

**KCMC Biotechnology
Laboratory, Microbiology**

**STANDARD
OPERATING
PROCEDURE**

Effective Date
9 May 2006

SOP-Number
MIC.025

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Date
9 May 2006

Title: BACITRACIN SUSCEPTIBILITY TEST

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Approvals/Date:

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This SOP has been read and understood by:

Name	Date
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Annual Review	
By	Date

Title: BACITRACIN SUSCEPTIBILITY TEST

Document History:

Version Number	Reason for Changes	Date

Copies distributed to:

Name	Date

Title: BACITRACIN SUSCEPTIBILITY TEST

PURPOSE

A screening test for the presumptive identification of Group A streptococci (*Streptococcus pyogenes*). May also be used to differentiate *Micrococcus* spp from *Staphylococcus* spp.

PRINCIPLE

Beta hemolytic colonies are subcultured to a blood agar plate and a 0.4 U bacitracin disk is placed in the center of the subculture. After overnight incubation the plate is observed for inhibition of growth around the disk. Bacitracin inhibits growth of Group A streptococci so there will be a zone of growth inhibition surrounding the disk. Most other species of hemolytic streptococci are not inhibited. If test is used to differentiate *Micrococcus* spp. they are susceptible to bacitracin, *Staphylococcus* spp. are resistant.

SCOPE

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

MATERIALS/STORAGE

Bacitracin disks (0.04 units)/Refrigerate
Blood Agar plate/ Refrigerate
18-24 hr culture of test organism
Forceps

QUALITY CONTROL

Frequency – Each shipment/lot of disks

Control organisms/Acceptable Results:

Group A Strep/Susceptible - any zone of inhibition
Group B strep/Resistant – no zone of inhibition

Corrective Actions for Unacceptable QC results:

1. Repeat test using fresh control isolates.
2. If still unsatisfactory, do not use for patient testing.
3. Arrange for replacement.

Documentation:

1. Record results on BACITRACIN QC sheet.
2. Document corrective actions of unacceptable results on QC DEVIATION FORM and submit to supervisor for review.
3. Supervisor will review QC results monthly.

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TEST PROCEDURE

1. Subculture colony to one half of a Blood agar plate (heavy inoculum is advisable).
2. Remove a bacitracin disc using forceps and place in the center of the subculture.
Press lightly with forceps.
3. Incubate at 35°C in 5% CO₂ overnight.

INTERPRETATION

Examine for a zone of inhibition surrounding disk.

For beta hemolytic streptococci:

No zone of inhibition– Negative, not Group A strep.

Zone (any size) of inhibition – Positive, presumptive Group A strep*

****All positive, presumptive results must be confirmed using PYR test.***

For differentiation of *Micrococcus* vs *Staphylococcus* sp.:

Zone of ≥ 10 mm – *Micrococcus* sp.

Zone <10 mm – *Staphylococcus* sp.

RECORDING RESULTS

Enter results on POSITIVE CULTURE form.

TEST LIMITATIONS

1. Some strains of beta hemolytic streptococci Groups B, C, D, and G may also be bacitracin susceptible (1-8%).
2. Group A streptococci that are resistant to bacitracin have been reported.

REFERENCE

Ruoff, KL, etal. 2003. Streptococcus. In: Manual of Clinical Microbiology. 8th Ed. ASM Press, Washington, DC.

APPENDIX A – Bacitracin QC sheet

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APPENDIX A

**KCMC Biotechnology Laboratory
Microbiology**

**Quality Control
Bacitracin Test**

CONTROL ORGANISMS/ACCEPTABLE RESULTS:

Streptococcus pyogenes - Any zone of inhibition (+)

Streptococcus agalactiae: no zone (-)

YEAR : _____

DATE	LOT#/EXP DATE	<i>S. pyogenes</i>	<i>S. agalactiae</i>	BY

DOCUMENT ALL CORRECTIVE ACTION ON QC DEVIATION FORM

Supervisor Review :					
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BACITRACIN QC/QC SHEETS