

GROCOTT'S METHOD FOR FUNGI (GMS) (Microwave)

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of fungi and Pneumocystis Carinii.
PRINCIPLE:	Chromic Acid oxidizes the polysaccharides contained in the cell walls of fungus and other organisms to aldehydes. These aldehydes then reduce the Methenamine Silver to the visible metallic form.
CONTROL:	Tissue positive for fungi or Pneumocystis Carinii <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs013.</i>
SPECIMEN PREPARATION:	10% Buffered Formalin, air dried smears, touch preps, or cryostat sections fixed in Reagent Alcohol, paraffin embedded sections cut at 5 micrometers
SOLUTIONS:	<ol style="list-style-type: none"> Chromic Acid 4% Aqueous Item# s164 Methenamine Silver Nitrate Stock Sol Set (A & B) Item# s240 <i>Working Solution: Must be prepared fresh.</i> Methenamine Stock Solution A 12.5 mL Silver Nitrate Stock Solution B..... 12.5 mL Distilled Water 25 mL Borax 5% Aqueous..... 2 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% Aqueous 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. <i>Variations in timing may occur due to the power wattage of the microwave oven. Provided times and power levels are based on 1000 watt microwave oven.</i>
REFERENCE:	Luna, Lee G. <u>Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts</u> . American Histolabs Inc. Gaithersburg, MD. 1992. pp. 200-202.

STAINING PROCEDURE:

- Deparaffinize and hydrate slides to distilled water.
- Place 50 mL of Chromic Acid 4% Aqueous in a plastic coplin jar (loosely apply cap) and microwave for 20 seconds. Stir and re-microwave for 15–20 seconds. Stir again and add slides to hot solution for 1 1/2 minutes.
- Rinse well in distilled water.
- Place slides in Sodium Bisulfite 1% Aqueous for 1 minute (do not microwave).
- Rinse well in distilled water.
- Place 50 mL of freshly mixed Working Methenamine Silver Nitrate Stock Solution in a plastic coplin jar (loosely apply cap) and microwave for 20 seconds. Stir and re-microwave for 15 seconds. Stir again and place slides in hot solution for 1 1/2-2 minutes. Check control. If additional staining time is needed place slides back in hot solution until desired intensity is reached (control should be golden brown).
- Rinse well in distilled water.
- Place 50 mL of Gold Chloride 0.1% Aqueous in a plastic coplin jar (loosely apply cap) and microwave for 30 seconds. Stir and add slides to hot solution for 1 minute.
- Rinse well in distilled water.
- Place 50 mL of Sodium Thiosulfate 2% Aqueous in a plastic coplin jar (loosely apply cap) and microwave for 25–30 seconds. Stir and add slides to hot solution for 1 1/2 minutes.
- Rinse well in distilled water.
- Counterstain in Fast Green Substitute For Light Green 0.2% Aqueous for 30 seconds or until desired intensity is reached.
- Dehydrate, clear and mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Fungi.....	Black
Mucin.....	Taupe to Gray
Pneumocystis Carinii	Black
Background.....	Green

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