July 17, 2020

Yasser Estafanous
Director of Regulatory Affairs & Quality Assurance
3B Medical, Inc.
203 Avenue A NW, Suite 300
Winter Haven, FL 33881
yestafanous@3bproducts.com
www.3Bproducts.com

RE: Lumin® LM3000 Adenovirus 2 Bioburden Reduction Testing on Inoculated Facemask; Device Performance Validation study.

Dear Mr. Estafanos,

We have completed the requested virucidal efficacy validation study of the supplied UV irradiation device. The testing was performed as requested using the direct inoculation and treatment. The protocol used was based on guidance from ASTM E3135 (Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against microorganisms on Carriers with Simulated Soil). In the study, we evaluated the virucidal efficacy of the Lumin® LM3000 unit on Adenovirus type 2 inoculated onto a supplied facemask (3M p/n 1860)..

In the following pages, you will find a summary of the study, methodology used, and the results of the analysis. Should you have any questions, do not hesitate to contact me.

Respectfully,

George Lukasik, Ph.D.
Laboratory Director

Stock Virus and Cell Culture Infectivity Assay:

Human Adenovirus 2 (Strain: Adenoid 6 NIAID 202-001-014; ATCC VR-846) was propagated and enumerated using Human lung epithelial cell line A-549 (ATCC CCL-185) as the host. Cells were grown in T-25 cell culture flasks. Virus and host cells were obtained from ATCC. Virus was enumerated as infectious units as per methodology described in ASTM E1053 (Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces) and EPA /600/4-84/013. Briefly, aliquots of a sample containing the virus were inoculated onto freshly prepared monolayers of respective cell line (approximately 90% confluence) and incubated at room temperature for 2 hours. Each sample volume was inoculated in replicates of five at multiple dilutions. The cells were then incubated in Dulbecco's Modified Eagle's medium (dMEM, Mediatech Inc, USA) media containing 2% Fetal Bovine Serum (FBS, Mediatech, USA) at 35°C and 5% CO_2 for up to 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 the number of foci of infectivity were counted if possible. 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

BCS Laboratories
4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com

iu/ml) was thawed rapidly in a 35°C water bath. The virus suspension was adjusted to a soil load described in ASTM E1053; briefly 100µL of 5% Bovine Serum Albumin (BSA), 400µL of 0.4% mucin, and 140µL of 5% yeast extract stock were added to 1360µL of the thawed virus stock. Virus suspension was titered by performing serial one hundred-fold dilutions in Phosphate Buffer Water (PBW) and inoculated onto A549 cells as described previously.

Test Material:

The UV unit "Lumin® LM3000" was supplied by 3B Medical Inc. The UV unit was assigned the BCS ID 2006241. Three face masks were also provided by 3B Medical Inc. The masks were marked "3M 1860 N95 Lot27214". The mask were each assigned BCS ID 2007004, 2007005, and 2007006.

Challenge Study: July 04, 2020

The study was conducted in accordance with BCS Laboratories disinfection efficacy SOP D-1. Study design was adapted from protocol described in ASTM E3135 (Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil) and client requested parameters. Using a permanent marker, each mask was given an identifying label and marked into

BCS Laboratories
4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com

approximately 5 mm round areas on both their convex and concave surfaces. Additionally areas on the plastic strap were marked. The data of this report describes methodology and data for two masks; one masked was exposed to the Lumin® device irradiation (designated as “Treated #2”; BCS ID 2007006) and another was used as the recovery control (designated as “Untreated”; BCS ID 2007004). Each face mask received 15 individual Adenovirus 2 inoculations onto the surface. The inoculations were placed into separate designated marked area. Furthermore, a BCS laboratory supplied mask served as a negative treatment control (Uninoculated and subjected to treatment). For inoculation, 20µL of the virus suspension were inoculated on eight marked areas on the front of the mask and two area on the strap. The virus inoculum was allowed to dry over approximately a 20-30 minute period before the mask was flipped over and five additional areas on the concave side of the mask were inoculated with 20µL of virus. A total of 300µL was applied to each of the “Treated #2” and “Untreated” marked masks. Concurrently, the Lumin® unit was operated for 3 consecutive “On” cycles immediately prior to testing of the referenced mask. Following the drying of the inoculum, “Treated #2” mask was placed into the Lumin® unit (concave side facing down) and the instructions for use were followed. Briefly, the unit was closed and the ON button was depressed to allow UV exposure for a full cycle (approximately 5 minutes). Following, the unit was opened and the mask was flipped onto its other side (concave side facing up) and the treatment cycle was repeated. Immediately

BCS Laboratories
4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH #E82924, ISO/IEC 17025:2017 L2422 (ANAB/ANSI), EPA# FL01147
File: 3B Products Lumin UV Mask Study Adenovirus 2 07 04 20 BCS ID 2006241r

following treatment, the mask was removed and placed in a sterile sample collection bag (Stomacher bag, Seward) containing 100mL sterile D/E Neutralizing Broth. The “Untreated” mask was also placed into a separate stomacher bag containing 100mL sterile D/E Neutralizing Broth. The bags were placed onto an orbital shaker and agitated at medium to high low speed for 15 minutes. After agitation, the bags were agitated thoroughly and one hundred-fold dilutions of suspensions were performed in PBS. The number of infectious virus units in the samples was determined by the Most Probable Number (MPN) assay procedure described previously. Table 1 present the virucidal efficacy results of the study. Cytotoxicity and negative controls were conducted using uninoculated treated material (Table 2). The infectivity data for all analyzed samples are presented in table 3.

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

BCS Laboratories
4609 NW 6TH Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
WWW.MICROBIOSERVICES.COM
FL DOH #E82924, ISO/IEC 17025:2017 L2422 (ANAB/ANSI), EPA# FL01147

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 (FL DOH) 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.

BCS Laboratories
4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com

Table 1. Virucidal efficacy results of UV treatment by the Lumin® LM3000 unit against Adenovirus 2 (ATCC VR-846) inoculated on supplied 3M P/N Petragon (p/n: 1860) face masks#.

Sample ID	Viral Infectious Units (IU) recovered from each face mask*	Percent reduction
Untreated Mask (Recovery Control; BCS ID 2007004)	7.8×10^6	NA
Treated #2 Mask (Lumin® exposed for 2 cycles#; BCS ID 2007006)	4.9×10^3	99.94

***Most Probable Number (MPN) of Viral Infectious Units (IU) was calculated using the MPNCalc software as per method EPA /600/4-84/013. Enumeration was performed by inoculating aliquots of sample dilutions onto freshly prepared monolayers of A549 cells in T25 flasks and monitoring for Cytopathic Effect (CPE) development during a 14-day incubation period. Cells were incubated at 35°C in a 5% CO_2 atmosphere. The IU MPN numbers represent recovery from each of the carriers used in the study.**
 # Virus inoculated face mask was placed into unit and treated for a cycle with the convex side facing up and then again immediately for another cycle with the concave side facing up. Prior to treatment procedure, the unit was operated consecutively for 3 cycles

BCS Laboratories
4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH #E82924, ISO/IEC 17025:2017 L2422 (ANAB/ANSI), EPA# FL01147

Table 2. Adenovirus 2 recovery data of control samples performed during the Lumin® LM3000 study.

Sample ID	Average Viral Infectious Units (IU) recovered per mask/sample	Notes
Negative Control (No Inoculum)/Cytotoxicity Control	None Detected <1.8	Data is in conformance
Cell Culture Assay Negative Control	<1.0; Negative CPE	Negative control data is in conformance
Cell Culture Assay Positive Control	>10; Positive CPE	Positive control data is in conformance
Virus stock titer (initial inoculum)	5.4×10^7/mL	Viral (IU) Inoculated per mask: $1.6 \times 10^{7\#}$
Untreated Mask (Recovery Control; BCS ID 2007004)	7.8×10^6	Data is in conformance

***Most Probable Number (MPN) of Viral Infectious Units (IU) was calculated using the MPNCalc software as per EPA /600/4-84/013. Enumeration was performed by inoculating aliquots of sample dilutions onto freshly prepared monolayers of A549 cells in T25 flasks and monitoring for Cytopathic Effect (CPE) development during a 14-day incubation period. Cells were incubated at 35°C in a 5% CO_2 atmosphere. The IU MPN numbers represent recovery from each of the carriers used in the study.**
 # calculated value based on 300 uL total virus inoculum added to each mask

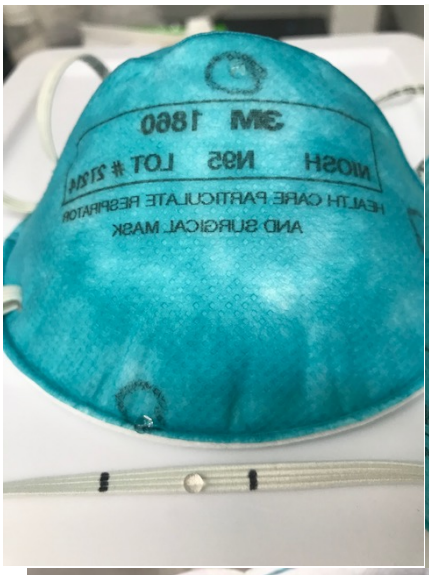
BCS Laboratories
4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH #E82924, ISO/IEC 17025:2017 L2422 (ANAB/ANSI), EPA# FL01147
File: 3B Products Lumin UV Mask Study Adenovirus 2 07 04 20 BCS ID 2006241r

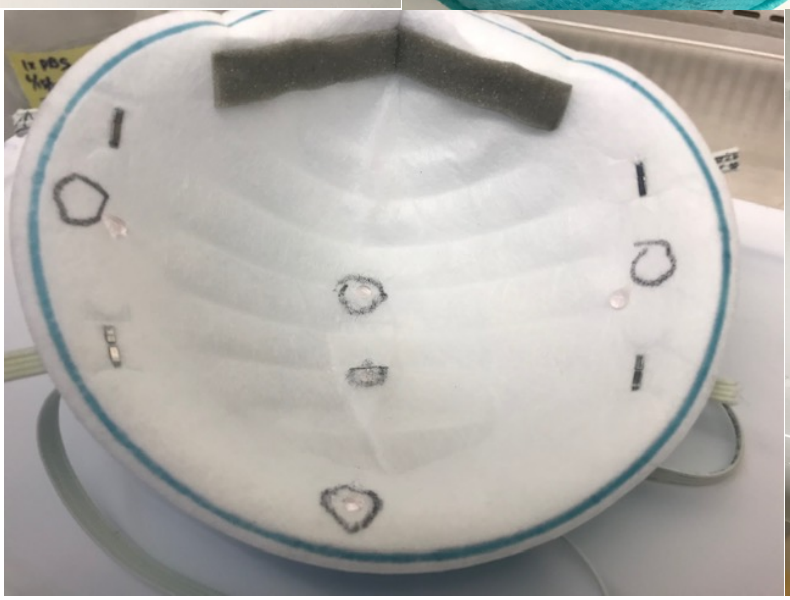
Table 3. Cell culture CPE results for the virucidal efficacy of UV treatment by the Lumin® LM3000 unit against Adenovirus 2 (ATCC VR-846)

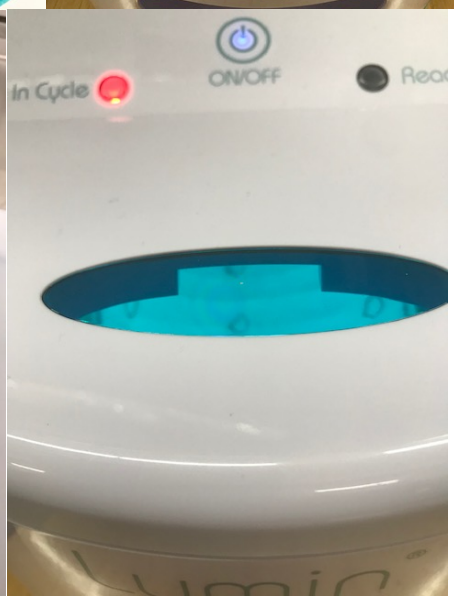
Sample ID	Sample volume Inoculated (mL) at indicated dilution								
	1.0 @ 10^{0}	0.1 @ 10^{0}	1.0 @ 10^{-2}	0.1 @ 10^{-2}	1.0 @ 10^{-4}	0.1 @ 10^{-4}	1.0 @ 10^{-6}	0.1 @ 10^{-6}	1.0 @ 10^{-8}
Virus Stock Inoculum	ND	ND	ND	ND	ND	5/5	5/5	5/5	2/5
Negative Control/ Cytotoxicity	0/5	0/5	0/5	ND	ND	ND	ND	ND	ND
Cell Culture Negative Control (PBS)	0/5	0/5	ND	ND	ND	ND	ND	ND	ND
Cell Culture Assay Positive Control (Adenovirus 2)	5/5	5/5	ND	ND	ND	ND	ND	ND	ND
Treated #2 Mask (Lumin® exposed for 2 cycles#; BCS ID 2007006)	5/5	5/5	2/5	0/5	ND	ND	ND	ND	ND
Untreated Mask (Recovery Control; BCS ID 2007004)	ND	ND	ND	5/5	5/5	3/5	0/5	0/5	ND

Flasks in replicates of five were each inoculated with different volumes and dilutions of each sample from the virucidal efficacy study. Cytopathic Effects (CPE) positive and negative results of inoculated A549 cells were used to calculate the MPN presented in Table 1. The number in the numerator is the number of inoculated cell culture flasks demonstrating positive CPE (virus presence) and the number in the denominator is the total number of cell culture flasks inoculated with the indicated volume. ND: Not Done

BCS Laboratories
4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH #E82924, ISO/IEC 17025:2017 L2422 (ANAB/ANSI), EPA# FL01147
File: 3B Products Lumin UV Mask Study Adenovirus 2 07 04 20 BCS ID 2006241r







BCS Laboratories
4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH #E82924, ISO/IEC 17025:2017 L2422 (ANAB/ANSI), EPA# FL01147
File: 3B Products Lumin UV Mask Study Adenovirus 2 07 04 20 BCS ID 2006241r