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| **Masson Trichrome Stain** |
| **Purpose** | Trichrome stains are used primarily for distinguishing collagen from muscle tissue.  |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. |
| **Principle** | The use of trichrome staining, which distinguishes collagen from muscle, is useful for indicating fibrotic change (or increased amounts of collagen). Fibrotic change can occur, for example, in cirrhosis of the liver and in various renal diseases. Trichrome methods are also useful for distinguishing histologic changes that occur in neuromuscular diseases and in distinguishing tumors that have arisen from muscle cells and from tumors that have arisen from fibroblasts. |
| **Materials** | **Supplies** | **Reagents** |
|  | • PPE • Coplin jars with lids  • Graduated cylinders  | •Bouin fluid•Ferric Chloride•Hematoxylin, 1%•Beibrich Scarlet- Acid Fuchsin•Phosphomolybdic-Phosphotungstic Acid•Aniline Blue•Acetic Acid, 0.5% |
| **Sample** | FFPE tissue |
| **Quality Control** | Kidney, liver, and muscle are positive controls. |
| **Special Safety Precautions** | Use Bouin’s Solution under a fume hood and avoid inhalation of any fumes. |
| **Stock Solutions** | Bouin fluidFerric ChlorideHematoxylin, 1%Beibrich Scarlet- Acid FuchsinPhosphomolybdic-Phosphotungstic AcidAniline BlueAcetic Acid, 0.5% |
| **Working Solutions** | **Weigert Iron Hematoxylin- made fresh every time** Ferric Chloride........................20 mL Hematoxylin 1%.....................20 mL**All other Reagents are ordered from Newcomer Supply ready to use.** |
| **Procedure** | **Step** | **Action** |
|  | 1 | Deparaffinize and hydrate to water |
|  | 2 | Pre-heat Solution A: Bouin Fluid (**use fresh each time)** in a plastic Coplin jar, in a water bath set at 56-60°C. Bouin’s solution comes to temperature in about 5 minutes |
|  | 3 | Mordant slides in Bouin’s solution at 56° C for..................**1** hour**. Keep covered.** Cool at room temperature for 5-10 minutes |
|  | 4 | Rinse in running tap water........................**10** minutes |
|  | 5 | Stain in fresh Weigert Iron Hematoxylin (mix well) for.................**10** minutes Solution B: Ferric Chloride, Acidified...........20 mL Solution C: Hematoxylin 1%, Alcoholic.......20 mL |
|  | 6 | Rinse in running tap water for **10** minutes, rinse in Distilled water\* See Procedure note |
|  | 7 | Stain in Solution D: Biebrich Scarlet-Acid Fuchsin for.............. **2** minutes |
|  | 8 | Rinse in Distilled water |
|  | 9 | Place slides in Solution E: Phosphomolybdic /Phosphotungstic Acid solution…**5** minutes |
|  | 10 | Transfer slides directly into Solution F: Aniline Blue for ............ **5** minutes |
|  | 11 | Rinse slides well in Distilled water |
|  | 12 | Place slides in Solution G: Acetic Acid, 0.5% ………………………. **3-5** minutes |
|  | 13 | Rinse slides in running tap water |
|  | 14 | Dehydrate slides rapidly to **avoid leaching of Aniline Blue**. Clear and coverslip |
| **Results** | Nuclei……….............Blue/blackCytoplasm…….....….RedMuscle fibers........….RedKeratin.......................RedCollagen……….........Blue |
| **Procedure Notes** | Do not allow sections to dry out at any point during staining procedure.If Weigert Iron Hematoxylin is not completely washed from tissue sections, nuclear and cytoplasmic staining may be compromised. |
| **Result Reporting** | By Pathologists |
| **References** | Histotechnology A Self-Instructional Text, F.Carson 1990Theory and Practice of Histotechnology, by Sheehan and Hrapchak, 2nd edition, Mosby,  1980. |

**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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