

KILL-TIME STUDIES

Antimicrobial Activity of Advanced Cellular Silver (ACS) 200

Using *Candida albicans*

Test Solution: ACS 200

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PREPARED FOR:

Results RNA

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I. PURPOSE

The purpose of this Study was to determine the antimicrobial activity of ACS 200 on the organism *Candida albicans*. 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 *Candida albicans* ATCC 10231 in Sabouraud Dextrose Broth at 37 °C, for 24 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 five ml sterile water.

B. Neutralizes

The Neutralizer solution consisted of 9 ml tubes of 12.7% Tween 80, 6.0% Tamol, 1.7% lecithin, 1% Peptone, 1.0% Cysteine and 500 mM Tris (pH 8.0).

C. Kill-Time Procedure

1. A 9.9 ml aliquot of the ACS 200 was placed in a 50 ml polypropylene sterile centrifuge tube. The tube was equilibrated in a 20 °C water bath.
2. The tube of ACS 200 was inoculated with 0.1 ml of the *C. albicans* suspension at time zero.
3. After the specified contact times, one ml of *C. albicans*/ACS 200 disinfectant suspension was removed to 9.0 ml of neutralizer. The tube was mixed thoroughly.
4. After two minutes, 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 Sabouraud 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 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 $1:1 \times 10^5$ dilution of the titer. This produced about 1 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:		
	<u>1:1x10⁶</u>	<u>1:1x10⁷</u>	<u>1:1x10⁸</u>
	5	2	0
	11	0	0

ACS 200:

(Received 8/18/08)

Dilution of *C. albicans*/ACS 200 disinfectant suspension:

Time	1:1x10 ²	1:1x10 ³	1:1x10 ⁴
1 min	TNC	110	1
	TNC	95	1
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	Undiluted	1:10	125
0	0	0.8	0.08	
2	0			

Sterility Controls:

Material	Counts
PSS	0
ACS 200	0
Water	0
Neutralizer	0
Sabouraud Dextrose Agar	0

IV. DISCUSSION

Results of the titer showed a viable *C. albicans* concentration of 8.0×10^6 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 8.0×10^4 *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) $PK = (1 - (S/S_0)) \times 100$. These values are shown below.

<u>Solution</u>	<u>Contact Time</u>	Log Reduction (LR)	Percent Kill (PC)
ACS 200	1 min.	0.89	87.3
	3 min	>4.20	>99.994
	5 min	>4.20	>99.994

Neutralization control data revealed that the neutralizer was able to adequately neutralize ACS 200 (neutralizer controls produced >100% of the expected counts).

V. CONCLUSION

ACS 200 had rapid fungicidal kill, producing a 0.89 log reduction in one minute and greater than a 4.2 log reduction equaling total kill of *C. albicans* within 3 minutes.

Test Dates: August 29-31, 2008