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Histology Special Stain - Acetylcholinesterase - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of nerve fibers and ganglia. The study is indicated in situations dealing with intestinal neuronal dysplasia, intestinal pseudo-obstruction syndrome, and cases atypical for Hirschsprung's Disease.

II. PRINCIPLE:

The acetylthiocholine iodine reacts with the acetylcholinesterase to form thiocholine iodine. This reacts with the copper sulfate to form copper thiocholine iodine. The ethopropazine inhibits pseudocholinesterase. The DAB precipitates to a brown dye to indicate the site of enzyme activity.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Fresh tissue.

B. Processing
    1. None.

C. Section Thickness
    1. Cut frozen sections at 10µ placing 2 sections per slide.

D. Slide Drying
    1. None.

E. Type of Slide

1. Plus slides.

IV. PROCEDURE FOR SNAP FREEZING SPECIMENS:

A. Obtain rectal biopsy specimen from the Pathologists' Assistant (PA) in Surgical Pathology.

B. Place the rectal biopsy specimen, on edge, directly onto the freezing well bar and fill well bar with OCT.

C. Place a chuck on top of the OCT (ensure the OCT is filling the well enough to reach the chuck so it can freeze together).

D. Let the OCT freeze to harden, then remove the chuck with the specimen from the well bar.

E. Place the chuck/specimen into the cryostat and let it come to cryostat temperature; about 30 minutes.

F. Face into the block until enough tissue is reached to check for orientation; the first slide should consist of a good cross-section of tissue.

G. Do a quick H&E stain by hand on the first slide and check orientation under the microscope.

H. Cut 10 serial frozen sections slides with 2 sections per slide.

I. Label slides 1 through 10.

J. Stain the slides labeled 1, 3, 5, and 7 with H&E stain.

K. Show stained sections to pathologist; the pathologist will choose 2 of the remaining slides to perform the ACE procedure on.

L. Any additional slides are kept in the freezer as extras.

V. REAGENTS:

A. **The following are stock solutions, stable for months:**

1. **4% Formaldehyde with 0.1 M Calcium Acetate**

Calcium Acetate	**15.8 gm**
40% formaldehyde	**40.0 ml**
Distilled water	**960.0 ml**

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

2. **Solution 1 stock – 0.2 M Sodium Phosphate Dibasic**

Sodium Phosphate Dibasic	**28.39 gm**
Distilled Water	**1000.0 ml**

Dissolve together. Store in refrigerator; stable for months.

3. **Solution 2 stock– 0.2 M Sodium Phosphate Monobasic**

Sodium phosphate monobasic	**27.6 gm**
Distilled Water	**1000.0 ml**

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

4. **0.1 M Sodium Citrate**

Sodium Citrate	**2.94 gm**

Distilled water **100.0 ml**

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

5. **0.03 M Copper Sulfate**

Copper Sulfate **0.75 gm**
Distilled water **100.0 ml**

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

B. **The following solutions must be made fresh:**

1. **0.1 M Phosphate Buffer Ph 6.0**

Solution 1 stock **3.6 ml**
Solution 2 stock **26.4 ml**
Distilled water **30.0 ml**

Make fresh each time. Can be adjusted to the proper pH with acetic acid or HCL.

2. **0.005M Potassium Ferricyanide**

Potassium Ferricyanide **0.0334 gm**
Distilled water **20.0 ml**

Must be made fresh each time.

3. **0.004 M Ethopropazine (Stored in freezer)**

Ethopropazine **0.014 gm**
Distilled water **10.0 mL**

Must be made fresh each time.

4. **Incubating Substrate:**

A – Acetylthiocholine iodide **0.025 gm**
B – 0.1 M Phosphate Buffer, pH 6.0 **32.5 ml**
C – 0.1 M Sodium Citrate **2.5 ml**
D – 0.03 M Copper Sulfate **5.0 ml**
E – 0.005 M Potassium Ferricyanide **5.0 ml**
F – 0.004 M Ethopropazine **1.0 ml**

Add each ingredient in order as above, mixing well after each.

5. **0.01M Sodium Phosphate Dibasic (Solution A)**

0.2 M Sodium phosphate dibasic (Solution 1 stock) **5.0 ml**
Distilled water **95.0 ml**

Made fresh each time.

6. **0.01M Sodium Phosphate Monobasic (Solution B)**

0.2 M Sodium phosphate monobasic (Solution 2 stock) **5.0 ml**
Distilled water **95.0 ml**

Made fresh each time.

7. **0.005 Phosphate Buffer (for DAB)**

0.01 M Sodium Phosphate Dibasic (Solution A above) **49.0ml**
0.01M Sodium Phosphate Monobasic (Solution B above) **51.0 ml**
Distilled water **100.0 ml**

Must be made fresh each time.

8. **0.05% DAB Phosphate Buffer**

DAB	**0.05 gm (use powder only)**
0.005 M Phosphate buffer	**100.00 mL**

Must be made fresh just before use. Will have brown color; if dark brown, discard. **DAB must be discarded according to the procedure "Disposal of Diaminobenzadine (DAB) Waste", found in the histology procedure manual (pour into "DAB waste" container in cabinet below muscle staining counter; when container is full follow above procedure.)**

9. **Hematoxylin**
   Use Hematoxylin from H&E set-up.

VI. EQUIPMENT:

A. Mettler balance
B. Magnetic stirrer
C. Cryostat

VII. SUPPLIES:

A. Erlenmeyer flasks
B. Graduated cylinders
C. Coplin jars
D. Weigh boats
E. Beakers
F. Forceps
G. OCT
H. Well-bars
I. Chucks

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Formaldehyde
   1. Causes skin and eye burns.
   2. May cause allergic respiratory reaction.
   3. Possible carcinogen.
   4. Vapor irritating.

B. Calcium Acetate
   1. This chemical is not considered hazardous.

C. Sodium Phosphate Dibasic
   1. This chemical is not considered hazardous.

D. Sodium Phosphate Monobasic
    1. This chemical is not considered hazardous.

E. Sodium Citrate
    1. This chemical is not considered hazardous.

F. Cupric Sulfate
    1. Causes skin and eye irritation.

G. Potassium Ferricyanide
    1. This chemical is not considered hazardous.

H. Ethopropazine
    1. Harmful if inhaled or swallowed.
    2. Attacks Central Nervous System.

I. Acetylthiocholine Iodide
    1. Harmful if inhaled, absorbed through skin, or swallowed.

J. Diaminobenzidine Tetrahydrochloride (DAB)
    1. Harmful if inhaled or swallowed.
    2. Dispose of DAB following procedure in the Histology Manual.

IX. QUALITY CONTROL (QC):

Frozen section of normal rectum

X. LIMITATIONS:

A. The rectal mucosa is to be oriented perpendicular (on edge) when frozen.

B. Tissue can be surrounded with OCT compound to enhance sectioning.

C. Increased positive staining may be seen in Hirschsprung's Disease and Intestinal Neuronal Dysplasia.

XI. PROCEDURE:

Step	Action	Time	Notes
1	Follow above frozen section procedure.		
2	Fix ACE slides in 4% formaldehyde/ 0.1M calcium acetate.	30 seconds	
3	Wash in distilled water.	10 seconds	
4	Prepare incubating substrate just		

	before use.		
5	Incubate slides at room temperature.	1.5 hours	
6	Wash briefly in distilled water.		
7	Prepare DAB/Phosphate Buffer just before use.		
8	Incubate slides in DAB/Buffer.	1.5 hours	Check microscopically until acceptable staining results are achieved.
9	Rinse in tap water.		
10	Counterstain in Hematoxylin.	30 seconds	
11	Wash in running water to blue.	5 minutes	
12	Dehydrate through graded alcohols and clear in xylene.		
13	Coverslip using a synthetic mounting media.		

XII. RESULTS:

A. Nerve fiber and ganglia - **rose-brown**

B. Nuclei - **blue**

XIII. REFERENCES:

A. Acetylcholinesterase Histochemistry and the Diagnosis of Hirschsprung's Disease: A3 and ½ year experience. Paul E. Wakely Jr., James McAdams. Pediatr Pathol 2: 35-46, 1984.

B. Intestinal Aganglionosis; A Histologic and Acetylcholinesterase Histochemical Study. Chen-Chih J. Sun, Donna A. Caniano, J. Laurance Hill. Pediatr Pathol 7: 421-435, 1987.

C. Neuronal Intestinal dysplasia, alois, Scharli. Pediatr Surg Int (1992) 7: 2-7.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak