

**KCMC Biotechnology  
Laboratory, Microbiology**

**STANDARD  
OPERATING  
PROCEDURE**

**Effective Date**

**SOP-Number  
MIC.005**

**Page 1 of 7**

**Date**

**Title: Auramine AFB Stain**

**Author/Date: Anne Morrissey, 9 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:**

<b>Name</b>	<b>Date</b>
<b>1.</b>	
<b>2.</b>	
<b>3.</b>	
<b>4.</b>	
<b>5.</b>	
<b>6.</b>	
<b>7.</b>	
<b>8.</b>	
<b>9.</b>	
<b>10.</b>	

<b>Annual Review</b>	
<b>By</b>	<b>Date</b>

**KCMC Biotechnology  
Laboratory, Microbiology**

**STANDARD  
OPERATING  
PROCEDURE**

**Effective Date**

**SOP-Number  
MIC.005**

**Page 2 of 7**

**Date**

**Title: Auramine AFB Stain**

**Document History:**

<b>Version Number</b>	<b>Reason for Changes</b>	<b>Date</b>

**Copies distributed to:**

<b>Name</b>	<b>Date</b>

**Title: Auramine AFB Stain**

### **PURPOSE**

For the staining of acid-fast bacteria (AFB) in clinical material and cultures.

### **PRINCIPLE**

Mycobacteria species retain the fluorescent dye auramine stain after decolorization with 0.5% HCl in ethanol and appear as yellow to orange fluorescent rods when viewed using a fluorescent microscope.

### **SCOPE**

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

### **CLINICAL APPLICATION**

The Auramine Acid-Fast stain is an aid in the diagnosis of mycobacterial infections by demonstrating acid-fast organisms in clinical specimens and blood cultures.

### **SAFETY PRECAUTIONS**

- Wear gloves when handling patient specimens.
- Prepare slides from blood cultures and clinical specimens in a biohazard hood.
- Use care when handling unstained slides to avoid touching infectious material.

### **REAGENTS/MATERIALS/STORAGE**

Glass slides

Syringe with needle (for aliquoting blood cultures)

Pasteur pipettes (for preparing slides from clinical material and blood culture aliquots)

Slide warmer set at position #7 (70° C)

Light microscope

Staining reagents:

Auramine stain

Potassium permanganate

0.5% HCl in ethanol (prepared in-house)

Preparation of 0.5% Acid Alcohol:

95% Alcohol            740 ml

Distilled Water        260 ml

Concentrated HCl\*    5.0 ml

***\*Add acid to alcohol, not alcohol to acid.***

All reagents are stored at room/ambient temperature.

**Title: Auramine AFB Stain**

### **QUALITY CONTROL**

**Frequency** - each day of use.

**Control Organisms/Acceptable Results:**

Positive – *Mycobacterium* sp./bright yellow, to yellow-orange fluorescence

Negative – *E. coli* ATCC 25922/no fluorescence

**Corrective actions for Unacceptable QC results:**

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

**Documentation:**

1. Record results on AURAMINE QC sheet.
2. Document corrective actions on QC DEVIATION form.
3. Review QC results monthly.

### **PREPARATION OF SMEARS**

**From blood culture:**

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.
3. Place on slide warmer set at position #7 to dry and fix for 2 hours.

**From digested sputum:**

1. Using a loop or Pasteur pipette remove a small portion of specimen and spread thinly as possible over the slide.
2. Place on slide warmer set at position #7 to dry and fix for 2 hours.

### **STAIN PROCEDURE**

1. Heat fix slide for 2 hours on slide warmer .
2. Flood slide with auramine for 10 minutes.
3. Rinse with tap water.
4. Decolorize with 0.5% acid alcohol for approximately 10 seconds or until colorless.
5. Rinse with tap water.
6. Flood slide for 2 minutes with 0.5% potassium permanganate.

**NOTE:** Over-exposure to potassium permanganate will cause loss of brilliance of AFB.

**Title: Auramine AFB Stain**

7. Rinse with tap water.
8. Dry slide on slide warmer.
9. Place a drop of FA mounting fluid on the slide over the stained material.
10. Cover with coverslip. Try to avoid bubble formation.

#### **EXAMINATION OF SMEARS**

1. Examine smears on fluorescent microscope first using 25X magnification, moving to greater magnification to observe suspicious organisms more closely.
2. Examine 30 fields at 25X before reporting negative findings.
3. AFB will stain bright yellow/yellow orange against a black background.

#### **REPORTING RESULTS**

##### **From blood cultures:**

1. Examine 100 fields before reporting negative findings.
2. Report either positive (AFB seen) or negative (no AFB seen).
3. Confirm all suspected positive smears with Kinyoun stain (prepare additional slide). Refer to MIC.004 KINYOUN STAIN procedure.

Note: If low numbers of organisms (1-2/30 fields) are observed on auramine stain they may be difficult to locate on Kinyoun stain. Remove a 2 ml aliquot from blood culture and centrifuge at 1500xg/3500 rpm for 15 minutes, prepare slide from sediment and restain. If AFB are still not observed, consult supervisor for further action and reporting.

4. Notify ISAAC Study Coordinator of all AFB positive smears and report results on Microbiology lab report form.
5. Record results on Positive Culture Form

**Title: Auramine AFB Stain****From digested sputum:**

1. Count the number of organisms per low (x250) or high (x400) power fields and report based on the guidelines in the following table:

<b>REPORT</b>	<b># Orgs/low power field (x250)</b>	<b># Orgs/high power field (x400)</b>
No AFB observed	0	0
Doubtful/repeat	1-2/30 F	1-2/70 F
1+	1-9/10 Fields (F)	2-18/70 F
2+	1-9/ F	4-36/10 F
3+	10-90/F	4-36/ F
4+	>90/F	>36/F

2. Confirm all auramine positive smears with Kinyoun stain. (prepare additional slide). Refer to MIC.004 KINYOUN STAIN procedure.

Note: If low numbers of organisms (1-2/30 fields) are observed on auramine stain they may be difficult to locate on Kinyoun stain. Remove a 2 ml aliquot from blood culture and centrifuge at 1500xg/3500 rpm for 15 minutes, prepare slide from sediment and restain. If AFB are still not observed, consult supervisor for further action and reporting.

3. Notify ISAAC Study Coordinator of all AFB positive smears and report results on Microbiology lab report form.
4. Record results on Positive Culture Form.

**REFERENCE:**

Pfyffer,GB, Elliott, BB, Wallace, RJ. 2003. Mycobacterium; General characteristics, Isolations and Staining. In: Manual of Clinical Microbiology. 8<sup>th</sup> Ed. American Society for Microbiology, Washington, DC.

**APPENDIX A – Auramine QC sheet**

**KCMC Biotechnology  
Laboratory, Microbiology**

**STANDARD  
OPERATING  
PROCEDURE**

Effective Date

SOP-Number  
MIC.005

Page 7 of 7

Date

Title: Auramine AFB Stain

**APPENDIX A**

**KCMC Biotechnology Laboratory  
Microbiology**

**Quality Control  
AURAMINE STAIN**

**ORGANISM/ACCEPTABLE RESULT:**

**YEAR :** \_\_\_\_\_

*M. scrofulaceum* (ATCC 19981)/fluorescent bacilli (+)

*E. coli* (ATCC 25922)/ no fluorescent bacilli (-)

QC DATE	LOT # AURAMINE	LOT# KMnO <sub>4</sub>	RESULT		BY
			POS (+)	NEG (-)	

**DOCUMENT ALL CORRECTIVE ACTION ON QC DEVIATION FORM**

<b>Supervisor Review :</b>					
--------------------------------	--	--	--	--	--

AURAMINE QC/QC SHEETS