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Beaumont

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Area	Laboratory-Histology
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Histology Special Stain - Sulfonated Alcian Blue - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of amyloid in tissue sections.

II. PRINCIPLE:

The reaction appears to stain the mucopolysaccharide portion of amyloid, rather than the protein component of amyloid. The alcian blue is the dye which stains the amyloid. Acetic acid is used to lower the pH to around 1.0, making it more specific for amyloid. The sodium sulfate changes the tertiary structure of the amyloid so that reactive sites are laid out in a fashion amenable to binding by the dye. As a neutral salt, it also suppresses background staining. The borax alkalizes the tissue, rendering fast the color. Weigert's hematoxylin stains the nuclei. Picric acid stains the muscle, cytoplasm, and red blood cells, and changes the color of the stained amyloid from blue to green. Acid fuchsin stains collagen.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well fixed tissue.
    2. B-5 fixative or prolonged fixation in 10% NBF may reduce stain intensity.

B. Processing
    1. Standard processing.

C. Section Thickness

1. Routine specimens-7-8µm.
2. Frozen section muscle biopsies-10µm.

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

E. Type of slide
   1. Plain

IV. REAGENTS:

A. **Acetic alcohol**

95% ethanol	**45.0 mL**
Distilled water	**45.0 mL**
Acetic acid	**10.0 mL**

Just before use, mix together. Make fresh each time.

B. **Stock 1% Alcian Blue (8GX)**

Alcian blue	**5.0 gm**
95% ethanol	**500.0 mL**

Dissolve together. Store at room temperature. Stable for months.

C. **Stock 1% Sodium Sulfate**

Sodium sulfate	**5.0 gm**
Distilled water	**500.0 mL**

Dissolve together. Store at room temperature. Stable for months.

D. **Working Sulfonated Alcian Blue (SAB)**

Stock 1% alcian blue (8GX)	**45.0 mL**
Stock 1% sodium sulfate	**45.0 mL**
Acetic acid	**10.0 mL**

Mix together. Allow to stand for 30 minutes before use. Make fresh each time.

E. **Saturated Borax in 80% Alcohol**
On a mechanical stirrer, mix together borax (sodium borate) in 80% ethanol, until no more borax dissolves into the alcohol. Stable at room temperature for months. Discard after use.

F. **1% Alcoholic Hematoxylin – Solution A**

Hematoxylin	**1.0 gm**
Distilled water	**5.0 mL**
Absolute ethanol	**95.0 mL**

Dissolve hematoxylin in distilled water. Add absolute alcohol. Store at room temperature in a dark brown bottle. Stable for months.

G. **29% Ferric Chloride**

Ferric chloride	**29.0 gm**
Distilled water	**100.0 mL**

Dissolve together. Store at room temperature. Stable for months.

H. **Iron Chloride – Solution B**

29% ferric chloride	**4.0 mL**

Distilled water **95.0 mL**
Hydrochloric acid, concentrated **1.0 mL**

Mix together 29% ferric chloride and distilled water. Slowly add hydrochloric acid, drop by drop, with stirring, to solution.
Store at room temperature. Stable for months.

I. **Working Weigert**

1% alcoholic hematoxylin (Solution A) **20.0 mL**
Iron chloride (Solution B) **20.0 mL**

Mix together. Store at room temperature. Stable for 2-3 days.

J. **Saturated Picric Acid in 80% Ethanol (may use vendor pre-made)**

On a mechanical stirrer, mix together picric acid in 80% ethanol, until no more picric acid will dissolve into the alcohol. Stable at room temperature for months. Discard after use.

K. **1% Aqueous Acid Fuchsin**

Acid fuchsin **1.0 gm**
Distilled water **100.0 mL**

Dissolve together. Store at room temperature. Stable for months.

L. **Saturated Aqueous Picric Acid (may use vendor pre-made)**

Picric acid
Distilled water **100.0 mL**

Add picric acid, a gram at a time, to the distilled water and stir using a mechanical stirrer. When dissolved, add another gram and stir. Continue doing this until no more picric acid will dissolve into the water. Store at room temperature. Stable for months.

M. **Van Gieson Counterstain**

1% aqueous acid fuchsin **5.0 mL**
Saturated aqueous picric acid **100.0 mL**

Mix together. Store at room temperature. Stable for months. May be re-used until weak.

V. EQUIPMENT:

A. Balance
B. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks
B. Graduated cylinders
C. Coplin jars
D. Forceps

VII. QUALITY CONTROL:

Run a control slide that is a known positive for amyloid.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Acetic Acid
    1. Add drop by drop to water.
    2. May cause skin and eye burns.

B. Alcian Blue
    1. Is an irritant

C. Sodium Sulfate
    1. Is an irritant

D. Borax (Sodium Borate)
    1. Is an irritant

E. Hematoxylin
    1. Is incompatible with oxidizers & alkalies.
    2. Store separate from these.

F. Ferric Chloride
    1. Is a corrosive.
    2. May cause skin and eye burns.
    3. Can be irritation to respiratory tract.

G. Hydrochloric Acid
    1. Add slowly, drop by drop, to solution.
    2. May cause severe skin and eye burns.

H. Picric Acid
    1. Is toxic, highly reactive (4) and an extreme fire hazard (4).
    2. Keep picric acid moist at all times.
    3. If dry around top of jar wash off dry particle before opening.
    4. Store in an explosion proof cabinet.

I. Acid Fuchsin
    1. Is an irritant

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Place in acetic alcohol.	1-2 minutes	

3	Stain in SAB solution.	2 hours	
4	Place in acetic alcohol.	1 minute	
5	Wash in running tap water.	1 minute	
6	Place in 80% ethanol saturated with Borax.	30 minutes	
7	Wash in running tap water.	1 minute	
8	Rinse in distilled water, 2-3 changes.	5-10 seconds each	
9	Stain in Weigert's hematoxylin.	5 minutes	
10	Wash in running tap water.	5 minutes	
11	Place in 80% ethanol saturated with picric acid.	20 seconds	
12	Rinse briefly with distilled water.	10 seconds	
13	Stain in Van Gieson.	2 minutes	
14	Dehydrate and clear.		
15	Coverslip.		

X. RESULTS:

A. New amyloid - **bright green**
B. Old amyloid - **pale green or non-reactive**
C. Collagen, Stroma - **red**
D. Muscle, Cytoplasm, Red Blood Cells - **yellow**
E. Nuclei - **black**

XI. REFERENCES:

A. John Bancroft and Harry Cook, Manual of Histological Techniques, Churchill Livingstone, 1984.

Approval Signatures

Step Description	Approver	Date
Medical Director	Kurt Bernacki: System Med Dir, Surgical Path	11/2/2022
Policy and Forms Steering Committee (if needed)	Gail Juleff: Project Mgr Policy	11/2/2022

Policy and Forms Steering Committee (if needed)	Sharon Scalise: Supv, Laboratory	11/1/2022
	Amy Knaus: Dir, Lab Operations C	10/31/2022
	Jennifer Lehmann: Mgr Laboratory	10/28/2022
	Sharon Scalise: Supv, Laboratory	10/27/2022

Applicability

Royal Oak