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Beaumont

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Histology Special Stain - Thioflavin T - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of amyloid and/or juxtaglomerular granules in tissue sections.

II. PRINCIPLE:

Thioflavin T is a fluorochrome dye that attaches to the P component of amyloid. Hematoxylin is used to quench nuclear fluorescence.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue.
    2. 10% NBF preferred.

B. Processing
    1. Standard processing.

C. Section Thickness
    1. Routine specimens-7-8µm.

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

E. Type of slide

1. Plain

IV. REAGENTS:

A. **Hematoxylin**
Use the hematoxylin from the H&E set up.

B. **1% Thioflavin T Solution**

Thioflavin T	**1.0 gm**
Distilled water	**100.0 mL**

Dissolve together. Store in refrigerator (3^{o} C.) Stable for several months. May be reused until weak. Let stabilize to room temperature before use

C. **1% Acetic Acid Solution**

Acetic acid, concentrated	**1.0 mL**
Distilled Water	**99.0 mL**

Just before use, mix together.

D. **Modified Apathy Mounting Medium**

Acacia (gum Arabic)	**50.0 gm**
Cane sugar	**50.0 gm**
Distilled water	**100.0 mL**
Sodium chloride	**10.0 gm**
Thymol	**0.1 gm**

Bring water to a boil. Slowly add acacia and cane sugar, stirring until both are dissolved. Restore the volume with distilled water. Add and dissolve the sodium chloride. Add the thymol. Store in a refrigerator (3°C). Stable for months.

V. EQUIPMENT:

A. Balance
B. Magnetic stirrer
C. Fluorescence microscope

VI. SUPPLIES:

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

VII. QUALITY CONTROL:

A. Use two control slides and two unknown slides
B. One of each will be stained in the Thioflavin T while the other set is unstained.
C. The unstained set, when viewed with the fluorescence microscope, will demonstrate those

components that are auto-fluorescing.

VIII. SPECIAL SAFETY PRECAUTIONS:

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

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

C. Gum Arabic (Acacia)
    1. May cause skin and eye irritation.

D. Thymol
    1. May cause skin and eye burns.
    2. May be irritating to respiratory tract.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Stain in hematoxylin.	30 seconds	
3	Rinse in distilled water.	5 minutes	
4	Place one control and one patient slide in one coplin jar with distilled water.		
5	Place second control and patient slide into another coplin jar with distilled water.		
6	Stain first coplin jar in Thioflavin T.	8 minutes	
7	Rinse in distilled water, 2 changes.	5 seconds each	
8	Wash in running water.	5 minutes	
9	Mount with Apathy mounting medium.		Any non-fluorescing mounting media may be used.
10	Seal edges of coverslip with clear fingernail		

	polish.		

X. RESULTS:

A. With a fluorescence microscope:
    1. Amyloid - **yellow**
    2. Juxtaglomerular granules - **yellow**
    3. Mast cell granules - **yellow**
    4. Keratin, collagen, fibrinoid - **yellow**
    5. Intestinal muciphages - **yellow**
    6. Paneth cells - **yellow**
    7. Zymogen granules - **yellow**
    8. Background - **black**

XI. LIMITATIONS:

A. This stain is a very quick procedure and shows minimal deposits of amyloid.

XII. REFERENCES:

A. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd edition. New York, NY, Churchill Livingstone, 1990.

B. Carson FL: Histotechnology: A Self-Instructional Text. ASCP Press. 1990.

C. Sheehan Dc, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, OH, Batelle Press, 1980.

D. Janigan DT: Fluorochrome staining of juxtaglomerular cell granules. Arch Pathol Lab Med. 79: 370, April 1965.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak