Subject
STR Solution Test report
by Microbac
(related with SARS-CoV-2/COVID 19)

To
Swisstec 3D

Purpose
For submission to Austria government

Document No. 20201215.S.001

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FINAL REPORT

Virucidal Efficacy Hard-Surface Test for STR-solution, A Space Sterilization Purifier Ionizer Device - per ASTM E1053 - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

Test Substance
STR-Solution (Device)

Lot Number
N/A

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
11/30/20

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

Laboratory Project Identification Number
1035-101

Protocol Identification Number
STR.1.07.13.20

Sponsor
STR-Solution
23, Cheondeoksan-ro, Namsa-myeon, Cheoin-gu
Yongin-si, Gyeonggi-clo, 17118
Republic of Korea

TABLE OF CONTENTS

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, Brandon G. Narvaez

Study Director:

Cory Chiossone — 11/30/2020 Date

QUALITY ASSURANCE UNIT STATEMENT

The Quality Assurance Unit of Microbac has inspected Project Number 1035-101 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	09/09/20 09/14/20	09/14/20	09/14/20
In Process	09/11/20	09/14/20	09/14/20
Final Report	10/20/20	10/20/20	10/20/20

Lucas Thurn, RQAP-GLP
Quality Assurance Associate III

11/30/2020
Date

TEST SUBSTANCE CHARACTERIZATION

Test Substance characterization as to the identity, strength, purity, solubility and composition, as applicable, according to 40 CFR, Part 160, Subpart F [160.105] was documented prior to its use in the study. The Test Substance Certificate of Analysis Reports, provided by the sponsor, are found in Appendix I.

TEST SUMMARY

Study Title:	Virucidal Hard Surface Efficacy Test for a UV Device - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)
Project No.:	1035-101
Protocol No.:	STR.1.07.13.20
Test Method:	ASTM International E1053-11 “Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces”
Sponsor:	STR-Solution 23, Cheondeoksan-ro, Namsa-myeon, Cheoin-gu Yongin-si, Gyeonggi-clo, 17118 Republic of Korea
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 substance to be labeled as a virucide by determining the potential of the test substance 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, as well as the Health Canada “Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs” (January 2014).
Study Dates:	Study Initiation: 09/11/20 Experimental Start: 09/11/20 Experimental End: 09/17/20 Study Completion: See page 1

TEST SUMMARY (continued)

Test Substance:
- STR-Solution (Device), Lot No.: N/A, Received: 06/26/20, and assigned DS No. K896
- Physical Description: Device
- Storage Condition: Ambient
- Active Ingredients: N/A

Test Conditions:
Organic Soil Load: 5.0% Fetal Bovine Serum (FBS)
Contact Times: 1 hour, 2 hours, 4 hours
Contact Temperature: 21°C
Contact Relative Humidity: 45-48%

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: 6 days)

Incubation Temperature:
36 ± 2°C with 5 ± 3% CO_2

Dilution Medium (DM):
Minimum Essential Medium (MEM) + 2% Newborn Calf Serum (NCS)

Recovery Medium:
MEM + 10% Newborn Calf Serum (NCS)

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 12 – 30 hours 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 Substance:

The test device was placed 1-2 m 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 23 minutes at 21°C with 53-64% Relative Humidity (RH). Fifteen carriers were prepared for each contact time using virus. Fifteen carriers were prepared for the virus recovery control using virus. Additionally, one carrier was prepared for the cytotoxicity controls using dilution media in lieu of virus as the inoculum.

Test Substance Application and Exposure Conditions:

Three carriers per contact time were evaluated. The carriers were placed about 60 cm above the surface with the device placed 1-2 m away from the carriers. The device remained on and focused on the carriers at the temperature and for the time specified by the sponsor. The test substance was tested at 21°C with 45-48% RH.

TEST PROCEDURES (continued)

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 6 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 6 days of incubation at 36 ± 2°C with 5 ± 3% the plates were removed, scored, and recorded for test-substance specific cytotoxic effects and/or virus-specific cytopathic effect (CPE).

Cytotoxicity Control (CT):

This control was performed to assess the cytotoxic effects of the test substance 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 substance application and neutralization, 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 substance results to evaluate the viral reduction by the test substance. 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 longest contact time. 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. 1.0 mL of DM was added to 4 wells of indicator cells and incubated in an identical manner as the test samples.

TEST PROCEDURES (continued)

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.

PROTOCOL CHANGES

Protocol Amendments:

1. The miscellaneous information section of the protocol does not include the test substance's lot number or active ingredient. Per Sponsor email the active ingredient is positive / negative Radical ions and the lot number is N/A. This amendment serves to include the active ingredient and lot number of the test substance to the miscellaneous information section of the protocol.

Protocol Deviations:

No protocol deviations occurred during this study.

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, from 09/11/20 to 09/17/20. The study director signed the protocol on 09/11/20. The study completion date is the date the study director signed the final report. The individual test dates are as follows:

- Testing started at 1:03 pm on 09/11/20 and ended at 3:47 pm on 09/17/20.

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 substance 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 substance evaluation due to the criteria below being satisfied:

- The average viral load recovered from the Initial PRC must be ≥ 4.0-log10
- 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 = 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			6.93		-
Theoretical load [a]					6.23
Initial Plate Recovery Control (T = 0 hours)	1	0 hours	6.18	0.2	5.48
	2		6.43	0.2	5.73
	3		6.43	0.2	5.73
	Average				5.65
Final Plate Recovery Control (T = 4 hours)	1	4 hours	6.05	0.2	5.35
	2		6.05	0.2	5.35
	3		5.68	0.2	4.98
	Average				5.23
STR-Solution (Device)	1	1 hour	5.68	0.2	4.98
	2		5.80	0.2	5.10
	3		5.55	0.2	4.85
	1	2 hours	5.43	0.2	4.73
	2		5.18	0.2	4.48
	3		5.30	0.2	4.60
	1	4 hours	5.18	0.2	4.48
	2		4.80	0.2	4.10
	3		4.80	0.2	4.10

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

RESULTS (continued)

Table 2
Cytotoxicity Controls

Dilution of the Neutralized Sample	Cytotoxicity Control
10^{-1}	no cytotoxicity observed in 4 out of 4 wells
10^{-2}	no cytotoxicity observed in 4 out of 4 wells
10^{-3}	no cytotoxicity observed in 4 out of 4 wells

Table 3
Viral Reduction

Test Agent	Contact Time	Replicate Number	Initial Viral Load* ($Log_{10}TCID_{50}$)	Output Viral Load ($Log_{10}TCID_{50}$)	Log_{10} Reduction	Percent Reduction
STR-Solution (Device)	1 hour	1	5.65	4.98	0.67	78.62%
		2		5.10	0.55	71.82%
		3		4.85	0.80	84.15%
		Mean Reduction			**0.67**	78.62%
	2 hours	1	5.65	4.73	0.92	87.98%
		2		4.48	1.17	93.24%
		3		4.60	1.05	91.09%
		Mean Reduction			**1.05**	91.09%
	4 hours	1	5.65	4.48	1.17	93.24%
		2		4.10	1.55	97.18%
		3		4.10	1.55	97.18%
		Mean Reduction			**1.42**	96.20%

* Results represent the average of three replicates.

CONCLUSIONS

When tested as described STR-Solution (Device), 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 substance for 1, 2 or 4 hours at 21°C and 45-48% RH.

All controls met the criteria for a valid test. These conclusions are based on observed data.

REFERENCES

1. ASTM E1053-11, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2011.

2. 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.

3. 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.

4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSPP 810.2000, 810.2100, and 810.2200.

APPENDIX I

MICROBAC

Microbac Protocol

Virucidal Efficacy Hard-Surface Test for STR-solution, A Space Sterilization Purifier Ionizer Device - per ASTM E1053 -

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
STR-Solution
23, Cheondeoksan-ro, Namsa-myeon, Cheoin-gu
Yongin-si, Gyeonggi-clo, 17118
Republic of Korea

July 13, 2020

Microbac Protocol: STR.1.07.13.20
Microbac Project: 1035-101

Microbac Laboratories, Inc.
105 Carpenter Drive | Sterling, VA 20164 | 703.925.0100 p | 703.925.9366 f | www.microbac.com

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 Petri dish glass carriers under ambient temperature. Three carriers will be used for device treatment contact time 1, three carriers will be used for device treatment contact time 2, and three carriers will be used for device treatment contact time 3. 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 longest contact time without device treatment.

The test carriers will be placed about 60 cm above a surface and 1-2 m from the device. The carriers are positioned vertically with the dried virus films facing upwards. The device is powered "on" in accordance with the manufacturer or Sponsor's instruction. After each 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. The average viral load from three test carriers will be compared to the average of the control carriers to determine the Log_{10} and percent reduction at each of the contact times.

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 15 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.

Nine carriers will be prepared for test device treatment, three for each contact time. 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.

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 60 cm above a surface with the device placed 1-2 m away from the carriers. The carriers are positioned vertically with the dried virus films facing upwards. The device is powered "on" in accordance with the manufacturer or Sponsor's instruction. 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 longest 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} $TCID_{50}$)

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

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-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	1 hr	Virus	Test device treated, T= 1 hr, Rep. 1
2				Test device treated, T= 1 hr, Rep. 2
3				Test device treated, T= 1 hr, Rep. 3
4		2 hr		Test device treated, T= 2 hr, Rep. 1
5				Test device treated, T= 2 hr, Rep. 2
6				Test device treated, T= 2 hr, Rep. 3
7		4 hr		Test device treated, T= 4 hr, Rep. 1
8				Test device treated, T= 4 hr, Rep. 2
9				Test device treated, T= 4 hr, Rep. 3
10	None	0 hr	Virus	Initial Plate Recovery Control, T = 0 hr, Rep. 1
11				Initial Plate Recovery Control, T = 0 hr, Rep. 2
12				Initial Plate Recovery Control, T = 0 hr, Rep. 3
13		4 hr		Final Plate Recovery Control, T = 4 hr, Rep. 1
14				Final Plate Recovery Control, T = 4 hr, Rep. 2
15				Final Plate Recovery Control, T = 4 hr, Rep. 3
16	Test Device	4 hr	DM	Cytotoxicity Control
17	N/A	N/A	N/A	Cell viability control
18	N/A	N/A	N/A	Virus stock titer control

Microbac Laboratories, Inc. 105 Carpenter Dr., Sterling, Virginia 20164

Date Issued: 09/09/20	Project Sheet No. 1	Page No. 1	Laboratory Project Identification No. 1035-101

STUDY TITLE: Virucidal Efficacy Hard-Surface Test for STR-solution, A Space Sterilization Purifier Ionizer Device - per ASTM E1053 - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)	**STUDY DIRECTOR:** Cory Chiossone Signature 09/11/20 Date		
TEST SUBSTANCE: STR-Solution (Device)	**LOT NO.:** NA	**DATE RECEIVED:** 06/26/20	**DS NO.:** K896
PERFORMING DEPARTMENT (S): Virology and Toxicology	**STORAGE CONDITIONS:** Location: DS room floor ☐ 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: 09/09/20 **TERMINATION DATE:** 09/18/20

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

SPONSOR: STR-Solution 23, Cheondeoksan-ro, Namsa-myeon, Cheoin-gu Yongin-si, Gyeonggi-clo, 17118 Republic of Korea	**CONTACT PERSON:**

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:	positive / negative Radical ions
Organic Load:	5.0% serum in virus inoculum
Dilution Medium:	Minimum Essential Medium (MEM) + 2% Newborn Calf Serum (NCS)
Neutralizer:	MEM + 10% NCS
Contact times:	1 hour, 2 hours, 4 hours
Contact temperature(s):	Room Temperature (20±2C)
Incubation time:	4 – 9 days
Incubation temperature(s):	36±2°C and 5±3% CO_2

PROTOCOL AMENDMENTS:

1. The miscellaneous information section of the protocol does not include the test substance's lot number or active ingredient. Per Sponsor email the active ingredient is positive / negative Radical ions and the lot number is N/A. This amendment serves to include the active ingredient and lot number of the test substance to the miscellaneous information section of the protocol.