

**A-123 A COMBINED HEMATOXYLIN AND EOSIN /  
METHENAMINE SILVER STAIN FOR THE HISTOLOGICAL  
DIAGNOSIS OF FUNGI IN TISSUE SECTIONS**

**FIXATION:** 10% Buffered Neutral Formalin (NBF)

**SECTIONS:** 3-4 um

**STAINING PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. Oxidize in **Chromic Acid, 10%** (A-123-1) for 10 minutes.
3. Rinse with 3-4 changes of tap water.
4. Rinse in **Sodium Bisulfite, 1%** (A-123-2) for 1 minute to remove any residual Chromic Acid.
5. Wash in running water, 5-10 minutes.
6. Rinse with 3-4 changes of distilled water.
7. Place in **freshly** mixed working methenamine Silver Nitrate Solution in a water bath @ 58-60°C, for 15-25 minutes or until sections turn yellowish brown.

Methenamine Silver Nitrate Stock:

- a. **Silver Nitrate, 5% Aq.** (A-123-3) . . . 5ml
- b. **Methenamine, 3% Aq.** (A-123-4) . . . 100ml

Use chemically clean glassware. Shake well to dissolve precipitate  
Store in refrigerator. Then to prepare Methenamine Silver Nitrate

Working solution:

- a. Methenamine Silver Nitrate Stock Solution . . . 25ml
- b. Water . . . . . 25ml
- c. **Sodium Borate, 5% Aq.** (A-123-5) . . . . . 4ml

8. Rinse in 6 changes of distilled water.
9. Tone in **Gold Chloride, 0.2%** (A-123-6) for 2-5 minutes.
10. Rinse in 3-4 changes of distilled water.
11. Remove unreduced Silver with **Sodium Thiosulfate, 2%** (A-123-7) for 2-5 minutes.
12. Wash thoroughly in tap water for 10 minutes.
13. Place in **Harris' Hematoxylin** (A-123-8) for 10 minutes.
14. Rinse in 3-4 changes of tap water.
15. Place in **Acetic Acid, 3%** (A-123-9) for 1 minute.
16. Rinse in 2 changes tap water.
17. Place in **Ammonia Water, 0.25%** (A-123-10) 10 minutes.
18. Rinse in 2 changes tap water.
19. Place in 95% alcohol, 1 minute.
20. Place in **Eosin Y, 1% Alcoholic** (A-123-11) for 5 minutes.
21. Dehydrate in 95% alcohol, absolute alcohol and clear in xylene, 2 changes each.
22. Mount with Permount.

**Stain Results:**

Organisms	Blue-Black
Background	Rose

**References:**

Lamps, Laura W. et al A Combined Hematoxylin and Eosin/Methenamine Silver Stain for the Histological Diagnosis of Fungi in Tissue Sections., J. of Histotechnology 23:4 p. 341-345