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A-130 G IEMSA STAIN - RICKETTSIA

Fixation: **10% Buffered Neutral Formalin** (F-113) OR **Zenker's** (F-155)

Sections: Cut paraffin section @ 6 microns

Staining:

1. Deparaffinize and hydrate to distilled water.

2. If Zenker's is used as a fixative, remove the "Zenker Crystals" (mercuric chloride) by placing in **Lugol's Iodine** (A-130-5) or **Gram's Iodine** (A-130-5A) for 15 minutes. Rinse in tap water and place in **Sodium Thiosulfate, 5%** (A-130-6) for 3 minutes. Wash in tap water for 15 minutes. Rinse in distilled water.

3. Mordant in **Phosphate Buffer Solution** (A-130-3) for one hour.

4. Leave in **Giemsa Working Solution** (A-130-1A) overnight.
   To prepare Giemsa Working:
   Mix: 1 part **Giemsa Stock** (A-130-1)
   50 parts **Phosphate Buffer-pH 6.8** (A-130-3)

5. Rinse in the **Phosphate Buffer Solution** (A-130-3).

6. Dip in **Acetic Acid, 0.2%** (A-130-4) for 1 minute.

7. Rinse again in the **Phosphate Buffer Solution** (A-130-3).

8. Differentiate in **Rosin Alcohol Working Solution** (A-130-2A). Carefully check the sections with a microscope to observe the coloration of the rickettsiae to violet granules. This may take up to 3 minutes.
9. Dehydrate in absolute alcohol and clear in Xylene. Perform three changes of each.

10. Mount in **Permount** (M-18).

Stain Results:

Rickettsia	Violet
Nuclei	Blue
Cytoplasm, connective tissue	Pink
Erythrocytes	Salmon

Note: The differentiation of step 8 is the most critical and only if this is properly done can one expect good results. An electron microscope may be necessary as the rickettsiae are very small, 0.2-0.3 μ wide to 0.2-1.0 μ long.

References:

AFIP Manual of Histological Staining Methods, 3rd ed., Ed. L. Luna: New York McGraw Hill Publications, c. 1968, p. 235.

Clark, G.: Staining Procedures, Williams and Wilkins Co., Baltimore, 3rd Ed., c. 1973, p. 289.

Reviewed and Approved
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