Diagnostic BioSystems

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GMS Stain Kit

Catalog Number: KT015

**This data sheet is applicable to all sizes (volume) of product. Actual volume is indicated on vial.

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Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
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): Rose
Hyphae (inner): Rose
Background: Light Green

Control Tissue
Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981: Sheehan & Hrapchak, 1980) for further details on specimen preparation.

1. Cut sections, usually 3 to 5 µm and pick the sections up on glass slides.
2. Bake the slides for at least 30 minutes at approximately 70°C.
3. Allow to cool.

Reagents Provided

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	125 mL	15-30°C
Light Green Solution	125 mL	15-30°C

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.

Storage and Handling
Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Staining 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:
   i. 25 mL Silver Nitrate Solution (0.2%)
   ii. 25 mL Methenamine Solution
   iii. 2 mL Borax Solution
   Note: Mixed solution may not be stored for reuse later.
7. Place working GMS solution in 60°C 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 in distilled water.

Staining 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:
   i. 25 ml Silver Nitrate
   ii. 25 ml Methenamine
   iii. 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.

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10. 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.
11. Rinse in 4 changes of distilled water.
12. Incubate slide in Gold Chloride solution for 15-30 seconds.
13. Rinse in 4 changes of distilled water.
14. Incubate slide in Sodium Thiosulfate for 2 minutes.
15. Rinse in tap water followed by 2 changes of distilled water.
16. Incubate slide in Light Green Solution for 2 minutes.
17. Rinse in distilled water.
18. Dehydrate through graded alcohols.
19. Clear, and mount in synthetic resin.

Limitations of the Procedure

1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining.
3. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide.
5. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.

Precautions

1. 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.
2. 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.
3. Consult local and/or state authorities with regard to recommended method of disposal.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
5. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
6. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
7. If reagent contacts these areas, rinse with copious amounts of water.
8. Do not ingest or inhale any reagents.
9. Handle all components with care, wearing gloves and eye protection.
10. Use in a chemical fume hood whenever possible.

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

References

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