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Beaumont

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Histology Special Stain - Von Kossa - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of calcium but is not specific for calcium. It is specific for the anionic part of calcium compounds, phosphate and carbonate being the most common, though it may also react with oxalate, urate, chloride, and sulfate. Abnormal deposits of calcium can be found in arteries, lung infections (such as TB), and in lymph nodes.

II. PRINCIPLE:

The silver nitrate will bind with the anionic part of the calcium, forming a new compound, such as silver carbonate or silver phosphate. These, when exposed to light, will reduce to a black color. The sodium thiosulfate will remove any unreduced silver. Nuclear fast red is used as a counterstain.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue.
    2. Avoid fixatives with acid or an acid pH.
    3. Avoid fixatives with calcium.

B. Processing
    1. Standard, overnight processing.

C. Section Thickness
    1. Cut frozen section at 10µ.

2. Cut paraffin sections at 5µ.

D. Slide Drying
    1. 30 minutes at 60°C.

E. Type of Slide
    1. Plain slides

IV. REAGENTS:

A. **5% Silver Nitrate**

Silver nitrate	**5.0 mL**
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. Store in the refrigerator (3°C); stable for 4-6 months. Discard if precipitate forms, or if solution is no longer clear.

B. **5% Sodium Thiosulfate**

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

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

C. **5% Aluminum Sulfate**

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

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

D. **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.

E. **Dilute Ammonia Water**
Use dilute ammonia water used in H&E set-up.

V. EQUIPMENT:

A. Balance
B. Magnetic stirrer
C. Bright lamp

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 Thiosulfate
    1. Is an irritant.

C. Aluminum Sulfate
    1. is a corrosive.
    2. Can cause serious eye damage.

D. Nuclear Fast Red
    1. Is an irritant to skin and eyes.
    2. Is toxic.

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

VIII. QUALITY CONTROL (QC):

Section of tissue with calcium.

IX. LIMITATIONS:

A. Avoid fixatives with acids (even acetic acid) or an acid pH, such as unbuffered formalin, or old neutral buffered formalin. These will decalcify the tissue, resulting in a false negative.

B. Avoid fixatives with calcium, as false positive staining may occur.

C. Lugol iodine, used to remove mercury from tissue, may remove some of the calcium salts from the tissue.

X. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate sections through graded alcohol to distilled water.		
2	Place slides in 5% silver nitrate.		Slides must be placed in acid-clean glass coplin

			jar.
3	Place coplin jar of slides in front of strong light.	20-60 minutes	Use a bright light or sunshine, or the black color may not develop. Using a strong fluorescent light at close range the stain will develop in about 60-90 minutes.
4	Rinse in distilled water, 2 changes.	5-10 seconds each	
5	Place in 5% sodium thiosulfate.	1 minute	
6	Wash in running water.	5 minutes	
7	Counterstain in nuclear fast red.	1-5 minutes	Always rinse with tap water after staining with nuclear fast red. Aluminum sulfate dissolves in water but does not dissolve in alcohol. If the slide is placed in alcohol directly after staining, a white film of aluminum salts will remain on the slide/tissue. This can be removed by hydrating the slides back to water, then dehydrating and clearing. Other counterstains, such as light green SF yellowish or eosin, may also be used.
8	Rinse in distilled water, 2 changes.	5-10 seconds each	
9	Dehydrate through graded alcohols, clear with xylene.	10 seconds	
10	Coverslip.		

XI. RESULTS:

A. Copper deposits - **orange/red**
B. Heavy metals (mercury, silver, cadmium) - **orange/red**
C. Bile - **yellow**
D. Red blood cells - **yellow**
E. Lipofuscin - **golden**
F. Nuclei - **blue**

XII. REFERENCES:

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

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Approval Signatures

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Applicability

Royal Oak