ACRIDINE ORANGE STAIN

PRODUCT:

Bottle:

Acridine Orange Stain, item no. R5800

PURPOSE:

Acridine Orange (AO) is a stain used for demonstrating bacteria in blood culture broths, cerebrospinal fluid, urethral smears, and other exudates when bacteria are present in low numbers ($<10^4$ cfu/ml).

PRINCIPLE:

Acridine Orange is a fluorochrome stain originally developed by soil microbiologists to demonstrate bacteria in soil samples. It has also been used in conjunction with fluorochrome dyes, particularly for *Mycobacterium* species, in staining the background orange and allowing for efficient screening of bacteria at low magnifications.[7]

Acridine Orange Stain is recommended in the routine examination of blood culture broths and has been suggested as a replacement of blind subcultures.[1] It has also been used for the examination of cerebrospinal fluid, screening urine samples for significant bacteriuria, and for the detection of *Trichomonas vaginalis* in vaginal specimens.[2]

Acridine Orange absorbs ultraviolet light and emits visible light. The compound stains the nucleic acids of bacteria and other cells. Under certain conditions, RNA stains orange, and DNA stains green. The stain is more sensitive than the Gram reaction. Organisms may be seen at low magnification, making smears easy to interpret and aesthetically pleasing to examine.

FORMULA:

Approximate ingredients per liter.

Acridine Orange (Stock Solution)	10.0 ml
Walpole Acetate Buffer	990.0

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.

Storage: Upon receipt, store at 10-30°C in the dark. Stains should not be used if there are signs of deterioration or if the expiration date has passed.

Limitations: The Acridine Orange Stain requires a fluorescent microscope.

Inexperienced technologists can make misinterpretation of smears. Granules of disintegrating leukocytes may be mistaken for cocci and dead bacteria and contaminants may stain and, therefore, lead to erroneous results. Under certain conditions the Acridine Orange Stain may be overly sensitive.

The AO Stain is a screening procedure in most cases. Positive smears must be Gram-stained to determine Gram stain reaction. A Gram stain may be done directly over the AO smear.

The formula for the counterstain for mycobacteria is not the same as used above. Consult appropriate references for details.

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 the details in preparing a primary stain. Procedures differ according to the type of specimen.[4,6]

Method of Use: In general, smears are made on clean slides and allowed to air dry, followed by methanol fixation for 2 minutes. The slides are then air dried and stained for 1 minute using the AO stain. The slides are then rinsed with water, air dried, and viewed under a high dry objective of a fluorescent microscope. Positive and negative control slides are included in each run.

The method for Acridine Orange as a counterstain for mycobacteria can be found in appropriate references.[7]

Interpretation:

Positive: The presence of bacteria on the slide after examination is indicative of a positive test. These slides should be confirmed by Gram stain for quantitation, morphology, and Gram reaction.

Negative: No bacteria seen on the slide is considered a negative test.

Materials Required but Not Provided: Standard microbiological supplies and equipment such as loops, needles, pipettes, glass slides, and fluorescent microscope are not provided.

QUALITY CONTROL:*

The effectiveness of the stain and the staining technique is controlled by using prepared smears of known gram-positive and gram-negative organisms. Using control slides, stain simultaneously with test slides. Any inconsistencies in the control slides is 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 stain by including quality control slides with each test run.

BIBLIOGRAPHY:

1. Burdash, N. M., et al., *J. Clin. Microbiol.*, 17:463-465, 1983.
2. Fripp, P. J., et al., *J. Parasitol.*, 61:966-967, 1975.
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4. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology*, 3rd ed., J. B. Lippincott, Philadelphia, 1988.
5. Lauer, B., et al., *J. Clin. Microbiol.*, 14:2, 201-205, 1981.
6. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1985.
7. Smithwick, R., et al., *Tubercle*, 52:226-231, 1971.

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

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