**Trichrome Stain, Masson, Aniline Blue - Technical Memo**

**SOLUTION:**

<table>
<thead>
<tr>
<th>Aniline Blue Stain, Aqueous</th>
<th>250 ml</th>
<th>500 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part 10072B</td>
<td>Part 10072C</td>
<td></td>
</tr>
</tbody>
</table>

**Additionally Needed:**

- Trichrome, Liver Control Slides Part 4690 or Trichrome, Multi-Tissue Control Slides Part 4693
- Xylene, ACS Part 1445
- Alcohol, Ethyl Denatured, 100% Part 10841
- Alcohol, Ethyl Denatured, 95% Part 10842
- Bouin Fluid Part 1020
- Hematoxylin Stain Set, Weigert Iron Part 1409
- Biebrich Scarlet-Acid Fuchsia Stain, Aqueous Part 10161
- Phosphomolybdic-Phosphotungstic Acid, Aqueous Part 1332
- Acetic Acid 0.5%, Aqueous Part 100121
- Coplin Jar, Plastic Part 5184 (for microwave modification)

**APPLICATION:**

Newcomer Supply Trichrome Stain, Masson, Aniline Blue procedure, with included microwave modification, is used to differentially demonstrate connective tissue elements, collagen and muscle fibers.

**METHOD:**

**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)

**Technique:** Paraffin sections cut at 5 microns

- **Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

**STAINING PROCEDURE:**

1. Preheat Bouin Fluid (1020) to 56-60°C in oven or water bath. **(Skip if using overnight method or microwave procedure.)**
2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water. **a. See Procedure Note #2 and #3.**
3. Mordant in preheated Bouin Fluid for one hour at 56-60°C or overnight at room temperature. Cool at room temperature for 5-10 minutes.
   - **a. Skip Step #3 if tissue was originally Bouin fixed.**
4. **Microwave Modification:** See Procedure Note #4.
   - b. Place slides in a plastic Coplin jar containing Bouin Fluid and microwave for 5 minutes at 60°C. Allow slides to sit an additional 10 minutes in solution.
5. Wash well in running tap water; rinse in distilled water.
6. Prepare fresh Weigert Iron Hematoxylin; combine and mix well.
   - a. Solution A: Ferric Chloride, Acidified 20 ml
   - b. Solution B: Hematoxylin 1%, Alcoholic 20 ml
7. Stain slides in fresh Weigert Iron Hematoxylin for 10 minutes.
8. Wash in running tap water for 10 minutes; rinse in distilled water. **a. See Procedure Note #5.**
9. Place slides in Biebrich Scarlet-Acid Fuchsia Stain, Aqueous for 2 minutes.
10. Rinse in distilled water.
11. Place slides in Phosphomolybdic-Phosphotungstic Acid, Aqueous for 10 to 15 minutes.
12. Transfer slides directly into Aniline Blue Stain, Aqueous for 5 minutes.
13. Place slides in Acetic Acid 0.5%, Aqueous for 3 to 5 minutes.
14. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

**RESULTS:**

- Collagen and mucin Blue
- Muscle fibers, cytoplasm and keratin Red
- Nuclei Blue/black

**PROCEDURE NOTES:**

1. Using ammonium hydroxide to soak or face tissue blocks will alter the pH of tissue sections and greatly diminish trichrome staining.
2. Drain staining rack/slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during staining procedure.
4. The suggested microwave procedure has been tested at Newcomer Supply using an “EB Sciences”, 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer's instructions, to prevent exposure to chemical vapors.
5. If Weigert Iron Hematoxylin is not completely washed from tissue sections, nuclear and cytoplasmic staining may be compromised.
6. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

**REFERENCES:**

5. Modifications developed by Newcomer Supply Laboratory.
# Trichrome Stain, McLetchie, Aniline Blue - Technical Memo

**SOLUTION:**

<table>
<thead>
<tr>
<th>Solution</th>
<th>250 ml</th>
<th>500 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline Blue Stain, Aqueous</td>
<td>Part 10072B</td>
<td>Part 10072C</td>
</tr>
</tbody>
</table>

Additionally Needed:

<table>
<thead>
<tr>
<th>Item</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichrome, Liver Control Slides</td>
<td>Part 4690</td>
</tr>
<tr>
<td>Xylene, ACS</td>
<td>Part 1445</td>
</tr>
<tr>
<td>Alcohol, Ethyl Denatured, 100%</td>
<td>Part 10841</td>
</tr>
<tr>
<td>Alcohol, Ethyl Denatured, 95%</td>
<td>Part 10842</td>
</tr>
<tr>
<td>Biebrich Scarlet-Acid Fuchsian Stain, Aqueous</td>
<td>Part 10161</td>
</tr>
<tr>
<td>Iodine, Weigert &amp; Lugol, Aqueous</td>
<td>Part 12092</td>
</tr>
<tr>
<td>Phosphotungstic Acid 2%, Alcoholic</td>
<td>Part 13342</td>
</tr>
</tbody>
</table>

**APPLICATION:**

Newcomer Supply Trichrome Stain, McLetchie, Aniline Blue procedure is useful for the demonstration of collagen and muscle fibers, has excellent staining results with bone marrow and renal biopsies and provides time effective trichrome results. This modified protocol differs from a standard trichrome procedure by not using a Bouin Fluid mordant or a hematoxylin nuclear stain.

**METHOD:**

**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)

**Technique:** Paraffin sections cut at 5 microns
- See Procedure Note #1.

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

**STAINING PROCEDURE:**

1. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
   - See Procedure Notes #2 and #3.
2. Place slides in Biebrich Scarlet-Acid Fuchsin Stain, Aqueous (10161) for 5 minutes.
3. Rinse slides in several changes of distilled water.
4. Place slides in Iodine, Weigert & Lugol, Aqueous (12092) for 2 minutes.
5. Rinse slides in several changes of distilled water.
6. Differentiate slides one at a time in Phosphotungstic Acid 2%, Alcoholic (13342) for 15-30 seconds. Gently agitate slides once.
   - To deter over-differentiation do not exceed the 30 second timing in Phosphotungstic Acid 2%, Alcoholic.
   - If sections are over-differentiated, wash slides well in distilled water and repeat Steps #2 through #6.
7. Rinse slides immediately in several changes of distilled water.
8. Place slides in Aniline Blue Stain, Aqueous for 1-3 minutes.
9. Rinse slides in several changes of distilled water.
10. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

**RESULTS:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Staining Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>Blue</td>
</tr>
<tr>
<td>Muscle fibers, cytoplasm and keratin</td>
<td>Magenta to red</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Dark red</td>
</tr>
</tbody>
</table>

**PROCEDURE NOTES:**

1. Using ammonium hydroxide to soak or face tissue blocks will alter the pH of tissue sections and greatly diminish trichrome staining.
2. Drain staining rack/slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during staining procedure.
4. The nuclear detail with this method is dark red with crisp definition.
5. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

**REFERENCES:**

3. Modifications developed by Newcomer Supply Laboratory.