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# Histology Special Stain - Methenamine for Urates - Royal Oak



Document Type: Procedure

# I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of uric acid and urates. These are deposited in tissue (gouty tophus) or around joints in people suffering with gout, which is a disorder in uric acid metabolism.

# II. PRINCIPLE:

Silver nitrate stains the urates. The methenamine, upon heating, breaks down into formaldehyde and ammonia. The formaldehyde reduces the silver ions to black metallic silver. The ammonia is an unwanted by-product, which will raise the pH, and cause the sections to fall off the slides. To control the pH, sodium borate (borax) is added. Sodium thiosulfate removes the unreduced silver. Nuclear fast red is the counterstain.

# III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Absolute alcohol or acetone required.
    2. Avoid all water.

B. Processing
    1. Standard, overnight processing.

C. Section Thickness

1. Cut paraffin sections at 5µ.

D. Slide Drying
    1. 30 minutes at 60°C.

E. Type of Slide
    1. Plain slides

# IV. REAGENTS:

A. **5% Borax (Sodium Borate)**

| | |
|---|---|
| **Borax (sodium borate)** | **25.0 gm** |
| **Distilled water** | **500.0 mL** |

Dissolve together with the aid of gentle heat. Cool. Store at room temperature; stable for months.

B. **5% Silver Nitrate**

| | |
|---|---|
| **Silver nitrate** | **5.0 gm** |
| **Distilled water** | **100.0 mL** |

Stir together with magnetic stirrer. Store in brown glass bottle; store in refrigerator (3°C.); stable for 2-3 month.

C. **3% Methenamine**

| | |
|---|---|
| **Methenamine (hexamethylenetetramine)** | **3.0 mL** |
| **Distilled water** | **100.0 mL** |

Dissolve together. Store in refrigerator (3°C.); stable for months.

D. **Stock Methenamine-Silver Solution**

| | |
|---|---|
| **5% silver nitrate** | **5.0 mL** |
| **3% methenamine** | **100.0 mL** |

Mix together in acid-clean glassware. A white precipitate forms, but will disappear with mixing. Store in refrigerator (3°C.) in dark glass bottle; stable for 1-3 months.

E. **Working Methenamine-Silver Solution**

| | |
|---|---|
| **STOCK methenamine-silver solution** | **25.0 mL** |
| **Distilled water** | **25.0 mL** |
| **5% borax (sodium borate)** | **2.0 mL** |

JUST BEFORE USE, mix together in the order listed above. Warm to room temperature before use; use only once.

F. **5% Sodium Thiosulfate**

| | |
|---|---|
| **Sodium thiosulfate** | **5.0 gm** |
| **Distilled water** | **100.0 mL** |

Dissolve together. Store at room temperature; stable for months.

G. **5% Aluminum Sulfate**

| | |
|---|---|
| **Aluminum sulfate** | **5.0 gm** |
| **Distilled water** | **100.0 mL** |

Dissolve together. Store at room temperature; stable for months.

H. **Nuclear Fast Red**

| | |
|---|---|
| **Nuclear fast red** | **0.1 gm** |

| | |
|---|---|
| **5% aluminum sulfate** | **100.0 mL** |

Dissolve together with the aid of gentle heat. Cool. Filter. Add a few crystals of thymol. Store at room temperature or in
refrigerator (3°C.); stable for months.

# V. EQUIPMENT:

A. Balance

B. Magnetic stirrer

# VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Non-metal forceps

# VII. SPECIAL SAFETY PRECAUTIONS:

A. Sodium Borate (Borax)

1. Has low hazard for recommended handling.

B. Silver Nitrate

1. Is an oxidizer.
2. Store separately from other material.
3. Is poisonous and may be fatal if swallowed.
4. Causes skin and eye burns.
5. Is an irritant to the respiratory system.

C. Methenamine (Hexamethylenetetramine)

1. Is an irritant to eye, skin, and respiratory system.

D. Sodium Thiosulfate

1. Is an irritant.

E. Aluminum Sulfate

1. Is corrosive.
2. May cause serious eye damage.

F. Nuclear Fast Red

1. Corrosive to skin.
2. Irritant to eyes.
3. Toxic to respiratory system.

G. Thymol

1. May cause eye burns.
2. May be irritating to respiratory tract and skin.

# VIII. QUALITY CONTROL (QC):

Section of tissue with urates or uric acid.

# IX. LIMITATIONS:

Use chemically cleaned coplin jars and non-metal forceps.

# X. PROCEDURE:

| Step | Action | Time | Notes |
|---|---|---|---|
| 1 | Deparaffinize and hydrate sections through graded alcohol to distilled water. | | |
| 2 | Place slides in WORKING methenamine-silver solution in 60°C oven. | 15-60 minutes | Make working solution just before use. Keep in oven until urates are black; check with microscope. Can also microwave on high for 45 seconds. Allow to set on counter 5 minutes, or until urates are black. Check with microscope. |
| 3 | Rinse in distilled water, 3-5 changes each. | 5-10 seconds | |
| 4 | Place in 5% sodium thiosulfate. | 5 minutes | |
| 5 | Rinse in running water. | 1-2 minutes | |
| 6 | Stain in nuclear fast red. | 1-5 minutes | |
| 7 | Wash in distilled water, 2-3 changes. | 5-10 seconds | |
| 8 | Dehydrate through graded alcohols, clear with xylene. | 10 seconds | |
| 9 | Coverslip. | | |



# XI. RESULTS:

A. Urates and uric acid - **black**
B. Calcium phosphate and carbonate, if large - **black**
C. Argentaffin structures (melanin, nuclei, etc.) - **black**
D. Nuclei - **pink**

E. Background - **pale pink**

# XII. REFERENCES:


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B. Carson FL: Histotechnology: A Self-Instructional Text, Chicago, IL, ASCP Press, 1990.

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## Approval Signatures

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|---|---|---|
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## Applicability

Royal Oak