Status **Active** PolicyStat ID **12427356**

Beaumont

Origination	10/12/2022
Last Approved	10/12/2022
Effective	10/12/2022
Last Revised	10/12/2022
Next Review	10/11/2024

Document Contact	Sharon Scalise: Supv, Laboratory
Area	Laboratory-Histology
Applicability	Royal Oak

Histology Special Stain-Alcian Blue, pH 2.5-Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of sulfated or carboxylated acid mucopolysaccharides in tissue sections.

II. PRINCIPLE

Alcian blue is a large molecular weight molecule which will penetrate and stain components with low density, such as mucin. It contains copper, which gives it a blue color. It is a polyvalent basic dye, which will bind electrostatically to certain tissue polyanions (i.e. acid mucopolysaccharides) with carboxyl or sulfate groups. Acetic acid is used to obtain a pH of 2.5. Nuclear fast red is the counterstain.

III. SPECIMEN COLLECTION AND HANDLING:

A. **Fixation**
   1. Any well fixed tissue.
   2. 10% neutral buffered formalin or Bouin preferred

B. **Processing**
   1. Standard, overnight processing

C. **Section Thickness**
   1. Routine specimens-5um

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

E. **Type of slide**

1. Plain

IV. REAGENTS:

A. **Alcian Blue, pH 2.5**

Alcian blue	**1.0 gm**
Distilled water	**97.0 ml**
Acetic Acid	**3.0 ml**

Dissolve together. Adjust pH to 2.5 using more acetic acid. Filter and add a few crystals of thymol. Stable at room temperature for 6 months. May be reused until weak.

B. **5% Aluminum Sulfate**

Aluminum Sulfate	**25.0 gm**
Distilled Water	**500.0 ml**

Dissolve together. Store at room temperature; stable for 6 months.

C. **Nuclear Fast Red**

Nuclear fast red (Kernechtrot)	**0.1 gm**
5% aluminum sulfate	**100.0 ml**

Dissolve together with the aid of gentle heat; do not boil. Cool and filter. Add a few crystals of thymol. Stable at room temperature for 6 months. May be reused until weak.

V. EQUIPMENT:

A. Balance

B. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinder

C. Filter paper

D. Coplin jars

VII. QUALITY CONTROL (QC):

A. Use umbilical cord as positive control tissue, especially if staining for hyaluronic acid (carboxylated acid mucopolysaccharide).

B. pH is critical, alcian blue solution should be checked periodically for pH.

C. To demonstrate hyaluronic acid, proceed with step 1 with 2 controls and 2 unknowns. Place one of the controls and one of the unknowns in hyaluronidase for 1 hour at room temperature. Keep the other control and unknown in distilled water. At the end of 1 hour, gently rinse off the hyaluronidase, combine all slides together, and continue with step 2. Carboxylated hyaluronic acid mucopolysaccharides will NOT stain with alcian blue. All other acid mucopolysaccharides will continue to stain.

D. If sulfated acid mucopolysaccharides need to be demonstrated, use the Alcian Blue pH 1.0 procedure. This will differentiate sulfated from carboxylated acid mucopolysaccharides.

E. Always rinse with tap water after counterstaining with nuclear fast red. Aluminum sulfate will remain as streaks on the slide if the water rinse is skipped, as aluminum sulfate does not dissolve in alcohol.

F. To save on the amount of nuclear fast red used, keep some in the refrigerator. When the solution being used become weak, add some of the fresh solution from the refrigerator.

G. Instead of counterstaining with nuclear fast red, a PAS or PASH stain may be used. Do Steps 1 through 4 of the Alcian blue procedure, then start with the periodic acid step in the PAS procedure. With the PAS counterstain, carboxylated and sulfated acid mucopolysaccharides are stained blue; polysaccharides, neutral mucopolysaccharides, mucoproteins, glycoproteins, and mucolipds are stained pink; and mixtures of both neutral and acid mucopolysaccharides are stained purple.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. **Alcian Blue**
No hazards listed

B. **Acetic Acid**
Add drop by drop to solutions. May cause skin and eye burns.

C. **Nuclear Fast Red**
Material Safety Data (MSD) sheet not available

D. **Aluminum Sulfate**
No hazards listed

E. **Thymol**
May cause eye burns. Is irritating to skin and respiratory tract. Is a suspected carcinogen.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate through graded alcohols to distilled water		
2	Place in alcian blue	**30 minutes**	
3	Wash in running tap water	**10 minutes**	
4	Rinse in distilled water	**10 seconds**	
5	Counterstain in nuclear fast red	**1-5 minutes**	
6	Rinse in running tap water	**10 seconds**	
7	Dehydrate through graded alcohols, clear in xylene		
8	Coverslip		

X. RESULTS:

A. **Acid mucopolysaccharides- blue**
(intestinal goblet cells, umbilical cord, cartilage, cornea, dermis, mast cell granules, calcium salts, some fungi)

B. **Nuclei- pink**

XI. REFERENCES:

1. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. New York, NY, Churchill Livingstone, 1990.
2. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.
3. Carson FL: Histotechnology: A Self-Instructional Text. ASCP Press. 1990.
4. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology - 3rd edition, Luna LG editor, New York, McGraw-Hill Book Co., 1968

Approval Signatures

Step Description	Approver	Date
Medical Director	Kurt Bernacki: System Med Dir, Surgical Path	10/12/2022

Policy and Forms Steering Committee (if needed)	Gail Juleff: Project Mgr Policy	10/12/2022
Policy and Forms Steering Committee (if needed)	Sharon Scalise: Supv, Laboratory	10/11/2022
	Amy Knaus: Dir, Lab Operations C	10/11/2022
	Jennifer Lehmann: Mgr Laboratory	10/11/2022
	Sharon Scalise: Supv, Laboratory	10/11/2022

Applicability

Royal Oak