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July 6, 2009

DRAFT FINAL REPORT #090312-201

**AN EVALUATION WITH STATISTICAL ANALYSIS OF ONE TEST PRODUCT AT THREE  
CONCENTRATIONS FOR ITS ANTIMICROBIAL PROPERTIES WHEN CHALLENGED WITH  
*STAPHYLOCOCCUS AUREUS* MRSA USING AN IN-VITRO TIME-KILL METHOD**

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Prepared for:

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Prepared by:

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## EXECUTIVE SUMMARY

An In-Vitro Time-Kill evaluation of one test product tested at three concentrations and one control product was performed versus a challenge suspension of *Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSA7). The test product was evaluated at concentrations of 99% (v/v), 10% (v/v), and 0.1% (v/v). The control product was evaluated at a 99% (v/v) concentration. All testing was performed in duplicate, and all agar-plating was performed in duplicate. The percent and log<sub>10</sub> reductions from the initial population of the challenge species were determined following 60-second and 90-second exposures to each test product concentration and to the control product.

The Test Product, Silverdyne, at 99% (v/v) concentration produced a greater than 6.0 log<sub>10</sub> reduction in *Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSA7) following 60-second exposures, reducing the organism by 1,339,999,000 CFU/mL (from 1.340 x 10<sup>9</sup> CFU/mL to less than 1.00 x 10<sup>3</sup> CFU/mL, the detection limit of this test).

The Test Product at 10% (v/v) and 0.1% (v/v) concentrations produced no reductions in *Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSA7) following 60-second or 90-second exposures.

The Control Product, HIBICLENS® (Lot Number 801629), produced a 4.9 log<sub>10</sub> reduction in *Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSA7) following 60-second exposures, as expected. The Control Product produced a 5.7 log<sub>10</sub> reduction and a greater than 6.0 log<sub>10</sub> reduction in *Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSA7) following 90-second exposures, as expected.

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- 1.0**    **TITLE:**                    **AN EVALUATION WITH STATISTICAL ANALYSIS OF ONE TEST PRODUCT AT THREE CONCENTRATIONS FOR ITS ANTIMICROBIAL PROPERTIES WHEN CHALLENGED WITH *STAPHYLOCOCCUS AUREUS* MRSA USING AN IN-VITRO TIME-KILL METHOD**
- 2.0**    **SPONSOR:**                    **WORLD HEALTH ALLIANCE INTERNATIONAL, INC.**  
3052 Wolfe Court  
Fremont, California 94555
- 3.0**    **TESTING FACILITY:**    **BIOSCIENCE LABORATORIES, INC.**  
300 N. Willson Avenue  
Bozeman, Montana 59715
- 4.0**    **STUDY DIRECTOR:**    Esther Campbell
- 5.0**    **PURPOSE:**

This study used an In-Vitro Time-Kill Method to evaluate the antimicrobial properties of one test product and one control product when challenged with *Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSa7). All testing was performed in accordance with Good Laboratory Practices, as specified in 21 CFR Part 58, with the exception that the characterization of the identity, strength, purity, composition, stability, and solubility of the test and control products remained the responsibility of the Study Sponsor and was not performed by the Testing Facility (GLP 58.105).

**6.0**    **SCOPE:**

An In-Vitro Time-Kill evaluation of one test product tested at three concentrations and one control product was performed versus a challenge suspension of *Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSa7). The test product was evaluated at concentrations of 99% (v/v), 10% (v/v), and 0.1% (v/v). The control product was evaluated at a 99% (v/v) concentration. All testing was performed in duplicate, and all agar-plating was performed in duplicate. The percent and log<sub>10</sub> reductions from the initial population of the challenge species were determined following 60-second and 90-second exposures to each test product concentration and to the control product. The statistical analysis described in the Study Protocol was not required to analyze the data collected. The Study Protocol, included as Addendum I of this Final Report, presents the study methodology, in detail, as do the General Data Gathering Forms (Form No. 91-L-002) in Addendum V of this Final Report. No deviations from the Study Protocol or from applicable BioScience Laboratories, Inc., Standard Operating Procedures occurred during the course of this evaluation.

**7.0**    **STUDY DATES:**

**STUDY INITIATION DATE:**                    04/27/09

**EXPERIMENTAL START DATE:**                04/29/09

**EXPERIMENTAL END DATE:**                    05/07/09

**STUDY COMPLETION DATE:**                    To Be Determined

## 8.0 CHALLENGE MICROORGANISM:

*Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSa7)

MRSA = Methicillin-Resistant *Staphylococcus aureus*

NARSA = Network on the Antimicrobial Resistance of *Staphylococcus aureus* (NARSA Program, Herndon, VA)

## 9.0 TEST MATERIALS:

The test product evaluated was provided to the Testing Facility by the Study Sponsor. The control product evaluated was provided by the Testing Facility. Responsibility for the determination of the identity, strength, purity, composition, stability, and solubility of the test and control products remained with the Study Sponsor, as did the responsibility for the retention of the test and control products.

Test Product: Silverdyne

Control Product: HIBICLENS® (chlorhexidine gluconate solution, 4% [w/v])

Lot Number: 801629

Expiration Date: 03/2010

## 10.0 EQUIPMENT AND SUPPLIES:

The equipment and supplies used in this study are as described in the Study Protocol in Addendum I of this Final Report. Additional details are recorded on the Equipment Tracking Forms (Form No. 98-L-007) in Addendum VI of this Final Report.

## 11.0 MEDIA:

The growth media and diluting fluids used in this study are as described in the Study Protocol in Addendum I of this Final Report. Additional details are recorded on the Media/Diluent Tracking Forms (Form No. 97-L-007) in Addendum VI of this Final Report.

## 12.0 NEUTRALIZATION STUDY:

A neutralization study was performed versus *Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSa7) to ensure that the neutralizing solution employed (Butterfield's Phosphate Buffer solution with product neutralizers [BBP++]) was effective in neutralizing the antimicrobial properties of the test and control products, and was non-toxic to the challenge species. This neutralization procedure was based on guidelines set forth in ASTM E 1054-08, *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*. All data resulting from the Neutralization Assay are included in Addendum IV of this Final Report.

## 13.0 RESULTS – TABLES I THROUGH IV:

13.1 Table I presents the initial population (CFU/mL) and the post-exposure populations (CFU/mL) of the challenge strain, and the log<sub>10</sub> and percent reductions produced by the Test Product, Silverdyne, at a 99% (v/v) concentration, at each time of exposure.

13.2 Table II presents the initial population (CFU/mL) and the post-exposure populations (CFU/mL) of the challenge strain, and the log<sub>10</sub> and percent reductions produced by the Test Product, Silverdyne, at a 10% (v/v) concentration, at each time of exposure.

13.3 Table III presents the initial population (CFU/mL) and the post-exposure populations (CFU/mL) of the challenge strain, and the log<sub>10</sub> and percent reductions produced by the Test Product, Silverdyne, at a 0.1% (v/v) concentration, at each time of exposure.

13.4 Table IV presents the initial population (CFU/mL) and the post-exposure populations (CFU/mL) of the challenge strain, and the log<sub>10</sub> and percent reductions produced by the Control Product, HIBICLENS® (Lot Number 801629), at a 99% (v/v) concentration, at each time of exposure.

**TABLE I**

**Test Product: Silverdyne at a 99% (v/v) concentration vs.**

***Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSa7)**

Initial Population (CFU/mL)	Replicate	Exposure Time	Post-Exposure Population (CFU/mL)	Log <sub>10</sub> Reduction	Percent Reduction
1.340 x 10 <sup>9</sup>	1	60 sec.	⊖ < 1.00 x 10 <sup>3</sup>	6.1271	99.9999%
	2	60 sec.	⊖ < 1.00 x 10 <sup>3</sup>	6.1271	99.9999%
	1	90 sec.	⊖ < 1.00 x 10 <sup>3</sup>	6.1271	99.9999%
	2	90 sec.	⊖ < 1.00 x 10 <sup>3</sup>	6.1271	99.9999%

⊖ Detection limit of this test.

**TABLE II**

**Test Product: Silverdyne at a 10% (v/v) concentration vs.**

***Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSa7)**

Initial Population (CFU/mL)	Replicate	Exposure Time	Post-Exposure Population (CFU/mL)	Log <sub>10</sub> Reduction	Percent Reduction
1.340 x 10 <sup>9</sup>	1	60 sec.	1.4250 x 10 <sup>9</sup>	0.0000	0.0000%
	2	60 sec.	1.620 x 10 <sup>9</sup>	0.0000	0.0000%
	1	90 sec.	1.610 x 10 <sup>9</sup>	0.0000	0.0000%
	2	90 sec.	1.7250 x 10 <sup>9</sup>	0.0000	0.0000%

**TABLE III**

**Test Product: Silverdyne at a 0.1% (v/v) concentration vs.**

***Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRSa7)**

Initial Population (CFU/mL)	Replicate	Exposure Time	Post-Exposure Population (CFU/mL)	Log <sub>10</sub> Reduction	Percent Reduction
1.340 x 10 <sup>9</sup>	1	60 sec.	1.6750 x 10 <sup>9</sup>	0.0000	0.0000%
	2	60 sec.	1.690 x 10 <sup>9</sup>	0.0000	0.0000%
	1	90 sec.	1.490 x 10 <sup>9</sup>	0.0000	0.0000%
	2	90 sec.	1.530 x 10 <sup>9</sup>	0.0000	0.0000%

**TABLE IV**  
**Control Product: HIBICLENS<sup>®</sup> at a 99% (v/v) concentration vs.**  
***Staphylococcus aureus* MRSA (Clinical Isolate; NARSA Strain NRS384 [USA300]; BSLI #120607MRsa7)**

Initial Population (CFU/mL)	Replicate	Exposure Time	Post-Exposure Population (CFU/mL)	Log <sub>10</sub> Reduction	Percent Reduction
1.340 x 10 <sup>9</sup>	1	60 sec.	1.450 x 10 <sup>4</sup>	4.9657	99.9989%
	2	60 sec.	1.60 x 10 <sup>4</sup>	4.9230	99.9988%
	1	90 sec.	⊙ < 1.00 x 10 <sup>3</sup>	6.1271	99.9999%
	2	90 sec.	2.50 x 10 <sup>3</sup>	5.7292	99.9998%

⊙ Detection limit of this test.

**14.0 ORIGIN OF CLINICAL ISOLATE - TABLE V:**

Table V presents the origin of the bacterial isolate used for this evaluation.

Organism	Date Isolated	Specimen	Patient Age/Sex	Source	BSLI ID No.
<i>Staphylococcus aureus</i> MRSA (USA300; NARSA Strain NRS384)	unknown	Wound	unknown	NARSA	120607MRsa7

MRSA = Methicillin-Resistant *Staphylococcus aureus*

NARSA = Network on the Antimicrobial Resistance in *Staphylococcus aureus* (NARSA Program, Herndon, VA)

**15.0 STATISTICAL ANALYSIS:**

The Two Factor Analysis of Variance (ANOVA) with two factors of A: Exposure Time and B: Percent Concentration of the Test Product specified in the Study protocol was not required to analyze the log<sub>10</sub> reduction of the microorganism attributable to the Test Product, since the Test Product at 99% (v/v) concentration produced the same reduction (greater than 6.0 log<sub>10</sub>) at both time points, and the Test Product at 10% (v/v) or 0.1% (v/v) concentrations produced no reduction at both time points.

**16.0 QUALITY ASSURANCE AUDITS/FINDINGS:**

The Quality Assurance Unit (QAU) conducted in-phase audits of the critical test procedures over the course of testing, and advised the Study Director and Management of the outcomes. On completion of testing, the QAU performed an audit of the raw data and of the Final Report, in its entirety. No deviations from the Study Protocol or from applicable Bioscience Labs, Inc., Standard Operating Procedures occurred during the course of this evaluation.

**17.0 LABORATORY PERSONNEL:**

The following employees of BioScience Laboratories, Inc., were involved in the testing or ancillary support of this Study. The laboratory personnel have been appropriately trained, and their training records are on-file in the Quality Assurance Unit at the Testing Facility.

STUDY DIRECTOR: Esther Campbell  
Microbiologist

**LABORATORY PERSONNEL:**

Stephanie Cebulla Laboratory Support Technician	Stephanie Scarff Laboratory Support Technician
Kendra Drake Microbiologist	Amanda Shaffer Microbiologist
Collette Duley Microbiologist	Jessica Sheehy Microbiologist
Patricia Mays Suko Supervisor of Laboratory Support	Clare Wilson Microbiologist

**18.0 QUALITY ASSURANCE PERSONNEL:**

Alicia Bogert Quality Assurance Associate/Product Handling	John A. Mitchell, Ph.D. Director of Quality Assurance
Scott D. Ferraro Manager of Quality Control	Janis Smoke Quality Assurance Associate
Amy L. Juhnke Manager of Quality Assurance/Document Control	

**19.0 DOCUMENTATION AND RECORD-KEEPING:**

All documentation and records were compiled, analyzed, and will be retained by BioScience Laboratories, Inc. at its facility in Bozeman, Montana. All raw data for this study, as well as the Final Report, will be retained in safe storage by the Testing Facility for a period of at least 3 years. BioScience Laboratories, Inc., will notify the Study Sponsor before any documents or records are destroyed.

20.0 **ACCEPTANCE:**

**BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)**  
300 N. Willson Avenue  
Bozeman, Montana 59715

President

And CEO: \_\_\_\_\_  
Daryl S. Paulson, Ph.D.

\_\_\_\_\_  
Date

Study Director: \_\_\_\_\_  
Esther Campbell

\_\_\_\_\_  
Date of Study Completion

**QUALITY ASSURANCE STATEMENT:**

This study was inspected by the Quality Assurance Unit, and reports were submitted to the Study Director and Management in accordance with Standard Operating Procedures, as follows:

<u>Phase</u>	<u>Date</u>
Neutralization Assay	04/29/09
Product Testing	05/05/09
Data Audit	05/18/09
Draft Final Report Review	05/21/09
Final Report Review	To Be Determined
Reports to Study Director and Management	04/29/09, 05/06/09, 05/21/09, and To Be Determined

This study was conducted in compliance with Good Laboratory Practices standards, as described by the FDA (21 CFR Part 58), with the following exception: test article preparations were not analyzed at BioScience Laboratories, Inc., to confirm concentration, stability, or homogeneity.

Director of

Quality Assurance: \_\_\_\_\_  
John A. Mitchell, Ph.D.

\_\_\_\_\_  
Date

## INDEX OF ADDENDA

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