



FINAL STUDY REPORT

STUDY TITLE

Standard Test Method for Efficacy of Sanitizers
Recommended for Inanimate Non-Food Contact Surfaces

Test Organism:

Staphylococcus aureus - MRSA (ATCC 33592)

PROTOCOL NUMBER

SRC27022404.NFS.5

PRODUCT IDENTITY

ACCEL TB
Lot 2-3646-REG-US and Lot 3-3647-REG-US

DATA REQUIREMENTS

U.S. EPA 40 CFR Part 158
"Data Requirements for Registration"
Pesticide Assessment Guidelines - Subdivision G, 91-2 (j)

AUTHOR

Sally Nada, B.S.
Study Director

STUDY COMPLETION DATE

July 8, 2004

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PREPARED FOR

Virox Technologies
6705 Mill Creek Road Unit 4
Mississauga, Ontario L5N5M4

SPONSOR REPRESENTATIVE

Scientific & Regulatory Consultants, Inc.
102 1/2 South Chauncey Street
Columbia City, IN 46725-2306

PROJECT NUMBER

A02075

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B), or (C).

Company: Virox Technologies

Company Agent: Sally Hayes

Agent for Virox Technologies

Title

Sally Hayes

Signature

Date: 09/20/04

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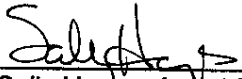
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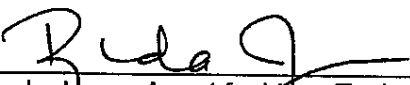
GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160.


The studies not performed by or under the direction of ATS Labs are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the compound(s).

Submitter: 
Sally Hayes, Agent for Virox Technologies

Date: 09/20/04

Sponsor: 
Rhonda Jones, Agent for Virox Technologies

Date: 7-14-04

Study Director: 
Sally Nada, B.S.

Date: 7/8/04

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QUALITY ASSURANCE UNIT SUMMARY

Study: Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date	Study Director	Management
Critical Phase	April 14, 2004	April 14, 2004	April 27, 2004
Draft Report	April 23, 2004	April 26, 2004	
Final Report	July 7, 2004	July 7, 2004	July 8, 2004

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: Rachelle L. Ewman Date: 07/08/04

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STUDY PERSONNEL

STUDY DIRECTOR: Sally Nada, B.S.

Professional personnel involved:

Douglas G. Anderson, Ph.D.	- President
Karen M. Ramm, B.A.	- Technical Director
David Rottjakob, M.T.	- Microbiology Program Manager
Barbara Bailey, A.A.	- Microbiology Laboratory Supervisor
Sally Nada, B.S.	- Research Scientist I
Adam W. Pitt, B.S.	- Research Assistant II
Scott R. Steinagel, B.S.	- Research Assistant I
Matthew Sathe, B.S.	- Research Assistant I
Lisa Slusser, B.S.	- Research Assistant I

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STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces

Project Number: A02075

Protocol Number: SRC27022404.NFS.5

Sponsor: Virox Technologies
6705 Mill Creek Road Unit 4
Mississauga, Ontario L5N5M4

Sponsor Representative: Scientific & Regulatory Consultants, Inc.
102 1/2 South Chauncey Street
Columbia City, IN 46725-2306

Test Facility: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance Name: ACCEL TB

Lot/Batch(s): Lot 2-3646-REG-US and Lot 3-3647-REG-US

Test Substance Characterization

Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor.

STUDY DATES

Date Sample Received: March 11, 2004
Study Initiation Date: April 1, 2004
Experimental Start Date: April 14, 2004
Experimental End Date: April 16, 2004
Study Completion Date: July 8, 2004

OBJECTIVE

The objective of this assay was to evaluate the antimicrobial efficacy of sanitizers on pre-cleaned inanimate, nonporous, non-food contact surfaces in compliance with the U.S. EPA requirements set forth in the Pesticide Assessment Guidelines.

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SUMMARY OF RESULTS

Test Substance: ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US)
 Dilution: Ready to use (RTU)
 Test Organism: *Staphylococcus aureus* - MRSA (ATCC 33592)
 Exposure Time: 30 seconds
 Exposure Temperature: 20.0°C
 Organic Soil Load: 5% fetal bovine serum
 Efficacy Result: Two lots of ACCEL TB demonstrated efficacy against *Staphylococcus aureus* - MRSA as required by the U.S. EPA for non-food contact label claims.

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium
<i>Staphylococcus aureus</i> - MRSA	33592	Synthetic Broth

The microorganism used in this study was obtained from the American Type Culture Collection, Manassas, Virginia.

Recovery Media

Neutralizing Subculture Medium: Lethen Broth with 1.0% Sodium Thiosulfate and 0.05% Catalase
 Agar Plate Medium: Tryptic Soy Agar with 5% Sheep Blood (BAP)

Reagents

Organic Soil Load Description: 5% fetal bovine serum (FBS)

Carriers

Glass square carriers (1 inch x 1 inch) were dipped in 95% alcohol, rinsed with deionized water, and air dried before sterilization. For each organism used, a sufficient number of carriers were placed into a large glass petri dish and sterilized in a hot air oven for 2 hours at 180°C. After sterilization, each carrier was placed into an individual petri dish using sterile forceps. Five carriers are tested per batch/organism.

Constant Humidity Chamber (Desiccator)

A controlled humidified chamber was used to dry the carriers.

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ATS LABS

TEST METHOD

Preparation of Test Substance

The test substance was ready to use (RTU), as received from the Sponsor. The test substance was homogenous as determined by visual observation.

Preparation of Inocula

From stock cultures, the tubes of Synthetic Broth were inoculated and incubated for 24±2 hours at 35-37°C. Using a 4-mm inside diameter wire transfer loop, at least three consecutive daily transfers of cultures were made prior to use as inoculum. Two loopfuls of culture were transferred to 10 mL broth medium and incubated for 48±4 hours. Transfers which were more than 15 days away from stock culture were not used.

The 48±4 hour culture of test organism was thoroughly mixed using a "vortex" mixer, then allowed to settle for ≥ 15 minutes. The upper two thirds of this suspension was removed and used as the inoculum for testing. To this supernatant, 5% (v/v) sterile fetal bovine serum was added. This suspension, containing 5% sterile fetal bovine serum, was used as the inoculum for testing. Inoculum dilution was not employed to achieve the requested carrier count range. Antimicrobial susceptibility testing was performed utilizing a representative culture from the day of testing to verify the antimicrobial resistance pattern stated.

Addition of Organic Soil Load

A 0.25 mL aliquot of FBS was added to 4.75 mL of broth culture to yield a 5% fetal bovine serum soil load.

Inoculation of Test and Control Carriers

The sterile glass carriers were inoculated with 0.01 mL of 48±4-hour culture using a calibrated pipettor. The inoculum was spread to within 1/8 inch of the edges of the carrier.

All plates containing the inoculated carriers were placed in the humidity chamber. The carriers were allowed to remain at 35-37°C and at a relative humidity of 40±2% for 30 minutes.

Treatment of Inoculated Test Carriers

After the inoculated carriers were dried at 36.0°C and 41% relative humidity for 30 minutes, all carriers were removed from the humidity chamber and placed at room temperature. The five test carriers and three control carriers were transferred to sterile jars using sterile forceps. The test carriers were medicated with 5.0 mL of the test substance and exposed at 20.0°C for 30 seconds.

Neutralization and Subculture

Following the Sponsor specified exposure period of 30 seconds, 20 mL of the appropriate neutralizer solution was transferred to the jar and rotated vigorously on an even plane approximately 50 rotations to suspend the surviving organisms in the neutralizer solution. Subsequent carriers were neutralized using staggered intervals and agitated each in turn.

Within 30 minutes after addition of the neutralizer to the test solution, 1.0 mL of the 10⁰ and 10⁻¹ dilution of the neutralizer solution from each of the five test carriers and three control carriers jars was plated in duplicate using the standard spread plate technique and BAP.

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Incubation and Observation

The neutralized subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth.

Representative neutralized subcultures showing growth were subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism.

STUDY CONTROLS

Carrier Quantitation Control

The test was performed using three inoculated carriers and a 0.01% Triton X-100 solution in place of test substance. The inoculated control carriers were exposed to the 0.01% Triton X-100 solution for 30 seconds at 20.0°C. 20 mL of neutralizing broth was transferred to the jar containing the treated carrier and the jars were rotated as in the test. Ten-fold serial dilutions of each neutralizing broth were made through 10⁻⁵ dilution. An aliquot (1.0 mL) was plated in duplicate on BAP from the 10⁻² through 10⁻⁵ dilutions. The plates were incubated as in the test.

The acceptance criterion for this study control is a minimum geometric mean of 1.0 x 10⁵ - 5.0 x 10⁶ CFU/carrier.

Dry Control

An inoculated dry carrier was added to a 20 mL jar of neutralizing broth and vortex mixed. Ten-fold serial dilutions of the neutralized broth were prepared. One (1.0) mL of 10⁻¹ through 10⁻⁵ were plated in duplicate to yield countable numbers. Plates were incubated as in the test and enumerated. This control is for information purposes only and therefore has no acceptance criterion.

Neutralization Confirmation Control

A neutralization confirmation control was performed to demonstrate the neutralizer's ability to inactivate the test substance. The neutralization of the test substance was confirmed by exposing sterile carriers to the test substance and neutralizing as in the test procedure. An aliquot (1.0 mL) of a diluted suspension of the test organism yielding 10-100 CFU/mL of neutralized solution was transferred to the jar and mixed. An aliquot (1.0 mL) of this mixed solution was plated in duplicate. A numbers control was performed, utilizing a sterile solution in place of the test substance. The resulting plates were incubated as in the test and enumerated. The acceptance criterion for this study control is growth within 1 log₁₀ of the numbers control.

Purity Control

A "streak plate for isolation" was performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control

The serum used for soil load was cultured, incubated, and observed for lack of growth. The acceptance criterion for this study control is lack of growth.

Inoculum Count Control

Ten-fold serial dilutions of the initial suspension were prepared. An aliquot (1.0 mL) of the dilutions 10⁻⁷ through 10⁻⁸ were plated in duplicate using BAP. The plates were incubated as in the test. This control was for informational purposes only and therefore has no acceptance criterion.

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Neutralizing Subculture Medium Sterility Control

A representative sample of uninoculated neutralizing subculture medium was incubated and visually examined. The acceptance criterion for this study control was lack of growth.

Carrier Sterility Control

A representative uninoculated carrier was added to the neutralizing subculture medium. The subculture medium containing the carrier was incubated and examined. The acceptance criterion for this study control is lack of growth.

Viability Control

A representative inoculated carrier was added to the subculture medium. The subculture medium containing the carrier was incubated and examined for growth. The acceptance criterion for this study control is growth.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria

The test substance must meet the EPA efficacy data requirements that a 99.9% reduction in numbers of the test organism(s) was obtained as compared to the carrier quantitation control.

Control Acceptance Criteria

The study controls must perform according to the criteria detailed in the study controls description section.

PROTOCOL CHANGES

Protocol Amendments:

This protocol is amended per Sponsor's request to state that the percent reduction will be reported using six digits.

Protocol Deviations:

The culture was not diluted for testing to achieve the standardization range as stated in the protocol. Daily culture transfers displayed homogeneity and typical counts of approximately 10^8 CFU/mL, consequently no adjustment of the culture was necessary on the day of testing. Therefore, this had no impact on the study.

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DATA ANALYSIS

Calculations

Number of Organisms Surviving per Carrier

$$\text{CFU/carrier} = \frac{(\text{average number colonies/plate @ dilution}) \times (\text{dilution factor}) \times (\text{volume neutralized solution})}{(\text{volume plated})}$$

The carrier population was calculated and reported using data from the most appropriate dilution(s).

Geometric Mean of Number of Organisms Surviving on Control Carrier:

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_3}{3}$$

where X equals CFU/control carrier

Geometric Mean of Number of Organisms Surviving on Test Carrier:

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}Y_1 + \text{Log}_{10}Y_2 + \text{Log}_{10}Y_3 + \text{Log}_{10}Y_4 + \text{Log}_{10}Y_5}{5}$$

where Y equals CFU/test carrier

Percent Reduction

$$\% \text{ reduction} = [(a - b) / a] \times 100$$

where:

- a = geometric mean of the number of organisms surviving on the inoculated control carriers.
- b = geometric mean of the number of organisms surviving on the test carriers.

$$\text{Recovery Log}_{10} \text{ Difference} = (\text{Log}_{10} \text{ Numbers Control}) - (\text{Log}_{10} \text{ Test Results})$$

Statistical Methods

Geometric Mean and Percent Reduction.

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STUDY RETENTION

Record Retention

All of the original raw data developed exclusively for this study shall be archived at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. These original data include, but are not limited to, the following:

1. Certified copy of final study report.
2. Original signed protocol.
3. Any protocol amendments.
4. All handwritten raw data for control and test substances including, but not limited to notebooks, data forms and calculations.
5. All measured data used in formulating the final report.
6. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
7. Study specific SOP deviations made during the study.

Test Substance Retention

The test substance will be discarded following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test material.

REFERENCES

1. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, Efficacy Data Requirements Sanitizer Test (for inanimate, non-food contact surfaces), DIS/TSS-10, January 7, 1982.
2. U.S. Environmental Protection Agency Pesticide Assessment Guidelines, Subdivision G, Section 91-2; Item j Sanitizers (for non-food contact surfaces) and Section 91-30(d) (8) Recommended Methods for Sanitizers – Non-food Contact Surfaces.
3. ASTM Test Method, Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces, E1153, July 2003.
4. Official Methods of Analysis of the AOAC, Seventeenth Edition, 2000. Chapter 6 – Disinfectants, 961.02. Germicidal Spray Products as Disinfectants.

RESULTS

Control and Neutralization Results (Tables 1-5)

All data measurements/controls including the carrier quantitation, neutralization confirmation, culture purity, organic soil load sterility, carrier sterility, viability and neutralizing subculture medium sterility controls were within acceptance criteria.

Test Results (Table 6-7)

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ANALYSIS

ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US), ready to use, demonstrated >99.9983 percent reduction of *Staphylococcus aureus* - MRSA.

STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum soil load, ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US), ready to use, is an effective sanitizer against *Staphylococcus aureus* - MRSA for inanimate non-food contact surfaces after a 30 second contact period at 20.0°C.

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.

The use of the ATS Labs name, logo or any other representation of ATS Labs without the written approval of ATS Labs is prohibited. In addition, ATS Labs may not be referred to in any form of promotional materials, press releases, advertising or similar materials (whether by print, broadcast, communication or electronic means) without the express written permission of ATS Labs.

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TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Type of Control	Results
	<i>Staphylococcus aureus</i> - MRSA (ATCC 33592)
Purity Control	Pure
Viability Control	Growth
Organic Soil Sterility Control	No Growth
Neutralizing Subculture Medium Sterility Control	No Growth
Carrier Sterility Control	No Growth

TABLE 2: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Dilution Plated	Neutralization Confirmation Numbers Control (# Survivors/mL)	Neutralization Confirmation Results (# Survivors/mL)	± 1.0 Log ₁₀	Pass/Fail
ACCEL TB, Lot 2-3646-REG-US	<i>Staphylococcus aureus</i> - MRSA	10 ⁻⁶	48, 43	46, 43	-0.01	Pass
ACCEL TB, Lot 3-3647-REG-US		10 ⁻⁶		15, 22	0.38	Pass

TABLE 3: INOCULUM COUNT RESULTS

Test Organism	Initial Suspension*
<i>Staphylococcus aureus</i> - MRSA (ATCC 33592)	6.9 x 10 ⁸ CFU/mL

CFU = Colony Forming Unit

* No dilution was employed to obtain this value.

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TABLE 4: EVALUATION OF CONTROL CARRIER DATA

Test Organism	Carrier #	#Survivors/mL Neutralizer Solution	#Survivors/Carrier	Log ₁₀	Geometric Mean (CFU/Carrier)
<i>Staphylococcus aureus</i> - MRSA	1	7.7×10^4	1.9×10^6	6.28	1.82×10^8
	2	7.0×10^4	1.8×10^6	6.26	
	3	6.6×10^4	1.7×10^6	6.23	

TABLE 5: EVALUATION OF DRY CONTROL CARRIER DATA

Test Organism	Carrier #	#Survivors/mL Neutralizer Solution	#Survivors/Carrier	Log ₁₀
<i>Staphylococcus aureus</i> - MRSA	1	8.0×10^4	2.0×10^6	6.30

TABLE 6: EVALUATION OF TEST CARRIER DATA

Test Substance	Test Organism	Sample Dilution*	Carrier Number	Number of Survivors (Neutralizer Solution)			
				1.0 mL plated of 10^0 dilution		1.0 mL plated of 10^{-1} dilution	
ACCEL TB, Lot 2-3646-REG-US	<i>Staphylococcus aureus</i> - MRSA	RTU	1	0,0	Avg. <1	0,0	Avg. <1
			2	0,0	Avg. <1	0,0	Avg. <1
			3	0,0	Avg. <1	0,0	Avg. <1
			4	0,0	Avg. <1	0,0	Avg. <1
			5	2,0	Avg. 1	0,0	Avg. <1
ACCEL TB, Lot 3-3647-REG-US			1	0,0	Avg. <1	0,0	Avg. <1
			2	0,0	Avg. <1	0,0	Avg. <1
			3	0,0	Avg. <1	0,0	Avg. <1
			4	0,0	Avg. <1	0,0	Avg. <1
			5	0,0	Avg. <1	0,0	Avg. <1

* RTU = Ready to use

A value of <1 was used in place of zero for calculation purposes only.

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TABLE 7: CALCULATED VALUES

Test Substance: ACCEL TB (Lot 2-3646-REG-US)						
Test Organism	Carrier #	# Survivors/ Carrier*	Log ₁₀	Average Log ₁₀	Geometric Mean	Percent Reduction
<i>Staphylococcus aureus</i> - MRSA	1	<3 x 10 ¹	<1.48	<1.48	<30.2	>99.9983
	2	<3 x 10 ¹	<1.48			
	3	<3 x 10 ¹	<1.48			
	4	<3 x 10 ¹	<1.48			
	5	3 x 10 ¹	1.48			
Test Substance: ACCEL TB (Lot 3-3647-REG-US)						
Test Organism	Carrier #	# Survivors/ Carrier*	Log ₁₀	Average Log ₁₀	Geometric Mean	Percent Reduction
<i>Staphylococcus aureus</i> - MRSA	1	<3 x 10 ¹	<1.48	<1.48	<30.2	>99.9983
	2	<3 x 10 ¹	<1.48			
	3	<3 x 10 ¹	<1.48			
	4	<3 x 10 ¹	<1.48			
	5	<3 x 10 ¹	<1.48			

*Calculated method detection limit values.

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ATTACHMENT I

Verification of Antibiotic Resistance

The following organism, *Staphylococcus aureus* - MRSA (ATCC 33592), was purchased from the American Type Culture Collection (ATCC) by ATS Labs. ATS Labs verified that the organism was resistant by performing a Kirby Bauer Susceptibility assay under GLP conditions. The organism was subcultured onto a BAP plate and was incubated for approximately 24 hours at 35-37°C. Following incubation, a suspension of the test organism equal to a 0.5 McFarland Standard was made in 0.85% sterile saline. The suspension was streaked onto Mueller Hinton agar. A oxacillin disc was placed in the center of the inoculated Mueller Hinton plate. The plate was inverted and incubated for ≥ 24 hours at 35-37°C. Following incubation, the zone of inhibition was measured using a calibrated caliper. A control organism, *Staphylococcus aureus* (ATCC 25923), was run concurrently with the test organism to confirm the validity of the assay. The interpretation of the zone of inhibition is based on established NCCLS performance standards.

Organism (ATCC)	Zone of Inhibition (mm)	NCCLS* Resistant Range (mm)
<i>Staphylococcus aureus</i> – MRSA (ATCC 33592)	6	≤ 10
Quality Control Organism	Zone of Inhibition (mm)	NCCLS* Acceptable Range (mm)
<i>Staphylococcus aureus</i> (ATCC 25923)	19	18-24

*NCCLS = National Committee for Clinical Laboratory Standards.