

Beaumont

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Applicability Royal Oak

Histology Special Stain-Basic Blue 41 - 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 or in a skin disease known as urticaria pigmentosa.

II. PRINCIPLE:

Mast cells contain cells granules composed of histamine and heparin. The heparin contains sulfonated acid mucopolysaccharides, to which the Basic blue 41 binds. Nuclear fast red is used as the counterstain.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation

1. Any well-fixed tissue.
2. 10% neutral buffered formalin preferred.

B. Processing

1. Standard processing.

C. Section Thickness

1. Routine specimens- 5μ.

D. Slide Drying

1. 60 minutes at 60°C.

- E. Type of slide
 - 1. Plain slides

IV. REAGENTS:

A. 0.5% Basic Blue Solution

Basic Blue 41	0.5 gm
Distilled water	100.0 mL

Dissolve together. Store at room temperature. Stable for 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 several 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. Filter. Add a few crystals of thymol. Stable at room temperature for months. May be reused until weak.

V. EQUIPMENT:

- A. Balance
- B. Magnetic stirrer

VI. SUPPLIES:

- A. Erlenmeyer flasks
- B. Graduated cylinders
- C. Acid clean coplin jars
- D. Forceps

VII. QUALITY CONTROL:

Use a section containing mast cells as a positive control.

VIII. SPECIAL SAFETY PRECAUTIONS:

- A. Basic Blue 41
 - 1. Is an irritant.
- B. Nuclear Fast Red
 - 1. Is an irritant.
- C. Aluminum Sulfate
 - 1. Is an irritant and a corrosive.

D. Thymol

1. May cause eye burns.
2. Is irritating to skin and respiratory tract.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Place in 0.5% Basic Blue 41 solution.	5-10 minutes	
3	Wash in running tap water.	3 minutes	DO NOT extend time at this step.
4	Rinse in distilled water, 2-3 changes.	5 seconds each	
5	Counterstain in Nuclear Fast Red.	1-5 minutes	
6	Rinse slides in tap water, 2-3 changes.	5 seconds	
7	Dehydrate through graded alcohols.		
8	Clear in two changes of xylene.		
9	Coverslip.		

X. LIMITATIONS:

- A. Dye is soluble in alcohol and may be removed from the sections through the alcohol rinses. If dye is removed, re-hydrate slides and re-stain in the 0.5% Basic Blue 41 Solution.
- B. After staining in Nuclear Fast Red (NFR), always rinse with tap water to remove excess aluminum sulfate. Aluminum sulfate salts do not dissolve in alcohols and may leave white streaks on the slides if they are taken from NFR directly to alcohol.

XI. RESULTS:

- A. Mast cell granules - **Dark blue to purple**

XII. REFERENCES:

- A. Aldrich Handbook of Fine Chemicals and Laboratory Equipment. 2000-2001.
- B. Carson FL: Histotechnology: A Self-Instructional Text, 2nd Edition. Chicago: ASCP Press, 1996.
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- D. Majno G and Joris E: Cells, Tissue, and Disease Principles of General Pathology, Massachusetts: Blackwell Science, 1994.

- E. Pearse AGE: Histochemistry: Theoretical and Applied, Vol. 1, 3rd Edition. Baltimore, Williams and Wilkins, 1968.

Approval Signatures

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Royal Oak