remel

PHENOLIC ACRIDINE ORANGE DECOLORIZER

INTENDED USE

Remel Phenolic Acridine Orange Decolorizer is a reagent recommended for use in qualitative procedures with Phenolic Acridine Orange Stain for detection of acid-fast bacilli in clinical specimens.

SUMMARY AND EXPLANATION

In 1977, Kronvall and Myhre described the use of acridine orange stain to detect microorganisms in direct smears prepared from clinical specimens.[1] They reported acridine orange buffered at a low pH produced differential staining of bacteria and background material in clinical specimens. Katila et al. evaluated acridine orange in an acid-fast staining procedure which required a prestaining acidification step and found it could be used in place of auramine O; however, the stain components were limited by a ten-day shelf life.[2] In 1995, Smithwick et al. described an acid-fast method which incorporated an acridine orange solution containing phenol and a decolorizing reagent, with methylene blue as a counterstain.[3] They compared Phenolic Acridine Orange Stain with auramine O in the acid-fast staining procedure. Phenolic Acridine Orange Stain was found to produce less nonspecific fluorescence of background material, making it superior to auramine O for detection of acid-fast bacilli in clinical specimens.

PRINCIPLE

Acridine orange is a dye which binds to the nucleic acid of bacteria and other cells, either in the native or the denatured state. Phenol enhances penetration of acridine orange through the mycobacterial cell wall. Glycerol increases the viscosity of the solution, which tends to keep the stain on the smear during the staining procedure. Methylene blue is added to Phenolic Acridine Orange Decolorizer to reduce background fluorescence in stained smears.

REAGENTS (CLASSICAL FORMULA)*

Methylene Blue (CAS 61-73-4)	2.0 g
95% Ethyl Alcohol (CAS 64-17-5)	740.0 ml
Hydrochloric Acid, Concentrated (CAS 7647-01-0)	5.0 ml
Demineralized Water (CAS 7732-18-5)	260.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, media, and test materials after use. Directions should be read and followed carefully. Refer to the Material Safety Data Sheet for additional information on reagent chemicals.

DANGER! Flammable, keep away from heat, sparks, and flame. May cause irritation to skin, eyes, and respiratory tract. Avoid breathing vapor and eye/skin contact.

STORAGE

This product is ready for use, and no further preparation is necessary. Store product in its original container at room temperature until used. Protect from light.

PRODUCT DETERIORATION

This product should not be used if (1) the color has changed from a bright blue, clear liquid, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.[4]

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Phenolic Acridine Orange Stain (REF R40180), (7) Sterile disposable pipettes, (8) Glass slides, (9) Bunsen burner or slide warmer, (10) Demineralized water, (11) Fluorescent microscope, immersion oil.

PROCEDURE

Follow established laboratory safety procedures when working with acid-fast cultures and specimens. Consult appropriate references when necessary for detailed procedural information on specimen processing.[4,5]

1. Transfer a portion of the specimen onto a clean, labeled microscope slide using a swab, loop, or pipette. For concentrated specimens, use the sediment obtained from the concentration process. For unconcentrated specimens, use bloody or necrotic material if present. (**Note:** For CSF sediment, use a sterile Pasteur pipette to place one drop on the center of a clean glass slide. Allow the slide to air dry. Repeat two more times, each time placing the new drop directly over the previous drop.)
2. Spread the specimen uniformly to make a thin smear covering an area approximately 1 by 2 cm. Allow the smear to air dry.
3. Heat-fix the smear by passing the slide through the flame of a Bunsen burner or place it on a slide warmer (65-75°C) for 2 hours. Slides should be considered potentially infectious and handled accordingly as heat fixing does not always kill mycobacteria.
4. Flood the slide with Phenolic Acridine Orange Stain.
5. Allow the slide to stain at room temperature for 15 minutes.
6. Rinse with demineralized water and drain.
7. Flood the slide with Phenolic Acridine Orange Decolorizer.
8. Allow the slide to decolorize at room temperature for 2 minutes.
9. Rinse with demineralized water, drain, and air dry. Do not blot.
10. Examine smears with a fluorescent microscope using 200 x magnification. Confirm the morphology of organisms using 600 x magnification.

INTERPRETATION

Positive - Bright-red to orange fluorescent bacilli with characteristic morphology confirmed at 600 x magnification

Negative - No bright-red to orange fluorescent bacilli observed after examining 30 fields at 200 x magnification

Note: Background material may fluoresce pale yellow-green. Red-fluorescing background material may be seen but is distinguishable from acid-fast bacilli based on morphology at 600 x magnification.

QUALITY CONTROL

All lot numbers of Phenolic Acridine Orange Decolorizer have been tested and found to be acceptable. Positive and negative control slides should be included whenever the acid-fast staining procedure is performed and upon receipt of new lot numbers of staining reagents.[5] Quality control procedures should be established by each laboratory according to applicable regulatory guidelines. If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

1. Slides subjected to prolonged light exposure may demonstrate reduced fluorescence.

BIBLIOGRAPHY

1. Kronvall, G. and E. Myhre. 1977. Acta. Path. Microbiol. Scand. Sec. B. 85:249-254.
2. Katila, M.L. and R.A. Mantyjarvi. 1982. Eur. J. Clin. Microbiol. 1:351-353.
3. Smithwick, R.W., M.R. Bigbie, Jr., R.B. Ferguson, M.A. Karlix, and C.K. Wallis. 1995. J. Clin. Microbiol. 33:2763-2764.
4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.
5. Garcia, L.S. 2010. Clinical Microbiology Procedures Handbook. 3rd ed. ASM Press, Washington, D.C.

PACKAGING

REF R40181, Phenolic Acridine Orange Decolorizer ..... 250 ml/Btl

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

CAS (Chemical Abstracts Service Registry No.)

IFU 40181, Revised September 16, 2010 Printed in U.S.A.

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