AFB Rapid Staining Kit (Cold Method) RDM-K-AFB-01

Principle

The AFB rapid staining kit (Cold Method) is a differential staining technique. 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 the identification of *Mycobacteria* species, especially *Mycobacteria tuberculosis* and *Mycobacteria leprae.* In this kit, the primary stain is Rapid AFB stain A, which is composed of pararosaniline, phenol, and detergent. In the presence of phenol and detergent, pararosaniline stains acid fast bacilli (AFB) and retains its red- color. The use of surface-active detergents catalyzes the penetration of stain-resistant bacilli. The counterstain is Rapid AFB stain B, which contains decolorizers (sulfuric acid and propanol) and counterstain methylene blue. The acid is decolorizing the primary stain and simultaneously methylene blue staining non-acid fast bacilli. All backgrounds get a bluish to light red color except acid-fast bacilli, which remain red colored. This simplicity of staining makes Rapid an ideal screening tool.

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

Contents*

Ingredients	
Rapid AFB stain A	**100 mL**
Pararosaniline Chloride	30 mMol/L
Methanol	1.5 mMol/L
Phenol	265 mMol/L
Detergent (Contains Stabilizer and preservatives)	1.00 %
Rapid AFB stain B	**100 mL**
Methylene blue	30 mMol/L
Sulfuric acid	7.0 Mol/L
Propanol	5.0 Mol/L

* 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 by gentle warming. then flood the smear with primary stain Rapid AFB stain A and wait for 5-6 minute and then wash in running tap water. Apply counter stain Rapid AFB stain B for 1-1½ minute. Wash in running tap water for 1-2 minutes. Dr y in the air, then examine under an oil immersion objective

Specimens' types analyzed

Clinical (Note: all samples should be free from blood) and non-clinical samples

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.

Note: Acid-fast bacilli other than the Mycobacterium group may be found red colored in the stained smears. The stain may be weakened by continuous staining on many slides. Periodical checking with known positive and negative smears must be performed as a quality control measure to establish the acceptability of staining results.

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.
Rapid AFB stain A – Red, clear to slightly opalescent solution.
Rapid AFB stain B – Colorless, clear solution.

Different Microbial Response
Acid fast bacterial:
Mycobacterium tuberculosis (ATCC: 25618): Dark to pale red color observes 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.
5. Path. Tech., W.B. Saunders & Co., Philadelphia PA 1938, P276.

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