April 22, 2021

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 Biozone AC® unit from BioZone Scientific; BCS ID 2104054.

Dear Mr. Anthony,

We have completed the virucidal efficacy study for ozone output representative of the Biozone AC® unit. In the study we utilized the surrogate Coronavirus OC43 (ATCC VR-1558) to assess the virucidal properties of the unit against SARS-CoV-2 in an enclosed area. Virus was inoculated onto non-porous carriers and they were placed in an enclosed 0.28 m^3 glass chamber containing the BioZone AC® unit. The unit was run continuously in the enclosed chamber for 30 minutes. The virucidal efficacy was measured following the 30 minutes of exposure.

The study concluded that continuous ozone exposure for 30 minutes in the sealed chamber resulted in measured virus inactivation on inoculated non porous surface. 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

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 T-25 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 HRT18G cells (approximately 90% confluence). Each sample volume was inoculated in replicates of five. 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% CO_2 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 10^8 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 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 HRT18G cells as described.

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Test Unit:

A BioZone AC® test unit was delivered by study sponsor on April 08, 2021. The unit was assigned BCS ID 2104053.The unit was placed in a 0.28 m^3 glass chamber that was connected to an ozone concentration monitor (2B Technologies, Model 106-L. Co, USA). A fan was placed inside the chamber to ensure uniform dispersion of ozone. The unit was operated continuously throughout the study.

Challenge Study: April 08, 2021

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). Three carriers were used for each exposure time period. Carriers inoculated, placed into the chamber, and not exposed to Ozone served as recovery controls. Uninoculated carriers served as negative controls. Exposure was performed at a contact time of 30 minutes. The ambient temperature during the study was maintained at 20.0-22.0°C. Following exposure of carriers to ozone, each was aseptically transferred to a tube containing 10-ml sterile D/E Neutralizing Broth. The tubes

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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 obtained 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

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Table 1. Efficacy of continuous Ozone production of Biozone AC® in an enclosed area on the inactivation of Coronavirus OC43 (ATCC VR-1558) inoculated onto glass carriers. The efficacy was determined at various exposure times in the 0.28 m^3 glass chamber containing unit. 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	1.7×10^6		
Recovery from Control Carriers (Not Exposed to Ozone)	2.2×10^5		
	5.4×10^5		
Recovery from Carriers Following 30-minute Continuous Exposure	2.2×10^3	99.4%	99.3%
	5.4×10^3	98.6%	
	4.7×10^2	99.9%	

***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 flasks and monitoring for Cytopathic Effect (CPE) development during a 10-14 day incubation period. Cells were incubated at 36.5°C in a 5% CO_2 atmosphere. The IU MPN numbers represent recovery from each of the carriers used in the study.**

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Table 2. Raw data of inoculated HRT18G culture. Flasks in replicates of five 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	5/5	4/5	1/5	ND
Assay Negative Control (Cytotoxicity Control)	0/5	0/5	ND	ND	ND	ND	ND	ND	ND	ND
Cell Culture Positive Control	5/5	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cell Culture Negative Control	0/5	ND	ND	ND	ND	ND	ND	ND	ND	ND
Neutralization Control	5/5	ND	ND	ND	ND	ND	ND	ND	ND	ND
Recovery from Control Carriers	ND	ND	5/5	5/5	4/5	2/5	ND	ND	ND	ND
	ND	ND	5/5	5/5	5/5	2/5	ND	ND	ND	ND
	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Efficacy at 30 Minutes Exposure	5/5	5/5	4/5	2/5	ND	ND	ND	ND	ND	ND
	5/5	5/5	5/5	2/5	ND	ND	ND	ND	ND	ND
	5/5	5/5	4/5	2/5	ND	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

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