April 23, 2020

FINAL REPORT #2003113-450

EVALUATION OF THE VIRUCIDAL PROPERTIES OF A TREATED FABRIC MATERIAL WHEN CHALLENGED WITH CORONAVIRUS

Prepared for:

NOBLE BIOMATERIALS (SPONSOR)
300 Palm Street
Scranton, Pennsylvania 18505

Prepared by:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718
(406) 587-5735

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EXECUTIVE SUMMARY

STUDY NUMBER: **2003113-450**

TITLE: **EVALUATION OF THE VIRUCIDAL PROPERTIES OF A TREATED FABRIC MATERIAL WHEN CHALLENGED WITH CORONAVIRUS**

SPONSOR: **NOBLE BIOMATERIALS**
300 Palm Street
Scranton, Pennsylvania 18505

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

STUDY INITIATION DATE: 04/01/2020

STUDY COMPLETION DATE: 04/23/2020

This study evaluated the virucidal properties of one treated fabric test material when challenged with Human Coronavirus strain OC43 (ZeptoMetrix Corp. #0810024CF). Samples of fabric were evaluated following 4-hour, 12-hour, and 24-hour exposures to the virus. Testing was based on the International Organization for Standardization (ISO) method ISO 18184:2019(E), *Textiles — Determination of antiviral activity of textile products*. 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 material and untreated control material remained the responsibility of the Study Sponsor and was not performed by the Testing Facility (GLP 58.105 and GLP 58.113).

Table 1 presents the summary of test results.

Table 1: Summary of Test Results for Metallized Cerex Fabric

Test Fabric Designation	Timed Exposure	Log_{10} $TCID_{50}$ Reduction	Standard Deviation	Percent Reduction
Metalized Cerex Fabric	4 hours	**1.625**	0.072	**97.63**
	12 hours	**2.333**	0.125	**99.54**
	24 hours	**3.208**	0.217	**99.94**

STUDY CONCLUSION:

Under conditions of this evaluation, Test Material, Metalized Cerex Fabric, reduced infectivity of Coronavirus OC43 by an average of 1.625 log_{10} (97.63%) following a 4-hour exposure, by an average of 2.333 log_{10} (99.54%) following a 12-hour exposure, and by an average of 3.208 log_{10} (99.94%) following a 24-hour exposure. In accordance with ISO18184, the Test Material, Metalized Cerex Fabric, may be categorized to have a Good antiviral effect ($\geq$ 2.0 log_{10}) following a 12-hour exposure and an Excellent antiviral effect ($\geq$ 3.0 log_{10}) following a 24-hour exposure.

April 23, 2020

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1.0 TITLE: **EVALUATION OF THE VIRUCIDAL PROPERTIES OF A TREATED FABRIC MATERIAL WHEN CHALLENGED WITH CORONAVIRUS**

2.0 SPONSOR: **NOBLE BIOMATERIALS**
300 Palm Street
Scranton, Pennsylvania 18505

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 was to evaluate virucidal activity of one treated fabric test material and one untreated control material when challenged with Human Coronavirus. Testing was conducted based on the International Organization for Standardization (ISO) method ISO 18184:2019(E), *Textiles — Determination of antiviral activity of textile products*. 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 material and untreated control material remained the responsibility of the Study Sponsor and was not performed by the Testing Facility (GLP 58.105 and GLP 58.113).

6.0 SCOPE:

One treated test material and one untreated control material was used for this evaluation. Samples were inoculated with Human Coronavirus strain OC43 (ZeptoMetrix Corp. #0810024CF) and evaluated following 4-hour, 12-hour, and 24-hour exposures. Untreated control material was also evaluated immediately or insofar as possible after inoculation. Three replicates of test and control were performed. Virus was eluted and plated onto susceptible cells in 8 replicates. Calculations of the estimated virus concentrations were performed using the Behrens-Kärber method.

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. The Study Protocol was amended once to use ISO18184 instead of AATCC 100 and to include exposure times of 4 hours ± 1 minute, 12 hours ± 1 minute, and 23 to 24 hours. The protocol was replaced in its entirety. Protocol Amendment 01 is included in Addendum 1 of this Final Report.

7.0 JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM:

The Sponsor requested an efficacy test against Coronaviruses using Coronavirus strain OC43. Coronavirus strain OC43 is a Beta-coronavirus and genetically is the closest human virus to the other Beta-coronaviruses like SARS and MERS. Coronaviruses are large RNA enveloped viruses. Viral envelopes are the major target of surface-active biocidal formulations (antiseptics and disinfectants). Destruction of lipid envelopes leads to a virus inactivation and inability to infect susceptible hosts. The viral envelope structure and composition is very conserved within a family of viruses due to the cellular origin of envelopes. That is why the biocidal formulations effective against one strain of enveloped virus representing the virus family are effective against the whole family of viruses. With the purpose to provide guidance for successful and safe testing of biocide efficacy, regulatory agencies such as the US EPA and Health Canada created a list of surrogate viruses that possess equivalent susceptibility and belong to the same virus family as the viruses desired for a disinfectant or antiseptic efficacy claim. Coronavirus OC43 is an appropriate and a safe choice for efficacy testing of biocide treated fabric material.

8.0 STUDY DATES:

STUDY INITIATION DATE: 04/01/2020

EXPERIMENTAL START DATE: 04/02/2020

EXPERIMENTAL END DATE: 04/13/2020

STUDY COMPLETION DATE: 04/23/2020

9.0 TEST MATERIALS:

The test and control materials evaluated were provided to the Testing Facility by the Study Sponsor. Responsibility for determination of the identity, strength, purity, composition, and stability of the test materials, as well as responsibility for retention of the test materials, remained with the Study Sponsor.

Treated Test Material:	Metalized Cerex Fabric
Active Ingredients:	Silver
Lot Number:	Not Provided
Expiration Date:	Not Provided
Manufacture Date:	Not Provided

Untreated Control Material:	Non-Metallized Cerex Fabric
Active Ingredients:	Not Applicable
Lot Number:	Not Provided
Expiration Date:	Not Provided
Manufacture Date:	Not Provided

10.0 CHALLENGE VIRAL STRAIN:

Human Coronavirus (Betacoronavirus), strain OC43 (ZeptoMetrix Corp. #0810024CF)

11.0 HOST CELLS:

HCT-8 (ATCC #CCL-244; human colon adenocarcinoma, epithelial)
ATCC = American Typed Culture Collection

12.0 TEST CONDITIONS:

Test Material Exposure:	4 hours ± 1 minute (T=4) 12 hours ± 1 minute (T=12) 23 hours to 24 hours (T=24)
Control Material Exposure:	4 hours ± 1 minute (T=4) 12 hours ± 1 minute (T=12) 23 hours to 24 hours (T=24) Immediate (T=0)
Exposure Temperature:	25°C ± 1°C T=0 at ambient temperature
Test and Control Replicates:	3
Elution/Neutralization Solution:	Dey-Engley Neutralizing Broth (D/E)

13.0 SUPPLIES AND EQUIPMENT:

13.1 Portable Pipetter
13.2 CO_2 Incubator, Temperature Range 37 ± 2 °C, 5% to 6% CO_2
13.3 CO_2 Incubator, Temperature Range 33 ± 2 °C, 5% to 6% CO_2
13.4 Thermometers
13.5 Refrigerator, 2 – 8 °C
13.6 Laminar Biological Flow hood
13.7 Inverted Compound Microscope
13.8 Vortex Mixers
13.9 Continuously Adjustable Pipettors, 100 µL - 1000 µL Capacity
13.10 Continuously Adjustable Pipetters, 20 µL - 200 µL Capacity
13.11 Calibrated Minute/Second Timers
13.12 NIST-Traceable Clocks
13.13 Sterile Disposable Pipettes
13.14 Sterile Disposable Petri Plates
13.15 Sterile Tissue Culture Treated Multi-well Plates
13.16 Sterile Test Tubes
13.17 Sterile Universal 1.0 mL and 0.2 mL Pipette Tips
13.18 Powder-free Gloves
13.19 Sterile Flasks
13.20 Sterile Reservoirs
13.21 Sterile Disposable Specimen Cups
13.22 Waste Pan
13.23 Forceps, alcohol sterilized.

14.0 MEDIA:

14.1 1X RPMI-1640 or other appropriate medium;
14.2 Growth Medium (GM): RPMI-1640 or other media with 10% FBS and 1% Antibiotic and L-glutamine;
14.3 Maintenance Medium (MM): RPMI-1640 or other media with 2% FBS and 1% Antibiotic and L-glutamine;
14.4 Trypsin-EDTA;
14.5 Antibiotics (e.g., Penicillin-Streptomycin-Amphotericin B);
14.6 Fetal Bovine Serum (FBS).
14.7 Neutralizing/eluting solution: Dey-Engley Broth

15.0 HOST CELL PREPARATION:

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 inoculation with the virus.

16.0 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 thawed at ambient temperature.

17.0 TEST VIRUS IDENTIFICATION:

Virus specific cytopathic effect (vacuolization of cytoplasm and sloughing) in susceptible to the virus cell culture (HCT-8).

18.0 PREPARATION OF TEST MATERIALS:

18.1 Square swatches 2.0 cm x 2.0 cm ± 0.1 cm were cut from the test and control fabric materials.

18.2 The swatches weighted 0.40 g ± 0.05 g. The number of swatches per specimen was 29 (test material) and 30 (control material).

18.3 Test and Control materials were autoclaved for sterilization as per BSLI SOP L-2017, *Preparation of dry goods for sterilization.*

18.4 Swatches were placed aseptically in a specimen cup.

18.5 The swatches weighting 0.40 g in a specimen cup represented one specimen.

19.0 TEST METHOD:

19.1 Test Procedure

19.1.1 An aliquot of 0.2 mL of the test virus was deposited onto specimens of test and control materials at 12 points of the specimen. Caps of the containers were closed. The specimens were transferred to an incubator. The exposure time commenced following virus application and concluded upon contact with elution/neutralizing solution.

19.1.2 Following exposures, 20 mL of D/E Broth was added to the specimens. Caps were closed and containers vortexed for 5 seconds 5 times to wash out the virus from the specimens. Wash-out solution was diluted 10-fold in MM and plated onto cells.

19.2 Controls

19.2.1 *Baseline Control (Wash-out immediately after deposit).* Three specimens of control materials were inoculated with 0.2 mL of the test virus at 12 points of the specimen. D/E broth was added immediately after inoculation (T=0). Elution was performed as described in Section 19.1.2. Wash-out solution was diluted 10-fold in MM and plated onto cells.

19.2.2 *Neutralization Control (Verification of cytotoxicity by cell sensitivity to virus and the inactivation of antiviral activity).* 20 mL of D/E broth was added to three test and three control specimens. Elution was performed as described in Section 19.1.2. Wash-out solution was dispensed into tubes or wells of dilution reservoirs. Dilution 10^{-1} of test virus was added to the first tube to obtain ~ 10^5 $TCID_{50}$/mL virus particles. Ten-fold virus titration was performed in dispensed wash-out solution and incubated for at least 30 minutes at 25 °C.

19.2.3 *Initial Population (Test virus titration).* Test virus was diluted in MM and plated onto cells.

19.2.4 *Cell Culture Control.* Intact cell culture monolayers served as the control of cell culture viability. The GM was replaced by MM in all Cell Culture Control wells (minimum four wells).

19.2.5 Wash-out samples and dilutions were plated onto cells in multi-well plates in eight replicates. The plates were incubated for 10 and 11 days at 33 ± 2 °C in a CO_2 incubator. Cytopathic/cytotoxic effect was monitored using an inverted compound microscope.

20.0 CALCULATIONS:

20.1 *Viral and cytotoxicity 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 (Behrens-Kärber Method) – was applied.

$$Y = X \times 10a$$
$$a = \Sigma p - 0.5$$

Where:
Y is the infective titre ($TCID_{50}$/0.1 ml);
X is the dilution rate of the base virus suspension;
P is the ratio of the cytopathic effect at the respective dilution of the virus suspension.
Σp is the sum of values of p.

Infectivity titre: A (TCID50/ml):
$A = Y \times 10$

The infectivity titre ($TCID_{50}$/specimen) will be calculated using:
$V = A \times C$

Where:
V is the infective titre (TCID50/specimen);
C is the amount of wash-out virus suspension (mL).

20.2 Calculation of Antiviral Activity value:

$Mv = \log_{10}(Va/Vc) = \log_{10}(Va) - \log_{10}(Vc)$

Where:
Mv is the antiviral activity value;
$\log_{10}(Va)$ is average of 3 infectivity titre values immediately after inoculation of the control specimen (*Baseline*);
$\log_{10}(Vc)$ is average of 3 infectivity titre values after exposure time point with the Test specimen (*Test*).

20.3 Percent Reduction: $\%\text{Reduction} = \left[1 - \frac{\text{TCID}_{50}\ test}{\text{TCID}_{50}\ baseline}\right] \times 100$

20.4 Standard Deviation: $S_{N-1} = \sqrt{\frac{1}{N-1}\sum(x_i - \bar{x})^2}$

Where:
N = number of replicates;
χ_i = each value;
$\bar{\chi}$ = average.

21.0 TEST ACCEPTANCE CRITERIA:

The following test acceptance criteria were met: 1) The virus infectivity titre in the Initial Population was $\geq$ 7 $TCID_{50}$/mL; 2) The difference between $\log_{10}$ recoveries from Neutralization Control of Control specimen and Neutralization Control of Test specimen was $\leq$ 0.5; 3) Reduction value of infective titre of Control specimen following 4-hour and 12-hour exposures was $\leq$ 1.0 $\log_{10}$ and $<$ 2.0$\log_{10}$ after 24 hours of exposure; 4) Cells in the Cell Control wells were viable and attached to the bottom of the well.

22.0 RESULTS – TABLE 2:

22.1 Table presents the data from the Test and Control material post-exposure infectivity ($TCID_{50}$), the $\log_{10}$ reductions observed following a 4-hour, 12-hour, and 24-hour exposures to Coronavirus strain OC43, virus recoveries for Neutralization, Baseline Control, and Initial Population.

TABLE 2

Test Product: Metalized Cerex Fabric (treated fabric)
Control Product: Non-Metalized Cerex Fabric (untreated fabric)
Virus: Coronavirus strain OC43 ZeptoMetrix #0810024CF
Host Cell Line: HCT-8 Host Cell Line ATCC #CCL-244

Designation	Replicate	Timed Exposure	Dilutions, log_{10}							$TCID_{50}$, log_{10}/0.1mL	$TCID_{50}$, log_{10}/mL	$TCID_{50}$, log_{10}/specimen	Log_{10} Reduction
			2	3	4	5	6	7	8				
Test (treated fabric)	1	4 hours	44444444	44440044	44000400	00000000	00000000	00000000	NT	3.625	4.625	5.926	1.583
	2		44444444	44440440	04000404	00000000	00000000	00000000	NT	3.625	4.625	5.926	1.583
	3		44444444	44444004	44000000	00000000	00000000	00000000	NT	3.500	4.500	5.801	1.708
									Average:	3.583	4.583	5.884	**1.625**
	1	12 hours	44444444	44000044	00000000	00000000	00000000	00000000	NT	3.000	4.000	5.301	2.208
	2		44444444	44000000	00000000	00000000	00000000	00000000	NT	2.750	3.750	5.051	2.458
	3		44444444	40004004	00000000	00000000	00000000	00000000	NT	2.875	3.875	5.176	2.333
									Average:	2.875	3.875	5.176	**2.333**
	1	24 hours	44444000	00000000	00000000	00000000	00000000	00000000	NT	2.125	3.125	4.426	3.083
	2		00444440	00000000	00000000	00000000	00000000	00000000	NT	2.125	3.125	4.426	3.083
	3		44000000	00000000	00000000	00000000	00000000	00000000	NT	1.750	2.750	4.051	3.458
									Average:	2.000	3.000	4.301	**3.208**
Control (untreated fabric)	1	4 hours	44444444	44444444	44444444	00440440	00000000	00000000	NT	5.000	6.000	7.301	0.208
	2		44444444	44444444	44444444	04040040	00000000	00000000	NT	4.875	5.875	7.176	0.333
	3		44444444	44444444	04444444	44040004	00000000	00000000	NT	4.875	5.875	7.176	0.333
									Average:	4.917	5.917	7.218	0.291
	1	12 hours	44444444	44444404	44440444	00000000	00000000	00000000	NT	4.250	5.250	6.551	0.958
	2		44444444	44444444	44444444	00000000	00000000	00000000	NT	4.500	5.500	6.801	0.708
	3		44444444	44444440	04400444	00000000	00000000	00000000	NT	4.000	5.000	6.301	1.208
									Average:	4.250	5.250	6.551	0.958
	1	24 hours	04444444	04400444	00000004	00000000	00000000	00000000	NT	3.125	4.125	5.426	2.083
	2		44440444	44440444	00000440	00000000	00000000	00000000	NT	3.500	4.500	5.801	1.708
	3		44444444	40444400	04000000	00000000	00000000	00000000	NT	3.250	4.250	5.551	1.958
									Average:	3.292	4.292	5.593	1.916
Baseline		Immediate	44444444	44444444	44444444	44044400	00000000	00000000	NT	5.125	6.125	7.426	
	2		44444444	44444444	44444444	04444400	40000000	00000000	NT	5.250	6.250	7.551	
	3		44444444	44444444	44444444	40044444	00000000	00000000	NT	5.250	6.250	7.551	
									Average:	5.208	6.208	7.509*	
NC (treated fabric)	1	NA	44444444	44444444	34444444	04444400	00000000	NT	NT	5.125	6.125		
	2		44444444	44444444	44444440	40044044	00000000	NT	NT	5.000	6.000		
	3		44444444	44444444	44044440	44400444	00400000	NT	NT	5.125	6.125		NA
									Average:	5.083	6.083		
NC (untreated fabric)	1	NA	44444444	44444444	04404444	44004404	40000000	NT	NT	5.000	6.000	NA	
	2		44444444	44444444	44444444	00440440	00000040	NT	NT	5.125	6.125		
	3		44444444	44444444	04444444	44404440	00000000	NT	NT	5.125	6.125		
									Average:	5.083	6.083		
IP	NA	NA	NT	44444444	44444444	44444444	44044004	00000000	00000000	6.125	7.125		

4	-	Monolayer completely destroyed by the virus or because of cytotoxicity
3	-	Substantial CPE due to virus; however, monolayer still present
0	-	No CPE present
NA	-	Not Applicable
NT	-	Not Tested
NC	-	Neutralization Control
IP	-	Initial Population
*	-	Used to calculate log reduction

23.0 STUDY CONCLUSION:

Under conditions of this evaluation, Test Material, Metalized Cerex Fabric, reduced infectivity of Coronavirus OC43 by an average of 1.625 $\log_{10}$ (97.63%) following a 4-hour exposure, by an average of 2.333 $\log_{10}$ (99.54%) following a 12-hour exposure, and by an average of 3.208 $\log_{10}$ (99.94%) following a 24-hour exposure. In accordance with ISO18184, the Test Material, Metalized Cerex Fabric, may be categorized to have a Good antiviral effect ($\geq$ 2.0 $\log_{10}$) following a 12-hour exposure and an Excellent antiviral effect ($\geq$ 3.0 $\log_{10}$) following a 24-hour exposure.

24.0 STATISTICAL ANALYSIS:

The Quantal test (Behrans-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.

25.0 QUALITY ASSURANCE AUDITS:

Quality Assurance (QA) conducted an in-phase audit of the critical test procedures over the course of testing and advised the Study Director and Management of the outcomes of these. On completion of testing, the QA performed an audit of the raw data and of the Final Report, in its entirety. No deviations from the Study Protocol or from applicable BioScience Laboratories, Inc., Standard Operating Procedures were observed.

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
Jared Montana Microbiologist	Marc Charnholm Manager of Laboratory Support
Terah Rash Microbiologist	Brooke Kapalka Laboratory Support Technician
Alexander Stanley Laboratory Technician	Stephanie Cebulla Laboratory Support Technician
Kameron Kohn Microbiologist	Dakotah Olson Product Handler
Aubrie Cornell Microbiologist	Mauri Erickson Microbiologist
Sarah Franklin Microbiologist	Kelly Burningham Virologist

27.0 QUALITY ASSURANCE AND QUALITY CONTROL PERSONNEL:

Jeremy Duley Systems Administrator/QC Specialist	Renee LaFond, M.S. Quality Assurance Specialist
Danielle Goveia Quality Assurance Specialist	Carl Schmidt ISO Technical Manager (QC, Safety)
Amy L. Juhnke, RQAP-GLP Director of Quality Assurance	

28.0 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 5 years. BioScience Laboratories, Inc., will notify the Study Sponsor before any documents or records are destroyed.

29.0 ACCEPTANCE:

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

Study Director: _____ 04-23-2020
Volha Teagle, Ph.D. 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	04/02/2020 04/03/2020	04/09/2020	04/09/2020
Data Audit	04/21/2020, 04/22/2020	04/22/2020	04/23/2020
Final Report Review	04/22/2020	04/22/2020	04/23/2020

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 materials were not performed by BioScience Laboratories, Inc. This statement also serves to confirm that the Final Report reflects the raw data.

Quality Assurance Specialist: _____ 04/23/2020
Danielle Goveia Date

ADDENDUM

Protocol #2003113-450.01
Protocol Amendment Form (Template Form G-AMEND-PR)

March 30, 2020

PROTOCOL #2003113-450.01

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Prepared by:

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(406) 587-5735

CONFIDENTIAL

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March 30, 2020

PROTOCOL #2003113-450.01

1.0 TITLE: **EVALUATION OF THE VIRUCIDAL PROPERTIES OF A TREATED FABRIC MATERIAL WHEN CHALLENGED WITH CORONAVIRUS**

2.0 SPONSOR: **NOBLE BIOMATERIALS**
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3.0 TESTING FACILITY: **BIOSCIENCE LABORATORIES, INC.**
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4.0 STUDY DIRECTOR: Volha Teagle, Ph.D.

5.0 PURPOSE:

This study will evaluate the virucidal properties of one treated fabric test material and one untreated control material when challenged with Human Coronavirus. Testing will be conducted based on the International Organization for Standardization (ISO) method ISO 18184:2019(E), *Textiles — Determination of antiviral activity of textile products.* 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 treated test material and untreated control material remains the responsibility of the Study Sponsor and will not be performed by the Testing Facility (GLP 58.105 and GLP 58.113).

6.0 SCOPE:

One treated test material and one untreated control material will be used for this evaluation. Samples will be inoculated with Human Coronavirus strain OC43 (ZeptoMetrix Corp. #0810024CF) and evaluated following 4-hour, 12-hour, and 24-hour exposures. Untreated control material will be also evaluated immediately or insofar as possible after inoculation. Three replicates of test and control will be performed. Virus will be eluted and plated onto susceptible cells in 8 replicates. Calculations of the estimated virus concentrations will be performed using the Behrens-Kärber method.

7.0 JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM:

The Sponsor requested an efficacy claim against Coronaviruses including 2019 novel strain SARS-CoV-2, the cause of COVID-19, using Coronavirus strain OC43 for testing of efficacy. Coronavirus strain OC43 is a Beta-coronavirus and genetically is the closest human virus to the other Beta-coronaviruses like SARS and MERS. Coronaviruses are large RNA enveloped viruses. Viral envelopes are the major target of surface-active biocidal formulations (antiseptics and disinfectants). Destruction of lipid envelopes leads to a virus inactivation and inability to infect susceptible hosts. The viral envelope structure and composition is very conserved within a family of viruses due to the cellular origin of envelopes. That is why the biocidal formulations effective against one strain of enveloped virus representing the virus family are effective against the whole family of viruses. With the purpose to provide guidance for successful and safe testing of biocide efficacy, regulatory agencies such as the US EPA and Health Canada created a list of surrogate viruses that possess equivalent susceptibility and belong to the same virus family as the viruses desired for a disinfectant or antiseptic efficacy claim. Coronavirus OC43 (BioSafety Level 2) is an appropriate and a safe choice for efficacy testing of biocide treated fabric material.

8.0 TEST AND CONTROL MATERIALS:

The treated test material and untreated control material will be provided by the Study Sponsor and cut into square swatches by the Testing Facility, prior to use in testing. Responsibility for determination of the identity, strength, purity, composition, and stability of the treated test material and untreated control material, as well as responsibility for retention of all materials, rests with the Sponsor.

Treated Test Material:	Metalized Cerex Fabric
Lot Number:	Not Provided
Expiration Date:	Not Provided
Manufacture Date:	Not Provided
Untreated Control Material:	Non-Metallized Cerex Fabric
Lot Number:	Not Provided
Expiration Date:	Not Provided
Manufacture Date:	Not Provided

9.0 CHALLENGE VIRUS:

Human Coronavirus (Betacoronavirus), strain OC43 (ZeptoMetrix Corp. #0810024CF)

10.0 HOST CELLS :

HCT-8 (ATCC #CCL-244; human colon adenocarcinoma, epithelial)
ATCC = American Typed Culture Collection

11.0 EQUIPMENT:

11.1 Portable Pipetter
11.2 CO_2 Incubator, Temperature Range 37 ± 2 °C
11.3 CO_2 Incubator, Temperature Range 33 ± 2 °C
11.4 Thermometers
11.5 Refrigerator, 2 – 8 °C
11.6 Laminar Biological Flow hood
11.7 Inverted Compound Microscope
11.8 Vortex Mixers
11.9 Continuously Adjustable Pipettors, 100 µL - 1000 µL Capacity
11.10 Continuously Adjustable Pipetters, 20 µL - 200 µL Capacity
11.11 Calibrated Minute/Second Timers
11.12 NIST-Traceable Clocks

12.0 SUPPLIES:

12.1 Sterile Disposable Pipettes
12.2 Sterile Disposable Petri Plates
12.3 Sterile Tissue Culture Treated Multi-well Plates
12.4 Sterile Test Tubes
12.5 Sterile Universal 1.0 mL and 0.2 mL Pipette Tips
12.6 Powder-free Gloves
12.7 Virus suspension
12.8 Sterile Flasks
12.9 Sterile Reservoirs
12.10 Sterile Disposable Specimen Cups
12.11 Waste Pan
12.12 Forceps, alcohol sterilized or autoclaved.

13.0 MEDIA:

13.1 1X RPMI-1640
13.2 Growth Medium (GM): RPMI-1640 with 10% FBS and 1% Antibiotic and L-glutamine
13.3 Maintenance Medium (MM): RPMI-1640 with 2% FBS and 1% Antibiotic and L-glutamine
13.4 Trypsin/EDTA
13.5 Antibiotics (e.g., Penicillin-Streptomycin-Amphotericin B)
13.6 Fetal Bovine Serum (FBS)

14.0 HOST CELL PREPARATION:

HCT-8 cells, obtained from American Type Culture Collection (ATCC), will be 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 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 inoculation with the virus. The growth medium (GM) and maintenance medium (MM) will be RPMI-1640.

15.0 TEST VIRUS PREPARATION:

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

16.0 PREPARATION OF TEST MATERIALS:

16.1 Square swatches 2.0 cm x 2.0 cm ± 0.1 cm will be cut from the test and control fabric materials.

16.2 The swatches to be used for testing will weigh 0.40g± 0.05g. The number of swatches will be counted.

16.3 Test and Control material will be autoclaved for sterilization as per BSLI SOP L-2017, *Preparation of dry goods for sterilization.*

16.4 Swatches will be placed aseptically in a specimen cup or other appropriate container with a screw cap.

16.5 The swatches weighting 0.4g in a specimen cup will represent one specimen.

17.0 TEST CONDITIONS :

17.1	Test Material Exposure:	4 hours ± 1 minute (T=4) 12 hours ± 1 minute (T=12) 23 hours to 24 hours (T=24)
17.2	Control Material Exposure:	4 hours ± 1 minute (T=4) 12 hours ± 1 minute (T=12) 23 hours to 24 hours (T=24) Immediate (T=0)
17.3	Exposure Temperature:	25°C ± 1°C T=0 at ambient temperature
17.4	Test and Control Replicates:	3
17.5	Elution/Neutralization Solution:	Dey-Engley Neutralizing Broth (D/E)

18.0 TEST METHOD:

18.1 Test Procedure:

18.1.1 An aliquot of 0.2mL of the test virus will be deposited onto specimens of test and control materials at several points of the specimen. Caps of the containers will be closed. The specimens will be transferred to an incubator. The exposure time will commence following virus application and conclude upon contact with elution/neutralizing solution.

18.1.2 Following exposure(s), 20 mL of D/E Broth will be added to the specimens. Caps will be closed and containers vortexed for 5 seconds 5 times to wash out the virus from the specimens. Wash-out solution will be diluted 10-fold in MM and plated onto cells.

18.2 Controls:

18.2.1 *Baseline Control (Wash-out immediately after deposit).* Three specimens of control materials will be inoculated with 0.2mL of the test virus at several points of the specimen. D/E broth will be added immediately after inoculation (T=0). Elution will be performed as described in Section 18.1.2. Wash-out solution will be diluted 10-fold in MM and plated onto cells.

18.2.2 *Neutralization Control (Verification of cytotoxicity by cell sensitivity to virus and the inactivation of antiviral activity).* 20ml of D/E broth will be added to three test and three control specimens. Elution will be performed as described in Section 18.1.2. Wash-out solution will be dispensed into tubes or wells of dilution reservoirs. Dilution 10^{-1} of test virus will be added to the first tube to obtain ~ 10^5 $TCID_{50}$/mL virus particles. Ten-fold virus titration will be performed in dispensed wash-out solution. Tubes or reservoir will be incubated for at least 30 minutes at 25°C.

18.2.3 *Initial Population (Test virus titration).* Test virus will be diluted in MM and plated onto cells.

18.2.4 *Cell Culture Control.* Intact cell culture monolayers will serve as the control of cell culture viability. The GM will be replaced by MM in all Cell Culture Control wells (minimum four wells).

18.2.5 Wash-out samples and dilutions will be plated onto cells in multi-well plates in eight replicates. The plates will be incubated for 10 to 14 days at 33 ± 2 °C in a CO_2 incubator. Cytopathic/cytotoxic effect will be monitored using an inverted compound microscope.

19.0 CALCULATIONS:

19.1 *Viral and cytotoxicity 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 (Behrens-Kärber Method) – will be applied.

$$Y = X \times 10a$$
$$a = \Sigma p - 0.5$$

Where:
Y is the infective titre ($TCID_{50}$/0.1 ml);
X is the dilution rate of the base virus suspension;
P is the ratio of the cytopathic effect at the respective dilution of the virus suspension.
Σp is the sum of values of p.

Infectivity titre: A (TCID50/ml):
$A = Y \times 10$

The infectivity titre ($TCID_{50}$/specimen) will be calculated using:
$V = A \times C$

Where:
V is the infective titre (TCID50/specimen);
C is the amount of wash-out virus suspension (ml).

19.2 *Calculation of Antiviral Activity value:*

$Mv = \log_{10}(Va/Vc) = \log_{10}(Va) - \log_{10}(Vc)$

Where:
Mv is the antiviral activity value;
$\log_{10}(Va)$ is average of 3 infectivity titre values immediately after inoculation of the control specimen (*Baseline*);
$\log_{10}(Vc)$ is average of 3 infectivity titre values after exposure time point with the Test specimen (*Test*).

20.0 TEST ACCEPTANCE CRITERIA:

A valid test requires: 1) The virus infectivity titre in the *Initial Population* is ≥ 7 $TCID_{50}$/mL; 2) Neutralization Control of Control specimen – Neutralization Control of Test specimen ≤ 0.5; 3) Reduction value of infective titre of Control specimen following 4-hour and 12-hour exposures is ≤ 1.0 $\log_{10}$ and is $< 2.0\log_{10}$ after 24 hours of exposure; 4) Cells in the Cell Control wells be viable and attached to the bottom of the well.

21.0 STATISTICAL ANALYSIS:

Descriptive statistics, including the means and standard deviations will be provided.

22.0 ANTIVIRAL PERFORMANCE OF MATERIAL:

The antiviral textile products may be evaluated by the categories according to Table F.1 of ISO18184.

TABLE – Antiviral Performance Standard

Item	Antiviral efficacy value, M_v	Standard
Tested textile material	$3.0\ \log_{10} > M_v \geq 2.0\ \log_{10}$	Good Effect
	$M_v \geq 3.0\ \log_{10}$	Excellent Effect

23.0 FINAL REPORT:

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

24.0 EXCEPTIONAL CONDITIONS:

The Study 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).

17.0 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 safe storage by the Testing Facility for a period of at least 5 years. BioScience Laboratories, Inc. will notify the Study Sponsor before any documents or records are destroyed.

18.0 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, the QA will perform an audit of the data and the Final Report in accordance with 21 CFR Part 58.

19.0 REFERENCE:

ISO 18184:2019(E), *Textiles — Determination of antiviral activity of textile products*.

20.0 LIABILITY AND INDEMNIFICATION:

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

21.0 ACCEPTANCE:

EVALUATION OF THE VIRUCIDAL PROPERTIES OF A TREATED FABRIC MATERIAL WHEN CHALLENGED WITH CORONAVIRUS

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

Study Director: _____ 04-01-2020
Volha Teagle, Ph.D. — Date of Initiation of Study

NOBLE BIOMATERIALS (SPONSOR)
300 Palm Street
Scranton, Pennsylvania 18505

_____ 03-30-2020
Representative — Date

Manger - Fabric Certification
Title

PROTOCOL AMENDMENT FORM

DATE: 03-27-2020 **AMENDMENT NUMBER:** 01

PROTOCOL NUMBER: 2003133-450

SPONSOR: NOBLE BIOMATERIALS

PROTOCOL TITLE: EVALUATION OF THE VIRUCIDAL PROPERTIES OF A TREATED FABRIC MATERIAL WHEN CHALLENGED WITH CORONAVIRUS

REASON FOR CHANGE(S): Sponsor requested to change standard method and exposure times.

CHANGE(S): Standard method will be ISO18184 instead of AATCC 100. Exposure times will be: 4 hours ± 1 minute, 12 hours ± 1 minute, and 23 to 24 hours.

APPROVALS:

Daryl S. Paulson	03-27-2020
CORPORATE MANAGEMENT	DATE
[signature]	03-27-2020
STUDY DIRECTOR / PRINCIPAL INVESTIGATOR	DATE
Not Applicable	Not Applicable
ASSOCIATE STUDY DIRECTOR / SUBINVESTIGATOR	DATE
Jodi Wallis	03-30-2020
SPONSOR	DATE
Not Applicable	Not Applicable
IRB COMMITTEE CHAIR *	DATE

* APPLICABLE: ☐ Yes ☐ No VT
Study Director / Principal Investigator Initials

REVIEWED BY:

Amy L. Juhnke	03-27-2020
QUALITY ASSURANCE	DATE

CITRIX

RightSignature

SIGNATURE CERTIFICATE

REFERENCE NUMBER
8220A775-87B5-4143-88C1-FDCA74D2CBB6

TRANSACTION DETAILS

Reference Number
8220A775-87B5-4143-88C1-FDCA74D2CBB6

Transaction Type
Signature Request

Sent At
03/27/2020 19:00 EDT

Executed At
03/30/2020 09:09 EDT

Identity Method
email

Distribution Method
email

Signed Checksum
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Signer Sequencing
Disabled

Document Passcode
Enabled

DOCUMENT DETAILS

Document Name
2003113-450 Amendment 01

Filename
2003113-450_amendment_01.pdf

Pages
1 page

Content Type
application/pdf

File Size
37.4 KB

Original Checksum
06c597ed8ddab51d7542bf78a71969762361b030182ffc4c447b9a4ada42d8bd

SIGNERS

SIGNER

Name
Jodi Wallis

Email
jwallis@x-static.com

Components
2

E-SIGNATURE

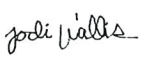
Status
signed

Multi-factor Digital Fingerprint Checksum
f3a7981c4bcfc3458ddb479ccc16472f9867310d9c249a51f64b106bddb3e03b

IP Address
72.28.57.19

Device
Microsoft Edge via Windows

Drawn Signature



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Signature Biometric Count
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Viewed At
03/30/2020 09:08 EDT

Identity Authenticated At
03/30/2020 09:09 EDT

Signed At
03/30/2020 09:09 EDT

Name
Volha Teagle, PhD

Email
vteagle@biosciencelabs.com

Components
2

Status
signed

Multi-factor Digital Fingerprint Checksum
4a56b17eb367783e03f3d01ad1832b0508164a57c62e986f2368cd185b36da02

IP Address
69.145.61.198

Device
Chrome via Windows

Drawn Signature

Signature Reference ID
074A670E

Signature Biometric Count
763

Viewed At
03/27/2020 21:32 EDT

Identity Authenticated At
03/27/2020 21:33 EDT

Signed At
03/27/2020 21:33 EDT

SIGNER	E-SIGNATURE	EVENTS
Name Daryl Paulson, MBA, PhD **Email** dpaulson@biosciencelabs.com **Components** 2	**Status** signed **Multi-factor Digital Fingerprint Checksum** f52299d8969f3ad04fd68ea526140f7a8fa2ea046c3da2a16d694d13f67bb960 **IP Address** 64.79.34.58 **Device** Microsoft Edge via Windows **Typed Signature** Daryl S. Paulson **Signature Reference ID** A2B951E1	**Viewed At** 03/27/2020 19:04 EDT **Identity Authenticated At** 03/27/2020 19:05 EDT **Signed At** 03/27/2020 19:05 EDT
Name Amy Juhnke, RQAP-GLP **Email** ajuhnke@biosciencelabs.com **Components** 2	**Status** signed **Multi-factor Digital Fingerprint Checksum** f8509f27028194554ae6c3099caa8eccc9e6ceef0759333004f4630d49a62870 **IP Address** 64.79.34.58 **Device** Chrome via Windows **Typed Signature** Amy L. Juhnke **Signature Reference ID** 3FD32673	**Viewed At** 03/27/2020 19:08 EDT **Identity Authenticated At** 03/27/2020 19:09 EDT **Signed At** 03/27/2020 19:09 EDT

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