

GROCOTT'S METHOD FOR FUNGI (GMS)

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of fungi and Pneumocystis Carinii in tissue.
PRINCIPLE:	Chromic Acid oxidizes the polysaccharides contained in the cell walls of fungus and other organisms to aldehydes. These aldehydes then reduce Silver from the Methenamine Silver to the visible metallic form. Gold replaces some of the Silver toning it. A counterstain is applied for contrast.
CONTROL:	Any tissue known positive for fungi or Pneumocystis <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs013.</i>
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 4-6 micrometers
SOLUTIONS:	<ol style="list-style-type: none"> Chromic Acid 4% Aqueous Item# s164 Methenamine Silver Nitrate Stock Solution Set (A & B) Item# s240 <i>Working Solution: Must be made fresh.</i> Methenamine Stock Solution A 12.5 mL Silver Nitrate Stock Solution B..... 12.5 mL Distilled Water25.0 mL Borax 5% Aqueous..... 2.0 mL Sodium Bisulfite 1% Aqueous Item# s1893 Gold Chloride 0.1% Aqueous Item# s201 Sodium Thiosulfate 2% Aqueous Item# s280 Fast Green Substitute for Light Green 0.2% Aq Item# s232 Borax 5% Aqueous Item# s128 <p><i>Solutions can be purchased separately from Poly Scientific.</i></p>
NOTES:	Do not use any metal.
REFERENCE:	Grocott, R. G. "Grocott's Method for Fungi". <u>Amer. J. Clin. Path.</u> 25: 1955. pp. 975-979.

STAINING PROCEDURE:

- Deparaffinize and hydrate to distilled water.
- Oxidize in Chromic Acid 4% Aqueous for 1 hour.
- Wash in tap water for a few seconds.
- Place in Sodium Bisulfite 1% Aqueous for 1 minute to remove any residual Chromic Acid.
- Wash in running water for 5-10 minutes.
- Rinse with 3-4 changes of distilled water.
- Place in freshly mixed Methenamine Silver Nitrate Working Solution in oven set at 58–60°C for 60 minutes or until sections turn yellowish brown.
- Rinse in 6 changes of distilled water.
- Tone in Gold Chloride 0.1% Aqueous for 2-5 minutes.
- Rinse in distilled water.
- Remove unreduced Silver with Sodium Thiosulfate 2% Aqueous for 2-5 minutes.
- Wash thoroughly in tap water.
- Counterstain Fast Green Substitute for Light Green 0.2% Aqueous for 30-45 seconds.
- Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
- Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Fungi Sharply Delineated in Black
Mucin Taupe to Dark Gray
Inner Parts of Mycelia & Hyphae Old Rose
Background Pale Green

Poly Scientific R&D Corp.

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