



Fluorescent Stains Kit for Mycobacteria

K021

Intended Use

Fluorescent Stains Kit for Mycobacteria is used for microscopic observation of Acid Fast bacilli by fluorescence microscopy.

Composition**

Ingredients

Phenolic auramine O (S042)	-
Auramine O	0.300 gm
Phenol	3.000 gm
Distilled Water	100.000 ml
Mycobacteria decolourizer (S043)	-
75% v/v aqueous Ethanol	99.500 ml
Sodium chloride	0.500 gm
Hydrochloric acid	0.500 ml
Potassium permanganate (S044)	-
Potassium permanganate	0.100 gm
Distilled Water	100.000 ml

**Formula adjusted, standardized to suit performance parameters

Directions

- 1) Thoroughly flame one side of a clean glass slide to be used for the smear and allow to cool before smearing.
- 2) Prepare a smear from the specimen on the flamed side of the slide and allow to air dry.
- 3) Fix the smear by passing the slide through a flame.
- 4) Flood the slide with phenolic auramine(S042) Heat to steaming for 10-15 minutes with a low flame; do not boil the stain and do not permit drying of the smear.
- 5) Rinse with distilled water and drain off excess liquid.
- 6) Flood the slide with decolorizer (S043) for 2-3 min. Slide will still appear pink.
- 7) Rinse thoroughly with distilled water, drain off excess.
- 8) Flood slide with counterstain (S044) for 3-4 min. Do not allow slide to dry.
- 9) Rinse thoroughly with distilled water and allow to air dry.
- 10) Examine the slide by fluorescent microscope.

Principle And Interpretation

This differential staining technique is useful for identification of the tubercle bacillus, other Mycobacteria, and Nocardia, which depends on the chemical composition of the bacterial cell wall. Because of the difficulty in staining these organisms with ordinary dyes, basic dyes in the presence of controlled amounts of acid are used. Generally, heat must be applied during the staining procedure. The phenolated fluorescent dye is used to stain acid fast bacilli. The advantage of a fluorescent procedure is that the acid-fast organisms appear as bright fluorescence against a dark background. Mycobacteria decolourizer is used for decolorizing the non-acid fast bacteria or other cellular matter ; while Potassium permanganate is used as counter stain.

Type of Specimen

Clinical specimen - Sputum, pus, CSF, body fluids, enriched cultures from clinical materials or histological sections

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3). Sputum and other mucilaginous fluids should be pretreated to dissolve the mucus and other artefacts as per standard protocol (2,3). After use, contaminated materials must be sterilized by autoclaving before discarding

Limitations :

1. Thick smears will interfere with proper decolorisation, and counterstain may mask the presence of AFB. Additionally, thick smears have a tendency to flake, resulting in loss of smear material and possible transfer of material to other slides.
2. Strong counterstain may mask the presence of AFB.
3. Smears that have been examined by Fluorescence microscopy may be restained by Z-N staining to confirm observations.
4. Fluorescent stained smear are to be read within 24 hours of staining because of fading.

Performance and Evaluation

Performance of the product is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Microscopic examination

Fluorescent staining is carried out and observed under Fluorescence microscope. Slides can be screened under high power (400 X) and verified under oil immersion lens.

Results

Acid fast bacilli : luminous yellow rods in dark field

Storage and Shelf Life

Store between 10- 30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Staining Procedures 4th edition: Edited by George Clark
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
3. 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.

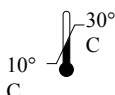
IVD

In vitro diagnostic
medical device

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CE
Marking



Storage
temperature



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is damaged



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