December 15, 2020

Yasser Estafanous
Director of Regulatory Affairs & Quality Assurance
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RE: Lumin® Wand LW5000 Efficacy Testing on Human Influenza H1N1 on Hard Non-Porous Surface. BCS ID 2011457.

Dear Mr. Estafanos,

We have completed the requested virucidal efficacy validation study of the supplied handheld UV irradiation device. The testing was performed as requested using direct inoculation of carriers with virus and exposure to radiation. 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) and sponsor's requested test parameters. In the study, we evaluated the virucidal efficacy of the Lumin® Wand LW5000 unit on Influenza A (H1N1; ATCC VR-1469) inoculated onto a glass carriers at a distance of 0.5" and an exposure time of 5 seconds.

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 Influenza A (H1N1; ATCC VR-1469) virus was propagated and enumerated as Most Probable Numbers (MPN) using Madin-Darby Canine Kidney type I (MDCK) cell monolayers (ATCC CCL-34). 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 cells (approximately 80-90% 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 supplemented with Bovine Serum Albumin and TPCK treated trypsin at 36.5°C and 5% CO2 for 10-14 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. Virus suspension was diluted at 1:10 ratio with Phosphate Buffered Dilution Water (PBW) prior to use in the study. The virus suspension was adjusted to a soil load described in ASTM E1053; briefly 100µL of 5% Bovine Serum



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Albumin (BSA), 400µL of 0.4% mucin, and 140µL of 5% yeast extract stock were added to 1360µL of the thawed virus stock. The virus suspension was used in the study and was enumerated by performing serial ten-fold dilutions in PBS and inoculated onto MDCK cells as described.

**Test Material:**

The UV Lumin® Wand LW5000" was supplied by 3B Medical Inc and received at BCS Laboratories on November 27th, 2020. The UV unit was assigned the BCS ID 2011457. The unit was operated as per supplied directions.

**Challenge Study: December 01, 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 test parameters. Sterile glass carriers (20 x 26 x 0.4 mm) were each inoculated with the previously described virus suspension in soil load. The inoculum was allowed to dry over approximately a 20-minute period in a biological containment cabinet. A Total of 5 carriers were each inoculated in the center with 100 µL of virus suspension: 2 served as recovery



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controls and 3 were exposed to UV treatment. Briefly, the Lumin Wand was turned on and allowed a minimum of 30 seconds for warm up. The wand was placed suspended horizontally at 0.5" from the surface. Each carrier was aseptically placed on the surface directly below the center of the Wands' UV lamp. A NIST traceable timer was used to measure time. The carriers were each sequentially subjected to 5 second exposure. The ambient temperature during the study was maintained at 20.0-22.0°C. Following exposure to radiation, each carrier was aseptically transferred to a tube containing 10-ml sterile D/E Neutralizing Broth. Carriers inoculated with virus but not exposed to UV radiation were also similarly placed into tubes containing 10-ml sterile D/E Neutralizing Broth. Additionally, uninoculated carriers were placed similarly into tubes 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 MDCK cell line. Table 1 and 2 present the results of the study. Cytotoxicity and negative controls were performed as per standard protocols requirement.

Material descriptions and names were obtained from the submitted documents. The analysis was authorized and commissioned by the client or client's representative. The



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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 ISO17025:2017 practices. Viral analysis was performed in accordance with laboratory procedures set-forth by ISO 17025:2017 and NELAP/TNI (FL DOH) accreditation standards unless otherwise noted. BCS Laboratories makes no express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or product.



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**Table 1. Efficacy of Lumin® Wand LW5000 on inactivation of Human Influenza H1N1 (ATCC VR-1469) virus inoculated onto glass carriers placed at 0.5" distance from unit following a 5 second exposure time.**

| Sample | MPN of Infectious Units of Virus Recovered per Carrier* | Percent Reduction Vs. Recovery Control Carriers | Average Percent Reduction of Exposed Carriers Vs. Recovery Control Carriers |
|---|---|---|---|
| Viral Infectious Units Inoculated per carrier | $3.1 \times 10^5$ | | |
| Recovery Control Carriers (Not Exposed to UV) | $1.4 \times 10^5$ | | |
| | $1.8 \times 10^5$ | | |
| Efficacy at 5 Second Exposure | $1.4 \times 10^1$ | 99.991% | 99.98% |
| | $6.1 \times 10^1$ | 99.96% | |
| | $4.2 \times 10^1$ | 99.98% | |

***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 MDCK (CCL-34) 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% $CO_2$ 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 MDCK cell 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 | 5/6 | 0/6 | 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 | 6/6 | 0/6 | ND | ND | ND | ND |
| | ND | ND | 6/6 | 6/6 | 4/6 | 3/6 | ND | ND | ND | ND |
| **Efficacy at 1.0 Second Exposure** | 1/6 | 0/6 | 0/6 | 0/6 | 0/6 | ND | ND | ND | ND | ND |
| | 2/6 | 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 |

* 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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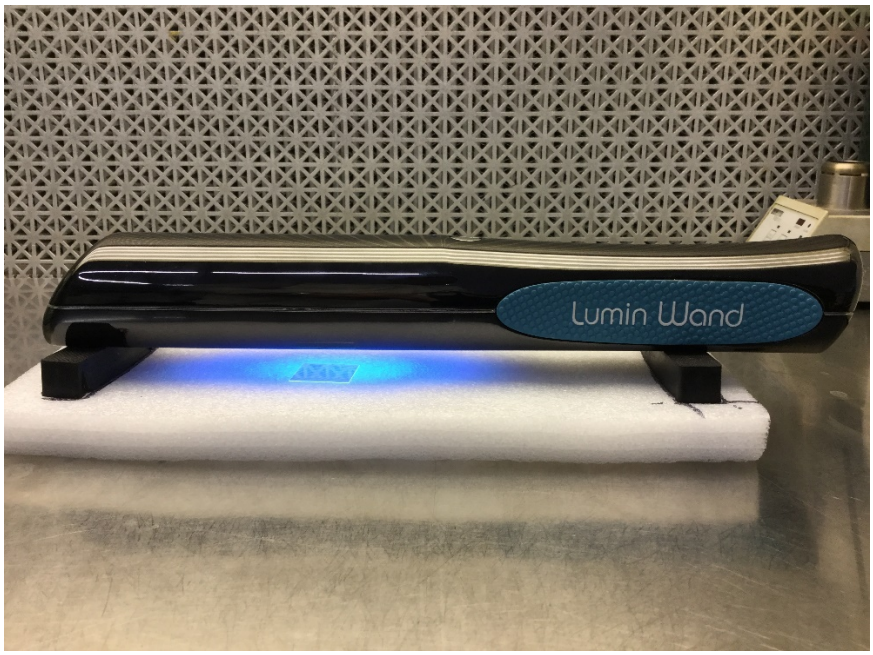


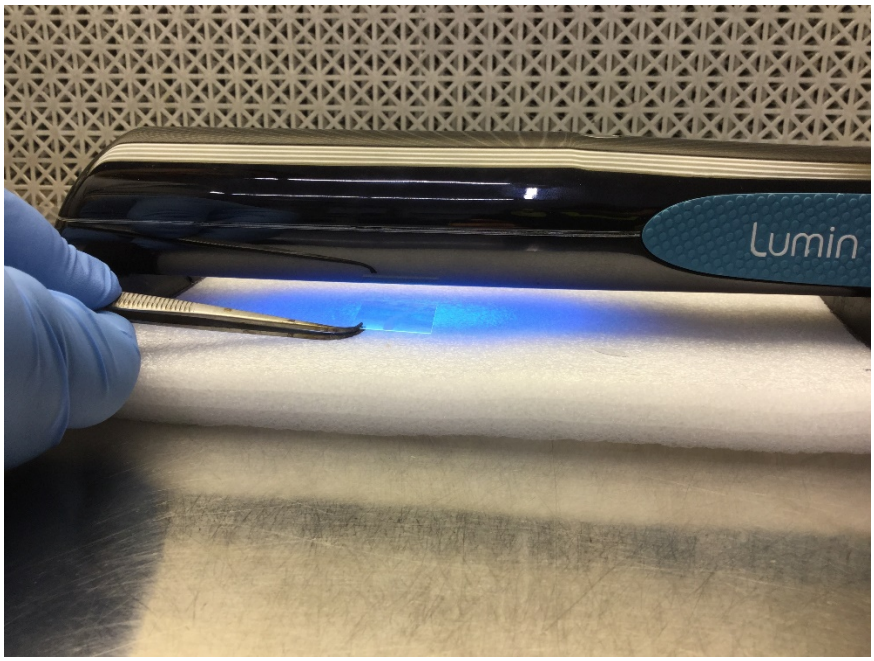




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