

ZIEHL-NEELEN FOR ACID FAST BACTERIA

Purpose Demonstration of acid fast bacillus.

Principle The lipoid capsule of the organism takes up carbol-fuchsin and resists decolorization with dilute mineral acid.

Fixative 10% neutral buffered formalin.

Reagents

Ziehl-Neelsen Carbol Fuchsin (Purchased)

1% Acid Alcohol

70% alcohol ... 99.0 ml

Concentrated hydrochloric acid ... 1.0 ml

Methylene Blue (stock)

Methylene blue ... 1.4 g

95% alcohol ... 100.0 ml

Methylene Blue (working)

Methylene blue (stock) ... 2.5 ml

Tap water ... 22.5 ml

Quality Control Use a control slide with any tissue with acid fast bacteria.

Procedure

1. Deparaffinize and hydrate to distilled water.
 2. Carbol Fuchsin solution for 30 minutes.
 3. Wash well in running water.
 4. Decolorize with 1% acid alcohol until sections are pale pink.
 5. Wash thoroughly in running water for 8 minutes.
 6. Counterstain by dipping one slide at a time into working Methylene blue solution. Sections should be pale blue.
 7. Wash with tap water.
 8. Dehydrate in 95% alcohol, absolute alcohol and clear in xylene, two changes each.
- Mount with synthetic resin.

Results

Acid-fast bacilli ... bright red

Erythrocytes ... yellowish-orange

Other tissue elements ... pale blue

References

Manual of Histologic Staining Methods, Armed Forces Institute of Pathology, 3rd Edition, p. 200.