December 15, 2020

FINAL REPORT #2009692-404

A NON-GLP VIRUCIDAL EFFICACY EVALUATION OF ONE HARD SURFACE DISINFECTANT SUBSTANCE

Prepared for:

NANO AND ADVANCED MATERIALS INSTITUE, LTD. (SPONSOR)
Units 517-518, Lakeside 1,
No. 8 Science Park West Avenue,
Hong Kong Science Park, Shatin,
N.T., Hong Kong, China

Prepared by:

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

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EXECUTIVE SUMMARY

STUDY NUMBER:	**2009692-404**
TITLE:	**A NON-GLP VIRUCIDAL EFFICACY EVALUATION OF ONE HARD SURFACE DISINFECTANT SUBSTANCE**
SPONSOR:	**NANO AND ADVANCED MATERIALS INSTITUE, LTD.** Units 517-518, Lakeside 1, No. 8 Science Park West Avenue, Hong Kong Science Park, Shatin, N.T., Hong Kong, China
TESTING FACILITY:	**BIOSCIENCE LABORATORIES, INC.** 1755 South 19th Avenue Bozeman, Montana 59718
STUDY INITIATION DATE:	December 2, 2020
STUDY COMPLETION DATE:	December 15, 2020

This study evaluated the virucidal efficacy of one disinfectant substance when challenged with Human Coronavirus strain 229E (ATCC #VR-740). Testing was based upon methods described as specified in the American Society for Test Materials (ASTM) test methods designated E1053-20, *Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surface*. The test substance was **not** evaluated in the presence of an Organic Soil Load. A challenge suspension was used to inoculate the bottom part of 100 mm X 15 mm glass Petri Dish carriers. Non-porous glass carriers were treated using 2.0 mL of the test substance at room temperature for a 30-minute exposure time. Following the timed exposure, a neutralizing solution appropriate for test substance was added to the carriers. An aliquot of the neutralized suspension was serially diluted in medium and assayed for the presence of viable viruses using the susceptible to the virus cell culture. The viral titers were determined using a 50% tissue culture infectious dose (TCID50) calculation -- the Quantal test (Spearman-Kärber Method). All testing was **not** performed in accordance with Good Laboratory Practices, as specified in 40 CFR 160.

STUDY CONCLUSION:

Under the conditions of this evaluation, Test Substance, ABV Nano EO handrub (Lot # F33B-20201019) reduced the infectivity of Coronavirus strain 229E (ATCC #VR-740) by 3.25 $\log_{10}$ following a 30-minute exposure. The Test Substance, ABV Nano EO handrub demonstrated the required ≥ 3 $\log_{10}$ reduction of Coronavirus strain 229E on non-porous test surface.

1.0 TITLE: **A NON-GLP VIRUCIDAL EFFICACY EVALUATION OF ONE HARD SURFACE DISINFECTANT SUBSTANCE**

2.0

3.0 SPONSOR: **NANO AND ADVANCED MATERIALS INSTITUE, LTD.**
Units 517-518, Lakeside 1,
No. 8 Science Park West Avenue,
Hong Kong Science Park, Shatin,
N.T., Hong Kong, China

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

5.0 STUDY DIRECTOR: Volha Teagle, Ph.D.

6.0 PURPOSE:

The purpose of this study was to evaluate the virucidal efficacy of one disinfectant substance when challenged with Human Coronavirus strain 229E. Testing was based upon methods described as specified in the American Society for Test Materials (ASTM) test methods designated E1053-20, *Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surface.* All testing was **not** be performed in accordance with Good Laboratory Practices, as specified in 40 CFR 160.

7.0 SCOPE:

This study evaluated the virucidal efficacy of one disinfectant test substance, when used on dry, non-porous, inanimate surfaces. The test substance was evaluated versus Human Coronavirus strain 229E (ATCC #VR_740). The test substance was **not** evaluated in the presence of an Organic Soil Load. The disinfectant test substance was provided as a ready to use formulations. A challenge suspension was used to inoculate the bottom part of 100 mm X 15 mm glass Petri Dish carriers to yield a minimum of $10^{4.8}$ viruses per carrier following drying. After drying, carrier was exposed to 2.0 mL of the test substance(s) at room temperature for exposure time. Following the timed exposure, the neutralizer appropriate for the test substance was added to the carrier. An aliquot of the neutralized suspension was serially diluted in medium and assayed for the presence of viable viruses using the cell culture susceptible to the virus. The viral titers were determined using a 50% tissue culture infectious dose (TCID50) calculation -- the Quantal test (Spearman-Kärber Method). This study evaluated the virucidal efficacy of two disinfectant spray substance, when used on dry, non-porous, inanimate surfaces.

The Study Protocol, included as Addendum of this Final Report, presents the study methodology, in detail. No deviations from the Study Protocol and from applicable Standard Operating Procedures occurred during the course of this evaluation.

8.0 JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM:

The Sponsor requested an antimicrobial surface test for human Coronavirus. Human Coronavirus strain 229E was used for testing.

9.0 STUDY DATES:

STUDY INITIATION DATE:	December 2, 2020
EXPERIMENTAL START DATE:	December 4, 2020
EXPERIMENTAL END DATE:	December 11, 2020
STUDY COMPLETION DATE:	December 15, 2020

10.0 TEST MATERIALS:

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

Test Substance:	ABV Nano EO handrub
Active Ingredients:	Chitosan, chlorhexidine
Lot Number:	F33B-20201019
Manufacture Date:	2020/10/19
Expiration Date:	2021/10/18

11.0 CHALLENGE VIRAL STRAIN:

Coronavirus strain 229E (ATCC #VR-740)
ATCC = American Type Culture Collection

12.0 HOST CELLS:

MRC-5 (ATCC #CCL-171; human lung fibroblasts)

13.0 TEST CONDITIONS:

12.1	Exposure Time:	30 minutes ± 5 seconds
12.2	Exposure Temperature:	Ambient (actual, 22.7 °C to 23.1 °C)
12.3	Relative Humidity:	Ambient (21%)
12.4	Organic Soil Load:	None
12.5	Diluent:	None

14.0 SUPPLIES AND EQUIPMENT:

The supplies and equipment were as specified in the Study Protocol in the Addendum to this Final Report.

15.0 MEDIA:

The media were as specified in the Study Protocol in the Addendum to this Final Report.

16.0 HOST CELL PREPARATION:

MRC-5 cells were obtained from American Type Culture Collection (ATCC) and were maintained as monolayers in disposable cell culture labware in accordance with BSLI SOP L-2084, "Procedure for Subculturing of Cells." Prior to testing, host cell cultures were seeded onto multi-well cell culture treated plates. Cell monolayers were 80% confluent and less than 48 hours old before use in testing.

17.0 TEST VIRUS PREPARATION:

Virus propagated and stored per BSLI SOP L-2102, Procedure for Production of High-Titered Virus Stock, was used for this study. On the day of use, aliquots of a stock virus suspensions were removed from a -70°C freezer and quickly thawed.

18.0 TEST VIRUS IDENTIFICATION:

Virus specific cytopathic effect (cell rounding and sloughing) in susceptible to the virus cell culture (MRC-5).

19.0 TEST SUBSTANCE PREPARATION:

The Test Substance was not tested in compliance with the EPA 810.2000 Lower Certified Limit policy.

20.0 TEST PROCEDURE:

The test procedure was as described in the Study Protocol in the Addendum to this Final Report.

21.0 CALCULATIONS:

The calculations were performed as described in the Study Protocol in the Addendum to this Final Report.

22.0 TEST ACCEPTANCE CRITERIA:

The following test acceptance criteria were met:

1) at least 4.8 $\log_{10}$ of $TCID_{50}$ per carrier was recovered from the Plate Recovery Control; 2) the virus titer from the Plate Recovery Control was sufficient to show at least 3 $\log_{10}$ reduction above the cytotoxicity level (*e.g.*, 5.5 $\log_{10}$ Plate Recovery – 2.5 $\log_{10}$ Cytotoxicity = 3.00 $\log_{10}$ Reduction); 3) cells in the Cell Control wells were viable and attached to the bottom of the well; 4) the medium was free of contamination in all wells of the plate; 5) when cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer could be demonstrated beyond the cytotoxic level; 6) the test substance(s) was fully neutralized, so the difference between the test virus titer of Virus Stock and Neutralization Control does not exceed 1.0 $\log_{10}$.

23.0 RESULTS – TABLE 1:

Table 1 presents data from the Hard Surface Disinfection Test of Test Substance, ABV Nano EO handrub/Lot # F33B-20201019, performed using 100 mm x 15 mm glass Petri dish carriers inoculated with Coronavirus strain 229E (ATCC #VR-740).

TABLE 1

Test Substance #1: ABV Nano EO handrub, Lot# F33B-20201019
Virus: Coronavirus, strain 229E (ATCC #VR-740)
Host Cell Line: MRC-5 (ATCC #CCL-171)
Volume Plated per Well: 1.0 mL
Volume Inoculated onto Carriers: 0.2 mL
Exposure Time: 30 minutes

Dilution (- Log_{10})	Plate Recovery Control	Test	Neutralization Control	Cytotoxicity Control	Virus Stock Titer	Virus Control	CC	NCC
							0000	0000
-2	NT	CT	NT	++++	NT	NT	NA	
-3	++++	0000	++++	0000	++++	++++		
-4	++++	0000	++++	0000	++++	++++		
-5	++++	0000	+0++	NT	++++	++++		
-6	+000	0000	+0++	NT	0++0	++++		
-7	0000	0000	0000	NT	0000	0000		
$TCID_{50}$/mL (log_{10})	5.75	2.50	6.00	2.50	6.00	6.50		
$TCID_{50}$/ carrier (log_{10})	5.05	1.80	NA					
Log_{10} Reduction	NA	**3.25**						
Percent Reduction		99.94						

\+ Virus infected cells present
0 Virus infected cells not detected
CT Cytotoxicity
NT Not tested
NA Not applicable
CC Cell Control
NCC Neutralizer Cytotoxicity Control

The virus Plate Recovery Control was 5.05 log_{10}/carrier, meeting the acceptance criterion. Cells in the Cell Culture Control wells and Neutralizer Cytotoxicity Control were viable and attached to the bottom of the wells. The medium was free of contamination in all wells of the plates.

24.0 STUDY CONCLUSION:

Under the conditions of this evaluation, Test Substance, ABV Nano EO handrub (Lot # F33B-20201019) reduced the infectivity of Coronavirus strain 229E (ATCC #VR-740) by 3.25 $\log_{10}$ following a 30-minute exposure. The Test Substance, ABV Nano EO handrub demonstrated the required ≥ 3 $\log_{10}$ reduction of Coronavirus strain 229E on non-porous test surface.

25.0 STATISTICAL ANALYSIS:

The Quantal test (Spearman-Kärber Method) was applied to calculate virus titer. No control of bias was performed.

26.0 LABORATORY PERSONNEL:

The following employees of BioScience Laboratories, Inc., were involved in the testing or ancillary support of this Study. The laboratory personnel have been appropriately trained, and their training records are on-file in the Quality Assurance Unit at the Testing Facility.

STUDY DIRECTOR:	Volha Teagle, Ph.D. Principal Scientist
Mauri Erickson Microbiologist	Brooke Kapalka Laboratory Support Technician
Marc Charnholm Manager of Laboratory Support	Stephanie Cebulla Laboratory Support Technician
Raphaelle Bassak Product Handler	

27.0 DOCUMENTATION AND RECORD KEEPING:

All documentation and records were compiled, analyzed, and will be retained by BioScience Laboratories, Inc. at its facility in Bozeman, Montana. All raw data for this study, as well as the Final Report, will be retained in safe storage by the Testing Facility for a period of at least 2 years. BioScience Laboratories, Inc., will notify the Study Sponsor before any documents or records are destroyed.

28.0 ACCEPTANCE:

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

Study Director: V Teagle
Volha Teagle, Ph.D.

12-15-2020
Date of Study Completion

ADDENDUM

Protocol #2009692-450

December 1, 2020

PROTOCOL #2009692-404

A NON-GLP VIRUCIDAL EFFICACY EVALUATION OF ONE HARD SURFACE DISINFECTANT SUBSTANCE

Prepared for:

NANO AND ADVANCED MATERIALS INSTITUE, LTD. (SPONSOR)
Units 517-518, Lakeside 1,
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Prepared by:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
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safe storage by the Testing Facility for a period of at least 2 years. BioScience Laboratories, Inc. will notify the Study Sponsor before any documents or records are destroyed.

30.0 TEST SUBSTANCE DISPOSITION:

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

21.3 The Log_{10} and percent (%) of infectivity reductions will be calculated as follows:

$$\% \text{ Reduction} = \left[1 - \frac{TCID_{50}\ test}{TCID_{50}\ plate\ recovery\ control}\right] \times 100$$

Log_{10} Reduction = (Log_{10} $TCID_{50}$ of the Plate Recovery Control) - (Log_{10} $TCID_{50}$ of the Test)

22.0 TEST ACCEPTANCE CRITERIA:

A valid test requires: 1) at least 4.8 log_{10} of $TCID_{50}$ per carrier will be recovered from the Plate Recovery Control; 2) the virus titer from the Plate Recovery Control should be sufficient to show at least 3 log_{10} reduction above the cytotoxicity level (*e.g.*, 5.5 log_{10} Plate Recovery – 2.5 log_{10} Cytotoxicity = 3.00 log_{10} Reduction); 3) cells in the Cell Control wells be viable and attached to the bottom of the well; 4) the medium be free of contamination in all wells of the plate; 5) when cytotoxicity is evident, at least a 3 log_{10} reduction in titer be demonstrated beyond the cytotoxic level; 6) the test substance(s) be fully neutralized, so the difference between the test virus titer of Virus Stock and Neutralization Control does not exceed 1.0 log_{10}.

23.0 STATISTICAL ANALYSIS:

The Quantal test (Spearman-Kärber Method) will be applied to calculate virus titer. No control of bias will be performed.

24.0 PROTOCOL DEVIATIONS AND AMENDMENTS:

Amendments to the approved protocol and the reasons will be documented, signed and dated by the Study Director and Sponsor, and maintained with the protocol. Deviations will be documented by the Study Director, signed and dated and maintained with the protocol.

25.0 FINAL REPORT:

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

26.0 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).

27.0 LIABILITY AND INDEMNIFICATION:

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

28.0 REFERENCE:

ASTM E1053-20, *Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces*

29.0 DOCUMENTATION AND RECORD-KEEPING:

All documentation and records will be compiled, analyzed, and retained by BioScience Laboratories, Inc., at its facility in Bozeman, Montana. All raw data for this study, as well as the Final Report, will be retained in

20.3.2 After the exposure time has elapsed, the appropriate amount of the neutralizer (18 mL) will be added to the Petri dish and the virus test substance mixture will be scraped from the surface of the carrier using a sterile cell scraper. Subsequent 10-fold dilutions will be made in MM and plated in four replicates.

20.3.3 Plate Recovery Control. One carrier will be used for the virus recovery control. The test virus will be dried as described in Section 23.2.1 and 23.2.2. A total of 2.0 mL of MM will be added to the contaminated carrier. The carrier will be exposed to MM at ambient temperature for the specified for each virus exposure time, timed using a calibrated minute/second timer. The appropriate neutralizer will be added to the carriers and the virus will be scraped from the surface. Subsequent 10-fold dilutions will be made in MM and plated in four replicates.

20.3.4 Virus Stock Titer. The test virus will be diluted 10-fold in MM. Each dilution will be plated in four replicates.

20.3.5 Neutralization and Cytotoxicity Controls. A 0.2 mL aliquot of medium will be transferred to the bottom inside surface of a sterilized 100 mm x 15 mm glass Petri plate carrier. A sterile cell scraper will be used to spread the medium uniformly. The medium will be air-dried at room temperature until visibly dry. After drying, the carrier will be treated as described in Section 23.3.1. After the exposure time has elapsed, the appropriate neutralizer will be dispensed into the carriers. The neutralized liquid will be used for the Neutralization control (virus added) and Cytotoxicity Control (no virus added). The Neutralization Control will receive an aliquot of a test virus, followed by exposure for at least the specified test exposure time. Subsequent 10-fold dilutions will be made in MM and plated in four replicates. The Cytotoxicity Control will receive no virus, will be diluted (10-fold) in MM, and plated in four replicates.

20.3.6 Virus Control. A dilution of the test virus will be added to a Neutralizer and exposed for at least the exposure time. Subsequent 10-fold dilutions will be made in MM and plated in four replicates.

20.3.7 Neutralizer Cytotoxicity Control. Neutralizing solution will be plated onto cell cultures in at least 4 wells

20.3.8 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 four wells).

20.3.9 The plates will be incubated for 5 to 14 days.

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

21.0 CALCULATIONS:

21.1 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 ($TCID_{50}$) calculation - the Quantal test (Spearman-Kärber Method) - will be applied.

Negative Log of $TCID_{50}$/mL = -log of 1st dilution assayed – [((Σ of % mortality at each dilution/100) – 0.5) × (log of dilution)]

21.2 Virus recoveries will be presented per assay volume and per carrier inoculum volume.

TCID50/carrier = (Antilog of $TCID_{50}$/mL) ×0.2 = Log_{10}/carrier

14.0 CHALLENGE VIRAL STRAIN:

Coronavirus strain 229E (ATCC #VR-740)
ATCC = American Type Culture Collection

15.0 HOST CELLS:

MRC-5 (ATCC #CCL-171; human lung fibroblasts)

16.0 HOST CELL PREPARATION:

The cell lines will be maintained as monolayers in disposable cell culture labware and will be used for testing of each virus. Cell monolayers will be allowed to grow for less than 48 hours until approximately 80% to 90% confluent. Growth Medium (GM) will be replaced with Maintenance Medium (MM).

17.0 TEST VIRUS PREPARATION:

The viral suspensions used for this study will originate from BSLI high titer virus stock. On the day of use, an aliquot of the virus will be thawed and used in testing.

18.0 TEST VIRUS IDENTIFICATION:

Cytotoxic viruses will be identified via Cytopathic Effect (CPE) in susceptible cell cultures using inverted compound microscope.

19.0 TEST SUBSTANCE PREPARATION:

The Test Substances will **not** be tested in compliance with the EPA 810.2000 Lower Certified Limit policy.

20.0 TEST PROCEDURE:

20.1 Preparation of Carriers

Sterilized glass Petri plates (100 mm x 15 mm) will be used as the carriers for this evaluation.

20.2 Contamination of Carriers

20.2.1 A 0.2 mL aliquot of the prepared virus suspension will be transferred to the bottom inside surface of a sterilized 100 mm x 15 mm glass Petri plate carrier. A sterile cell scraper will be used to spread the inoculum uniformly.

20.2.2 The virus suspension(s) will be air-dried at room temperature until visibly dry. Drying conditions (time, temperature, and relative humidity) will be documented.

20.2.3 One carrier per batch of the test substance(s) will be used for testing.

20.3 Test

20.3.1 After the inoculated carrier(s) has dried, the carrier(s) will be treated with 2.0 mL of the test substance(s). The carrier(s) will be exposed to the test substance(s) at ambient temperature for the specified exposure time, timed using a calibrated minute/second timer. Timing will commence after the liquid substance is spread over the entire surface of the contaminated carrier(s). The treated carrier(s) will be kept undisturbed for the duration of the contact time. Test conditions (time, temperature, and relative humidity) will be documented.

8.0 TEST CONDITIONS:

8.1	Exposure Time:	30 minutes ± 5 seconds
8.2	Exposure Temperature:	Ambient (nominally, 18 °C to 25 °C)
8.3	Relative Humidity:	Ambient (<60%)
8.4	Organic Soil Load:	None
8.5	Diluent:	None

9.0 EQUIPMENT:

9.1 CO_2 Incubator, Temperature Range 37 °C ± 2 °C with 4 % to 6% CO_2
9.2 CO_2 Incubator, Temperature Range 35 °C ± 2 °C with 4 % to 6% CO_2
9.3 Thermometers
9.4 Portable Pipetter
9.5 Continuously Adjustable Pipettes, 100 µL – 1000 µL Capacity
9.6 Continuously Adjustable Pipettes, 20 µL – 200 µL Capacity
9.7 Inverted Compound Microscope
9.8 Laminar Flow Biological Safety Cabinet
9.9 Waste Pan
9.10 Calibrated Minute/Second Timers
9.11 Hygrometer

10.0 SUPPLIES:

10.1 Sterile Disposable Pipettes
10.2 Sterile Polystyrene Test Tubes
10.3 Sterile Universal 1.0 mL and 0.2 mL Pipette Tips
10.4 Powder-Free Gloves
10.5 Sterile Tissue Culture Treated 24-Well Plates
10.6 Viral Suspension(s)
10.7 Sterile Flasks
10.8 Sterile 50 mL Centrifuge Tubes
10.9 Sterile Pipette Reservoir
10.10 Non-Sterile Waste Beaker for discarded tips, etc.
10.11 Sterile Cell Scrapers
10.12 Sterile Glass Petri Dishes

11.0 MEDIA:

11.1 1X Eagle's Minimum Essential Medium (EMEM), or other appropriate medium
11.2 Growth Medium: EMEM with 10% FBS, 1% penicillin-streptomycin-amphotericin B, 1% of L-Glutamine (when necessary)
11.3 Maintenance Medium: EMEM with 2% FBS, 1% penicillin-streptomycin-amphotericin B, 1% of L-Glutamine (when necessary)
11.4 Trypsin/EDTA for cell culture maintenance
11.5 Antibiotics for medium
11.6 Appropriate Neutralizer
11.7 Fetal Bovine Serum (FBS)

12.0 ORGANIC SOIL LOAD:

None.

13.0 DILUENT:

No diluent will be used.

December 1, 2020

PROTOCOL #2009692-404

1.0 TITLE: **A NON-GLP VIRUCIDAL EFFICACY EVALUATION OF ONE HARD SURFACE DISINFECTANT SUBSTANCE**

2.0 SPONSOR: **NANO AND ADVANCED MATERIALS INSTITUE, LTD.**
Units 517-518, Lakeside 1,
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N.T., Hong Kong, China

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

4.0 STUDY DIRECTOR: Volha Teagle, Ph.D.

5.0 PURPOSE:

The purpose of this study is to evaluate the virucidal efficacy of one disinfectant test substance when challenged with Human Coronavirus strain 229E. Testing will be based upon methods described as specified in the American Society for Test Materials (ASTM) test methods designated E1053-20, *Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surface.* All testing will **not** be performed in accordance with Good Laboratory Practices, as specified in 40 CFR 160.

6.0 SCOPE:

This study will evaluate the virucidal efficacy of one disinfectant test substance, when used on dry, non-porous, inanimate surfaces. The test substances will be evaluated versus Human Coronavirus strain 229E (ATCC #VR_740). The test substance will **not** be evaluated in the presence of an Organic Soil Load. The disinfectant test substance will be provided as a ready to use formulations. A challenge suspension will be used to inoculate the bottom part of 100 mm X 15 mm glass Petri Dish carriers to yield a minimum of $10^{4.8}$ viruses per carrier following drying. After drying, each carrier will be exposed to 2.0 mL of the test substance(s) at room temperature for exposure time. Following the timed exposure, the neutralizer appropriate for the test substances will be added to the carrier. An aliquot of the neutralized suspension will be serially diluted in medium and assayed for the presence of viable viruses using the cell culture susceptible to the virus. The viral titers will be determined using a 50% tissue culture infectious dose ($TCID_{50}$) calculation -- the Quantal test (Spearman-Kärber Method).

7.0 TEST SUBSTANCE:

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

Test Substance:	ABV Nano EO handrub
Active Ingredients:	Chitosan, chlorhexidine
Lot Number:	F33B-20201019
Manufacture Date:	2020/10/19
Expiration Date:	2021/10/18

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31.0 ACCEPTANCE:

A NON-GLP VIRUCIDAL EFFICACY EVALUATION OF ONE HARD SURFACE DISINFECTANT SUBSTANCE

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

Study Director: _____ 12-02-2020
Volha Teagle, Ph.D. Date of Study Initiation

ACCEPTED BY: NANO AND ADVANCED MATERIALS INSTITUE, LTD. (SPONSOR)
Units 517-518, Lakeside 1,
No. 8 Science Park West Avenue,
Hong Kong Science Park, Shatin,
N.T., Hong Kong, China

_____ Neil CHEUK. 21/12/2020
Representative Date

Senior Engineer
Title