

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.

