**F-365, LUNA-ISHAKE METHOD FOR BILE CANALICULI**

**Fixation:** 10% Buffered Neutral Formalin (F-113)

**Sections:** Paraffin @ 6 microns

**Staining:**
1. Deparaffinize and hydrate to distilled water.
2. Stain in **Celestine Blue Solution** (F-365-1) for 30 minutes. Rinse in distilled water.
3. Place for 15 minutes in **Phosphotungstic Acid, 1%** (F-365-2) Wash in water for 3 minutes.
4. Place in working Acid Fuchsin solution * for 8 minutes.

Prepare working stain by mixing:

- **Acid Fuchsin, 1%** (F-365-3A) 10 ml
- **Oxalic Acid, 1%** (F-365-3B) 1 ml
- Distilled Water 80 ml

5. Dehydrate rapidly** in 95% alcohol, absolute alcohol, and clear in xylene, two changes each.

**Note:** Water and alcohol will remove red dye on prolonged exposure.


**Results:**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Bile canaliculi</td>
<td>Pink to red</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Blue</td>
</tr>
<tr>
<td>Bile</td>
<td>Yellowish-green</td>
</tr>
<tr>
<td>Iron</td>
<td>Brown with some red inclusions</td>
</tr>
<tr>
<td>Background</td>
<td>Bluish-pink</td>
</tr>
</tbody>
</table>

**References:**
Luna, L.G. (ed), Manual of Histologic Staining Methods of the AFIP, 3rd edition