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12 JAN 2023

FINAL REPORT #2211575-402

EVALUATION OF ONE TEST ARTICLE FOR VIRUCIDAL PROPERTIES BASED UPON THE ASTM E1052-20 METHOD

Prepared for:

AVADIM HEALTH, INC. (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
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EXECUTIVE SUMMARY

STUDY NUMBER	**2211575-402**
TITLE	**EVALUATION OF ONE TEST ARTICLE FOR VIRUCIDAL PROPERTIES BASED UPON THE ASTM E1052-20 METHOD**
SPONSOR	**AVADIM HEALTH, INC.** 4 Old Patton Cove Road Swannanoa, North Carolina 28778
TESTING FACILITY	**NESLON LABORATORIES BOZEMAN, LLC** 1755 South 19th Avenue Bozeman, Montana 59718
STUDY INITIATION DATE	16 DEC 2022
STUDY COMPLETION DATE	12 JAN 2023

This study evaluated virucidal properties of one test article when challenged with Respiratory Syncytial Virus (ATCC #VR-26). The testing was based upon ASTM E1052-20, *Standard Practice to Assess the Activity of Microbicides against Viruses in Suspension.* All testing was performed in accordance with Food and Drug Administration 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).

Table 1 presents a summary of results.

TABLE 1

Exposure Time	Log_{10} Reduction	Percent Reduction
30 minutes	≥4.00	≥99.99%
3 hours	≥4.25	>99.99%
6 hours	≥3.75	≥99.98%

12 JAN 2023

FINAL REPORT #2211575-402

1.0 TITLE **EVALUATION OF ONE TEST ARTICLE FOR VIRUCIDAL PROPERTIES BASED UPON THE ASTM E1052-20 METHOD**

2.0 SPONSOR **AVADIM HEALTH, INC.**
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 Mauri Erickson, M.S.

5.0 PURPOSE

This study evaluated virucidal properties of one test article when challenged with Respiratory Syncytial Virus. The testing was based upon ASTM E1052-20, *Standard Practice to Assess the Activity of Microbicides against Viruses in Suspension.* All testing was performed in accordance with Food and Drug Administration 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).

6.0 SCOPE

This study was designed to evaluate the virucidal properties of one test article versus Respiratory Syncytial Virus strain Long (ATCC #VR-26) using a Virucidal Suspension Test (*In-Vitro* Time-Kill method) based upon ASTM E1052-20, *Standard Practice to Assess the Activity of Microbicides against Viruses in Suspension.* The percent and $\log_{10}$ reductions from the initial population of the viral strain were determined following exposure to the test article for 30 minutes, 3 hours and 6 hours. Testing was performed in one replicate. Plating was performed in four replicates.

The protocol, included in the addendum to this final report, presents the study methodology, in detail. No deviations from the protocol or from applicable standard operating procedures occurred during the course of this evaluation.

7.0 STUDY DATES

STUDY INITIATION DATE	16 DEC 2022
EXPERIMENTAL START DATE	22 DEC 2022
EXPERIMENTAL END DATE	30 DEC 2022
STUDY COMPLETION DATE	12 JAN 2023

8.0 TEST ARTICLE

The test article evaluated was provided to the testing facility by the sponsor. Responsibility for determination of the identity, strength, purity, composition, solubility, and stability of the test article, as well as responsibility for retention of the test article, remained with the sponsor. The test article was evaluated as received from the sponsor.

Test Article:	Theraworx Protect Foam
Active Ingredient(s):	N/A
Lot Number:	520669
Manufacture Date:	Not Provided
Expiration Date:	Not Provided

9.0 CHALLENGE VIRAL STRAIN

Respiratory Syncytial Virus strain Long (ATCC #VR-26)
ATCC = American Type Culture Collection

10.0 HOST CELLS

HEp-2 (CCL-23; Human adenocarcinoma, HeLa contaminant, epithelial)

11.0 SUPPLIES AND EQUIPMENT

The equipment and supplies used in this study are as described in the protocol in the addendum to this final report. All applicable equipment and instrumentation were calibrated in accordance with Nelson Laboratories Bozeman, LLC, Standard Operating Procedures.

12.0 MEDIA

The growth media and diluting fluids used in this study are as described in the protocol in the addendum to this final report.

13.0 HOST CELL PREPARATION

Cells, obtained from American Type Culture Collection (ATCC), were maintained as monolayers in disposable cell culture labware in accordance with SOP L-2084, "*Procedure for Subculturing of Cells.*" Prior to testing, host cell cultures were seeded onto 24-well cell culture treated plates. Cell monolayers were at least 24 hours old before inoculation with each virus. HEp-2 cells were approximately 80% confluent before inoculation with Respiratory Syncytial Virus. The growth medium (GM) was replaced by maintenance medium (MM) to support virus propagation.

14.0 TEST VIRUS PREPARATION

The test virus used for this study was from high titer virus stock. On the day of use, aliquots of the stock virus were removed from a -70°C freezer and thawed.

15.0 TEST ARTICLE PREPARATION

The test article was a ready-to-use product and was tested as provided by the sponsor. The test article was shaken well prior to use in testing.

16.0 VIRUCIDAL SUSPENSION TEST

16.1 The procedure for virucidal suspension test was performed as described in the protocol in the addendum to this final report. The virucidal suspension test included the following parameters (Table 2):

TABLE 2

Parameters of Virucidal Suspension Test

Parameter	Summary	Replicates
Virucidal Suspension Test	Virus + Test Article → Exposure → Neutralization → Dilution → Plating	4 per group
Virus Control	Virus + Diluent → Exposure → Dilution → Plating	4 per group
Cytotoxicity Control	Test Article + Diluent → Neutralization → Dilution → Plating	4 per group
Neutralization Control	Test Article + Diluent → Neutralization → Virus inoculation → Dilution → Plating	4 per group
Neutralizer Toxicity Control	Virus + Diluent → Neutralization → Dilution → Plating	4 per group
Cell Culture Control	Maintenance medium	4 per group

17.0 CALCULATIONS

Calculations were performed as described in the protocol in the addendum to this final report.

18.0 TEST ACCEPTANCE CRITERIA

The test acceptance criteria for this study were met:

1) at least 4 $\log_{10}$ of $TCID_{50}$ was recovered from the Virus Control;
2) cells in the cell culture wells were viable and attached to the bottom of the well;
3) the medium was free of contamination in all wells of the plate;
4) at least a 3 $\log_{10}$ reduction in titer could be demonstrated beyond the cytotoxic level;
5) the test articles were fully neutralized after the timed exposure such that the difference in virus titer for the Neutralization Control, Neutralizer Toxicity Control and Virus Control did not exceed 1.0 $\log_{10}$ (Tables 3 through 5).

19.0 RESULTS – TABLES 3 THROUGH 5

19.1 Table 3 presents the data from the virus control infectivity ($TCID_{50}$) and the post-exposure infectivity ($TCID_{50}$); the $\log_{10}$ and percent reductions observed following a 30-minute exposure of Respiratory Syncytial Virus strain Long (ATCC #VR-26) to Test Article, Theraworx Protect Foam (Lot #520669).

TABLE 3

Test Article: Theraworx Protect Foam (Lot #520669)
Virus: Respiratory Syncytial Virus strain Long (ATCC #VR-26)
Host Cell Line: HEp-2 (ATCC #CCL-23)

Dilution (- Log_{10})	Virus Control	Test	Neutralization Control	Neutralizer Toxicity Control	Cytotoxicity Control	Cell Control
		30 Minutes				0000
-2	NT	0000	NT	NT	0000	N/A
-3	++++	0000	++++	++++	0000	
-4	++++	0000	++++	++++	0000	
-5	++++	0000	00++	+0+0	NT	
-6	0000	0000	0000	0000	NT	
-7	0000	0000	0000	0000	NT	
$TCID_{50}$ (log_{10})	5.50	≤1.50	5.00	5.00	≤1.50	
Log_{10} Reduction	N/A	≥4.00	N/A			
Percent Reduction		**≥99.99%**				

\+ Virus infected cells present
0 Virus infected cells not detected
NT Not tested
N/A Not applicable

19.2 Table 4 presents the data from the virus control infectivity ($TCID_{50}$) and the post-exposure infectivity ($TCID_{50}$); the log_{10} and percent reductions observed following a 3-hour exposure of Respiratory Syncytial Virus strain Long (ATCC #VR-26) to Test Article, Theraworx Protect Foam (Lot #520669).

TABLE 4

Test Article: Theraworx Protect Foam (Lot #520669)
Virus: Respiratory Syncytial Virus strain Long (ATCC #VR-26)
Host Cell Line: HEp-2 (ATCC #CCL-23)

Dilution (- Log_{10})	**Virus Control**	**Test**	**Neutralization Control**	**Neutralizer Toxicity Control**	**Cytotoxicity Control**	**Cell Control**
		3 Hours				0000
-2	NT	0000	NT	NT	0000	N/A
-3	++++	0000	++++	++++	0000	
-4	++++	0000	++++	++++	0000	
-5	++++	0000	+++0	++++	NT	
-6	+000	0000	0000	0000	NT	
-7	0000	0000	0000	0000	NT	
$TCID_{50}$ (log_{10})	5.75	≤1.50	5.25	5.50	≤1.50	
Log_{10} Reduction	N/A	≥4.25	N/A			
Percent Reduction		**>99.99%**				

\+ Virus infected cells present
0 Virus infected cells not detected
NT Not tested
N/A Not applicable

19.3 Table 5 presents the data from the virus control infectivity ($TCID_{50}$) and the post-exposure infectivity ($TCID_{50}$); the log_{10} and percent reductions observed following a 6-hour exposure of Respiratory Syncytial Virus strain Long (ATCC #VR-26) to Test Article, Theraworx Protect Foam (Lot #520669).

TABLE 5

Test Article: Theraworx Protect Foam (Lot #520669)
Virus: Respiratory Syncytial Virus strain Long (ATCC #VR-26)
Host Cell Line: HEp-2 (ATCC #CCL-23)

Dilution (- Log_{10})	Virus Control	Test	Neutralization Control	Neutralizer Toxicity Control	Cytotoxicity Control	Cell Control
		6 Hours				0000
-2	NT	0000	NT	NT	0000	N/A
-3	++++	0000	++++	++++	0000	
-4	++++	0000	++++	++++	0000	
-5	++0+	0000	+0+0	++00	NT	
-6	0000	0000	0000	0000	NT	
-7	0000	0000	0000	0000	NT	
$TCID_{50}$ (log_{10})	5.25	≤1.50	5.00	5.00	≤1.50	
Log_{10} Reduction	N/A	≥3.75	N/A			
Percent Reduction		**≥99.98%**				

\+ Virus infected cells present
0 Virus infected cells not detected
NT Not tested
N/A Not applicable

20.0 STUDY CONCLUSIONS

Under the conditions of this evaluation, the Test Article, Theraworx Protect Foam (Lot #520669), reduced the infectivity of Respiratory Syncytial Virus strain Long (ATCC #VR-26) by ≥4.00 log_{10} (≥99.99%) following a 30-minute exposure, by ≥4.25 log_{10} (>99.99%) following a 3-hour exposure, and by ≥3.75 log_{10} (≥99.98%) following a 6-hour exposure.

21.0 STATISTICAL ANALYSIS

A statistical analysis was not performed on the data derived from this study.

22.0 QUALITY ASSURANCE AUDITS

Quality Assurance (QA) conducted an in-phase audit of the critical test procedures over the course of testing and advised the study director and management of the outcomes of these. On completion of testing, QA performed an audit of the raw data and of the final report, in its entirety.

23.0 LABORATORY PERSONNEL

The following employees of Nelson Laboratories Bozeman, LLC, 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 — Mauri Erickson, M.S.

SUPERVISORS

Chelsey Allison, CCRC
Sr. Manager Laboratory Operations

Penny Chamnongpol, Ph.D.
Manager Laboratory Operations

SCIENTISTS

Kelly Burningham
Cecelia K. McAffee
Amelia Smith

24.0 QUALITY ASSURANCE AND QUALITY CONTROL PERSONNEL

Kevin Crawford
Maintenance Technician

Jeremy Duley
Maintenance Manager

Danielle Goveia
Quality Assurance Manager

Selinda Kiefer
Maintenance Technician

Renee LaFond, MS
Quality Assurance Specialist

Robert McDonald
Maintenance Technician

Dario Rodriguez Steinhardt
Quality Assurance Specialist

Robert Sukhu
Quality Assurance Specialist

Tom Woods
Maintenance Technician

25.0 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 five years. Nelson Laboratories Bozeman, LLC will notify the sponsor before any documents or records are destroyed.

26.0 ACCEPTANCE

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

Study Director: Mauri Erickson — 12 JAN 2023
Mauri Erickson, M.S. — Date of Study Completion

QUALITY ASSURANCE STATEMENT

This study was inspected by Quality Assurance, 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
Test Article Testing	22 DEC 2022	27 DEC 2022	27 DEC 2022
Data Audit	11 JAN 2023	12 JAN 2023	12 JAN 2023
Final Report Review	12 JAN 2023	12 JAN 2023	12 JAN 2023

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 articles was not performed by Nelson Laboratories Bozeman, LLC. No deviations from the protocol and no deviations from applicable Nelson Laboratories Bozeman, LLC, Standard Operating Procedures were observed. This statement also serves to confirm that the final report reflects the raw data.

Quality Assurance Specialist: Dario Rodriguez Steinhardt — 12 JAN 2023
Date

ADDENDUM

Protocol #2211575-402

Nelson Labs
A Sotera Health company

13 DEC 2022

PROTOCOL #2211575-402

EVALUATION OF ONE TEST ARTICLE FOR VIRUCIDAL PROPERTIES BASED UPON THE ASTM E1052-20 METHOD

Prepared for:

AVADIM HEALTH, INC. (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

CONFIDENTIAL

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13 DEC 2022

PROTOCOL #2211575-402

1.0 TITLE — **EVALUATION OF ONE TEST ARTICLE FOR VIRUCIDAL PROPERTIES BASED UPON THE ASTM E1052-20 METHOD**

2.0 SPONSOR

AVADIM HEALTH, INC.
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 — Mauri Erickson, M.S.

5.0 PURPOSE OF STUDY

The purpose of this GLP study is to evaluate the virucidal efficacy of one test article when challenged with Respiratory Syncytial Virus. All testing will be performed in accordance with Food and Drug Administration 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).

6.0 SCOPE

This study will determine the virucidal efficacy of one test article when challenged Respiratory Syncytial Virus strain Long (ATCC #VR-26) using a virucidal suspension test (*In-Vitro* time-kill method) based upon ASTM E1052-20, *Standard Practice to Assess the Activity of Microbicides against Viruses in Suspension*. The test article is a ready-to-use product and will be tested neat (undiluted). Calculations of the estimated virus concentrations will be performed using a 50% tissue culture infectious dose ($TCID_{50}$) calculation — the Quantal test (Spearman-Kärber Method). The log_{10} reductions from the initial population of the viral strain(s) will be determined following exposure to the test article for 30 minutes, 3 hours and 6 hours. Testing will be conducted in one replicate. Plating will be performed in four replicates.

7.0 TEST ARTICLE

The test article to be evaluated will be provided to the testing facility by the sponsor, complete with appropriate documentation. If there are any changes in test article identity below, the protocol will be amended and corrections will be provided in the final report. Responsibility for the determination of the identity, strength, purity, composition, and stability of the test article, as well as the retention of the test article, rests with the sponsor. The test article will be evaluated as received from the sponsor.

Test Article:	Theraworx Protect Foam
Active Ingredient(s):	N/A
Lot Number:	520669
Manufacture Date:	Not Provided
Expiration Date:	Not Provided

8.0 TEST CONDITIONS

8.1	Exposure Times:	30 minutes ± 1 minute 3 hours ± 15 minutes 6 hours ± 15 minutes
8.2	Exposure Temperature:	Ambient (nominally 18 °C to 25 °C)

9.0 CHALLENGE VIRAL STRAIN

Respiratory Syncytial Virus strain Long (ATCC #VR-26)
ATCC = American Type Culture Collection

10.0 HOST CELLS

HEp-2 (CCL-23; Human adenocarcinoma, HeLa contaminant, epithelial)

11.0 EQUIPMENT

11.1 Ultralow temperature freezer, temperature range ≤ -70°C
11.2 CO_2 incubator, temperature range 37 °C ± 2 °C
11.3 Water bath, 37 °C ± 2 °C
11.4 Incubator thermometers
11.5 Continuously adjustable pipettes, 100 µL – 1000 µL capacity
11.6 Continuously adjustable pipettes, 20 µL – 200 µL capacity
11.7 Portable pipetter
11.8 Inverted compound microscope
11.9 Laminar flow biological safety cabinet
11.10 Calibrated minute/second timers
11.11 NIST traceable clock

12.0 SUPPLIES

12.1 Personal protective equipment
12.2 Sterile disposable pipettes
12.3 Sterile polystyrene test tubes
12.4 Sterile universal 1000 µL pipette tips
12.5 Powder-free gloves
12.6 Sterile tissue culture treated multi-well plates
12.7 Viral suspension
12.8 Sterile flasks
12.9 Sterile 15 mL centrifuge tubes
12.10 Sterile reservoirs
12.11 Waste pan
12.12 Non-sterile waste beaker for discarded tips, etc.

13.0 MEDIA AND REAGENTS

13.1 1X Minimum Essential Medium (MEM) or other appropriate medium (e. g. Advanced MEM or EMEM)
13.2 Growth Medium (GM): MEM or other media with 4% serum and 1% antibiotic and L-glutamine; EMEM or other media with 10% serum and 1% antibiotic and L-glutamine (when necessary).
13.3 Maintenance Medium (MM): MEM or other media with 2% serum, 1% antibiotic and L-glutamine (when necessary).
13.4 Trypsin/EDTA

13.5 Antibiotics (e.g., penicillin-streptomycin-amphotericin B [10,000 units/mL of penicillin, 10,000 µg/mL of streptomycin, and 25 µg/mL amphotericin B])
13.6 Fetal bovine serum (FBS)
13.7 Appropriate neutralizer (e.g. Dey-Engley [D/E] Neutralizing Broth)

14.0 HOST CELL PREPARATION

Cells will be maintained as monolayers in disposable cell culture labware as per SOP L-2084, *Procedure for Subculturing of Cells.* Prior to testing, host cell cultures will be seeded onto multi-well cell culture treated plates. Cell monolayers will be 80% to 90% confluent and at least 24 hours old before use in testing. Growth Medium (GM) and Maintenance Medium (MM) will be MEM, or other medium with appropriate supplements as described in 13.2 and 13.3.

15.0 TEST VIRUS PREPARATION

The virus suspension(s) used for this study will originate from high titer virus stock, propagated and stored per SOP L-2102, *Procedure for Production of High-Titered Virus Stock.* On the day of use, the virus aliquot will be removed from storage in a -70°C freezer and thawed for use in testing.

16.0 TEST ARTICLE PREPARATION

The test article is a ready-to-use product and will be tested as provided by the sponsor. The test article will be shaken well prior to use in testing.

17.0 EVALUATION OF TEST ARTICLE

17.1 The virucidal suspension test will include the following parameters:

Parameter	Summary	Replicates
Virucidal Suspension Test	Virus + Test Article → Exposure → Neutralization → Dilution → Plating	4 per group
Virus Control	Virus + Diluent → Exposure → Dilution → Plating	4 per group
Cytotoxicity Control	Test Article + Diluent → Neutralization → Dilution → Plating	4 per group
Neutralization Control	Test Article + Diluent → Neutralization → Virus inoculation → Dilution → Plating	4 per group
Neutralizer Toxicity Control	Virus + Diluent → Neutralization → Dilution → Plating	4 per group
Cell Culture Control	Maintenance medium	4 per group

17.2 *Test.* A 0.5 mL aliquot of test virus will be added to a vial containing 4.5 mL of the test article(s). The test virus(s) will be exposed to the test article(s) for 30 minutes, 3 hours and 6 hours, timed using a calibrated minute/second timer or NIST traceable clock. The calibrated minute/second timer will be started within ± 1 second of adding the challenge suspension. Immediately after exposure, the test virus/test article suspension(s) will be neutralized in D/E Neutralizing Broth (or another appropriate neutralizer) and mixed thoroughly. Subsequent 10-fold dilutions will be made in MM. Each dilution will be plated in four replicates.

17.3 *Virus Control.* A 0.5 mL aliquot of test virus(s) will be added to 4.5 mL of MM and exposed for 30 minutes, 3 hours and 6 hours at ambient temperature. Subsequent 10-fold dilutions will be made in MM. Each dilution will be plated in four replicates.

17.4 *Neutralizer Toxicity Control.* A 0.5 mL aliquot of test virus(s) will be added to 4.5 mL of neutralizer and exposed for at least 30 minutes, 3 hours, and 6 hours. Subsequent 10-fold dilutions will be made in MM. Each dilution will be plated in four replicates.

17.5 *Cytotoxicity Control.* A 0.5 mL aliquot of MM will be added to a vial containing 4.5 mL of the test article(s). The MM/test article suspension(s) will be neutralized in D/E Neutralizing Broth (or another appropriate neutralizer) and mixed thoroughly. Subsequent 10-fold dilutions will be made in MM. Each dilution will be plated in four replicates.

17.6 *Neutralization Control.* A 0.5 mL aliquot of MM will be added to a vial containing 4.5 mL of the test article(s). The MM/test article suspension(s) will be neutralized in D/E Neutralizing Broth (or another appropriate neutralizer) and mixed thoroughly. An aliquot of the virus will be added to the neutralized test article(s), thoroughly mixed and exposed to the neutralized test article(s) for at least 30 minutes, 3 hours and 6 hours. Subsequent 10-fold dilutions will be made in MM. Each dilution will be plated in four replicates.

Note: In the case of excessive cytotoxicity of the test article(s), a higher dilution in a neutralizer (1:100) or sephadex gel filtration will be applied. If sephadex gel filtration is utilized, samples from the critical test parameters will be subjected to filtration.

17.7 *Cell Culture Control.* Intact cell culture will serve as the control of cell culture viability. The GM will be replaced by MM in all cell control wells.

17.8 The plates will be incubated in a CO_2 incubator for 5 to 14 days at a temperature appropriate for the virus. Cytopathic/cytotoxic effect will be monitored using an inverted compound microscope. In cases when viral CPE is undetectable using an inverted compound microscope, additional immunostaining with virus specific antibodies may be performed.

18.0 CALCULATIONS

18.1 Viral titers will be expressed as $-Log_{10}$ of the 50% titration end point for infectivity. To calculate the viral titer, a 50% tissue culture infectious dose ($TCID_{50}$) calculations for Cytopathic viruses - the Quantal test (Spearman-Kärber Method) - will be applied.

$$\log_{10} TCID_{50} = L - d\,(s - 0.5)$$

Where:

L = $-\log_{10}$ of the lowest dilution;
d = difference between dilution steps;
s = sum of proportions of positive wells.

18.2 The $\log_{10}$ of infectivity reduction will be calculated as follows:

$\log_{10}$ Reduction Formula:

$\log_{10}$ Reduction = ($\log_{10}$ $TCID_{50}$ of the Virus Control) - ($\log_{10}$ $TCID_{50}$ of the Virucidal Suspension Test)

18.3 The percent reduction will be calculated as follows:

$$\% \text{ Reduction} = \left[1 - \frac{\text{TCID}_{50}\ test}{\text{TCID}_{50}\ virus\ control}\right] \times 100$$

19.0 TEST ACCEPTANCE CRITERIA

A valid test requires that:

19.1 At least 4 $\log_{10}$ of $TCID_{50}$ be recovered from the Virus Control;
19.2 Cells in the cell culture wells be viable and attached to the bottom of the well;
19.3 The medium be free of contamination in all wells of the plate;
19.4 When cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer be demonstrated beyond the cytotoxic level; and
19.5 The test article be fully neutralized after the timed exposure such that the difference in virus titer for the Neutralization Control, Neutralizer Toxicity Control and Virus Control does not exceed 1.0 $\log_{10}$.

20.0 STATISTICAL ANALYSIS

A statistical analysis will not be performed on the data derived from this evaluation.

21.0 PROTOCOL DEVIATIONS AND AMENDMENTS

Amendments to an approved protocol and the reasons will be documented, signed, and dated by the study director and sponsor, and maintained with the protocol per current Standard Operating Procedures. Deviations will be documented by the study director, signed and dated and maintained with the protocol per current Standard Operating Procedures.

22.0 FINAL REPORT

A final report will be prepared by Nelson Laboratories Bozeman, LLC, describing the results of the study in a clear and concise manner.

23.0 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).

24.0 LIABILITY AND INDEMNIFICATION

The testing facility's liability to the sponsor under this protocol shall be limited to the price of this evaluation. The sponsor shall be responsible to study participants (when applicable) and to other third parties for the fitness of the test article for use as defined in the protocol.

25.0 REFERENCES

ASTM E1052-20, Standard Practice to Assess the Activity of Microbicides against Viruses in Suspension.

26.0 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 five years. Nelson Laboratories Bozeman, LLC, will notify the sponsor before any documents or records are destroyed.

27.0 ARTICLE DISPOSITION

It is the responsibility of the sponsor to retain a sample of the test article for future audit or evaluation. All unused test article will be returned to the sponsor following study completion, unless otherwise indicated by the sponsor prior to initiation of the study.

28.0 QUALITY ASSURANCE AUDITS

Quality Assurance (QA) will conduct in-phase audits of critical processes in testing at least once and advise the Study Director and management of the outcomes. On completion of testing, QA will perform an audit of the data and of the final report in its entirety.

29.0 ACCEPTANCE

EVALUATION OF ONE TEST ARTICLE FOR VIRUCIDAL PROPERTIES BASED UPON THE ASTM E1052-20 METHOD

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

Study Director: Mauri Erickson
Mauri Erickson, M.S.

16 DEC 2022
Date of Study Initiation

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

Jared Buckner
Representative

12-16-2022
Date

V.P. Business Development
Title