**F-363, HEIDENHAIN’S “AZAN” MODIFICATION**

**Fixation:** Zenker’s (F-155), Helly’s (F-153), Bouin’s (F-40) or Carnoy’s (F-60)

**Sections:** Paraffin @ 6 microns

**PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. If formalin fixed, mordant in Zenker’s overnight, then remove Mercuric Chloride by placing in *Lugol’s Iodine* (F-363-6) or *Gram’s Iodine* (F-363-6A) for 15 minutes. Rinse in tap water and place in *Sodium Thiosulfate, 5%* (F-363-7) for 3 minutes. Wash in tap water for 10 minutes longer. Rinse in distilled water.
3. Place in preheated *Azocarmine B Solution* (F-363-1) for 15 minutes @ 56°C. Rinse in distilled water.
4. Differentiate in *Aniline Alcohol Solution* (F-363-3) until cytoplasm and connective tissue are pale pink and nuclei stand out sharply. Control differentiation by rinsing in *Acetic Acid Alcohol* (F-363-4).
5. Mordant in *Phosphotungstic Acid, 5%* (F-363-2) for 15 minutes. Rinse in distilled water.
6. *Aniline Blue Orange G Solution* (F-363-2) for 15 minutes. Rinse in distilled water.
7. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, two changes each, two minutes each.
8. Mount with *Permount* (M-18).

**NOTE:** Clark omits steps #1 and #2 and times in Step #3,#5 and #6 are substantially longer: i.e.; #3, 30-60 minutes @ 50-55°C plus 1-2 hours @ 37°C; #5, 30 minutes to 3 hours; #6, 1-3 hours.

**Stain Results:**

<table>
<thead>
<tr>
<th>Stain Category</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatin, osteocytes, neuroglia</td>
<td>Red</td>
</tr>
<tr>
<td>Collagen, reticulum</td>
<td>Blue</td>
</tr>
<tr>
<td>Muscle</td>
<td>Red to yellow</td>
</tr>
<tr>
<td>Osteoid Material in Decalcified Sections</td>
<td>Red</td>
</tr>
</tbody>
</table>

**References:**