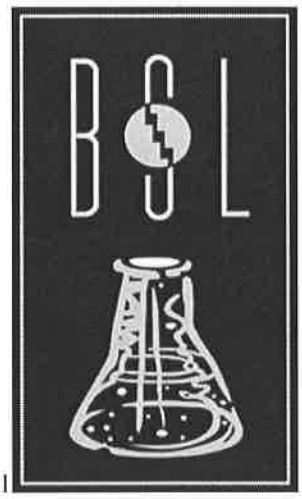

June 9, 2017

FINAL REPORT #1703130-201

AN EVALUATION OF ONE TEST PRODUCT FOR ITS ANTIMICROBIAL PROPERTIES WHEN CHALLENGED WITH THREE MICROORGANISMS USING AN IN-VITRO TIME-KILL METHOD

Prepared for:

HEALTH MATTERS (SPONSOR)
12279 Marin Road
Fayetteville, Arkansas 72704

Prepared by:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718
(406) 587-5735

TABLE OF CONTENTS

SECTION		PAGE
	EXECUTIVE SUMMARY	3
1.0	TITLE	4
2.0	SPONSOR	4
3.0	TESTING FACILITY	4
4.0	STUDY DIRECTOR	4
5.0	PURPOSE	4
6.0	SCOPE	4
7.0	STUDY DATES	4
8.0	CHALLENGE MICROORGANISMS	5
9.0	TEST PRODUCT	5
10.0	EQUIPMENT AND SUPPLIES	5
11.0	MEDIA	5
12.0	NEUTRALIZATION STUDY – RESULTS (TABLE 1)	5
13.0	IN-VITRO TIME-KILL EVALUATION – RESULTS (TABLES 2 THROUGH 4)	7
14.0	STATISTICAL ANALYSIS	7
15.0	QUALITY ASSURANCE AUDITS	7
16.0	LABORATORY PERSONNEL	8
17.0	QUALITY ASSURANCE AND QUALITY CONTROL PERSONNEL	8
18.0	DOCUMENTATION AND RECORD-KEEPING	8
19.0	ACCEPTANCE	9
	ADDENDUM 1	10

EXECUTIVE SUMMARY

STUDY NUMBER:	**1703130-201**
TITLE:	**AN EVALUATION OF ONE TEST PRODUCT FOR ITS ANTIMICROBIAL PROPERTIES WHEN CHALLENGED WITH THREE MICROORGANISMS USING AN IN-VITRO TIME-KILL METHOD**
SPONSOR:	**HEALTH MATTERS** 12279 Martin Road Fayetteville, Arkansas 72704
TESTING FACILITY:	**BIOSCIENCE LABORATORIES, INC.** 1755 South 19th Avenue Bozeman, Montana 59718
STUDY INITIATION DATE:	04/11/2017
STUDY COMPLETION DATE:	06/09/2017

An In-Vitro Time-Kill evaluation of one test product was performed versus three microorganisms -- *Candida auris* (AR-Bank #0385), *Candida auris* (AR-Bank #0389), and *Candida auris* (AR-Bank #0390). All testing was performed based upon the method described in ASTM E2783-11, *Standard Test Method for Assessment of Antimicrobial Activity of Water Miscible Compounds Using a Time-Kill Procedure*. The percent and $\log_{10}$ reductions from the numbers control population of the challenge microorganism was determined following exposure to the test product for 4 hours, 8 hours, and 24 hours. All agar-plating was performed in duplicate.

Test Product, Broad Spectrum Hygiene Management, 4 oz Foam (Lot #16180-1), reduced the populations of the three challenge microorganisms -- *Candida auris* (AR-Bank #0385), *Candida auris* (AR-Bank #0389), and *Candida auris* (AR-Bank #0390) by greater than 0.5 $\log_{10}$ following a 4 hour exposure time, by greater than 1.0 $\log_{10}$ following a 8 hour exposure time, and by greater than 6.0 $\log_{10}$ following a 24 hour exposure time.

June 9, 2017

FINAL REPORT #1703130-201

1.0 TITLE: **AN EVALUATION OF ONE TEST PRODUCTS FOR ITS ANTIMICROBIAL PROPERTIES WHEN CHALLENGED WITH THREE MICROORGANISMS USING AN IN-VITRO TIME-KILL METHOD**

2.0 SPONSOR: **HEALTH MATTERS**
12279 Martin Road
Fayetteville, Arkansas 72704

3.0 TESTING FACILITY: **BIOSCIENCE LABORATORIES, INC.**
1755 South 19th Avenue
Bozeman, Montana 59718

4.0 STUDY DIRECTOR: Alyssa Yeik

5.0 PURPOSE:

This study used an In-Vitro Time-Kill Method to evaluate the antimicrobial activity of one test product when challenged with *Candida auris* (AR-Bank #0385), *Candida auris* (AR-Bank #0389), and *Candida auris* (AR-Bank #0390). 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 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 was performed versus *Candida auris* (AR-Bank #0385), *Candida auris* (AR-Bank #0389), and *Candida auris* (AR-Bank #0390). All testing was performed based upon the method described in ASTM E2783-11, *Standard Test Method for Assessment of Antimicrobial Activity of Water Miscible Compounds Using a Time-Kill Procedure*. The percent and $\log_{10}$ reductions from the numbers control population of the challenge microorganism was determined following exposure to the test product for 4 hours, 8 hours, and 24 hours. All agar-plating was performed in duplicate.

The Study Protocol, included as Addendum 1 of this Final Report, presents the study methodology, in detail. 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:	04/11/2017
EXPERIMENTAL START DATE:	05/16/2017
EXPERIMENTAL END DATE:	05/19/2017
STUDY COMPLETION DATE:	06/09/2017

8.0 CHALLENGE MICROORGANISMS:

The challenge species (FDA-CDC Antimicrobial Resistance Bank) evaluated is designated below:

Candida auris (AR-Bank #0385)
Candida auris (AR-Bank #0389)
Candida auris (AR-Bank #0390)

9.0 TEST PRODUCT:

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

Test Product:	Broad Spectrum Hygiene Management (4 oz Foam)
Lot Number:	16180-1
Manufacture Date:	06/28/2016
Expiration Date:	Not Provided

10.0 EQUIPMENT AND SUPPLIES:

The equipment and supplies used in this study are as described in the Study Protocol in Addendum 1 of this Final Report. All applicable equipment and instrumentation were calibrated in accordance with BioScience Laboratories, Inc., Standard Operating Procedures.

11.0 MEDIA:

The growth media and diluting fluids used in this study are as described in the Study Protocol in Addendum 1 of this Final Report.

12.0 NEUTRALIZATION STUDY – RESULTS (TABLE 1):

A neutralization study of the test product was performed versus *Candida auris* (AR-Bank #0390) to ensure that the neutralizing solution employed (Butterfield's Phosphate Buffer solution with product neutralizers [BBP I+]) was effective in neutralizing the antimicrobial properties of the test product and was non-toxic to the challenge strain. These neutralization procedures were based on guidelines set forth in ASTM E1054-08(2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*. All results from the Neutralization Validation Study are presented in Table 1. When challenged with *Candida auris* (AR-Bank #0390), BBP I+ was demonstrated to be non-toxic to this challenge species and to effectively neutralize the antimicrobial properties of the test product.

TABLE 1
Neutralization Evaluation - Results

Organism	Replicate	Neutralization Phase	Post-Exposure Population (Log_{10})	Log_{10} Average Post-Exposure Population	Results of Neutralization
Candida auris (AR-Bank #0390)	1	Initial Population (Test C)	2.6767	2.6716	N/A
	2		2.6435		
	3		2.6946		
	1	Neutralizer Efficacy (Test A) Test Product Broad Spectrum Hygiene Management (4 oz Foam) Lot # 16180-1	2.6484	2.6315	Neutralizer Efficacious ❶
	2		2.6385		
	3		2.6075		
	1	Neutralizer Toxicity (Test B) Neutralizing Formulation: BBP++	2.6435	2.6325	Neutralizer Non-Toxic ❶
	2		2.5682		
	3		2.6857		

❶ =The 95% Confidence Interval for this population overlapped that of the Initial Population and/or the mean log_{10} population was not more than 0.2 log_{10} lower than the initial population.

13.0 IN-VITRO TIME-KILL EVALUATION – RESULTS (TABLE 2):

Table 2 presents the Initial Populations (CFU/mL), the Numbers Control Populations (CFU/mL), and the Post-Exposure Populations (CFU/mL) for the challenge microorganism, and the log_{10} reductions produced by the Test Product, (Broad Spectrum Hygiene Management (4oz Foam) Lot #16180-1) when tested at three exposure times.

TABLE 2
Test Product: Broad Spectrum Hygiene Management (4 oz Foam)
Lot Number: 16180-1

Microorganism Species (ATCC #)	Initial Population (CFU/mL)	Exposure Time	Numbers Control Population (CFU/mL)	Post-Exposure Population (CFU/mL)	Log_{10} Reduction	Percent Reduction
Candida auris (AR-Bank #0385)	5.050×10^9	4 hours	5.00×10^7	1.320×10^7	0.5784	73.6000%
		8 hours	5.150×10^7	4.250×10^6	1.0834	91.7476%
		24 hours	4.00×10^7	$< 1.00 \times 10^1$	6.6021	99.9999%
Candida auris (AR-Bank #0389)	5.10×10^9	4 hours	5.950×10^7	9.350×10^6	0.8037	84.2857%
		8 hours	6.250×10^7	3.90×10^5	2.2048	99.3760%
		24 hours	5.750×10^7	$< 1.00 \times 10^1$	6.7597	99.9999%
Candida auris (AR-Bank #0390)	5.950×10^9	4 hours	4.00×10^7	1.4550×10^6	1.4392	96.3625%
		8 hours	5.10×10^7	1.4050×10^4	3.5599	99.9725%
		24 hours	4.80×10^7	$< 1.00 \times 10^1$	6.6812	99.9999%

14.0 STATISTICAL ANALYSIS:

A statistical analysis of the data derived from the Neutralization Studies was performed. A statistical analysis of the data derived from the Time-Kill Evaluation was not performed.

15.0 QUALITY ASSURANCE AUDITS:

The Quality Assurance Unit (QAU) conducted in-phase audits of the critical test procedures at least once during testing, and advised the Study Director and Management of the outcomes of these audits. 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 Laboratories, Inc., Standard Operating Procedures occurred during the course of this evaluation.

16.0 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 at the Testing Facility.

STUDY DIRECTOR:

Alyssa Yeik
Study Director, In-Vitro Laboratory

LABORATORY PERSONNEL:

Stephen Antonich
Microbiologist

Christopher Hill
Laboratory Technician

Dylan Kempf
Laboratory Technician

Stephanie Cebulla
Laboratory Support Technician

Michelle Chandler
Product Handler

Marc Charnholm
Manager of Laboratory Support

Kien Lim, M.S.
Microbiologist

Wayne Lin, M.S.
Laboratory Technician

Grady Wertman
Microbiologist

Sarah Franklin
Microbiologist

Jessica McDonnell-Philipp
Microbiologist

Megan Landes
Laboratory Support Technician

17.0 QUALITY ASSURANCE AND QUALITY CONTROL PERSONNEL:

Jeremy Duley
QC/Maintenance Specialist

Danielle Goveia
Quality Assurance Specialist

Amy L. Juhnke, RQAP-GLP
Director of Quality Assurance

Renee LaFond
Quality Assurance Specialist

Lisa Lehman
Quality Assurance Specialist

Kim Potter
Quality Assurance Associate

Carl Schmidt
ISO Technical Manager (QC; Training, Safety)

18.0 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 5 years. BioScience Laboratories, Inc., will notify the Study Sponsor before any documents or records are destroyed.

19.0 ACCEPTANCE:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718

President and CEO: Daryl S Paulson — Daryl S. Paulson, Ph.D.

06-09-17
Date

Study Director: Alyssa M Yeik — Alyssa Yeik

06/09/17
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:

Phase Inspected	Audit Date	Date reported to Study Director	Date reported to Management
Neutralization	05/16/2017	05/16/2017	05/18/2017
Product Testing	05/16/2017	05/16/2017	05/18/2017
Data Audit	05/24/2017 05/25/2017	06/01/2017	06/01/2017
Final Report Review	05/24/2017 05/25/2017	06/01/2017	06/01/2017

This study was conducted in compliance with Good Laboratory Practices standards, as described by the FDA (21 CFR Part 58), with the exception that the characterization of the identity, strength, purity, composition, stability, and solubility of the test products was not performed by BioScience Laboratories, Inc. No deviations to the Study Protocol or from applicable BioScience Laboratories, Inc., Standard Operating Procedures occurred. This statement also serves to confirm that the Final Report reflects the raw data.

Quality Assurance Specialist: — Lisa Lehman

06-09-17
Date

ADDENDUM 1

Protocol #1703130-201

April 06, 2017

PROTOCOL #1703130-201

AN EVALUATION OF ONE TEST PRODUCT FOR ITS ANTIMICROBIAL PROPERTIES WHEN CHALLENGED WITH THREE MICROORGANISMS USING AN IN-VITRO TIME-KILL METHOD

Prepared for:

HEALTH MATTERS (SPONSOR)
12279 Martin Road
Fayetteville, Arkansas 72704

Prepared by:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718
(406) 587-5735

CONFIDENTIAL

TABLE OF CONTENTS

SECTION		PAGE
1.0	TITLE	4
2.0	SPONSOR	4
3.0	TESTING FACILITY	4
4.0	STUDY DIRECTOR	4
5.0	PURPOSE OF STUDY	4
6.0	SCOPE	4
7.0	CHALLENGE MICROORGANISMS	4
8.0	TEST PRODUCT	4
9.0	EQUIPMENT	5
10.0	SUPPLIES	5
11.0	MEDIA	5
12.0	NEUTRALIZATION STUDY	5
13.0	METHODOLOGY	7
	TABLE 1: CHALLENGE MICROORGANISMS	9
14.0	CALCULATIONS	9
15.0	STATISTICAL ANALYSIS	10
16.0	REFERENCES	10
17.0	FINAL REPORT	10
18.0	EXCEPTIONAL CONDITIONS	10
19.0	DOCUMENTATION AND RECORD-KEEPING	10
20.0	QUALITY ASSURANCE AUDITS	10
21.0	LIABILITY AND INDEMNIFICATION	10
22.0	ACCEPTANCE	11

April 06, 2017

PROTOCOL #1703130-201

1.0 TITLE: **AN EVALUATION OF ONE TEST PRODUCT FOR ITS ANTIMICROBIAL PROPERTIES WHEN CHALLENGED WITH THREE MICROORGANISMS USING AN IN-VITRO TIME-KILL METHOD**

2.0 SPONSOR: **HEALTH MATTERS**
12279 Martin Road
Fayetteville, Arkansas 72704

3.0 TESTING FACILITY: **BIOSCIENCE LABORATORIES, INC.**
1755 South 19th Avenue
Bozeman, Montana 59718

4.0 STUDY DIRECTOR: Alyssa Yeik

5.0 PURPOSE OF STUDY:

This study will use an In-Vitro Time-Kill Method to evaluate the antimicrobial properties of one test product when challenged with suspensions of three microorganisms. All testing will be 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 product remains the responsibility of the Study Sponsor and will not be performed by the Testing Facility (GLP 58.105 and GLP 58.113).

6.0 SCOPE:

An In-Vitro Time-Kill evaluation of one test product will be performed versus suspensions of three microorganisms. The percent and $\log_{10}$ reduction from the numbers control population of each challenge microorganism will be determined following exposure to the test product for 4 hours, 8 hours, and 24 hours. All agar plating will be performed in duplicate.

7.0 CHALLENGE MICROORGANISMS:

The challenge species (FDA-CDC Antimicrobial Resistance Bank) evaluated are designated below:

7.1 *Candida auris* (AR-Bank #0385)
7.2 *Candida auris* (AR-Bank #0389)
7.3 *Candida auris* (AR-Bank #0390)

8.0 TEST PRODUCT:

The test product to be evaluated will be provided to the Testing Facility by the Study Sponsor, complete with appropriate documentation. Responsibility for determination of the identity, strength, purity, composition, stability, and solubility of the test product, as well as responsibility for retention of the test product, rests with the Study Sponsor.

Test Product:	Broad Spectrum Hygiene Management (4 oz Foam)
Lot Number:	16180-1
Manufacture Date:	06/28/2016
Expiration Date:	Not Provided

9.0 EQUIPMENT:

9.1 Incubator, Temperature Range 30 °C ± 2 °C
9.2 Incubator Thermometers
9.3 Refrigerators, 2 °C to 8 °C
9.4 Refrigerator Thermometers
9.5 Water Bath, 47 °C ± 2 °C
9.6 Water Bath Thermometer
9.7 Vortex Mixers
9.8 Laminar Biological Flowhood
9.9 Steam Autoclaves
9.10 Continuously Adjustable Pipetters, 100 µL - 1000 µL Capacity
9.11 Continuously Adjustable Pipetters, 20 µL - 200 µL Capacity
9.12 Microman® Positive Displacement Pipetters, 10 µL - 100 µL Capacity
9.13 Microman® Positive Displacement Pipetters, 100 µL - 1000 µL Capacity
9.14 Calibrated Minute/Second Timers
9.15 Portable Pipetters

10.0 SUPPLIES:

10.1 Sterile Disposable Pipettes
10.2 Sterile Universal 1.0 mL and 0.2 mL Pipette Tips
10.3 Sterile 0.1 mL and 1.0 mL Positive Displacement Tips
10.4 Sterile Syringes
10.5 Sterile Disposable Petri Plates
10.6 Glass Test Tubes, Sterilized
10.7 Sterile 4 oz Disposable Specimen Containers
10.8 Glass Beakers, Sterilized
10.9 Hand Tally Counters
10.10 Inoculating Loops

11.0 MEDIA:

11.1 Sabouraud Dextrose Agar (SDA)
11.2 Sabouraud Dextrose Agar with product neutralizers (SDA+)
11.3 0.9% Sodium Chloride Irrigation, USP (SCI)
11.4 Butterfield's Phosphate Buffer solution with product neutralizers (BBP++)

12.0 NEUTRALIZATION STUDY:

A neutralization study of the test product will be performed versus *Candida auris* (AR-Bank #0390) to ensure that the neutralizing solution employed (Butterfield's Phosphate Buffer solution with product neutralizers [BBP++]) effectively neutralizes the antimicrobial properties of the test product and is non-toxic to this challenge strain.

Inoculum Preparation

12.1 Approximately 48 hours prior to testing, inocula from cryogenic vials will be suspended in 0.9% Sodium Chloride Irrigation, USP (SCI), inoculated onto the surface of Sabouraud Dextrose Agar (SDA) contained in Petri plates, and incubated at 30 °C ± 2 °C for approximately 24 hours, or until sufficient growth is observed.

12.2 Approximately 24 hours prior to testing, a suspension of each microorganism will be prepared by rinsing the plates of SDA with sterile SCI. Aliquots of each suspension will then be spread-plated onto the surface of additional SDA contained in Petri plates, and incubated at 30 °C ± 2 °C for approximately 24 hours, or until sufficient growth is observed. This will produce lawns of the bacteria on the surface of the agar plates, and growth from these will be used to prepare the challenge suspensions.

Challenge Suspension

12.3 A Neutralization Challenge Suspension will be prepared in sterile SCI, by suspending the growth from the solid medium to achieve a suspension concentration of approximately 1 x 10^9 CFU/mL and then diluting the prepared suspension in additional SCI as necessary. This suspension will contain approximately 1 x 10^4 CFU/mL and will be utilized in all phases of the neutralization validation assay.

Neutralization Effectiveness Evaluation (Test A)

12.4 Three replicates of this procedure will be performed.

12.5 A 0.1 mL aliquot of a Neutralization Challenge Suspension will be transferred to a test tube containing 8.9 mL of BBP++ (10^0 dilution). A 1.0 mL aliquot of test product will be added to the tube containing inoculum/BBP++ and mixed thoroughly.

12.6 The inoculum/BBP++/product mixture will be exposed for greater than or equal to 15 minutes, timed using a calibrated minute/second timer. Following exposure, a 10-fold dilution (e.g., 10^{-1}) will be prepared in BBP++ and mixed thoroughly.

12.7 1.0 mL and/or 0.1 mL aliquots of each suspension will be pour-plated, in duplicate, using Sabouraud Dextrose Agar with product neutralizers (SDA+) to produce final plated dilutions of e.g., 10^0, 10^{-1}, and 10^{-2}. The plates will be incubated at 30 °C ± 2 °C for approximately 48 hours, or until sufficient growth is observed.

Neutralizer Toxicity Evaluation (Test B)

12.8 Three replicates of this procedure will be performed.

12.9 A 0.1 mL aliquot of a Neutralization Challenge Suspension will be transferred to a test tube containing 8.9 mL of BBP++ (10^0 dilution). A 1.0 mL aliquot of SCI will be added to the tube containing inoculum/BBP++ and mixed thoroughly (10^0 dilution).

12.10 The inoculum/BBP++/SCI mixture will be exposed for greater than or equal to 15 minutes, timed using a calibrated minute/second timer. Following exposure, a 10-fold dilution (e.g., 10^{-1}) will be prepared in BBP++ and mixed thoroughly.

12.11 1.0 mL and/or 0.1 mL aliquots of each suspension will be pour-plated, in duplicate, using SDA+ to produce final plated dilutions of e.g., 10^0, 10^{-1}, and 10^{-2}. The plates will be incubated at 30 °C ± 2 °C for approximately 48 hours, or until sufficient growth is observed.

Test Organism Viability (Test C)

12.12 Three replicates of this procedure will be performed.

12.13 A 0.1 mL aliquot of a Neutralization Challenge Suspension will be transferred to a test tube containing 9.9 mL of SCI and mixed thoroughly (10^0 dilution).

12.14 The inoculum/SCI mixture will be exposed for greater than or equal to 15 minutes, timed using a calibrated minute/second timer. Following exposure, a 10-fold dilution (e.g., 10^{-1}) will be prepared in SCI and mixed thoroughly.

12.15 1.0 mL and/or 0.1 mL aliquots of each suspension will be pour-plated, in duplicate, using SDA to produce final plated dilutions of e.g., 10^{0}, 10^{-1}, and 10^{-2}. The plates will be incubated at 30 °C ± 2 °C for approximately 48 hours, or until sufficient growth is observed.

Data Collection

12.16 Following incubation, the colonies on the plates will be counted manually using a hand-tally counter. Counts in the range of 30 to 300 colony-forming units (CFU) will be used preferentially in the data calculations. If no counts in this range are observed, those plates with colony counts closest to those ranges will be used for the data calculations.

Acceptance Criterion

12.17 The Log_{10} of the number of survivors of the challenge strain from Test A and Test B will be statistically compared to those from Test C using a One-Way Analysis of Variance (ANOVA). If the 95% Confidence Interval of Test A for the product overlaps that of Test C, neutralization will be considered effective for that product. If the 95% Confidence Interval of Test B overlaps that of Test C, the neutralizing formulation (BBP++) will be considered non-toxic to the challenge microorganism.

12.18 Because low variability of the recovery data can affect interpretation of the results from the statistical analysis, an alternative method of assessing the neutralization outcomes may be necessary. As referenced by ASTM E1054-08 (Note 10), the data may be considered equivalent if the microbial recovery populations (the average of the three replicates) are no more than 0.2 log_{10} lower than those of Test C (the average of the three replicates).

13.0 METHODOLOGY:

Inoculum Preparation

13.1 Approximately 48 hours prior to testing, inocula from cryogenic vials will be suspended in SCI, inoculated onto the surface of SDA contained in Petri plates, and incubated at 30 °C ± 2 °C for approximately 24 hours, or until sufficient growth is observed.

13.2 Approximately 24 hours prior to testing, a suspension of each microorganism will be prepared by rinsing the plates of appropriate agar with sterile SCI. Aliquots of each suspension will then be spread-plated onto the surface of additional plates of SDA, and incubated at 30 °C ± 2 °C for approximately 24 hours, or until sufficient growth is observed. This will produce lawns of the bacteria on the surface of the agar plates, and growth from these will be used to prepare the challenge suspensions.

Challenge Suspensions

13.3 Immediately prior to initiating the test procedure, challenge suspensions of each microorganism will be prepared in SCI, by suspending the microorganisms from the solid media previously prepared (reference Section 13.2) to achieve challenge suspension concentrations of approximately 1 x 10^{9} CFU/mL. These suspensions will be utilized in all phases of testing.

Initial Population Determinations

13.4 Prior to use in testing, the initial population of each challenge suspension will be determined by preparing 10-fold dilutions (e.g., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}) in BBP++. Using SDA+, pour-plates will be prepared, in duplicate, from the inoculum dilutions by plating 0.1 mL of the final dilutions, e.g., 10^{-6}, 10^{-7}, and 10^{-8}, to achieve plated dilutions of, e.g., 10^{-7}, 10^{-8}, and 10^{-9}. The plates will be incubated at 30 °C ± 2 °C for approximately 48 hours, or until sufficient growth is observed.

Numbers Control

13.5 A 0.1 mL aliquot of a challenge suspension containing approximately 1 x 10^9 CFU/mL will be transferred to a sterile tube containing 10.0 mL of SCI and mixed thoroughly using a vortex mixer and/or positive displacement pipetter (10^0 dilution).

13.6 Each challenge microorganism will be exposed to the SCI for 4 hours, 8 hours, and 24 hours, timed using a calibrated minute/second timer, at 30 ± 2° C.

13.7 After each exposure time has elapsed, 1.0 mL will be transferred from each tube containing SCI/challenge suspension to separate sterile test tubes containing 9.0 mL BBP++ (10^{-1} dilution), and mixed thoroughly using a vortex mixer. 10-fold dilutions (e.g., 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) of the suspensions of each challenge species will be prepared in BBP++, mixing thoroughly using a vortex mixer between dilutions.

13.8 From the final dilutions of the SCI/neutralizer/challenge suspension, 0.1 or 1.0 mL aliquots will be pour-plated, in duplicate, using SDA+, producing final plated dilutions of, e.g., 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. The plates will be incubated at 30 ± 2 °C for 48 to 72 hours, or until sufficient growth is observed (reference Table 1).

Time-Kill Testing Procedure

13.9 A 0.1 mL aliquot of a challenge suspension containing approximately 1 x 10^9 CFU/mL will be transferred to a sterile test tube containing 10 mL of the test product and mixed thoroughly using a vortex mixer and/or positive displacement pipetter (10^0 dilution).

13.10 Each challenge microorganism will be exposed to the test product for 4 hours, 8 hours, and 24 hours, timed using a calibrated minute/second timer.

13.11 Upon elapse of each exposure time, a 1.0 mL aliquot will be transferred from each tube containing test product/challenge suspension to separate sterile test tubes containing 9.0 mL BBP++ (10^{-1} dilution), and mixed thoroughly using a vortex mixer. Additional 10-fold dilutions (e.g., 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) will be prepared in BBP++, mixing thoroughly using a vortex mixer between dilutions.

13.12 From the dilutions of the test product/neutralizer/challenge suspension, 1.0 mL and/or 0.1 mL aliquots will be pour-plated, in duplicate, using SDA+, resulting in final plated dilutions of, e.g., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. The plates will be incubated at 30 °C ± 2 °C for approximately 48 hours, or until sufficient growth is observed.

Data Collection

13.13 Following incubation, the colonies on the plates will be counted manually using a hand-tally counter. Counts in the range of 30 to 300 colony-forming units (CFU) will be used preferentially in the data calculations. If no counts in this range are observed, those plates with colony counts closest to this range will be used for the data calculations.

TABLE 1: CHALLENGE MICROORGANISMS

No.	Microorganisms	AR-Bank #	Incubation Time	Incubation Temperature	Media
1	*Candida auris*	0385	24 to 72 hours	30 °C ± 2 °C	SDA/SDA+
2	*Candida auris*	0389	24 to 72 hours	30 °C ± 2 °C	SDA/SDA+
2	*Candida auris*	0390	24 to 72 hours	30 °C ± 2 °C	SDA/SDA+

Note: Incubation times are nominal, but in practice, incubation will continue until good growth is observed.

14.0 CALCULATIONS:

14.1 The Initial Population (IP) of each challenge suspension will be calculated as follows:

Log_{10} (IP) = Log_{10} (C_i x 10^{-D})

CFU/mL (IP) = (C_i x 10^{-D})

Where:

C_i = Average of the Two Plates Counted
D = Dilution Factor of the Plates Counted

14.2 The Numbers Control (NC) population recovery (CFU/mL and Log_{10} CFU/mL) will be calculated for each challenge suspension following each timed exposure as follows:

Log_{10} (NC) = Log_{10} (C_i x 10^{-D})

CFU/mL (NC) = (C_i x 10^{-D})

Where:

C_i = Average of the Two Plates Counted
D = Dilution Factor of the Plates Counted

14.3 The Post-Exposure Population (P_{EX}) of each challenge suspension following each timed exposure to the test product will be calculated as follows:

Log_{10} (P_{EX}) = Log_{10} (C_i x 10^{-D})

CFU/mL (P_{EX}) = (C_i x 10^{-D})

Where:

C_i = Average of the Two Plates Counted
D = Dilution Factor of the Plates Counted

14.4 The Percent Reduction attributable to the test product will be calculated for each exposure time of testing as follows:

$$\text{Percent Reduction} = \frac{NC - P_{EX}}{NC} \times 100$$

Where:

NC = Numbers Control Population (CFU/mL)
P_{EX} = Post-Exposure Population (CFU/mL)

15.0 STATISTICAL ANALYSIS:

A One-Way Analysis of Variance (ANOVA) will be performed on the data derived from the Neutralization Study. A statistical analysis will not be performed on the data derived from the Time-Kill portion of this evaluation.

16.0 REFERENCES:

16.1 ASTM E1054-08 (2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents.*

16.2 ASTM E2783-11 (2008), *Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure.*

17.0 FINAL REPORT:

A Final Report will be prepared by BioScience Laboratories, Inc., presenting the results of the study in a clear and concise manner.

18.0 EXCEPTIONAL CONDITIONS:

The Study Sponsor will be notified by telephone, email, and/or letter of any exceptions encountered in this study. The exceptional conditions or occurrences will be detailed in full and formally recorded. Exceptional conditions that occur and are not addressed in this Protocol will be subject to Out-of-Scope charges (see Proposal/Contract).

19.0 DOCUMENTATION AND RECORD-KEEPING:

All documentation and records will be compiled, analyzed, and 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 5 years. BioScience Laboratories, Inc., will notify the Sponsor before any documents or records are destroyed.

20.0 QUALITY ASSURANCE AUDITS:

The Quality Assurance Unit (QAU) will conduct in-phase audits of critical testing processes at least once during testing and will advise the Study Director and Management of the outcomes of these audits. On completion of testing, the QAU will perform an audit of the data, and of the Final Report in its entirety.

21.0 LIABILITY AND INDEMNIFICATION:

Test Facility's liability to Sponsor under this Protocol shall be limited to the price of this evaluation. Sponsor shall be responsible to Study Participants (when applicable) and to other third parties for the fitness of the product for use as defined in the Study Protocol.

ACCEPTANCE:

AN EVALUATION OF ONE TEST PRODUCT FOR ITS ANTIMICROBIAL PROPERTIES WHEN CHALLENGED WITH THREE MICROORGANISMS USING AN IN-VITRO TIME-KILL METHOD

ACCEPTED BY: BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718

Study Director: Alyssa M Yeik
Alyssa Yeik

04/11/17
Date of Study Initiation

ACCEPTED BY: HEALTH MATTERS (SPONSOR)
12279 Martin Road
Fayetteville, Arkansas 72704

Dana Gallagher
Representative

4-8-17
Date