

KILL-TIME STUDIES
Antimicrobial Activity of Advanced Cellular Silver (ACS) 200
Using Methicillin-resistant *Staphylococcus aureus* (MRSA)

Test Solution: ACS 200
Submitted August 19, 2008

September 4, 2008

PREPARED FOR:
Results RNA
1272 South 1380 West
Orem, UT 84058

I. PURPOSE

The purpose of this study was to determine the antimicrobial activity of ACS 200 on methicillin-resistant *Staphylococcus aureus* (MRSA). This was accomplished by performing a standard kill-time suspension test using 1, 3, and 5 minute contact times.

II. MATERIALS AND METHODS

A. Test organism

The test suspension was prepared by growing a 5 ml culture of Methicillin-resistant *Staphylococcus aureus*, ATCC 43300 in Trypticase Soy Broth at 37° C for 20 hr. Five ml of culture was pelleted by centrifugation, washed with five ml sterile 18 MΩ water, centrifuged again, and re-suspended in a final volume of two ml sterile water.

B. Neutralizers

The Neutralizer solution consisted of 9ml tubes of 12.7% Tween 80, 6.0% Tamol, 1.7% lecithin, 1% Peptone, 1.0% Cysteine and 500 mM Tris (pH 7.0).

C. Kill-Time Procedure

1. A tube (50 ml polypropylene sterile centrifuge tube) containing 9.9 ml of ACS 200 was equilibrated in a 20 °C water bath. At time zero, 0.1 ml of the MRSA suspension was added.
2. After the specified contact times, one ml of *S. aureus*/ACS 200 disinfectant suspension was removed to 9.0 ml of neutralizer. The tube was mixed thoroughly.
3. After two minutes, the neutralized suspension was serially diluted 1:10, in physiological saline solution (PSS).
4. The number of viable organisms in selected dilution tubes was assayed by membrane filtration. One ml aliquots were plated in duplicate. The membranes were washed with about 100 ml of sterile PSS and removed to Columbia Agar plates. The plates were incubated at 37 °C for 24 and 48 hours.
5. The number of colonies on each filter was counted and log reduction and percent kill values were computed.

D. Controls

1. A titer of the test suspension was computed by performing membrane filtration assays on selected 1:10 dilutions in PSS of the test suspension.
2. A neutralizer control for each disinfectant was performed by inoculating a mixture of 9.0 ml of neutralizer and 1 ml of ACS 200 disinfectant with 0.1 ml of the 1:1x10⁵ dilution of the titer. This produced about 215 CFU / ml in the tube, which was allowed to stand for 20 minutes prior to dilution and assay by membrane filtration using duplicate 1 ml samples.

III. RESULTS

Methicillin-resistant *Staphylococcus aureus*:

Titer.

Number of colonies:

Dilution:		
<u>1:1x10⁶</u>	<u>1:1x10⁷</u>	<u>1:1x10⁸</u>
TNC	154	20
TNC	215	30

ACS 200:

(Received 8/19/08)

Dilution of *S. aureus*/ACS 200 disinfectant suspension:

Time	1:1x10 ¹	1:1x10 ²	1:1x10 ³
1 min	265	26	1
	251	22	0
3 min	0	0	0
	0	0	0
5 min	0	0	0
	0	0	0

Neutralization Control		Expected Counts:		Percent of Expected:
Undiluted	1:10	<u>Undiluted</u>	<u>1:10</u>	200
TNC	38	215	22	
TNC	50			

Sterility Controls:

Material	Counts
PSS	0
ACS 200	0
Water	0
Neutralizer	0
Columbia Agar	0

IV. DISCUSSION

Results of the titer showed a viable *S. aureus* concentration of 2.17×10^9 organisms per ml in the original suspension. Inoculation of 9.9 ml of ACS 200 disinfectant with 0.1 ml of this suspension produced an initial concentration of 2.17×10^7 *S. aureus* per ml in the assay tube.

Results from these procedures allowed log reduction (LR) and percent kill (PK) values to be calculated using the formulas: 1) $LR = -\log(S/S_0)$; where S = concentration of viable organisms after the specified contact time; and S_0 = the initial concentration of viable organisms at time zero. 2) $PK = (1 - (S/S_0)) \times 100$. These values are shown below.

<u>Solution</u>	<u>Contact Time</u>	<u>Log Reduction (LR)</u>	<u>Percent Kill (PC)</u>
ACS 200	1 min.	3.92	99.988
	3 min	>6.64	>99.99998
	5 min	>6.64	>99.99998

Neutralization control data revealed that the neutralizer was able to adequately neutralize ACS 200. Observed counts were 200 percent of those expected.

V. CONCLUSION

ACS 200 had excellent bactericidal activity against Methicillin-resistant *S. aureus*, producing a 3.92 log reduction in one minute and complete kill (>6.6 log reduction) within 3 minutes.

Date of test: September 04, 2008

Performed By:

Emily Moore
Research Associate

Supervised by:

A handwritten signature in black ink, appearing to read "Richard A. Robison". The signature is written in a cursive style with a horizontal line underneath it.

Richard A. Robison, Ph.D.
Professor
851 WIDB
Brigham Young University
Provo, Utah 84602

Please Note: This report does not constitute endorsement by Richard A. Robison or Brigham Young University, of the tested products in any way. The names 'Richard A. Robison' and/or 'Brigham Young University' may not be used in any type of promotional published material, either written or electronic, without express written permission from both parties.