
**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-122-1), 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-122-4), 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-122-3A) – 5ml

Methenamine, 3%, Aq. (A-122-3B) – 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-122-2) 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.** (A-122-5) for 10 seconds.
10. Rinse slides in distilled water.
11. Place in **Sodium Thiosulfate, 2% Aq.** (A-122-6) for 1 minute.
12. Wash in running tap water for 2 minutes.
13. Place in **Light Green Stock Solution, 0.2%** (A-122-7), 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:

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AFIP Manual of Histologic Staining Methods; ed. G. Luna, 3rd. ; New York:
McGraw-Hill Publications, c. 1968, p. 230