PATHOLOGY CORE

Special Stain Protocol: Perl's Iron

Purpose:
The purpose is to detect iron in the form of hydroxide $Fe(OH)_3$, by dilute hydrochloric acid.

Principle:
The iron will react with the dilute potassium ferrocyanide solution to produce an insoluble blue color.

Positive Control Tissue:
Liver tissue

Tissue Fixative:
10% Formalin fixed tissue

Reagents Required:
Potassium ferrocyanide

- Vendor- Fisher Chemical
- Lot number- 178712
- Catalog number- P236-500

Hydrochloric acid

- Vendor- Fisher Chemical
- Lot number- 169287
- Catalog number- A144-212

Nuclear Fast Red (Kernechtrot)

- Vendor- Fluka Chemika
- Catalog number- 60700

Solution Preparation:
10% potassium ferrocyanide: 10 gram of potassium ferrocyanide + 100ml of DI water
10% hydrochloric acid: 10ml of concentrated hydrochloric acid + 90ml of DI water
(Freshly prepared)

Protocol:

1. Deparaffinize slides (xylene→distilled water)
2. Immerse slides in 10% potassium ferrocyanide for 5-10 minutes
3. Immerse slides in equal parts of 10% potassium ferrocyanide and 20% hydrochloric acid for 30 minutes (Do not mix these two solutions together until just before use)
4. Wash slides thoroughly in distilled water
5. Counterstain with nuclear fast red for 7 minutes
6. Wash slides with cold running tap water for 3 minutes
7. Dehydrate slides (95% ethanol→xylene)
8. coverslip

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Interpretation:
Iron= Blue
Background/Nuclei=Light red/Pink
References:
Theory and Practice of Histotechnology

Notes:

- Separate solutions should not be stored for more than 1 week.