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

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

Technical Discussion

Microtomy
Cut paraffin sections at 4-6 microns. Frozen sections, smears and touch preps may also be used.

Fixation
10% Neutral Buffered Formalin is adequate. Fix frozen sections or cytology specimens in Pen-Fix or alcoholic formalin for 10 minutes.

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

Technical Procedure

Working Methenamine Silver Solution
Methenamine Borax……..1 Capsule

> **Note:** Wearing gloves, empty 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

Standard Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Oxidize sections in Chromic Acid Solution 5% for 10 minutes.
3. Rinse sections well in deionized water (5-6 changes).
4. Place sections in Sodium Metabisulfite Solution 1% for 1 minute at room temperature.
5. Rinse sections well in deionized water (5-6 changes).
6. 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 jiroveci*. Return sections to Working Methenamine Silver Solution and continue checking every 5 minutes until sections appear golden brown and organisms are sharply defined.
7. Rinse sections well in deionized water (3-5 changes).
8. Tone sections in Gold Chloride Solution for 30 seconds to 1 minute until sections lose their golden brown color and appear light gray.
9. Rinse sections in deionized water for 30 seconds.
10. Rinse sections in Sodium Thiosulfate Solution for 1 minute.
11. Rinse sections well in deionized water for 1 minute.
12. Counterstain sections in Fast Green Solution for 30 seconds to 1 minute to achieve the desired intensity.
13. Dehydrate sections in 95% alcohol for 1 minute.
14. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
15. 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 Chromic Acid Solution 5%: Place sections in a plastic coplin jar containing 50 mL of Chromic Acid; microwave for 30 seconds at 90% power to reach an optimal temperature of 60-65° C. Avoid overheating solution as tissue loss may occur. Allow sections to remain in hot Chromic Acid for an additional 1 minute.
3. Rinse sections well in deionized water (5-6 changes).
4. Place sections in Sodium Metabisulfite Solution 1% for 1 minute at room temperature.
5. Rinse sections well in deionized water (5-6 changes).
6. 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 or agitate solution to equalize the temperature for more uniform staining.
7. Microwave an 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* appear dark brown to black and sharply defined. If desired staining intensity has not been achieved, allow sections to stand in hot silver solution and recheck every 5-10 seconds.
8. Rinse sections in deionized water (3-5 changes).
9. Tone sections in Gold Chloride Solution for 30 seconds to 1 minute until sections lose their golden brown color and appear light gray.
10. Rinse sections in deionized water for 30 seconds.
11. Place sections in Sodium Thiosulfate Solution for 1 minute.
12. Rinse sections well in deionized water for 1 minute.
13. Counterstain sections in Fast Green Solution for 30 seconds to 1 minute to achieve desired intensity.
14. Dehydrate sections in 95% alcohol for 1 minute.
15. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
16. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results

Fungi – Brown to Black
Pneumocystis jiroveci – Black
Histoplasmosis – Black
Background – Light Green

Discussion

Pneumocystis jiroveci (previously classified as *Pneumocystis carinii)* was renamed in 2002. The acronym PCP is still acceptable for describing Pneumocystic Pneumonia.

All staining reagents should be stored in a refrigerator at 2-8° C. The Modified Methenamine Silver staining reagents used in this kit 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 recommended storage parameters. It is recommended that the Chromic Acid, Sodium Metabisulfite and Working Methenamine Silver Solution be discarded after use. The Gold Chloride Solution, Sodium Thiosulfate Solution, and Fast Green Stain Solution may be saved and reused. All dyes used in these formulations are certified by the Biological Stain Commission.

Technical Comments

Plastic or coated 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 oven 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. Treatment with either Periodic Acid or Chromic Acid oxidizes the polysaccharides to form aldehyde groups; however use of Chromic Acid will oxidize the newly formed aldehyde groups to further breakdown products that will not react with the silver and therefore are not demonstrated in the final product. This further oxidation results in a reduction in background staining of basement membranes and collagen fibers. Chromic Acid will also prove more effective than Periodic Acid for demonstration of *Pneumocystis jiroveci* and Histoplasmosis. After oxidation the Sodium Metabisulfite rinse removes traces of Chromic Acid left in the tissue.

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, allowing for visualization of the fungi. After Methenamine silver impregnation the sections are toned in Gold Chloride. Gold toning deposits gold at the site of the 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. Stringer, J.R., Beard, C.B., Miller, R.F., Wakefield, A.E. A New Name (*Pneumocystis* jiroveci) for *Pneumocystis* from Humans. Emerg Infect Dis 2002; 8:891-896.
2. Bancroft, J.D. and Stevens, A. Theory and Practice of Histological Techniques. Churchill Livingstone, New York, NY, 1977.
3. Sheehan, D.C. and Hrapchak, B.B. Theory and Practice of Histotechnology, 2nd Edition. Mosby, St. Louis, MO, 1980.
4. Thompson, C.C. Selected Histochemical and Histopathological Methods. Springfield, IL, 1966.
5. Lillie, R.D., H.J. Conn's Biological Stains. Williams & Wilkins, Baltimore, MD, 1972.

Order Information

Product	Size	Qty.	REF
Modified Grocott's Methenamine Silver (Chromic Acid) Kit		1 Kit	1 87024
Methenamine/Borax (capsules)	6 caps.	2	88023
Fast Green Stain Solution	125 mL	1	88024
Sodium Thiosulfate Solution (5%)	125 mL	1	88025
Sodium Metabisulfite Solution (1%)	500 mL	1	88042
Gold Chloride Solution (0.1%)	125 mL	1	88026
Chromic Acid Solution (5%)	500 mL	1	88041
Silver Nitrate Solution (5%)	30 mL	1	88036

Anatomical Pathology

2 C (36 F) – 8 C (46 F)

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