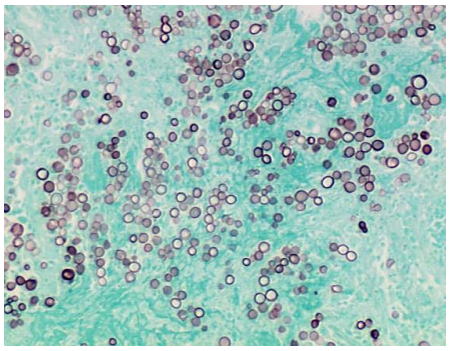
GMS Stain Kit
(Modified Gomori Methenamine-Silver Nitrate Stain for Fungus and Pneumocystis carinii)

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.

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.



Results:

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

Kit Contents:

Item #	Kit Contents	Volume	Storage
SSC-SNS125	Silver Nitrate Solution (0.2%)	125 ml	2-8° Centigrade
SSC-MET125	Methenamine Solution	125 ml	2-8° Centigrade
SSC-GCB125	Gold Chloride Solution (0.2%)	125 ml	2-8° Centigrade
SSC-BOR015	Borax Solution	15 ml	Room Temperature
SSC-SBC125	Sodium Bisulfite Solution	125 ml	Room Temperature
SSC-CHR125	Chromic Acid Solution	125 ml	Room Temperature
SSC-STB125	Sodium Thiosulfate Solution (5%)	125 ml	Room Temperature
SSC-LGA125	Light Green Solution	125 ml	Room Temperature

Mixed Storage Conditions. Separate Contents.

For information regarding ordering individual components, please contact us at: 800-442-3573.
Control Slides Available. Catalog: CS-FUNG/25, Fungus, 25/pack

Precautions: Keep away from open flame.
Avoid all contact with skin and eyes.
May cause burns.
Harmful if swallowed or inhaled.
Follow all Federal, State, and local regulations regarding disposal.
Use in chemical fume hood whenever possible.
Statlab Medical Products may not be held liable for injury due to mishandling.

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. Pre-warm 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.

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.

16. Rinse in distilled water.

17. Dehydrate through graded alcohols.

18. 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.

2. Koski, J.P. 1981. Silver methenamine-borate (SMB); Cost reduction with technical improvement in silver nitrate-gold chloride impregnation's. Journal of Histotechnology 4:115.

3. 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.

4. 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.

5. Sale, G.E. 1978. Rapid Methenamine Silver Stain. Arch Path Lab Med, 1978, 102, pp 351-352.

6. Sheehan, D.C., Hrapchak, B.B. 1980. Theory and Practice of Histotechnology, 2nd edition, CV Mosby Company, St. Louis, MO.

Lot-to-Lot Validation Form
Gomori Methenamine Silver (GMS) Catalog:SSK-GMS/GOMORI

Kit Lot Number: _____
Kit Expiration Date: _____
Date Tested: _____
Control Tissue (#) _____
Approved for Use: Y/N _____
Date put into use:

If not approved, corrective actions taken:	

Approved by: _____

Kit Component	**Lot #**
Silver Nitrate (for GMS)	
Methenamine Silver (for GMS)	
Gold Chloride 0.2%	
Borax Solution (for GMS)	
Sodium Bisulfite Sol (for GMS)	
Chromic Acid (For GMS)	
Sodium Thiosulfate 5%	
Light Green Solution	

Replacement Component if used	Replacement Date	Lot #	Accepted Y/N	Comments
Silver Nitrate (for GMS)				
Methenamine Silver (for GMS)				
Gold Chloride 0.2%				
Borax Solution (for GMS)				
Sodium Bisulfite (for GMS)				
Chromic Acid (for GMS)				
Sodium Thiosulfate 5%				
Light Green Solution				
Approved by:				

StatLab is providing this form to assist with reagent lot validation as stated in CLIA'88 Standard 493.1256-For reagent(s), the laboratory must do the following: Check each batch (prepared in-house), lot number (commercially prepared) and shipment of reagents, stains, and identification systems (systems using two or more substrates or two or more reagents, or a combination) when prepared or opened for positive and negative reactivity, if applicable.