



SANI-SHIELD PRODUCT VALIDATION STUDY

SUBMITTED TO: UNELKO CORPORATION

LEGEND PROJECT NO. 7051333

JUNE 21, 2007

SUBMITTED BY:

LEGEND TECHNICAL SERVICES OF ARIZONA

17631 N. 25th Avenue, Phoenix, Arizona 85023

June 21, 2007

Mr. Howard Ohlhausen
Director
Unelko Corporation
14641 N. 74th Street
Scottsdale, AZ 85260

RE: Sani-Shield Product Validation Testing
LEGEND Project No. 7051333

Dear Mr. Ohlhausen:

1.0 INTRODUCTION AND BACKGROUND

Legend Technical Services of Arizona, Inc. (LEGEND) was contacted by Mr. Howard Ohlhausen (Study Sponsor) to conduct product testing on Sani-Shield 3-in-1 Surface Care for hard surfaces (also marketed as 1-Step Clean and Shield Surface Care for household surfaces). The purpose of this study was to reconfirm and further validate the efficacy of Sani-Shield against three common types of bacteria.

The scope of LEGEND's services was limited to product validation testing for Unelko Corporation's "spray and wipe" and "barrier durability" antibacterial performance claims on surfaces. These claims are:

Sani-Shield – "Cleans Organic Dirt and Grime With The Antiseptic Power of Hydrogen Peroxide – Protects With Invisible Barrier On Which Bacteria, Mold and Mildew Will Not Grow!"

Clean & Shield – "Easily Cleans Dirt, Grease & Everyday Household Spills and Splatters – Provides Long-Lasting Invisible Barrier On Which Bacteria, Mold & Mildew Will Not Grow"

When questioned by LEGEND regarding the meaning of "organic dirt and grime," Unelko responded, "In its simplest definition, it is any form of surface soil containing carbon compounds and microorganisms."

Testing was divided into two stages: A spray and wipe correlation study (Stage 1) and a residual antibacterial study to assess barrier durability (Stage 2).

2.0 STUDY OVERVIEW

Study Preparation

LEGEND was provided a supply of Sani-Shield product (at least 60 days old), sterile water, and 2.5% citric acid aqueous solution to be used in the study.

For the study, Sani-Shield was evaluated against three types of bacteria that are potentially pathogenic to humans and commonly used in laboratory studies:

- *Escherichia coli* strain 0157:H7 (ATCC No. 35150)
- *Staphylococcus aureus* (ATCC No. 25923)
- *Enterobacter aerogenes* (ATCC No. 13048)

Bacterial cultures were first prepared using Trypticase Soy Agar with 5% Sheep Blood (BAP). Stock cultures were incubated at 35°C for 48 hours to obtain heavy growth for preparation of the inoculum. Tubes of sterile Butterfield's buffer solution were inoculated with each type of bacteria to obtain a turbid culture. Aerobic plate counts were performed on each tube of bacteria to determine the number of bacteria as Colony-Forming Units (CFU)/mL.

Test Procedure

Stage 1 – Spray and Wipe Correlation Study

Sterile Petri dishes were inoculated with a quantity of 1,000,000 CFU of each type of bacteria used in the study. The inoculum contained 5% fetal bovine serum to represent the "soil load" (i.e. surface soil with germs). Positive and negative control (media blank) samples were prepared for each batch of samples. After 15 minutes of inoculum contact time, each of the Petri dishes was sprayed with one of three different test liquids (#1-Sterile water; #2-Sani-Shield; #3- 2.5% Citric acid solution) and hand wiped using sterile latex gloves with a sterile polyester wipe until dry and polished as per the instructions for the use of the Sani-Shield Product. Tests using the Sani-Shield solution were prepared in triplicate.

In this study, analyses of residual, viable bacteria were performed on the wipes used to clean and shield the inoculated surfaces as well as on the swabs employed to test the cleaned surfaces. For the wipe analysis, the wipes used to clean and dry each plate were analyzed using an aerobic plate count procedure (Tables 1 and 5). For the swab tests, a sterile cotton swab was used to swab the surface of each plate after cleaning of the inoculums with the different solutions. The swabs were analyzed for viable bacteria using an aerobic plate count procedure (Tables 2 and 6).

An aerobic plate count is a standard microbiological test used to determine the level of bacteria in a product that are able to grow in the presence of oxygen. This test procedure is also known as a Standard Plate Count. Aerobic plate counts typically involve the plating of a sample at multiple dilutions on a nutrient-rich medium formulated to isolate a wide range of common bacteria. Plating is performed using a "pour-plate" procedure in which the samples is added to the sterile Petri dish, and tempered, liquid agar is added to the dish and swirled gently to distribute the sample. Culture plates are incubated at 35°C for 48 +/- 3 hours. After incubation, the resulting bacterial colonies are counted using a darkfield colony counter. Plates with counts in excess of 300 colonies are

reported as greater than (>) the upper count limit (300 colonies) multiplied by the dilution factor.

In addition to the test of the wipes and analysis of the swabs, a separate set of Petri dish test plates was inoculated with the various bacteria, dried for 15 minutes, then sprayed with Sani-Shield and wiped dry. To test for viable residual bacteria, the Petri dishes were then immersed in 100 mL of sterile buffered water in a sterile plastic bag. The bag was agitated by hand for approximately one minute to dislodge bacteria adhering to the surface of the Petri dish, and an aerobic plate count was performed on the rinse liquid (Tables 4 and 8) and on the wiping cloths for each sample (Tables 3 and 7).

Stage 2 – Residual Antimicrobial Study

For the residual antimicrobial barrier durability study, Petri dishes treated with Sani-Shield were evaluated at 24, 48, 72, 96, and 120 hours after Sani-Shield treatment, with separate dishes used for each organism.

Each dish was treated with Sani-Shield two times (sprayed and wiped until dry using sterile latex gloves and sterile polyester wipes) and allowed to dry for an additional 15 minutes. Each dish was then inoculated with the test bacteria/soil and allowed to air dry for 24 hours.

After air drying the bacterial inoculums, each dish was swabbed, and aerobic plate counts were performed on the swab sample. This procedure of re-inoculating the Sani-Shield-treated dish with the soil/bacteria – and swab testing the inoculated dishes 24 hours later – was repeated at 48, 72, and 96 hours for each organism. The Sani-Shield was not reapplied after the initial applications. Both positive (3) and negative (media blank) control samples were analyzed. Test plates were maintained at approximately 25°C and 30% relative humidity (See Tables 9-13).

3.0 ANALYTICAL RESULTS

Appendix A, Tables 1 through 18 report the Aerobic Plate Count and “Percent Kill” results.

4.0 DISCUSSION AND CONCLUSIONS

Stage 1 – Spray and Wipe Correlation Study

The Spray and Wipe Correlation Study tested the efficacy of Sani-Shield against three types of bacteria using a variety of assessment methods. The aerobic plate count data for swab tests performed on the treated surfaces indicated percent kill rates of 99.0 to >99.8% for Sani-Shield, compared to kill rates ranging from 78.0 to 90.6% for 2.5% Citric Acid solution and 84.4 to 92.0% for sterile water (Tables 5-8). As indicated by the fairly high “kill rate” associated with the Sterile Water Control, significant bacterial kill can be attributed to the “bio-mechanical” act of spraying and wiping the surfaces until dry.

To address this observation, this study also included an analysis of the sterile wipe cloth used in the cleaning and in the cleaning and shielding procedures. Percent kill rate comparisons between Sani-Shield, Citric Acid, and the Sterile Water showed much higher deviations between the test groups, indicating that much of the surface bacteria, while removed from the surface, is deposited on the wipe. Only the Sani-Shield product yielded kill rates ranging from 99.8 to >99.9% for bacteria analyzed from the surfaces by swab-testing them and from the wipes used for cleaning and shielding the surfaces (Tables 5-8).

In addition to the testing of the swabs and the wipes, an immersion procedure was also performed on the cleaned and on the cleaned and shielded surfaces as a further means of assessing the efficacy of Sani-Shield. The cleaned and the cleaned and shielded (Sani-Shield) test plates were immersed in a solution of sterile water, which was then analyzed for residual aerobic bacteria. Testing of the immersion solution yielded mixed results for the different types of bacteria. The *E. coli* exhibited the greatest reduction in concentration following treatment, with a kill rate of >99.7%. The *S. aureus* indicated a kill rate of 50.0% when treated with Sani-Shield, and the *E. aerogenes* was not detected in the positive control. From the observed results, it appears that low detection of the target organisms in the rinse solution may have biased the kill rates low for all test groups (Tables 3 and 4, 7 and 8).

The wipe testing following the immersion procedure yielded similar results to that performed in the swab procedure. Percent kill rates for Sani-Shield-treated surfaces ranged from 99.2 to >99.9%. Those for citric acid ranged from 0.0 to 85.0% while kill rates for sterile water ranged from 0.0 to 30.8%.

Stage 2 – Residual Antimicrobial Study

To determine the residual efficacy of Sani-Shield-treated surfaces, surfaces were reinoculated with the three test groups of bacteria at different intervals and evaluated using a swab procedure. Sani-Shield surfaces – when inoculated and re-inoculated with *E. coli* – consistently yielded kill rates of >99.9% when swabbed at 24, 48, 72, 96, and 120 hours after inoculation. Percent kill rates for *E. aerogenes* were >99.9% when the organism was also detected in the positive control sample. Similarly, kill rates ranged from 85.7 to 97.7% for *S. aureus* when the positive control samples yielded significant detection of the target organism (Tables 9-18). The data indicates that the test procedure may require some amendments (i.e. testing at shorter intervals after inoculation) to ensure greater detection of the target organisms in the positive control samples. Indeed, the concentrations of the bacteria in the positive control samples likely biased the kill rates low for *E. aerogenes* and *S. aureus*.

Summary of Results

Both the Spray and Wipe Correlation Study and the Residual Antimicrobial Study yielded results that validate the efficacy of Sani-Shield against *E. coli*, *E. aerogenes*, and *S. aureus* bacteria. While subtle differences in the kill rates between the target organisms were observed, the efficacy of Sani-Shield has been demonstrated using a variety of methodologies. Sani-Shield has been demonstrated to provide significantly higher rates of “killing” effectiveness when

compared to Citric Acid solution and Sterile Water. The Sani-Shield barrier has also been demonstrated to have significant residual antibacterial effectiveness on surfaces that are repeatedly inoculated with the target organisms over a period of at least five (5) days.

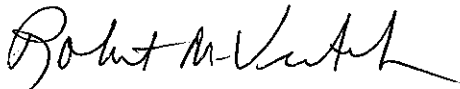
For those everyday surfaces and objects that cannot be immersed in – or readily flooded by – disinfectants or sterilants to make them free of bacterial contamination, the “spray and wipe” organism-cleaning technique appears efficacious and can be confirmed by “swab-testing” the cleaned and shielded surface or by analyzing the “cloth wipe” employed to “clean and shield” with significant correlation of results.

5.0 REMARKS

If there are any additional questions regarding the final results or the methodology used for this project, or if further testing is elected please contact LEGEND at (602) 324-6100.

Prepared By:

LEGEND TECHNICAL SERVICES OF ARIZONA, INC.



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APPENDIX A

APPENDIX A - AEROBIC PLATE COUNT DATA (AFTER SPRAY AND WIPE PROCEDURES)

Stage 1 Results: Spray and Wipe Correlation Study

Table 1: Wipe Tests

Plate Counts (CFU/mL)

Test Organism	Sterile Water	Sani-Shield (A)	Sani-Shield (B)	Sani-Shield (C)	Citric Acid	Positive Control
<i>E. coli</i>	>3000	<1	<1	<1	430	>3000
<i>E. aerogenes</i>	260	<1	<1	<1	340	400
<i>S. aureus</i>	420	<1	<1	<1	420	620

Table 2: Swab Tests

Plate Counts (CFU/mL)

Test Organism	Sterile Water	Sani-Shield (A)	Sani-Shield (B)	Sani-Shield (C)	Citric Acid	Positive Control
<i>E. coli</i>	760	<10	<10	<10	1100	5600
<i>E. aerogenes</i>	500	<10	<10	<10	300	3200
<i>S. aureus</i>	80	<10	<10	<10	220	1000

Table 3: Wipe Tests

Plate Counts (CFU/mL)

Test Organism	Sterile Water	Sani-Shield (A)	Sani-Shield (B)	Sani-Shield (C)	Citric Acid	Positive Control
<i>E. coli</i>	>3000	<1	<1	<1	450	>3000
<i>E. aerogenes</i>	150	<1	<1	<1	320	130
<i>S. aureus</i>	830	<1	<1	<1	840	1200

Table 4: Immersion Tests

Plate Counts (CFU/mL)

Test Organism	Sterile Water	Sani-Shield (A)	Sani-Shield (B)	Sani-Shield (C)	Citric Acid	Positive Control
<i>E. coli</i>	200	<1	<1	2	14	310
<i>E. aerogenes</i>	1	<1	<1	<1	14	<1
<i>S. aureus</i>	10	<1	<1	<1	<1	2

* Media blanks analyzed for all Aerobic Plate Counts. Results are <1 CFU/plate unless otherwise specified.

APPENDIX A - AEROBIC PLATE COUNT DATA (BARRIER DURABILITY)

Stage 2 - Residual Antimicrobial Study

Table 9: Swab Test 24 Hours:

Test Organism	Plate Counts (CFU/mL)	
	Sani-Shield	Positive Control
<i>E. coli</i>	10	>30000
<i>E. aerogenes</i>	<10	<10
<i>S. aureus</i>	<10	<10

Table 10: Swab Test 48 Hours:

Test Organism	Plate Counts (CFU/mL)	
	Sani-Shield	Positive Control
<i>E. coli</i>	<10	>30000
<i>E. aerogenes</i>	<10	<10
<i>S. aureus</i>	<10	440

Table 11: Swab Test 72 Hours:

Test Organism	Plate Counts (CFU/mL)	
	Sani-Shield	Positive Control
<i>E. coli</i>	20	>30000
<i>E. aerogenes</i>	<10	>30000
<i>S. aureus</i>	<10	260

Table 12: Swab Test 96 Hours:

Test Organism	Plate Counts (CFU/mL)	
	Sani-Shield	Positive Control
<i>E. coli</i>	<10	>30000
<i>E. aerogenes</i>	<10	>30000
<i>S. aureus</i>	<10	240

Table 13: Swab Test 120 Hours:

Test Organism	Plate Counts (CFU/mL)	
	Sani-Shield	Positive Control
<i>E. coli</i>	<10	>30000
<i>E. aerogenes</i>	<10	>30000
<i>S. aureus</i>	<10	70

* Media blanks analyzed for all Aerobic Plate Counts. Results are <1 CFU/plate unless otherwise specified.

APPENDIX A - "PERCENT KILL" DATA (AFTER SPRAY AND WIPE PROCEDURES)

Stage 1 Results: Spray and Wipe Correlation Study

Table 5: Wipe Tests

Percent Kill

Test Organism	Sterile Water	Sani-Shield (A)	Sani-Shield (B)	Sani-Shield (C)	Citric Acid
<i>E. coli</i>	0.0	>99.9	>99.9	>99.9	85.7
<i>E. aerogenes</i>	35.0	>99.8	>99.8	>99.8	15.0
<i>S. aureus</i>	32.2	>99.8	>99.8	>99.8	32.2

Table 6: Swab Tests

Percent Kill

Test Organism	Sterile Water	Sani-Shield (A)	Sani-Shield (B)	Sani-Shield (C)	Citric Acid
<i>E. coli</i>	86.4	>99.8	>99.8	>99.8	80.4
<i>E. aerogenes</i>	84.4	>99.7	>99.7	>99.7	90.6
<i>S. aureus</i>	92.0	>99.0	>99.0	>99.0	78.0

Table 7: Wipe Tests

Percent Kill

Test Organism	Sterile Water	Sani-Shield (A)	Sani-Shield (B)	Sani-Shield (C)	Citric Acid
<i>E. coli</i>	0.0	>99.9	>99.9	>99.9	85.0
<i>E. aerogenes</i>	0.0	>99.2	>99.2	>99.2	0.0
<i>S. aureus</i>	30.8	>99.9	>99.9	>99.9	30.0

* No bacteria detected in positive control sample. Percent Kill not applicable.

Table 8: Immersion Tests

Percent Kill

Test Organism	Sterile Water	Sani-Shield (A)	Sani-Shield (B)	Sani-Shield (C)	Citric Acid
<i>E. coli</i>	35.5	>99.7	>99.7	>99.7	95.5
<i>E. aerogenes</i>	*	*	*	*	*
<i>S. aureus</i>	0	>50.0	>50.0	>50.0	>50.0

APPENDIX A - "PERCENT KILL" DATA (BARRIER DURABILITY)

Stage 2 - Residual Antimicrobial Study

Table 14: Swab Test 24 Hours:

Percent Kill	
Test Organism	Sani-Shield
<i>E. coli</i>	>99.9
<i>E. aerogenes</i>	*
<i>S. aureus</i>	*

Table 15: Swab Test 48 Hours:

Percent Kill	
Test Organism	Sani-Shield
<i>E. coli</i>	>99.9
<i>E. aerogenes</i>	*
<i>S. aureus</i>	>97.7

Table 16: Swab Test 72 Hours:

Percent Kill	
Test Organism	Sani-Shield
<i>E. coli</i>	>99.9
<i>E. aerogenes</i>	>99.9
<i>S. aureus</i>	>96.2

Table 17: Swab Test 96 Hours:

Percent Kill	
Test Organism	Sani-Shield
<i>E. coli</i>	>99.9
<i>E. aerogenes</i>	>99.9
<i>S. aureus</i>	>95.8

Table 18: Swab Test 120 Hours:

Percent Kill	
Test Organism	Sani-Shield
<i>E. coli</i>	>99.9
<i>E. aerogenes</i>	>99.9
<i>S. aureus</i>	>85.7

* No bacteria detected in positive control sample. Percent Kill not applicable.