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

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 Buin’s Fluid is adequate. 

Quality Control 
A section-containing fungus 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 entire contents of capsule into distilled or deionized water and dissolve. 

Distilled or Deionized water, ……………50ml 
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 at 56-60°C water bath or oven. Check sections for staining intensity after 30 minutes. 
5. Rinse sections in deionized water (3-5 changes). 
6. Rinse sections in deionized water for 1 minute. 
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 Thiocyanate Solution for 1 minute. 
10. Rinse sections well in distilled 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 56-60°C water bath or oven. Check sections for staining intensity after 30 minutes. 
5. Rinse sections in deionized water (3-5 changes). 
6. Rinse sections in deionized water for 1 minute. 
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 Thiocyanate Solution for 1 minute. 

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 Material 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 Staining Commission. 

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 Thiocyanate. Sodium Thiocyanate 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 


Order Information 

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<th>Product</th>
<th>Size</th>
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<th>REF</th>
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