remel

BactiDrop™ Acridine Orange

(English)

INTENDED USE

Remel BactiDrop™ Acridine Orange stain is recommended for use in qualitative procedures in the fluorescent microscopic detection of microorganisms from clinical specimens and blood cultures.

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 10^4 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,[4] *Neisseria gonorrhoeae* in cervical and urethral smears,[5] *Helicobacter pylori* in gastric biopsies,[6] and the enumeration of mycoplasmas in broth culture.[7]

PRINCIPLE

Fluorochrome acridine orange is a 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)*

Sodium Acetate (CAS 127-09-3)	44.26	g
Acridine Orange (CAS 10127-02-3)	0.1	g
Hydrochloric Acid (CAS 7647-01-0)	38.0	ml
Demineralized Water (CAS 7732-18-5)	962.0	ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

WARNING! May cause eye, skin, and respiratory tract irritation. Corrosive to metal.

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. Refer to Material Safety Data Sheet for additional information.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 20-25°C until used. Do not freeze or overheat. Protect from light.

PRODUCT DETERIORATION

This product should not be used if (1) there is evidence of dehydration, (2) the color has changed, (3) the expiration date has passed, or (4) there are other signs of deterioration. The expiration date applies to the product in its intact container when stored as directed. Discard remaining portion of partially used ampule at end of workday.

SPECIMEN COLLECTION, STORAGE, 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, blood culture media, (5) Quality control organisms, (6) Glass slides, coverslips, (7) Methanol, (8) Fluorescent microscope, (9) Immersion oil, (10) Gram stain reagents, (11) Microscope, (12) Xylene.

PROCEDURE

Place dropper in the assembled, reusable ampule crusher provided. Hold the dropper/crusher in an upright position and lightly tap the bottom to dislodge any bubbles that may have formed. Grasp the middle of the dropper/crusher with the thumb and forefinger, and with the tip pointing away, press gently to crush the ampule. Invert dropper and squeeze slightly to dispense in a dropwise fashion.

Test Procedure:

1. Prepare a smear of the specimen on a clean, glass slide and allow smear to air dry.
2. Fix the smear in methanol for 2 minutes. Allow the smear to air dry.
3. Flood slide with Acridine Orange and allow stain to remain on surface for 2 minutes.
4. Rinse with demineralized water and allow smear to air dry.
5. Examine the slide under 100 x to 400 x magnification using a fluorescent microscope. Confirm by examination at 1000 x under oil immersion.

NOTE: Smears may be Gram stained directly, without prior decolorization, as long as all immersion oil is removed with xylene.[10] Consult appropriate reference for Gram stain procedure.

INTERPRETATION

Background:	Black, yellow, or pale green
Leukocytes:	Pale apple-green
Erythrocytes:	Non-fluorescent
Bacteria and Yeast:	Bright red-orange
Inclusion Leukocytes:	Apple-green, yellow, orange, or red

QUALITY CONTROL

All lot numbers of BactiDrop™ Acridine Orange have been tested using the following quality control organisms and have been found to be acceptable. Testing with a known, fresh, reference specimen 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	RESULTS
Escherichia coli ATCC® 25922	Bright red-orange fluorescent rods
Staphylococcus aureus ATCC® 25923	Bright red-orange fluorescent cocci

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.[10]
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 (1000 x).[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

BactiDrop™ Acridine Orange (0.75 ml/Ampule):
REF 21502..... 50 Ampules/Pk

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)
EC REP	European Authorized Representative

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