Trichrome Stain, Gomori One-Step, Aniline Blue - Technical Memo

**SOLUTION:**

<table>
<thead>
<tr>
<th>Trichrome Stain, Gomori One-Step, Aniline Blue</th>
<th>250 ml</th>
<th>500 ml</th>
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<tbody>
<tr>
<td>Part 1403C</td>
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<tr>
<td>Part 1403B</td>
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**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
- Acetic Acid 0.5%, Aqueous: Part 100121
- Coplin Jar, Plastic: Part 5184 (for microwave modification)

For storage requirements and expiration date refer to individual product labels.

**APPLICATION:**

Newcomer Supply Trichrome Stain, Gomori One-Step, Aniline Blue procedure, with included microwave modification, uses a one-step solution combining a plasma stain and a connective tissue stain to differentially demonstrate collagen and muscle fibers.

**METHOD:**

- **Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)
- **Technique:** Paraffin sections cut at 5 microns
  - a. See Procedure Note #1.
- **Solutions:** All solutions 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 Notes #2 and #3.
3. Mordant in Bouin Fluid for 1 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. Stain in Trichrome Stain, Gomori One-Step, Aniline Blue for 20 minutes.
10. Directly differentiate in Acetic Acid 0.5%, Aqueous (100121) for 2 minutes.
11. Rinse quickly in distilled water.
12. 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.