ACID-FAST STAINS AND REAGENTS

PRODUCT:

Bottle:[a]

Auramine-Rhodamine Fluorescent Stain, item no.R5881
Auramine-Rhodamine Decolorizer, item no. R5882
Auramine-O Flourescent Stain, item no. R5803
Auramine-O and Auramine-Rhodamine Flourescent Counterstain, item no. R5804
Kinyoun Carbol Fuchsin Stain, item no. R5895 (250ml), R5896 (1 liter)
Kinyoun Decolorizer, item no. R5915 (250ml), R5916 (1 liter)
Methylene Blue Counterstain,item no. R5920 (250ml) R5921 (1 liter)
Kinyoun Carbol Fuchsin Stain Set, item no. R5925

[a]see catalog for ordering options

PURPOSE:

Acid-fast stains are used to stain acid-fast organisms, including *Mycobacterium* species, in specimens and cultures.

PRINCIPLE:

In 1882 Ziehl et al.[8] demonstrated that certain bacteria stained with hot fuchsin resisted decolorization with acid alcohol. These organisms were identified as acid-fast. Kinyoun et al.[1] modified the technique so that heat was not required. Acid- fast characteristics (resistance to the acid decolorizer) are due to the high lipid and wax content of mycobacteria. Phenol-fuchsin has greater solubility in cellular lipids and waxes than the decolorizer. Methylene blue acts as a counterstain staining the background blue.

Later, the fluorochrome dyes auramine and rhodamine were used to demonstrate acid-fastness in bacilli.[4,5] These stains selectively dye mycobacteria by binding to the mycolic acid of the cell wall. This method is a better screening method than the conventional acid-fast stain, and allows for screening at a lower magnification. A modification of the stain allows for screening fluorochrome-stained mycobacteria against an orange background.[6]

FORMULAS:

Approximate ingredients per liter.

(1) Kinyoun Carbol Fuchsin Stain:

Water, Deionized	1000.00 ml
Ethanol, Denatured	200.00
Basic Fuchsin	26.67 g
Phenol	66.67

(2) Kinyoun Decolorizer:

Ethanol, Denatured	970.0 ml
Hydrochloric Acid, 12 N	30.0

(3) Methylene Blue Counterstain:

Water, Deionized	1000.0 ml
Methylene Blue	3.0 g

(4) Auramine-Rhodamine Fluorescent Stain:

Water, Deionized	375.00 ml
Glycerol	562.50
Auramine O	11.25 g
Rhodamine B	5.63
Phenol	75.00

(5) Auramine-Rhodamine Decolorizer:

Water, Deionized	300.0 ml
Ethanol, Denatured	700.0
Hydrochloric Acid, 12 N	5.0

(6) Auramine O and Auramine-Rhodamine Flourescent Counterstain:

Water, Deionized	1000.0 ml
Potassium Permanganate	5.0 g

(7) Auramine O Flourescent Stain:

Water, Deionized	870.0 ml
Auramine O	1.0 g
Ethanol, denatured	100.0 ml
Phenol	30.0 g

PRECAUTIONS:*

For in vitro diagnostic use. Observe all safety precautions consistent with the hazard(s) stated on the product label and/or Material Safety Data Sheet. Kinyoun Carbol Fuchsin and Auramine-Rhodamine Fluorescent Stain are carcinogenic and should be handled with caution.

Storage: Upon receipt store at 10-30°C away from direct light. Kinyoun Carbol Fuchsin Stain, Methylene Blue Counterstain, and Auramine-Rhodamine Fluorescent Stain should be stored in the dark. Stains should not be used if there are signs of deterioration or if the expiration date has passed.

Limitations: A fluorochrome stain provides a good means for screening smears for acid-fast bacilli, but requires a fluorescent scope for evaluation.

Cryptosporidium species oocysts can be stained with acid-fast stains; for further information refer to Cryptosporidium Stain Set, Data #890.

Fluorochrome-stained smears cannot be used to estimate inoculum size for drug susceptibility testing.

The Ziehl-Nielsen or Kinyoun Stain should confirm all fluorochrome-positive stains.

Slides should be stained individually since mass staining in a common container may allow cross-transfer of acid-fast bacilli.

Take care to wipe the lens after each examination when viewing under oil immersion, especially if the previous smear showed acid-fast bacilli, since the organisms can be transferred from slide to slide.

PROCEDURE:*

Specimen Collection: Information on specimen collection and transport is found in standard reference material on the subject. In general, specimens should be delivered to the laboratory without delay. Consult appropriate references for details in preparing a primary stain.[2,3,7] Procedures may differ according to the type of specimen.

Method of Use, Kinyoun: Smears should be heat-fixed on a slide-warmer at 65-75°C for 2 hours or overnight at room temperature. For the Kinyoun stain, flood the heat-fixed smear with Carbol Fuchsin for 2 minutes at room temperature. Rinse with tap water. Decolorize with the acid-alcohol until the pink color fades. Rinse with tap water. Counterstain with Methylene Blue for 20-30 seconds. Rinse with tap water. Air dry and examine microscopically using low and oil-immersion lenses.

Method of Use, Auramine-Rhodamine: Flood the slides with fluorescent stain and let stand 15 minutes at room temperature. DO NOT HEAT. Rinse with tap water. Decolorize 2 minutes with the fluorescent decolorizer. Rinse with tap water. Counterstain for 3 minutes with 0.5% aqueous potassium permanganate or Acridine Orange. Rinse with tap water. Drain and air dry. Examine under a fluorescent microscope with low and high-dry magnification.

Interpretation: For Kinyoun stain: Acid-fast bacilli stain red against a blue background. The smear should be examined with an oil immersion lens. Three long lines the length of the smear or nine short lines the width of the smear should be examined before considering the smear negative.

For Fluorescent Stain: Acid-fast bacilli appear as bright greenish-yellow fluorescing bacilli against a green-black background in the case of potassium permanganate counterstain, or an orange background in the case of acridine orange counterstain.

Materials Required but Not Provided: Standard microbiological supplies and equipment such as loops, needles, pipettes, incinerators, microscope slides, and safety hood, as well as counterstains for fluorescent staining, are not provided.

QUALITY CONTROL:*

Using prepared smears of known positive and negative organisms controls the effectiveness of the stain and the staining technique. Using control slides, stain simultaneously with test slides. Any inconsistencies in the control slides are an indication that the technique was incorrect or that the stain is defective.

User Quality Control: Check for signs of deterioration. Check the performance of the reagents by including quality control slides with each test run.

BIBLIOGRAPHY:

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3. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington D. C., 1985.
4. Manfield, R. E., *Am. J. Clin. Pathol*., 53:394, 1970.
5. Morse, W. C., et al., *Mycobacter. Lab. Methods*, Report No. 317., U. S. Army Medical Research and Nutrition Laboratory, Denver, Co.
6. Smithwick, R. W., et al., *Tubercle*, 52:226-231, 1971.
7. Vestal, A. L., *Procedures for the Isolation and Identification of Mycobacteria*, USDHEW, Public Health Service No. 1995, U.S. Government Printing, Washington, D. C., 1969.
8. Ziehl, F., *Deustch. Med. Wchnschr*. 33:451, 1882.

*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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