Status **Active** PolicyStat ID **12853685**

Beaumont

Origination	1/5/2023
Last Approved	1/5/2023
Effective	1/5/2023
Last Revised	1/5/2023
Next Review	1/4/2025

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

Histology Special Stain - Toluidine Blue - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of mast cells in tissue sections. Mast cells are found in areas of inflammation.

II. PRINCIPLE:

This procedure is a metachromatic stain. Toluidine blue is a pure dye. When each dye molecule is separate in solution, it is called a monomer. When several dye molecules join together, they form larger dye compounds called dimers, trimers, and polymers. The larger dye compounds will exhibit a different color than the monomers. Mast cells contain cells granules composed of histamine and heparin. These granules will stain with the larger dye molecules, giving a metachromatic purple color. The background (nuclei) will stain with the smaller dye molecules, giving an orthochromatic blue color. Eosin is used as the counterstain.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue.

B. Processing
    1. Standard, overnight processing.

C. Section Thickness
    1. Cut routine paraffin sections at 5µ.

D. Slide Drying
    1. 30 minutes at 60°C.

E. Type of Slide
    1. Plain slides.

IV. REAGENTS:

A. **Toluidine Blue-Acid**

Toluidine Blue	**0.05 g**
Distilled Water	**99.00 mL**

Dissolve together. Adjust to pH 4.0 with hydrochloric acid. Store at room temperature; stable for months. May be reused until weak.

B. **Eosin Y Solution**
Use the eosin Y solution with acetic acid found in the H&E set-up.

V. EQUIPMENT:

A. Balance
B. 60°C oven
C. Magnetic stirrer

VI. SUPPLIES:

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

VII. SPECIAL SAFETY PRECAUTIONS:

A. Toluidine Blue
    1. This chemical is not considered hazardous.

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

C. Eosin Y
    1. Is a possible irritant.

D. Acetic Acid
    1. Is an acid.
    2. Add drop by drop to solution.

3. May cause skin or eye burns.

VIII. QUALITY CONTROL(QC):

Use a section of tissue with mast cells as a control.

IX. LIMITATIONS:

A. pH of the solution must be at 4.0.

B. Metachromasia of the mast cells is stable so the sections can be dehydrated through alcohols after staining.

C. Use a very pale eosin counterstain, so as not to mask the mast cells.

X. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate sections through graded alcohol to distilled water.		
2	Place in Toluidine Blue solution.	1 hour	
3	Rinse in 2-3 changes of distilled water.	10 seconds each	
4	Rinse in 70% alcohol.	10 seconds	
5	Counterstain very lightly in eosin Y.	1-2 seconds	
6	Dehydrate in absolute alcohol, 2 changes each.	5-10 seconds	
7	Clear in xylene, 2 changes.	10 seconds each	
8	Coverslip.		

XI. RESULTS:

A. Mast Cell Granules - **blue-purple**

B. Nuclei - **pale blue or unstained**

C. Background - **pink**

XII. REFERENCES:

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

B. Vacca LL: Laboratory Manual of Histochemistry. New York, NY, Raven Press, 1985

Approval Signatures

Step Description	Approver	Date
Medical Director	Kurt Bernacki: System Med Dir, Surgical Path	1/5/2023
Policy and Forms Steering Committee (if needed)	Gail Juleff: Project Mgr Policy	1/5/2023
Policy and Forms Steering Committee (if needed)	Sharon Scalise: Supv, Laboratory	1/4/2023
	Amy Knaus: Dir, Lab Operations C	1/4/2023
	Jennifer Lehmann: Mgr Laboratory	1/3/2023
	Sharon Scalise: Supv, Laboratory	12/19/2022

Applicability

Royal Oak