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GMS Stain Kit

(Modified Gomori Methenamine-Silver Nitrate Stain for Fungus and Pneumocystis carinii)

Description: The Modified Gomori Methenamine-Silver Nitrate Stain (GMS Stain Kit) 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

Uses/Limitations:
For In-Vitro Diagnostic use only.
Histological applications.
Air-dried smears.
Do not use past expiration date.
Use caution when handling these reagents.

Availability/Contents:

Kit Contents	Volume	Storage
Silver Nitrate Solution (0.2%)	125 ml	2-8°C
Methenamine Solution	125 ml	2-8°C
Gold Chloride Solution (0.2%)	125 ml	2-8°C
Borax Solution	15 ml	15-30°C
Sodium Bisulfite Solution	125 ml	15-30°C
Chromic Acid Solution	125 ml	15-30°C
Sodium Thiosulfate Solution (5%)	125 ml	15-30°C
Light Green Solution	125 ml	15-30°C

Precautions: This product is a single-use, non-sterile, in vitro diagnostic device.
Chromic Acid is a strong oxidizer. Contact with other material may cause fire. Contact with skin and eyes can cause burns. Harmful if swallowed. Harmful to respiratory and gastrointestinal system. Can cause cancer and birth defects.

Borax solution is irritating to skin, eyes, and respiratory system. Target organs are central nervous system and kidneys. Harmful if swallowed. Possible risk to unborn child.

Storage: 2° C 30° C

Mixed Storage Conditions.
Separate Contents.

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Handle all components with care, wearing gloves and eye protection.

Diagnostic Biosystems may not be held liable for injury due to mishandling.

Follow all Federal, State, and local regulations regarding disposal.

Use in chemical fume hood whenever possible.

Important Notes:

1. All glassware used in this procedure should be chemically cleaned and rinsed thoroughly in distilled water.
2. 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.
3. Do not use metal forceps to remove slides from reagents. Use plastic forceps only.
4. Prewarm all reagents to room temperature prior to use.

Procedure (Standard):

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.

Storage: 2° C 30° C

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13. Rinse in tap water followed by 2 changes of distilled water.
14. Incubate slide in Light Green Solution for 2 minutes.
15. Rinse in distilled water.
16. Dehydrate through graded alcohols.
17. 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 the 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.

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16. Rinse in distilled water.
17. Dehydrate through graded alcohols.
18. Clear, and mount in synthetic resin.

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