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Beaumont

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Histology Special Stain - Wilder Reticulin - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of reticulin 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 a high sugar content. Phosphomolybdic acid oxidizes the sugars to aldehydes. Uranyl nitrate 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 is added to the silver to increase the pH to between 11 and 12. Sodium hydroxide is added to the silver solution to produce a precipitate of silver hydroxide. The addition of more ammonium hydroxide results in the clearing of the precipitate, and the formation of a diamine silver complex, $Ag(NH_3)_2^+$. The silver ions bind to the aldehydes. 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 the 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 -5µ.
2. Cut liver biopsies and lymph nodes at 4µ.

D. Slide Drying

1. 60 minutes at 60°C.

E. Type of Slide

1. Plain slides.

IV. REAGENTS:

A. **10% Phosphomolybdic Acid**

Phosphomolybdic acid	**10.0 gm**
Distilled water	**100.0 mL**

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

B. **1% Uranyl Nitrate**

Uranyl nitrate	**1.0 gm**
Distilled water	**100.0 mL**

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

C. **10% Silver Nitrate**

Silver nitrate	**10.0 gm**
Distilled water	**100.0 mL**

Dissolve together. Use acid clean glassware. Store in refrigerator; stable for several months. Store in brown bottle.

D. 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.

E. **Wilder Ammoniacal Silver Solution**

JUST BEFORE USE (use acid clean glassware):

10% Silver nitrate	**15.0 mL**

1. Add 28% (concentrated) ammonium hydroxide, drop by drop, until precipitate which is formed, almost dissolves.
2. Add 3% sodium hydroxide **5.0 mL**
3. Add a few drops of 28% ammonium hydroxide until the resulting precipitate is just barely dissolved.
4. Add enough distilled water to bring solution up to 50 mL.

F. **Reducing Solution**

Distilled water	**50.0 mL**
40% formaldehyde	**0.5 mL**
1% urnayl nitrate	**1.5 mL**

JUST BEFORE USE, mix together in order listed.

G. **Stock 1% Gold Chloride (may use vendor pre-made)**

Gold chloride	**1.0 gm**
Distilled water	**100.0 mL**

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

H. **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.

I. **5% Sodium Thiosulfate**

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

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

J. **5% Aqueous Aluminum Sulfate**

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

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

K. **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:

Not needed usually, as all tissue has some reticulin. If needed, use liver.

VIII. SPECIAL SAFETY PRECAUTIONS:

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

B. Uranyl Nitrate
    1. Is an oxidizer.
    2. Store separately from other chemicals.

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

D. 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.

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

F. 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.

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

H. Sodium Thiosulfate
    1. Is an irritant.

I. Nuclear Fast Red
    1. Is an irritant.

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

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Place slides in 10% phosphomolybdic acid.	1 minute	
3	Rinse in running tap until yellow color is removed.	1-5 minutes	
4	Place slides in 1% uranyl nitrate.	1 minute	
5	Rinse in distilled water, 2-3 changes.	5-10 seconds	
6	Place in Wilder ammoniacal silver solution.	1 minute	Make working silver solution JUST BEFORE USE. Better staining is obtained if two changes of silver solution are used, and gentle agitation also increases staining.
7	Rinse very quickly in 95% ethanol.	2-3 seconds	
8	Place slides in reducing solution.	1 minute	Better staining is obtained if two changes of the reducing solution are used, and gentle agitation also increases staining. Check development of silver with a microscope.
9	Rinse slides in distilled water, 2-3 changes each.	10 seconds	
10	Tone in Working 0.2% Gold Chloride until sections lose their yellow color.	15-60 seconds	Check with microscope.
11	Rinse in distilled water, 2-3 changes each.	5-10 seconds	
12	Place slides in 5% sodium thiosulfate.	1 minute	

13	Rinse slides in running water.	1 minute	
14	Counterstain, if desired, in nuclear fast red.	1-5 minutes	Other counterstains can also be used, such as eosin, hematoxylin, or light green SF yellowish. Follow standard staining procedures after the sodium thiosulfate step. Counter stain may be omitted, especially if the pattern of the reticulin is important.
15	Rinse in distilled water, 2-3 changes each.	5-10 seconds	
16	Dehydrate through graded alcohols, clear with xylene.	10 seconds	
17	Coverslip.		

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 running 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

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Applicability

Royal Oak