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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: FFPE  Hematological smears: Formaldehyde fumes or Methanol; fix 5 minutes and air-dry  Cytology preparations: Fix in 95% Ethanol and air-dry | | |
| **Quality Control** | Paraffin sections: liver or spleen with hemochromatosis  Hematological smears: Iron positive smears  Cytology 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 use  10% 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 water  Exposure 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 Blue  Nuclei ……………….Red  Cytoplasm…….…….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. 1972  Sheehan, 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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