## Modified Verhoeff Elastic-Masson Trichrome Stain – Technical Memo

### SOLUTIONS:

<table>
<thead>
<tr>
<th>Solution</th>
<th>250 ml</th>
<th>500 ml</th>
<th>1 Liter</th>
<th>1 Gallon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picric Acid, Saturated Alcohol or Bouin Fluid</td>
<td>Part 10856A</td>
<td>Part 10856B</td>
<td>Part 10856C</td>
<td>Part 1020A</td>
</tr>
<tr>
<td>Ferric Chloride 10%, Aqueous</td>
<td>Part 1016A</td>
<td>Part 1016B</td>
<td>Part 1016C</td>
<td>Part 1020A</td>
</tr>
<tr>
<td>Hematoxylin 5%, Alcoholic</td>
<td>Part 1332A</td>
<td>Part 1332B</td>
<td>Part 1332C</td>
<td>Part 10072C</td>
</tr>
<tr>
<td>Iodine, Weigert &amp; Lugol, Aqueous</td>
<td>Part 10072B</td>
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<tr>
<td>Biebrich Scarlet-Acid Fuchsin Stain, Elastic-Trichrome, Aqueous</td>
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<tr>
<td>Phosphomolybdic-Phosphotungstic Acid, Aqueous</td>
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<tr>
<td>Aniline Blue Stain, Aqueous</td>
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<tr>
<td>Acetic Acid 1%, Aqueous</td>
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<tr>
<td>Additional Needed:</td>
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<tr>
<td>Xylene, ACS</td>
<td>Part 1445</td>
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<tr>
<td>Alcohol, Ethyl Denatured, 100%</td>
<td>Part 10841</td>
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<td></td>
</tr>
<tr>
<td>Alcohol, Ethyl Denatured, 95%</td>
<td>Part 10842</td>
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</tbody>
</table>

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

### APPLICATION:

Newcomer Supply Modified Verhoeff Elastic-Masson Trichrome Stain combines elastic and trichrome staining techniques for demonstration and clear definition of elastic fibers of all sizes, connective tissue and nuclei in a single tissue section. This procedure can be useful for identification of normal tissue morphology as well as heart, liver, lung and kidney pathologic conditions.

### METHOD:

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

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

**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. 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 #1 and #2.
   - b. See Procedure Notes #3 and #4.
   - c. Skip Step #2 if tissue was originally Bouin fixed.
2. Mordant in Picric Acid, Saturated Alcoholic (1337) for 5 minutes or Bouin Fluid (1020) at 56º C for 1 hour.
   - a. See Procedure Notes #3 and #4.
   - b. Bouin Fluid mordant; Cool at room temperature for 5-10 minutes before proceeding.
   - c. Drain staining rack/slides after staining time. Bouin Fluid mordant requires longer exposure.
3. Wash well in running tap water; rinse in distilled water.
4. Prepare Verhoeff Working Solution:
   - a. Hematoxylin 5%, Alcoholic (11623) 20 ml
   - b. Ferric Chloride 10%, Aqueous (10856) 12 ml
   - c. Iodine, Weigert & Lugol, Aqueous (12092) 8 ml
6. Rinse in several changes of tap water.
7. Prepare fresh Ferric Chloride 2%, Aqueous.
   - a. Ferric Chloride 10%, Aqueous 10 ml
   - b. Distilled Water 40 ml
8. Differentiate each slide individually in fresh Ferric Chloride 2%, Aqueous with agitation; 2-10 dips.
   - a. Check differentiation; rinse well in tap water and check microscopically for black elastic staining with gray background. Repeat in Ferric Chloride 2%, Aqueous until desired elastic differentiation is achieved.
9. Wash well in running tap water.
10. Stain in Biebrich Scarlet-Acid Fuchsin Stain, Elastic-Trichrome, Aqueous (1016) for 3 minutes.
11. Rinse in distilled water for 10 minutes.
12. Differentiate slides in Phosphomolybdic-Phosphotungstic Acid, Aqueous (1332) for 15 minutes.
   - a. Check slides microscopically until collagen is colorless but muscle remains red.
13. Transfer directly into Aniline Blue Stain, Aqueous (10072) for 3 minutes.
14. Differentiate in Acetic Acid 1%, Aqueous (10012) for 3 minutes.
15. 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:

- Elastin: Blue-black
- Muscle, keratin & cytoplasm: Red
- Collagen: Blue
- Nuclei: Red-brown to blue-black

### PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. The use of Picric Acid, Saturated Alcoholic mordant will reduce overall staining time. Bouin Fluid mordant requires longer exposure but will enhance Biebrich Scarlet-Acid Fuchsin staining (Step #10).
4. Dispose of Picric Acid, Saturated Alcoholic or Bouin Fluid as hazardous waste and/or according to local and state environmental regulations.
5. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

### REFERENCES:

4. Modifications developed by Newcomer Supply Laboratory.