

Ziehl-Neelson Staining

Principle: The cell surface component of some bacteria contain waxes and phospholipids (Mycolic acid) so they do not stain with ordinary stains. They can be stained by Z.N staining method.

Materials / Requirements:

Primary dye: Carbol fuchsin.

Decolorizer: HCl 3% v/v or H₂SO₄ 20%.

Counter stain: Malachite green or Methylene blue.

Steps:

1. Sterilize the wire loop properly. Transfer purulent material /sputum on the slide. Spread the specimen evenly covering an area of about 15-20 mm diameter on the slide. Sterilize loop properly. Dry the smear in air and fix it by applying gentle heat or alcohol. Label the slide.
2. Cover the smear with corbol fuchsin stain.
3. Heat the stain until vapours rise. (60°C). Do not over heat. Allow the heated stain remain on slide for 5 minutes.
4. Wash with water.
5. Cover the slide with 20% H₂SO₄ or 3% HCL, until smear is sufficiently decolorized. i.e. pale pink.
6. Wash the slide well in water.
7. Counter stain it with methylene blue or malachite green for 1-2 minutes.
8. Wash it with water.
9. Let it dry and observe under oil immersion lens for Acid Fast Bacilli.

Result:

Acid fast bacilli stain as red, straight or highly covered rods occurring singly or in small groups. While the tissue cells and other organisms are stained blue or green according to the counter stain used.

Quality Control:

At regular intervals and always, when a new batch of stain is started. Two sputum smears of known high and low AFB positively should be stained. To check that carbol fuchsin staining method and microscopical examination of smear are satisfactory.

Acid Fast Bacteria:

Mycobacterium tuberculosis
Mycobacterium ulcerans
Mycobacterium bovis

Weakly Acid Fast Organism:

Certain organisms are weakly acid fast. For staining of these organisms 1HCL or 5% H_2SO_4 is used as decolorizer.

Nocardia

Mycobacterium leprae

Candida spores

Actinomyces

Cryptosporidium

WHO Reporting:

More than 10 AFB/field= Report as +++

1-10 AFB/field=Report as ++

10-100 AFB/100 field=Report as +

1-9 AFB/100 field= Report the exact number