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Histology Special Stain-Jones (Periodic Acid Methenamine Silver) Stain - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of basement membrane in tissue. It may be used to demonstrate thickening of glomerular basement membrane in kidney diseases.

II. PRINCIPLE:

This is an argyrophilic silver reaction. The basement membrane contains polysaccharides. Periodic acid oxidizes the polysaccharides to aldehydes. The silver nitrate is the source of silver ions. The silver ions bind to the aldehydes. Methenamine is used to reduce the silver ions. When heated, methenamine will breakdown into formaldehyde and ammonia. The formaldehyde reduces the silver ions to visible silver. The ammonia is an unwanted by-product, as it increases the pH, causing the sections to fall off of the slides. Borax (sodium borate) is used to control the pH. Gold chloride tones the stain. Sodium thiosulfate removes unreduced silver. Chromotrope 2R is the counterstain.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue. 10% neutral buffered formalin, DB or Bouin preferred. Avoid all mercuric fixatives.

B. Processing
    1. Standard, overnight processing.

C. Section Thickness

1. Routine specimens- 4-5 µm. Cut kidney biopsies at 2-3 µm.

D. Slide Drying

1. 60 minutes at 60°C.

E. Type of slide

1. Plain Slides

IV. REAGENTS:

A. **5.0% Stock Periodic Acid**

Periodic acid 25.0 gm
Distilled water 500.0 mL

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

B. **0.5% Working Periodic Acid Solution**

STOCK 5.0% periodic acid 50.0 mL
Distilled Water 450.0 mL

Mix together. Store at room temperature; stable for several months.

C. **3% Methenamine Solution**

Methenamine (Hexamethylenetetramine) 15.0 gm
Distilled water 500.0 mL

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

D. **5% Silver Nitrate**

Silver nitrate 5.0 gm
Distilled water 100.0 mL

Dissolve together. Store in a dark brown bottle in refrigerator (3°C.); stable for months. Discard if discolored, or if black precipitate is present.

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

Sodium borate (borax) 25.0 gm
Distilled water 500.0 mL

Warm distilled water on either a hot plate or in the microwave oven, until water is steaming. It does not need to boil.
Dissolve in sodium borate. Cool. Store at room temperature; stable for months.

F. **Working Methenamine Silver Solution**

3% methenamine 50.0 mL
5% silver nitrate 2.5 mL
5% sodium borate (borax) 6.0 mL

JUST BEFORE USE, mix together. Filter; discard after use.

G. **Stock 1% Gold Chloride**

Gold chloride 1.0 gm
Distilled water 100.0 mL

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

H. **Working 0.1% Gold Chloride**

Stock 1% gold chloride	**5.0 mL**
Distilled water	**45.0 mL**

Mix together. Store at room temperature; may be reused until weak. Can be filtered if precipitate forms.

I. **5% Sodium Thiosulfate**

Sodium thiosulfate	**25.0 gm**
Distilled water	**500.0 mL**

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

J. **Phosphotungstic Acid**

Phosphotungstic acid crystals	**1.0 gm**
Distilled water	**100.0 mL**

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

K. **1/10 N Hydrochloric Acid**

Hydrochloric acid	**3.7 mL**
Distilled water	**1000.0 mL**

This solution may be kept for six months at room temperature.

L. **Chromotrope 2R Solution**

Chromotrope 2 R	**2.0 gm**
1/10N Hydrochloric acid	**100.0 mL**

Dissolve together. Store at room temperature; may be reused until weak.

V. EQUIPMENT:

A. Balance

B. Microwave oven or 60°C water bath

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Funnel

D. Filter paper

E. Coplin jars

F. Magnetic stirrer

VII. QUALITY CONTROL:

Not usually needed; all tissue has some basement membrane. If needed, use kidney with glomeruli.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Periodic Acid

1. Is a strong oxidizer.

2. Store separately from all other chemicals.

B. Methenamine (Hexamethylenetetramine)

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

C. Silver Nitrate

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

D. Borax (Sodium Borate)

1. Is an irritant.

E. Gold Chloride

1. May cause skin and eye irritation.

F. Sodium Thiosulfate

1. Is an irritant.

G. Hydrochloric Acid

1. Add drop by drop to solution.
2. May cause skin/eye burns.

H. Phosphotungstic Acid

1. Causes skin and eye irritation.
2. Do not inhale vapor

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate through graded alcohols to distilled water.		
2	Place slides in WORKING 0.5% periodic acid.	30 minutes	
3	Rinse in distilled water, 2-3 changes.	10 seconds each	
4	Place in WORKING methenamine silver solution at 60°C. water bath until ½ to ¾ of the capillary loops in the glomeruli are dark brown/black.	30-60 minutes	Use microscope to check.
5	Rinse in distilled water, 2-3 changes.	10 seconds each	

6	Dip in WORKING 0.1% gold chloride until sections are no longer brown or yellow. Capillary loops and basement membrane should be black.	10-60 seconds	Use microscope to check.
7	Rinse in distilled water, 2-3 changes.	10 seconds each	
8	Place in 5% sodium thiosulfate.	1 minute	
9	Rinse in running tap water.	1 minute	
10	Place in 1% phosphotungstic acid solution.	1 minute	
11	Counterstain in Chromotrope 2R.	10 minutes	
12	Rinse in distilled water.	30 seconds	
13	Dehydrate through graded alcohols and clear in xylene.		
14	Coverslip.		

X. LIMITATIONS:

A. The following may influence the validity of test results:

1. Overheating the methenamine silver solution will cause the methenamine to breakdown quickly. This will increase the amount of silver ions to be reduced, causing the silver to stain the background, slide, and coplin jar. An increased amount of ammonia will also be formed. This will increase the pH of the solution, causing the sections to fall off the slides.
2. Other counterstains can also be used, such as eosin, H&E, light green SF yellowish, or metanil yellow. Follow standard staining procedures after the sodium thiosulfate step, skip the phosphotungstic acid step.
3. For chromotrope 2R, if tissue is not fixed in Bouin's or DB, tissue must be post-mordanted in Bouin's before counter staining. After the water rinse after sodium thiosulfate (step 9), place the slides in Bouin's in the 60°C oven for 35-60 minutes. Rinse in running water to remove yellow color. Proceed with step 10.

XI. RESULTS:

A. Basement Membrane - **Black**

B. Background - **Light pink to red**

XII. REFERENCES:

A. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

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

Approval Signatures

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Applicability

Royal Oak