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**IRON - PRUSSIAN BLUE REACTION - MALLORY'S METHOD**

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**PURPOSE:** To demonstrate ferric iron in tissue sections. Small amounts of iron are found normally in spleen and bone marrow. Excessive amounts are present in hemochromatosis, with deposits found in the liver and pancreas, hemosiderosis, with deposits in the liver, spleen, and lymph nodes.

**PRINCIPLE:** The reaction occurs with the treatment of sections in acid solutions of ferrocyanides. Any ferric ion (+3) in the tissue combines with the ferrocyanide and results in the formation of a bright blue pigment called "Prussian blue" or ferric ferrocyanide.

**CONTROL:** A known positive control tissue.

**FIXATIVE:** 10% formalin

**TECHNIQUE:** Cut paraffin sections 4 $\mu$ .

**EQUIPMENT:** Microwave oven, acid-cleaned glassware, non-metallic forceps.

**REAGENTS:**

**5% Potassium Ferrocyanide:**  
Potassium ferrocyanide 25.0 gm  
Distilled water 500.0 ml

Mix well, pour into an acid-cleaned brown bottle. Stable for 6 months.

**CAUTION:** Low toxicity if not heated..

**Nuclear-fast Red:**  
See Retic

**5% Hydrochloric Acid:**  
Hydrochloric acid, conc. 25.0 ml  
Distilled water 475.0 ml

Mix well, pour into brown bottle, stable for 6 months.

**CAUTION:** Corrosive, avoid contact and inhalation.

**Working Solution:**  
5% potassium ferrocyanide 25.0 ml  
5% hydrochloric acid 25.0 ml

Make fresh, discard after use.

**CAUTION:** Avoid contact and inhalation.

**SAFETY:** Wear gloves, goggles and lab coat. Avoid contact and inhalation.

Potassium ferrocyanide; Low toxicity as long as it is not heated, it will release cyanide gas.

Hydrochloric acid; target organ effects on reproductive system and fetal tissue. Irritant to skin eyes and respiratory sytem.

**PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. \*Working solution, \* microwave, 30 seconds. Allow slides to stand in solution for 5 minutes, in the fume hood.
3. Rinse in distilled water.
4. Nuclear-fast red, 5 minutes.
5. Wash in tap water.
6. Dehydrate, clear, and coverslip.  
\*Conventional method: room temperature for 30 minutes.

**RESULTS:**

Iron (hemosiderin)	blue
Nuclei	red
Background	pink

**REFERENCES:**

Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp217-218, Battelle Press, Ohio  
Luna L, Manual of Histologic Staining Methods of the AFIP, 3rd Ed, 1968, pp 183, McGraw-Hill, NY  
Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: \_\_\_\_\_ By: \_\_\_\_\_

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## PROCEDURE CARD

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DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

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**CAUTION:** Corrosive, avoid contact and inhalation.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**NUCLEAR-FAST RED**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_