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FINAL REPORT

TESTING ANTIMICROBIAL ACTION ON HARD SURFACES

PROTOCOL NO. 200428005-01

LABORATORY NO. 274841

PREPARED FOR:

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LABORATORY NUMBER:	274841
PROTOCOL NUMBER:	200428005-01
SAMPLE SOURCE:	Advanced Vapor Technologies, LLC
SAMPLE IDENTIFICATION:	Dry Steam Vapor System, 2400 Series by Advanced Vapor Techologies, LLC Surfaces: Tile (Clay)
DEVIATIONS:	None
DATA ARCHIVE LOCATION:	Sequentially by lab number
PROTOCOL APPROVAL DATE:	11 Oct 2004
SAMPLE RECEIVED DATE:	11 Oct 2004
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REFERENCES:

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United States Pharmacopeial Forum, 2003. <1072> Disinfectants and Antiseptics. United States Pharmacopeial Convention, Inc., Rockville, MD.

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INTRODUCTION:

Validation of sanitizing agents for efficacy against organisms is increasingly an area of concern to manufacturers and regulatory agencies alike. Two ways to validate a disinfectant program include testing the disinfectant by performing a time kill procedure of the use dilution in a liquid test matrix, or testing the kill rate after the disinfectant is applied to contaminated hard surfaces. The

procedure that follows was designed to test the kill rate after the disinfectant is applied to contaminated hard surfaces and is based on the AOAC Official Method 961.02 Germicidal Spray Products, with the exception that this is a quantitative test. Additional controls for neutralization determination and organism concentration have been included.

This report describes the procedures for determining the effectiveness of a steam cleaning device tested against representative challenge organisms. The device was tested against designated organisms that were dried onto a representative porous coupon surface. Each combination was tested in duplicate with positive controls against the organisms listed below.

PROCEDURES:

TEST ORGANISM PREPARATION:

Organisms were inoculated into the appropriate media and grown according to the following Table:

ORGANISM	MEDIA	TEMPERATURE	TIME
Staphylococcus aureus ATCC #6538	SCDB	30-35°C	24-48 Hours
Staphylococcus epidermidis ATCC #12228	SCDB	30-35°C	24-48 Hours
Pseudomonas aeruginosa ATCC #15442	SCDB	30-35°C	24-48 Hours
Escherichia coli ATCC #8739	SCDB	30-35°C	24-48 Hours
Enterococcus faecium ATCC #19434	SCDB	30-35°C	24-48 Hours
Listeria monocytogenes ATCC #19111	SCDB	30-35°C	24-48 Hours
Salmonella choleraesuis ATCC #10708	SCDB	30-35°C	24-48 Hours

The *P. aeruginosa* test culture was decanted aseptically leaving the pellicle behind.

C. albicans ATCC #10231 was transferred to plates of Sabouraud dextrose agar (SDEX) and incubated at 20-25°C for 3-4 days. The culture was harvested with Physiological Saline Solution 0.9% (PHSS).

A. niger ATCC #16404 was inoculated onto Sabouraud dextrose agar (SDEX) and incubated at 20-25°C for 6-10 Days. *A. niger* was harvested by removing the mycelial mats from the surface using a sterile spatula. The mycelia were placed into a sterile funnel containing moist cotton and rinsed with SALT, a solution of 0.9% saline with 0.05% Tween®.

T. mentagrophytes ATCC #9533 was inoculated onto plates of glucose agar (FUAGA) and incubated at 25-30°C for 6-10 days. The mycelial mats were removed from the agar surface using a sterile spatula. The mycelia were transferred to a sterile tissue grinder and macerated using PHSS. The suspension was filtered through a sterile funnel containing moist cotton and standardized with PHSS.

TEST PRODUCT PREPARATION:

The Dry Steam Vapor System, 2400 Series was prepared by sponsor.

COUPON PREPARATION:

The clay tile coupons were sterilized in an autoclave at 121+°C for a minimum of 20 minutes. Coupons were prepared in duplicate for each organism/product application. Additional coupons were prepared to represent positive and negative coupons.

TEST PROCEDURE:

The prepared cultures of each test organism were thoroughly shaken. Coupons were inoculated with 0.01-0.03 mL of prepared culture. The culture was immediately spread uniformly over an approximate 1" x 1" area. Two positive control coupons were inoculated for each organism and surface type. The negative control coupons were left uninoculated.

The coupons were dried for 30-40 minutes at 37 ± 2°C. Positive control coupons were immediately transferred to 100 mL of Letheen broth (LETH) after the drying time.

Three applications were performed as follows:

Application One: A nozel brush with a towel placed over the brush was applied to the coupon. The brush was placed on coupons with a back and forth motion while steam from Dry Steam Vapor System, 2400 Series was activated. The exposure time for application one was 7 seconds.

Application Two: The Tri-brush with a towel placed over the brush was applied to the coupon. The brush was placed on coupons with a back and forth motion while steam from Dry Steam Vapor System, 2400 Series was activated. The exposure time for the application was 10 seconds.

Application Three: The Tri-brush with a towel placed over the brush was applied to the coupon. The brush was placed on coupons with a back and forth motion while steam from Dry Steam Vapor System, 2400 Series was activated. The exposure time for the application was 30 seconds.

After the exposure time, the coupons were placed into 100 mL of LETH. The bottles were shaken manually for 1 minute or 100 times through a 12 inch path to extract surviving organism. The positive controls were extracted in the same manner. After the first extraction of the positive controls, the coupons were transferred to new bottles containing 100 mL of LETH and a second extraction was performed. This was repeated a third time. Appropriate dilutions of the positive controls were made in LETH to achieve plates within a countable range. The extract was serially diluted in LETH blanks. Plate counts were performed in triplicate by plating 0.5 mL onto appropriate agar. The remaining extraction fluid was filtered through a sterile 0.45 μm filter membrane to determine total kill. The filter was plated onto the appropriate agar. Soybean casein digest agar (SCDA) for bacteria samples, Sabouraud Dextrose Agar (SDEX) for yeast and mold, and 1% Glucose Agar (FUAGA) was used for fungus. Bacterial Samples were incubated at 30-35°C for 48-72 hours. Mold test samples were incubated at 20-25°C for 3-7 days. Yeast test samples were incubated at 20-25°C for 3-5 days. Fungi samples were incubated at 25-30°C for 4-10 days. The test procedures was repeated for all possible organism and cleaning application combinations.

CALCULATIONS:

For the positive controls, the percent efficiency is obtained by dividing the number of organisms recovered in the first extraction by the total number of organisms recovered from the coupon over all three extractions. The positive control titer is corrected for the percent efficiency by dividing the number recovered in the first extraction by the percent efficiency.

Log reductions were calculated using the following formula:

$$\log \textit{reduction} = \log U - \log C$$

Where U = Average corrected positive control titer
Where C = Average corrected recovered counts

Percent reductions were calculated using the following formula:

$$\% \ reduction = 1 - \frac{1}{10^{(\log \ reduction)}} \ x \ 100\%$$

ACCEPTANCE CRITERIA:

In order to be considered passing for each surface, the application(s) must demonstrate a 3 log reduction against vegetative organisms and at least a 2 log reduction against spore forming organisms. Specific criteria for pass/fail of the application surface combination must be determined by the sponsor.

STATEMENT OF UNCERTAINTY:

The uncertainty of this test is based on the standard deviation of the Nelson Laboratories, Inc.'s general plate count proficiency test data. The combined standard uncertainty for a plate count is 0.05 log units. The expanded uncertainty for a plate count, at a 95% confidence level, is 0.10 log units.

RESULTS:

The data demonstrates effective kill for all organism types and applications in 7 seconds with some organisms demonstrating even greater kill at 10 and 30 seconds. The counts of recovered organisms, percent reductions and $\log_{10}$ reductions for the test materials can be found in Table 1. Extraction Efficiency data for each material and organism can be found in Table 2.

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22 Nov 2004
Study Completion Date

BEW/ejb

TABLE 1. Results

SAMPLE IDENTIFICATION	EXPOSURE INTERVALS	AVERAGE CONTROL TITER AT TIME ZERO (CFU)	AVERAGE RECOVERED (CFU)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
Application #1 with *S. aureus*	7 Seconds	2.5×10^7	$\sim 2.8 \times 10^4$	~99.89	~2.95
Application #2 with *S. aureus*	10 Seconds	2.5×10^7	$< 1.0 \times 10^0$	>99.999996	>7.40
Application #3 with *S. aureus*	30 Seconds	2.5×10^7	$< 1.0 \times 10^0$	>99.999996	>7.40

TABLE 1. Results (Cont.)

SAMPLE IDENTIFICATION	EXPOSURE INTERVALS	AVERAGE CONTROL TITER AT TIME ZERO (CFU)	AVERAGE RECOVERED (CFU)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
Application #1 with *S. epidermidis*	7 Seconds	1.1×10^7	$<1.0 \times 10^0$	>99.999991	>7.04
Application #2 with *S. epidermidis*	10 Seconds	1.1×10^7	$<1.0 \times 10^0$	>99.999991	>7.04
Application #3 with *S. epidermidis*	30 Seconds	1.1×10^7	$<1.0 \times 10^0$	>99.999991	>7.04

TABLE 1. Results (Cont.)

SAMPLE IDENTIFICATION	EXPOSURE INTERVALS	AVERAGE CONTROL TITER AT TIME ZERO (CFU)	AVERAGE RECOVERED (CFU)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
Application #1 with *P. aeruginosa*	7 Seconds	1.8×10^7	$<1.0 \times 10^0$	>99.999994	>7.26
Application #2 with *P. aeruginosa*	10 Seconds	1.8×10^7	$<1.0 \times 10^0$	>99.999994	>7.26
Application #3 with *P. aeruginosa*	30 Seconds	1.8×10^7	$<1.0 \times 10^0$	>99.999994	>7.26

TABLE 1. Results (Cont.)

SAMPLE IDENTIFICATION	EXPOSURE INTERVALS	AVERAGE CONTROL TITER AT TIME ZERO (CFU)	AVERAGE RECOVERED (CFU)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
Application #1 with *S. cholerasuis*	7 Seconds	1.4×10^7	$<1.0 \times 10^0$	>99.999993	>7.15
Application #2 with *S. cholerasuis*	10 Seconds	1.4×10^7	$<1.0 \times 10^0$	>99.999993	>7.15
Application #3 with *S. cholerasuis*	30 Seconds	1.4×10^7	$<1.0 \times 10^0$	>99.999993	>7.15

TABLE 1. Results (Cont.)

SAMPLE IDENTIFICATION	EXPOSURE INTERVALS	AVERAGE CONTROL TITER AT TIME ZERO (CFU)	AVERAGE RECOVERED (CFU)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
Application #1 with *E. coli*	7 Seconds	1.8×10^7	$<1.0 \times 10^0$	>99.999994	>7.26
Application #2 with *E. coli*	10 Seconds	1.8×10^7	$\sim 1.3 \times 10^1$	~99.999931	~6.16
Application #3 with *E. coli*	30 Seconds	1.8×10^7	$<1.0 \times 10^0$	>99.999994	>7.26

TABLE 1. Results (Cont.)

SAMPLE IDENTIFICATION	EXPOSURE INTERVALS	AVERAGE CONTROL TITER AT TIME ZERO (CFU)	AVERAGE RECOVERED (CFU)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
Application #1 with *L. monocytogenes*	7 Seconds	4.6×10^6	$\sim 1.6 \times 10^1$	~99.99966	~5.47
Application #2 with *L. monocytogenes*	10 Seconds	4.6×10^6	$\sim 1.0 \times 10^0$	~99.999978	~6.66
Application #3 with *L. monocytogenes*	30 Seconds	4.6×10^6	$< 3.5 \times 10^0$	>99.999924	>6.12

TABLE 1. Results (Cont.)

SAMPLE IDENTIFICATION	EXPOSURE INTERVALS	AVERAGE CONTROL TITER AT TIME ZERO (CFU)	AVERAGE RECOVERED (CFU)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
Application #1 with *E. faecium*	7 Seconds	2.1×10^7	$<1.0 \times 10^0$	>99.999995	>7.30
Application #2 with *E. faecium*	10 Seconds	2.1×10^7	$<1.0 \times 10^0$	>99.999995	>7.30
Application #3 with *E. faecium*	30 Seconds	2.1×10^7	$<1.0 \times 10^0$	>99.999995	>7.30

TABLE 1. Results (Cont.)

SAMPLE IDENTIFICATION	EXPOSURE INTERVALS	AVERAGE CONTROL TITER AT TIME ZERO (CFU)	AVERAGE RECOVERED (CFU)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
Application #1 with *C. albicans*	7 Seconds	2.9×10^7	$<2.0 \times 10^0$	>99.999993	>7.16
Application #2 with *C. albicans*	10 Seconds	2.9×10^7	$<2.0 \times 10^0$	>99.999993	>7.16
Application #3 with *C. albicans*	30 Seconds	2.9×10^7	$<1.0 \times 10^0$	>99.999997	>7.46

TABLE 1. Results (Cont.)

SAMPLE IDENTIFICATION	EXPOSURE INTERVALS	AVERAGE CONTROL TITER AT TIME ZERO (CFU)	AVERAGE RECOVERED (CFU)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
Application #1 with *A. niger*	7 Seconds	4.9×10^5	$<1.0 \times 10^0$	>99.99980	>5.69
Application #2 with *A. niger*	10 Seconds	4.9×10^5	$<1.0 \times 10^0$	>99.99980	>5.69
Application #3 with *A. niger*	30 Seconds	4.9×10^5	$<1.0 \times 10^0$	>99.99980	>5.69

TABLE 1. Results (Cont.)

SAMPLE IDENTIFICATION	EXPOSURE INTERVALS	AVERAGE CONTROL TITER AT TIME ZERO (CFU)	AVERAGE RECOVERED (CFU)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
Application #1 with *T. mentagrophytes*	7 Seconds	1.8×10^6	$<1.0 \times 10^0$	>99.999944	>6.26
Application #2 with *T. mentagrophytes*	10 Seconds	1.8×10^6	$<1.0 \times 10^0$	>99.999944	>6.26
Application #3 with *T. mentagrophytes*	30 Seconds	1.8×10^6	$<1.0 \times 10^0$	>99.999944	>6.26

TABLE 2. Extraction Efficiency

COUPON TYPE	ORGANISM	EXTRACTION EFFICIENCY (%)
Tile	*S. aureus*	97.2
Tile	*S. epidermidis*	99.1
Tile	*P. aeruginosa*	95.5
Tile	*S. cholerasuis*	94.0
Tile	*E. coli*	99.0
Tile	*L. monocytogenes*	99.3
Tile	*E. faecium*	97.3
Tile	*C. albicans*	96.1
Tile	*A. niger*	99.2
Tile	*T. mentagrophytes*	98.9