Microbial Study Results Using Violumas UVC LEDs

Introduction

Ultraviolet light has been proven to have germicidal properties and has been used for many water, air and surface disinfection applications. Violumas' ultraviolet light-emitting diodes (UV LEDs) with no mercury content, instantaneous warm-up times, narrow band emissions, and form-factor adaptability offer high optical output and longer lifetimes with the use of the patented 3-PAD technology. In this study, the inactivation efficacy of Violumas UVC-LEDs emitting at 265 nm wavelength was determined by a third-party laboratory using the microbe MS2 Bacteriophage. The reported log reduction values have been obtained based on the report from the third-party laboratory.

Violumas' UVC LED System

Violumas developed a custom UVC LED chamber for this microbial study. The chamber consisted of 4 custom chip-on-board (COB) LEDs specifically designed and manufactured for this test, ensuring a minimum of 2.5 mw/cm^2 optical intensity on all surfaces at a working distance of 9 cm from the COB. A tray module was used for disinfection object placement and the tray walls were coated with Al to increase reflectivity. Figure 1 shows the dimensions of the system box prototyped for this study.

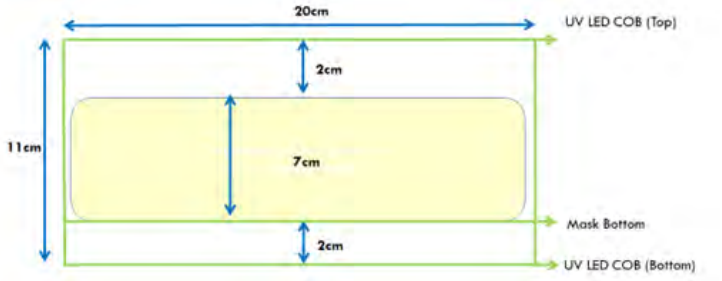

Figure 1: Cross section of system box along with dimensions. The surrogate disinfection object was placed at the bottom of the tray.

Each LED COB consisted of 36 pieces of 265 nm UVC LEDs, placed at the top as well as the bottom of the chamber. The chamber also incorporated the electrical as well as the thermal management (heatsinks and fans) elements required to operate the UVC LED COBs, at a junction temperature of less than 70°C. The LEDs were operated at a chip current of 700 mA. The complete device can be seen in Figure 2 below.

Figure 2: Violumas' UVC LED system

Optical Intensity Measurements

In order to estimate the dosage at the 9 cm distance, optical intensity measurements were made with the top UV LEDs turned on, keeping the testing conditions the same as the microbial tests. The measurement grid is shown in Figure 3 below and has been chosen to determine the intensity based on the chip placement on the COB.

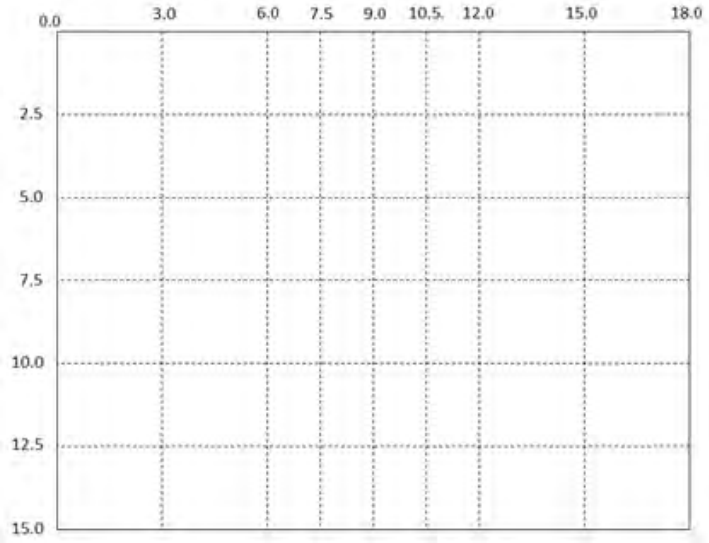

Figure 3: Measurement grid used to measure the optical intensity inside the cavity.

The optical measurement data is shown in Table 1. The average intensity was measured to be 2.5 mW/cm^2. The minimum to maximum intensity ratio was 0.8, implying good uniformity throughout the measurement area.

Grid Location (cm)	3.00	6.00	7.50	9.00	10.50	12.00	15.00
2.50	2.18	2.37	2.39	2.42	2.39	2.38	2.24
5.00	2.37	2.60	2.65	2.65	2.62	2.56	2.35
7.50	2.42	2.70	2.74	2.74	2.70	2.63	2.38
10.00	2.46	2.69	2.74	2.74	2.69	2.62	2.41
12.50	2.46	2.62	2.65	2.65	2.59	2.53	2.34

Table 1: Optical intensity measurements in mW/cm^2 performed with the device at various grid locations at a throw distance of 9 cm.

Microbial Testing Methodology

Violumas UVC LEDs were tested against MS2 bacteriophage. The MS2 bacteriophage is considered as a surrogate for influenza viruses [1]. It is a hardy virus and exhibits high UV resistance, representing the worst-case scenario for UV efficacy testing. This bacteriophage is quick to propagate and is easier to handle in a Level 2 safety laboratory. This implies rapid and cost-effective efficacy testing.

The UV LEDs were tested against a modified version of ASTM E3135-18 "Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil" test method at GAP EnviroMicrobial Services Ltd. (GAP), in Ontario, Canada. The carriers used for this testing were 10 mm x 10 mm glass slides painted on one side, which were used as a surrogate for non-porous opaque surfaces.

The glass slides were sterilized prior to testing, then 20 µL of inoculum was spread across the surface of the slide. Slides were then dried in a biosafety cabinet for approximately 15 minutes, until no liquid remained visible, before testing was initiated. Carriers were then placed in the LED disinfection unit for specified time periods. The placement of the glass slide inside the tray is shown in Figure 4.

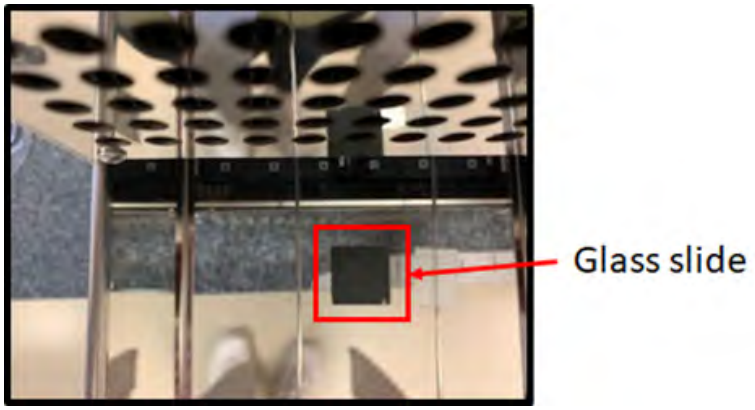

Figure 4: Slide placement with carrier inside the LED disinfection device.

The LED current settings were verified to be approximately 700 mA/chip for each test. After exposures were complete, the carriers were placed in 20 mL sterile phosphate buffered water and vortexed for one minute to elute the surviving microbes. Enumeration of the surviving microbes was achieved by membrane filtration. Log and percent reductions were calculated by comparison to control slides that were not exposed to the LED device. For this testing only the top bank of UV LEDs were turned on.

Challenge Organism: Bacteriophage MS2 (ATCC 15597-B1)

The MS2 Bacteriophage was prepared at GAP laboratories using a proprietary procedure, identified internally as "SOP #40: Preparation and Storage of Concentrated Bacteriophage". In short, a host E. coli was grown in Tryptic Soy Broth to log phase in a shaking incubator. A small volume of bacteriophage (1 transfer removed from ATCC) was added at this time, and the broth mixture remained shaking overnight. The E. coli was subsequently removed via centrifugation, leaving the bacteriophage in solution.

These time points were chosen in order to achieve at least 50 mJ/cm^2 in 23 seconds and 300 mJ/cm^2 in 2 minutes, ensuring a greater than log 2 or log 3 reduction for some of the commonly occurring microbes in a healthcare environment such as E.Coli, Staph. Aureus, Klebsiella pneumoniae and even hardier microbes such as the Adenovirus [2].

Time Points Reported in this Study

1. 0 seconds (Control)
2. 23 seconds
3. 2 minutes

Microbial Population Quantification

Enumeration of the surviving microbial population was achieved using GAP laboratories' internal method "BACTPHAGE-0001: Quantitative Recovery of Bacteriophage Used for Disinfection Equipment Validation". The media used was Tryptone Yeast Extract Glucose agar (TYGA) containing triphenyl tetrazolium chloride (TTC) and incubation was 35±0.5°C.

Antimicrobial Activity Calculation

The antimicrobial activity (R-value) of the test agent to each microbe was calculated according to the following calculation:

$$R = A_0 - A_t$$

Where R is the value of antimicrobial activity, or log reduction of the test agent.
A_0 is the logarithm of the number of viable bacteria, in PFU/swatch (or PFU/coupon), initially (T=0) recovered from the phosphate buffered water control.
A_t is the logarithm of the number of viable bacteria, in PFU/swatch (or PFU/coupon), recovered from the treated test agent after the specified contact time.

Test Results

Table 2 shows the testing results as obtained at 23 second (58 mJ/cm^2) and at 2 minute (300 mJ/cm^2) cycle time. Based on these microbial testing results, we could validate that a log reduction of **2.71 (99.8%)** could be obtained under a UV dosage of 58 mJ/cm^2 and a log reduction of **3.37 (99.96%)** could be obtained under a UV dosage of 300 mJ/cm^2 using Violumas' 265 nm UVC LEDs.

UV Dosage	Control (0 sec)	58 mJ/cm^2 (23 sec)	300 mJ/cm^2 (120 sec)
Carrier 1 (PFU/Carrier)	445,000	1,030	3,560
Carrier 2 (PFU/Carrier)	880,000	320	50
Carrier 3 (PFU/Carrier)	1,005,000	8,800	170
Log Rep 1	5.65	3.01	3.55
Log Rep 2	5.94	2.51	1.70
Log Rep 3	6.00	3.94	2.23
Average	5.87	3.15	2.49
A_0	5.87		
A_t		3.15	2.49
$R = A_0 - A_t$		2.71	3.37
% Reduction		99.8054%	99.9575%

Table 2: Glass slide (carrier) test results where only the upper bank of lamps is illuminated.

According to published literature [2], UV dosage of up to 70 mJ/cm^2 has been reported for 2 log reduction of MS2, which is comparable with our results. The dosage of 300 mJ/cm^2 is significantly higher than reported values and was chosen to ensure a higher log reduction with hardy microbes as shown in Table 3.

Microbe Type	Log Reduction	Dosage (mJ/cm^2)
Adenovirus	4	222
Rotavirus	4	200
Bacillus pumilus (spores)	4	272
Thermoactinomyces vulgaris	4	140

Table 3: UV dosage requirements for hardy microbes as presented in [2].

In addition, such high intensities may be required for disinfection of porous objects, where some of the UV light may be absorbed by the material. For example, CDC recommends 1000 mJ/cm^2 dosage for disinfection of an N95 mask [3]. Disinfection times or UV intensities can be increased to obtain these intensities. In this case, the device would need to be operated for less than 9 minutes to obtain 1000 mJ/cm^2.

References

[1] Coulliette, A. D., Perry, K. A., Fisher, E. M., Edwards, J. R., Shaffer, R. E., & Noble-Wang, J. (2014). MS2 Coliphage as a Surrogate for 2009 Pandemic Influenza A (H1N1) Virus (pH1N1) in Surface Survival Studies on N95 Filtering Facepiece Respirators. Journal of the International Society for Respiratory Protection, 21(1), 14–22.

[2] Haji Malayeri A, Mohseni M, Cairns W, Bolton JR (2016) Fluence (UV dose) required to achieve incremental log inactivation of bacteria, protozoa, viruses and algae. International Ultraviolet Association Inc News 18(3):4– 6 and supplemental tables

[3] Zhao Z, Zhang Z, Lanzarini-Lopes M, et al. Germicidal Ultraviolet Light Does Not Damage or Impede Performance of N95 Masks Upon Multiple Uses. Environ Sci Technol Lett. 2020;acs.estlett.0c00416. Published 2020 Jun 24.