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B-156 PRICE'S GIEMSA STAIN METHOD

Fixation: 10% Buffered Neutral Formalin or Zenker's. Use no fixation technique that will destroy erythrocytes.

Sections: Cut **paraffin** @ 4 microns

Staining:

1. Deparaffinize with two changes at 2 minutes each in xylene. Rinse in absolute alcohol for two changes, 2 minutes each.
2. Rinse in 95% Alcohol for two changes after a one minute immersion. If necessary to further remove mercuric chloride crystals, use an **Alcoholic Iodine Solution** (B-156-4), which will be further removed in successive alcohols.
3. Rinse through 80%, 70%, 50%, alcohols for one change, 1 minute each.
4. Rinse in distilled water for 15 seconds.
5. Buffer in the **Phosphate Buffer Solution**, (B-156-2) for 30 minutes.
6. Stain in the **Giemsa Working** (B-156-1A) overnight.
   To prepare Giemsa Working:
   . **Geimsa Stock** (B-156-1)…….3 parts
   Phosphate Buffer pH 7.0 (B-156-2)….97 parts
7. Rinse in the buffer solution.
8. Rinse in **Acetic Acid, 0.2%** (B-156-3), for one minute, absolute alcohol for two changes, 15 seconds each. This step should take only 90 seconds!!!
9. Dip in xylene for two changes, two minutes each.
10. Mount in Permount (M-18).

Cells:

Blue	Malarial parasites
Blue	Tissue Nuclei
Dark Blue	Bacteria
Black	Malarial Pigment
Blue	Schistosomic egg shells
Pale Pink	Collagen, etc.
Pink-Rose	Erthrocytes

References:

AFIP Manual of Histological Staining Methods, 3rd ed., L.Luna: New York: McGraw Hill
Publications, c. 1968, p. 127
Price, D.L., Mil.Med. 133:363-367, 1968.