

## **A-103 ZIEHL-NEELSEN METHOD FOR ACID FAST BACTERIA**

**Fixation:** Any well-fixed tissue.

**TECHNIQUE:** Cut paraffin at 6 microns.

**Staining:** Use control slide.

**Procedure:**

1. Deparaffinize and hydrate to distilled water.
2. **Ziehl Neelson Carbol Fuchsin** (A-103-1) for 30 minutes.
3. Wash in running water.
4. Decolorize with **Acid Alcohol, 1%** (A-103-3) or **Sulfuric Acid, 1%** (A-103-4) until sections are pale pink.
5. Wash thoroughly in running water for 8-10 minutes.
6. Counterstain with **Methylene Blue Working** (A-103-2A), dipping one slide at a time. Sections should be pale blue.

*To prepare working solution, mix:*

<b>Methylene Blue Stock</b> (A-100-2).	10 ml
Distilled water.	90 ml

7. Wash with tap water, then rinse in distilled water.
8. Dehydrate in two changes each of 95% alcohol.
9. Clear in two changes of **Xylene** (C-120).
10. Mount with **Permunt** (M-18).

**Stain Results:**

Acid Fast Bacilli	Bright Red
Erythrocytes	Yellowish Orange
Other Tissue Elements	Pale Blue

**References:**

AFIP Manual of Histological Staining Techniques, 3<sup>rd</sup>. ed. G. Luna, ed., New York, McGraw-Hill Publications, c 1968, p. 220.