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## TECHNICAL DATA SHEET 864

Page 1 of 2

# Grocott Methenamine Silver Stain (GMS) for Fungus and PCP - Room Temperature & Microwave Kit

### DESCRIPTION

Grocott's alkaline hexamine-silver solution allows silver ions upon reduction, to appear dark or black, also known as "argentaftin reaction". the ability of a silver complex solution to blacken a tissue element without the need of a reducing bath.

In fungal infections are becoming more common in the form of opportunistic infections in patients that are immunocompromised. Fungi stain weakly with hematoxylin, they are also PAS positive due to carbohydrate found in fungal cell walls.

### KIT COMPONENTS

25087A: 3.8% chromium trioxide aqueous solution – 100.0 mL  
25087B: 1% sodium bisulfite aqueous solution – 100.0 mL  
25087C: 5% silver nitrate aqueous solution – 9.0 mL  
25087D: Methenamine solution – 180.0 mL

25087E: 5% Borax aqueous solution – 50.0 mL (warm to 40°C and shake before use.)  
25087F: 0.2% gold chloride aqueous solution – 100.0 mL  
25087G: 2% Sodium thiosulfite aqueous solution – 100.0 mL  
25087H: 0.5% Light Green stock solution – 20.0 mL

### NOTES

1. A known positive control section must be used to ensure correct differentiation has been achieved.
2. Microwave method : Volume of chromic acid must be measured
3. When microwaving solutions, ensure lids are not put on tightly to allow for expansion.
4. Prior to leaving slides to stain in microwaved solutions ensure solutions are thoroughly mixed to ensure even temperature distribution.
5. Note solutions removed from the microwave oven may spontaneously boil.
6. Times may vary according to type and power of micro-wave.
7. Combination of the original method and microwave method can be used. *ie the microwave technique for silver used with the original method for chromic acid or vice-versa.*

### METHOD

1. Rehydrate sections to distilled water, use acid cleaned glassware and non metal forceps.
2. Oxidize with solution A at room temperature for 1 hour (as good time management tool, after 30 minutes, start preparing for preheated working methenamine silver solution below, allow for the waterbath to get to temperature, 60°, before making the pre-heated working solution)
3. Wash in water for a few seconds.
4. Treat sections with solution B for 1 minute
5. Wash in running tap water for 3 minutes
6. Rinse thoroughly in distilled water
7. Place in pre-heated working silver solution in a waterbath at 60 °C for 15 to 20 minutes until section turns yellowish-brown (Check microscopically after washing in distilled water – fungi should be dark brown).

### Preparation of Preheated Working Solution

Prepare working silver solution fresh each time add in the following order in an acid cleaned coplin jar, and do not use metal forceps

Solution D ----- 23 ml  
Solution C ----- 1.25 ml  
Solution E ----- 3 ml (Shake before use.)  
Distilled H<sub>2</sub>O ----- 25 ml

8. Rinse thoroughly in distilled water.
9. Tone sections with solution F for 2 minutes
10. Rinse in distilled water.
11. Treat sections with solution G for 2 minutes
12. Wash with running tap water 5 minutes
13. Counterstain in working solution H 15 seconds
14. Rinse excess light green off slide with alcohol
15. Dehydrate, clear and mount

**MICROWAVE METHOD**

1. Bring sections to distilled water.
2. Place slides in 40 ml solution A in a loosely covered plastic coplin jar. Microwave at 150 Watt (see technical note 7) 2 min 30 sec
3. Dip slides up and down in coplin jar and leave to stand for a further 2 minutes
4. Wash in running tap water 30 seconds
5. Treat sections with solution B for 30 seconds
6. Wash in running tap water 30 seconds
7. Preheat working silver solution (loosely lidded plastic coplin jar) at 450 Watt 60 seconds

**Preparation of Preheated Working Solution for Microwave**

Prepare working silver solution fresh each time add in the following order in an a plastic coplin jar, and do not use metal forceps, to stir solution, use an orange-wood stick

- Solution D ----- 23 ml
  - Solution C ----- 1.25 ml
  - Solution E ----- 3 ml
  - Distilled H<sub>2</sub>O ----- 25 ml
8. Tone sections with solution F for a minute
  9. Rinse in distilled water
  10. Treat sections with solution G for one minute
  11. Wash with running tap water 15 seconds

**Working Light Green Solution Preparation**

- Solution H ----- 10 ml
- Distilled H<sub>2</sub>O ----- 40 ml

12. Counterstain in working light green 15 sec
13. Rinse excess light green off slide with alcohol
14. Dehydrate, clear and mount.

**RESULTS**

- fungi, Pneumocystis carinii, histoplasma spp ----- black
- inner parts of mycelia and hyphae ----- old rose
- leishmania spp, toxoplasma spp ----- negative
- mucin ----- dark grey
- background ----- pale green

**STORAGE, SAFETY AND HANDLING**

**Storage**

Store at room temperature - Solution A, B, E, G  
 Store at 4°C - Solution C, D, F, H

**Safety**

Wear protective clothing, gloves and safety glasses when preparing reagents. Refer to MSDS for more details.

**ORDERING INFORMATION**

Cat. #	Description	Size
25087-1	Grocott Methenamine Silver Stain (GMS) for Fungus and PCP - Room Temperature & Microwave Kit	1 kit

**TO ORDER**

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