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Area	Laboratory-Histology
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Histology Special Stain - Reticulin-Gordon and Sweet - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of reticulin (Collagen Type III) in tissue. Reticulin is a small irregular connective tissue, usually found in single strands. It is the supporting stoma in bone marrow, lymph nodes, spleen, liver, and smooth muscle. This stain is used to indicate a change from the normal reticulin pattern, which may indicate sarcoma, leukemia, or cirrhosis

II. PRINCIPLE:

This is an argyrophilic silver reaction. The reticulin fibers have high sugar content. Potassium permanganate oxidizes the sugars to aldehydes. Oxalic acid is used to remove the excess potassium permanganate in the tissue. Ferric ammonium sulfate is the sensitizer. It forms a metal-organic compound with the tissue. The metal is subsequently replaced by silver. Silver nitrate is the source of silver ions. Ammonium hydroxide and sodium hydroxide are added to the silver to increase the pH to between 11 and 12, and to form a diamine silver complex, $Ag(NH_3)_2^+$. The silver ions bind to the aldehydes on the reticular fibers. Formaldehyde reduces the silver ions to a visible metal. Gold chloride tones the stain. Sodium thiosulfate removes unreduced silver. Nuclear fast red may be used as counterstain.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation

1. Any well-fixed tissue.

2. 10% NBF preferred.

B. Processing

1. Standard, overnight processing.

C. Section Thickness

1. Routine specimens-5um.
2. Cut liver biopsied and lymph nodes at 4 um.

D. Slide Drying

1. 60 minutes at 60°C.

E. Type of Slide

1. Plain slides

IV. REAGENTS:

A. **1.0% Potassium Permanganate**

Potassium permanganate ($KMnO_4$) 5.0gm
Distilled water 500.0mL

Dissolve together. Store in brown bottle; store at room temperature; stable for months.

B. **1% Oxalic Acid**

Oxalic acid $(COOH)_2$*$2H_2O$) 5.0gm
Distilled water 500.0mL

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

C. **2.5% Ferric Ammonium Sulfate**

Ferric ammonium sulfate ($FeNH_4(SO_4)_2X12H_2O$) 12.5gm
Distilled water 500.0mL

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

D. **10% Silver Nitrate**

Silver nitrate 10.0 gm
Distilled water 100.0 mL

Dissolve together. Use acid clean glassware. Store in refrigerator (3°C.); stable for several months; store in brown bottle.

E. **3% Sodium Hydroxide**

Sodium hydroxide 3.1 gm
Distilled water 100.0 mL

Carefully add sodium hydroxide to distilled water. Dissolve together. Store at room temperature; stable for several months.

F. **Working Gordon and Sweet Ammoniacal Silver Solution**

JUST BEFORE USE, combine:

10% Silver Nitrate 5.0 mL
Concentrated ammonium hydroxide (NH_4OH)

1. Place the 10% silver nitrate in an Erlenmeyer flask.

2. Add concentrated ammonium hydroxide, drop by drop, while shaking the container continuously, until the precipitate is completely dissolved. Do NOT add any excess ammonium hydroxide.
3. Add 5mL of 3% sodium hydroxide solutions, and cautiously re-dissolve the precipitate with concentrated ammonium hydroxide until only a faint cloudiness remains. (If this step is carried too far and no cloudiness remains, add 10% silver nitrate solution, drop by drop with agitation, until ion drop causes the solution to become permanently cloudy.)

G. **10% Formalin**

Formaldehyde (37-40%)(HCHO)	**50.0 mL**
Distilled water	**450.0 mL**

H. **Stock 1% Gold Chloride**

Gold chloride ($AuCl_3X3H_2O$	**1.0 gm**
Distilled water	**100.0 mL**

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

I. **Working 0.2% Gold Chloride**

Stock 1% gold chloride	**10.0 mL**
Distilled water	**40.0 mL**

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

J. **5% Sodium Thiosulfate**

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

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

K. **5% Aqueous Aluminum Sulfate**

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

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

L. **Nuclear Fast Red**

Nuclear fast red	**0.1 gm**
5% aqueous aluminum sulfate	**100.0 mL**

Dissolve together with the aid of heat. Cool. Filter. Add a few crystals of thymol. Store at room temperature; stable for months; may be reused until weak.

V. EQUIPMENT:

A. Balance

B. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Coplin jars
D. Forceps
E. Funnel
F. Filter paper

VII. QUALITY CONTROL:

Use liver as a positive control

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Potassium Permanganate
    1. Is an oxidizer.
    2. Store separately from all other chemicals.
    3. May cause skin and eye irritation.

B. Oxalic Acid
    1. Is a strong reducing agent.
    2. Contact with other material may cause fire.
    3. May cause skin and eye burns.
    4. Irritating to respiratory system.

C. Ferric Ammonium Sulfate
    1. Is an irritant and is toxic.

D. 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.

E. Ammonium Hydroxide
    1. May cause severe skin and eye burns.
    2. Vapors are irritating to eyes and respiratory tract.
    3. Harmful if swallowed or inhaled.

F. Sodium Hydroxide
    1. May cause severe skin and eye burns.
    2. Harmful if inhaled.

G. Formaldehyde
    1. Is a poison.

2. May be fatal or cause blindness if swallowed.
3. Cannot be made non-poisonous.
4. Possible cancer hazard.
5. Irritating to eyes, skin and respiratory tract.
6. Can cause severe eye burns.

H. Gold Chloride
1. May cause skin and eye irritation.

I. Sodium Thiosulfate
1. Is an irritant.

J. Nuclear Fast Red
1. Is an irritant.

K. Aluminum Sulfate
1. Has low hazard for recommended handling.

L. Thymol
1. Is an irritant to skin and respiratory tract.
2. May burn eyes.

M. Sodium Thiosulfate
1. Is an irritant.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Place slides in 1.0% potassium permanganate.	5 minutes	
3	Rinse in running tap water.	2 minutes	
4	Place in 1.0% Oxalic acid.	2 minutes	
5	Rinse in running tap water.	2 minutes	
6	Place in 2.5% ferric ammonium sulfate.	1 minute	
7	Wash in running tap water.	2 minutes	
8	Rinse in distilled water, 2 changes.	10 seconds	

9	Place in WORKING Gordon and Sweet ammoniacal silver solution.	2 minutes.	Make just before use.
10	Rinse in distilled water, 2-3 changes.	10 seconds	
11	Reduce in 10% formalin.	2 minutes	
12	Wash in running tap water.	3 minutes	
13	Rinse in distilled water, 2-3 changes.	10 seconds	
14	Place in WORKING 0.2% gold chloride until sections are no longer brown or yellow, and reticulin is black.	10-60 seconds	Check with microscope.
15	Rinse in distilled water, 2-3 changes.	10 seconds	
16	Place in 5% sodium thiosulfate.	1 minute	
17	Wash in running tap water.	2 minutes	
18	Counterstain, if desired, in nuclear fast red.	1-5 minutes	
19	Rinse in distilled water, 2-3 changes.	5 seconds	
20	Dehydrate through graded alcohols, clear with xylene.	10 seconds	
21	Coverslip with a synthetic mounting media.		

X. LIMITATIONS:

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

1. Non-metal forceps must be used, or a silver precipitate may be formed on the slides and tissue.
2. Acid-clean glassware must be used, or a silver precipitate may be formed on the slides and tissue.
3. If nuclear fast red is used as a counterstain, wash slides with tap water after staining. Aluminum sulfate does not dissolve in alcohol. Placing the slides directly from nuclear fast red to alcohol with result in a white precipitate forming on the slides and tissue, which can be removed by returning the slides to water.

XI. RESULTS:

A. Reticulin fibers - **black**

B. Background, if nuclear fast red is used - **pink**

C. Nuclei, if nuclear fast red is used - **pink**

XII. REFERENCES:

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

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

C. Vacca LL: Laboratory Manual of Histochemistry. Raven Press. 1985.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak