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FINAL REPORT

Evaluation of Virucidal Efficacy on Hard Surface by a Device - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

Test Substance
P3000/PS3000

Lot Number
Not Applicable

Test Organism
Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), Strain: USA-WA1/2020 Source: BEI Resources, NR-52281

Test Guidelines
EPA (2018) Guidelines 810.2000 and 810.2200 (G)

Author
Cory Chiossone

Study Completion Date
08/04/21

Performing Laboratory
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Laboratory Project Identification Number
1073-102

Protocol Identification Number
1073.V.21.001

Sponsor
Puraclenz LLC
30 Butler Lane
New Canaan, CT 06840

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR § 160 with the following exceptions:

- Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test article resides with the sponsor of the study.

The following technical personnel participated in this study:

Cory Chiossone, Calli Tang

Study Director:

Cory Chiossone — Date: 08/04/2021

QUALITY ASSURANCE UNIT STATEMENT

The Quality Assurance Unit of Microbac has inspected Project Number 1073-102 to be in compliance with current Good Laboratory Practice regulations, (40 CFR § 160).

The dates that inspections were made and the dates that findings were reported to management and to the study director are listed below.

Phase Inspected	Date of Inspection	Date Reported to Study Director	Date Reported to Management
Protocol	02/25/21 03/01/21	03/01/21	03/01/21
In Process	03/01/21	03/01/21	03/01/21
Final Report	03/31/21	04/05/21	04/05/21

Danielle Downs, RQAP-GLP
Quality Assurance Specialist III

08/04/2021
Date

TEST SUMMARY

Study Title: Evaluation of Virucidal Efficacy on Hard Surface by a Device - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

Project No.: 1073-102

Protocol No.: 1073.V.21.001

Test Method: ASTM International E1053-20 “Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces”

Sponsor: Puraclenz LLC
30 Butler Lane
New Canaan, CT 06840

Testing Facility: Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Study Objective: This test was performed in order to substantiate virucidal efficacy claims for a test device to be labeled as a virucide by determining the potential of the test device to disinfect hard surfaces contaminated with SARS-CoV-2. This test was designed to simulate consumer use and was performed in conformance to EPA OCSPP 810.2000 (2018) and 810.2200 (2018) Product Performance Test Guidelines, Frequent Questions for the 2018 Series 810 – Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines.

Study Dates: Study Initiation: 02/26/21
Experimental Start: 02/26/21
Experimental End: 03/05/21
Study Completion: See page 1

Test Device:

- P3000/PS3000 (Device), Lot No.: Not Applicable, Received: 02/08/21, and assigned DS No. L166
- Physical Description: Device
- Storage Condition: Ambient
- Active Ingredients: Not Applicable

TEST SUMMARY (continued)

Test Conditions:	Organic Soil Load: 5.0% Fetal Bovine Serum (FBS) Contact Times: 1 hour Contact Temperature: Ambient (20 ± 2°C) Contact Relative Humidity: 25%
Challenge Virus:	Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus) • Strain: USA-WA1/2020 • Source: BEI Resources, NR-52281
Indicator Cells:	Vero E6 cells • Source: ATCC CRL-1586
Incubation Time:	4 – 9 days (Actual: 7 days)
Incubation Temperature:	36 ± 2°C with 5 ± 3% CO_2
Dilution Medium (DM):	Dulbecco's Modified Eagle Medium (DMEM) + 10% Fetal Bovine Serum (FBS)
Recovery Medium:	MEM + 10% Fetal Bovine Serum (FBS)
Study Design:	This study was performed according to the signed protocol and project sheet(s) issued by the Study Director (see Appendix I).

TEST PROCEDURES

Indicator Cells:

Vero E6 cells were obtained from ATCC and maintained in cell culture at 36 ± 2°C with 5 ± 3% CO_2 prior to seeding. The indicator cell plates were prepared the day prior to inoculation with test sample. The cells were seeded in 96-well plates at a density of 8 x 10^4 cells/mL at 0.150 mL per well.

Virus Inoculum:

The stock virus was propagated in Vero E6, the cell supernatant was clarified, aliquoted, and stored at -60 to -90°C. Frozen viral stock was thawed on the day of the test.

Challenge Virus:

Original stock virus contained a 5.0% organic load (FBS).

Test Device:

The test device was placed 10 ft away from the prepared test carriers.

Test Carriers:

Glass Petri dish carriers were inoculated with 0.2 mL of virus inoculum spread with a cell scraper over a 4 in^2 area. The virus was dried for 30 minutes at 22°C with 25-26% Relative Humidity (RH). Nine carriers were prepared for the test and the plate recovery control using virus. Additionally, one carrier was prepared for the cytotoxicity controls using dilution media in lieu of virus as the inoculum.

Test Device Application and Exposure Conditions:

The test device was located in a laboratory space (see figure 1 for details including the presence of furniture and pieces of large equipment). The flooring was a laboratory-grade, chemical resistant vinyl flooring material. All equipment in the laboratory had both plastic and metal parts on their exterior. The laboratory contained drywall on the surfaces, both on the walls and on the ceiling. The carriers were placed about 10 feet from the test device on a metal cart with the below dimensions:

Length: 27.1 inches
Width: 18 inches
Height from floor: 33.5 inches
Height from bottom shelf to top shelf: 28.5 inches

TEST PROCEDURES (continued)

The HVAC system for the laboratory space did not have filtration, only that which is within the exhaust hoods. Fresh air was drawn from outside and exhaust air was released in the space above the ceiling. The RTU serving the BSL-3 suite was isolated / dedicated and does not connect to spaces external to the lab. For safety reasons the HVAC system could not be shut off during testing with SARS-CoV-2 (COVID-19) or any other BSL-3 organisms.

The fronts of the laminar flow hoods were turned off and sealed during the test to minimize the impact on ion distribution during the test.

The device was turned on for a period of approximately 12 hours in the laboratory space before the virus carriers were placed and prior to testing. The laboratory doors were sealed during this period of time so no one entered the space during pre-conditioning.

When the virus carriers were being placed in the laboratory prior to testing, the device continued to run. The technician minimized the time that the laboratory door remained open when they entered the room. When exiting the room, after placing the carriers in position, the technician again minimized the time that the laboratory door remained open.

Three carriers were evaluated. The carriers were placed with the device placed 10 ft away from the carriers. The device remained on at the test temperature for 1 hour. The test substance was tested at 22°C with 25% RH.

Recovery of Samples:

After the contact times, the samples were recovered with 2.0 mL of recovery medium. The carriers were scraped with a cell scraper to remove residual virus. This post-neutralized sample (PNS) was considered the 10^{-1} dilution. An aliquot of the PNS was ten-fold serially diluted in DM.

Selected dilutions were inoculated onto 96-well host cell plates at 0.05 mL per well, 8 wells per dilution. The inoculated cells were incubated at 36 ± 2°C with 5 ± 3% CO_2 for 7 days.

Infectivity Assay:

The residual infectious virus in both test and controls was detected by viral-induced cytopathic effect (CPE). CPE is defined as cell rounding and sloughing off of the cell monolayer. After 7 days of incubation at 36 ± 2°C with 5 ± 3% CO_2 the plates were removed, scored, and recorded for test-substance specific cytotoxic effects and/or virus-specific cytopathic effect (CPE).

TEST PROCEDURES (continued)

Cytotoxicity Control (CT):

This control was performed to assess the cytotoxic effects of the test device on indicator cells. The CT was prepared identically to the test sample except DM was used in lieu of virus inoculum to inoculate the carrier. After test device application and recovery, the PNS was serially diluted and selected dilutions were inoculated onto indicator cells plates and incubated in an identical manner as the test sample.

Plate Recovery Control (PRC):

This control was performed to establish the input viral load to compare with the test device results to evaluate the viral reduction by the test device. The PRC was prepared identically to the test sample except no treatment was done to the dried virus inoculum before either being immediately recovered or being held for the contact time. To prevent the device from inadvertently treating these controls they were placed in a separate room from the test device with similar conditions. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples.

Cell Viability Control (CVC):

This control was performed to demonstrate that the indicator host cells remained viable and to confirm the sterility of the media employed throughout the incubation period. 0.05 mL of DM was added to 8 wells of indicator cells and incubated in an identical manner as the test samples.

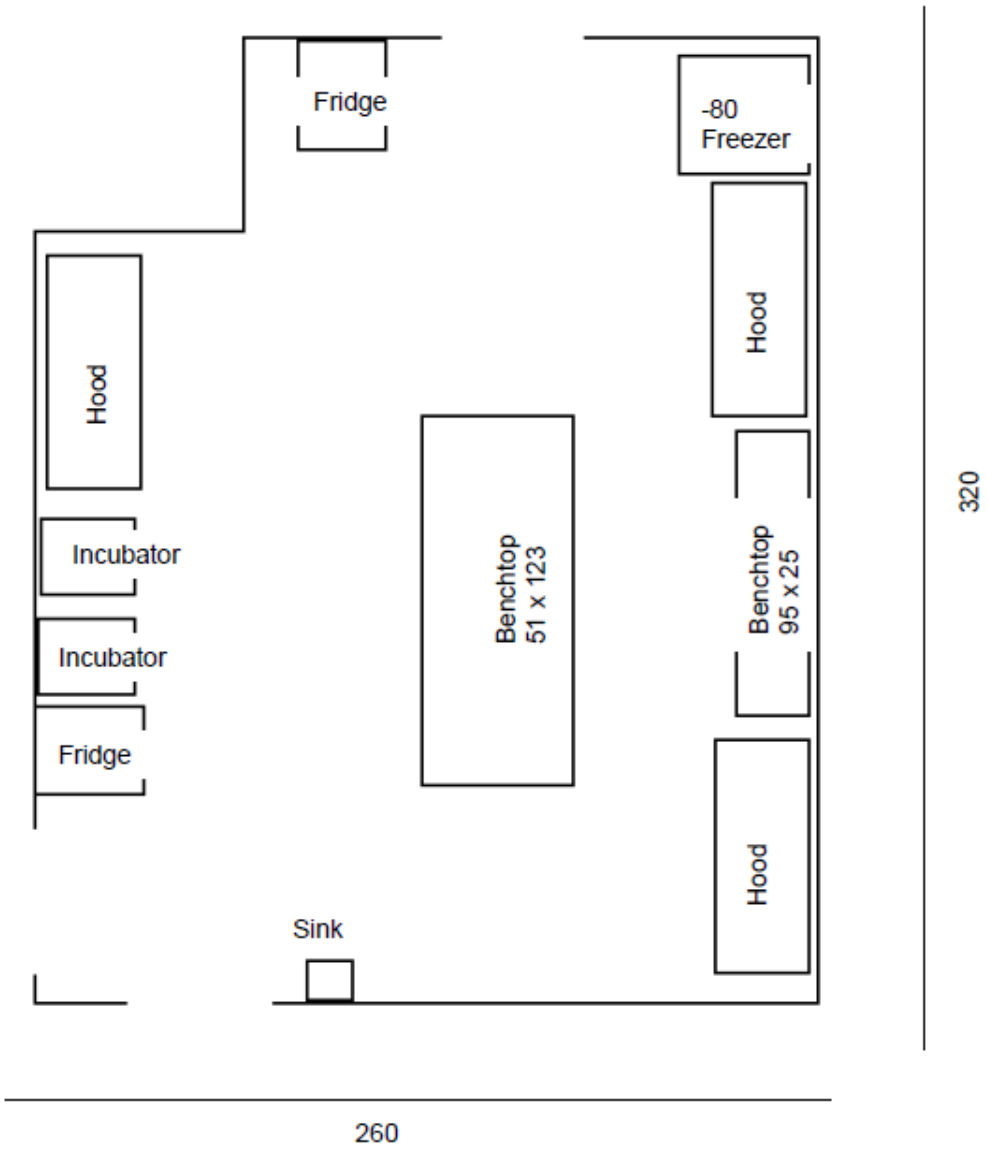
Virus Stock Titer Control (VST):

This control was performed to demonstrate that the titer of the stock virus was appropriate for use and that the viral infectivity assay was performed appropriately. An aliquot of the virus inoculum used in the study was ten-fold serially diluted in DM. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples.

TEST PROCEDURES (continued)

Figure 1

All measurements are in inches
-80 freezer: 40 x 44
Fridges: 30 x 37
Incubators: 26 x 33
Hoods: 32 x 78



PROTOCOL CHANGES

Protocol Amendments:

1. Protocol page 14 does not say where the client intends to submit the study. This amendment serves to clarify that field will be non-applicable.

2. All mentions of the title for ASTM E1053 in the protocol should be changed from, "Standard Test Method to [...]" to "Standard Practice to […]" and the year "2020" given for the references. Also, any reference to ASTM E1053-11 should instead refer to ASTM E1053-20. This amendment serves to update the title and reference number of ASTM E1053 in the protocol.

3. Project Sheet No. 1 states the Test Substance was received on 02/09/21. It should state the Test Substance was received 02/08/21. This amendment serves to correct the Test Substance received date on Project Sheet No. 1.

4. The Study Title is listed in the Protocol and Project Sheets No. 1 and 2 as, "Evaluation of Virucidal Efficacy on Hard Surface by a Disinfecting Device - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)". Per Sponsor request the title should be changed to, "Evaluation of Virucidal Efficacy on Hard Surface by a Device - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)". This amendment serves to change the Study Title listed in the Protocol and Project Sheets No. 1 and 2.

Protocol Deviations:

1. Project Sheet No. 1 lists the dilution medium as MEM + 2% FBS. The dilution medium used for this study was DMEM + 10% FBS. As the controls still showed the expected and proper results this deviation does not have an adverse effect on study data quality or integrity.

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, from 02/26/21 to 03/05/21. The study director signed the protocol on 02/26/21. The study completion date is the date the study director signed the final report. The individual test dates are as follows:

- Testing started at 11:23 am on 02/26/21 and ended at 4:24 pm on 03/05/21.

All changes or revisions of the protocol were documented, signed by the study director, dated and maintained with the protocol.

RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modifications, test device records, the final report, and correspondence between Microbac and the sponsor will be stored in the archives at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

TEST ACCEPTANCE CRITERIA

The test was considered acceptable for test device evaluation due to the criteria below being satisfied:

- The average viral load recovered from the Initial PRC must be ≥ 4.0-$\log_{10}$
- Viral-induced cytopathic effect must be distinguishable from test substance-induced cytotoxic effects (if any).
  Cell viability control and cytotoxicity control must be negative for infectivity.

CALCULATIONS

Titer Calculation:

The 50% Tissue Culture Infectious Dose per mL ($TCID_{50}$/mL) was determined using the Spearman-Karber method using the following formula:

$$m = x_k + \left(\frac{d}{2}\right) - d\sum p_i$$

where: m = the logarithm of the dilution at which half of the wells are infected relative to the test volume
x_k = the logarithm of the smallest dosage which induces infection in all cultures
d = the logarithm of the dilution factor
p_i = the proportion of positive results at dilution i
$\sum p_i$ = the sum of p_i (starting with the highest dilution producing 100% infection)

The values were converted to $TCID_{50}$/mL using a sample inoculum of 0.05 mL.

Viral Load Calculation:
Virus Load (Log_{10} $TCID_{50}$) per carrier = Virus Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [volume per sample (mL)]

Viral Reduction Calculation:
Log_{10} Reduction = Initial Viral Load (Log_{10} $TCID_{50}$*) – Output Viral Load (Log_{10} $TCID_{50}$*)
* per assayed volume and per carrier

The percentage of virus inactivation was calculated in the following manner:
[1-Output Viral Load / Initial Viral Load] x 100 = 1-10^ (Log_{10}Reduction Factor) x 100

RESULTS

Results are presented in Tables 1 – 3.

Table 1
Titer Results

Sample	Replicate	Contact time	Titer ($Log_{10}TCID_{50}$/mL)	Volume (mL)	Viral Load ($Log_{10}TCID_{50}$)
Cell viability/media sterility control	NA		no virus detected, cells viable; media sterile		
Virus Stock Titer Control			6.18	-	-
Theoretical load [a]					5.48
Initial Plate Recovery Control (T = 0 hours)	1	0 hours	5.80	0.2	5.10
	2		5.93	0.2	5.23
	3		6.05	0.2	5.35
	Average				5.23
Final Plate Recovery Control (T = 1 hour)	1	1 hour	5.93	0.2	5.23
	2		5.93	0.2	5.23
	3		6.05	0.2	5.35
	Average				5.27
P3000/PS3000	1	1 hour	5.43	0.2	4.73
	2		6.18	0.2	5.48
	3		5.55	0.2	4.85

[a] The theoretical load is determined based on the Virus Stock Titer control and the volume of virus challenged per carrier.
NA = Not applicable

Table 2
Cytotoxicity Controls

Dilution of the Neutralized Sample	Cytotoxicity Control
10^{-1}	no cytotoxicity observed in 8 out of 8 wells
10^{-2}	no cytotoxicity observed in 8 out of 8 wells
10^{-3}	no cytotoxicity observed in 8 out of 8 wells

RESULTS (continued)

Table 3
Viral Reduction

Test Device	Contact Time	Replicate Number	Initial Viral Load* ($Log_{10}TCID_{50}$)	Output Viral Load ($Log_{10}TCID_{50}$)	Log_{10} Reduction	Percent Reduction
P3000/PS3000	1 hour	1	5.23	4.73	0.50	68.13
		2		5.48	No Reduction	No Reduction
		3		4.85	0.38	58.31

* Results represent the average of three replicates.

CONCLUSIONS

When tested as described, Puraclenz's P3000/PS3000 demonstrated the viral reductions shown in Table 3 when Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), containing 5% Fetal Bovine Serum, was exposed to the test device for 1 hour at 22°C and 25% RH.

All controls met the criteria for a valid test.

APPENDIX I

MICROBAC

Microbac Protocol

Evaluation of Virucidal Efficacy on Hard Surface by a Disinfecting Device -

Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

Testing Facility
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
Puraclenz LLC
30 Butler Lane
New Canaan, CT 06840

January 5, 2021

Microbac Protocol: 1073.V.21.001
Microbac Project: 1073-102

OBJECTIVE:

This test is designed to substantiate virucidal effectiveness claims for a test device to be labeled as a virucide. It determines the potential of the test device to disinfect hard surfaces contaminated with the test virus. The test is designed to simulate consumer use and conforms to EPA OCSPP 810.2000 (2018) and 810.2200 (2018) Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSPP 810.2000, 810.2100, and 810.2200, and follows the procedure outlined in the ASTM International test method designated E1053-11, "Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces".

TESTING CONDITIONS:

Virus will be dried on glass Petri dish carriers under ambient temperature. Three carriers will be used for the device treatment contact time. Additionally, three carriers will be used as the Initial Plate Recovery Control without device treatment or holding; and three carriers will be used as the Final Plate Recovery Control with virus dried and held for the contact time without device treatment.

The test carriers will be placed about 10 feet from the device. The carriers are positioned vertically with the dried virus films facing upwards. The device set upright and powered "on" in accordance with the manufacturer or Sponsor's instruction. After the exposure (contact time), a virus recovery medium (= neutralizer) will be added onto each carrier and the virus particles will be scraped off from the surface and assayed to determine the quantity of remaining infectious virus. Multiple carriers may be treated simultaneously by the same device.

MATERIALS:

A. Test, control and reference substances, as applicable, will be supplied by the sponsor of the study (see last page). As per CFR 40.160.105:

- The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance, as applicable, shall be determined for each batch and shall be documented by the sponsor before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented and retained by the sponsor.

- When relevant to the conduct of the study the solubility of each test, control, or reference substance shall be determined by the sponsor before the experimental start date. The stability of the test, control, or reference substance shall be determined by the sponsor before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis of each batch.

The test substance will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test substance such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures Microbac testing facility management that the test substance has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

Microbac will retain all unused chemical test substances for a period of one year upon completion of the test, and then discard them in a manner that meets the approval of the safety officer, or return them to the Sponsor. The test materials and the paper records will be retained in accordance to FIFRA. Microbac will contact the Study Sponsor to arrange for transfer of records when/if the test substance is returned to the Sponsor.

B. Materials supplied by Microbac, including, but not limited to:

1. Challenge virus (requested by the Sponsor of the study): Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), Strain: USA-WA1/2020, Source: BEI Resources, NR-52281

2. Host cell line: Vero E6 cells, ATCC CRL-1586

3. Laboratory equipment and supplies.

- Clean, sterile 100 x 15 mm plastic Petri dishes
- Disposable sterile cell scrapers
- Sterile serological pipettes
- Micro-pipettors and sterile pipette tips
- 24-well cell culture plates
- Cell incubators
- Autoclave
- Certified clock
- Certified digital timer

4. Media and reagents:

- Cell culture medium (= Virus Recovery Medium)
- Dilution medium
- Sterile deionized water

Details of the media and reagents relevant to the virus-host system and test substance being tested will be documented in the first project sheet and data pack.

B. Materials supplied by the sponsor:

1. Test device

TEST SYSTEM IDENTIFICATION:

All applicable carriers, dilution tube racks, and host-containing apparatus will be appropriately labeled with the following information: virus, host, and test substance and/or project number.

EXPERIMENTAL DESIGN:

The procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at Microbac. SOPs and Logs are referred to in the raw data and are required as part of GLP regulations. The study process is described in the following sections.

A. Inoculum preparation:

Viral stocks are purchased from reputable sources that identify them by scientifically accepted methods and may have been propagated at Microbac. Records are maintained that demonstrate the origin of the virus. The virus stocks are stored at an ultra-low temperature.

Frozen viral stocks will be thawed on the day of the test. Serum will be added to viral stock to achieve an organic load of 5.0% (if not already 5.0%), unless otherwise directed by the Sponsor and pre-agreed by Microbac. If the challenge virus culture is standardized by concentration or dilution, or if a column is used, these manipulations must be documented and reported.

Note: A 3-5 Log_{10} reduction window is targeted for this study.

B. Carrier preparation:

A total of 9 sterile Petri dish glass carriers will be prepared by adding **0.2 mL virus inoculum** per carrier. The virus inoculum will be spread as much as possible with a cell scraper over an area of approximately 4 in^2 that has been marked on the underside of pre-sterilized glass Petri dishes. All inoculated carriers are incubated under ambient temperature in a biosafety cabinet in sterile plastic Petri dishes until visually dry. The clock start and stop time will be recorded for the drying time of virus. The temperature and humidity will also be recorded.

Three carriers will be prepared for test device treatment. Three carriers will be used for the Initial Plate Recovery Control. Three carriers will be used for the Final Plate Recovery Control.

Additionally, one carrier will be prepared for the cytotoxicity control using dilution medium (DM) in lieu of virus as the inoculum. No neutralizer effectiveness/viral interference control is applicable as the test material is not a chemical.

C. Test device preparation and handling:

The test device will be assembled, if required, and operated safely according to the manufacturer or sponsor's instructions as provided.

The test device will be located in a laboratory space (see figure 1 for details including the presence of furniture and pieces of large equipment). The flooring is a laboratory-grade, chemical resistant vinyl flooring material. All equipment in the laboratory has both plastic and metal parts on their exterior. The laboratory contains drywall on the surfaces, both on the walls and on the ceiling. The carriers will be placed about 10 feet from the test device on a metal cart with the below dimensions:

Length: 27.1 inches
Width: 18 inches
Height from floor: 33.5 inches
Height from bottom shelf to top shelf: 28.5 inches

The HVAC system for the laboratory space does not have filtration, only that which is within the exhaust hoods. Fresh air is drawn from outside and exhaust air is released in the space above the ceiling. The RTU serving the BSL-3 suite is isolated / dedicated and does not connect to spaces external to the lab. For safety reasons the HVAC system cannot be shut off during testing with SARS-CoV-2 (COVID-19) or any other BSL-3 organisms. However, the temperature and humidity level of the laboratory will be monitored and reported.

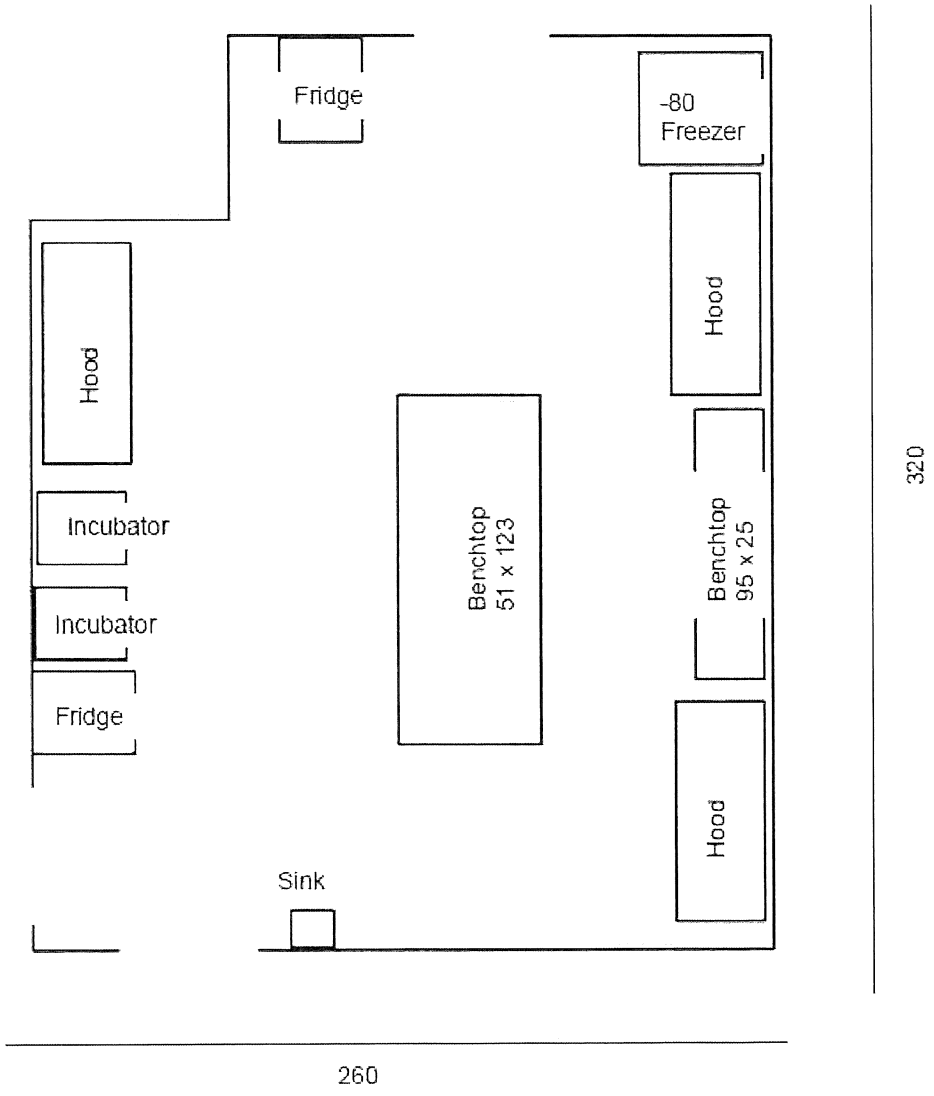
The fronts of the laminar flow hoods will be either turned off or sealed during the test in order to minimize the impact on ion distribution during the test.

The device will be turned on for a period of approximately 12 hours in the laboratory space before the virus carriers are placed and prior to testing. The laboratory doors will be sealed during this period of time so no one enters the space during pre-conditioning.

When the virus carriers are being placed in the laboratory prior to testing, the device will continue to be run. The technician will minimize the time that the laboratory door remains open when they enter the room. When exiting the room, after placing the carriers in position, the technician will again minimize the time that the laboratory door remains open.

Figure 1

All measurements are in inches
-80 freezer: 40 x 44
Fridges: 30 x 37
Incubators: 26 x 33
Hoods: 32 x 78



D. Test:

Note: The temperature and humidity level of the laboratory during the test phase will be monitored and reported.

After the inoculation and drying, the test carriers will be placed about 10 feet away from the carriers. The carriers are positioned vertically with the dried virus films facing upwards. The carriers will be exposed to the test device throughout the entire exposure (contact time). Note: Multiple carriers may be treated simultaneously by the same device. After each contact time, **2.0 mL** virus recovery medium (= neutralizer) will be added onto each carrier and the virus/neutralizer mixture will be scraped off from the surface of the carrier with a cell scraper. This "post-neutralized sample" (PNS), considered 10^{-1} dilution from the original viral inoculum, will be serially 10-fold diluted in DM. Selected dilutions of the sample will be inoculated onto cultured cell monolayers as described in "Infectivity Assay" section.

E. Controls:

1. Initial Plate recovery control (Initial PRC):

This control will be performed in three replicates concurrently with the test substance runs. The virus inoculum will be spread over the surface of the carrier and left to dry at ambient temperature. Immediately after drying – without device treatment or holding - each carrier will be applied with 2.0 mL of the virus recovery medium and processed as the test. Selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in the "Infectivity Assay" section. This control will determine the relative loss in virus infectivity resulting from drying and neutralization alone.

The average viral load from the three Initial PRC carriers will be used as the baseline and compared with the test carrier results to determine the Log_{10} and percent reduction by the test device.

2. Final Plate recovery control (Final PRC):

This control will be performed in three replicates concurrently with the test

substance runs. The virus inoculum will be spread over the surface of the carrier and left to dry at ambient temperature. The carrier will then be held for the same contact time as for the test carriers but without any device treatment. Post contact time, the carrier will be applied with 2.0 mL of the virus recovery medium and processed as the test. Selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in the "Infectivity Assay" section. This control will determine the relative loss in virus infectivity resulting from drying and neutralization alone.

3. Cytotoxicity control:

Although the test material is not chemical, a cytotoxicity control is necessary to confirm that the cytopathic effect (CPE) observed, if any, is from viral infection and not from non-specific cytotoxicity.

One carrier will be used for this control. Dilution medium, in lieu of virus, will be spread over the surface of the carrier and dried. The carrier will be placed next to the three virus-inoculated test carriers and subject to the same device treatment for the longest contact time (as a worst-case scenario for the shorter contact times). After the treatment, 2.0 mL of virus recovery medium will be added to the carrier and the residues will be scraped off from the carrier into a collection dish. The sample will be serially 10-fold diluted. Selected dilutions will be added to cultured cell monolayers at four wells per dilution, and incubated along with the other test and control samples. At the end of the incubation, it will be observed for cell condition.

4. Cell viability control:

This control will demonstrate that cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the DM employed throughout the assay period. At least four wells of cells will receive only DM and will be incubated and processed with both test and other controls. This will serve as the negative control.

5. Virus Stock Titer control (VST):

An aliquot of the virus used in the study will be directly serially diluted and inoculated onto the host cells at 4 or 8 replicate wells per dilution to confirm the titer of the stock virus. This control will demonstrate that the titer of the stock virus is appropriate for use and that the viral infectivity assay is performed appropriately.

F. Infectivity assay:

The residual infectious virus in all test and control samples will be detected by viral-induced cytopathic effect (CPE).

Selected dilutions of the recovered inoculum mixture (test samples) and control samples will be added to cultured host cells (at least four wells per dilution, per reaction mixture) and incubated at 36±2°C with 5±3% CO_2 for total 4 – 9 days. The host cells may be washed twice with phosphate buffered saline prior to inoculation. The inoculated culture will be observed and refed with fresh media as necessary, during the incubation period. These activities, if applicable, will be recorded. The host cells will then be examined microscopically for presence of infectious virions. The resulting virus-specific CPE and test device-specific cytotoxic effects will be scored by examining all test and control samples. These observations will be recorded.

G. Calculation:

The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber. The test results will be reported as the reduction of the virus titer due to treatment with test substance expressed as log_{10}. No statistical analysis will be used for this test.

The Virus Load will be calculated in the following manner:
Virus Load (Log_{10} $TCID_{50}$) = Virus Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [Volume per sample (mL)]

The Log_{10} Reduction Factor (LRF) will be calculated in the following manner:
Log_{10} Reduction Factor = Initial viral load (Log_{10} $TCID_{50}$) – Output viral load (Log_{10} $TCID_{50}$)

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- The average viral load recovered from the Initial PRC must be $\geq 4.0\text{-log}_{10}$
- Viral-induced cytopathic effect must be distinguishable from test substance-induced cytotoxic effects (if any).
- Cell viability control and cytotoxicity control must be negative for infectivity.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at Microbac, 105 Carpenter Drive, Sterling, VA 20164.

PROTOCOL AMENDMENTS AND DEVIATIONS:

Any protocol amendment(s) and protocol deviation(s) identified will be reported in project sheet(s) and included in the final report. The sponsor will sign the project sheet(s) to acknowledge the change in the protocol.

STATISTICAL ANALYSIS:

No statistical analysis will be performed in this study.

REPORT FORMAT:

Microbac employs a standard report format for each test design. Each report will provide at least the following information:

- Sponsor identification
- Test device identification

- Type of assay and project number
- Log10 and percent viral reduction
- Test results presented in tabular form and conclusions
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)
- Certificate of Analysis (GLP studies only; if provided by the Sponsor)

RECORDS TO BE MAINTAINED:

For all GLP studies, the original signed final report will be sent to the Sponsor. A draft report will be provided to Sponsor for review prior to finalization of the report

All raw data, protocol, protocol modifications, test substance records, copy of final report, and correspondence between Microbac and the sponsor will be stored in the archives at Microbac, 105 Carpenter Drive, Sterling, VA 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test substance; challenge virus and host cell line monolayers used and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

SUMMARY OF SAMPLES TO BE ASSAYED:

No.	Treatment	Contact time	Inoculum	Description
1	Test Device		Virus	Test device treated, Rep. 1
2				Test device treated, Rep. 2
3				Test device treated, Rep. 3
4	None	0 hr	Virus	Initial Plate Recovery Control, T = 0 hr, Rep. 1
5				Initial Plate Recovery Control, T = 0 hr, Rep. 2
6				Initial Plate Recovery Control, T = 0 hr, Rep. 3
7				Final Plate Recovery Control, Rep. 1
8				Final Plate Recovery Control, Rep. 2
9				Final Plate Recovery Control, Rep. 3
10	Test Device		DM	Cytotoxicity Control
11	N/A	N/A	N/A	Cell viability control
12	N/A	N/A	N/A	Virus stock titer control

MISCELLANEOUS INFORMATION:

The following information is to be completed by the sponsor prior to initiation of the study:

Test substance information and test conditions:

Test device name	P3000 / PS3000
Active ingredient(s)/Technology	
Lot No.	
Exposure Distance / Clearance	10 feet
Contact time	1 hour
Contact temperature	Ambient (20±2C)
No. of test carriers per condition	Three (N=3)
Organic Load in viral inoculum	5% serum

The sponsor intends to submit this information to: ☐ _____

Study Conduct: ☒ GLP

PROTOCOL APPROVAL BY SPONSOR:

Sponsor Signature: _____ Date: 1/11/2021

Printed Name: CHRISTOPHER BOOLEY

PROTOCOL APPROVAL BY STUDY DIRECTOR (Microbac):

Study Director Signature: _____ Date: 02/26/21

Printed Name: CORY CHRISTENSEN

Date Issued: 02/26/21	Project Sheet No. 1	Page No. 1	Laboratory Project Identification No. 1073-102

STUDY TITLE: Evaluation of Virucidal Efficacy on Hard Surface by a Disinfecting Device - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)	**STUDY DIRECTOR:** Cory Chiossone Signature 02/26/21 Date		
TEST SUBSTANCE: P3000/PS3000	**LOT NO.:** N/A	**DATE RECEIVED:** 02/09/21	**DS NO.:** L166
PERFORMING DEPARTMENT (S): Virology and Toxicology	**STORAGE CONDITIONS:** Location: E3 ☐ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		

PROTECTIVE PRECAUTION REQUIRED: MSDS ☐ Yes / ■ No

PHYSICAL DESCRIPTION: ☐ Solid ☐ Liquid ☐ Aerosol ■Other: Device

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 02/26/21 **TERMINATION DATE:** 03/05/21

CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other:

SPONSOR: Puraclenz LLC 30 Butler Lane New Canaan, CT 06840	**CONTACT PERSON:** Chris Dooley Email: cdooley@puraclenz.com

TEST CONDITIONS:

Challenge organism:	Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), Strain: USA-WA1/2020, Source: BEI Resources, NR-52281
Host cell line:	Vero-E6 cells, ATCC CRL-1586
Active ingredient:	N/A
Organic Load:	5% serum
Dilution Medium:	Minimum Essential Medium (MEM) + 2% Newborn Calf Serum (NCS)
Neutralizer:	MEM + 10% NCS
Contact times:	1 hour
Contact temperature(s):	Ambient (20±2C)
Incubation time:	4 – 9 days
Incubation temperature(s):	36±2°C and 5±3% CO_2

PROTCOL AMENDMENTS:

1. Protocol page 14 does not say the test material lot number, test material active ingredient and where the client intends to submit the study. This amendment serves to clarify that these fields will be non-applicable.

Date Issued: 05/19/21 Project Sheet No. 2 Page No. 1 Laboratory Project Identification No. 1073-102

STUDY TITLE: Evaluation of Virucidal Efficacy on Hard Surface by a Disinfecting Device - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)	**STUDY DIRECTOR:** Cory Chiossone Signature 05/19/21 Date		
TEST SUBSTANCE: P3000/PS3000	**LOT NO.:** N/A	**DATE RECEIVED:** 02/08/21	**DS NO.:** L166
PERFORMING DEPARTMENT (S): Virology and Toxicology	**STORAGE CONDITIONS:** Location: E3 ☐ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other:			
SPONSOR: Puraclenz LLC 30 Butler Lane New Canaan, CT 06840	**CONTACT PERSON:** Chris Dooley Email: cdooley@puraclenz.com		

PROTCOL AMENDMENTS:

2. All mentions of the title for ASTM E1053 in the protocol should be changed from, "Standard Test Method to [...]" to "Standard Practice to [...]" and the year "2020" given for the references. Also, any reference to ASTM E1053-11 should instead refer to ASTM E1053-20. This amendment serves to update the title and reference number of ASTM E1053 in the protocol.

3. Project Sheet No. 1 states the Test Substance was received on 02/09/21. It should state the Test Substance was received 02/08/21. This amendment serves to correct the Test Substance received date on Project Sheet No. 1.

PROTOCOL DEVIATIONS:

1. Project Sheet No. 1 lists the dilution medium as MEM + 2% FBS. The dilution medium used for this study was DMEM + 10% FBS. As the controls still showed the expected and proper results this deviation does not have an adverse effect on study data quality or integrity.

Date Issued: 06/01/21 Project Sheet No. 3 Page No. 1 Laboratory Project Identification No. 1073-102

STUDY TITLE: Evaluation of Virucidal Efficacy on Hard Surface by a Device - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)	**STUDY DIRECTOR:** Cory Chiossone Signature 06/04/21 Date		
TEST SUBSTANCE: P3000/PS3000	**LOT NO.:** N/A	**DATE RECEIVED:** 02/08/21	**DS NO.:** L166
PERFORMING DEPARTMENT (S): Virology and Toxicology	**STORAGE CONDITIONS:** Location: E3 ☐ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other:			
SPONSOR: Puraclenz LLC 30 Butler Lane New Canaan, CT 06840	**CONTACT PERSON:** Chris Dooley Email: cdooley@puraclenz.com		

PROTCOL AMENDMENTS:

4. The Study Title is listed in the Protocol and Project Sheets No. 1 and 2 as, "Evaluation of Virucidal Efficacy on Hard Surface by a Disinfecting Device - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)". Per Sponsor request the title should be changed to, "Evaluation of Virucidal Efficacy on Hard Surface by a Device - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)". This amendment serves to change the Study Title listed in the Protocol and Project Sheets No. 1 and 2.