F-386 GOLDNER’S TRICHRONE METHOD

Fixation: 10% Buffered Neutral Formalin (F-113) or Bouin’s Solution (F-40)

Sections: Paraffin, frozen or celloidin

Microtomy: Cut paraffin sections at 6 micrometers.

Procedure:

1. Decerate slides in Xylene, 2 changes, 2 minutes each. Place in absolute alcohol, 2 changes, 2 minutes each; 95% alcohol, 2 changes, 2 minutes each; then rinse in distilled water.
2. Mordant in Bouin’s Fluid (F-386-1) solution for 1 hour at 56°C, or overnight at room temperature, if formalin fixed.
3. Cool and wash in running tap water until yellow color disappears.
4. Rinse in distilled water.
5. Place in Weigert’s Hematoxylin for 10 minutes.
   *To prepare Weigert’s Solution, mix equal parts of Weigert’s Iron Hematoxylin A (F-386-2) and Weigert’s Iron Hematoxylin B (F-386-3) stir vigorously.
6. Wash in running tap water for 10 minutes.
7. Rinse in distilled water.
8. Stain in Ponceau Acid Fuchsin (F-386-4) for 5 minutes.
9. Wash in Acetic Acid, 1% (F-386-5).
10. Place in Phosphomolybdic Acid-Orange G solution (F-386-6) until collagen is decolorized.
11. Rinse in Acetic Acid, 1% (F-386-5) for 30 seconds.
12. Stain in Light Green Stock Solution (F-386-7) for 5 minutes.
13. Rinse in Acetic Acid, 1%, (F-386-5) for 5 minutes.
14. Blot sections but do not allow to dry.
15. Dip quickly in absolute alcohol.
16. Dehydrate slides in 95% alcohol and absolute alcohol 3 changes each. Clear in Xylene, 3 changes.
17. Mount coverglass with appropriate medium (refractive index 1.48-1.56).

Stain Results:

<table>
<thead>
<tr>
<th>Component</th>
<th>Color</th>
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</thead>
<tbody>
<tr>
<td>Nuclear chromatin</td>
<td>Brownish black</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Bright red</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>Orange</td>
</tr>
<tr>
<td>Muscle</td>
<td>Red</td>
</tr>
<tr>
<td>Collagen</td>
<td>Green</td>
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</tbody>
</table>