FINAL STUDY REPORT

Study Title
Custom Virucidal Efficacy of an Ultraviolet Light Disinfection Device

Product(s) Identity
Test Device Name: KR615 and KR615-AUTO

Test Microorganism
Human coronavirus, 229E strain, ATCC VR-740

Data Requirements
U.S. EPA OCSPP 810.2200

Author
Victoria Zarate, B.S.
Study Director

Study Completion Date
25AUG2021

Testing Facility
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, Texas 78681

Study Sponsor
AUVS Advanced Ultra-Violet Systems
2427 Craig Mill Road
South Hill, VA 23970

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA section 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: _____

Agent/Submitter: _____

Title: _____

Date: _____

Signature: _____

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets U.S. Environmental Protection Agency's Good Laboratory Practice Standards and requirements for 40 CFR Part 160 with the following exception(s):

> Per the signed protocol, the Study Sponsor indicated that the applicable identity, strength, purity, stability, and uniformity testing had not been or would not be completed prior to efficacy testing. The Study Sponsor also indicated that stability testing had not been or would not be completed prior to efficacy testing or concomitantly with efficacy testing.

Records concerning test substance characteristics (i.e. composition, purity, stability, strength, solubility) and test device characteristics (i.e. model, manufacturing, materials, history, etc.) are maintained by the Study Sponsor.

The test device, provided by the Study Sponsor, was calibrated by the Study Sponsor. Additional information concerning device functionality and calibration is maintained by the Study Sponsor.

Study Director

Company: Microchem Laboratory
Name: Victoria Zarate, B.S.
Title: Study Director

Signature: _____ Date: 25AUG2021

Study Sponsor

Company: AUVS Advanced Ultra-Violet Systems
Name: David Henderson
Title: Study Sponsor

Signature: _____ Date: _____

Submitter

Company:
Name:
Title:

Signature: _____ Date: _____

QUALITY ASSURANCE STATEMENT

The following quality assurance audits were conducted in accordance with Good Laboratory Practice Standards outlined in 40 CFR §160 and reported to management and the Study Director:

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
In Phase	21JUL2021	21JUL2021	22JUL2021
Draft Report Audit	24AUG2021	24AUG2021	24AUG2021
Final Report Audit	25AUG2021	25AUG2021	25AUG2021

Signature: _____ Date: 25AUG2021

Name: Audrey Landrum, B.S.
Title: Specialist I, Quality Assurance

PERSONNEL INVOLVED IN THE STUDY

Study Director

Name: Victoria Zarate, B.S.
Title: Team Lead, Virology

Professional or Supervisory Personnel

Name: Madhuri Patil, M.S.
Title: Analyst II

Name: Jose Vides, B.S.
Title: Associate Analyst

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FINAL STUDY REPORT SUMMARY

Study Title: Custom Virucidal Efficacy of an Ultraviolet Light Disinfection Device

Study Identification Number: GLP2834

Test Microorganism: Human coronavirus, 229E strain, ATCC VR-740

Host Cell: MRC-5 cells (ATCC CCL-171)

Test Device(s): KR615 and KR615-AUTO

Test Device Serial Number(s): 3697 and 3692

Test Device Receipt Date: Date Received: 21MAY2021

Organic Soil Load: 5% fetal bovine serum (FBS)

Carrier Type: RD100 Fused Quartz Petri Dishes (100 mm x 15 mm) provided by Study Sponsor

Number of Carriers Per Lot: Two

Contact Time(s): 30 seconds and 60 seconds

Exposure Temperature: Room temperature (22.9 °C) and 48–49% Relative Humidity (RH)

Neutralization Method: 2% FBS EMEM test media (2.0 ml)

Study Results:

Description	Assay Results			
Device	KR615		KR615-AUTO	
Contact Time	30 seconds		60 seconds	
Test Replicate	1	2	1	2
$TCID_{50}$ per 0.1 ml	$\leq 0.50 \log_{10}$	$\leq 0.50 \log_{10}$	$\leq 0.50 \log_{10}$	$\leq 0.50 \log_{10}$
$TCID_{50}$ per Carrier	$\leq 0.80 \log_{10}$	$\leq 0.80 \log_{10}$	$\leq 0.80 \log_{10}$	$\leq 0.80 \log_{10}$
Avg. $TCID_{50}$ per Carrier	$\leq 0.80 \log_{10}$		$\leq 0.80 \log_{10}$	
Avg. Recovery Control per Carrier	$5.55 \log_{10}$		$5.87 \log_{10}$	
Log_{10} Reduction per Carrier	$\geq 4.75 \log_{10}$		$\geq 5.07 \log_{10}$	

STUDY DATES

Study Initiation Date: 21JUL2021
Experimental Start Date/Time: 21JUL2021/1337
Experimental End Date/Time: 28JUL2021/0841
Study Completion Date: 25AUG2021

TEST DEVICE

Device Name: KR615

Serial Number: 3697
Date of Manufacture: 15MAR2021
Date Received: 21MAY2021

Device Name: KR615-AUTO

Serial Number: 3692
Date of Manufacture: 15MAR2021
Date Received: 21MAY2021

Form: Ultraviolet light disinfection device

Storage Conditions: Ambient room temperature under fluorescent lighting.

PROTOCOL CHANGES

Protocol Amendment(s)

No amendments to the approved protocol were made for this study.

Protocol Deviation(s)

There were no deviations from the approved protocol during the conduct of this study.

TEST OBJECTIVE

The purpose of this study was to document the virucidal efficacy of the test device against the test system (microorganism) under the test parameters specified in the protocol. The test protocol was in compliance with the requirements of and may be submitted to one or more of the following agencies as indicated by the Study Sponsor: U.S. Environmental Protection Agency (EPA) and Health Canada.

TEST PROCEDURE

Test System (Microorganism)

Human coronavirus, 229E strain, ATCC VR-740, originally received from the American Type Culture Collection (ATCC), Manassas, VA was used in this study. The Microchem Laboratory lot number used in testing was HCoV_21MAR2021C.

ATCC® microorganisms are used under commercial license. The ATCC trademark and trade name and any and all ATCC catalog numbers are trademarks of the American Type Culture Collection.

Preparation of the Test Virus

The test virus was propagated internally by Microchem Laboratory personnel by inoculating the virus into cell culture flasks containing the appropriate host cell line and incubating at the appropriate conditions. Once the cell culture flasks displayed approximately 75–100% cytopathic effect (as determined by microscopic evaluation), the flasks were subjected to freeze-thaw cycles to release virus from infected cells. The contents of the cell culture flasks were collected and centrifuged in order to remove the cell debris. The test virus was then aliquoted and stored at ≤-70 °C.

On the day of testing, an aliquot of the stock virus was removed from cryostorage and thawed for use in the assay. The test virus contained 2% fetal bovine serum (FBS) organic soil load. The test virus was adjusted to contain 5% FBS organic soil load by adding 0.06 ml of FBS to 1.90 ml of test virus.

Host Cell-Line

MRC-5 cells (ATCC CCL-171), originally received from the ATCC, were utilized in the assay. The cells were subcultured by Microchem Laboratory personnel and seeded into 24-well cell culture plates. The plates were incubated at 36±2 °C in a humidified atmosphere of 6±1% CO_2 until they reached the desired confluence required for testing. On the day of use, the cells were microscopically examined to verify the appropriate confluency and health of the cells.

ATCC® microorganisms are used under commercial license. The ATCC trademark and trade name and any and all ATCC catalog numbers are trademarks of the American Type Culture Collection.

Test Medium

The test medium utilized in the assay was Eagle's Minimum Essential Medium (EMEM) supplemented with 2% FBS, 40 mM HEPES buffer, 125 µM non-essential amino acids, 1 mM sodium pyruvate, plus antibiotics [antibiotic-antimycotic solution (100 units/ml penicillin G, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B)]. Concentrations based on preparation of 1 L of Eagle's Minimal Essential Medium.

TEST PROCEDURE (cont.)

Preparation of Test Carriers

Eight of Study Sponsor-provided RD100 fused quartz petri dishes (100 x 15 mm) free from scratches, chips, or cracks were soaked in 70-95% reagent alcohol for ≥ 30 minutes to remove oil and film. The carriers were then thoroughly rinsed using two separate deionized (DI) water rinses and dried. The dry carriers were placed in single layers on autoclavable trays lined with absorbent towels and autoclave sterilized on a fast/dry cycle for ≥20 minutes at approximately 121°C.

Preparation of Virus Films

The test virus was vortexed thoroughly and a 0.200 ml aliquot of virus was placed on the inside bottom surface of eight 100 mm x 15 mm sterile glass Petri dishes which served as the test carriers and plate recovery control. The inoculum was spread over the entire area of the carriers using a sterile bent pipette tip without touching the sides of the Petri dish. The virus films were dried in an environmental chamber for 26 minutes at 20.0 °C in a relative humidity of 30%.

Preparation of the Test Device

Each test device was set up and operated per the Study Sponsor-provided instructions. Carrier holders were UV bulbs and these were not wiped with reagent alcohol. Each device was turned on and a warm-up cycle was performed for 30 seconds prior to use in testing.

Exposure of Virus Films to the Test Substance

For each device tested, two dried virus film carriers were placed uncovered inside the test device and the door to the device was shut and secured. Each device was allowed to run for the Study Sponsor-requested contact time, as measured by a digital timer (Unit KR615, serial number 3697 tested at 30 seconds and Unit KR615-AUTO, serial number 3692 tested at 60 seconds, at at an exposure temperature of 22.9 °C in a relative humidity of 48–49%. At the conclusion of the contact time and once each device had been turned off, the carriers were removed from the device and transferred to a biological safety cabinet. A 2.0 ml aliquot of test media was added to each carrier. The carriers were gently rotated to ensure complete coverage of the solution over the entirety of the surface. Using sterile cell scrapers, the carriers were scraped to re-suspend the viral films and the suspensions were transferred into sterile vessels. Serial 10–fold dilutions using 0.1 ml of test media and 0.9 ml of test assay media were prepared to the appropriate dilution.

STUDY CONTROLS

Plate Recovery Control

Two plate recovery control films per contact time were prepared to determine the baseline dried virus titer. The plate recovery control film was generated as described above in "Preparation of Virus Films." The dried carriers were allowed to dwell uncovered in ambient conditions. Following the Study Sponsor-requested contact time, a 2.0 ml aliquot of test media was added to each control film. The carriers were gently rotated to ensure complete coverage of the solution over the entirety of the surface. A sterile cell scraper was used to re-suspend each viral film and the suspensions were transferred to sterile vessels. Serial 10-fold dilutions using 0.1 ml of test media and 0.9 ml of test assay media were prepared to the appropriate dilution.

Cytotoxicity Control

Unlike chemical germicides, ultraviolet light should not produce cellular toxicity, nor should the sterile fused quartz carriers used, and nor should the recovery medium. Therefore this control was appropriately not included in the study.

Test Substance Neutralization Control

Unlike chemical germicides, antimicrobial activity of ultraviolet light should not persist after the conclusion of the contact time. Therefore this control was appropriately not included in the study.

Cell Culture Control

To ensure that the host cells were not contaminated with bacteria, fungi, or any cytopathogenic viruses, and to confirm the viability of the cells during the incubation period of the assay, at least four cell monolayers were left untreated and microscopically examined periodically throughout the incubation period. Any obvious contamination or degeneration in such monolayers could invalidate the virucidal efficacy assay.

Virus Inoculum Titer Control

To confirm that the host cell-line monolayers were susceptible to the test virus and to confirm the titer of the viral inoculum, an aliquot of the virus inoculum employed in the test was serially diluted 10-fold in test media.

Infectivity Assay

A 0.1 ml aliquot of all test and control dilutions was inoculated into the host cell cultures (which contained test medium) in quadruplicate. The cell culture control wells contained just test medium. The assay plates were incubated at 33±2 °C in a humidified atmosphere of 6±1% CO_2 for 7 days. The assay plates were examined microscopically periodically throughout the incubation period with any changes to the monolayers including viral cytopathic effects (CPE), cytotoxicity, or contamination clearly documented in the raw data. Data obtained from the final reading are documented in the "Results" section of this report.

SUCCESS CRITERIA

The following measures are met to ensure the acceptability of virucidal efficacy data:

- The virus titer control demonstrates obvious and or typical cytopathic effects on the monolayers unless a detection method other than cytopathic effect is used.
- A minimum of 4.80 $\log_{10}$ infective units/control carrier is recovered from each plate recovery control film(s).
- Quantification of the test and control parameters is conducted at a minimum of four determinations per dilution.
- The cell controls are negative for infectivity and demonstrate typical cell morphology.

Product Performance Criteria

- The log and percent reduction of the test virus following exposure to the test substance are calculated however, there is no minimum reduction level to qualify as "passing" or an "efficacious" product.
- For liquid/spray/towelette products, the U.S. EPA performance criteria for disinfection follows:
  - In the presence or absence of cytotoxicity, the product should demonstrate a $\geq$3.00-$\log_{10}$ reduction in viral titer on each surface.

CALCULATIONS AND STATISTICAL ANALYSIS

- The $TCID_{50}$ (Tissue Culture Infectivity Dose) represents the endpoint dilution where 50% of the cell cultures exhibit cytopathic effects due to infection by the test virus. The endpoint dilution at which 50% of the host cell monolayers exhibit cytotoxicity is termed the Tissue Culture Dose (TCD_{50}). The $TCID_{50}$ and TCD_{50} were determined using the Spearman-Kärber method and calculated as follows:

  Negative logarithm of endpoint titer =

  [– Log of first dilution inoculated] – [((sum of % mortality at each dilution/100) – 0.5) x Logarithm of dilution]

- The result of this calculation is expressed as $TCID_{50}$/volume of dilution inoculated (e.g. 0.1 ml) for the test and plate recovery control.

- Determination of viral titer per carrier is established by accounting for the volume of viral inoculum per carrier.

Calculation of the Log_{10} Reduction

- The log_{10} reduction in viral titer was calculated as follows:

  Control Log_{10} $TCID_{50}$/0.1 ml – Virus-Test Device Log_{10} $TCID_{50}$/0.1 ml

- If multiple plate recovery control and test replicates were performed, the average $TCID_{50}$ of each parameter was calculated and the average result used to calculate the log_{10} reduction in viral titer following:

  Average $TCID_{50}$/Carrier (double replicate) = $\log_{10}[((10^{TCID50\ rep\ 1}) + (10^{TCID50\ rep\ 2}\ 2))/2]$

Statistical Analysis

Not applicable.

Methods for the Control of Bias

Not applicable.

DATA AND SAMPLE RETENTION

Study Record Retention

The original (or certified copy) of the study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory indefinitely For studies not meeting the performance criteria for submission or for studies that have been canceled prior to the generation of valid data, the original (or certified copy) of the final study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory for a minimum of two years following the study completion date at which time they may be removed from the archive or transferred to the Sponsor's archive at their expense.

If requested by the Study Sponsor (or Sponsor Representative), the study file may be transferred to the Study Sponsor's archive at the Study Sponsor's expense prior to the time frames listed.

All test facility records including, but not limited to, standard operating procedures, quality assurance inspection records, temperature and equipment records including maintenance, inspection and calibration, and employee training records will be maintained at Microchem Laboratory indefinitely.

Test Substance Retention

The test substance (or test control, test article, test device, as applicable) may be returned to the Study Sponsor at the Study Sponsor's request and expense following study completion unless otherwise requested to be returned earlier. If the Study Sponsor does not request return of the sample, it will be disposed >90 days following the study completion. Arrangements may be made for extended storage as necessary, at the Sponsor's request and expense.

RESULTS

Table 1: Plate Recovery Control Results

	Plate Recovery Control			
Device	KR615		KR615-AUTO	
Contact Time	30 seconds		60 seconds	
Replicate	1	2	1	2
Dilution 10^{-1}	+ + + +	+ + + +	+ + + +	+ + + +
Dilution 10^{-2}	+ + + +	+ + + +	+ + + +	+ + + +
Dilution 10^{-3}	+ + + +	+ + + +	+ + + +	+ + + +
Dilution 10^{-4}	+ + + +	+ + + +	+ + + +	+ + + +
Dilution 10^{-5}	+ + 0 +	+ + 0 +	+ + 0 +	+ + + +
Dilution 10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0	0 + 0 0
$TCID_{50}$ per 0.1 ml	5.25 log_{10}	5.25 log_{10}	5.25 log_{10}	5.75 log_{10}
$TCID_{50}$ per Carrier	5.55 log_{10}	5.55 log_{10}	5.55 log_{10}	6.05 log_{10}
Avg. $TCID_{50}$ per Carrier	5.55 log_{10}		5.87 log_{10}	

Table 2: Test Results

	Test Results			
Device	KR615		KR615-AUTO	
Contact Time	30 seconds		60 seconds	
Replicate	1	2	1	2
Dilution 10^{-1}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Dilution 10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Dilution 10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Dilution 10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Dilution 10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Dilution 10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml	≤0.50 log_{10}	≤0.50 log_{10}	≤0.50 log_{10}	≤0.50 log_{10}
$TCID_{50}$ per Carrier	≤0.80 log_{10}	≤0.80 log_{10}	≤0.80 log_{10}	≤0.80 log_{10}
Avg. $TCID_{50}$ per Carrier	≤0.80 log_{10}		≤0.80 log_{10}	
Avg. Log_{10} Reduction per Carrier	≥4.75 log_{10}		≥5.07 log_{10}	

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Cytotoxicity observed; N/A = not applicable

RESULTS (cont.)

Table 3: Virus Inoculum Titer Control

		Virus Inoculum Titer Control
Cell Control		0 0 0 0
Dilution	10^{-1}	N/A
	10^{-2}	+ + + +
	10^{-3}	+ + + +
	10^{-4}	+ + + +
	10^{-5}	0 + + +
	10^{-6}	0 0 0 0
$TCID_{50}$ per 0.1 ml		5.25 log_{10}

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Cytotoxicity observed; N/A = not applicable

STUDY CONCLUSION

The purpose of the study was to determine the virucidal efficacy of KR615 (S/N: 3697) for a contact time of 30 seconds and KR615-AUTO (S/N: 3692) for a contact time of 60 seconds against Human coronavirus, 229E strain, ATCC VR-740, supplemented with a 5% FBS soil load, at an exposure temperature of room temperature (22.9 °C) and 48–49% RH).

The Plate Recovery Control demonstrated an average viral titer of 5.55 $\log_{10}$ $TCID_{50}$ per carrier at a contact time of 30 seconds and 5.87 $TCID_{50}$ per carrier at a contact time of 60 seconds, thereby satisfying U.S. EPA study acceptance criteria of a minimum of 4.80 $\log_{10}$ infective units per control carrier.

The evaluated test device, KR615 (S/N: 3697) demonstrated an average $\geq$4.75 $\log_{10}$ reduction in viral titer, as compared to the titer of the corresponding plate recovery control.

The evaluated test device, KR615-AUTO (S/N: 3692) demonstrated an average $\geq$5.07 $\log_{10}$ reduction in viral titer, as compared to the titer of the corresponding plate recovery control.

No microbial contamination of any host cell cultures was observed during the course of the study.

KR615 (S/N: 3697) for a contact time of 30 seconds and KR615-AUTO (S/N: 3692) for a contact time of 60 seconds met the U.S. EPA Product Performance Guidelines for Disinfectants for Use on Hard Surfaces outlined in U.S. EPA OCSPP 810.2200 and the success criteria detailed in the approved protocol when tested against Human coronavirus, 229E strain, ATCC VR-740.

This study was carried out in compliance with the approved protocol. All experimental controls met the established acceptance criteria unless otherwise noted in the Protocol Changes section of this Report.

There were no circumstances that may have affected the quality or the integrity of the data.

REFERENCES

- *Annual Book of ASTM Standards*, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, Designation E1053, current edition. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

- Annual Book of ASTM Standards, Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, Designation E1482, current edition. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.

- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.

- U.S. Environmental Protection Agency, Frequent Questions for the 2018 Series 810 – Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines. 2019.

- Guidance Document – Disinfectant Drugs. Health Canada. April 2020.

- Guidance Document – Safety and Efficacy Requirement for Hard Surface Disinfectant Drugs. Health Canada. April 2020.

PROTOCOL

MICROCHEM LABORATORY

Protocol Number: P3382 Study ID Number: GLP 2834

21JUL2021 VN2

Protocol Title
Custom Virucidal Efficacy of an Ultraviolet Light Disinfection Device

Test Microorganism
Human coronavirus, 229E strain, ATCC VR-740

Data Requirements
U.S. EPA OCSPP 810.2200

Study Sponsor
AUVS Advanced Ultra-Violet Systems
2427 Craig Mill Road
South Hill, VA 23970

Testing Facility
Microchem Laboratory
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Prepared By:
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Date
25JUN2021

PROTOCOL (cont.)

Custom Virucidal Efficacy of an Ultraviolet Light Disinfection Device

Protocol Number: P3382

I. Introduction

This document details the materials and procedure for evaluating the virucidal efficacy of the Study Sponsor's submitted test device using a modified ASTM E1053 test method. Testing will be performed in accordance with Good Laboratory Practice Standards (GLPS) stipulated by U.S. EPA 40 CFR Part 160 as well as the U.S. EPA Product Performance Test Guidelines outlined in OCSPP 810.2200. This document also explains the terms and conditions of testing.

II. Purpose

The purpose of this study is to document the virucidal efficacy of the test device against the test system (microorganism) under the test parameters specified in this protocol. The test protocol is in compliance with the requirements of and may be submitted to one or more of the following agencies as indicated by the Study Sponsor: U.S. Environmental Protection Agency (EPA) and Health Canada.

III. Justification for the Selection of the Test System (Microorganism)

The United States Environmental Protection Agency (U.S. EPA) requires that specific antimicrobial claims made for disinfecting devices sold in the United States be supported by relevant test systems (microorganisms) outlined in the EPA Product Performance Test Guidelines, OCSPP 810.2200, Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing.

IV. Terms and Conditions

Studies by Microchem Laboratory are conducted in accordance with general terms and conditions posted on www.MicrochemLab.com/terms.

Prior to study initiation, Microchem Laboratory should receive the approved and signed protocol, test substance and applicable payment. Changes to the signed, approved protocol will require amendment and may incur additional fees. Cancellation of the study any time after the protocol is signed by the Study Sponsor will result in Sponsor being charged for work completed in addition to up to 50% of the cost of the uncompleted testing, to be determined by laboratory management at its sole discretion.

Microchem Laboratory may repeat studies at its cost in the event of an unintended protocol non-conformance that affects the study outcome, or for studies which yield invalid control results. If the Sponsor requests a specific neutralizer to be utilized in testing and test controls indicate incomplete or inadequate neutralization, repeat testing will be at the Study Sponsor's expense for applicable testing. Repeat testing may be conducted under the current initiated protocol and Microchem Laboratory GLP study identification number. In addition, the Study Sponsor is responsible for the cost of all studies performed to confirm the outcome of a previous study and for ensuring that the study will meet their regulatory objectives.

The Study Sponsor must obtain written consent from Microchem Laboratory to use or publish its protocols, study reports (or parts thereof), logo or employee names for marketing purposes.

V. Test Device Characterization and Handling

As stated in 40 CFR Part 160 Subpart F [160.105], each batch (lot) of test substance/device shall be characterized as to identity, strength, purity, composition, and solubility (as applicable), and shall be documented prior to use in this assay. Stability of the test formula shall be determined prior to or concomitantly with this study. If the requirements set forth in 40 CFR Part 160 Subpart F [160.105] have not been met, this will be noted in the Good Laboratory Practice compliance statement in the study report. Certificates of Analysis (C of A) will be appended to the study report, if provided by the Study Sponsor.

PROTOCOL (cont.)

Test devices are handled as follows unless otherwise requested by the Study Sponsor:

- The test device is stored at ambient (room) temperature under fluorescent lighting or in a cabinet.
- The test device is handled safely in accordance with the chemical, electrical, or mechanical risks it may pose, as stated in the SDS/operation manual or by the Study Sponsor during the course of pre-study communication.

VI. Study Dates

The listed proposed experimental start and completion dates are estimates based on the current laboratory schedule and may change based on when the test device, sponsor signed protocol, and payment (if applicable) are received at the testing laboratory. To avoid scheduling delays, assure that all paperwork is completed fully and accurately.

Proposed Experimental Start Date: 06JUL2021
Proposed Experimental Termination Date: 13JUL2021

VII. Procedure for Identification of Test System

Microchem Laboratory maintains Standard Operating Procedures which outline the procedures for receipt, storage, and tracking of microorganisms. The vessels, racks, and trays containing the test system are labeled with microorganism identifiers to maintain microorganism traceability. Information regarding the microorganism identity, strain, propagation procedure, media utilized, etc. is documented in the study raw data. All studies are assigned a unique identification number which is labeled on the test and control vessels, racks, trays, etc. These procedures are followed to identify and document the test system.

VIII. Test System (Microorganism) and Host Cell-Line

Test System: Human coronavirus, 229E strain, ATCC VR-740

The virus to be used in this study was originally obtained from the American Type Culture Collection (ATCC), Manassas, Virginia. The source of the virus will be documented in the raw data and report.

Host Cell: MRC-5 cells, ATCC CCL-171

IX. Procedure

Preparation of the Test Virus

- The test virus is propagated internally by Microchem Laboratory personnel by inoculating the virus into cell culture flasks containing the appropriate host cell line and incubating at the appropriate conditions.
- Once the cell culture flask(s) display approximately 75-100% cytopathic effect (as determined by microscopic evaluation), the flask(s) are subjected to freeze thaw cycles to release virus from infected cells.
- The contents of the cell culture flask(s) are collected and centrifuged in order to remove the cell debris.
- The test virus is then aliquoted and stored at ≤-70 °C.
- Alternate methods of propagation and harvesting may be utilized as necessary for the test virus. The propagation procedure is documented and will be reported.

PROTOCOL (cont.)

- On the day of testing, the appropriate number of virus stock suspension vials are removed from cryostorage and thawed. The test virus may be standardized by dilution as needed to target a recoverable plate recovery control of ≥4.8 log_{10} infective units per recovery control or 3-5 log_{10} beyond the level of cytotoxicity.
- If the Study Sponsor requests an organic soil to be incorporated into the test virus, it will be added following any standardization of the test virus.

Host Cell-Line

- MRC-5 cells (ATCC CCL-171) originally received from ATCC will be utilized in the assay. If necessary, cells received from an alternate source may be utilized. The original source of the cells will be documented in the raw data and reported.
- The cells will be subcultured by Microchem Laboratory personnel and seeded into 24 well cell culture plates.
- The plates are incubated at 36 ± 2 °C in a humidified atmosphere of 6 ± 1% CO_2 until they have reached the desired confluence required for testing.
- Cell culture passage documentation including cell culture source, passage number, seeding densities, etc. is retained.

Test Medium

- The test medium to be utilized in the assay is Eagle's Minimum Essential Medium (EMEM) or Dulbecco's Modified Eagle Medium (DMEM) which has been supplemented with 0-10% heat-inactivated fetal bovine serum (FBS). The test medium may also contain additional supplements such as antibiotics, fungizone, L-glutamine, trypsin, non-essential amino acids, etc., depending on the requirements of the test virus and/or host cells. The final composition of the test media utilized in the assay will be documented in the raw data and reported.

Preparation of Sephadex LH-20 Gel Filtration Columns

- Sephadex LH-20 gel filtration columns may be utilized to neutralize and/or to reduce the cytotoxicity of the test substance following exposure to the test virus by separating the virus from the test substance via filtration.
- On the day of testing, the prepared Sephadex slurry is aseptically added to prepared column units (sterile syringe) to completely fill the column.
- Just prior to testing, the syringe is centrifuged at approximately 100 x g for 3-4 minutes to clear the void volume.
- Alternatively, Sephacryl may be utilized in place of Sephadex, in which case, preparation will be conducted according to internal SOP, documented, and reported.

Preparation of Test Carriers

- An appropriate number of Study Sponsor-provided RD100 fused quartz petri dishes (100 x 15 mm) free from scratches, chips, or cracks are soaked in 70-95% ethyl alcohol (ethanol, reagent alcohol) or Isopropyl alcohol for ≥ 30 minutes to remove oil and film.
- The carriers are then thoroughly rinsed using two separate DI water rinses and dried.
- The dry carriers are placed in single layers on autoclavable trays lined with absorbent towels and autoclave sterilized on a fast/dry cycle for ≥20 minutes at approximately 121°C.

Preparation of Virus Films

- The test virus is vortexed thoroughly and a 0.2 ml aliquot is placed on the inside bottom surface of the appropriate number of 100 x 15 mm sterile glass Petri dishes which serve as the test carriers.
  - A larger inoculum volume may be used as necessary in order to ensure an appropriate viral inoculum titer. The volume of test virus utilized will be documented in the raw data and reported.

PROTOCOL (cont.)

- The inoculum is then spread over the entire area of the carriers using a sterile cell scraper tool or bent pipette tip without touching the sides of the Petri dish.
- The virus films are dried in a biological safety cabinet or other suitable chamber at the temperature and humidity conditions appropriate to lessen the levels of virus inactivation due to drying with the Petri dish covers removed. The viral inoculum is allowed to dry until the surface appears to be visibly dry. The temperature, relative humidity, and drying time period will be recorded in the raw data and reported.

Preparation of the Test Device

- The test device is set up and operated per the Study Sponsor-provided instructions/device manual, if available.
- If applicable, the test device is hung at an appropriate height for the Study Sponsor-requested treatment distance. Otherwise, the test device is placed on a flat surface (i.e. floor, bench top).
- Information regarding device set up will be documented in the raw data. Additionally, photos of the set up may be taken.
- If requested by Study Sponsor, warm up cycle(s) may be performed using the device prior to testing.

Treatment of Virus Films by the Test Device

- If the device is a chamber unit, test carriers are placed uncovered in the test device, and the door of the device is shut and secured.
  Note: Specific placement and/or orientation will be documented in the raw data and in the final report.
- If the device is a hanging unit, the carriers are positioned uncovered under the device at the Study Sponsor-requested distance.
- The device is turned on and allowed to run for the Study Sponsor-requested contact time. The device exposure time is measured using a digital timer, and the ambient room temperature and humidity are recorded in the raw data and reported.
- At the conclusion of the contact time, once the device has been turned off, the carriers are removed from the device (if applicable), covered with the lid, and transferred to a biological safety cabinet.
- A 2.0 ml aliquot of test media, or other media as appropriate, is added to each carrier. The carriers are gently rotated to ensure complete coverage of the solution over the entirety of the surface.
- Using sterile cell scrapers, the carriers are scraped to re-suspend the viral films, and the suspensions are transferred to sterile vessels.
  - Alternatively, the suspensions may be transferred into gel filtration columns. A syringe plunger is used to pass the contents of the re-suspended test carrier through the column. Alternatively, the re-suspended contents may be passed through the gel filtration column by centrifugation at 100 x *g* for 3-4 minutes.
- Serial 10-fold dilutions using 0.1 ml of appropriate recovery fluid (test media) and 0.9 ml of test assay media are prepared to the appropriate dilution.

Plate Recovery Control

- An appropriate number of control carriers will be prepared to determine the baseline dried virus titer. The control carriers will be generated as described above in "Preparation of Virus Films."
- The dried carriers are allowed to dwell uncovered in ambient conditions
- Following the Study Sponsor-requested contact time, a 2.0 ml aliquot of test media, or other media as appropriate, is added to each control film. The carriers are gently rotated to ensure complete coverage of the solution over the entirety of the surface.
- Using sterile cell scrapers, the carriers are scraped to re-suspend the viral films and the suspensions are transferred to sterile vessels.
  - If used for the test carriers, the suspensions may be transferred into gel filtration columns. A syringe plunger is used to pass the contents of the re-suspended test carrier through the column. Alternatively, the re-suspended contents may be passed through the gel filtration column by centrifugation at 100 x *g* for 3-4 minutes.
- Serial 10-fold dilutions using 0.1 ml of appropriate recovery fluid (test media) and 0.9 ml of test assay media are prepared to the appropriate dilution.

PROTOCOL (cont.)

Cytotoxicity Control

- Unlike chemical germicides, ultraviolet light should not produce cellular toxicity, nor should the sterile fused quartz carriers used, nor should the recovery medium. Therefore this control is appropriately not included in the study.

Test Substance Neutralization Control

- Unlike chemical germicides, antimicrobial activity of ultraviolet light should not persist after the conclusion of the contact time. Therefore this control is appropriately not included in the study.

Cell Culture Control

- To ensure that the host cells are not contaminated with bacteria, fungi, or any cytopathogenic viruses, and to confirm the viability of the cells during the incubation period of the assay, at least four host cell monolayers are left untreated, and will be microscopically examined periodically throughout the incubation period. Any obvious contamination or degeneration in such monolayers may invalidate the virucidal efficacy assay.

Virus Inoculum Titer Control

- To confirm that the host cell-line monolayers are susceptible to the test virus and to confirm the titer of the viral inoculum, an aliquot of the test virus inoculum is serially diluted (10-fold) in test media.

Infectivity Assay

- A 0.1 ml aliquot of all test and control dilutions will be inoculated into the host cells cultures (which contain test medium) in quadruplicate.
- To facilitate virus-host cell adsorption, an adsorption step may be performed by inoculating the dilutions into the host cell cultures which do not contain test medium. The assay plates are incubated at 33 ± 2 °C in a humidified atmosphere of 6 ± 1% CO_2 for a minimum of 30 minutes. The plates may also be placed upon an orbital rotator during this incubation period.
  - Following the optional adsorption, each well receives an approximate 1.0 ml aliquot of test medium via pipette delivery.
- The assay plates are incubated at 33 ± 2 °C in a humidified atmosphere of 6 ± 1% CO_2 for approximately 7 days.
- If necessary, test medium may be replaced during the incubation period to maintain the health of the host cell cultures.
- The assay plates will be examined microscopically periodically throughout the incubation period with any changes to the monolayers including viral cytopathic effects (CPE) or contamination clearly documented in the raw data.

X. Calculations

- The $TCID_{50}$ (Tissue Culture Infectivity Dose) represents the endpoint dilution where 50% of the cell cultures exhibit cytopathic effects due to infection by the test virus. The endpoint dilution at which 50% of the host cell monolayers exhibit cytotoxicity is termed the Tissue Culture Dose (TCD_{50}). The $TCID_{50}$ and TCD_{50} are determined using the Spearman-Kärber method and calculated as follows:

  Negative logarithm of endpoint titer =

  [–Log of first dilution inoculated] – [((sum of % mortality at each dilution/100) – 0.5) x logarithm of dilution]

  The result of this calculation is expressed as $TCID_{50}$/volume of dilution inoculated (e.g. 0.1 ml) for the test and plate recovery control.
- Determination of viral titer per carrier is established by accounting for the volume of viral inoculum per carrier.

PROTOCOL (cont.)

Calculation of the Log_{10} Reduction

- The log_{10} reduction in viral titer will be calculated as follows:

  Control log_{10} $TCID_{50}$/0.1 ml – Virus-Test Device log_{10} $TCID_{50}$/0.1 ml

- If multiple plate recovery control and test replicates are performed, the average $TCID_{50}$ of each parameter will be calculated as follows, and the average result used to calculate the log_{10} reduction in viral titer.

  Average $TCID_{50}$ (double replicate) = log_{10} $[(10^{TCID50\ rep\ 1} + 10^{TCID50\ rep\ 2})/2]$

XI. Statistical Analysis

Not applicable.

XII. Methods for the Control of Bias

Not applicable.

XIII. Success Criteria

- The following measures are met to ensure the acceptability of virucidal efficacy data:
  - The virus liter control demonstrate obvious and or typical cytopathic effects on the monolayers unless a detection method other than cytopathic effect is used.
  - A minimum of 4.80 log_{10} infective units/control carrier is recovered from each plate recovery control film(s).
  - Quantification of the test and control parameters are conducted at a minimum of four determinations per dilution.
  - The cell controls are negative for infectivity and demonstrate typical cell morphology.

XIV. Product Performance Criteria

- The product performance criteria follows:
  - The log and percent reduction of the test virus following exposure to the test substance are calculated however, there is no minimum reduction level to qualify as "passing" or an "efficacious" product.
- For liquid/spray/towelette products, the U.S. EPA performance criteria for disinfection follows:
  - In the presence or absence of cytotoxicity, the product should demonstrate a ≥3.00-log10 reduction in viral titer on each surface.

XV. Protocol Changes

- If changes or revisions to the approved protocol are required, they will be documented in the form of a protocol amendment that also includes the reason for the change or revision and the amendment will be signed and dated, minimally, by the Study Director. The protocol amendment will be retained with the protocol. The Study Sponsor will be notified of the changes or revisions.

XVI. Reporting

- Results are reported accurately and fully, in accordance with EPA GLP (40 CFR Part 160.185). A draft report may be provided for review by the Study Sponsor prior to study completion.

PROTOCOL (cont.)

XVII. Study Record and Test Substance Retention

- The original (or certified copy) of the study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory indefinitely. For studies not meeting the performance criteria for submission or for studies that have been canceled prior to the generation of valid data, the original (or certified copy) of the final study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory for a minimum of two years following the study completion date at which time they may be removed from the archive or transferred to the Sponsors archive at their expense.
- If requested by the Study Sponsor (or Sponsor Representative), the study file may be transferred to the Study Sponsor's archive at the Study Sponsor's expense prior to the time frames listed.
- All test facility records including, but not limited to, standard operating procedures, quality assurance inspection records, temperature and equipment records including maintenance, inspection and calibration, and employee training records will be maintained at Microchem Laboratory indefinitely.
- The test substance (or test control, test article, test device, as applicable) may be returned to the Study Sponsor at the Study Sponsor's request and expense following study completion unless otherwise requested to be returned earlier. If the Study Sponsor does not request return of the sample, it will be disposed >90 days following the study completion. Arrangements may be made for extended storage as necessary, at the Sponsor's request and expense.

XVIII. Quality Assurance

- The study is conducted in accordance with Microchem Laboratory's Quality Management System and 40 CFR Part 160 and will undergo a full quality assurance review. All protocol amendments will be fully recorded and reported, as well as any deviations from the protocol.

XIX. References

- *Annual Book of ASTM Standards*, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, Designation E1053, current edition. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.
- Annual Book of ASTM Standards, Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, Designation E1482, current edition. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
- U.S. Environmental Protection Agency, Frequent Questions for the 2018 Series 810 – Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines. 2019.
- Guidance Document – Disinfectant Drugs. Health Canada. April 2020.
- Guidance Document – Safety and Efficacy Requirement for Hard Surface Disinfectant Drugs. Health Canada. April 2020.

PROTOCOL (cont.)

Specific Testing Parameters to be completed by the Study Sponsor/Representative
- all fields need to be completed before testing may commence

Test Device Name	KR615 & KR615-AUTO
Test Device Lot Numbers	Serial Number: 3692 (30s) & 3697 (60s)
Manufacture Date(s)	3692- 15MAR2021 3697- 15MAR2021
Expiration Date(s)	N/A
Test Device Shipment Status	☑ Use test device already present at Microchem. ❑ Test device will be shipped. Estimated arrival date, if known:
Test Device Storage	☑ Room temperature (default for all packages unless otherwise advised) ❑ 2 – 8 °C ❑ Other:
Test Device Hazards	❑ None known ❑ SDS attached ☑ Other: UVC Exposure
Organic Soil Load	❑ No additional organic soil load supplementation, virus will be tested as propagated (<5% organic challenge). Organic soil level will be reported ☑ 5% fetal bovine serum supplementation ❑ Other:
Contact Time(s)	30 seconds (3692) and 60 seconds (3697) *Note: The contact time includes a range of ±5 seconds for carrier manipulation*
Exposure Temperature	☑ Ambient Room Temperature ❑ Other:
Exposure Distance(s)	☑ N/A (chamber enclosure) ❑ Other:
Number of Test and Plate Recovery Control Carriers Per Test Device Per Distance	❑ 1 ☑ 2 ❑ 3 ❑ Other:
EPA 40 CFR Part 160.31(d) requires testing facility management to assure that the test, control, and reference substances have been appropriately tested for identity, strength, purity, stability and uniformity, as applicable.	Applicable identity, strength, purity, stability, and uniformity testing has been or will be completed prior to efficacy testing: ❑ Yes ☑ No Performed under 40 CFR Part 160 regulations? ❑ Yes ☑ No Stability testing of the formulation has been or will be completed prior to efficacy testing or concomitantly with efficacy testing: ❑ Yes ☑ No Performed under 40 CFR Part 160 regulations? ❑ Yes ☑ No If no is marked, compliance status will be noted in the GLP compliance statement in the final report.

PROTOCOL (cont.)

Custom Virucidal Efficacy of an Ultraviolet Light Disinfection Device

Protocol Number: P3382

Continuation of Specific Testing Parameters to be completed by the Study Sponsor/Representative - all fields need to be completed before testing may commence

Certificate of Analysis (CoA)	☐ CoA for each lot provided. CoA will be appended in the final report. ☑ CoA will not be provided.
Device Use Instructions:	☑ Device Use Instructions: • Instructions includes with device • Perform | Warm up cycle for each test • Prior to carrier insertion.
Additional Instructions:	1. Clean visible dirt, moisture or liquid residue from items to be disinfected 2. Place items in UV Box 3. Do not stack or allow sides of items to touching 4. Close the door and turn knob to lock. Press red button to begin decontamination cycle. 5. When the red button and LED indicators turn off, remove items use normally.
Protocol Modifications:	☑ Testing is to be performed as outlined in the protocol. ☐ The following protocol modifications are to be performed: KR615 = S/N 3692 for 30 seconds KR615-AUTO= S/N 3697 for 60 seconds
Regulatory Agency(s) that report may be submitted to	☑ EPA ☐ Other:

PROTOCOL (cont.)

XVIII. Authorized Personnel

Due to Microchem Laboratory confidentiality policy, study information will only be released to the Study Sponsor/Sponsor Representative who has signed the protocol unless otherwise noted in writing. Please list any additional personnel authorized to receive information regarding this study.

1. Jim Psihas
2. Kalleh Kneeland
3. _____
4. _____

XIX. Protocol Approval

"I, the Study Sponsor/Sponsor Representative, have read and understand the study protocol. By signing this protocol I am certifying that the information and parameters accurately describe the test[s] to be completed in accordance with Good Laboratory Practice Standards (GLPS) stipulated by 40 CFR Part 160. I have also read, understand and agree to the terms and conditions listed in the protocol."

Study Sponsor/Sponsor Representative Signature Approving Protocol

DAVID HENDERSON
Study Sponsor/Sponsor Representative Printed Name

David Henderson
Study Sponsor/Sponsor Representative Signature

07/21/2021
Date

DavidHenderson602@gmail.com
Email Address

(404) 433-4687
Phone

Microchem Laboratory Study Director

Victoria Zarate
Study Director Printed Name

Study Director Signature

21JUL2021
Date