Status **Active** PolicyStat ID **12427259**

Beaumont

Origination	10/11/2022
Last Approved	10/11/2022
Effective	10/11/2022
Last Revised	10/11/2022
Next Review	10/10/2024

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

Histology Special Stain- Bielschowsky-Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of axons to determine whether a small round cell tumor is a neuroblastoma. It also distinguishes multi-sclerosis plaques which contain neurons and axons from an infarct which lacks these structures. The Bielschowsky stain also demonstrates neurofibrillary tangles and senile plaques associated with Alzheimer's disease and senile dementia.

II. PRINCIPLE:

Sections are first impregnated with silver nitrate followed by an ammoniacal silver treatment. The silver is deposited on the neurofibrils, axons, and senile plaques and reduced to visible metallic silver by the action of the formalin reducing agent. Then sodium thiosulfate is used to remove the unreacted silver from the tissue and stop the silver impregnation.

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-8-10um.

D. **Slide Drying**

1. 60 minutes at 60^0 C.

E. **Type of slide**

1. Charged (+) slides

IV. REAGENTS:

A. **20% Silver Nitrate**

Silver nitrate	**10.0 gm**
Distilled water	**50.0 ml**

Dissolve together.

Must be made fresh each time. Warm to 37^oC just before use.

B. **Developer**

37-40% Formaldehyde	**20.0 ml**
Distilled Water	**100.0 ml**
Nitric Acid, concentrated	**1 drop**
Citric Acid	**0.5 gm**

Mix together always adding acid to water.

C. **Ammonium Hydroxide (concentrated)**

D. **0.5% Gold Chloride**

Gold Chloride	**0.5 gm**
Distilled Water	**100.0 ml**

Dissolve together. Store in brown bottle in fridge. Re-use until weak.

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° Oven

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Acid clean coplin jars

D. Non-metal forceps

VII. QUALITY CONTROL (QC):

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

B. Silver solutions which contain ammonia and silver nitrate only are more easily controlled. In these solutions, after the precipitate has been dissolved, a drop or two of silver nitrate can be added to absorb any excess ammonia present.

C. It is important to check slides microscopically to ensure the proper development of fibers. To do this, remove slides individually and dip in 0.1% ammonium hydroxide. This solution stops silver development. If fibers are not dark enough, return slides to the silver solution.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. **Silver nitrate**
Is harmful if inhaled.

B. **Formaldehyde**
Is a poison. May be fatal or cause blindness if swallowed. Cannot be made non-poisonous. Possible cancer hazard. Irritating to eyes, skin and respiratory tract. Can cause severe eye burns.

C. **Citric acid**
May cause skin and eye burns. Irritating to respiratory tract.

D. **Nitric acid**
May cause skin and eye burns. Irritating to respiratory system.

E. **Sodium thiosulfate**
Is an irritant.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Place in 20% silver nitrate for in 37^{o} oven (save this solution)	**30 minutes**	
3	Wash in 2 changes of distilled water (more is better)		
4	Add ammonium hydroxide drop by drop to the silver nitrate solution, stirring until the precipitate which forms disappears		
5	Filter solution		
6	Place slides in silver nitrate solution in 37o oven	**30 minutes**	
7	Wash in 2 changes of distilled water (more is better)		
8	Add 3-4 drops of the developer to the silver solution, mix well. Place slides in this solution until the sections turn black with a golden brown background		
9	Wash in 2 changes of distilled water		

10	Dip slides in 2% sodium thiosulfate	**1-2 dips**	
11	Wash in distilled water		
12	Dehydrate through graded alcohols		
13	Clear in two changes of xylene		
14	Coverslip		

X. RESULTS:

A. Neurofibrils- **black**
B. Senile plaques- **black**
C. Axons, dendrites- **black**
D. Other structures- **brown-tan**
E. Background- **purple-gray**

XI. LIMITATIONS:

A. Use acid-cleaned coplin jars and non-metal forceps or a dirty background may appear.
B. The more developer used, the quicker the development, and the darker the background staining.
C. In addition, there is greater chance of precipitate formation when larger quantities of developer are used.

XII. REFERENCES:

1. Vacca, Linda L., Laboratory Manual of Histochemistry, 1985, pages 385-386.

Approval Signatures

Step Description	Approver	Date
Medical Director	Kurt Bernacki: System Med Dir, Surgical Path	10/11/2022
Policy and Forms Steering Committee (if needed)	Sharon Scalise: Supv, Laboratory	10/11/2022
Policy and Forms Steering Committee (if needed)	Gail Juleff: Project Mgr Policy	10/4/2022
	Amy Knaus: Dir, Lab Operations C	10/3/2022

Jennifer Lehmann: Mgr Laboratory	9/30/2022
Sharon Scalise: Supv, Laboratory	9/26/2022

Applicability

Royal Oak