

ZIEHL-NEELSEN STAIN WITH METHYLENE BLUE (NON-CARBOL)

Principle

Ziehl-Neelsen stain is a useful differential staining to detect acid-alcohol resistant bacteria among them the identification of mycobacteria, although it does not allow the differentiation of different species. The acid-resistance characteristic of these microorganisms makes this coloration a rapid method in the presumptive diagnosis of mycobacterial infection. Mycobacteria are colored red by Fuchsin and retain color despite the action of acid and alcohol.

Material

Well-fixed tissues in paraffin sections.

Reagents

Code	Description
251333	Ziehl-Neelsen Carbol-Fuchsin Basic solution for clinical diagnosis (*)
251804	Alcohol-Hydrochloric 8:2 for clinical diagnosis
251170	Methylene Blue (C.I. 52015) for clinical diagnosis (*)
251769	Xylene, mixture of isomers for clinical diagnosis (*)
251085	Ethanol 96% v/v for clinical diagnosis (*)
251086	Ethanol absolute for clinical diagnosis (*)
253681	Eukitt [®] , mounting medium for clinical diagnosis

Preparation of solutions

1. Solution of 1.4% Methylene Blue.
Dissolve 1.4 g of Methylene Blue (C.I. 52015) in 100 ml of 96% ethanol.
2. Diluted methylene blue solution.
Dilute 10 ml of 1.4% Methylene Blue Solution in 90 ml of water.

Procedure

1. Deparaffinate and hydrate until distilled water is reached.
2. Color with Ziehl-Neelsen Carbol-Fuchsin Basic solution according to Ziehl for 30 minutes at room temperature.
3. Rinse thoroughly with running water.
4. Decolor with 8: 2 alcohol-hydrochloric acid until the sections appear pale pink.
5. Rinse thoroughly with running water for 8 minutes.
6. Contrast by immersing the foil in the solution of methylene blue diluted for 30 seconds. The sections should be pale blue. Excessive contrast can mask bacilli.
7. Wash with water and then with distilled water.

8. Dehydrate rapidly with 96% Ethanol and Absolute Ethanol 2 changes each, rinse with 2 xylene changes, 2 minutes each.
9. Mount with mounting medium.
10. Observe under a microscope.

Results

Acid-fast bacilli: Red

Erythrocytes: Orange yellow

Other Tissue Elements: Blue

Technical note

The microscope used should correspond to the requirements of a clinical diagnostic laboratory. If an automatic staining device is used, the operating instructions of the appliance manufacturer and the software must be observed.

Sample preparation

All samples should be treated according to the state of the technology. All samples must be unambiguously labeled.

Diagnostics

Diagnosis should be established only by authorized and qualified persons. Each application should involve appropriate controls to rule out erroneous results.

Storage

The staining solution should be stored at room temperature.

Expiration

The product stored at the indicated temperature and in a tightly closed container is usable until the expiration date indicated on the package.

Notes on use

To avoid errors, the staining must be carried out by specialized personnel. For professional use only. The national directives on safety at work and quality assurance must be complied with.

Advise on disposal of waste

Solutions used and expired solutions should be disposed of as hazardous waste and local waste disposal regulations must be observed. If further questions are asked about disposal, they may be processed through E-Mail: info.es@itwreagents.com. Inside the EU are valid the requirements based on Council Directive 67/548/EEC on the approximation of the laws, regulations and laws, regulations and administrative provisions relating to the classification, packaging and labeling of dangerous substances in the relevant version.

Classification of hazardous substances

Observe the classification of dangerous substances on the label and the information on the safety data sheet.

Manufacturer

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(*) Sanitary product for In Vitro Diagnostics

