BIO-SHIELD
BioShield Technologies Inc.

PROJECT NAME
Residual Self-Sanitizing Efficacy

PRODUCT IDENTITY
BSTI 1:16 Disinfectant Spray
NoviGuard RTU Spray

TEST INITIATION DATE
May 16, 2001 – 24 Hour

RELEVANT TESTING DATES
May 18, 2001 – 72 Hour
May 23, 2001 – 7 Day
May 30, 2001 – 14 Day
May 30, 2001 – Repeat 24 Hour
June 13, 2001 – 28 Day

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This study was not performed under
EPA Good Laboratory Practice Regulations

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Test System Growth Media

Pseudomonas aeruginosa ATCC# 15442 - Tryptic Soy Broth - Incubate at 35-37°C
-The microorganism used in this study was obtained from the American Type Culture Collection, Manassas, Virginia.

Recovery Media

Neutralizing Subculture Medium: Letheen Broth
Agar Plate Medium: Trypticase Soy Broth

Test Method

Carriers:
Approximately 75 x 25 mm glass slides were placed in glass 250 ml beakers and sterilized at 121°C for 15 minutes. Individual sterile plastic petri dishes were matted with filter paper. Four sterile glass slides were transferred into each of the matted petri dishes.

Medication:
The carriers were allowed to equilibrate to ambient temperature prior to medication. Each carrier was then treated at 4-6" from the carrier. The surface was sprayed for three seconds at which point the carriers were completely wet. The carries were allowed to air dry under a fume hood until solution was completely evaporated.

Holding Periods:
These carriers will be held for periods of 24 hours, 72 hours, 7 days, 14 days, and 28 days.

Preparation of Test Organism:
A 24 hour broth culture of *P. aeruginosa* was prepared. Two ml of this culture was applied to the surface of two TSA plates and incubated at 37 °C for 44-48 hours. 3 ml of sterile 0.9% saline solution was applied to each plate. This solution was recovered and vortex mixed until uniformly turbid. A 1:50 dilution was made in sterile 0.9% saline solution.

Carrier contamination:
After the specified holding time, 0.01 ml (or 10ul) of the test organism suspension was added to the designated glass coupon at staggered intervals and spread evenly over surface. The test organism was allowed to contact the product residue for a fifteen minute exposure time at ambient temperature.

Subculture:
Following the fifteen minute exposure period, each carrier was transferred at identical staggered intervals to 40 ml of letheen broth and vortex mixed for one minute. The number of survivors from each carrier was determined by standard pour plate method done on the above letheen broth in the case of the treated carriers. The control carriers were serially diluted in 0.9% sterile saline solution and pour plated.

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Incubation and Observation:
All subculture plates and tubes were incubated for 48 hours at 37 °C. Following incubation, the subculture plates and tubes were visually examined for growth. The colonies present on each plate were counted and recorded and the number of colony forming units, or CFU, present on each carrier or into each suspension was calculated.

Neutralization Confirmation:
No neutralization confirmation was performed in this experiment, as it is known that letheen broth neutralized BSTI products. In addition, letheen broth was demonstrated to neutralize the NoviGuard product by ViroMed laboratories.

Study Acceptance Criteria:
The EPA requires the results to show a bacterial reduction of at least 99.9% over the parallel control.

Data Analysis
Calculations:
The colonies present on each plate were counted and recovered and the number of CFU present on each carrier or in each suspension was calculated.

$$\text{CFU/Carrier} = \frac{\text{CFU (average of two plates)} \times \text{Dilution Factor} \times \text{Volume of LB}}{\text{Number of carriers tested} \times \text{volume plated}}$$

Percent Reduction: The following equation was used to calculate percent reduction:

$$\% \text{ reduction} = [(A-B)/A] \times 100$$

where:
A = CFU/carrier average of the number of survivors on the control carriers
B = CFU/carrier average of the number of survivors on the test carriers

Results

Qualitative Growth Results (48 Hours from initiation)

Sample	24 Hour	72 Hour	7 Day	14 Day	28 Day
Control	+,+,+	+,+,+	+,+,+	+,+,+	+,+,+
BSTI	-,+,+	-,-,-	-,-,-	+,+,+	+,-,+
NoviGuard	-,-,-	-,-,-	+,-,-	-,-,+	-,+,+

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Quantitative Results:

Control Carrier

Carrier #	24 Hour	72 Hour	7 Day	14 Day	28 Day
1	$1.81x10^5$	$6.00x10^4$	$1.333x10^5$	$4.00x10^5$	$1.87x10^5$
2	$1.46x10^5$	$4.00x10^4$	$6.67x10^4$	$4.33x10^5$	$2.93x10^5$
3	$1.47x10^4$	$4.67x10^4$	$1.00x10^5$	$2.13x10^5$	$2.00x10^5$
Average	$1.14x10^5$	$4.89x10^4$	$1.00x10^5$	$3.49x10^5$	$2.27x10^5$

BioShield Carrier

Carrier #	24 Hour	72 Hour	7 Day	14 Day	28 Day
1	<1	<1	<1	<1	<1
2	6.67	<1	<1	<1	<1
3	<1	<1	<1	$4.66x10^1$	<1
Average	2.23	<1	<1	$1.55x10^1$	<1

NoviGuard Carrier

Carrier #	24 Hour	72 Hour	7 Day	14 Day	28 Day
1	<1	<1	<1	<1	<1
2	<1	<1	<1	<1	$2.67x10^1$
3	<1	<1	<1	<1	$1.47x10^2$
Average	<1	<1	<1	<1	$<5.79x10^1$

Calculated Reduction Values:

BioShield

Time	Control Carrier	Treated Carrier	% Reduction
24 hours	$1.14x10^5$	2.23	99.9998%
72 hours	$4.89x10^4$	<1	>99.9999%
7 days	$9.99x10^4$	<1	>99.9999%
14 days	$3.49x10^5$	$1.55x10^1$	99.996
28 days	$2.27x10^5$	<1	>99.9999%

NoviGuard

Time	Control Carrier	Treated Carrier	% Reduction
24 hours	$1.14x10^5$	<1	>99.9999%
72 hours	$4.89x10^4$	<1	>99.9999%
7 days	$9.99x10^4$	<1	>99.9999%
14 days	$3.49x10^5$	<1	>99.9999%
28 days	$2.27x10^5$	$<5.79x10^1$	99.97%

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Repeat 24 Hour Trial

Note – the BioShield carriers were still moist at the testing time of 12:00 p.m. This may be due to over-saturation of the filter paper lining the petri dish.

Qualitative Results

Sample	24 Hour
Control	+,+,+
BSTI	-,-,-
NoviGuard	-,-,+

Quantitative Results

Carrier #	Control	BSTI	NoviGuard
1	$2.53x10^5$	<1	<1
2	$3.47x10^5$	<1	<1
3	$2.00x10^4$	<1	<1
Average	$2.07x10^5$	<1	<1

Calculated reduction values

BioShield Carriers

Time	Control Carrier	BSTI Treated	% Reduction
24 hours	$2.07x10^5$	<1	99.9999%

NoviGuard Carriers

Time	Control Carrier	NoviGuard Treated	% Reduction
24 hours	$2.07x10^5$	<1	99.9999%

Conclusion:

Under the conditions of this study, both BioShield 1:16 disinfectant spray and NoviGuard RTU performed at or above EPA standards at 24 hours, 72 hours, 7 days, 14 days, and 28 days. There is a concern with the result of day 14, where BioShield resulted in the growth of three out of three positive tubes after 48 hours. But, on all test days, the calculations suggest greater than the 99.9% reduction required for this study. The positive growth in these tubes was confirmed to be Gram-negative rods, suggesting that the proliferating organism in the positive tubes is *Pseudomonas aeruginosa* (the test organism). This has been determined only by morphology characteristics, but are consistent with expected results.

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