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Thermo Scientific™ Richard-Allan™ Scientific Chromaview™ – Advanced Testing Modified Grocott's Methenamine Silver Stain (Periodic Acid) Instructions for Use

For in vitro diagnostic use.
For use as a kit in special stain techniques.

Technical Discussion

Fungus and Pneumocystis
Cut sections at 4-6 microns.

Basement Membranes
Cut sections at 1-4 microns.

Fixation
No special requirements; formalin fixation or Bouin's Fluid is adequate.

Quality Control
A section containing fungi should be used; if staining for *Pneumocystis,* a *Pneumocystis* control should be used.

Technical Procedure

Working Methenaimine Silver Solution
Methenamine-Borax……1 Capsule

Note: Wearing gloves, empty entire contents of capsule into distilled or deionized water and dissolve. Discard the empty capsule. Use of a stir plate will accelerate dissolution.

Distilled or Deionized water……50 mL
Silver Nitrate Solution……1 mL
Mix Well

Note: For Histoplasmosis demonstration, incubate sections in Periodic Acid Solution (Step 2) for 1 hour at 56-60° C and continue through procedure. Histoplasmosis will stain black.

Standard Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Oxidize sections in Periodic Acid Solution for 5 minutes.
3. Rinse sections well in deionized water (5-6 changes).
4. Place sections in coplin jar containing freshly prepared Working Methenamine Silver Solution. Apply lid loosely and place in 56-60° C water bath or oven. Check sections for staining intensity after 30 minutes. When checking microscopically, rinse sections in hot deionized water and look for dark brown to black fungi and/or *Pneumocystis carinii*.
5. Return sections to Working Methenamine Silver Solution and continue checking every 5 minutes until sections appear golden brown and organisms are sharply defined.
6. Rinse sections in deionized water (3-5 changes).
7. Tone sections in Gold Chloride Solution for 30 seconds to 1 minute until sections lose their golden brown color and appear light gray.
8. Rinse sections in deionized water for 30 seconds.
9. Place sections in Sodium Thiosulfate Solution for 1 minute.
10. Rinse sections well in deionized water for 1 minute.
11. Stain sections in Fast Green Stain Solution for 30 seconds to 1 minute to achieve the desired intensity.
12. Dehydrate sections in 95% alcohol for 1 minute.
13. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
14. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Microwave Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Oxidize sections in Periodic Acid Solution for 5 minutes.
3. Rinse sections well in deionized water (5-6 changes).
4. Place sections in coplin jar containing freshly prepared Working Methenamine Silver Solution. Apply lid loosely and place in microwave oven and heat for 20 seconds on high power. Stir solution to equalize the temperature for more uniform staining.
5. Microwave additional 20 seconds at 70% power. Do not exceed 80° C. Sections should appear golden brown. When checking microscopically, rinse sections in hot deionized water. Fungi and *Pneumocystis carinii* appear dark brown to black and sharply defined. If sections have not reached desired staining intensity, allow to stand in hot silver solution and recheck every 5-10 seconds.
6. Rinse sections in deionized water (3-5 changes).
7. Tone sections in Gold Chloride Solution for 30 seconds to 1 minute until sections lose their golden brown color and appear light gray.
8. Rinse sections in deionized water for 30 seconds.
9. Place sections in Sodium Thiosulfate Solution for 1 minute.
10. Rinse sections well in deionized water for 1 minute.
11. Stain sections in Fast Green Stain Solution for 30 seconds to 1 minute to achieve the desired intensity.
12. Dehydrate sections in 95% alcohol for 1 minute.
13. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
14. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results

Fungi – Brown to Black
Basement Membranes – Black
Pneumocystis carinii – Black
Background – Light Green

Discussion

Pneumocystis jiroveci (previously classified as *Pneumocystis carinii)* was renamed in 2002. The acronym PCP is still acceptable for describing Pneumocystis Pneumonia. All staining reagents should be stored in a refrigerator at 2-8° C. The Modified Methenamine Silver staining reagents are for "In Vitro" use only. Some of the reagents used in this kit are considered toxic. Refer to the Safety Data Sheet for Health and Safety Information. All reagents are stable and should not form precipitants under ordinary storage parameters. It is recommended that the Working Methenamine Silver Solution be discarded after use. The Fast Green Stain Solution can be filtered and reused. All dyes used in these formulations are certified by the Biological Stain Commission.

Technical Comments

Plastic forceps or paraffin coated metal forceps should be used to prevent the formation of a silver precipitate. Staining dishes should be thoroughly acid-washed and then rinsed in several changes of deionized water to eliminate the occurrence of the silver precipitate and ensure the primary reaction will occur. The microwave protocol was developed using a 1200 watt microwave oven. Microwave frequencies vary from model to model. It may be necessary to adjust power levels or times to achieve desired results.

Probable Mode of Action

Fungal cell walls are rich in polysaccharides. The Periodic Acid oxidizes the polysaccharides to form aldehydes. The aldehyde groups are reduced by the silver ions present in the methenamine silver. The reduction of silver ions in alkaline solutions form metallic silver on the aldehyde groups. The formation of the metallic silver allows for visual examination of the fungi. After methenamine silver impregnation the sections are toned in Gold Chloride. Gold toning deposits gold at the site of reduced silver (metallic silver). The Gold Chloride intensifies the reduced silver by conjugating with it. The sections are then placed in Sodium Thiosulfate. Sodium Thiosulfate removes unreduced silver from the tissue sections. The Fast Green Stain Solution exhibits a light green background to enhance the contrast of the preparation and to further pronounce the positive staining organisms.

References

1. Bancroft, J.D. and Stevens, A. Theory and Practice of Histological Techniques. Churchill Livingstone, New York, NY, 1977.
2. Sheehan, D.C. and Hrapchak, B.B. Theory and Practice of Histotechnology, 2nd Edition. Mosby, St. Louis, MO, 1980.
3. Thompson, C.C. Selected Histochemical and Histopathological Methods. Springfield, IL, 1966.
4. Lillie, R.D., H.J. Conn's Biological Stains. Williams & Wilkins, Baltimore, MD, 1972.
5. Carson, F. L. Histotechnology: A self-Instructional Text, 2nd Edition. ASCP Press, Chicago, 1997.

Order Information

Product	Size	Qty.	REF
Modified Grocott's Methenamine Silver (Periodic Acid) Kit	1 Kit	1	87008
Methenamine/Borax (capsules)	6 caps.	2	88023
Fast Green Stain Solution	125 mL	1	88024
Sodium Thiosulfate Solution (5%)	125 mL	1	88025
Gold Chloride Solution (0.1%)	125 mL	1	88026
Periodic Acid Solution (1%)	125 mL	1	88027
Silver Nitrate Solution (5%)	30 mL	1	88036

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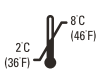
Anatomical Pathology



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