

**KCMC Biotechnology
Laboratory, Microbiology**

**STANDARD
OPERATING
PROCEDURE**

Effective Date

**SOP-Number
MIC.004**

Page 1 of 7

Date

Title: Kinyoun Acid-Fast 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:

| Name | Date |
|-------------|-------------|
| 1. | |
| 2. | |
| 3. | |
| 4. | |
| 5. | |
| 6. | |
| 7. | |
| 8. | |
| 9. | |
| 10. | |

| Annual Review | |
|----------------------|-------------|
| By | Date |
| | |
| | |
| | |
| | |
| | |

**KCMC Biotechnology
Laboratory, Microbiology**

**STANDARD
OPERATING
PROCEDURE**

Effective Date

**SOP-Number
MIC.004**

Page 2 of 7

Date

Title: Kinyoun Acid-Fast Stain

Document History:

| Version Number | Reason for Changes | Date |
|-----------------------|---------------------------|-------------|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

Copies distributed to:

| Name | Date |
|-------------|-------------|
| | |
| | |
| | |

Title: Kinyoun Acid-Fast Stain

PURPOSE

For the staining of mycobacteria species in clinical material and cultures.

PRINCIPLE

Mycobacteria species retain carbolfuchsin stain after decolorization with 3% acid alcohol and appear as red beaded rods against a blue background. Other microorganisms are decolorized, and stain blue.

CLINICAL APPLICATION

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

SCOPE

This Standard Operating Procedure applies to the staining of smears from blood cultures and clinical specimens with Kinyoun stain by all technical staff in the microbiology laboratory that have been trained and are competent in performing this procedure.

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 & EQUIPMENT

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:

Carbolfuchsin KF (Kinyoun carbolfuchsin)

3% acid alcohol:

95% alcohol 2910 ml

concentrated HCl* 90 ml

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

Methylene Blue

Store all reagents at room temperature.

Title: Kinyoun Acid-Fast Stain

QUALITY CONTROL

Frequency - each day of use.

Control Organisms/Acceptable Results:

Positive – *M. tuberculosis* H37Ra / Red bacilli

Negative – *E. coli* ATCC 25922/Blue bacilli

Corrective actions:

1. Do not interpret or report patient results.
2. Restain new control slide and all patient slides.

Documentation:

1. Record results on KINYOUN QC sheet.
2. Document corrective actions on QC DEVIATION form and submit to supervisor for review.
3. Supervisor will 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 to dry and fix slide for 2 hours.

From undigested sputum (direct smear):

1. Select purulent or bloody material.
2. Using a loop or Pasteur pipette remove a small portion of specimen and spread thinly as possible over the slide.
3. Place on slide warmer 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 to dry and fix for 2 hours.

Title: Kinyoun Acid-Fast Stain

STAIN PROCEDURE

1. Flood slides with Kinyoun carbolfuchsin for 2-3 minutes.
2. Rinse with tap water.
3. Decolorize with 3% acid alcohol by dripping over the slide until the alcohol flows colorless from the slide. Do not overdecolorize.
4. Rinse with tap water.
5. Counterstain with methylene blue for 20-30 seconds.
6. Rinse with tap water.
7. Air dry, gently blot or place on slide warmer to dry.

EXAMINATION OF SMEARS

1. Examine with a light microscope at 1000X magnification (oil immersion). Organisms appear red against a blue background.
2. Wipe objective clean after each positive slide to prevent carry-over of organisms.

REPORTING RESULTS

From blood culture bottles:

1. Examine 100 fields before reporting negative findings.
2. Report either positive (AFB seen) or negative (no AFB seen).

From smears of clinical specimens:

1. Examine 300 fields before reporting negative findings.
2. Enumerate AFB on positive smears using these guidelines:

| REPORT | # Orgs/oil immersion (1000x) |
|-----------------|------------------------------|
| No AFB observed | 0 |
| Doubtful/repeat | 1-2/300 F |
| 1+ | 1-9/100 Fields (F) |
| 2+ | 1-9/10 F |
| 3+ | 1-9/F |
| 4+ | 1-90/F |

Title: Kinyoun Acid-Fast Stain

3. Notify ISAAC Study Coordinator of all positive AFB smears/cultures.
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.8th Ed. American Society for Microbiology, Washington, DC.

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 – Kinyoun QC sheet

Title: Kinyoun Acid-Fast Stain

APPENDIX A

KCMC Biotechnology Laboratory
Microbiology

Quality Control
KINYOUN STAIN

ORGANISM/ACCEPTABLE RESULT:
M. kansasii/red bacilli (+)
E. coli (ATCC 25922)/blue bacilli (-)

YEAR: _____

| QC DATE | LOT # CARBOLFUCHSIN | LOT# METHYLENE BLUE | RESULT | | BY |
|---------|------------------------|------------------------|---------|---------|----|
| | | | POS (+) | NEG (-) | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

DOCUMENT ALL CORRECTIVE ACTION ON QC DEVIATION FORM

| | | | | | |
|------------------------|--|--|--|--|--|
| Supervisor Review : | | | | | |
|------------------------|--|--|--|--|--|

KINYOUN QC/QC