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A-104 WADE'S METHOD FOR ACID-FAST ORGANISMS

FIXATION: **10% Buffered Neutral Formalin** (F-113) or **Zenker's Modified** (F-222).
TECHNIQUE: Cut paraffin at 6 microns

STAINING PROCEDURE: Use control slide

1. Deparaffinize in a solution made from 2 parts of rectified turpentine and 1 part liquid Petrolatum. Use 2 changes, 5 minutes each.
2. Drain; wipe off excess liquid, blot to opacity.
3. Wash in water for 2 minutes.
4. Stain in **Carbol New Fuchsin Solution** (A-104-1) overnight.
5. Wash in water for 2 minutes.
6. Place in **Formaldehyde, 37-40%,** (A-104-4) for 5 minutes.* Wash in running water for 2 minutes.

   *The bacilli become blue while the sections may turn blue or remain mostly reddish. If total demonstration of bacilli is in question, step 1 may be lengthened to 4-6 hours for "restoration" of poorly stained bacilli.

5. Place for 1 minute in **Sulfuric Acid, 5%,** (A-104-5). Wash thoroughly for 5 minutes in running water.
6. Place for 3 minutes in **Potassium Permanganate, 1%, Aqueous** (A-104-6). Rinse in water.
7. Bleach in **Oxalic Acid, 2%, Aqueous** (A-104-7) for 30-60 seconds and wash well in running water.
8. Counterstain in **Modified Van Gieson Solution** (A-104-2) for 3 minutes.
9. Dehydrate rapidly in 95% alcohol.
10. Clear in **Xylene** (C-120), two changes each.
11. Mount with **Permount** (M-18).

RESULTS:

Acid-fast bacilli	deep ultramarine blue or blue-black
Connective Tissue Elements	red
Background	yellowish

REFERENCES:

Wade, H.W., Amer. J. Path., 28:157, 1952.

Luna, L.G. (ed.), Manual of Histologic Staining Methods of the AFIP, 3rd edition, McGraw-Hill, N.Y., p. 220, c. 1968.