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Beaumont

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Area	Laboratory-Histology
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Histology Special Stain - Prussian Blue - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of the ferric (Fe^{+3}) form of iron in tissues. It may be used to demonstrate normal amounts found in tissue such as bone marrow and spleen. It may be used to demonstrate the absence of stainable iron in cases of anemia or malabsorption. Large deposits of ferric iron, such as those found in hemochromatosis (over-absorption in the gut) or hemosiderosis (spleen, bone marrow, liver). Heart failure cells, found in the lungs of congestive heart failure, are macrophages that have digested red blood cells, and will also stain. Asbestos, or ferruginous bodies, are coated with iron in the body, and may also be demonstrated with this stain.

II. PRINCIPLE:

The hydrochloric acid releases the loosely bound ferric iron, such as in hemosiderin. Potassium ferrocyanide will react with the ferric ions, forming an insoluble blue pigment, ferric ferrocyanide, or Prussian blue. Nuclear fast red is used as the counterstain. Tightly bound iron, such as in hemoglobin, will not react.

III. SPECIMEN COLLECTION AND HANDLING:

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

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. **10% Potassium Ferrocyanide**

Potassium ferrocyanide	**10.0 gm**
Distilled water	**100.0 mL**

Stir together with magnetic stirrer. Store at room temperature; stable for 1 month.

B. **10% Hydrochloric Acid**

Hydrochloric acid, concentrated	**10.0 mL**
Distilled water	**90.0 mL**

Add slowly, drop by drop, to distilled water. Stir. Store at room temperature; stable for 1 month.

C. **Working Potassium Ferrocyanide-Hydrochloric Acid Solution**

10% potassium ferrocyanide	**20.0 mL**
10% hydrochloric acid	**20.0 mL**

JUST BEFORE USE, mix together. Good for one day only.

D. **5% Aluminum Sulfate**

Aluminum sulfate	**5.0 gm**
Distilled water	**100.0 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 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. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Non-metal forceps

VII. SPECIAL SAFETY PRECAUTIONS:

A. Potassium Ferrocyanide
    1. No hazards identified.

B. Hydrochloric Acid
    1. Is an acid.
    2. Add slowly, drop by drop, to solution.
    3. May causes severe eye and skin burns.

C. Aluminum Sulfate
    1. Is a corrosive.
    2. May cause serious eye damage.

D. Nuclear Fast Red
    1. Is corrosive to skin.
    2. Is an irritant to eyes.
    3. May be toxic to respiratory system.

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

VIII. QUALITY CONTROL (QC):

Section of tissue with iron.

IX. LIMITATIONS:

A. Use chemically cleaned coplin jars and non-metal forceps. If jars have previously contained iron solutions, a diffuse background staining may occur.

B. Iron may dissolve out in decalcifying solutions and acidic fixatives.

X. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate sections through graded alcohol to distilled water.		
2	Place slides in WORKING potassium ferrocyanide-hydrochloric acid.	20-30 minutes	
3	Rinse in distilled water, 3-5	5-10	Use distilled water. If tap water contains iron, a

	changes.	seconds	diffuse background staining or iron precipitate may occur.
4	Stain 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.
5	Wash in distilled water, 2-3 changes.	5-10 seconds	
6	Dehydrate through graded alcohols, clear with xylene.	10 seconds	
7	Coverslip.		

XI. RESULTS:

A. Iron deposits (hemosiderin) - **blue**

B. Heart failure cells - **blue**

C. Asbestos bodies - **blue**

D. Nuclei - **pink**

E. 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. Vacca L: Laboratory Manual of Histochemistry. New York, NY, Raven Press, 1985.

Approval Signatures

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Applicability

Royal Oak