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GMS Staining Kit

(Cat# K1427-125; Store at Multiple Temperatures)

I. Introduction:

The GMS Staining Kit, (Modified Gomori Methenamine-Silver Nitrate) is intended for use in the histologic visualization of fungi, basement membrane and some opportunistic organisms such as Pneumocystis carinii. Pneumocystis carinii is an opportunistic pathogen that causes severe pulmonary disease in humans, dogs, rats, mice and other vertebrate species with acquired, induced, or inherited immune deficiency syndromes. In addition, this procedure will demonstrate Actinomyces and related species, Nocardia asteroids, and certain encapsulated bacteria.

Fungi: Black; P. Carinii: Black; Mucin: Gray; Mycelia (inner): Grey to Black; Hyphae (inner): Grey to Black; Background: Light Green

II. Application:

- Histological applications (IHC-Fr, IHC-P)
- For *in vitro* diagnostic use

III. Sample Type:

- Formalin-fixed, paraffin-embedded (5 microns) or frozen sections.
- Control Tissue: Any Fungus infected tissue.

IV. Kit Contents:

Components	K1427-125	Part Number	Storage Temperature
Silver Nitrate Solution (0.2%)	125 ml	K1427-125-1	2-8°C
Methenamine Solution	125 ml	K1427-125-2	2-8°C
Gold Chloride Solution (0.2%)	125 ml	K1427-125-3	2-8°C
Borax Solution	15 ml	K1427-125-4	RT
Sodium Bisulfite Solution	125 ml	K1427-125-5	RT
Chromic Acid Solution	125 ml	K1427-125-6	RT
Sodium Thiosulfate Solution (5%)	125 ml	K1427-125-7	RT
Light Green Solution	125 ml	K1427-125-8	RT

V. User Supplied Reagents and Equipment:

- Distilled water
- Coplin jars
- Plastic forceps
- Absolute alcohol
- Synthetic resin

VI. Shipment and Storage:

All the reagents are shipped at room temperature (RT) and stored at multiple temperatures.

VII. Reagent Preparation:

- Do not use if reagents become cloudy.
- Do not use past expiration date.
- Use caution when handling reagents.
- All glassware used in this procedure should be chemically cleaned and rinsed thoroughly in distilled water.
- Failure to adequately remove the alcohol used in deparaffination will result in reduction of the chromic acid solution.
- Reduction of the chromic acid solution will result in a change in color from orange to brown. Discard the reagent if color change is noted.
- **Do not use metal forceps** to remove slides from reagents. Use plastic forceps only.
- Prewarm all reagents to room temperature prior to use.

VIII. <u>Procedure (Standard):</u>

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Incubate slide in Chromic Acid Solution for 10 minutes.
3. Rinse in tap water followed by 2 changes of distilled water.
4. Incubate slide in Sodium Bisulfite Solution for 1 minute (to remove any residual chromic acid).
5. Rinse in tap water followed by 2 changes of distilled water.
6. Combine the following for a working GMS solution:
    - **25 ml Silver Nitrate Solution (0.2%)**
    - **25 ml Methenamine Solution**
    - **2 ml Borax Solution**

Note: Mixed solution may not be stored for reuse later.

7. Place working GMS solution in 60° centigrade water bath and allow temperature to equilibrate.
8. Incubate slide in working GMS solution for 10-15 minutes. Using plastic forceps, dip slide in distilled water and check under a microscope for evaluation of silver impregnation. Fungi should be dark brown. If color is not sufficient, return the slide to working GMS solution for 2-3 minutes and check again.
9. Rinse in 4 changes of distilled water.
10. Incubate slide in Gold Chloride Solution for 15-30 seconds.
11. Rinse in 4 changes of distilled water.
12. Incubate slide in Sodium Thiosulfate Solution (5%) for 2 minutes.
13. Rinse in tap water followed by 2 changes of distilled water.
14. Incubate slide in Light Green Solution for 2 minutes.
15. Rinse slide using absolute alcohol.
16. Dehydrate in 2 changes of absolute alcohol, clear, and mount in synthetic resin.

Procedure (Microwave):

Note: These instructions were developed using a standard 500-watt microwave oven. Heating times should be modified as needed depending on the microwave oven used.

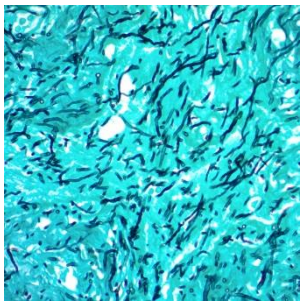
1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Place slide in plastic coplin jar filled with Chromic Acid solution. Cap jar loosely!
3. Place jar in microwave oven and heat on high power for 10 seconds. Allow slide to remain in warm solution for 3 minutes.
4. Rinse in tap water followed by 2 changes of distilled water.
5. Incubate slide in Sodium Bisulfite solution for 1 minute (to remove any residual chromic acid).
6. Rinse in tap water followed by 2 changes of distilled water.
7. Combine the following for a working GMS solution:

25 ml Silver Nitrate
25 ml Methenamine
2 ml Borax Solution

Note: Mixed solution may not be stored for reuse later.

8. Place working GMS solution (loosely capped) in microwave oven for 40 seconds. Remove and pour several times between coplin jar and a clear graduated cylinder to mix thoroughly (use protective glove to avoid burning hand). Mixed solution remains in coplin jar.
9. Incubate slide in working GMS solution (heated) for 2-6 minutes until the tissue is medium brown in color. Using plastic forceps, dip slide in distilled water and check under a microscope for evaluation of silver impregnation. Fungi should be dark brown. If color is not sufficient, return he slide to working GMS solution for 1-2 minutes and check again. Reheat solution if needed.
10. Rinse in 4 changes of distilled water.
11. Incubate slide in Gold Chloride solution for 15-30 seconds.
12. Rinse in 4 changes of distilled water.
13. Incubate slide in Sodium Thiosulfate for 2 minutes.
14. Rinse in tap water followed by 2 changes of distilled water.
15. Incubate slide in Light Green Solution for 2 minutes.
16. Rinse slide using absolute alcohol.
17. Dehydrate in 2 changes of absolute alcohol, clear, and mount in synthetic resin.

IX. Data:



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