Status **Active** PolicyStat ID **12853530**

Beaumont

Origination	1/5/2023
Last Approved	1/5/2023
Effective	1/5/2023
Last Revised	1/5/2023
Next Review	1/4/2025

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

Histology Special Stain - Modified Churukian-Schenk - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of argyrophilic granules in carcinoid tumors and neurosecretory cells and tumors. All argentaffin granules will also be stained by this manner.

II. PRINCIPLE:

Tissue components that are argyrophilic will bind silver ions from a silver nitrate solution. To reduce the silver ions to black metallic silver, a separate reducing solution must be used. Silver nitrate is the source of silver ions. Sodium sulfite and hydroquinone reduce the silver ions to a black metallic precipitate. Nuclear fast red is the counterstain.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. 10% neutral buffered formalin preferred.
    2. Avoid the use of mercuric fixatives

B. Processing
    1. Standard, overnight processing.

C. Section Thickness
    1. Cut paraffin sections at 5µ.

D. Slide Drying

1. 30 minutes at 60°C.

E. Type of Slide

1. Plain slides

IV. REAGENTS:

A. **Stock 10% Silver Nitrate**

Silver nitrate	**10.0 gm**
Distilled water	**100.0 mL**

In an acid-cleaned flask, dissolve together with the aid of a magnetic stirrer. Store in a brown glass bottle in the refrigerator(3°C); stable for 4-6 months. Discard if precipitate forms, or if solution is no longer clear.

B. **Working 1% Silver Nitrate Solution**

STOCK 10% silver nitrate	**4.0 mL**
Distilled water	**36.0 mL**

Mix together. May be made fresh, just before use, or may be stored in refrigerator (3°C) in brown glass bottle; stable for 2-4 months.

C. **Reducing Solution**

Sodium sulfite	**5.0 gm**
Hydroquinone	**1.0 gm**
Distilled water	**100.0 mL**

JUST BEFORE USE, dissolve together completely.

D. **5% Aluminum Sulfate**

Aluminum sulfate	**25.0 gm**
Distilled water	**500.0 mL**

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

E. **Nuclear Fast Red**

Nuclear fast red	**0.1 gm**
5% aluminum sulfate	**100.0 mL**

Dissolve together will the aid of gentle heat. Cool. Filter. Add a few crystals of thymol. Store at room temperature or in refrigerator (3°C.); stable for months.

V. EQUIPMENT:

A. Balance

B. 60°C oven, water bath or microwave oven

C. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Acid clean coplin jars

D. Non-metal forceps

VII. SPECIAL SAFETY PRECAUTIONS:

A. Silver Nitrate

1. Is an oxidizer.
2. Store separately from other material.
3. Is poisonous and may be fatal if swallowed.
4. Causes skin and eye burns.
5. Is an irritant to the respiratory system.

B. Sodium Sulfite

1. Has low hazard for recommended handling.

C. Hydroquinone

1. Is irritating to eye, skin, and respiratory tract.
2. May cause allergic skin reaction.
3. Harmful if swallowed.

D. Aluminum Sulfate

1. Is a corrosive.
2. May cause serious eye damage.

E. Nuclear Fast Red

1. Corrosive to skin.
2. Irritant to eyes.
3. Toxic to respiratory system.

F. Thymol

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

VIII. QUALITY CONTROL(QC):

Section of tissue with argyrophilic carcinoid tumor or pancreas with islet cells.

IX. LIMITATIONS:

A. Use acid-clean glassware, to avoid silver precipitate.

B. Use non-metal forceps, or a silver precipitate may be formed on the slides and tissue.

C. Use distilled water. Any metals in tap water may cause silver precipitate to be formed on the

slides and the tissue.

D. To differentiate between argyrophil and argentaffin substances, a duplicate slide should be stained with the Fontana-Masson procedure, and the results compared.

E. If nuclear fast red is used as a counterstain, wash slides with water after staining. Aluminum sulfate does not dissolve in alcohol. Placing slides directly from nuclear fast red into alcohol ill result in a white precipitate forming on the slides, which can be removed by returning the slides to water.

X. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate sections through graded alcohol to distilled water.		
2	Place slides WORKING 1% silver nitrate solution.	1-2 hours	Incubate in 60°C oven. Or microwave on high for 45 seconds and allow to sit on counter for 1 minute. Save hot silver solution.
3	Rinse in distilled water, 3 changes.	5-10 seconds each	
4	Place slides in previously heated (60°C.) reducing solution.	5 minutes	
5	Wash in running tap water.	5 minutes	
6	Rinse in distilled water, 2-3 changes.	5-10 seconds	
7	Return slides to same coplin jar of 1% silver solution in 60°C oven.	10 minutes	Or heat slides in same 1% silver solution in microwave on high for 30 seconds.
8	Rinse slides in distilled water, 2-3 changes.	5-10 seconds	
9	Return slides to same coplin jar of reducing solution in 60°C oven.	1-2 hours	Or heat same reducing solution in microwave oven on high for 15 seconds. Return slides to heated solution for 1-2 minutes.
10	Rinse in distilled water, 2-3 changes each.	5-10 seconds	
11	Counterstain in nuclear fast red.	1-5 minutes	
12	Rinse in distilled water, 2 changes each.	5-10 seconds	
13	Dehydrate through graded	10	

	alcohols, clear with xylene.	seconds each	
14	Coverslip.		

XI. RESULTS:

A. Argyrophilic granules (carcinoid tumors, neurosecretory granules) - **black**

B. All argentaffin granules (melanin, argentaffin cells) - **black**

C. Nuclei - **red**

D. Background - **pale pink**

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. Microwave Ovens for Metallic Histologic Staining: A New Concept. Paper presented at National Society of Histotechnology Symposium. Modified by William Beaumont Hospital, Royal Oak, MI.

Approval Signatures

Step Description	Approver	Date
Medical Director	Kurt Bernacki: System Med Dir, Surgical Path	1/5/2023
Policy and Forms Steering Committee (if needed)	Gail Juleff: Project Mgr Policy	1/5/2023
Policy and Forms Steering Committee (if needed)	Sharon Scalise: Supv, Laboratory	1/4/2023
	Amy Knaus: Dir, Lab Operations C	1/4/2023
	Jennifer Lehmann: Mgr Laboratory	1/3/2023
	Sharon Scalise: Supv, Laboratory	12/19/2022

Applicability

Royal Oak