July 09, 2020

FINAL REPORT #2004279-402

EVALUATION OF ONE TEST PRODUCT FOR ITS VIRUCIDAL PROPERTIES USING THE ASTM E1052 METHOD

Prepared by:

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

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EXECUTIVE SUMMARY

STUDY NUMBER: 2004279-402

TITLE: EVALUATION OF ONE TEST PRODUCT FOR ITS VIRUCIDAL PROPERTIES USING THE ASTM E1052 METHOD

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

STUDY **INITIATION DATE:** 05/29/2020

STUDY COMPLETION DATE: 07/09/2020

This study evaluated the virucidal properties of one test product when challenged with Coronavirus stain 229E (ATCC #VR-740). A Virucidal Suspension Test (In-Vitro Time-Hill method) based upon the ASTM E1052-11, *"Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension"* was used. The percent and logio reductions from the initial population of the viral stain was determined following exposure to the test product for 30 seconds and 60 seconds. 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 test product remained the responsibility of the Study Sponsor and was not performed by the Testing Facility (GLP 58.105).

Under the conditions of this evaluation Test Product #1, Disinfect & Shield Advanced Hand Sanitizer (Lot#05l22020-HS-A) reduced the infectivity of Coronavirus by >3.00 log,, (99.909a) following a 30-second and a 60-second exposure.

July 09, 2020

FINAL REPORT #2004279-402

1.0 TITLE: **EVALUATION OF ONE** TEST PRODUCT FOR ITS **VIRUCIDAL PROPERTIES**

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

4.0 STUDY DIRECTOR: Kelly Burningham

5.0 PURPOSE:

This study evaluated the virucidal properties of one test product when challenged with Coronavirus strain 229E (ATCC #VR-740), using a Virucidal Suspension Test (In-Vitro Time-Hill Method) based on ASTM E1052-11, *Standard Test Method to Assess the Activity of microbicides against Viruses in Suspension.* 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 test product remained the responsibility of the Study Sponsor and was not performed by the Testing Facility (GLP 58.105).

6.0 SCOPE:

A Virucidal Suspension Test (In-Vitro Time-Kill method) based upon the ASTM E1052-11 Method, *Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension* was used to evaluate the virucidal properties of one test product versus Coronavirus stain 229E (ATCC #VR-740). The percent and $\log_{10}$ reductions from the initial population of the viral strain was determined following exposure to the test product for 30 seconds and 60 seconds. Plating was performed in four replicates. The viral titers were determined using a 50% tissue culture infectious dose ($TCID_{50}$) calculation -- the Quantal test (Spearman-Kärber Method).

The Study Protocol, included as Addendum 1 of this Final Report, presents the study methodology, in detail. No deviations from the Study Protocol and no deviations from applicable Standard Operating Procedures occurred during the course of this evaluation.

7.0 STUDY DATES:

STUDY INITIATION **DATE:**	05/29/2020
EXPERIMENTAL START **DATE:**	06/05/2020
EXPERIMENTAL END DATE:	06/28/2020
STUDY COMPLETION DATE:	07/09/2020

8.0 TEST PRODUCT:

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

Test Product #1:	Disinfect & Shield Hand Sanitizer Benzalkonium Chloride 0.13%
Active Ingredients:	05122020-HS-A
Lot Number:	05/12/2020
Manufacture Date:	05/12/2022
Expiration Date:	

9.0 CHALLENGE VIRAL STRAIN:

Coronavirus strain 229E (ATCC #VR-740)

10.0 HOST CELLS:

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

11.0 SUPPLIES AND EOUIPMENT:

The equipment and supplies used in this study are as described in the Study Protocol in Addendum I of this Final Report.

12.0 MEDIA:

The growth media and diluting fluids used in this study are as described in the Study Protocol in Addendum 1 of this Final Report.

13.0 HOST CELL PREPARATION:

MRC-5 cells were maintained as monolayers in disposable cell culture labware and were used for the Virucidal Suspension Test for testing Coronavirus. Prior to testing, host cell cultures were seeded onto 24-well cell culture plates. Cell monolayers were sufficiently confluent (90%) and less than 48 hours old before inoculation with the virus. The growth medium (GM) was replaced by maintenance medium (MM) to support virus propagation.

14.0 TEST VIRUS PREPARATION:

Test virus used for this study was from BSLI high titer virus stock. On the day of use, aliquots of a stock virus were removed from a -70°C freezer and thawed prior to use in testing.

15.0 TEST PRODUCT PREPARATION:

The test product was used as received. Test concentrations were 90%.

16.0 VIRUCIDAL SUSPENSION TEST:

The Virucidal Suspension Test included the following parameters (Table 1):

TABLE 1

Parameters of Virucidal Suspension Test

Parameter	Summary	Replicates
Virucidal suspension test	Virus+ Test Product ➔ Exposure ➔ Neutralization ➔ Dilution ➔ Plating	4 per group
Virus Control	Virus + Diluent ➔ Exposure ➔ Dilution ➔ Plating	4 per group
Cytotoxicity Control	Test Product + Diluent ➔ Neutralization ➔ Dilution ➔ Plating	4 per group
Neutralization Control	Test Product + Diluent ➔ Neutralization ➔ Virus inoculation ➔Dilution ➔ Plating	4 per group
Neutralizer Toxicity Control	Virus + Diluent ➔ Neutralization ➔ Dilution ➔ Plating	4 per group
Cell Culture Control	Maintenance medium	4 per group

17.0 RESULTS-TABLE 2:

Table 2 presents the data from the Virus Control infectivity (TCIDso) and the post-exposure infectivity (TCIDso); the $\log_{10}$ and percent reductions observed following a 30-second and 60-second exposure of Coronavirus strain 229E to Test Product #1, Disinfect & Shield /Glan Health Advanced Hand Sanitizer (Lot #05122020-HS-A).

TABLE 2

Test Product #1 Disinfect & Shield Hand Sanitizer
Virus: Coronavirus Strain 229E (ATCC#VR-740)
Host Cell Line: MRC-5 (ATCC #CCL-171)
Volume Plated per Well: 1.0 mL

Dilution (- Log10)	Virus Control	test		Neutralization Control	NTC	Cytotoxicity Control	Cell Control
		30 sec	1 min				0000
-2	NT	CT	CT	NT	NT	++++	
-3	++++	CT	CT	CT	++++	++++	
-4	++++	0000	0000	++++	++++	0000	
-5	++++	0000	0000	++++	++++	NT	
-6	+++0	0000	0000	00++	+0+0	NT	
-7	00+0	0000	0000	0000	0000	NT	
TCID 50 (-Log10)	6.5	≤ 3.5	≤ 3.5	6.00	6.00	3.5	
Log10 Reduction	NA	≥ 3.00	≥ 3.00				
Percentage Reduction		99.90%	99.90%				

+	CPE(cytopathiclcytotoxic effect) present
0	CPE (cytopathiclcytotoxic effect) not detected
NT	Not tested
NIA	Not applicable
CT	Cytotoxicity
NTC	Neutralization Toxicity Control

NIA

18.0 **STUDY CONCLUSIONS**

Under the conditions of this evaluation Test Product #1 Disinfect & Shield Advanced Hand Sanitizer (Lot#05122020-HS-A), Reduced the infectivity of Coronavirus strain 229E by >.3.00 $\log_{10}$ (99.90%) following a 30-second and a 60-second exposure.

19.0

JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM:

The Sponsor requested an efficacy evaluation against Coronaviruses including 2019 novel strain SARS-CoV- 2, the cause ofCOVID-19, using Coronavirus strain 229E for testing of efficacy. 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, regulato1y agencies such as the US EPA and Health Canada created a list ofsurrogate viruses that possess equivalent susceptibility and belong to the same virus family as the viruses desired for a disinfectant or antiseptic efficacy claim. Coronavirus 229E (BioSafety Level 2) is an appropriate and a safe choice for efficacy testing of hand sanitizers.

#05
30- 122020-HS-A), reduced the infec
second and a 60-second exposure.

20.0 STATISTICAL ANALYSIS:

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

21.0 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 fi'oin the Study Protocol and no deviations from applicable BioScience Laboratories, Inc., Standard Operating Procedures were observed.

22.0 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:

Kelly Burninghain
Virologist

Rachel Byrd, M.S.
Microbiologist

Jared Montana, M.S.
Microbiologist

Stephanie Cebulla
Laboratory Support Technician

Dakotah Olson
Product Handler

Marc Charnholm
Manager of Laboratory Suppoit

Terah Rash
Microbiologist

Brooke Kapalka
Laboratory Support Technician

Alexander Stanley
Microbiologist

Kameron I4ohn
Microbiologist

23.0 QUALITY ASSURANCE AND QUALITY CONTROL PERSONNEL:

Jeremy Duley
Systems Administrator/QC Specialist

Renee LaFond, M.S.
Quality Assurance Specialist

Danielle Goveia
Quality Assurance Specialist

Chet Trumbull
QC Specialist

Amy L. Juhnke, RQAP-GLP
Director of Quality Assurance

24.0 DOCUMENTATION AND RECORD KEEPING:

All documentation and records were compiled, analyzed, and will be retained by BioScience Laboratories, Inc. at its facility in Bozeinan, 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.

25.0 ACCEPTANCE:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South l9th Avenue
Bozeinan, Montana 59718

Study Director: _____
Kelly Burningham

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	06/05/2020	06/08/2020	06/09/2020
Data Audit	07/07/2020	07/08/2020	07/08/2020
Final Report Review	07/07/2020	07/08/2020	07/08/2020

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 material were not performed by BioScience Laboratories, Inc. No deviations from the Protocol or from applicable Standard Operating Procedures occurred. This statement also serves to confirm that the Final Report reflects the raw data.

Director of Quality Assurance: _____
Amy L. Juhnke, RQAP-GLP

07/09/2020
Date

ADDENDUM

Protocol #2004279-402

May 29, 2020

PROTOCOL #2004279-402

EVALUATION OF ONE TEST PRODUCT FOR ITS VIRUCIDAL PROPERTIES USING THE ASTM E1052 METHOD

Prepared by:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19"' Avenue
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CONFIDENTIAL

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PROTOCOL #2004279-402

1.0 TITLE: EVALUATION OF ONE TEST PRODUCT FOR ITS VIRUCIDAL PROPERTIES USING THE ASTM E1052 METHOD

3.0 TESTING FACILITY: BIOSCIENCE LABORATORIES, INC.
1755 South 19"' Avenue
Bozeinan, Montana 59718

4.0 STUDY DIRECTOR:

Kelly Buminghain

5.0 PURPOSE OF STUDY:

This study will evaluate the virucidal properties of one test product when challenged with Coronavirus. The testing will be based upon ASTM E1052-11, *Standai'd Test Method to Assess the Activi3› of Microbicides against Viruses in Suspension.* 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 product(s) remains the responsibility of the Study Sponsor and will not be performed by the Testing Facility (GLP 58.105).

6.0 SCOPE:

This study is designed to evaluate the virucidal properties of one test product versus Coronavirus using a Virucidal Suspension Test (In-Vitro Time-Kill method). The percent and logl0 reductions from the initial population of the viral strain(s) will be determined following exposure to the test product(s) for 30 seconds and 60 seconds. Plating will be performed in four replicates.

7.0 TEST PRODUCT:

The test product 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, and stability of the test product(s), as well as the retention of the test product(s), rests with the Sponsor.

Test Product #1:	Disinfect & Shield Advanced Hand Sanitizer Lot
Number:	05122020-HS-A
Manufacture Date:	05/12/2020
Expiration Date:	05/12/2022

8.0 CHALLENGE VIRAL STRAIN:

Coronavirus strain 229E (ATCC#VR-740)

HOST CELLS:

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

10.0 EQUIPMENT:

10.1 Ultralow Temperature Freezer, Temperature Range < -70°C
10.2 CO2 Incubator, Temperature Range 37 °C ± 2 °C
10.3 COC Incubator, Temperature Range 35 °C ± 2 °C
10.4 Refrigerators, 2 °C - 8 °C
10.5 Water Bath, 37 °C ± 2 °C
10.6 Incubator, Refrigerator, Freezer, and Water Bath Thermometers
10.7 Continuously Adjustable Pipettes, 100 µL — 1000 µL Capacity
10.8 Continuously Adjustable Pipettes, 20 µL — 200 µL Capacity
10.9 Portable Pipetter
10.10 Inverted Compound Microscope
10.11 Laminar Flow Biological Safety Cabinet
10.12 Calibrated Minute/Second Timers

11.0 SUPPLIES:

11.1 Sterile Disposable Pipettes
11.2 Sterile Polystyrene Test Tubes
11.3 Sterile Universal 1.0 and 0.2 mL Pipette Tips
11.4 Powder-free Gloves
11.5 Sterile Tissue Culture Treated Multi-well Plates
11.6 Viral suspension
11.7 Sterile 100 µL and 1000 µL Positive Displacement Tips
11.8 Sterile Flasks
11.9 Sterile 50 mL Centrifuge Tubes
11.10 Sterile Reservoirs
11.11 Waste Pan
11.12 Non-Sterile Waste Beaker for discarded tips, etc.

12.0 **MEDIA**:

12.1 lX Minimum Essential Medium (MEM) or other appropriate medium
12.2 Growth Medium: MEM or other appropriate medium with 10% non-heat inactivated FBS, 1% penicillin-streptomycin-amphotericin B, 1% of L-Glutamine (when necessary)
12.3 Maintenance Medium: MEM or other appropriate medium with 2% non-heat inactivated FBS, 1% penicillin-streptomycin-amphotericin B, 1% of L-Glutamine (when necessary)
12.4 Trypsin
12.5 Antibiotics (e.g., Penicillin-Streptomycin-Amphotericin B)
12.6 Fetal Bovine Serum (FBS)
12.7 D/E Neutralizing Broth

13.0 HOST CELL PREPARATION:

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 MEM or as appropriate for each cell culture.

14.0 TEST VIRUS PREPARATION:

Virus propagated and stored per BSLI SOP L-2102, *Procedure for Production of High-Titered Virus its 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 ft'eezer and thawed.

15.0 VIRUCIDAL SUSPENSION TEST:

15.1 The Virucidal Suspension Test will include the following parameters:

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

15.2 *Test.* A 0.5 mL aliquot of test virus(s) will be added to a vial containing 4.5 inL of the undiluted test product to achieve a 90% (v/v) concentration of the test product. The test virus(s) will be exposed to the test product for 30 seconds and 60 seconds, timed using a calibrated minute/second timer. The calibrated minute/second timer will be started within 1 second of adding the challenge suspension. Immediately after each exposure, the test virus(s)/product suspensions will be neutralized in D/E Neutralizing Broth, mixed thoroughly, and serially diluted in MM. Each dilution will be plated in four replicates.

15.3 Virus *Control.* A 0.5 mL aliquot of test virus(s) will be added to 4.5 inL of MM and exposed for 60 seconds at ambient temperature. The subsequent test virus dilution will be made in MM and serially diluted in MM. Each dilution will be plated in four replicates.

15.4 C*ytotoxicity Control.* A 0.5 mL aliquot of MM will be added to a vial containing 4.5 inL of the undiluted test product. The MM/product mixture will be neutralized in D/E Neutralizing Broth, mixed thoroughly and serially diluted in MM. Each dilution will be plated in four replicates.

15.5 *Neutralization Control.* A 0.5 mL aliquot of MM will be added to a vial containing 4.5 inL of the undiluted test product. The MM/product mixture will be diluted 1:10 in D/E Neutralizing Broth. An aliquot of the virus(s) will be added to the neutralized product and thoroughly mixed and exposed to the neutralized product for 10 to 20 minutes. Additionally, the effect of the neutralizer on virus infectivity will be assessed by adding virus to the neutralizer (D/E Neutralizing Broth) alone followed by exposure for 10 to 20 minutes. Subsequent 10-fold dilutions of neutralized test product/virus suspension will be made in MM. Each dilution will be plated in four replicates.

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

15.7 The plates will be incubated in a CO2 incubator for 10 to 14 days at 35 °C + 2 °C in a COC incubator. Cytopathic/cytotoxic effect will be monitored using an Inverted Compound Microscope.

Note: In cases when viral CPE is undetectable using Inverted Compound Microscope, additional iininunostaining with vii'us specific antibodies can be performed.

16.0 CALCULATIONS:

16.1 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 (TCIDso) calculation - the Quanta! test (Spearman-Karber Method) - will beapplied.

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

Where:
L = -log,o of the lowest dilution;
d - difference between dilution steps;
s = sum ofpropmtions of positive wells.

16.2 The log10 of infectivity reduction will be calculated as follows:

Log,o Reduction Formula:

Log,o Reduction = (log,o TCID*so* of the Virus Control) - (log,o TCIDso of the Virucidal Suspension Test)

16.3 The percent reduction will be calculated as follows:

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

17.0 STATISTICAL ANALYSIS:

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

18.0 TEST ACCEPTANCE CRITERIA:

A valid test requires that: 1) at least 4 log10 ofTCIDso be recovered from the Virus Control; 2) cells in the cell culture wells be viable and attached to the bottom of the well; 3) the medium be free of contamination in all wells of the plate; 4) when cytotoxicity is evident, at least a 3 log10 reduction in titer be demonstrated beyond the cytotoxic level, and 5) the test product be fully neutralized after the timed exposure such that the difference in virus titer for the Neutralization Control and Virus Control does not exceed 1.0 $\log_{10}$.

19.0 FINAL REPORT:

A Final Report will be issued that presents the results in a clear and concise manner.

20.0 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).

21.0 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 product for use as defined in the Study Protocol.

22.0 DOCUMENTATION AND RECORD-M'EPING:

All documentation and records will be compiled, analyzed, and retained by BioScience Laboratoi ies, 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 Sponsor before any records or documents are destroyed.

23.0 PRODUCT 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 returned to the Sponsor at the conclusion of the study.

24.0 QUALITY ASSURANCE AUDITS:

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

25.0 **ACCEPTANCE**:

EVALUATION OF ONE TEST PRODUCT FOR ITS VIRUCIDAL PROPERTIES USING TH E ASTM E1052 METHOD

ACCEPTED BY: BIOSCIENCE LABORATORIES, INC. (’TESTING FACILITY)
1 755 South l9th Avenue
Bozeman, Montana 59718

Study Director: KellyBurninghan Date of Study Initiation

ACCEPTED BY: DALRADA HEALTH PRODUCTS (SPONSOR)
600 La Terraza Boulevard
Escondido, California 92025

Representative Date