



EFFICACY OF THE DUO-2000 WITH PRO-CELL™ TECHNOLOGY DEVICE  
AGAINST SURFACE SARS-COV-2

**PROJECT: DUO-2000 SURFACE SARS-COV-2**

PRODUCT: DUO-2000 with PRO-Cell™ Technology

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

**CHALLENGE ORGANISM(S):**

SARS-CoV-2 USA-CA1/2020

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**Laboratory Project Number**

**1059**



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## Efficacy Study Summary

<b>Study Title</b>	EFFICACY OF THE DUO-2000 WITH PRO-CELL™ TECHNOLOGY DEVICE AGAINST SURFACE SARS-COV-2
<b>Laboratory Project #</b>	1059
<b>Guideline:</b>	Modified ISO standards as no international standards exist.
<b>Testing Facility</b>	Innovative Bioanalysis, Inc.
<b>Study Dates:</b>	
<b>Study Initiation Date:</b>	3/11/2021
<b>Study Completion Date:</b>	6/16/2021
<b>GLP Compliance</b>	All internal SOPs and processes follow GCLP guidelines and recommendations.
<b>Test Substance</b>	SARS-CoV-2 USA-CA1/2020
<b>Description</b>	The DUO-2000 with PRO-Cell™ Technology induct air purifier unit is designed to operate continuously when installed in a heating or air conditioning system to purify the air as it circulates through the household. This in vitro study conducted will focus on determining the effectiveness of the air purifier on surfaces against the known pathogen, SARS-CoV-2 USA-CA1/2020.
<b>Test Conditions</b>	The test was conducted in a sealed biosafety cabinet located in the BSL3 chamber complying to BSL3 standards. The conditions throughout the duration of the experiment were 74°F with a relative humidity of 32%. Both the BSL3 and biosafety cabinet were operating to prevent any unwanted exposure of viral media to the surrounding environment. The iris dampener allowed for automated closure of the aperture to block exposure to the UV-C lamp which was needed due to the short series of timepoints used for this experiment.
<b>Test Results</b>	Active SARS-CoV-2 was not detected on the surface with levels below the 120 TCID50/mL limit of quantification after 2.5 seconds of the unit operating.
<b>Control Results</b>	A single control test run was conducted to serve as a comparative baseline. A $6.32 \times 10^6$ TCID50/mL viral stock of SARS-CoV-2 USA-CA1/2020 was used for this experiment and pipetted onto a glass slide where it was evenly spread and left to air dry for 5 minutes. After 5 seconds, the concentration of active SARS-CoV-2 collected was 6,218,640 TCID50/mL.
<b>Conclusion</b>	The DUO-2000 with PRO-Cell™ Technology showed significant capabilities in neutralizing active SARS-CoV-2 USA-CA1/2020, indicating a 99.99% net reduction after 2.5 seconds of exposure to the UV-C lamp.



## Study Report

**Study Title:** EFFICACY OF THE DUO-2000 WITH PRO-CELL™ TECHNOLOGY DEVICE AGAINST SURFACE SARS-COV-2

**Sponsor:** Field Controls

**Test Facility:** Innovative Bioanalysis, Inc. 3188 Airway Ave Suite D, Costa Mesa, CA 92626

**Device Testing:** Testing the surface efficacy of the DUO-2000 with PRO-Cell™ Technology against a known pathogen, SARS-CoV-2

**Study Report Date:** 6/16/2021

**Experimental Start Date:** 4/20/2021

**Experimental End Date:** 4/20/2021

**Study Completion Date:** 6/16/2021

### Study Objective:

This in vitro study was designed to determine the effectiveness of the DUO-2000 with PRO-Cell™ Technology air purifier on surfaces against a known pathogen, SARS-CoV-2 USA-CA1/2020.

### Test Method:

#### Surface Inoculation:

For the control and viral challenge, each of the testing sites were equally subjected to a 1 mL inoculation of viral media containing a known titer of  $6.32 \times 10^6$  TCID<sub>50</sub>/mL\*. The viral solution was spread with a spatula to ensure even distribution and saturation of all materials and left to air dry for 5 minutes. The viral solution was splayed out on a sterile glass slide that is 3" x 1.5" and 0.125" thick.

#### Surface Sampling:

Swabs were moistened with viral media solution prior to collecting samples to maximize collection. Each slide was subjected to a 1 mL rinse in viral media and swabbed for residual pathogen material. After collection was completed, the swab and media were vortexed for 1 full minute. All samples collected were subjected to the same TCID<sub>50</sub> assay protocol to determine viral concentration.

#### Test System Strains: SARS-CoV-2 USA/CA-1/2020

The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-CA1/2020, NR-52382.

*\*The viral titer listed in the Certificate of Analysis is representative of the titer provided by BEI Resources. These viruses are grown on VeroE6 cells either inhouse or at a partner lab to the concentrations listed within the experiment design.*

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## Study Materials and Equipment:

**Equipment Overview:** The equipment arrived pre-packaged from the manufacturer and was inspected upon arrival. The device came with all required parts for assembling and a step-by-step instruction manual for assembly and installation was provided by the manufacturer. After assembly, the device was powered on to check for normal operations.

MANUFACTURER: Field Controls

MODEL: DUO-2000 with PRO-Cell™ Technology

SIZE: N/A

MAKE: DUO

SERIAL #: N/A



**Equipment Specifics:** The equipment arrived at the laboratory pre-packaged from the manufacturer and all device parts were inspected for damage upon arrival. The package included the DUO-2000 with PRO-Cell™ Technology air purifier, 120V power cord, UV-C germicidal lamp, a mounting plate, screws, and instruction manual. The DUO-2000 with PRO-Cell™ Technology was assembled on site using the instructions provided by the manufacturer and powered on to check for normal operations.

**Testing Chamber:** The test was conducted inside a metal and glass BSL2 biosafety cabinet located inside a BSL3 laboratory complying to BSL3 standards. The air temperature remained at approximately 74°F with a humidity of 38% throughout the course of the test inside the testing chamber and BSL3 lab. The BSL3 was designed to maintain a negative pressure environment with HEPA filters and the biosafety cabinet level 2 was operated in adjunction to prevent any release of testing media into the surrounding environment. All seals for the BSL3 chamber were confirmed and all equipment used had a function test completed to confirm working conditions. For calibrated equipment, the records were checked to confirm operational status.

**Design Layout:**

The test was conducted in a metal and glass biosafety cabinet with sealed seams measuring 72" x 32" x 32". The testing chamber is located in a BSL3 laboratory consisting of metal walls and epoxy floor equipped with viewing windows and a lockable antechamber complying to BSL3 standards. The temperature inside the BSL3 lab was approximately 74°F and had a relative humidity of 32% throughout the study.

The DUO-2000 with PRO-Cell™ Technology was positioned horizontally in the center of the testing chamber with the sample centered to the middle of the device and below the UV-C source. Samples were laser aligned and positioned three inches from the luminaire. The iris dampener system was placed between the device and the sample slides to restrict UV exposure to the samples. The iris dampener was manually opened for the start of the test and was set to automatically close at the appropriate timepoints by the ATC 304 GX Solid State timer.

Prior to testing, all internal lab systems were reviewed and determined to be functioning. The chamber was pressure tested for leaks by visual inspections using a colored smoking device. All seals for the chamber were confirmed and all equipment used had a function test to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

**Test Method:**

Field Controls supplied a DUO-2000 with PRO-Cell™ Technology air purifying system for testing purposes to determine its efficacy against viral pathogens. This study evaluated the efficacy of the device in its ability to inactivate the viral strain referred to as SARS-CoV-2. A pneumatic iris dampener was used in this experiment which allowed the closure of the aperture in an estimated ¼ of a second. It was connected to a timer so it would close on the timepoints, protecting the samples from UV-C over exposure.

**Exposure Conditions:**

1. Prior to the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. The temperature during all test runs was approximately 74°F with a relative humidity of 32%.
3. The sample slides were inoculated with 1mL of a known concentration of viral media and left to air dry for 5 minutes.
4. The DUO-2000 with PRO-Cell™ Technology was operated prior to being used to allow the UVC lamp to warm up.
5. An iris dampener was set to close on each pre-determined timepoint to protect samples from UVC exposure.
6. Samples were placed in the center so they would be equal distance from both ends of the device.

**Experimental Procedure:**

1. A 3"x1.5" sterile glass slide, 0.125" thick was inoculated with 1mL of  $6.32 \times 10^6$  TCID50/mL viral media for each timepoint.
2. Samples were placed 3 inches from the luminaire.
3. One slide sample was used for each timepoint and placed on the stainless-steel surface under the iris dampener, which closed at the eight following timepoints:
  - 0.5 secs
  - 1 sec
  - 1.5 secs
  - 2 secs
  - 2.5 secs
  - 3 secs
  - 3.5 secs
4. Upon collection of samples, the UV system would be turned off to remove the slide associated with the timepoint.
5. To maximize collection, a swab was moistened with viral media solution prior to collecting samples and rinsed.
6. All swabs were sealed after collection and provided to lab staff for analysis after study completion.
7. One control and viral challenge was conducted using the same methodology.

**Post Decontamination:**

At the conclusion of each viral challenge test, the UV system inside the biosafety chamber was activated for 30 minutes. After 30 minutes of UV exposure, all test equipment was cleaned the end of the day with 70% alcohol solution.

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## Preparation of The Pathogen

Viral Stock: SARS-CoV-2 USA-CA1/2020 (BEI NR-52382)

Test	Specifications	Results
Identification by Infectivity in Vero 6 cells	Cell Rounding and Detachment	Cell Rounding and Detachment
Next-Generation Sequencing (NGS) of the complete genome using Illumina® iSeq™ 100 Platform	≥ 98% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1	99.9% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1
Approx. 940 Nucleotides	≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1	100% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1
Titer by TCID50 in Vero E6 Cells by cytopathic effect	Report Results	2.8 X 10 <sup>5</sup> TCID50 per mL in 5 days at 37°C and 5% CO <sub>2</sub>
Sterility (21-Day Incubation)		
Harpos HTYE Broth, aerobic	No Growth	No Growth
Trypticase Soy Broth, aerobic	No Growth	No Growth
Sabourad Broth, aerobic	No Growth	No Growth
Sheep Blood Agar, aerobic	No Growth	No Growth
Sheep Blood Agar, anaerobic	No Growth	No Growth
Thioglycollate Broth, anaerobic	No Growth	No Growth
DMEM with 10% FBS	No Growth	No Growth
Mycoplasma Contamination		
Agar and Broth Culture	None Detected	None Detected
DNA Detection by PCR of extracted test article nucleic acid	None Detected	None Detected





**TCID50 Procedure:**

**Materials and Equipment:**

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips—20uL, 200 uL, 1000uL
- Inverted Microscope
- Tubes for dilution
- Hemocytometer with coverslip
- Cell media for infection
- Growth media appropriate for the cell line
- 0.4 % Trypan Blue Solution
- Lint-free wipes saturated with 70% isopropyl alcohol
- CO<sub>2</sub> Incubator set at 37°C or 34°C, or other temperature as indicated

**Procedure:**

1. One day before infection, prepare 96 well dishes by seeding each well with Vero E6 cells in DMEM plus 7.5% fetal bovine serum, 4mM Glutamine, and antibiotics.
2. On the day of infection, make dilutions of virus samples in PBS.
3. Make a series of dilutions at 1:10 of the original virus sample. Fill the first tube with 2.0 mL PBS and the subsequent tubes with 1.8mL.
4. Vortex the viral samples, then transfer 20 uL of the virus to the first tube, vortex, discard tip.
5. With a new tip, serial dilute subsequent tips transferring 200 uL.

**Additions of virus dilutions to cells**

1. Label the lid of a 96-well dish by drawing grid lines to delineate quadruplicates and number each grid to correspond to the virus sample and label the rows of the plate for the dilution, which will be plated.
2. Include four (4) negative wells on each plate which will not be infected.
3. Remove all but 0.1 mL of media from each well by vacuum aspiration.
4. Starting from the most dilute sample, add 0.1 mL of virus dilution to each of the quadruplicate wells for that dilution.
5. Infect four wells per dilution, working backward.



6. Allow the virus to absorb to the cells at 37°C for 2 hours.
7. After absorption, remove the virus inoculum. Start with the most dilute and work backward.
8. Add 0.5 mL infection medium to each well, being careful not to touch the wells with the pipette.
9. Place plates at 37°C and monitor CPE using the inverted microscope over a period of 1 to 4 weeks.
10. Record the number of positive and negative wells.

#### Protocol Changes:

Protocol Amendments: None

Protocol Deviations: None

#### Control Protocol

One control test was conducted with no UV-C exposure from the DUO-2000 with PRO-Cell™ Technology system in the testing chamber. Control samples were taken at the corresponding sample times used for the challenge trial to serve as a comparative baseline to assess the viral reduction when the DUO-2000 with PRO-Cell™ Technology was operated. This allows for the net reduction calculations to be made for the challenge trial. Furthermore, temperature and relative humidity were monitored inside the BSL3 lab and confirmed to be in relative range, +/-5% compared to control testing conditions prior to running the viral challenges.

#### Inoculation of Viral Media:

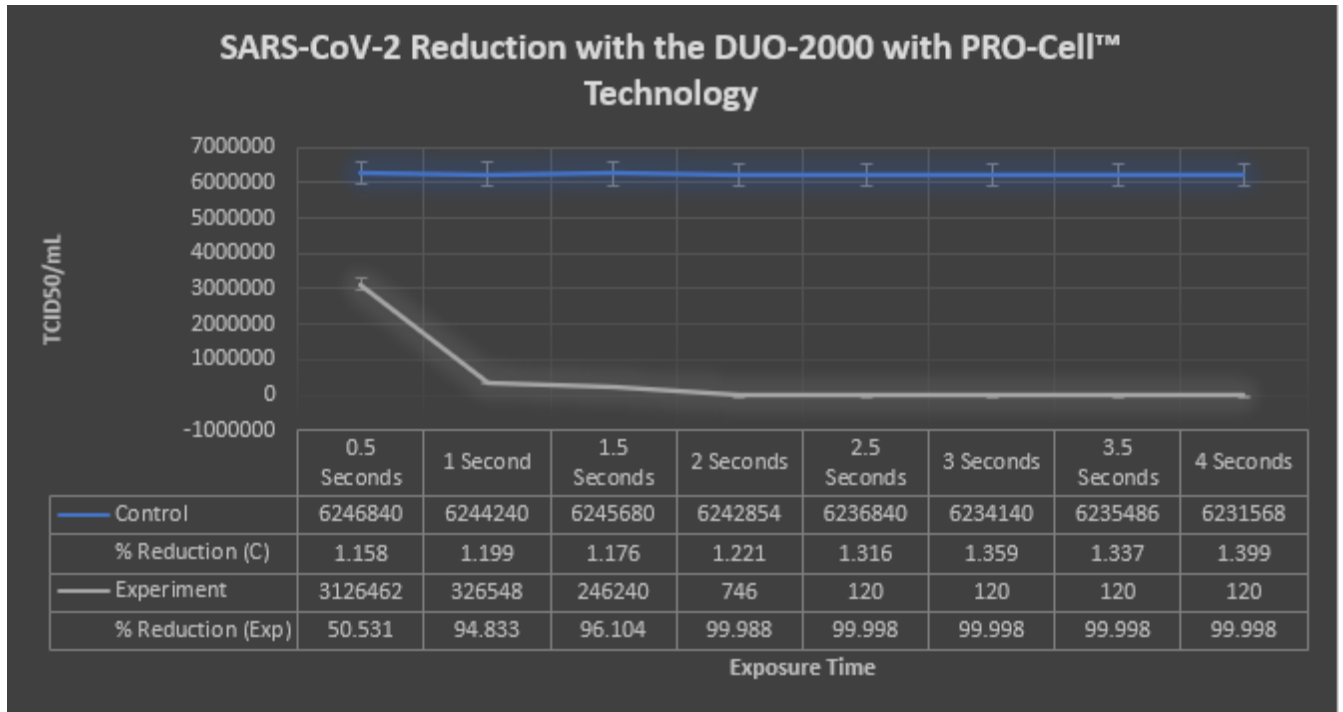
A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of  $6.32 \times 10^6$  TCID<sub>50</sub>/mL\* was used for this experiment. Each of the testing sites were equally subjected to a 1 mL inoculation of viral media and was spread evenly on the slide using a spatula before letting it air dry for 5 minutes. The viral solution was splayed out on a sterile glass slide that was 3" x 1.5" and 0.125" thick. The control samples were prepared and collected in the same manner as the viral test regarding the time points and collection rate.

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## Study Results

### RESULTS:



\*\*As it pertains to data represented herein, the value of 1.2E+02 indicates a titer that is lower than the specified limit of quantitation. The limit of quantitation for this assay is 1.2E+02.

\*\*\*As it pertains to data represented herein; the percentage error equates to an average of +/-5% of the final concentration.



### Conclusion:

The DUO-2000 with PRO-Cell™ Technology demonstrated the ability to reduce the concentration of active pathogen, SARS-CoV-2 from the surface. The DUO-2000 with PRO-Cell™ Technology equipment had an observed overall reduction of 99.99% of viral media after 2.5 seconds of exposure to the UV-C. When compared to control values, this is indicative of a 4-log reduction of SARS-CoV-2 on surfaces with an operating DUO-2000 with PRO-Cell™ Technology system. For the purpose of this experiment, it was observed that given enough exposure time, the overall viral neutralization was substantial.

When working with and collection microorganisms, some variables cannot be accounted for, namely, placement of microorganisms, collection volume, collection points, surface saturation, microorganism destruction upon collection, and possibly others. However, every effort was made to address these constraints with the design and execution of the trials. The efforts are reflected in the meaningful recovery of microorganisms in the control test. Furthermore, efforts were made to simulate a replicable environment in the chamber while taking into considerations the precautions needed when working with infectious microorganisms.

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## Disclaimer

The Innovative Bioanalysis, Inc. ("Innovative Bioanalysis") laboratory is not certified or licensed by the United States Environmental Protection Agency and makes no equipment emissions claims pertaining to ozone or byproduct of any Field Controls device. Innovative Bioanalysis, Inc. makes no claims to the overall efficacy of any DUO-2000. The experiment results are solely applicable to the device used in the trial. The results are only representative of the experiment design described in this report. Innovative Bioanalysis, Inc. makes no claims as to the reproducibility of the experiment results given the possible variation of experiment results even with an identical test environment, viral strain, collection method, inoculation, nebulization, viral media, cell type, and culture procedure. Innovative Bioanalysis, Inc. makes no claims to third parties and takes no responsibility for any consequences arising out of the use of, or reliance on, the experiment results by third parties.

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