

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

Fixation:

1. Smears: Draw a curretted fragment lightly across a sterile slide in a serpentine path. Don't allow the streak to dry completely. Start with step #4.
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 in 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:

Blue	Nuclei. Bacteria
Pink-Rose	Cytoplasm

Tones are redder than when stained with Giemsa alone.

References:

- Beck, R.C., Laboratory Manual of Hematological Technique, W.B. Saunders & Co., Philadelphia, c. 1938, pp 233-236.
- Clark, G. (ed) Staining Procedures, 3rd ed. Williams & Wilkins, Baltimore, c. 1973, pp 131-132.
- Strumia, M.M., J Lab Clin. Med 21:930-934, 1935-1936.
- Luna, L.G., (ed) Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed. McGraw-Hill, New York, c1968, pp121-122.

