

Ziehl-Neelsen Staining for Acid Fast Organisms

Reagents Needed:

Fuchsin-Carbol Stain, Ziehl-Neelsen
Methylene Blue, Loeffler

RICCA CHEMICAL COMPANY Cat. No. 3220
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Recommended Method:

1. Prepare an even, thin 3-4 cm² smear using a flame-sterilized loop, with as little mechanical manipulation as possible. Unnecessary movement of the loop may injure bacterial cells, which may interfere with proper staining.
2. Heat fix by heating gently in the flame, or by placing on a temperature-controlled slide warmer at 65°C for at least 2 hours (overnight fixation is acceptable).
3. Cover the fixed smear with a piece of filter paper just smaller than the slide, but covering the fixed smear.
4. Flood with Fuchsin-Carbol Stain solution. Heat gently to steaming (do not allow stain to boil) and keep hot for 5 minutes. Always keep the filter paper flooded with stain.
 Caution: Do not allow the solution to evaporate to the point at which the dye will crystallize out.
 Note: Overnight staining at room temperature gives acceptable results.
5. Remove filter paper with forceps and cool the slide.
6. Rinse briefly (5-10 sec) in tap water.
7. Decolorize with 3% Hydrochloric Acid in 95% Alcohol by allowing the decolorizer to run over the surface of the smear. Decolorization times are usually 5-30 seconds for thin smears and up to 2 minutes for thick smears.
8. Rinse briefly in tap water.
9. Flood the smear with Loeffler Methylene Blue for 1-2 minutes.
10. Rinse briefly in tap water.
11. Blot dry and examine.

Satisfactory Staining Results:

Acid-fast microorganisms stain bright Red.
Other organisms and the background stain Blue.

Possible causes of unsatisfactory staining:

Excessively long heating time during staining or allowing the smear to dry too much before decolorizing can result in smears staining too dark.

Insufficient heating time during staining or excessive decolorization can result in smears staining too light.

Excessive rinsing after the Fuchsin-Carbol can cause acid-fast bacteria to stain too light.

Excessive rinsing after the Methylene Blue can cause other organisms and the background to stain too light.

This is a typical staining procedure. These reagents may be suitable for other staining procedures. Consult staining reference books or standard operating procedures for other suitable uses of these products.