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| **Prussian Blue Iron Stain** |
| **Purpose** | To detect the presence of **iron** in specimens, such as deposits of storage ferritin in bone marrow biopsy samples. |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. |
| **Principle** | Hemosiderin, which occurs as yellow to brown intracellular granules, is a crystalline aggregate of ferritin. Small amounts of ferric iron are found normally in the spleen and bone marrow. Excessive amounts are present in hemochromatosis and hemosiderosis. There is a significant damage to the tissue because of this deposition.  |
| **Materials** | **Supplies** | **Reagents** |
|  | • Coplin jars with lids• PPE• Graduated cylinder• Non-metallic slide forceps | • Hydrochloric Acid• Potassium Ferrocyanide• Nuclear Fast Red |
| **Sample** | Tissue: FFPEHematological smears: Formaldehyde fumes or Methanol; fix 5 minutes and air-dryCytology preparations: Fix in 95% Ethanol and air-dry |
| **Quality Control** | Paraffin sections: liver or spleen with hemochromatosisHematological smears: Iron positive smearsCytology preparations: Iron positive specimens NO METAL EQUIPMENT |
| **Stock Solutions** | **Nuclear Fast Red****Hydrochloric Acid, 20%****Potassium Ferrocyanide, 10%** |
| **Working Solutions** | **Working Hydrochloric Acid-Potassium Ferrocyanide**20% Hydrochloric Acid ……..……….20 mL Make fresh each use10% Potassium Ferrocyanide………20 mL |
| **Procedure** | **Step** | **Action** |
|  | 1 | Deparaffinize slides and hydrate to water |
|  | 2 | Rinse in Distilled water |
|  | 3 | Incubate slides in freshly prepared Working Hydrochloric Acid-Potassium Ferrocyanide solution …….....**20** minutes |
|  | 4 | Rinse well in Distilled waterExposure to tap water at this time may cause artifact |
|  | 5 | Counterstain in Nuclear Fast Red……….**4** minutes |
|  | 6 | Rinse well in Distilled water |
|  | 7 | Dehydrate, clear and coverslip |
| **Results** | Iron pigments……….Bight BlueNuclei ……………….RedCytoplasm…….…….Pink |
| **Result Reporting** | By Pathologist |
| **References** | Bancroft, John D. and Stevens, Alan: Theory and Practice of Histological Techniques, c.1977 Preece, Ann: A Manual for Histologic Technicians, c. 1972Sheehan, D.C. and Hrapchak, B.B.: Theory and Practice of Histotechnology, 2nd edition,1980.Histotechnology a Self-Instructional Text Frieda Carson, second edition 1997 |

**Historical Record**

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| Version | Revised by | Effective Date | Summary of Revisions |
| 1 |  |  | Initial version. |
| 2 | A. Dubbelde | 6/27/19 | Update format, add version, and update to match current staining procedure used. |
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