Status Active PolicyStat ID 12589780

Beaumont

Origination	11/2/2022
Last Approved	11/2/2022
Effective	11/2/2022
Last Revised	11/2/2022
Next Review	11/1/2024

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

Histology Special Stain - Bodian - 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, nerve endings, and neurofibrils, which are aggregates of microtubules and neurofilaments found in the cell body, axon and dendrites of nerve cells.

II. PRINCIPLE:

The reaction is an argyrophilic silver stain. Protargol, a silver proteinate compound, impregnates the tissue sections. Copper, which is added to the incubating solution, is more reactive than the silver, and will remove the silver from the connective tissue. This allows for a greater differentiation between the neural fibers and the connective tissue. Hydroquinone and formaldehyde reduce the silver salts to visible metallic silver. Gold chloride tones the section. Oxalic acid is used to reduce the gold. This gives a darker stain, as the metallic gold is also deposited onto the tissue. Sodium thiosulfate removes excess unreduced silver.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
   1. Any well-fixed tissue.
   2. 10% neutral buffered formalin preferred.
   3. Avoid mercuric fixatives

B. Processing
   1. Standard processing.

C. Section Thickness

1. Routine specimens-8-10µm.

D. Slide Drying

1. 60 minutes at 60°C.

E. Type of slide

1. Plain slides

IV. REAGENTS:

A. **Protargol (silver proteinate) solution**

Protargol (silver proteinate) 0.5 gm
Distilled water 50.0 mL

1. Place the distilled water in a 50 mL beaker.
2. Sprinkle Protargol on the surface of the water.
3. Do NOT shake or stir.
4. Place in 37°C. oven for about 30 minutes, until it is dissolved.
5. Make solution just before use.
6. Discard after use.

B. **Reducing solution**

Hydroquinone 1.0 gm
Distilled Water 50.0 mL
Formaldehyde, 37-40% 2.5 mL

1. JUST BEFORE USE, dissolve together hydroquinone in distilled water.
2. Add formaldehyde.
3. Discard after use.

C. **1% gold chloride**

Gold chloride 1.0 gm
Distilled water 100.0 mL

Dissolve together. Store at room temperature. Stable for months. May be reused. Filter when necessary.

D. **2% oxalic acid**

Oxalic acid 2.0 gm
Distilled water 100.0 mL

Dissolve together. Store at room temperature. Stable for months.

E. **5% sodium thiosulfate**

Sodium thiosulfate 5.0 gm
Distilled water 100.0 mL

Dissolve together. Store at room temperature. Stable for months.

V. EQUIPMENT:

A. Balance

B. 37°C oven

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Acid clean coplin jars

D. Non-metal forceps

VII. QUALITY CONTROL:

A. Use a section of brain medulla or peripheral nerve as a positive control.

B. If copper shots appear "rusty", clean them by placing in a solution of aqua regia (15 mL hydrochloric acid, concentrated, and 5 mL nitric acid, concentrated). Use gloves and apron when preparing or handling this solution. Prepare and use under hood. Wash copper shots in running water, and then distilled water, before using in protargol solution.

C. Leave the protargol to dissolve from the surface downward. Do not disturb until dissolved. Do not allow to coagulate.

D. Nuclear fast red may be used as a counterstain. Nuclei will be stained pink.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Silver proteinate (protargol)

1. Is an irritant.
2. Is possibly toxic.

B. Hydroquinone

1. Is an irritant to eyes, skin and respiratory system.

C. Formaldehyde

1. Is a poison.
2. May be fatal or cause blindness if swallowed.
3. Cannot be made non-poisonous.
4. Possible cancer hazard.
5. Irritating to eyes, skin and respiratory tract.
6. Can cause severe eye burns.

D. Gold chloride

1. Is an irritant to eyes and skin.

E. Oxalic acid

1. Is a strong reducing agent.
2. Contact with other material may cause fire.
3. May cause skin and eye burns.
4. Irritating to respiratory system.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Place 7g copper shot into an acid-clean coplin jar.		
3	Pour protargol solution over copper shot.		
4	Place slides into protargol solution, incubate in 37°C oven.	24-72 hours	
5	Rinse in 3 changes of distilled water.	5 seconds each	
6	Place slides in reducing solution.	10 minutes	
7	Rinse in 3 changes of distilled water	5-10 seconds each	
8	Develop in 2% oxalic acid until background is grey and nerve fibers appear clearly black.	3-5 minutes	**Use microscope to check**
9	Rinse in 3 changes of distilled water.	5-10 seconds each	
10	Place slides in 5% sodium thiosulfate.	5 minutes	
11	Wash in distilled water.	10 minutes	
12	Dehydrate through graded alcohols.		
13	Clear in two changes of xylene.		
14	Coverslip.		

X. LIMITATIONS:

A. Prolonged treatment in oxalic acid will destroy the protargol reaction.

B. Use acid-cleaned coplin jars and non-metal forceps, or a dirty background may appear.

XI. RESULTS:

A. Nerve fibers - **black**

B. Connective tissue - **gray to black**

C. Background - **gray**

XII. REFERENCES:

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

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

C. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

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

Approval Signatures

Step Description	Approver	Date
Medical Director	Kurt Bernacki: System Med Dir, Surgical Path	11/2/2022
Policy and Forms Steering Committee (if needed)	Sharon Scalise: Supv, Laboratory	11/2/2022
Policy and Forms Steering Committee (if needed)	Gail Juleff: Project Mgr Policy	11/2/2022
	Amy Knaus: Dir, Lab Operations C	11/2/2022
	Jennifer Lehmann: Mgr Laboratory	11/2/2022
	Sharon Scalise: Supv, Laboratory	11/1/2022

Applicability

Royal Oak