

This procedure is available in kit form from:
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cat# cy016

GROCOTT'S METHENAMINE SILVER STAIN (CHURUKIAN SCHENK)

SOLUTIONS:

1. Methenamine Stock Solution A
2. Silver Nitrate Stock Solution B
- Methenamine Silver Working Solution:**
Methenamine Stock Solution12.5 mL
Silver Nitrate Stock Solution12.5 mL
Distilled Water 25 mL
Borax 5% Aqueous 2 mL
3. Fast Green 0.2% Solution
Fast Green Working Solution:
Fast Green 0.2% Solution..... 10 mL
Distilled Water 40 mL
4. Chromic Acid 5% Aqueous
5. Sodium Bisulfite 1% Aqueous
6. Gold Chloride 0.2% Aqueous
7. Sodium Thiosulfate 2% Aqueous
8. Borax 5% Aqueous

STAINING PROCEDURE:

Note: Start with step 1 for paraffin sections and with step 3 for smears.

1. Deparaffinize in two changes of Xylene, 5 minutes each.
2. Hydrate through two changes of Absolute Ethyl Alcohol, 95%, 80% and 70% Alcohols, 2 changes for 15 dips each.
3. Wash in distilled water for 15 dips.
4. Place slides in Chromic Acid 5% Aqueous in a 43°C water bath for 2 minutes.
5. Transfer coplin jar to a 58°C water bath for 5 minutes.
6. Wash in tap water for 15 dips.
7. Place slides in Sodium Bisulfite 1% Aqueous for 30 seconds.
8. Wash in running tap water for 15 seconds.
9. Wash in four changes of distilled water - 15 dips each.

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10. Place slides in freshly mixed Methenamine Silver Working Solution in a 32°C water bath for 2 minutes.
11. Transfer coplin jar to a 58°C water bath for 23 minutes.
12. Wash in four changes of distilled water - 15 dips each.
13. Place in Gold Chloride 0.2% Aqueous - 20 to 30 dips.
14. Wash in two changes of distilled water - 15 dips each.
15. Place in Sodium Thiosulfate 2% Aqueous for 1 minute.
16. Wash in running tap water for 15 seconds.
17. Stain in Fast Green Working Solution for 30 seconds.
18. Wash in running tap water for 15 seconds.
19. Wash in two changes of distilled water - 15 dips each.
20. Dehydrate in 95% Ethyl Alcohol, Absolute Ethyl Alcohol, 2 changes each - 15 dips.
21. Clear in Xylene, 2 changes - 15 dips each.
22. Mount with Poly Mount (cat# s2153) or any other acceptable mounting medium.

RESULTS:

Pneumocystis Carinii & Fungi.....Black
Glycogen and Mucin.....Rose to Gray
Background.....Pale Green