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Beaumont

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Histology Special Stain - Fontana-Masson - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of argentaffin substances, such as melanin, argentaffin cells of the intestine, and argentaffin granules of carcinoid tumors. It will also demonstrate lipofuchsin, chromaffin cells, formalin pigment, iron and some neurosecretory granules.

II. PRINCIPLE:

Tissue components that are argentaffin will bind silver ions from a silver nitrate solution, and then will reduce the silver ions to visible metallic silver without the use of an separate reducing solution. Silver nitrate is the source of silver ions. Ammonium hydroxide (NH_4OH) increases the pH to between 11 and 12, and creates a diamine silver complex $Ag(NH_3)_2^+$. Gold chloride tones the section. Sodium thiosulfate removes unreduced silver. Nuclear fast red is the counterstain.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue. (B-5 should not be used)

B. Processing
    1. Standard, overnight processing.

C. Section Thickness
    1. Routine specimens 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. **Stock Fontana-Masson Silver Solution**

Stock 10% silver nitrate	**95.0 mL**
Ammonium hydroxide (concentrated)	

UNDER THE HOOD: While swirling, to the 95 mL of Stock 10% silver nitrate, add ammonium hydroxide, drop by drop. A
precipitate will form. Continue to add ammonium hydroxide, until solution becomes clear.

C. **Stock 1% Gold Chloride**

Gold chloride	**1.0 gm**
Distilled water	**100.0 mL**

Dissolve together. Store at room temperature; stable of months to a year.

D. **Working 0.2% Gold Chloride**

Stock 1% gold chloride	**10.0 mL**
Distilled water	**30.0 mL**

Mix together. Store at room temperature; may be reused until weak. Filter solution if black precipitate appears.

E. **5% Sodium Thiosulfate**

Sodium thiosulfate	**5.0 gm**
Distilled water	**100.0 mL**

Dissolve together. Store at room temperature; stable for 6 months.

F. **5% Aluminum Sulfate**

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

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

G. **Nuclear Fast Red**

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

Dissolve together with 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
C. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks
B. Graduated cylinders
C. Acid-clean coplin jars
D. Non-metal forceps
E. Filter paper

VII. QUALITY CONTROL (QC):

Section of tissue with Cryptococcus.

VIII. 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. It is an irritant to the respiratory system.

B. Ammonium Hydroxide
    1. May cause severe skin and eye burns.
    2. Vapors are irritating to eyes and respiratory tract.
    3. Harmful if swallowed or inhaled.

C. Gold Chloride
    1. May cause skin and eye irritation.

D. Sodium Thiosulfate
    1. Is an irritant.

E. Aluminum Sulfate
    1. Has low hazard for recommended handling.

F. Nuclear Fast Red

1. Causes skin irritation.
2. Causes serious eye irritation.
3. May cause respiratory irritation.

G. Thymol

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

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate sections through graded alcohol to distilled water.		
2	Place slides in working Fontanna-Masson silver solution in 60ºC oven. Make just before use.	1-3 hours	Can also heat in microwave on high for 30 seconds, swirl, 30 seconds more, swirl and let sit on the counter for an additional 5 minutes.
3	Rinse in distilled water, 3 changes at room temperature.	5-10 seconds	
4	Tone in 0.3% gold chloride until sections are no longer yellow or brown.	10-60 seconds	Check with microscope.
5	Rinse in distilled water 2-3 changes.	5-10 seconds	
6	Place in 5% sodium thiosulfate.	1 minute	
7	Wash in running water.	5 minutes	
8	Counterstain in nuclear fast red.	1-5 minutes	If nuclear fast red is used as a counterstain, wash slides with tap water after staining. Aluminum sulfate does not dissolve in alcohol. Placing slides directly from nuclear fast red into alcohol will result in a white precipitate forming on the slides, which can be removed by returning the slides to tap water.
9	Rinse in distilled water, 2 changes.	5-10 seconds	
10	Dehydrate through graded alcohols, clear with xylene.		
11	Coverslip.		

X. LIMITATIONS:

A. The following may influence the validity of test results:
    1. Use acid-clean glassware to avoid silver precipitate.
    2. Allow Fontana-Masson silver solution to stand overnight before use.
    3. Use non-metal forceps or a silver precipitate may be formed on the slides and tissue.
    4. Use distilled water. Any metals in tap water may cause silver precipitate to be formed on the slides and the tissue.

XI. RESULTS:

A. Argentaffin substances (melanin, argentaffin cells). Lipofuchsin, chromaffin cells, some neurosecretory.
Granules, Iron, Formalin pigments (possibly) - **black**

B. Nuclei - **red**

C. Background - **pale pink**

XII. REFERENCES:

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F. Histopathology Laboratory Procedures of the Pathologic Anatomy Branch of National Cancer Institute.

Approval Signatures

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Applicability

Royal Oak