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G-452 <u>PHOSPHOTUNGSTIC ACID</u> <u>HEMATOXYLIN (PTAH)</u>

Fixation: Bouin's Fluid (F-40), Formalin-Acetic Acid (F-121)

Sections: Paraffin @ 3-5 microns

Staining:

- 1. Deparaffinize and hydrate to distilled water.
- 2. Oxidize in freshly prepared acidified-permanganate for 5-40 seconds.

 *Prepare oxidizing solution by adding 0.16 ml Sulfuric Acid, concentrated (G-452-2) to 100 ml of Potassium Permanganate, 0.3% (G-452-1).
- 3. Rinse in water.
- 4. Decolorize in **Potassium Metabisulfite**, **4%** (G-452-3) for 5-10 seconds or until sections are colorless. Wash in running tap water for 5 minutes. Rinse in distilled water.
- 5. Mordant in <u>Ferric Ammonium Sulfate, 4%</u> (G-452-4) for two minutes to two hours. Rinse in distilled water.
- 6. Stain in **PTAH Solution** (G-452-5) for 16-48 hours.
- 7. Pass slides quickly through graded alcohols to 95% alcohol or blot and take directly to 95% alcohol.
- 8. Rinse slides in 95% alcohol until no more stain runs from the tissue, about 10 seconds to 2 minutes.
- 9. Dehydrate in absolute alcohol, clear and mount.

Stain Results:

Alpha cell granules Deep blue

RBC's Deep blue black

Background Pale pink or colorless

Nuclei of all cells are distinctly, but lightly stained.

References:

Clark, G.: Staining Procedures, Williams and Wilkins Co., Baltimore, 3rd Ed., c. 1973, p. 173.

Levene, C. & Feng, P., Stain Techn., 39:39-44, 1964.

Mallory, F.B., Pathological Technique, W. B. Saunders Co., Philadelphia, 1938.