

remel

Acridine Orange

INTENDED USE

Remel Acridine Orange is a stain recommended for use in qualitative procedures in the fluorescent microscopic detection of microorganisms from 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 that Acridine Orange buffered at a low pH produced differential staining of bacteria and background material in clinical specimens. Human cells and tissue material stain a pale green to yellow, while bacteria stain a bright orange at a pH of 4.0. In 1980, McCarthy and Senne evaluated the use of Acridine Orange for the detection of microorganisms in blood cultures.² They found Acridine Orange to be a rapid, inexpensive alternative to blind subcultures and more sensitive than Gram stains for detecting microorganisms in smears. Detection levels were reported as low as 1 x 10⁴ colony forming units per ml. In 1981, Lauer, Reller, and Mirrett compared Acridine Orange with the Gram stain for detection of microorganisms in cerebrospinal fluid, other body fluids, tissues, and exudates.³ Their results supported the findings of Kronvall and Myhre showing the Acridine Orange stain to be more sensitive than the Gram stain and equally specific. Acridine Orange has also been used for the detection of *Trichomonas vaginalis* in genital tract specimens, *Neisseria gonorrhoeae* in cervical and urethral smears, *Helicobacter pylori* in gastric biopsies, and the enumeration of mycoplasmas in broth culture.^{4,5,6,7}

PRINCIPLE

Acridine Orange is a fluorochrome dye, which binds to nucleic acids of bacteria and other cells, either in the native or the denatured state. The low pH of the buffer solution results in an orange staining of bacteria and fungi, and a green to yellow staining of human epithelial and inflammatory cells and background debris.

REAGENTS (CLASSICAL FORMULA)*

Acridine Orange (CAS 10127-02-3).....0.1 g
0.2 M Acetate Buffer (pH 4.0)1000.0 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, and media after use. Directions should be read and followed carefully.

STORAGE

This product should be stored in its original container at 2-8°C. Protect product from light.

PRODUCT DETERIORATION

This product should not be used if (1) the color has changed from an orange-yellow, 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.^{8,9}

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) Absolute methanol (REF 40121) (optional), (7) Glass slides, (8) Bunsen burner or slide warmer, (9) Fluorescent microscope, (10) Immersion oil.

PROCEDURE

1. Prepare smears following laboratory procedures and allow to air dry.
2. Fix slide with absolute methanol for 2 minutes or heat fix.
3. Flood slide with Acridine Orange stain and allow to remain on surface for 2 minutes.
4. Rinse slide with water and allow to air dry.
5. Examine the slide under 100X to 400X magnification using a fluorescent microscope. Confirm by examination at 1000X under oil immersion.

INTERPRETATION

Bacteria and yeast will fluoresce bright orange against a green-fluorescing or dark background. The nuclei of blood cells may also fluoresce. Leukocytes stain pale apple green. Erythrocytes may not stain or stain pale green.

QUALITY CONTROL

All lot numbers of Acridine Orange have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

Escherichia coli ATCC® 25922
Staphylococcus aureus ATCC® 25923

RESULTS

Orange fluorescence
Orange fluorescence

LIMITATIONS

1. The presence of microorganisms in smears stained by the Acridine Orange method should be confirmed by culture.
2. A Gram stain must be performed to distinguish between gram-positive and gram-negative organisms. The Gram reaction may be determined by Gram staining directly over the Acridine Orange stain after removal of the immersion oil.

3. Avoid excessive exposure of stained smears to light as it may lower the intensity of fluorescence of the organisms.
4. The Acridine Orange stain is capable of detecting bacteria in concentrations of approximately 10^4 colony forming units per ml.⁵
5. Certain debris may fluoresce yellow, orange, or red. Examination at a higher magnification will differentiate on the basis of morphology.⁵
6. Nuclei or granules from disintegrating leukocytes may resemble cocci at lower magnifications. They may be distinguished on the basis of morphology at higher magnifications (1000X).³
7. Occasionally, bacteria appear as faint, gray silhouettes; other brightly staining organisms should be present in large numbers on the same smear.³

BIBLIOGRAPHY




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PACKAGING

REF 40010, Acridine Orange, 250 ml/Btl Ea

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)

Manufactured for Remel Inc.
 ATCC® is a registered trademark of American Type Culture Collection.
 CAS (Chemical Abstracts Service Registry No.)

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