FINAL STUDY REPORT

Study Title
Custom Virucidal Efficacy of an Ultraviolet Light Disinfection Device

Product Identity
Test Device Name: Lightcare Eclipse and Humphrey Visual Field Analyzer
Test Device Model: Prototype Device 2-01

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

Data Requirements
U.S. EPA OCSPP 810.2200

Author
Victoria Zarate, B.S.

Study Completion Date
26JUL2022

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

Study Sponsor
Breathh, Inc.
2215 Paseo De Las Americas, Suite 30
San Diego, CA 92154

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 exceptions:

> 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. Certificate of Analysis was not provided by 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: 26JUL2022

Study Sponsor

Company: Breathh, Inc.
Name: Adam Doherty
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	23JUN2022	24JUN2022	24JUN2022
Draft Report	05JUL2022	05JUL2022	05JUL2022
Final Report	25JUL2022	26JUL2022	26JUL2022

Signature: _____ Date: 26JUL2022

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: Ashley Grafe, 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: GLP3014

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

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

Test Device: Name: Lightcare Eclipse and Humphrey Visual Field Analyzer

Model: Prototype Device 2-01

Test Device Application: Carriers placed inside Device. Tested locations as follows: 1) chin rest, 2) behind chin rest, 3) side of concave unit, 4) back side of concave unit, and 5) top of concave unit, behind headrest.

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

Inoculum Volume: 0.100 ml

Carrier Type: Sterile Microchem-provided acrylic carriers (cut to approximately 1" x 3")

Number of Replicates per Device: Three

Contact Time: 3 minutes

Exposure Temperature: Ambient room temperature (24.5-24.6°C) and 31-33% relative humidity (RH)

Neutralization Method: Eagle's Minimum Essential Medium (EMEM) supplemented with 2% FBS test media (1.0 ml)

Study Results

Description	Assay Results					Recovery Control
	Lightcare Eclipse and Humphrey Visual Field Analyzer Model: Prototype Device 2-01					
	Location 1	Location 2	Location 3	Location 4	Location 5	
Avg. Log_{10} $TCID_{50}$ per 0.1 ml	1.30 log_{10}	≤0.60 log_{10}	≤0.50 log_{10}	≤0.74 log_{10}	≤0.50 log_{10}	5.68 log_{10} ($TCID_{50}$ per 0.1 ml)
Avg. Log_{10} Reduction per 0.1 ml	4.38 log_{10}	≥5.08 log_{10}	≥5.18 log_{10}	≥4.94 log_{10}	≥5.18 log_{10}	

STUDY DATES

Study Initiation Date: 23JUN2022
Experimental Start Date / Time: 23JUN2022 / 1452
Experimental Termination Date / Time: 30JUN2022/ 0926
Study Completion Date: 26JUL2022

TEST DEVICE

Device Name: Lightcare Eclipse and Humphrey Visual Field Analyzer
Model: Prototype Device 2-01
Date of Manufacture: 01JUN2022
Date Received: 06JUN2022
Expiration Date: N/A

Form: Ultraviolet light disinfection device

Storage Conditions: Ambient room temperature

Test Device Handling: The test devices were handled safely in accordance with the chemical, electrical, or mechanical risks potentially posed as stated in the SDS/operation manual or by the Study Sponsor during the course of pre-study communication.

PROTOCOL CHANGES

Protocol Amendment(s)

Protocol Amendment # 1
On 24JUN2022, the approved/signed protocol is hereby amended due to a typographical error of the device name used in testing. The test device name's correct spelling should read "Lightcare Eclipse" as stated in the instruction manual provided by the Study Sponsor.

Furthermore, at the discretion of the Study Director, clarification of the Study Sponsor provided devices are as follows: device one is the Lightcare Eclipse which is used for treatment and contains the UV bulbs, and the second device is the Humphrey Visual Field Analyzer which is used to hold the carriers and serve as an attachment place for the Lightcare Eclipse; both devices are used together.

All remaining testing parameters are to be followed as stated in the protocol.

Protocol Amendment # 2
At the request of the Study Sponsor, shipment of the test device will occur prior to the protocol requirement of maintaining test device for >90 days from the study completion.

All remaining testing parameters are to be followed as stated in Protocol P3770.

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 obtained from the American Type Culture Collection (ATCC), Manassas, VA., was used in this study. The Microchem Laboratory lot number used in testing was HCoV_15MAR2022.

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 in an ultra-low freezer.

On the day of testing, the appropriate number of virus stock suspension vials were removed from cryostorage and thawed for use in the assay. The test virus contained 0% FBS organic soil load. The test virus was adjusted to contain 5% FBS organic soil load by adding 0.150 ml of FBS to 2.850 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. Cell culture passage documentation including cell culture source, passage number, seeding densities, etc. was 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.

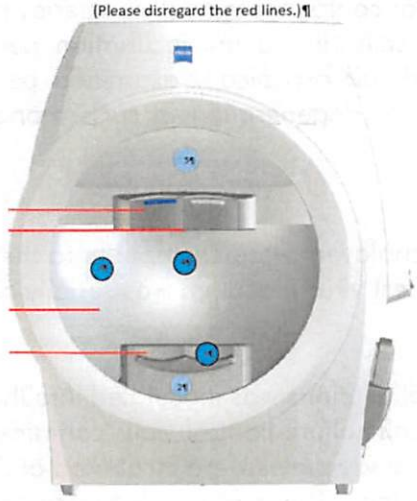
Preparation of Test Carriers
An appropriate number of Microchem-provided acrylic carriers were cut to approximately 1" x 3" and allowed to soak in 70-95% ethyl alcohol (ethanol, reagent alcohol) for ≥30 minutes. Following the 70-95% ethyl alcohol soak, the carriers were rinsed twice with deionized water. The carriers were placed onto a tray and UV sanitized for ≥15 minutes on each side and placed in sterile Petri dishes.

Preparation of Virus Films
For each preparation of virus films, the test virus was vortexed thoroughly and a 0.100 ml aliquot was placed on the surface of 7 of the approximately cut 1" x 3" acrylic carriers which served as both test carriers and recovery carriers. The inoculum was then spread over the entire area of the carriers using a sterile bent pipette tip without touching the sides of the acrylic carriers. The virus films were dried in an environmental chamber for 38-41 minutes at 20.0-20.4°C in a relative humidity of 25-42%.

Preparation of the Test Device
The test device was set up and operated per the Study Sponsor-provided instructions/device manual. The test device was pre-programmed to 6 minutes. A digital timer was used to determine contact time of 3 minutes. The stop button was pressed to end contact time. Proper function was confirmed and no errors displayed.

Treatment of Virus Films by the Test Device
For each device run, five dried virus film carriers were placed in the test device with the inoculated side facing the UV bulbs and adhered to the surface. Sterile alligator clips were used to hold carriers in place for treatment. The carriers were manipulated onto the clips using sterile gloves and/or sterile forceps and the door of the device was shut and secured. Per Study Sponsor specifications, the five test locations were: 1) on chin rest, 2) behind chin rest, 3) side of concave unit, 4) back side of concave unit, and 5) top of concave unit behind headrest. See Diagram 1 for illustration of carrier placement/tested locations. The device was turned on and allowed to run for the Study Sponsor-requested contact time of 3 minutes, at the requested exposure temperature of 24.5-24.6°C in a relative humidity of 31-33%.

Diagram 1: Illustration of carrier placement/tested locations.



At the conclusion of the contact time, once the device had been turned off, the carriers were removed from the device using sterile gloves and/or sterile forceps, placed into a new sterile Petri dish, and transferred to a biological safety cabinet. A 1.0 ml aliquot of test media was added to each carrier. Using sterile cell scrapers, the carriers were scraped to re-suspend the viral films, and the suspensions were transferred to sterile vessels. Serial 10-fold dilutions using 0.1 ml of appropriate recovery fluid (test media) and 0.9 ml of test assay media were prepared to the appropriate dilution.

STUDY CONTROLS

Recovery Control

For each device run, one recovery control film was prepared to determine the baseline dried virus titer. The control carriers were generated as described above in "Preparation of Virus Films." The dried carriers were allowed to dwell uncovered in ambient conditions for the duration of the contact time. Following the Study Sponsor-requested contact time, a 1.0 ml aliquot of test medium was added to each control film. Using sterile cell scrapers, the carriers were scraped to re-suspend the viral films and the suspensions were transferred to sterile vessels. Serial 10-fold dilutions using 0.1 ml of appropriate recovery fluid (test media) and 0.9 ml of test assay media were prepared to the appropriate dilution.

Cytotoxicity Control

Unlike chemical germicides, antimicrobial activity of ultraviolet light should not produce cellular toxicity, nor should the sterile acrylic carriers used, 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 may 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 test virus inoculum 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 cells cultures (which contained test medium) in quadruplicate. The cell culture control wells contained an approximate 1.0 ml aliquot of test medium via pipette delivery. The assay plates were incubated at 33±2 °C in a humidified atmosphere of 6±1% CO_2 for approximately 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.

- 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 $\log_{10}$ 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 as follows, and the average result used to calculate the log_{10} reduction in viral titer.

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

Statistical Analysis
Not applicable.

Methods for the Control of Bias
Not applicable.

STUDY RECORD AND TEST SUBSTANCE RETENTION

Study Record Retention

"Failing" Study Records – Reports and all associated documentation (other than test facility records) for studies that fail to meet the passing criteria specified by the protocol will be held in the archives of Microchem Laboratory for two (2) years from study completion, after which reasonable storage fees apply and will be billed annually. Sponsors may elect to transfer archived documentation to their own GLP-compliant archives at their own expense at any time. After two years, the Sponsor may grant Microchem permission in writing to destroy the file.

"Passing" Study Records – Reports and all associated documentation (other than test facility records) for studies that meet the passing criteria specified by the protocol will be held in the archives of Microchem Laboratory for five (5) years from study completion, after which reasonable storage fees apply and will be billed annually. Sponsors may elect to transfer archived documentation to their own GLP-compliant archives at their own expense at any time. If, after five years, the Sponsor indicates the study will not be used to support a product registration, it may grant Microchem permission in writing to remove it from the archives or destroy it.

All other GLP Records, including "Facility" Records - 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

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 unless otherwise required by law. Arrangements may be made for extended storage as necessary, at the Sponsor's request and expense. Unless otherwise agreed to in writing in advance, all disposal costs of test substances containing hazardous substances shall be the responsibility of Client and Client shall reimburse Microchem, within thirty (30) days of invoice, for any such costs. If Client wishes test substances to be returned to them, it will be the Client's responsibility to schedule pick up and provide Microchem with a shipping label for the package. Client must also provide packing materials and/or instructions to pack the return, if non-standard packing is necessary. Microchem is not responsible for any costs to return test substances to Client (e.g. freight fees including customs charges, packaging materials, etc.).

RESULTS

Table 1: Recovery Control Results

		Recovery Control		
		Replicate 1	Replicate 2	Replicate 3
Cell Control		0 0 0 0	N/A	N/A
Dilution	10^{-1}	+ + + +	+ + + +	+ + + +
	10^{-2}	+ + + +	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +	+ + + +
	10^{-4}	+ + + +	+ + + +	+ + + +
	10^{-5}	+ + + +	+ 0 + +	+ + + +
	10^{-6}	0 0 0 0	0 0 + 0	0 0 + 0
	10^{-7}	0 0 0 0	0 + 0 0	0 0 0 0
	10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		5.50 log_{10}	5.75 log_{10}	5.75 log_{10}
Avg. $TCID_{50}$ per 0.1 ml		5.68 log_{10}		

Table 2: Test Results: Location 1 – Chin rest

		Test Results		
		Replicate 1	Replicate 2	Replicate 2
Dilution	10^{-1}	0 0 + +	+ + + +	0 0 + 0
	10^{-2}	0 0 + 0	0 0 0 0	+ 0 0 0
	10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		1.25 log_{10}	1.50 log_{10}	1.00 log_{10}
Avg. $TCID_{50}$ per 0.1 ml		1.30 log_{10}		
Log_{10} Reduction		4.38 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: Test Results: Location 2 – Behind chin rest

		Test Results		
		Replicate 1	Replicate 2	Replicate 2
Dilution	10^{-1}	0 0 0 +	0 0 0 0	0 0 0 0
	10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		0.75 log_{10}	≤0.50 log_{10}	≤0.50 log_{10}
Avg. $TCID_{50}$ per 0.1 ml		≤0.60 log_{10}		
Log_{10} Reduction		≥5.08 log_{10}		

Table 4: Test Results: Location 3 – Side of concave unit

		Test Results		
		Replicate 1	Replicate 2	Replicate 2
Dilution	10^{-1}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-8}	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}
Avg. $TCID_{50}$ per 0.1 ml		≤0.50 log_{10}		
Log_{10} Reduction		≥5.18 log_{10}		

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

RESULTS (cont.)

Table 5: Test Results: Location 4 – Back side of concave unit

		Test Results		
		Replicate 1	Replicate 2	Replicate 2
Dilution	10^{-1}	+ 0 + 0	0 0 0 0	0 0 0 0
	10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		1.00 log_{10}	≤0.50 log_{10}	≤0.50 log_{10}
Avg. $TCID_{50}$ per 0.1 ml		≤0.74 log_{10}		
Log_{10} Reduction		≥4.94 log_{10}		

Table 6: Test Results: Location 5 – Top of concave unit, behind headrest

		Test Results		
		Replicate 1	Replicate 2	Replicate 2
Dilution	10^{-1}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-8}	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}
Avg. $TCID_{50}$ per 0.1 ml		≤0.50 log_{10}		
Log_{10} Reduction		≥5.18 log_{10}		

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

RESULTS (cont.)

Table 7: Virus Inoculum Titer Control

		Virus Inoculum Titer Control
Cell Control		0 0 0 0
Dilution	10^{-1}	+ + + +
	10^{-2}	+ + + +
	10^{-3}	+ + + +
	10^{-4}	+ + + +
	10^{-5}	+ + + +
	10^{-6}	0 0 + +
	10^{-7}	0 0 0 0
	10^{-8}	0 0 0 0
$TCID_{50}$ per 0.1 ml		6.00 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 Lightcare Eclipse and Humphrey Visual Field Analyzer (Prototype Device 2-01) against Human coronavirus, 229E strain, ATCC VR-740 supplemented with a 5% FBS soil load, at a contact time of 3 minutes and an exposure temperature of room temperature.

The Recovery Control demonstrated an average viral titer of 5.68 $\log_{10}$ $TCID_{50}$ per 0.1 ml.

The evaluated test device(s), Lightcare Eclipse and Humphrey Visual Field Analyzer demonstrated:

- An average 4.38 $\log_{10}$ reduction in viral titer in test location 1 – on chin rest.
- An average ≥5.08 $\log_{10}$ reduction in viral titer in test location 2 – behind chin rest.
- An average ≥5.18 $\log_{10}$ reduction in viral titer in test location 3 – side of concave unit.
- An average ≥4.94 $\log_{10}$ reduction in viral titer in test location 4 – back side of concave unit.
- An average ≥5.18 $\log_{10}$ reduction in viral titer in test location 5 – top of concave unit, behind headrest.

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

Lightcare Eclipse and Humphrey Visual Field Analyzer (Prototype Device 2-01) 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. A ≥3.00 log reduction is required for EPA submission of liquid/spray/towelette disinfectants for use on hard surfaces. This EPA requirement is used only as a frame of reference for the effectiveness of the Lightcare Eclipse device. The Lightcare Eclipse device achieved ≥4.00 log reduction in all areas tested and, in some areas, ≥5.00 log reduction for a 3-minute exposure time.

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 AMENDMENTS

MICROCHEM LABORATORY

Protocol Amendment for Protocol P3770 Study ID Number GLP3014

Protocol Amendment # 2

At the request of the Study Sponsor, shipment of the test device will occur prior to the protocol requirement of maintaining test device for >90 days from the study completion.

All remaining testing parameters are to be followed as stated in Protocol P3770.

Adam [signature]
Study Sponsor (Signature)

7/18/22
Date Signed

Adam Doherty
Study Sponsor (Print)

[signature]
Study Director (Signature)

19JUL2022
Date Signed

Victoria Zarate
Study Director (Print)

PROTOCOL AMENDMENTS (cont.)

MICROCHEM LABORATORY

Protocol Amendment for Protocol P3770 Study ID Number GLP3014

Protocol Amendment # 1

On 24JUN2022, the approved/signed protocol is hereby amended due to a typographical error of the device name used in testing. The test device name's correct spelling should read "Lightcare Eclipse" as stated in the instruction manual provided by the Study Sponsor.

Furthermore, at the discretion of the Study Director, clarification of the Study Sponsor provided devices are as follows: device one is the Lightcare Eclipse which is used for treatment and contains the UV bulbs, and the second device is the Humphrey Visual Field Analyzer which is used to hold the carriers and serve as an attachment place for the Lightcare Eclipse; both devices are used together.

All remaining testing parameters are to be followed as stated in Protocol P3770.

Adam Doherty — Study Sponsor (Signature) — 6/24/22 — Date Signed

Adam Doherty — Study Sponsor (Print)

Study Director (Signature) — 24JUN2022 — Date Signed

Victoria Zarate — Study Director (Print)

Page 1 of 1

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PROTOCOL

MICROCHEM LABORATORY

Protocol Number: P3770 Study ID Number: GLP 3014

23JUN2022VN2

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
Breathh, Inc.
2215 Paseo De Las Americas, Suite 30
San Diego, CA 92154

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

Prepared By:
Ashley Grafe, B.S.

Date
20JUN2022

PROTOCOL (cont.)

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 https://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 price quote is signed will result in Sponsor being charged for work completed in addition to up to 100% of the cost of the uncompleted testing (see Microchem's Terms & Conditions for details).

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 does not advise about specific required neutralization technique 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.

PROTOCOL (cont.)

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.

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

- The test device is stored at ambient (room) temperature.
- 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: **23JUN2022**
Proposed Experimental Termination Date: **30JUN2022**

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

PROTOCOL (cont.)

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 in an ultra-low freezer.
  - Alternately test virus may be stored in liquid nitrogen.
- Alternate methods of propagation and harvesting may be utilized as necessary for the test virus. The propagation procedure is documented and will be reported.
- 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 Test Carriers

- Microchem provided acrylic carriers are cut to approximately 1" X 3" and allowed to soak in 70-95% ethyl alcohol (ethanol, reagent alcohol) for ≥30 minutes.
- Following the 70-95% ethyl alcohol soak, the carriers are rinsed twice with deionized water.
- The carriers are placed onto a tray and UV sanitized for ≥15 minutes on each side and placed in sterile Petri dishes.

PROTOCOL (cont.)

Preparation of Virus Films

- The test virus is vortexed thoroughly and a 0.1 ml aliquot is placed on the surface of the appropriate number of the approximately cut 1" X 3" acrylic carriers which serve as the test carriers.
  - An alternative 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.
- 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 acrylic carriers.
- 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 on the acrylic carriers with the Petri dish lids off. 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.
- Information regarding device set up will be documented in the raw data, if applicable. 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

- Test carriers are placed in the test device with the inoculated side facing the UV bulbs and adhered to the surface, sterile alligator clips may be used. The carriers are manipulated onto the clips using sterile gloves and/or sterile forceps 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.
- 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 using sterile gloves and/or sterile forceps, placed into a new sterile Petri dish, and transferred to a biological safety cabinet.
- A 1.0 ml aliquot of test media, or other media as appropriate, is added to each carrier.
  - An alternate volume may be used. The volume will be documented in the raw data and reported.
- Using sterile cell scrapers, the carriers are scraped to re-suspend the viral films, and the suspensions are transferred to sterile vessels.
  - This process is repeated until all test replicates have been performed.
- 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.

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 condition for the duration of the contact time.
- Following the Study Sponsor-requested contact time, a 1.0 ml aliquot of test media, or other media as appropriate, is added to each control film.
- Using sterile cell scrapers, the carriers are scraped to re-suspend the viral films and the suspensions are transferred to sterile vessels.
- 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 acrylic 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.

PROTOCOL (cont.)

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.

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.

PROTOCOL (cont.)

XIII. Success Criteria

- The following measures are met to ensure the acceptability of virucidal efficacy data:
  - The virus titer 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 $\geq$3.00-$\log_{10}$ 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

- "Failing" Study Records – Reports and all associated documentation (other than test facility records) for studies that fail to meet the passing criteria specified by the protocol will be held in the archives of Microchem Laboratory for two (2) years from study completion, after which reasonable storage fees apply and will be billed annually. Sponsors may elect to transfer archived documentation to their own GLP-compliant archives at their own expense at any time. After two years, the Sponsor may grant Microchem permission in writing to destroy the file.
- "Passing" Study Records – Reports and all associated documentation (other than test facility records) for studies that meet the passing criteria specified by the protocol will be held in the archives of Microchem Laboratory for five (5) years from study completion, after which reasonable storage fees apply and will be billed annually. Sponsors may elect to transfer archived documentation to their own GLP-compliant archives at their own expense at any time. If, after five years, the Sponsor indicates the study will not be used to support a product registration, it may grant Microchem permission in writing to remove it from the archives or destroy it.
- All other GLP Records, including "Facility" Records - 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 by Microchem Laboratory, free of charge to Sponsors, 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 unless otherwise required by law. Arrangements may be made for extended storage as necessary, at the Sponsor's request and expense. Unless otherwise agreed to in writing in advance, all disposal costs of test substances containing hazardous substances shall be the responsibility of Client and Client shall reimburse Microchem, within thirty (30) days of invoice, for any such costs. If Client wishes test substances to be returned to them, it will be the Client's responsibility to schedule pick up and provide Microchem with a shipping label for the package. Client must also provide packing materials and/or instructions to pack the return, if non-standard packing is necessary. Microchem is not responsible for any costs to return test substances to Client (e.g. freight fees including customs charges, packaging materials, etc.).

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.

PROTOCOL (cont.)

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	Lightcare Elcipse
Test Device Lot Numbers	Prototype Device 2-01
Manufacture Date(s)	June 1st, 2022
Expiration Date(s)	None
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: UV light exposure. Do not look directly at the UV light. Do not use device if there are UV light leaks from the gasket.
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)	Device is programmed for 3 minutes *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: P3770

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: User manual was sent separately from device by email.
Additional Instructions:	Carrier placement: - Please refer to the powerpoint (Virology Testing Areas in HFA device) that was sent for carrier locations (slide 3).
Protocol Modifications:	☒ Testing is to be performed as outlined in the protocol. ☐ The following protocol modifications are to be performed:
Regulatory Agency(s) that report may be submitted to	☒ EPA ☒ Other: FDA

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. Amir Tafreshi
2. _____
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

Adam Doherty
Study Sponsor/Sponsor Representative Printed Name

Study Sponsor/Sponsor Representative Signature

6/22/2022
Date

Adam@Breathh.com
Email Address

310-926-7296
Phone

Microchem Laboratory Study Director

Victoria Zavate
Study Director Printed Name

Study Director Signature

23JUN2022
Date