IRON - PRUSSIAN BLUE REACTION - MALLORY'S METHOD

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 4µ.

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

REAGENTS:

5% Potassium Ferrocyanide: 5% Hydrochloric Acid:
Potassium ferrocyanide Hydrochloric acid, conc. 25.0 gm 25.0 ml
Distilled water Distilled water 500.0 ml 475.0 ml

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

CAUTION: Low toxicity if not heated. CAUTION: Corrosive, avoid contact and inhalation.

Nuclear-fast Red: Working Solution:
See Retic 5% potassium ferrocyanide 25.0 ml
5% hydrochloric acid 25.0 ml

Make fresh, discard after use.

CAUTION: Avoid contact and inhalation.
MINERALS AND PIGMENTS

IRON

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 system.

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:
Crookham, J, Dapson, R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: ______________________ By: ________________

Approved: ______________________ By: ________________

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PROCEDURE CARD

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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.

Working Solution:
5% potassium ferrocyanide 25.0 ml
5% hydrochloric acid 25.0 ml
Make fresh, discard after use.

CAUTION: Avoid contact and inhalation.

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.

Nuclear-fast Red:
See Retic
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.

DATE:__________________________

TECH:__________________________

EXPIRATION:____________________

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.

DATE:__________________________

TECH:__________________________

EXPIRATION:____________________

NUCLEAR-FAST RED

DATE:__________________________

TECH:__________________________