** (A-121-3B) – 100ml

Use chemically clean glassware. Store in refrigerator.

Then, to prepare **Methenamine – Silver Nitrate Working**

**Mix just prior to use.**

- a. Methenamine Silver Nitrate Stock – 25.0ml
- b. Distilled Water – 25.0ml
- c. **Sodium Borate, 5%, Aq.** (A-121-2) – 2.0ml

Rinse in 6-8 changes distilled water; further tone in **Gold Chloride, 0.1%, Aq.** (A-121-5), 2-5 minutes.

4. Rinse in 6-8 changes distilled water and transfer to **Sodium Thiosulfate, 2%, Aq.** (A-121-6) to remove unreduced silver, approximately 2-3 minutes. Wash thoroughly in tap water.
5. Counterstain in working \* Light Green Solution 30-45 seconds.

**\*Prepare working solution by diluting:**

**Light Green Stock, 0.2%, (A-121-7)**

1:5 with distilled water.

6. 8. Dehydrate with 95% and 100% alcohol, two changes. Clear in Xylene (C-120), two changes.
7. Mount with **Permount (M-18)**.

**RESULTS:**

Fungi.....black, sharp detail  
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