



inc. BIOLOGICAL CONSULTING SERVICES
OF NORTH FLORIDA, INC.

December 09, 2020

Mr. Adam Anthony
BioZone Scientific International, Inc.
7616 Southland Blvd., Suite 114
Orlando, Florida 32809 USA
Email: adam.anthony@biozonescientific.com

RE: SARS-CoV-2 Surrogate Inactivation by Ultraviolet Disinfection System from BioZone Scientific; BCS ID 2011334.

Dear Mr. Anthony,

We have completed the virucidal efficacy study for the supplied UV exposure chamber containing four low pressure mercury lamps (10-H36000; 87 W). In the study, in order to assess SARS-CoV-2, the surrogate Coronavirus OC43 (ATCC VR-1558) was used to evaluate the virucidal properties of the UV radiation at a 12-inch distance from the UV lamps. Virus was inoculated onto non-porous carriers and efficacy was determined for 0.25, 0.5, and 1.0 second exposure to UV radiation at a calculated direct irradiance of 7496 $\mu\text{W}/\text{cm}^2$. The study was conducted to conservatively estimate virucidal efficacy as virus particles passed through a UV air treatment unit. The study likely represents a worst-case scenario for exposure since the viruses are primarily being exposed on a single side due to their deposition onto a carrier. In a flow through system, particles would experience rotational movement that likely would result in radiation exposure to additional parts of the virus particle as it passes through.

The study concluded that UV exposure resulted in significant virus inactivation. The inactivation increased with the contact time. In the following pages, you will find a summary of the methodology used and the results of our analysis. Should you have any further concerns please do not hesitate to contact me.

Respectfully,

George Lukasik, Ph.D.
Laboratory Director

- Page 1 of 7-

BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE. A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630

WWW.MICROBIOSERVICES.COM

FL DOH #E82924, PA DEP 68-03950, ISO/IEC 17025:2005 L2422 (L-A-B), EPA# FLO1147
FILE: SARS-COV-2 SURROGATE INACTIVATION BY ULTRAVIOLET DISINFECTION SYSTEM FROM BIOZONE SCIENTIFIC BCSID
2011334R22011334

THIS DOCUMENT SHALL NOT BE REPRODUCED, EXCEPT IN FULL, WITHOUT THE WRITTEN CONSENT OF BCS LABORATORIES



Stock Virus and Cell Culture Infectivity Assay:

Human Coronavirus OC43 (ATCC VR-1558) virus was propagated and enumerated as Most Probable Numbers (MPN) using human ileocecal colorectal adenocarcinoma HRT-18G (ATCC® CRL-11663) as the host. Cells were grown in 6-well cell culture flasks. For enumeration, virus was enumerated as infectious units as per the assay methodology described in Standard Method 9510 (APHA, 2012) and EPA /600/4-84/013. Briefly, aliquots of a sample containing the virus were inoculated on freshly prepared monolayers of HCT8 cells (approximately 90% confluence). Each sample volume was inoculated in replicates of six. The cells were then incubated in Dulbecco's Modified Eagle's medium (dMEM, Mediatech Inc, USA) media 2% Fetal Bovine Serum (FBS, Mediatech, USA) at 36.5°C and 5% CO₂ for 14 days. Cells were microscopically monitored routinely for signs of degeneration. Cells in flasks demonstrating signs of infectivity (Cytopathic effects; CPE) were recorded as positive (+) and those that did not demonstrate any CPE were recorded as negative (-). The most probable number of infectious virus in a sample was then calculated using MPNCALC software (version 0.0.0.23). For challenge experiments, frozen viral stock (typically >1 x 10⁸ iu/ml) was thawed rapidly in a 35°C water bath. The virus suspension contained 2% FBS and was used within 15 minutes of thawing. Virus suspension was diluted at 1:1 ratio with Phosphate Buffered Dilution Water (PBW) prior to

- Page 2 of 7-

BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE. A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630

WWW.MICROBIOSERVICES.COM

FL DOH #E82924, PA DEP 68-03950, ISO/IEC 17025:2005 L2422 (L-A-B), EPA# FLO1147
FILE: SARS-COV-2 SURROGATE INACTIVATION BY ULTRAVIOLET DISINFECTION SYSTEM FROM BIOZONE SCIENTIFIC BCSID
2011334R22011334

THIS DOCUMENT SHALL NOT BE REPRODUCED, EXCEPT IN FULL, WITHOUT THE WRITTEN CONSENT OF BCS LABORATORIES



use in the study. The resulting dilution was used in the study and was enumerated by performing serial ten-fold dilutions in PBS and inoculated onto HCT-8 cells as described.

Test Unit:

The unit tested for virucidal efficacy was designed, built, and delivered by study sponsor on November 19, 2020. The unit consisted of a closed rectangular reflective chamber that contained 4 low pressure mercury quartz lamps horizontally mounted to the upper side of the chamber. At 12-inches below the lamps, an electronically controlled plastic chamber was placed. The chamber was used to control exposure time of inoculated carriers to the UV radiation emitted by the lamps. The specifications of lamps as supplied by the sponsor are: Lamp type: 10-H36000 at 87 W each; the calculated direct irradiance on carriers was 7496 $\mu\text{W}/\text{cm}^2$. The unit was assigned BCS ID 2011334.

Challenge Study: November 19, 2020

The study was conducted using BCS's disinfection efficacy SOP D-1. Study execution was adapted from protocol ASTM E3135–18 (Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil) and client requested parameters. Briefly, 25x26 mm sterile glass carriers were each inoculated with one hundred microliters of diluted virus solution stock solution (containing a final concentration of 1% FBS). The inoculum was allowed to dry in a

- Page 3 of 7-

BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE. A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630

WWW.MICROBIOSERVICES.COM

FL DOH #E82924, PA DEP 68–03950, ISO/IEC 17025:2005 L2422 (L-A-B), EPA# FLO1147
FILE: SARS-COV-2 SURROGATE INACTIVATION BY ULTRAVIOLET DISINFECTION SYSTEM FROM BIOZONE SCIENTIFIC BCSID
2011334R22011334

THIS DOCUMENT SHALL NOT BE REPRODUCED, EXCEPT IN FULL, WITHOUT THE WRITTEN CONSENT OF BCS LABORATORIES



biological cabinet. Three carriers were used for each exposure period. Carriers inoculated and not exposed to UV radiation served as recovery controls. Uninoculated carriers served as negative controls. Triplicate carriers were placed in the exposure chamber. The lamps were warmed up prior to exposing to the radiation. Exposure was performed via the PLC controlled shutter box. The ambient temperature during the study was maintained at 20.0-22.0°C. Following exposure of carriers to radiation, each was aseptically transferred to a tube containing 10-ml sterile D/E Neutralizing Broth. The tubes were placed onto an orbital shaker and agitated at low speed for 15 minutes. After agitation, ten-fold dilutions of suspensions were performed in PBW. The number of viable (infectious) virus units in the samples was determined by the Most Probable Number (MPN) assay procedure described previously using HRT18G cell line. Table 1 and 2 present the results of the study. Cytotoxicity and negative controls were conducted using uninoculated treated material.

Material descriptions and names were obtained from the submitted documents. The analysis was authorized and commissioned by the client or client's representative. The resulting data are representative of the analysis conducted on the collected samples and its/their condition at the time of analysis. The data provided is strictly representative of the study conducted under laboratory conditions using the material/samples/articles provided by the client (or client's representative) and its (their) condition at the time of test. The data

- Page 4 of 7-

BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE. A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630

WWW.MICROBIOSERVICES.COM

FL DOH #E82924, PA DEP 68-03950, ISO/IEC 17025:2005 L2422 (L-A-B), EPA# FLO1147
FILE: SARS-COV-2 SURROGATE INACTIVATION BY ULTRAVIOLET DISINFECTION SYSTEM FROM BIOZONE SCIENTIFIC BCSID
2011334R22011334

THIS DOCUMENT SHALL NOT BE REPRODUCED, EXCEPT IN FULL, WITHOUT THE WRITTEN CONSENT OF BCS LABORATORIES



tained may not be representative or indicative of a real-life process and/or application. The sample(s) were analyzed in accordance with the appropriate method, however due to the inherent limitations of methods, microorganisms may avoid detection. BCS Laboratories offers no express or implied warranties concerning the quality, safety, and/or purity of any sample, batch, source, or the process they are derived from. Quality assurance controls were performed as outlined in the method and as per Good Laboratory Practices. Viral analysis was performed in accordance with laboratory practices and procedures set-forth by ISO 17025:2017 and NELAP/TNI accreditation standards unless otherwise noted. BCS makes no express or implied warranty regarding the ownership, merchantability, safety, or fitness for a particular purpose of any such property or product. Detailed report format was requested by client in addition to standard report format that was submitted.

- Page 5 of 7-

BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE. A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630

WWW.MICROBIOSERVICES.COM

FL DOH #E82924, PA DEP 68-03950, ISO/IEC 17025:2005 L2422 (L-A-B), EPA# FLO1147
FILE: SARS-COV-2 SURROGATE INACTIVATION BY ULTRAVIOLET DISINFECTION SYSTEM FROM BIOZONE SCIENTIFIC BCSID
2011334R22011334

THIS DOCUMENT SHALL NOT BE REPRODUCED, EXCEPT IN FULL, WITHOUT THE WRITTEN CONSENT OF BCS LABORATORIES



Table 1. Efficacy of UV radiation on the inactivation of Coronavirus OC43 (ATCC VR-1558) on glass carriers placed at 12” distance. The efficacy was determined at various exposure times in the chamber containing four high pressure mercury bulbs. Test chamber provided by BioZone Scientific International, Inc.; study conducted as per sponsor’s request and guidance from ASTM E3135-18

Sample	Infectious Units of Virus Recovered per Carrier*	Percent Reduction Vs. Recovery Control Carriers	Average Percent Reduction Vs. Recovery Control Carriers
Viral Infectious Units Inoculated per carrier	2.3 x 10 ⁶		
Recovery Control Carriers (Not Exposed to UV)	2.4 x 10 ⁵		
	1.5 x 10 ⁵		
Efficacy at 0.25 Second Exposure	2.4 x 10 ³	98.8%	99.1%
	2.4 x 10 ³	98.8%	
	3.1 x 10 ²	99.8%	
Efficacy at 0.5 Second Exposure	3.6	99.998%	99.997%
	9.2	99.995%	
	1.6	99.9992%	
Efficacy at 1.0 Second Exposure	<1.5	>99.9992%	>99.9992%
	<1.5	>99.9992%	
	<1.5	>99.9992%	

*Most Probable Number (MPN) of Viral Infectious Units (IU) was calculated using the MPNCalc Software as per EPA 600/R95/178. Enumeration was performed by inoculating aliquots of sample dilutions onto freshly prepared monolayers of HRT18G (CCL-11663) cells in 6-well flasks and monitoring for Cytopathic Effect (CPE) development during a 14-day incubation period. Cells were incubated at 36.5°C in a 5% CO₂ atmosphere. The IU MPN numbers represent recovery from each of the carriers used in the study.

BCS LABORATORIES, INC. - GAINESVILLE
 4609 NW 6TH STREET, STE. A, GAINESVILLE, FLORIDA 32609
 TEL. (352) 377-9272, FAX. (352) 377-5630

WWW.MICROBIOSERVICES.COM

FL DOH #E82924, PA DEP 68-03950, ISO/IEC 17025:2005 L2422 (L-A-B), EPA# FLO1147
 FILE: SARS-COV-2 SURROGATE INACTIVATION BY ULTRAVIOLET DISINFECTION SYSTEM FROM BIOZONE SCIENTIFIC BCSID
 2011334R22011334

THIS DOCUMENT SHALL NOT BE REPRODUCED, EXCEPT IN FULL, WITHOUT THE WRITTEN CONSENT OF BCS LABORATORIES



Table 2. Raw data of inoculated HRT18G culture. Wells in replicates of six were inoculated with different volumes & dilutions of each sample from the virucidal efficacy study. Cytopathic Effects' (CPE) positive and negative results of inoculated cells flasks are used to calculate the MPN presented in Table 1.

Sample	Volume Inoculated (ml) at indicated dilution									
	1.0 @ 10 ⁰	0.1 @ 10 ⁰	1.0 @ 10 ⁻²	0.1 @ 10 ⁻²	1.0 @ 10 ⁻⁴	0.1 @ 10 ⁻⁴	1.0 @ 10 ⁻⁶	0.1 @ 10 ⁻⁶	1.0 @ 10 ⁻⁸	0.1 @ 10 ⁻⁸
Initial Inoculum	ND	ND	ND	ND	ND	ND	6/6	6/6	2/6	0/6
Assay Negative Control	0/6	0/6	ND	ND	ND	ND	ND	ND	ND	ND
Cell Culture Positive Control	6/6	6/6	ND	ND	ND	ND	ND	ND	ND	ND
Cell Culture Negative Control	0/6	0/6	ND	ND	ND	ND	ND	ND	ND	ND
Recovery Control Carriers	ND	ND	6/6	6/6	4/6	2/6	ND	ND	ND	ND
	ND	ND	6/6	6/6	6/6	0/6	ND	ND	ND	ND
Efficacy at 0.25 Second Exposure	6/6	6/6	6/6	0/6	0/6	ND	ND	ND	ND	ND
	6/6	6/6	6/6	0/6	0/6	ND	ND	ND	ND	ND
	6/6	6/6	1/6	0/6	0/6	ND	ND	ND	ND	ND
Efficacy at 0.5 Second Exposure	2/6	0/6	0/6	0/6	0/6	ND	ND	ND	ND	ND
	4/6	0/6	0/6	0/6	0/6	ND	ND	ND	ND	ND
	1/6	0/6	0/6	0/6	0/6	ND	ND	ND	ND	ND
Efficacy at 1.0 Second Exposure	0/6	0/6	0/6	0/6	0/6	ND	ND	ND	ND	ND
	0/6	0/6	0/6	0/6	0/6	ND	ND	ND	ND	ND
	0/6	0/6	0/6	0/6	0/6	ND	ND	ND	ND	ND

*The number in the numerator is the number of inoculated flasks demonstrating positive CPE and the number in the denominator indicates the total number of flasks inoculated with the indicated volume and dilution of sample. ND: Not Done

BCS LABORATORIES, INC. - GAINESVILLE
 4609 NW 6TH STREET, STE. A, GAINESVILLE, FLORIDA 32609
 TEL. (352) 377-9272, FAX. (352) 377-5630

WWW.MICROBIOSERVICES.COM

FL DOH #E82924, PA DEP 68-03950, ISO/IEC 17025:2005 L2422 (L-A-B), EPA# FLO1147
 FILE: SARS-COV-2 SURROGATE INACTIVATION BY ULTRAVIOLET DISINFECTION SYSTEM FROM BIOZONE SCIENTIFIC BCSID
 2011334R22011334

THIS DOCUMENT SHALL NOT BE REPRODUCED, EXCEPT IN FULL, WITHOUT THE WRITTEN CONSENT OF BCS LABORATORIES

