Nelson Labs®
A Sotera Health company

12 APR 2024

FINAL REPORT #2402064-201

A Time-Kill Evaluation of Protect Foam Formulation (Swab Solution) Against: *Candida auris* (AR-BANK #0381), *Staphylococcus aureus* MRSA (ATCC #33591), *Staphylococcus aureus* (ATCC #6538), & *Staphylococcus epidermidis* (ATCC #12228) Based Upon ASTM E2783-22

Prepared for:

AVADIM HEALTH (SPONSOR)
4 Old Patton Cove Road
Swannanoa, North Carolina 28778

Prepared by:

NELSON LABORATORIES BOZEMAN, LLC (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718
(406) 587-5735

TABLE OF CONTENTS

EXECUTIVE SUMMARY

STUDY NUMBER:	**2402064-201**
TITLE:	**A Time-Kill Evaluation of Protect Foam Formulation (Swab Solution) Against: Candida auris (AR-BANK #0381), Staphylococcus aureus MRSA (ATCC #33591), Staphylococcus aureus (ATCC #6538), & Staphylococcus epidermidis (ATCC #12228) Based Upon ASTM E2783-22**
SPONSOR:	**AVADIM HEALTH (SPONSOR)** 4 Old Patton Cove Road Swannanoa, North Carolina 28778
TESTING FACILITY:	**NELSON LABORATORIES BOZEMAN, LLC** 1755 South 19th Avenue Bozeman, Montana 59718
STUDY INITIATION DATE:	20 MAR 2024
STUDY COMPLETION DATE:	12 APR 2024

The $\log_{10}$ reduction in the microbial population of each challenge species produced by a 1-minute, 30-minute, 1-hour, 6-hour, and 12-hour exposure to the test article is summarized below.

TABLE 1 SUMMARY OF LOG_{10} REDUCTIONS **Test Article: Protect Foam Formulation (Swab Solution), Lot #522695**		
Challenge Microorganism	Exposure Time	Log_{10} Reduction
Candida auris (AR-BANK #0381)	1 minute	< 0.16
	30 minutes	< 0.16
	1 hour	0.29
	6 hours	0.92
	12 hours	2.10
Staphylococcus aureus (ATCC #6538)	1 minute	0.04
	30 minutes	0.93
	1 hour	1.97
	6 hours	> 4.80
	12 hours	> 4.57
Staphylococcus aureus MRSA (ATCC #33593)	1 minute	-0.06
	30 minutes	0.74
	1 hour	1.46
	6 hours	3.67
	12 hours	> 4.90
Staphylococcus epidermidis (ATCC #12228)	1 minute	-0.07
	30 minutes	0.11
	1 hour	0.30
	6 hours	2.37
	12 hours	4.06

1.0 TITLE: **A Time-Kill Evaluation of Protect Foam Formulation (Swab Solution) Against: Candida auris (AR-BANK #0381), Staphylococcus aureus MRSA (ATCC #33591), Staphylococcus aureus (ATCC #6538), & Staphylococcus epidermidis (ATCC #12228) Based Upon ASTM E2783-22**

2.0 SPONSOR: **AVADIM HEALTH (SPONSOR)**
4 Old Patton Cove Road
Swannanoa, North Carolina 28778

3.0 TESTING FACILITY: **NELSON LABORATORIES BOZEMAN, LLC**
1755 South 19th Avenue
Bozeman, Montana 59718

4.0 STUDY DIRECTOR: Keely Huth

5.0 PURPOSE AND SCOPE:

This study used an *in-vitro* time-kill method to evaluate the broad-spectrum antimicrobial properties of the test article when challenged with suspensions of challenge microorganisms. This procedure was based upon the methodology described in ASTM E2783-22, *Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure.* 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 article remained the responsibility of the sponsor and was not performed by the testing facility (GLP 58.105 and GLP 58.113).

The percent and $\log_{10}$ reduction in the microbial population of each challenge strain was determined following exposure to the test article for the times listed in Table 2. Testing of each challenge species versus the test article was performed for the number of replicates listed in Table 2; all agar-plating was performed in duplicate.

The protocol, included as the addendum to this final report, presents the study methodology, in detail.

TABLE 2 MICROORGANISM TEST MATRIX

Microorganism Species	Replicate(s)	Contact time(s) for test article(s)	Contact time(s) for numbers control
Candida auris (AR-BANK #0381)	1	1 minute, 30 minutes, 1 hour, 6 hours, and 12 hours	1 hour, 6 hours, and 12 hours
Staphylococcus aureus MRSA (ATCC #33591)			
Staphylococcus aureus (ATCC #6538)			
Staphylococcus epidermidis (ATCC #12228)			

NOTE: The 1-hour numbers control was compared to the test article contact times: 1 minute, 30 minutes, and 1 hour. The 6-hour numbers control was compared to the test article contact time: 6 hours. The 12-hour numbers control was compared to the test article contact time: 12 hours.

6.0 TEST ARTICLE:

The test article used for this evaluation was provided to the testing facility by the sponsor, complete with appropriate documentation. Responsibility for the determination of the identity, strength, purity, composition, stability, and solubility of the test article rests with the sponsor, as does responsibility for the retention of the test article. The test article was evaluated as received.

TABLE 3 TEST ARTICLE

Test Article Identification	Active Ingredients	Lot Number	Manufacture Date❶	Expiration Date
Protect Foam Formulation (Swab Solution)	N/A	522695	03/2023	03/2028

❶ Additional information provided on the Sample Submission Form (SSF).

7.0 CHALLENGE MICROORGANISMS:

The challenge microorganism species (American Type Culture Collection [ATCC] strains or CDC & FDA AR Isolate Bank [AR Bank]) strains evaluated are designated below:

TABLE 4 CHALLENGE MICROORGANISMS, GROWTH MEDIA, AND INCUBATION CONDITIONS

Microorganism Species	Microorganism Type	Incubation Temperature	Growth Media	Inoc. Prep. Incubation Time	Microbial Recovery Incubation Time
Candida auris (AR-BANK #0381)	Yeast	30 ± 2 °C	SDA	24 ± 4 hours	1 to 3 days
Staphylococcus aureus MRSA (ATCC #33591)	Vegetative Bacteria	35 ± 2 °C	TSA	24 ± 4 hours	1 to 3 days
Staphylococcus aureus (ATCC #6538)	Vegetative Bacteria	35 ± 2 °C	TSA	24 ± 4 hours	1 to 3 days
Staphylococcus epidermidis (ATCC #12228)	Vegetative Bacteria	35 ± 2 °C	TSA	24 ± 4 hours	1 to 3 days

8.0 STUDY DATES:

STUDY INITIATION DATE: 20 MAR 2024

EXPERIMENTAL START DATE: 22 MAR 2024

EXPERIMENTAL END DATE: 01 APR 2024

STUDY COMPLETION DATE: 12 APR 2024

9.0 SUPPLIES, EQUIPMENT, AND MEDIA:

The supplies, equipment, and media used in this study are as described in the protocol included as the addendum to this final report.

10.0 AMENDMENTS:

There were no amendments to the study protocol.

11.0 DEVIATIONS:

There were no deviations from the study protocol or from Nelson Laboratories Bozeman standard operating procedures that were observed during the course of the evaluation.

12.0 NEUTRALIZATION STUDIES – RESULTS (TABLES 5 AND 6):

12.1 Neutralization studies of the test article were performed versus *Candida auris* (AR-BANK #0381) and *Staphylococcus aureus* (ATCC #6538) to ensure that the neutralizing solution employed (Butterfield's Phosphate Buffer with product neutralizers [BBP++]) effectively neutralized the antimicrobial properties of the test article and was non-toxic to these representative microorganism strains. This neutralization procedure was based on guidelines set forth in ASTM E1054-22, *Standard Practices for Evaluation of Inactivators of Antimicrobial Agents*.

12.2 The results from the Neutralization Validation Studies are presented in Tables 5 and 6.

TABLE 5 NEUTRALIZATION EVALUATION Challenge Microorganism: *Candida auris* (AR-BANK #0381)							
Test Description	**Sample Size**	**Mean**	**Standard Deviation**	**Results of Equivalency Test to Test C Equivalence Interval (-0.5, 0.5)**			
				Difference	**SE**	**90% CI ❶**	**Conclusion**
Test C Test Organism Viability	3	3.9723	0.0115	N/A	N/A	N/A	N/A
Test B Neutralizer Toxicity Evaluation Neutralizing Solution: (BBP++)	3	3.9458	0.0200	-0.026437	0.013317	(-0.0548279, 0.0019538)	Equivalent
Test A Neutralizer Effectiveness Evaluation Test Article: Protect Foam Formulation (Swab Solution), Lot #522695	3	3.9249	0.0182	-0.047371	0.012435	(-0.0738813, -0.0208615)	Equivalent

TABLE 6 NEUTRALIZATION EVALUATION Challenge Microorganism: *Staphylococcus aureus* (ATCC #6538)							
Test Description	**Sample Size**	**Mean**	**Standard Deviation**	**Results of Equivalency Test to Test C Equivalence Interval (-0.5, 0.5)**			
				Difference	**SE**	**90% CI ❶**	**Conclusion**
Test C Test Organism Viability	3	2.1536	0.0314	N/A	N/A	N/A	N/A
Test B Neutralizer Toxicity Evaluation Neutralizing Solution: (BBP++)	3	2.0929	0.0254	-0.060633	0.023331	(-0.110371, -0.0108952)	Equivalent
Test A Neutralizer Effectiveness Evaluation Test Article: Protect Foam Formulation (Swab Solution), Lot #522695	3	2.0944	0.0157	-0.059162	0.020294	(-0.102425, -0.0158985)	Equivalent

❶ =The 90% Confidence Interval for the Difference (Mean Test A [or B] – Mean Test C) is contained in the equivalency interval (-0.5, 0.5).

13.0 IN-VITRO TIME-KILL EVALUATION - RESULTS (TABLE 7):

TABLE 7 TIME KILL EVALUATION RESULTS								
Test Article: Protect Foam Formulation (Swab Solution), Lot #522695								
Microorganism Species	Replicate	Initial Population (Log_{10})	Final Population (Log_{10})	Numbers Control (1 hour, 6 hours, & 12 hours)* (Log_{10})	Exposure Time	Post-Exposure Log_{10} Recovery	Log_{10} Reduction	Percent Reduction
Candida auris (AR-BANK #0381)	1	9.57	9.49	7.56	1 minute	> 7.40	< 0.16	< 32%
					30 minutes	> 7.40	< 0.16	< 32%
					1 hour	7.27	0.29	49%
				7.56	6 hours	6.64	0.92	88%
				7.51	12 hours	5.41	2.10	99.20%
Staphylococcus aureus (ATCC #6538)	1	8.08	7.95	5.91	1 minute	5.87	0.04	9%
					30 minutes	4.98	0.93	88%
					1 hour	3.93	1.97	98.9%
				5.80	6 hours	< 1.00	> 4.80	> 99.9984%
				5.57	12 hours	< 1.00	> 4.57	> 99.9973%

* = The 1-hour numbers control was compared to the test article contact times: 1 minute, 30 minutes, and 1 hour. The 6-hour numbers control was compared to the test article contact time: 6 hours. The 12-hour numbers control was compared to the test article contact time: 12 hours.

TABLE 7 (continued) TIME KILL EVALUATION RESULTS Test Article: Protect Foam Formulation (Swab Solution), Lot #522695								
Microorganism Species	Replicate	Initial Population (Log_{10})	Final Population (Log_{10})	Numbers Control (1 hour, 6 hours, & 12 hours) * (Log_{10})	Exposure Time	Post-Exposure Log_{10} Recovery	Log_{10} Reduction	Percent Reduction
Staphylococcus aureus MRSA (ATCC #33591)	1	8.16	8.12	6.05	1 minute	6.11	-0.06	-16%
					30 minutes	5.31	0.74	82%
					1 hour	4.59	1.46	96.6%
				5.91	6 hours	2.24	3.67	99.978%
				5.90	12 hours	< 1.00	> 4.90	> 99.9988%
Staphylococcus epidermidis (ATCC #12228)	1	9.12	9.14	7.10	1 minute	7.18	-0.07	19%
					30 minutes	6.99	0.11	23%
					1 hour	6.80	0.30	50%
				7.18	6 hours	4.81	2.37	99.57%
				7.14	12 hours	3.08	4.06	99.9912%

* = The 1-hour numbers control was compared to the test article contact times: 1 minute, 30 minutes, and 1 hour. The 6-hour numbers control was compared to the test article contact time: 6 hours. The 12-hour numbers control was compared to the test article contact time: 12 hours.

14.0 STATISTICAL ANALYSIS:

A statistical analysis of the data derived from the neutralization studies was performed as described in the protocol included as the addendum to this final report. A statistical analysis was not performed on the data derived from the time-kill evaluation.

15.0 STUDY PERSONNEL:

STUDY DIRECTOR:	Keely Huth Study Director I
SUPERVISORS:	Chelsey Allison Site Leader
	Paul Tewson Manager Laboratory Operations

16.0 DOCUMENTATION AND RECORD KEEPING:

All documentation and records were compiled, analyzed, and will be retained by Nelson Laboratories Bozeman, LLC, 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. Nelson Laboratories Bozeman, LLC, will notify the sponsor before any documents or records are destroyed.

17.0 ACCEPTANCE:

NELSON LABORATORIES BOZEMAN, LLC (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718

Study Director: _____ 12 APR 2024
Keely Huth — 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 Assay	22 MAR 2024	22 MAR 2024	22 MAR 2024
Product Testing	28 MAR 2024	28 MAR 2024	28 MAR 2024
Data Audit	11 APR 2024	12 APR 2024	12 APR 2024
Final Report Review	11 APR 2024 and 12 APR 2024	12 APR 2024	12 APR 2024

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 article was not performed by Nelson Laboratories Bozeman, LLC. This statement also serves to confirm that the final report reflects the raw data.

Quality Assurance: _____ 12 APR 2024
Lisa Lehman — Date

ADDENDUM

Protocol #2402064-201

Protocol #2402064-201

Nelson Labs®
A Sotera Health company

20 Mar 2024

PROTOCOL #2402064-201

A Time-Kill Evaluation of Protect Foam Formulation (Swab Solution) Against: *Candida auris* (AR-BANK #0381), *Staphylococcus aureus* MRSA (ATCC #33591), *Staphylococcus aureus* (ATCC #6538), & *Staphylococcus epidermidis* (ATCC #12228) Based Upon ASTM E2783-22

Prepared for:

AVADIM HEALTH (SPONSOR)
4 Old Patton Cove Road
Swannanoa, North Carolina 28778

Prepared by:

NELSON LABORATORIES BOZEMAN, LLC (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718
(406) 587-5735

Protocol #2402064-201

TABLE OF CONTENTS

PROTOCOL #2402064-201

1.0 TITLE: **A Time-Kill Evaluation of Protect Foam Formulation (Swab Solution) Against: Candida auris (AR-BANK #0381), Staphylococcus aureus MRSA (ATCC #33591), Staphylococcus aureus (ATCC #6538), & Staphylococcus epidermidis (ATCC #12228) Based Upon ASTM E2783-22**

2.0 SPONSOR: **AVADIM HEALTH**
4 Old Patton Cove Road
Swannanoa, North Carolina 28778

3.0 TESTING FACILITY: **NELSON LABORATORIES BOZEMAN, LLC**
1755 South 19th Avenue
Bozeman, Montana 59718

4.0 STUDY PERSONNEL:

Study Director – Keely Huth

5.0 PURPOSE AND SCOPE:

This evaluation will use an *in-vitro* time-kill method to evaluate the broad-spectrum antimicrobial properties of the test article(s) when challenged with suspensions of challenge microorganisms. This procedure is based upon the methodology described in ASTM E2783-22, *Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure*[1]. 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 article(s) remains the responsibility of the sponsor and will not be performed by the testing facility (GLP 58.105 and GLP 58.113).

The percent and $\log_{10}$ reduction in the microbial population of each challenge strain will be determined following exposure to the test article(s) for the times listed in Table 1. Testing of each challenge species versus a test article will be performed for the number of replicates listed in Table 1; all agar-plating will be performed in duplicate.

TABLE 1 MICROORGANISM TEST MATRIX

Microorganism Species	Replicate(s)	Contact time(s) for test article(s)	Contact time(s) for numbers control
Candida auris (AR-BANK #0381)	1	1 minute, 30 minutes, 1 hour, 6 hours, and 12 hours	1 hour, 6 hours, and 12 hours
Staphylococcus aureus MRSA (ATCC #33591)			
Staphylococcus aureus (ATCC #6538)			
Staphylococcus epidermidis (ATCC #12228)			

NOTE: The 1-hour numbers control will be compared to the test article contact times: 1 minute, 30 minutes, and 1 hour. The 6-hour numbers control will be compared to the test article contact time: 6 hours. The 12-hour numbers control will be compared to the test article contact time: 12 hours.

[1] Reference **Appendix A** for a summary of the proposed modifications included in this document.

6.0 TEST ARTICLE:

The test article will be provided to the testing facility by the sponsor, complete with the appropriate documentation. Responsibility for the determination of the identity, strength, purity, composition, solubility, and stability of the test article, as well as responsibility for retention of the test article, rests with the sponsor.

TABLE 2 TEST ARTICLE			
Test Article Identification	**Active Ingredients**	**Lot Number**	**Expiration Date**
Protect Foam Formulation (Swab Solution)	N/A	522695	03/2028

7.0 CHALLENGE MICROORGANISM SPECIES:

The challenge microorganism species (American Type Culture Collection [ATCC] strains) to be evaluated are designated below.

TABLE 3 CHALLENGE MICROORGANISMS, GROWTH MEDIA, AND INCUBATION CONDITIONS					
Microorganism Species	**Microorganism Type**	**Incubation Temperature**	**Growth Media**	**Inoc. Prep. Incubation Time**	**Microbial Recovery Incubation Time**
Candida auris (AR-BANK #0381)	Yeast	30 ± 2 °C	SDA	24 ± 4 hours	1 to 3 days
Staphylococcus aureus MRSA (ATCC #33591)	Vegetative Bacteria	35 ± 2 °C	TSA	24 ± 4 hours	1 to 3 days
Staphylococcus aureus (ATCC #6538)	Vegetative Bacteria	35 ± 2 °C	TSA	24 ± 4 hours	1 to 3 days
Staphylococcus epidermidis (ATCC #12228)	Vegetative Bacteria	35 ± 2 °C	TSA	24 ± 4 hours	1 to 3 days

8.0 EQUIPMENT, SUPPLIES, AND MEDIA:

The lists below contain a general list of equipment, supplies, and media that may be used, although each global lab location may have varying materials to perform testing. Applicable materials used will be documented in the raw data, as necessary. Other equipment, supplies, media, buffers, or agar may be used as deemed appropriate by the study director.

Equipment

8.1 Calibrated minute/second timer
8.2 Centrifuge
8.3 Continuously adjustable pipetter, 20 µl - 200 µl capacity and 100 µl - 1000 µl capacity
8.4 Environmental container system
8.5 Hand tally counter
8.6 Incubator
8.7 Laminar biological flow hood
8.8 Microman® positive displacement pipetter, 10 µl - 100 µl capacity and 100 µl - 1000 µl capacity
8.9 NIST traceable clock
8.10 Pipet aid

8.11 Refrigerator
8.12 Steam autoclave
8.13 Thermometer
8.14 Vortex mixer

Supplies
8.15 Sterile 1.0 ml and 0.1 ml positive displacement tips
8.16 Sterile disposable centrifuge tubes, 15 ml
8.17 Sterile disposable Petri plates
8.18 Sterile disposable pipettes
8.19 Sterile disposable specimen containers
8.20 Sterile disposable syringes
8.21 Sterile inoculating loops
8.22 Sterile test tubes
8.23 Sterile T-spreaders
8.24 Sterile universal 1.0 ml and 0.2 ml pipette tips

Media
8.25 0.9% sodium chloride irrigation, USP (SCI)
8.26 Neutralizer: Butterfield's Phosphate Buffer with product neutralizers (BBP++)
8.27 Tryptic soy agar (TSA)
8.28 Sabouraud Dextrose Agar (SDA)

9.0 INOCULUM PREPARATION:

Vegetative Bacteria and Yeast

9.1 Prior to testing, inoculum from a cryogenic vial, lyophilized vial, or stock culture plate will be suspended in SCI, inoculated onto the surface of the appropriate agar and incubated at the appropriate temperature for the appropriate amount of time (reference Table 3).

9.2 Following incubation, at least one daily transfer, but no more than two daily transfers, will be prepared by transferring microbial growth from the prepared solid media into sterile SCI. Aliquots of each suspension will then be inoculated onto the surface of the appropriate agar and incubated at the appropriate temperature for the appropriate amount of time.

9.3 Prior to initiating the test procedure, a challenge suspension of each species will be prepared by suspending growth from the solid media in SCI.

10.0 NEUTRALIZATION STUDIES:

10.1 Neutralization studies of the test article(s) will be performed versus the microorganisms listed in Table 4 to ensure that the neutralizing solution employed effectively neutralizes the antimicrobial properties of the test article(s) and is non-toxic to these representative challenge strains. This neutralization procedure is based on guidelines set forth in ASTM E1054-22, *Standard Practices for Evaluation of Inactivators of Antimicrobial Agents*[2].

10.2 Neutralization challenge suspensions prepared to target approximately 1 x 10^4 CFU/mL will be prepared by diluting the initial suspensions in the media listed below in Table 4 under the Test C column.

[2] Modifications to the ASTM Methodology may be necessary to inactivate the active drug component of the test substance. Reference **Appendix A** for a summary of the proposed modifications included in this document.

TABLE 4 SUMMARY OF NEUTRALIZATION MICROORGANISMS AND MEDIA				
Microorganism Species	Dilution Media			Hold Time (min)
	Test A	Test B	Test C	
Candida auris (AR-BANK #0381)	Neutralizer	Neutralizer	SCI	≥ 15
Staphylococcus aureus (ATCC #6538)	Neutralizer	Neutralizer	SCI	≥ 15

Neutralization Effectiveness Evaluation (Test A)

10.3 Three replicates of this procedure will be performed versus the test article(s) and each challenge microorganism listed in Table 4.

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

10.5 The mixture will be exposed for the hold time specified in Table 4 using a calibrated minute/second timer as necessary. Following exposure, a 10-fold dilution may be prepared in the appropriate dilution media and mixed thoroughly.

10.6 Duplicate 1.0 mL and/or 0.1 mL aliquots of each suspension will be plated using the appropriate agar (reference Table 3). If spread-plated the 1.0 mL aliquots will be split across two plates of the appropriate agar. The prepared plates will be incubated at the appropriate temperature and under the appropriate conditions.

Neutralizer Toxicity Evaluation (Test B)

10.7 Three replicates of this procedure will be performed versus each species listed in Table 4.

10.8 A 0.1 mL aliquot of a Neutralization challenge suspension will be transferred to a test tube containing 8.9 mL of neutralizer (10^0 dilution). A 1.0 mL aliquot of SCI will be added to the tube containing inoculum/neutralizer and mixed thoroughly.

10.9 The mixture will be exposed for the hold time specified in Table 4 using a calibrated minute/second timer as necessary. Following exposure, a 10-fold dilution may be prepared in the appropriate dilution media and mixed thoroughly.

10.10 Duplicate 1.0 mL and/or 0.1 mL aliquots of each suspension will be plated using the appropriate agar (reference Table 3). If spread-plated the 1.0 mL aliquots will be split across two plates of the appropriate agar. The prepared plates will be incubated at the appropriate temperature and under the appropriate conditions.

Test Organism Viability (Test C)

10.11 Three replicates of this procedure will be performed versus each species listed in Table 4.

10.12 A 0.1 mL aliquot of a Neutralization Challenge Suspension will be transferred to a test tube containing 9.9 mL of the appropriate dilution media as specified in Table 4 under the Test C column and mixed thoroughly (10^0 dilution).

10.13 The mixture will be exposed for the hold time specified in Table 4 using a calibrated minute/second

timer as necessary. Following exposure, a 10-fold dilution may be prepared in the appropriate dilution media and mixed thoroughly.

10.14 Duplicate 1.0 mL and/or 0.1 mL aliquots of each suspension will be plated using the appropriate agar (reference Table 3). If spread-plated the 1.0 mL aliquots will be split across two plates of the appropriate agar. The prepared plates will be incubated at the appropriate temperature and under the appropriate conditions.

Data Collection

10.15 Following incubation, the colonies on the plates will be counted manually using a hand-tally counter. Counts in the range of 25 to 250 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

10.16 The $\log_{10}$ of the number of survivors of each challenge strain from Test A and Test B will be statistically compared to those from Test C using a Two One-Sided t-Test performed using an $\alpha = 0.05$ and an equivalency margin $\delta = 0.5$ to calculate a 90% confidence interval on the difference between Test C and the other tests (A and B). The tests will be considered equivalent if the 90% confidence interval is between -0.5 and 0.5.

10.17 If the Test A recovered population is statistically equivalent to Test C, the neutralizer is considered effective for neutralizing the antimicrobial activity of the test article.

10.18 If the Test B recovered population is statistically equivalent to Test C, the neutralizer is considered non-toxic.

11.0 TIME-KILL METHODOLOGY:

Initial and Final Population Methodology

11.1 Prior to use, the initial population of each challenge suspension will be determined by preparing appropriate 10-fold dilutions in neutralizer. Duplicate 0.1 mL aliquots will be plated using the appropriate agar. The prepared plates will be incubated at the appropriate temperature and under the appropriate conditions (reference Table 3).

11.2 Following the completion of the appropriate testing procedures, the final population of each challenge suspension will be determined, as described above.

11.3 The initial and final population of each challenge suspension must be within 0.5 $\log_{10}$ of each other for the testing to be considered valid.

Numbers Control

11.4 A 0.1 mL aliquot of a challenge suspension containing no less than 1 x 10^8 CFU/mL will be transferred to a sterile tube containing 10 mL of SCI and mixed thoroughly using a vortex mixer (10^0 dilution).

11.5 Each challenge microorganism will be exposed to the SCI for the time(s) listed in Table 1, timed using a calibrated minute/second timer.

11.6 After the exposure time has elapsed, 1.0 mL will be transferred from the tube containing the

SCI/challenge suspension to a sterile test tube containing 9.0 mL of the neutralizer and mixed thoroughly using a vortex mixer (10^{-1} dilution). Ten-fold dilutions will be prepared in the neutralizer. Duplicate 0.1 mL aliquots of the appropriate dilutions will be plated. The prepared plates will be incubated at the appropriate temperature and under the appropriate conditions (reference Table 3).

Time-Kill Testing Methodology

11.7 A 0.1 mL aliquot of a challenge suspension containing no less than 1 x 10^8 CFU/mL will be transferred to a sterile tube containing 10 mL of a test article and mixed thoroughly using a vortex mixer and/or positive displacement pipettor (10^0 dilution).

11.8 Each challenge microorganism will be exposed to the test article(s) for the time listed in Table 1, timed using a calibrated minute/second timer.

11.9 After each exposure time has elapsed, 1.0 mL will be transferred from each tube containing test article/challenge suspension to separate sterile test tubes containing 9.0 mL of neutralizer and mixed thoroughly using a vortex mixer (10^{-1} dilution). Ten-fold dilutions of the suspensions of each challenge microorganism may be prepared in neutralizer, mixing thoroughly using a vortex mixer between dilutions.

11.10 Duplicate 1.0 mL and/or 0.1 mL aliquots of each suspension will be plated using the appropriate agar (reference Table 3). If spread-plated the 1.0 mL aliquots will be split across two plates of the appropriate agar. The prepared plates will be incubated at the appropriate temperature and under the appropriate conditions.

11.11 The procedures described in the Time-Kill testing methodology section will be performed for each challenge species versus a test article for the number of replicates listed in Table 1.

Data Collection

11.12 Following incubation, the colonies on the plates will be counted manually using a hand-tally counter. Counts in the range of 25 to 250 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.

12.0 CALCULATIONS:

12.1 The initial population (IP), the final population (FP), the numbers control (NC) population recovery, and post-exposure population (P_{EX}) following each timed exposure will be calculated as follows:

$$CFU/mL = C_i \times 10^{-D}$$

Where:

C_i = Average of the two plates counted
D = Dilution factor of the plates counted

12.2 The $\log_{10}$ reduction attributable to the test article(s) will be calculated for each replicate of testing as follows:

$\log_{10}$ reduction = $\log_{10}$ (NC) - $\log_{10}$ (P_{EX})

Where:
NC = Numbers control population (CFU/mL)
P_{EX} = Post-exposure population (CFU/mL)

12.3 The percent reduction attributable to the test article(s) will be calculated for each time of exposure and each replicate of testing as follows:

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

Where:
NC = Numbers control population (CFU/mL)
P_{EX} = Post-exposure population (CFU/mL)

12.4 If more than one replicate was performed, the mean reduction ($\log_{10}$) attributable to the test article(s) will be calculated for each time of exposure as follows:

$$\text{Mean reduction} = \log_{10}\left(CFU/mL\ (NC) - \left(\frac{\sum \text{CFU/mL}\ (P_{EX})}{N}\right)\right)$$

Where:
N = Number of replicates

13.0 STATISTICAL ANALYSIS:

Statistical analyses will be performed on the data derived from the neutralization studies. Statistical analyses will not be performed on the data derived from the time-kill evaluation.

14.0 FINAL REPORT:

A final report will be issued presenting the results of this evaluation in a clear, concise manner.

15.0 EXCEPTIONAL CONDITIONS:

The 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).

16.0 QUALITY ASSURANCE AUDITS:

Quality Assurance (QA) will conduct an in-phase audit of critical processes in testing at least once and advise the study director and management of the outcomes of this. On completion of testing, QA will perform an audit of the data and the Final Report in accordance with 21 CFR Part 58.

17.0 DOCUMENTATION AND RECORD-KEEPING:

All documentation and records will be compiled, analyzed, and retained by Nelson Laboratories Bozeman, LLC, 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. Nelson Laboratories Bozeman, LLC, will notify the sponsor before any documents or records are destroyed.

18.0 SERVICE TERMS AND CONFIDENTIALITY:

This document has been copyrighted by Nelson Laboratories Bozeman, LLC, and is considered confidential between Nelson Laboratories Bozeman, LLC and the sponsor. Permission to release the protocol and study results to the United States Food and Drug Administration (FDA) is explicitly granted.

Please refer to the Test Report Use Guidance on the Nelson Labs website (www.nelsonlabs.com/service-terms/) for use of the test report.

19.0 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 protocol.

20.0 REFERENCES:

ASTM E1054-22 (2022), *Standard Practice for Evaluation of Inactivators of Antimicrobial Agents*.

ASTM E2783-22 (2022) *Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure*

Protocol #2402064-201

21.0 ACCEPTANCE:

A Time-Kill Evaluation of Protect Foam Formulation (Swab Solution) Against: Candida auris (AR-BANK #0381), Staphylococcus aureus MRSA (ATCC #33591), Staphylococcus aureus (ATCC #6538), & Staphylococcus epidermidis (ATCC #12228) Based Upon ASTM E2783-22

ACCEPTED BY: NELSON LABORATORIES BOZEMAN, LLC (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718

Study Director:

Keely Huth

20 MAR 2024
Date of Study Initiation

ACCEPTED BY: AVADIM HEALTH (SPONSOR)
4 Old Patton Cove Road
Swannanoa, North Carolina 28778

Representative

V.P. R&D
Title

3-20-2024
Date

Protocol #2402064-201

SUMMARY OF MODIFICATIONS TO ASTM E2783-22 AND ASTM E1054-22

SUMMARY OF MODIFICATIONS TO ASTM E2783-22

1) The Numbers Control will be prepared using sterile 0.9% Sodium Chloride Irrigation, USP (SCI), rather than sterile deionized or distilled water.

2) Minor modifications to the inoculum preparation procedures will be made. These modifications include, but are not limited to, the use of agar plates (rather than agar slants) and the omission of centrifugation/rinsing procedures for the suspended bacterial cells.

3) The post-exposure sampling volume of certain bacterial species may be changed from 1.0 mL to 0.1 mL, to reflect the procedures utilized for the neutralization studies.

4) Serial ten-fold dilutions may be prepared by transferring 0.5 mL aliquots to tubes containing 4.5 mL of diluent, rather than using 9.0 mL dilution blanks.

SUMMARY OF MODIFICATIONS TO ASTM E1054-22

1) Sterile 0.9% Sodium Chloride Irrigation, USP (SCI) will be used as a diluent for Test C (Test Organism Viability), rather than Phosphate Buffered Saline Dilution Water (PBS).

2) Neutralization challenge suspensions with a concentration higher than specified by the method (e.g., approximately 10^4 CFU/mL) may be necessary and increased to approximately 10^5 to 10^6 CFU/mL. This modification may be necessary to produce valid plate counts at the test article dilution at which sub-inhibitory levels of the test articles are achieved. If higher microbial titers are used, additional dilutions of each test (Test C, Test A and Test B) may be prepared and plated for each species.

3) Test D will not be performed in this evaluation. Test D is utilized to determine if the test article has any antimicrobial activity for the neutralizer to inactivate. As this evaluation is being conducted in tandem with ASTM E2783-22, to determine the efficacy of the test article, performance of Test D is not necessary.