KILL-TIME STUDIES
Antimicrobial Activity of Advanced Cellular Silver (ACS) 200
Using *Candida albicans*
Test Solution: ACS 200
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PREPARED FOR:

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BY:

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I. PURPOSE.

The purpose of this study was to determine the antimicrobial activity of ACS 200 on *Candida albicans.* This was accomplished by performing standard kill-time suspension tests using a 2 minute contact time.

II. MATERIALS AND METHODS.

A. Test organism.

The test suspension was prepared by growing a 24hr culture of *Candida albicans* in 5 ml of Sabaroud Dextrose broth at 37 °C. The suspension was then centrifuged, washed and re-suspended in 1ml sterile water.

B. Neutralizers.

The Neutralizer solution consisted of 9ml tubes of 10% Tween 80, 6.0% Tamol, 1.7% lecithin, 1% Tryptone, 0.6% Sodium Thiosulfate, and 1.0 % Cysteine.

C. Kill-Time Procedure.

1. 9.9ml of the ACS 200 test solution was added to a 20x150mm sterile bacterial culture tube. This tube was equilibrated in a 20 °C water bath. Then, 100 µl of the *C. albicans* suspension was added at time zero.
3. After the specified contact time (2min), 1.0 ml of bacterial suspension and ACS 200 test solution was added to 9.0ml of neutralizer. The tube was mixed thoroughly.
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 Sabaroud Dextrose Agar plates. The plates were incubated at 37 °C for 24 and 48 hours.
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 on selected 1:10 dilutions in PSS of the test suspension.
2. A neutralizer control for the disinfectant was performed by adding 1.0 ml of ACS 200 test solution to 9 ml of neutralizer and then inoculating the mixture with 0.1 ml of the $1{:}1x10^{5}$ dilution of the titer. This produced about 44 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.

Candida albicans:
Titer.

Number of colonies:	Dilution:		
	$1:1x10^{7}$	$1:1x10^{8}$	$1:1x10^{9}$
	39	6	0
	50	4	0

Titer: 4.45 x 10^{8}cfu/ml
ACS 200: (Received 04/29/09)

Exposure Time: 2 min	Dilution of *C. albicans*/disinfectant suspension:			
	$1:1x10^{1}$	$1:1x10^{2}$	$1:1x10^{3}$	$1:1x10^{4}$
	0	0	0	0
	0	0	0	0

	Neutralization Control		**Expected Counts:**		**Percent of Expected**
	Undiluted	1:10	Undiluted	1:10	
ACS 200	54	3			106%
	39	3			

Sterility Controls:

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

IV. DISCUSSION.

Results of the titer showed a viable *C. albicans* concentration of 4.45×10^8 organisms per ml in the original suspension. Inoculation of 9.9 ml of the test solution with 0.1 ml of this suspension produced an initial concentration of 4.45×10^6 *C. albicans* 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) $PK = (1 - (S/S_0))^{x} 100$. These values are shown below.

Test Solution	**Contact Time**	**Log Reduction (LR)**	**Percent Kill (PK)**
ACS 200	2 min	>5.95	>99.99989

Neutralization control data revealed that the neutralizer was able to adequately neutralize each solution. Observed count was 106% of those expected for the ACS 200 solution. Sterility controls showed no contamination issues.

Dates: August 7- August 10, 2009

Performed By:

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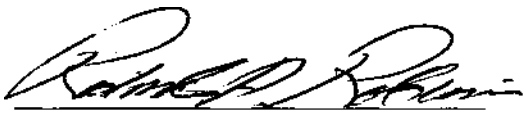

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