ZN Stains Kit (AFB stain) RDM-K-ZN-01

Principle

ZN (Ziehl-Neelsen staining) staining is a differential staining technique that was first developed by Ziehl and Neelsen. The main aim of this staining is to differentiate bacteria into acid fast bacteria (AFB) and non-acid-fast bacteria.

This differential staining technique is useful for identification of the *Mycobacteria* species, especially *Mycobacteria tuberculosis* and Nocardia, which depends on the chemical composition of the bacterial cell wall. The mycobacterial cell wall contains mycolic acids, which are difficult to stain due to the high lipid content (mycolic acid) in the cell walls. When the smear is stained with carbol fuchsin, it solubilizes the lipoidal material present in the *Mycobacterial* cell wall, but with the application of heat, carbol fuchsin further penetrates through the lipoidal wall and enters the cytoplasm, and all cells appear red. The cells were decolorized with a decolorizing agent (3% HCL in 95% alcohol), but the acid-fast cells were resistant due to the presence of a large amount of lipoidal material in their cell walls, which prevented the penetration of the decolorizing solution. The non-acid fast organism lacks the lipoidal material in their cell wall, due to which they are easily decolorized, leaving the cells colorless. The counterstain, methylene blue and only decolorized cells absorb the counterstain and take its color, which appears blue, while acid-fast cells retain the red color.

Use: To differentiate between acid fast and non-acid fast bacteria.

Contents*

Ingredients	
Carbol Fuchsin	
Basic Fuchsin	0.30 gm
Ethyl alcohol	10.0 ml
95% Phenol	5.0 gm
Distilled Water	95.0 ml
Acid Fast Decolorizer	
Hydrochloric acid, concentrated	3.0 ml
95% Ethyl alcohol	97.0 ml
Loeffler's - Methylene Blue	
Methylene Blue	0.30 gm
95% Ethyl alcohol	30.0 ml
Distilled Water	100.0 ml

* Formula adjusted for optimum performance and parameters

Directions:

Add one loopful of test organism suspension to a microscope slide and make a heavy smear. Allow it to air dry and heat fix, and then flood the smear with carbol fuchsin stain and heat to steam for 5 minutes. (Note: Continue to apply stain if the filter paper begins to dry.) Wash in running tap water and add acid fast decolorizer for 2 minutes to decolorize the cell. Wash with tap water and apply counterstain methylene blue for 30 seconds. Wash with tap water, dry in the air, then examine under an oil immersion objective.

Specimens' types analyzed

Bacterial samples etc.

Precautions to be taken

1. Reagents must be added in the order and the amounts specified or a weak-positive or false-negative reaction may occur.
2. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.
3. The test is an aid to identification and is not a confirmatory test. Complete identification should include determination of morphology, other biochemical and serological tests.
4. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.

Performance and Evaluation

The expected performance of the stain kit is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

Physical Parameters:

Physical state - Liquid.
Carbol Fuchsin – Red, clear solution.
Decolorizer – Colorless, clear solution.
Methylene Blue- Blue, clear solution.

Different Microbial Response

Acid fast bacteria: Bright red color observe under oil immersion lens microscope.
Non-acid fast bacteria: Blue color observe under oil immersion lens microscope.

Storage and Shelf Life: Keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label.

Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. Ogodo, A. C., Agwaranze, D. I., Daji, M., & Aso, R. E. (2022). Microbial techniques and methods: Basic techniques and microscopy. In *Analytical Techniques in Biosciences* (pp. 201-220). Academic Press.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
3. Nurzynska, K., Li, D., Walts, A. E., & Gertych, A. (2023). Multilayer outperforms single-layer slide scanning in AI-based classification of whole slide images with low-burden acid-fast mycobacteria (AFB). *Computer Methods and Programs in Biomedicine*, *234*, 107518.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

rdmsales@chaitanyagroupindia.com, mkt.cabt@chaitanyagroupindia.com, www.chaitanyagroupindia.com