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23 MAY 2023

FINAL REPORT #2303763-408B

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

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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:

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## EXECUTIVE SUMMARY

**STUDY NUMBER** 2303763-408B

**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 °C ± 1 °C 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<sub>10</sub> reductions were determined following the timed exposure. The viral titers were determined using a 50% tissue culture infectious dose (TCID<sub>50</sub>) 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 <sub>10</sub> Reduction/cm <sup>2</sup>	Standard Error of Log <sub>10</sub> Reduction (K <sub>R</sub> )	Percent Reduction
AGS-WB-side2 (Lot #1)	≥3.83	0.41	≥99.99%

## STUDY CONCLUSIONS

Under conditions of this evaluation, the Test Article AGS-WB-side2 (Lot #1), reduced infectivity of SARS-CoV-2 strain USA-WA1-2020 (BEI Resources #NR-52281) by ≥3.83 log<sub>10</sub> (≥99.99%) following a 24-hour exposure.

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- 2.0 SPONSOR** NANO AND ADVANCED MATERIALS INSTITUTE LIMITED  
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No. 10 Science Park West Avenue  
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<sub>10</sub> 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<sub>50</sub>) 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**

<b>STUDY INITIATION DATE</b>	27 APR 2023
<b>EXPERIMENTAL START DATE</b>	27 APR 2023
<b>EXPERIMENTAL END DATE</b>	10 MAY 2023
<b>STUDY COMPLETION DATE</b>	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-side2  
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<sub>2</sub> 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 pipettor
- 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<sub>10</sub> TCID<sub>50</sub>/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 °C ± 1 °C. 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 °C ± 1 °C. 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<sub>2</sub> 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<sub>50</sub>) calculation -- the Quantal test (Spearman-Kärber Method) -- was applied.

$$\text{Log TCID}_{50} = L - d (s - 0.5)$$

Where:

L = - log<sub>10</sub> of the lowest dilution;  
d = difference between dilution steps;  
s = sum of proportions of positive wells.

TCID<sub>50</sub> was calculated per plating volume and per volume of inoculum and the film square area:

$$\text{TCID}_{50}/\text{plating volume} \times 0.4 \text{ mL (volume of inoculum)}$$

$$\text{TCID}_{50}/\text{volume of inoculum} / 16 \text{ cm}^2 \text{ (square area of inoculation)} = \text{TCID}_{50}/\text{cm}^2$$

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<sub>0</sub> = Average of the log<sub>10</sub> TCID<sub>50</sub> recovered from the Input Control, in TCID<sub>50</sub>/cm<sup>2</sup>  
U<sub>t</sub> = Average of the log<sub>10</sub> TCID<sub>50</sub> recovered from the Control Test samples, in TCID<sub>50</sub>/cm<sup>2</sup>  
A<sub>t</sub> = log<sub>10</sub> TCID<sub>50</sub> recovered from the test, in TCID<sub>50</sub>/cm<sup>2</sup>

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

The standard error (*S<sub>E</sub>*) from virus titer was calculated:

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

Where:

*S<sub>E</sub>* = standard error of logarithmic titer  
*d* = logarithm of dilution factor  
*p<sub>i</sub>* = 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<sub>R</sub>* = standard error of log reduction  
*S<sub>EVC</sub>* = standard error of virus control logarithmic titer  
*S<sub>ET</sub>* = 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<sub>E</sub>*.

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:

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

### 23.0 **TEST ACCEPTANCE CRITERIA**

The following test acceptance criteria were met:

- 1) 6.50 log<sub>10</sub> to 8.00 log<sub>10</sub> TCID<sub>50</sub>/mL of virus was recovered from initial population (actual 6.50 log<sub>10</sub> TCID<sub>50</sub>/mL).
- 2) The logarithmic value of the virus recovered from the input control satisfied the requirement of:  $L_{\max} - L_{\min}/L_{\text{mean}} \leq 0.2$  where,  $L_{\max}$  maximum number virus recovered,  $L_{\min}$  minimum number of virus recovered, where  $L_{\text{mean}}$  mean of three replicates (actual value was 0.12).
- 3) TCID<sub>50</sub> 5.0 log<sub>10</sub>/mL to 6.50 log<sub>10</sub>/mL was recovered from the input control (actual recovery was 6.08 log<sub>10</sub>/mL, reference Table 2).
- 4) At least 2.95 log<sub>10</sub>/cm<sup>2</sup> 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<sub>10</sub>
- 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<sub>50</sub>), the log<sub>10</sub> reductions observed following a 24-hour exposure to SARS-CoV-2.

<b>TABLE 1: Test Article: AGS-WB-side2 (Lot #1)</b> <b>Control Article: Inert Glass Plate, 2" x 2"</b> <b>SARS-CoV-2 strain USA-WA1/2020 (BEI Resources #NR-52281)</b>													
Designation	Rep.	Dilutions, log <sub>10</sub>						TCID <sub>50</sub> , log <sub>10</sub> /mL	TCID <sub>50</sub> , log <sub>10</sub> /cm <sup>2</sup>	SE	Log <sub>10</sub> Reduction/cm <sup>2</sup>	K <sub>R</sub>	
		-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	++++	++++	++++	+0++	0000	0000	5.25	3.65	0.25	N/A		
	2	++++	++++	++++	+++0	0000	0000	5.25	3.65	0.25			
	3	++++	++++	++++	++++	0000	0000	5.50	3.90	0.00			
	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	0000	6.25	N/A	0.25			
	2	NT	++++	++++	++++	++00	0000	6.00		0.29			
	3	NT	++++	++++	++++	+0++	0000	6.25		0.25			
	Average:							6.17		0.26			
NC Untreated Control	1	NT	++++	++++	++++	+++0	0000	6.25		0.25			
	2	NT	++++	++++	++++	++0+	0000	6.25		0.25			
	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		N/A			0.00
	2	0000	0000	0000	NT	NT	NT	≤1.50					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<sub>R</sub> Standard error of log reduction



### **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 Uchida

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	01 MAY 2023
Data Audit	22 MAY 2023	22 MAY 2023	22 MAY 2023
Final Report Review	22 MAY 2023	22 MAY 2023	22 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:

Dario Rodriguez Steinhardt

23 May 2023  
Date

**ADDENDUM**

Protocol #2303763-408B



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20 APR 2023

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20 APR 2023

PROTOCOL #2303763-408B

- 1.0**    **TITLE**                    **AN EVALUATION OF THE VIRUCIDAL PROPERTIES OF TREATED NON-POROUS MATERIALS BASED UPON ISO 21702:2019(E)**
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- 3.0**    **TESTING FACILITY**        **NELSON LABORATORIES BOZEMAN, LLC**  
1755 South 19<sup>th</sup> 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<sub>10</sub> 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 (TCID<sub>50</sub>) 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-side2  
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  $\leq -70^{\circ}\text{C}$
- $\text{CO}_2$  incubator, temperature range  $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$
- Incubator, temperature range  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$
- Incubator thermometers
- Continuously adjustable pipettes, 100  $\mu\text{L}$  – 1000  $\mu\text{L}$  capacity
- Continuously adjustable pipettes, 20  $\mu\text{L}$  – 200  $\mu\text{L}$  capacity
- Portable pipetter
- Inverted compound microscope
- Laminar flow biological safety cabinet
- Calibrated minute/second timers
- NIST traceable clock
- Microman® positive displacement pipettes, 10  $\mu\text{L}$  - 100  $\mu\text{L}$  capacity
- Microman® positive displacement pipettes, 100  $\mu\text{L}$  - 1000  $\mu\text{L}$  capacity
- Hygrometer

**TABLE 2 SUPPLIES**

- Personal protective equipment
- Inert cover film (4 cm x 4 cm squares cut from 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  $\mu\text{L}$  and 1000  $\mu\text{L}$  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  $\mu\text{g}/\text{mL}$  of Streptomycin, and 25  $\mu\text{g}/\text{mL}$  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<sub>10</sub> to 8.00 log<sub>10</sub> TCID<sub>50</sub>/mL.

**Note:** Requirements for virus titer are converted from plaque forming units/mL (PFU/mL) to 50% tissue culture infectious dose (TCID<sub>50</sub>/mL) using the equation described in [www.atcc.org](http://www.atcc.org) (PFU/mL=TCID<sub>50</sub>/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<sub>10</sub> to 8.0 log<sub>10</sub> TCID<sub>50</sub>/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<sup>2</sup> and 1600 mm<sup>2</sup>. 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  $\pm$  1 minute at 25 °C  $\pm$  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  $\pm$  1 minute at 25 °C  $\pm$  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<sub>2</sub> incubator at 37 °C  $\pm$  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<sub>50</sub>) 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<sub>50</sub> will be calculated per plating volume and per volume of inoculum and the film square area:

$$\frac{\text{TCID}_{50}/\text{plating volume} \times 0.4 \text{ mL (volume of inoculum)}}{\text{TCID}_{50}/\text{volume of inoculum} / 16 \text{ cm}^2 \text{ (square area of inoculation)}} = \text{TCID}_{50}/\text{cm}^2$$

- 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<sub>0</sub> – the average of the  $\log_{10}$  TCID<sub>50</sub> recovered from the Input Control, in TCID<sub>50</sub>/cm<sup>2</sup>;

$U_i$  – the average of the  $\log_{10}$  TCID<sub>50</sub> recovered from the Control Test samples, in TCID<sub>50</sub>/cm<sup>2</sup>;  
 $A_i$  – the average of the  $\log_{10}$  TCID<sub>50</sub> recovered from the test, in TCID<sub>50</sub>/cm<sup>2</sup>

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<sub>50</sub>/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<sub>50</sub> 5.0  $\log_{10}$ /mL to 6.50/mL is recovered from the input control;
- 20.4 At least 2.95  $\log_{10}$ /cm<sup>2</sup> 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<sub>50</sub> 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 19<sup>th</sup> Avenue  
Bozeman, Montana 59718

Study  
Director: Mauri Erickson 27 APR 2023  
Mauri Erickson, M.S. 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 Yeh Yin 26 April 2023  
Representative 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.