KILL-TIME STUDIES
STAPHYLOCOCCUS AUREUS
SILVER MICROCLUSTERS
(Solution Submitted July 16, 2004)

July 27, 2004

PREPARED FOR:

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BY:

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PURPOSE.

The purpose of this study was to determine the effect of media components contained in minimal essential medium (MEM) on the antimicrobial activity of a Silver-based antimicrobial using the test organism *Staphylococcus aureus.* This was accomplished by performing a standard suspension test on the disinfectant using two *S. aureus* preparations; one suspended in water and the other in MEM. A 15 second contact time was evaluated.

MATERIALS AND METHODS.

A. Test organism.

The test suspensions were prepared by growing 5 ml cultures of *Staphylococcus aureus.* ATCC 6538 in Todd Hewitt Broth at 37°C, for 20 hr. Five ml of culture was pelleted by ccntrifugation, washed with five ml sterile 18 MQ water or MEM, centrifuged again, and re-suspended in a final volume of five ml sterile water or MEM.

B. Neutralizes

The Neutralizer consisted of 9ml tubes of 12.7% Twcen 80, 6.0% Tamol, 1.7% lecithin, 1% Peptone, and 0.1% Cystine, to which was added 10 µ1 of catalase solution (Sigma, C100. 42.300 units/mg).

C. Kill-Time Procedure.

1. Two 9.9 ml aliquots of the disinfectant were placed in sterile 20 mm x 150 mm tubes. The tubes were equilibrated in a 20°C water bath.
2. Each tube of disinfectant was inoculated with 100 µl of the test organism suspension at time zero. One tube was inoculated with organisms suspended in water and one tube was inoculated with organisms suspended in MEM.
3. After 15 seconds, one ml of organism/disinfectant suspension was removed to nine ml of neutralizer.
4. After two min, the neutralized suspension was serially diluted 1:10, in physiological saline solution (PSS).
5. 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 Tryptic Soy Agar plates. The plates were incubated at 37°C for 20 hr.
6. 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 of selected 1:10 dilutions ot the test suspension in PSS.
2. A neutralizer control was performed for each suspension by inoculating a mixture of 9 ml of neutralizer and 1 ml of disinfectant wiih 100 µl of a dilution of the titer containing about 6.000 CFU. This produced about 600 CFU / ml in the tube, which was allowed to stand for 20 minutes prior to dilution and assay of the tubes by membrane filtration using duplicate 1 ml samples.
3. Sterilization controls were performed by filtering 100 ml (PSS) or 1 ml (other fluids) samples of each solution used in this testing. Plates were incubated as above.

III. RESULTS.

A. Titer, *S. aureus* suspended in water.

	Dilution:		
	$1:1x10^5$	$1:1x10^6$	$1:1x10^7$
Number of colonies:	TNC	176	11
	TNC	139	13

Solution 7/16/04, *S. aureus* suspended in water.

	Dilution of staphylococcus/disinfectant suspension:			
Time	$1:1x10^1$	$1:1x10^2$	$1:1x10^3$	$1:1x10^4$
15 sec	0	0	0	0
	0	0	0	0

Neutralization Control (organisms in water)

$1:1x10^6$	$1:1x10^7$
TNC	51
TNC	74

B. Titer, *S. aureus* suspended in MEM.

	Dilution:		
	$1:1x10^5$	$1:1x10^6$	$1:1x10^7$
Number of colonies:	TNC	223	17
	TNC	245	30

Solution 7/16/04, *S. aureus* suspended in MEM.

	Dilution of staphylococcus/disinfectant suspension:			
Time	$1:1x10^1$	$1:1x10^2$	$1:1x10^3$	$1:1x10^4$
15 sec	0	0	0	0
	0	0	0	0

Neutralization Control (organisms in MEM)

$1:1x10^6$	$1:1x10^7$
TNC	187
TNC	162

C. Sterilization Controls.

Solution 7/16/04	0
Neutralizer	0
PSS	0
TSA medium	0
MEM	0

IV. DISCUSSION.

Results of the titers showed a viable staphylococcus concentration of 1.58×10^8 organisms per ml (water) and 2.34×10^8 organisms per ml (MEM) in the original suspensions. Inoculation of 9.9 ml of disinfectant with 100 µl of these suspensions produced initial concentrations of 1.58×10^6 and 2.34×10^6 organisms per ml respectively, in the assay tubes.

Results from these procedures did not allow accurate log reductions (LR) or percent kill (PK) values to be calculated because in each case there was complete kill resulting in zero counts. However, lower limits of activity could be estimated. Log reduction (LR) and percent kill (PK) values were calculated using the formulas: 1) LR = -Log(S/So), where S = concentration of viable organisms after 15 seconds; and So = the initial concentration of viable organisms at time zero. 2) PK = $(1 - (S/So)) \times 100$. These values are shown in the table below.

Solution	Diluent	Contact Time	Log Reduction (LR)	Percent Kill (PK)
Solution 7/16/04	Water	15 sec	>5.20	>99.9994
Solution 7/16/04	MEM	15 sec	>5.37	>99.9996

Neutralization control data showed that the disinfectant was adequately neutralized. Expected counts were similar to or slightly greater than those of the titer.

The disinfectant preparation tested here (solution 7/16/04) had significant bacteriocidal activity against *Staphylococcus aureus* in 15 seconds. However, since complete kill was observed with organisms suspended in each type of diluent, it was not possible to determine the effect of ingredients in MEM on the activity of this disinfectant.

Test Dates: July 22-23, 2004

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