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| **Dacie Iron Stain** | | | |
| **Purpose** | To demonstrate iron in blood smears. | | |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. | | |
| **Principle** | The Dacie Iron stain is often used in the assessment of erythroid precursors in various types of sideroblastic anemias. | | |
| **Materials** | **Supplies** | | **Reagents** |
|  | • PPE  • Coplin jars with lids  • Non-metallic forceps  • Graduated cylinders  • waterbath | | • Methanol  • Hydrochloric Acid,0.2 N  • Potassium Ferrocyanide |
| **Sample** | Bone marrow smears: concentrate smears (preferred), aspirate smears, or peripheral blood smears. Slides made from the fat and perivascular layer of the marrow are not to be used. | | |
| **Quality Control** | Use smears containing an increased number of sideroblasts and ringed siderocytes for controls. Slides may be stored fixed at room temperature for several months. Longer than 6 months storage in -70° freezer. The Iron tissue control may also be used if blood smear controls are not available.  Use of metal equipment, contaminated glassware or rinsing with tap water may cause artificial  staining.  Excessive heat or prolonged incubation in the Hydrochloride-Potassium Ferrocyanide solution  can alter the chemical reaction and cause artifact which cannot be removed. | | |
| Stock Solutions | **Hydrochloric Acid, 0.2 N**  Purchased through RICCA/ Cardinal Health.  See Manufacturer’s label for expiration date information.  **Potassium Ferrocyanide, 2%**  Potassium Ferrocyanide….…….0.5 gm  Distilled water……………..……..25.0 mL Shelf life: 6 months  *This solution is used in the routine Iron procedure* | | |
| Working Solutions | **Hydrochloric Acid-Potassium Ferrocyanide Solution**  0.2N Hydrochloric Acid (stock)……………25.0 ml  2% Potassium Ferrocyanide………………25.0 ml Make fresh each use    **Nuclear Fast Red**  Purchased as a working solution from Newcomers Supply, available in the Iron Stain bin | | |
| **Procedure:** | **Step** | **Action** | |
|  | 1 | Fix air-dried smears in absolute Methyl alcohol …….....….**10-15** minutes. Air dry.  Do NOT wash slides in distilled water.  While slides are fixing, prepare the working Dacie solution and place in a 56°C  waterbath. | |
|  | 2 | Incubate slides in freshly prepared working, preheated Hydrochloric Acid-Potassium Ferrocyanide solution ………………..**10** minutes | |
|  | 3 | Rinse slides in distilled water and then rinse slides in running tap water...................**5** minutes  Do not air-dry slides before counterstaining. This may cause artifact. | |
|  | 4 | Counterstain slides in Nuclear Fast Red……………………………...**3** minutes | |
|  | 5 | Rinse briefly with distilled water and air-dry | |
|  | 6 | Coverslip | |
| **Results** | Iron in Siderocytes/ Sideroblasts..........…….Deep blue-green  Nuclei …………………………………………Red  Red blood cells ………………………………Pale rose/brown | | |
| Result Reporting | By Pathologist | | |
| **References** | Homburger, H.A. and Batsakis, J.G.: Clinical Laboratory Annual, Appleton-Century-Crofts, 1984.  Beuerlein, F.J.: Testing Strategies for Anemias. Laboratory Management, 1988  Histotechnology A Self-Instructional Text, F.Carson 1990  Sheehan & Hrapchak:Theory and Practice of Histotechnology, 2nd edition 1980 Battelle | | |

**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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