Diagnostic BioSystems

Instructions For Use

KT 015-IFU

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GMS Stain Kit

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

Description and Principle

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 jiroveci. Pneumocystis jiroveci* 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 cell walls contain large amounts of polysaccharides that are oxidized to aldehyde groups by chromic acid. Aldehydes bind silver ions from a methenamine silver complex and reduce the silver to its visible metallic form. Background tissue elements such as collagen is overoxidized by chromic acid becomes non-reactive.

Expected Results

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

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

Suggested Controls *(not provided)*

Any Fungus infected tissue.

Uses/Limitations

For In-Vitro Diagnostic use only.
Do not use if reagents become cloudy or precipitate
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile
Intended for FFPE sections cut at 5-10µm.
This procedure has not been optimized for frozen sections.
Frozen sections may require protocol modification.

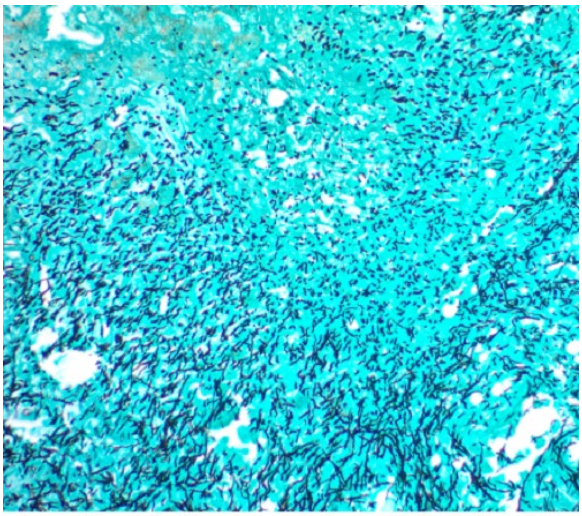
Storage

Mixed storage conditions. Store according to individual label instructions.

Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements. If there is any serious incident that has occurred in relation to the device, please contact the manufacturer: Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com. If required, please report to the Competent Authority of the Member State in which the user and/or patient is established.

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.



Aspergillus infection demonstrated with GMS Stain Kit (Modified Gomori Methenamine-Silver Nitrate Stain)

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.

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. Equilibrate all reagents to room temperature prior to use.

Procedure

1. Deparaffinize sections if necessary and hydrate to distilled water.

2. Incubate slide in Chromic Acid Solution for 10 minutes.

Note: Increasing incubation time and temperature of the Chromic Acid Solution reduces background silver staining. We suggest a 10 min incubation at 60°C if there is unwanted background staining.

3. Rinse in tap water followed by 2 changes of distilled water.

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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 10-20 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 excess stain off slide using absolute alcohol.

16. Dehydrate in 2 changes of absolute alcohol, clear, and mount in synthetic resin.

References

1. McManus, J.F.A. and Mowry, R. 1955. Staining Methods and Histologic and Histochemical. Grocott, pp 194-197.
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3. Sheehan, D.C., Hrapchak, B.B. 1980. Theory and Practice of Histotechnology, 2nd edition, CV Mosby Company, St. Louis, MO.
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5. Raab, S.S. et al. 1994. Utility of Gomori methenamine silver stains in bronchoalveolar lavage specimens. Modern Pathology, June 1994, Vol. 7, No. 5, pp 599-604.
6. Procop, G.W. et al. 2004. Detection of Pneumocystis jiroveci in Respiratory Specimens by Four Staining Methods. Journal of Clinical Microbiology. July 2004, Vol. 42, No. 7, pp 3333-3335.

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