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| **Gomori’s Reticulin Stain** |
| **Purpose** | To demonstrate reticulin fibers in tissue samples. |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. |
| **Principle** | Oxidation of the tissue enhances staining. The Ferric Ammonium Sulfate is used to sensitize the tissue and the Ammoniacal Silver will replace the Ferric Ammonium Sulfate. The 20% Formalin will reduce the Silver to visible brown, and Gold Chloride will tone it to a black-grey. The last step is a counterstain using Nuclear Fast Red. |
| **Materials** | **Supplies** | **Reagents** |
|  | **•** Coplin jars with lids• Graduated cylinders• Erlenmeyer flasks• Plastic slide forceps• PPE• Plastic transfer pipets | • Potassium Permanganate• Sodium Hydroxide• Ferric Ammonium Sulfate• Sodium Thiosulfate• Silver Nitrate• Ammonium Hydroxide• Oxalic Acid• Gold Chloride• 10% NB Formalin• Nuclear Fast Red |
| **Sample** | FFPE tissue |
| **Quality Control** | A section of normal liverAll plastic and glassware must be extremely clean. Rinse in distilled water before use. Do not use metal forceps. |
| **Special Safety Precautions** | Silver Nitrate may cause severe burns. Avoid contact with skin and eyes. Poison: avoid breathing in, corrosive.Potassium Metabisulfite and Ferric Ammomium Sulfate avoid contact with skin.Formaldehyde is an irritant to eyes and mucous membranes. Use under fume hood.   |
| **Stock Solutions** | **Silver Nitrate, 10%****Potassium Permanganate, 1%****Sodium Hydroxide, 3%****Ferric Ammonium Sulfate, 2.5%****Sodium Thiosulfate, 5%****Ammonium Hydroxide, 28-30%****Oxalic Acid, 1%****Gold Chloride, 0.2%****Formalin, 10% NB****Nuclear Fast Red** |
|  **Working Solutions** | **Working Ammoniacal Silver Solution: Make fresh**1. Place 5 mL of Solution D: Silver Nitrate, 10% in a flask2. Add Solution E: Ammonium Hydroxide, 28-30% drop by drop, continuosly swirling, until formed precipitate completely dissolves.3. Add 5 mL of Solution F: Sodium Hydroxide, 3%4. Re-dissolve formed precipitate with Solution E: Ammonium Hydroxide, 28-30% until faint cloudiness remains **\*Note**: If you’ve added too much Ammonia (the solution is very clear), add 10% Silver Nitrate drop by drop until the solution turns cloudy brown again.5. Bring solution volume to 50 mL with Distilled water6. Filter solution before use  |
| **Procedure** | **Step** | **Action** |
|  | 1 | Deparaffinize and hydrate slides with several changes of Distilled water |
|  | 2 | Oxidize sections in Solution A: Potassium Permanganate, 1%……….**5** minutes |
|  | 3 | Rinse slides in running water for 2 minutes then rinse in Distilled water |
|  | 4 | Bleach in Solution B: Oxalic Acid 1%……….**2** minutes or until colorless |
|  | 5 | Rinse slides in running water for 2 minutes then rinse in Distilled water |
|  | 6 | Sensitize sections in Solution C: Ferric Ammonium Sulfate, 2.5%…15-20 minutes |
|  | 7 | Rinse in several changes of Distilled water |
|  | 8 | Impregnate slides with the filtered Working Silver Solution……….**2** minutes |
|  | 9 | Rinse in Distilled water………**1** minute |
|  | 10 | Reduce sections in Solution G: Formalin, 10%……………...….**2** minutes Sections should turn brown/ black at this step |
|  | 11 | Rinse slides under running water.................**3** minutes |
|  | 12 | Check control slide microscopically for sufficient black reticular fiber development Repeat Silver if necessary |
|  | 13 | Tone sections in Solution H: Gold Chloride, 0.2%………..….**10** minutes  |
|  | 14 | Rinse well in Distilled water |
|  | 15 | Place slides in Solution I: Sodium Thiosulfate, 5%……….…..**1** minute |
|  | 16 | Rinse slides in running water for 2 minutes then rinse in Distilled water |
|  | 17 | Counterstain in Solution J: Nuclear Fast Red………**5** minutes |
|  | 18 | Rinse slides well in Distilled water |
|  | 19 | Dehydrate, clear and coverslip |
| **Interpretation/****Results/Alert Values** | Reticulum fibers……….....BlackBackground…………....…Red |
| **Result Reporting** | By Pathologists |
| **References** | Histotechnology A Self-Instructional Text, F.Carson 1990Sheehan & Hrapchak:Theory and Practice of Histotechnology, 2nd edition 1980 Battelle Press |

**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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