

**KCMC Biotechnology
Laboratory, Microbiology**

**STANDARD
OPERATING
PROCEDURE**

**Effective Date
10 May 2006**

**SOP-Number
MIC.024**

Page 1 of 6

**Date
10 May 2006**

Title: ACRIDINE ORANGE STAIN

Author/Date: Anne Morrissey, 10 May 2006

Approvals/Date:

John A. Crump, MB, ChB, Microbiology Laboratory Director

Anne Morrissey, MS, MT(ASCP)SM, Microbiology Lab Supervisor

This SOP has been read and understood by:

Name	Date
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Annual Review	
By	Date

Title: ACRIDINE ORANGE STAIN

Document History:

Version Number	Reason for Changes	Date

Copies distributed to:

Name	Date

Title: ACRIDINE ORANGE STAIN

PURPOSE

For staining of Gram stain negative blood cultures that have been flagged as positive by BacT/ALERT instrument.

PRINCIPLE

The fluorescent stain acridine orange stains nucleoproteins (DNA and RNA) of bacteria and some human cells. Bacteria present in small numbers or species that stain poorly with Gram stain (e.g. campylobacter, spirochetes) may be more easily seen using this stain. Slides are first fixed with methanol then stained with acridine orange and examined using a fluorescent microscope.

CLINICAL APPLICATION

Acridine Orange stain can be helpful in the diagnosis of disseminated bacterial infections by demonstrating bacteria in Gram stain negative blood cultures.

SCOPE

This Standard Operating Procedure applies to the staining of slides with Acridine Orange by all technical staff in the microbiology laboratory that have been trained and are competent in performing this procedure.

EQUIPMENT/REAGENTS

Glass slide
Syringe with needle (for aliquoting blood cultures)
Pasteur pipettes (for preparing slides from clinical material and blood culture aliquots)
Methanol
Acridine Orange stain
Fluorescent microscope

QUALITY CONTROL

Frequency - Each day of use.

Control Organisms/Acceptable Results:

Positive – Slide of blood culture containing bacteria/orange fluorescing organisms

Negative – NA

Corrective actions:

1. Do not interpret or report patient results.
2. Check that the correct cube with proper wave-length (FITC cube) is selected on fluorescent microscope
3. If still negative, repeat stain on fresh QC slide and patient slide.

Title: ACRIDINE ORANGE STAIN

Documentation:

1. Record results on ACRIDINE ORANGE QC sheet.
2. Document corrective actions on QC DEVIATION form.
3. Supervisor will review QC results monthly.

PREPARATION OF SMEARS

1. Remove an aliquot of blood culture into sterile test tube.
2. Using a Pasteur pipette or a loop, remove a small aliquot and place a drop or loopful onto a glass slide and spread to make a thin smear.
3. Allow slide to air dry (approximately 30 minutes or until it appears dry).

STAIN PROCEDURE.

1. Flood slide with methanol for two minutes and drain.
2. Allow to air dry.
3. Flood slide with Acridine Orange for 2 minutes.
4. Rinse with tap water and drain excess water.
5. Allow to air dry (approximately 30 minutes or until it appears dry).

EXAMINATION

1. Examine using fluorescent microscope and cube for FITC.
2. Scan slide first with low power (10X) objective, then switch to high dry (45X) or oil immersion (100X) to view morphological details.

INTERPRETATION

1. Bacteria stain bright red orange against a black background.
2. Cells and other specimen components stain yellow or yellow green.
Note: Eosinophilic granules also stain orange. Take care not to confuse with microorganisms.
3. If organisms are observed, reexamine original Gram stain (or restain another).
4. Consult with supervisor if organisms cannot be observed on Gram stain.
5. Record results of AO stain on POSITIVE CULTURE FORM.

Title: ACRIDINE ORANGE STAIN

TEST LIMITATIONS

1. Artifacts, eosinophilic granules and other non-specific fluorescence may be mistaken for bacteria.

REFERENCES

Mirrett, S., et al. 1982. Comparison of acridine orange, methylene blue and Gram stains for blood cultures. J. Clin. Micro. 15:562.

Chapin, KC, Lauderdale, T. 2003. Reagents, Stains and Media: Bacteriology. In: Manual of Clinical Microbiology. 8th Ed. American Society for Microbiology, Washington, DC.

APPENDIX A – Acridine Orange QC sheet

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Page 6 of 6

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APPENDIX A

**KCMC Biotechnology Laboratory
Microbiology
Stain**

**Quality Control
Acridine Orange**

CONTROL/ACCEPTABLE RESULTS:

Positive - blood culture smear/Fluorescing bacteria

YEAR : _____

Negative - NA

DATE	LOT#	POS CONTROL (+)	BY

DOCUMENT ALL CORRECTIVE ACTION ON QC DEVIATION FORM

Supervisor Review :					
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ACRIDINE ORANGE QC/QC SHEETS