23 MAY 2023

FINAL REPORT #2303763-408A

AN EVALUATION OF THE VIRUCIDAL PROPERTIES OF TREATED NON-POROUS MATERIALS BASED UPON ISO 21702:2019(E)

Prepared for:

NANO AND ADVANCED MATERIALS INSTITUTE LIMITED (SPONSOR)
Units 608-609, Lakeside 2
No. 10 Science Park West Avenue
Hong Kong Science Park, Hong Kong

Prepared by:

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

STUDY NUMBER **2303763-408A**

TITLE **AN EVALUATION OF THE VIRUCIDAL PROPERTIES OF TREATED NON-POROUS MATERIALS BASED UPON ISO 21702:2019(E)**

SPONSOR **NANO AND ADVANCED MATERIALS INSTITUTE LIMITED**
Units 608-609, Lakeside 2
No. 10 Science Park West Avenue
Hong Kong Science Park, Hong Kong

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

STUDY INITIATION DATE 27 APR 2023

STUDY COMPLETION DATE 23 MAY 2023

This study evaluated the virucidal property of one treated test article and one untreated control article when challenged with Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2). Testing was based upon ISO 21702:2019(E) "*Measurement of antiviral activity on plastics and other non-porous surfaces*." The virucidal efficacy of the test surface was compared with that of the untreated control surface. The test virus was inoculated onto the test and control surfaces and incubated at 25 ℃ ± 1 ℃ and ≥ 90% relative humidity for 24 hours. Following the timed exposure, the virus was eluted from the surfaces, diluted, and plated onto susceptible cells. Three replicates of the test and control were performed. Log_{10} reductions were determined following the timed exposure. The viral titers were determined using a 50% tissue culture infectious dose ($TCID_{50}$) calculation -- the Quantal test (Spearman-Kärber Method). All testing was performed in accordance with Good Laboratory Practices, as specified in 21 CFR Part 58.

SUMMARY OF RESULTS			
Test Article Designation	**Average Log_{10} Reduction/cm^2**	**Standard Error of Log_{10} Reduction (K_R)**	**Percent Reduction**
AGS-WB (Lot #1)	≥3.83	0.41	≥99.99%

STUDY CONCLUSIONS

Under conditions of this evaluation, the Test Article AGS-WB (Lot #1), reduced infectivity of SARS-CoV-2 strain USA-WA1-2020 (BEI Resources #NR-52281) by ≥3.83 log_{10} (≥99.99%) following a 24-hour exposure.

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2.0 SPONSOR **NANO AND ADVANCED MATERIALS INSTITUTE LIMITED**
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Hong Kong Science Park, Hong Kong

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

The purpose of this study was to evaluate the virucidal activity of one treated test article and one untreated control article when challenged with Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2). Testing was based upon ISO 21702:2019(E) "*Measurement of antiviral activity on plastics and other non-porous surfaces*." 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 treated test articles and untreated control articles remained the responsibility of the sponsor and was not performed by the testing facility (GLP 58.105 and GLP 58.113).

6.0 SCOPE

This study was designed to evaluate the virucidal properties of one treated surface. The virucidal efficacy of the test surface was compared with that of the untreated control surface. The test virus was inoculated onto the test and control surface and incubated at 25°C ± 1°C and ≥ 90% relative humidity. Following the timed contact, the virus was eluted from the surfaces, diluted, and plated onto susceptible cells. Three replicates of the test and control were performed. Log_{10} reductions were determined following the timed contact. Plating was performed in four replicates. The viral titers were determined using a 50% tissue culture infectious dose ($TCID_{50}$) calculation -- the Quantal test (Spearman-Kärber Method).

The protocol, included as addendum to this final report, presents the study methodology in detail. No deviations from the methodology described in the protocol and from applicable Nelson Laboratories Bozeman, LLC, Standard Operating Procedures occurred during this evaluation.

7.0 JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM

The sponsor requested an antimicrobial surface test per ISO 21702:2019(E) against SARS-CoV-2.

8.0 STUDY DATES

STUDY INITIATION DATE	27 APR 2023
EXPERIMENTAL START DATE	27 APR 2023
EXPERIMENTAL END DATE	10 MAY 2023
STUDY COMPLETION DATE	23 MAY 2023

9.0 TEST ARTICLES

The test article evaluated was provided to the testing facility by the sponsor. Responsibility for determination of the identity, strength, purity, composition, 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:	AGS-WB
Active Ingredient:	quaternary ammonium cations
Lot Number:	1
Manufacture Date:	10 Mar 2023
Expiration Date:	10 Mar 2026

Control Article:	Inert Glass Plate, 2" x 2"
Active Ingredient:	N/A
Cat. Number:	Flinn Scientific AP8263
Manufacture Date:	N/A
Expiration Date:	N/A

10.0 CHALLENGE VIRAL STRAIN

SARS-CoV-2 strain USA-WA1/2020 (BEI Resources #NR-52281)

BEI Resources = Biological and Emerging Infections Resources Program (BEI Resources) National Institute of Allergy and Infectious Diseases (NIAID)

11.0 HOST CELLS

Vero E6 (ATCC #CRL-1586; green monkey kidney cells, epithelial)

ATCC = American Type Culture Collection

12.0 TEST CONDITIONS:

Exposure Time:	24 hours (23 hours to 24 hours)
Exposure Temperature:	25°C ± 1°C
Exposure Relative Humidity (RH):	≥ 90%
Neutralizing Solution:	Growth Medium
Organic Soil Load:	None

13.0 EQUIPMENT

13.1 Ultralow temperature freezer, temperature range ≤ -70°C
13.2 CO_2 incubator, temperature range 37 °C ± 2 °C
13.3 Incubator, temperature range 25 °C ± 1 °C
13.4 Incubator thermometers
13.5 Continuously adjustable pipettes, 100 µL – 1000 µL capacity
13.6 Continuously adjustable pipettes, 20 µL – 200 µL capacity
13.7 Portable pipetter
13.8 Inverted compound microscope
13.9 Laminar Flow Biological Safety Cabinet
13.10 Calibrated minute/second timers
13.11 NIST traceable clock

13.12 Microman® positive displacement pipettors, 10 µL - 100 µL capacity
13.13 Microman® positive displacement pipettors, 100 µL - 1000 µL capacity
13.14 Hygrometer

14.0 SUPPLIES

14.1 Personal protective equipment
14.2 Inert Cover film (4 cm x 4 cm squares cut from Stomacher bag)
14.3 Petri plates
14.4 Sterile Disposable Pipettes
14.5 Sterile polystyrene test tubes
14.6 Sterile universal 1.0 and 0.2 mL pipette tips
14.7 Powder-free gloves
14.8 Sterile tissue culture treated multi-well plates
14.9 Viral suspension
14.10 Sterile 100 µL and 1000 µL positive displacement Tips
14.11 Sterile flasks
14.12 Sterile 50 mL centrifuge tubes
14.13 Sterile reservoirs
14.14 Waste pan

15.0 MEDIA

15.1 1X Eagle's Minimum Essential Medium (EMEM)
15.2 Growth Medium (GM): EMEM with 10% Fetal Bovine Serum (FBS) and 1% antibiotic/antimycotic
15.3 Maintenance Medium (MM): EMEM with 2% FBS and 1% antibiotic/antimycotic
15.4 Trypsin-EDTA
15.5 Antibiotics (e.g., Penicillin-Streptomycin-Amphotericin B)
15.6 Fetal Bovine Serum (FBS)

16.0 HOST CELL PREPARATION

Vero E6 cells were obtained from American Type Culture Collection (ATCC) and were maintained as monolayers in disposable cell culture labware in accordance with Work Instruction WI0465, "*Procedure for Subculturing of Cells*." Prior to testing, host cell cultures were seeded onto multi-well cell culture treated plates. Cell monolayers were 90% confluent and less than 48 hours old before use in testing.

17.0 TEST VIRUS PREPARATION

The virus was propagated and stored per Standard Operating Procedure SOP0787, "*Procedure for Production of High-Titered Virus Stock,"* was used for this study. On the day of use, aliquots of a stock virus suspension were removed from a -70 °C freezer and quickly thawed for use in testing. The virus initial population titer was 6.50 $\log_{10}$ $TCID_{50}$/mL, meeting the acceptance criteria.

18.0 TEST VIRUS IDENTIFICATION

Virus specific cytopathic effect in a cell culture susceptible to the virus: SARS-CoV-2 causes cytopathic effect in Vero E6 cells, such as cell rounding and sloughing.

19.0 TEST ARTICLE PREPARATION

Test articles were non-porous carriers treated with antimicrobial substance (test) and untreated (control). The articles were square carriers 50 ± 2 mm x 50 ± 2 mm, and the control article was a square inert glass carrier approximately 50 ± 2 mm x 50 ± 2 mm. The carriers were exposed to UV light in a laminar flow biological safety cabinet for 30 minutes before use in testing.

20.0 CONTAMINATION OF TEST ARTICLE

20.1 The virus from a Nelson Laboratories Bozeman, LLC high-titer virus collection was used for the study to simulate viral contamination.

20.2 Test and control carriers were placed in Petri plates.

20.3 A 0.4 mL aliquot of inoculum was transferred to the surface of the test and control surfaces. The test inoculum was covered with a piece of inert film that measured 40 mm ± 2 mm x 40 mm ± 2 mm. The inert film was gently pressed down so that the test inoculum spread to the edges, making sure the inoculum did not leak beyond the edges of the film. The exposure time commenced following film application.

21.0 TEST PROCEDURE

21.1 *Test.* Three test carriers were inoculated with the virus and exposed for the contact time and conditions as specified in Section 12.0. After the contact time elapsed, the virus was eluted from the surfaces with 10 mL of neutralizing/elution solution by pipetting the neutralizing solution at least four times. The eluate was transferred to a dilution reservoir. Subsequent 10-fold dilutions were made in MM and plated in 4 replicates.

21.2 *Control Carriers.* Three control carriers were inoculated with the virus and exposed for the contact time and conditions as specified in Section 12.0. After the contact time elapsed, the virus was eluted from the surfaces with 10 mL of neutralizing/elution solution by pipetting the neutralizing solution at least 4 times. The eluate was transferred to a dilution reservoir. Subsequent 10-fold dilutions were made in MM and plated in 4 replicates.

21.3 *Input Control.* Three control carriers were inoculated with the virus and eluted immediately from the surfaces with 10 mL of neutralizing/elution solution by pipetting the neutralizing solution at least four times. The eluate was transferred to a dilution reservoir and subsequent 10-fold dilutions were made in MM and plated in 4 replicates.

21.4 *Neutralization Verification.* Three test carriers and three control carriers were washed with 10 mL of neutralizing/elution solution by pipetting the neutralizing solution at least four times. The 4.5 mL of each eluate was transferred to a tube. A 0.5 mL aliquot of test virus was transferred to the eluates and exposed for 30 minutes ± 1 minute at 25 ℃ ± 1 ℃. Subsequent 10-fold dilutions were made in MM and plated in 4 replicates.

21.5 *Virus Control.* Virus was transferred to a neutralizing solution and exposed for 30 minutes ± 1 minute at 25 ℃ ± 1 ℃. After the contact time elapsed, subsequent 10-fold dilutions were made in MM and plated in 4 replicates.

21.6 *Cytotoxicity Control.* Three test carriers and three control carriers were washed with 10 mL of neutralizing/elution solution by pipetting the neutralizing solution at least four times. Each eluate was transferred to a dilution reservoir. Subsequent 10-fold dilutions were made in MM and plated in 4 replicates.

21.7 *Initial Population.* The test virus was diluted in MM. Dilutions were plated in 4 replicates.

21.8 *Cell Culture Control.* Intact cell culture monolayers served as the control of cell culture viability. The growth medium was replaced by MM in all cell culture control wells (minimum 4 wells).

21.9 The plates were incubated for 13 days at 37 °C ± 2 °C in a CO_2 incubator.

22.0 CALCULATIONS

22.1 Viral and toxicity titers were expressed as $-\log_{10}$ of the 50% titration end point for infectivity. To calculate the viral titer, a 50% tissue culture infectious dose ($TCID_{50}$) calculation -- the Quantal test (Spearman-Kärber Method) -- was applied.

$$\text{Log } TCID_{50} = L - d\,(s - 0.5)$$

Where:

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

$TCID_{50}$ was calculated per plating volume and per volume of inoculum and the film square area:

$TCID_{50}$/plating volume x 0.4 mL (volume of inoculum)
$TCID_{50}$/volume of inoculum / 16 cm^2 (square area of inoculation) = $TCID_{50}/cm^2$

22.2 The reduction of virus population (antiviral activity) was calculated as follows:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

Where:

R	=	Reduction (the antiviral activity)
U_0	=	Average of the $\log_{10}$ $TCID_{50}$ recovered from the Input Control, in $TCID_{50}/cm^2$
U_t	=	Average of the $\log_{10}$ $TCID_{50}$ recovered from the Control Test samples, in $TCID_{50}/cm^2$
A_t	=	$\log_{10}$ $TCID_{50}$ recovered from the test, in $TCID_{50}/cm^2$

22.3 The 95% Confidence Intervals for virus titer was calculated as follows:

The standard error (S_E) from virus titer was calculated:

$$S_E = \sqrt{d^2 \sum \{[p_i\,(1 - p_i)]/(n - 1)\}}$$

Where:

S_E	=	standard error of logarithmic titer
d	=	logarithm of dilution factor
p_i	=	observed reaction rate
n	=	number of test objects per dilution

22.4 The 95% Confidence Intervals for the log reductions were calculated as follows:

$$K_R = 2\sqrt{S_{EVC}^2 + S_{ET}^2}$$

Where:

K_R	=	standard error of log reduction
S_{EVC}	=	standard error of virus control logarithmic titer
S_{ET}	=	standard error of test logarithmic titer

22.5 If no virus was recovered from the test samples the following equation was used:

$$K_R = 2\sqrt{S_{EVC}^2}$$

95% confidence interval of the titer is approximately 2 S_E.

22.6 The 95% confidence interval of the average reduction R ($K_{R(mi)}$) was calculated as follows:

$$K_{R(mi)} = \sqrt{(K_{R(T1)}^2 + K_{R(T2)}^2 + K_{R(T3)}^2)/3}$$

Where:

$K_{R(mi)}$	=	95 % confidence interval of the average reduction
$K_{R(T1)}$	=	95 % confidence interval of the reduction of the first test replicate
$K_{R(T2)}$	=	95 % confidence interval of the reduction of the second test replicate
$K_{R(T3)}$	=	95 % confidence interval of the reduction of the third test replicate

22.7 Average percent reduction presented in the Executive Summary:

$$\text{Average reduction, \%} = 100 - \left[\left(\frac{1}{\text{TCID}_{50}\ Average\ Reduction}\right) \times 100\right]$$

23.0 TEST ACCEPTANCE CRITERIA

The following test acceptance criteria were met:

1) 6.50 $\log_{10}$ to 8.00 $\log_{10}$ $TCID_{50}$/mL of virus was recovered from initial population (actual 6.50 $\log_{10}$ $TCID_{50}$/mL).

2) The logarithmic value of the virus recovered from the input control satisfied the requirement of: L_{max} - $L_{min)}/L_{mean} \leq 0.2$ where, L_{max} maximum number virus recovered, L_{min} minimum number of virus recovered, where L_{mean} mean of three replicates (actual value was 0.12).

3) $TCID_{50}$ 5.0 $\log_{10}$/mL to 6.50 $\log_{10}$/mL was recovered from the input control (actual recovery was 6.08 $\log_{10}$/mL, reference Table 2).

4) At least 2.95 $\log_{10}/cm^2$ was recovered from control carriers.

5) The activity of the test articles was fully neutralized, so the difference between the virus titer in virus control and the neutralization verification for test and control carriers did not exceed 0.5 $\log_{10}$

6) Cells in the cell control wells were viable and attached to the bottom of the well.

7) The medium was free of contamination in all wells of the plate.

24.0 RESULTS (TABLE 1)

24.1 Table 1 presents the data from the test article and untreated control article post-exposure infectivity ($TCID_{50}$), the log_{10} reductions observed following a 24-hour exposure to SARS-CoV-2.

TABLE 1: Test Article: AGS-WB (Lot #1)
Control Article: Inert Glass Plate, 2" x 2"
SARS-CoV-2 strain USA-WA1/2020 (BEI Resources #NR-52281)

Designation	Rep.	Dilutions, log_{10}						$TCID_{50}$, log_{10}/mL	$TCID_{50}$, log_{10}/cm^2	*SE*	Log_{10} Reduction/ cm^2	K_R
		-2	-3	-4	-5	-6	-7					
Test Article	1	0000	0000	0000	0000	0000	0000	≤1.50	≤-0.10	0.00	≥3.83	0.41
	2	0000	0000	0000	0000	0000	0000	≤1.50	≤-0.10	0.00	≥3.83	0.41
	3	0000	0000	0000	0000	0000	0000	≤1.50	≤-0.10	0.00	≥3.83	0.41
							Average:	≤1.50	≤-0.10	0.00	≥3.83	0.41
Control Article	1	++++	++++	++++	++++	0000	0000	5.50	3.90	0.00		
	2	++++	++++	++++	+++0	0000	0000	5.25	3.65	0.25		
	3	++++	++++	++++	+0++	0000	0000	5.25	3.65	0.25		
							Average:	5.33	3.73	0.20		
Input Control	1	++++	++++	++++	++++	+000	0000	5.75	4.15	0.25		
	2	++++	++++	++++	++++	+0++	00+0	6.50	4.90	0.35		
	3	++++	++++	++++	++++	0++0	0000	6.00	4.40	0.29		
							Average:	6.08	4.48	0.30		
NC Test Article	1	NT	++++	++++	++++	+++0	00+0	6.50		0.35		
	2	NT	++++	++++	++++	0+++	0000	6.25		0.25		
	3	NT	++++	++++	++++	++0+	0000	6.25		0.25		
							Average:	6.33		0.29		
NC Untreated Control	1	NT	++++	++++	++++	+++0	0000	6.25		0.25		
	2	NT	++++	++++	++++	++0+	0000	6.25		0.25	N/A	
	3	NT	++++	++++	++++	+++0	0000	6.25		0.25		
							Average:	6.25		0.25		
CTC Test Article	1	0000	0000	0000	NT	NT	NT	≤1.50		0.00		
	2	0000	0000	0000	NT	NT	NT	≤1.50	N/A	0.00		
	3	0000	0000	0000	NT	NT	NT	≤1.50		0.00		
CTC Untreated Control	1	0000	0000	0000	NT	NT	NT	≤1.50		0.00		
	2	0000	0000	0000	NT	NT	NT	≤1.50		0.00		
	3	0000	0000	0000	NT	NT	NT	≤1.50		0.00		
Virus Control	1	NT	++++	++++	++++	0+0+	0000	6.00		0.29		
	2	NT	++++	++++	++++	+00+	0000	6.00		0.29		
	3	NT	++++	++++	++++	00++	0000	6.00		0.29		
							Average:	6.00		0.29		
IP	N/A	NT	NT	++++	++++	++++	0000	6.50		0.00		
CC	0000							N/A		N/A		

\+ Virus infected cells present
0 Virus infected cells not detected
CC Cell Control
CTC Cytotoxicity Control
IP Initial Population
NT Not tested
NC Neutralization Control
Rep. Replicate
SE Standard error of logarithmic titer
K_R Standard error of log reduction

25.0 STUDY CONCLUSION

Under the conditions of this evaluation, the Test Article AGS-WB (Lot #1), reduced the infectivity of SARS-CoV-2 strain USA-WA1-2020 (BEI Resources #NR-52281) by ≥3.83 $\log_{10}/cm^2$ (≥99.99%) following a 24-hour exposure.

26.0 STATISTICAL ANALYSIS

The Quantal test (Spearman-Kärber Method) was applied to calculate virus titer. The average of virus $TCID_{50}$ recoveries for test and control replicates and virus reductions were calculated and presented with standard error. No control of bias was performed.

27.0 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. On completion of testing, QA performed an audit of the raw data and the final report, in its entirety.

28.0 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 in Quality Assurance 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

Jeanine Allen, Ph.D.
Kelly Burningham
Jaana Goff
Cecelia K. McAfee
Amelia Smith

29.0 QUALITY ASSURANCE AND QUALITY CONTROL PERSONNEL

Kevin Crawford
Maintenance Technician

Jeremy Duley
Maintenance Manager

Kathryn Elkins
Quality Assurance Specialist

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

30.0 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.

31.0 ACCEPTANCE

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

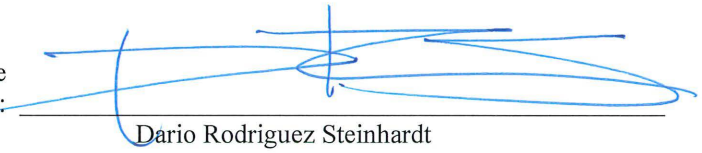
Study Director: Mani Mohan — 23 MAY 2023
Date of Study Completion

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
Product Testing	27 APR 2023 28 APR 2023	01 MAY 2023	22 MAY 2023
Data Audit	22 MAY 2023	22 MAY 2023	22 MAY 2023
Final Report Review	22 MAY 2023	22 MAY 2023	23 MAY 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 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 Specialist:  — 23 May 2023
Dario Rodriguez Steinhardt — Date

ADDENDUM

Protocol #2303763-408A

Nelson Labs
A Sotera Health company

20 APR 2023

PROTOCOL #2303763-408A

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Prepared by:

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PROTOCOL #2303763-408A

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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 study is to evaluate the virucidal activity of one treated test article and one untreated control article when challenged with Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2). Testing will be based upon ISO 21702:2019(E) "Measurement of antiviral activity on plastics and other non-porous surfaces." 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).

6.0 SCOPE

This study is designed to evaluate the virucidal property of one treated surface. The virucidal efficacy of the test surface will be compared with that of the untreated control surface. The test virus will be inoculated onto the test and control surface and incubated at 25°C ± 1°C and ≥90% relative humidity. Following the timed exposure, the virus will be eluted from the surfaces, diluted and plated onto susceptible cells. Three replicates of the test and control will be performed. Log_{10} reductions will be determined following timed contact. Plating will be performed in four replicates. The viral titers will be determined using a 50% tissue culture infectious dose (TCID50) calculation -- the Quantal test (Spearman-Kärber Method). Modifications from ISO 21702:2019(E) are listed in the Appendix to this protocol.

7.0 JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM

The sponsor requested an antimicrobial surface test per ISO 21702:2019(E) against SARS-CoV-2.

8.0 TEST ARTICLES

The test and control articles to be evaluated will be provided to the testing facility by the sponsor, complete with appropriate documentation. Certificates of analysis were not provided to the testing facility. Responsibility for the determination of the identity, strength, purity, composition, and stability of the test material, as well as the retention of the test material, rests with the sponsor. The test articles will be evaluated as received from the sponsor.

Test Article:	AGS-WB
Active Ingredients:	quaternary ammonium cations
Lot Number:	1
Manufacture Date:	10 Mar 2023
Expiration Date:	10 Mar 2026
Untreated Control Article:	Inert Glass Plate, 2" x 2"
Active Ingredients:	N/A
Cat. Number:	Flinn Scientific AP8263
Expiration Date:	N/A
Manufacture Date:	N/A

9.0 TEST CONDITIONS

Contact Time:	24 hours (23 hours to 24 hours)
Contact Temperature:	25°C ± 1°C
Contact Relative Humidity (RH):	≥90%
Neutralizing/Elution Solution:	Growth Medium
Organic Soil Load:	None

10.0 CHALLENGE VIRAL STRAIN(S)

SARS-CoV-2 strain USA-WA1/2020 (BEI Resources #NR52281)

BEI Resources = Biological and Emerging Infections Resources Program (BEI Resources) National Institute of Allergy and Infectious Diseases (NIAID).

11.0 HOST CELLS

Vero E6 (ATCC #CRL-1586; green monkey kidney cells, epithelial)
ATCC = American Type Culture Collection

12.0 MATERIALS

The tables below contain a general list of equipment, supplies, and media use, although each global lab location may have varying materials to perform testing. Applicable materials used will be documented in the raw data as necessary.

TABLE 1 EQUIPMENT
• Ultralow temperature freezer, temperature range ≤ -70°C
• CO_2 incubator, temperature range 37 °C ± 2 °C
• Incubator, temperature range 25 °C ± 1 °C
• Incubator thermometers
• Continuously adjustable pipettes, 100 µL – 1000 µL capacity
• Continuously adjustable pipettes, 20 µL – 200 µL capacity
• Portable pipetter
• Inverted compound microscope
• Laminar flow biological safety cabinet
• Calibrated minute/second timers
•NIST traceable clock
•Microman® positive displacement pipetters, 10 µL - 100 µL capacity
• Microman® positive displacement pipetters, 100 µL - 1000 µL capacity
• Hygrometer

TABLE 2 SUPPLIES
• Personal protective equipment
• Inert cover film (4 cm x 4 cm squares cut form stomacher bag)
• Petri plates
• Sterile disposable pipettes
• Sterile polystyrene test tubes
• Sterile universal 1.0 and 0.2 mL pipette tips
• Powder-free gloves
• Sterile tissue culture treated multi-well plates
• Viral suspension
• Sterile 100 µL and 1000 µL positive displacement Tips
• Sterile flasks
• Sterile 50 mL centrifuge tubes
• Sterile reservoirs
• Waste pan

TABLE 3 MEDIA
• 1X Eagle's Minimum Essential Medium (EMEM) or other appropriate medium
• Growth Medium (GM): EMEM or other media with 4-10% Serum and 1% Antibiotic/Antimycotic and 1% L-glutamine (when necessary)
• Maintenance Medium (MM): EMEM or other media with 2% Serum, 1% Antibiotic/Antimycotic and 1% L-glutamine (when necessary)
• Trypsin/EDTA
• Antibiotic/Antimycotic (e.g., Penicillin-Streptomycin-Amphotericin B [10,000 units/mL of Penicillin, 10,000 µg/mL of Streptomycin, and 25 µg/mL Amphotericin B])
• Fetal Bovine Serum (FBS), Horse Serum (HS) or other appropriate serum
• Appropriate neutralizer (e.g. Dey-Engley [D/E] Neutralizing Broth)
• Other media, and buffers as deemed appropriate by the study director.

13.0 HOST CELL PREPARATION

Cells will be maintained as monolayers in disposable cell culture labware per Work Instruction WI0465, *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 less than 48 hours old before use

in testing. Growth medium (GM) and maintenance Medium (MM) will be MEM or other media with appropriate supplements as described in 12.0.

14.0 TEST VIRUS PREPARATION

The virus suspension(s) will originate from high titer virus stock, propagated and stored per Standard Operating Procedure SOP0787, *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. The virus will be from 6.50 $\log_{10}$ to 8.00 $\log_{10}$ $TCID_{50}$/mL.

Note: Requirements for virus titer are converted from plaque forming units/mL (PFU/mL) to 50% tissue culture infectious dose ($TCID_{50}$/mL) using the equation described in www.atcc.org (PFU/mL=$TCID_{50}$/mL*0.7).

15.0 TEST VIRUS IDENTIFICATION

Virus specific cytopathic effect in a cell culture susceptible to the virus: SARS-CoV2 causes cytopathic effect in Vero E6 cells, such as cell rounding and sloughing.

16.0 TEST ARTICLE PREPARATION

Test articles will be non-porous carriers treated with antimicrobial substance (test) and untreated (control). Test and control articles will be provided by the sponsor. The articles will be square carriers 50 ± 2 mm x 50 ± 2 mm. The carriers will be exposed to UV light in a laminar flow biological safety cabinet for 30 minutes before use in testing.

17.0 CONTAMINATION OF TEST ARTICLES

17.1 The virus from Nelson Laboratories Bozeman, LLC high-titer virus collection will be used for the study to simulate viral contamination.

17.2 Test and control carriers will be placed in a Petri plate. The standard volume, 0.4 mL of test inoculum and the size of the cover film, 40 ± 2 mm x 40 ± 2 mm for the 50 ± 2 mm x 50 ± 2 mm test specimen, shall be adjusted in direct proportion and included in the final report.

17.3 An aliquot of inoculum approximately 6.5 $\log_{10}$ to 8.0 $\log_{10}$ $TCID_{50}$/mL will be transferred to the surface of the test and control articles and covered with an inert film with a surface area between 400 mm^2 and 1600 mm^2. Gentle pressure on the film will be applied so that the test inoculum spreads to the edges, making sure inoculum does not leak beyond the edges of the film. The time will commence following film application.

18.0 EVALUATION OF TEST ARTICLE

18.1 *Test.* Three test carriers will be inoculated with the virus(s) and exposed for the contact times and conditions as specified in Section 9.0. After the contact time has elapsed, the virus(s) will be eluted from the surfaces with 10 mL of neutralizing/elution solution by pipetting the neutralizing solution at least four times. The eluate will be transferred to a tube or dilution reservoir and subsequent 10-fold dilutions will be made in MM and plated in 4 replicates.

18.2 *Control Carriers.* Three control carriers will be inoculated with the virus(s) and exposed for the contact times and conditions as specified in Section 9.0. After the contact time has elapsed, the virus will be eluted from the surfaces with 10 mL of neutralizing/elution solution by pipetting the neutralizing solution at least four times. The eluate will be transferred to a tube or dilution reservoir and subsequent 10-fold dilutions will be made in MM and plated in 4 replicates.

18.3 *Input Control.* Three control carriers will be inoculated with the virus and eluted immediately from the surfaces with 10 mL of neutralizing/elution solution by pipetting the neutralizing solution at least four times. The eluate will be transferred to a tube or dilution reservoir and subsequent 10-fold dilutions will be made in MM and plated in 4 replicates.

18.4 *Neutralization Verification.* Three test carriers and three control carriers will be washed with 10 mL of neutralizing/elution solution by pipetting the neutralizing solution at least four times. The 4.5 mL of each eluate will be transferred to a tube or dilution reservoir. A 0.5 mL aliquot of the test virus will be transferred to the eluates and exposed for 30 minutes ± 1 minute at 25 °C ± 1 °C. Following contact, subsequent 10-fold dilutions will be made in MM and plated in 4 replicates.

18.5 *Virus Control.* Virus will be transferred to a neutralizing solution and exposed for 30 minutes ± 1 minute at 25 °C ± 1 °C. After the contact time has elapsed, subsequent 10-fold dilutions will be made in MM and plated in 4 replicates.

18.6 *Cytotoxicity Control.* Three test carriers and three control carriers will be washed with 10 mL of neutralizing/elution solution by pipetting the neutralizing solution at least 4 times. Each eluate will be transferred to a tube or dilution reservoir. Subsequent 10-fold dilutions will be made in MM and plated in 4 replicates.

18.7 *Initial Population.* The test virus will be diluted in MM. Dilutions will be plated in 4 replicates.

18.8 *Cell Culture Control.* Intact cell culture monolayers will serve as the control of cell culture viability. The growth medium will be replaced by MM in all cell culture control wells (minimum 4 wells).

18.9 The plates will be incubated for 5 to 20 days in a CO_2 incubator at 37 °C ± 2 °C.

18.10 *Evaluation of Virus Recovery.* Cytopathic/cytotoxic effect will be monitored using an inverted compound microscope.

19.0 CALCULATIONS

19.1 Viral and toxicity 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 ($TCID_{50}$) calculation -- the Quantal test (Spearman-Kärber Method) -- will be applied.

$$\text{Log } TCID_{50} = L - d\,(s - 0.5)$$

Where:

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

$TCID_{50}$ will be calculated per plating volume and per volume of inoculum and the film square area:

$TCID_{50}$/plating volume x 0.4 mL (volume of inoculum)
$TCID_{50}$/volume of inoculum / 16 cm^2 (square area of inoculation) = $TCID_{50}/cm^2$

19.2 The reduction of virus population (antiviral activity) will be calculated as follows:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

Where,
R – Reduction (the antiviral activity);
U_0 – the average of the $\log_{10}$ $TCID_{50}$ recovered from the Input Control, in $TCID_{50}/cm^2$;

U_t – the average of the $\log_{10}$ $TCID_{50}$ recovered from the Control Test samples, in $TCID_{50}/cm^2$;
A_t – the average of the $\log_{10}$ $TCID_{50}$ recovered from the test, in $TCID_{50}/cm^2$

19.3 The 95% Confidence Intervals for virus titer will be calculated as follows:

The standard error (S_E) from virus titer will be calculated:

$$S_E = \sqrt{d^2 \sum \{[p_i\,(1 - p_i)]/(n - 1)\}}$$

Where:

S_E	=	standard error of logarithmic titer
d	=	logarithm of dilution factor
p_i	=	observed reaction rate
n	=	number of test objects per dilution

19.4 The 95% confidence intervals for the log reductions will be calculated as follows:

$$K_R = 2\sqrt{S_{EC}^2 + S_{ET}^2}$$

Where:

K_R	=	standard error of log reduction
S_{EC}	=	standard error of control logarithmic titer
S_{ET}	=	standard error of test logarithmic titer

19.5 If no virus is recovered from the test samples the following equation will be used:

$$K_R = 2\sqrt{S_{EC}^2}$$

95 % confidence interval of the titer is approximately 2 S_E.

19.6 The 95% confidence interval of the average reduction R ($K_{R(mi)}$) will be calculated as follows:

$$K_{R(mi)} = \sqrt{(K_{R(T1)}^2 + K_{R(T2)}^2 + K_{R(T3)}^2)/3}$$

Where:

$K_{R(mi)}$	=	95 % confidence interval of the average reduction
$K_{R(T1)}$	=	95 % confidence interval of the reduction of the first test replicate
$K_{R(T2)}$	=	95 % confidence interval of the reduction of the second test replicate
$K_{R(T3)}$	=	95 % confidence interval of the reduction of the third test replicate

20.0 TEST ACCEPTANCE CRITERIA

A valid test requires:

20.1 6.50 $\log_{10}$ to 8.00 $\log_{10}$ $TCID_{50}$/mL of virus is recovered from initial population;
20.2 The logarithmic value of the virus recovered from the input control shall satisfy the requirement of: $(L_{max} - L_{min})/L_{mean} \leq 0.2$ (where, L_{max} maximum number virus recovered, L_{min} minimum number of virus recovered, L_{mean} mean of three replicates);
20.3 $TCID_{50}$ 5.0 $\log_{10}$/mL to 6.50/mL is recovered from the input control;
20.4 At least 2.95 $\log_{10}/cm^2$ recovered from the control carriers;
20.5 Activity of the test articles is fully neutralized, so the difference between the virus titer in the virus control and the neutralization verification for test and control carriers does not exceed 0.5 $\log_{10}$;
20.6 Cells in the cell control wells are viable and attached to the bottom of the well;
20.7 Medium is free of contamination in all wells of the plate.

21.0 STATISTICAL ANALYSIS

The Quantal test (Spearman-Kärber Method) will be applied to calculate virus titer. The average of virus $TCID_{50}$ recoveries for test and control replicates and virus reductions will be calculated and presented with standard error. No control of bias will be performed.

22.0 FINAL REPORT

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

25.0 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.

26.0 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 product for use as defined in the protocol.

27.0 PRODUCT DISPOSITION

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

28.0 REFERENCE

ISO 21702:2019(E), *Measurement of antiviral activity on plastics and other non-porous surfaces.*

29.0 ACCEPTANCE

AN EVALUATION OF THE VIRUCIDAL PROPERTIES OF TREATED NON-POROUS MATERIALS BASED UPON ISO 21702:2019(E)

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

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

27 APR 2023 — Date of Study Initiation

ACCEPTED BY: NANO AND ADVANCED MATERIALS INSTITUTE LIMITED (SPONSOR)
Unit 608-609, Lakeside 2
No. 10 Science Park West Avenue
Hong Kong Science Park, Hong Kong

Ng Chi Sze — Representative

26 April 2023 — Date

Senior Engineer — Title

APPENDIX A

SUMMARY OF MODIFICATION FROM ISO 21702:2019(E)

The following are modifications from procedures defined in ISO 21702:2019(E).

1) Virus not specified in the standard method will be used in testing. The acceptance criteria has been modified for the non-method virus tested including the following:

    a. Cell and virus culture media.

    b. The virus concentration (measured in $TCID_{50}$ log_{10}/mL) of the initial population.

    c. The average virus recovery (measured in $TCID_{50}$ log_{10}/mL) from the input control.

2) Virus recovered from each untreated control article after the contact time may be less than $TCID_{50}$ 2.95 log_{10}/cm^2, due to the achievable titer of the virus stock.