D-250 MAY-GRUNWALD/GEIMSA COMBINED STAIN FOR BONE MARROW

Fixation:
2. Sections: Zenker’s or other well-fixed tissue. Start with step #1.

Sections: Cut paraffin section 6 microns.

Staining:
1. Deparaffinize and hydrate to distilled water.
2. Remove mercuric chloride crystals with Lugol’s or Gram’s Iodine solution. Clear with Sodium Thiosulfate, 5% for three minutes.
3. Wash in tap water, 10 min. and rinse in distilled water for two changes.
4. Place promptly in Methyl Alcohol, two changes, three times each.
5. Stain with May Grunwald Solution (D-250-1) or Jenner Solution (D-250-1A) 3 minutes. Add an equal volume of distilled water and allow standing one minute. Drain, no rinsing. Alternatively, stain in working Jenner’s (dil. 1:1 with distilled water) 6 minutes.
6. a. Sections: Stain in working Geimsa (freshly stain in working Geimsa(freshly diluted 2.5 ml Geimsa Stock Solution (D-250-2) in 50ml distilled water, do not reuse), 45 minutes.
b. Smears: Cover for 12 minutes with working Geimsa (15 drops of Geimsa Stock (D-250-2) to 10 drops of distilled water).
7. A. Sections: Differentiate each slide individually in Acetic Acid, 1% with gentle agitation, checking often under the microscope until nuclei are well defined. Rinse quickly in distilled water.
b. Smears: Differentiate is distilled water, agitating for approximately 5 seconds and checking under the microscope.
8. (Sections only) Dehydrate quickly in 95% alcohol and absolute alcohol and clear in two changes of xylene.
9. a. Sections: Mount with Permount (M-18) or other synthetic mountant.
b. Smears: Blot with fine grain filter paper and mount.

Results:

<table>
<thead>
<tr>
<th></th>
<th>Blue</th>
<th>Pink-Rose</th>
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<tbody>
<tr>
<td>Nuclei.</td>
<td>Bacteria</td>
<td>Cytoplasm</td>
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Tones are redder than when stained with Giemsa alone.

References: