Evaluation of the SteriLumen® SLR-1 Disinfecting Bar against SARS-CoV-2

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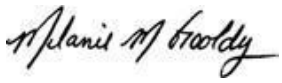
APPROVALS

Preparation

Melanie Gooldy		06/24/2021
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1. EXECUTIVE SUMMARY

CUBRC performed a laboratory experiment to evaluate the performance of the SteriLumen® SLR-1 Disinfecting Bar to inactivate SARS-CoV-2 on two (2) surface substrate materials (stainless-steel and ceramic) at a single distance (7 inches) at two (2) timepoints (5 minutes and 20 minutes). The results demonstrate that, on stainless steel, the SteriLumen® SLR-1 Disinfecting Bar achieved viral inactivation (i.e., viral particles rendered non-infectious) of >3.04-log reduction (>99.908%) at the 5-minute exposure time and >3.66-log reduction (>99.978%) at the 20-minute exposure time on stainless steel, at a distance of 7 inches. On ceramic, the viral inactivation was >3.60-log reduction (> 99.975%) at the 5-minute exposure time and >4.43-log reduction (>99.996%) at the 20-minute exposure time at a distance of 7 inches.

Testing was performed within CUBRC's CDC-permitted Biosafety Level 3 facility. All work was performed in accordance with external regulatory requirements and following approved internal safety and technical protocols. The experiments covered in this technical report were performed over the period beginning 2 April 2021 and ending 14 May 2021.

2. TECHNICAL APPROACH

CUBRC used a modified test protocol using ASTM E1153, *Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces*, as guidance to prepare an internal Standard Operating Procedure (SOP) that adapted the procedures in the referenced document for use with SARS-CoV-2 and UV-based disinfection products. The SOP, titled *Standard Operating Procedure for the Evaluation of UV-C and Ozone Sanitizers Recommended for Inanimate Non-Food Contact Surfaces* (Document ID No. SOP-ASTM-E1153-R00) was used to conduct the testing.

2.1 Test Matrix

Four test trials were performed to evaluate the SteriLumen® SLR-1 Disinfecting Bar to inactivate SARS-CoV-2 on two (2) surface substrate materials (stainless-steel and ceramic) at a single distance of 7-inches and at two (2) timepoints (5 minutes and 20 minutes).

2.2 Samples and Controls

To ensure that the test procedures produce dependable and defendable results, CUBRC included the following samples in the test trial: 1) Test Samples – these are the samples that receive the full test process to include inoculation, drying, disinfection, extraction and analysis; 2) Positive Control Samples – these samples serve to define the baseline challenge (contamination level), from which efficacy can be calculated, and receive inoculation, drying, extraction and analysis (i.e., no disinfection); and 3) Negative Control Samples – these serve to ensure that the test method doesn't introduce errant contamination and receive disinfection, extraction and analysis (i.e., no inoculation). Figure 1 presents the quantities of each sample type in the test trial.

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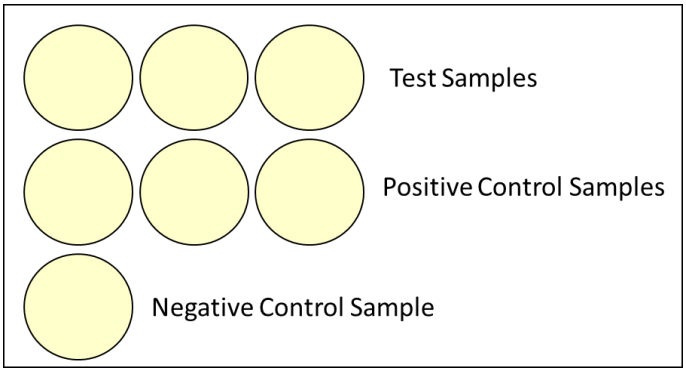

Figure 1: Sample Types and Quantities for a Test Trial.

3. TEST PROCEDURES

3.1 Test Preparation

One day before the test trial, one 12-well plate was prepared for each test sample by seeding each well with Vero E6 (ATCC CRL-1586) cells and incubating overnight to produce host cell monolayers in each well at approximately 90% confluency. On each plate, three wells were dedicated to controls and the remaining nine wells were used for triplicate analyses of each of undiluted test sample, 10-fold diluted test sample and 100-fold diluted test sample. Seven 12-well plates were prepared for the trial to accommodate the three test samples, the three positive controls and one negative control. Stainless steel test surfaces (coupons) were cleaned prior to testing by submerging in ethanol and sterilizing in an autoclave. Ceramic test surfaces (coupons) were cleaned prior to testing by submerging in ethanol. Once ceramic coupons were set up inside the Biological Safety Cabinet (BSC), they were sprayed with 70% EtOH. Additionally, antibiotic/antimycotic solution was added to media for ceramic testing only to mitigate contamination.

3.2 Test Procedure

On the day of the test, small circular stainless steel or ceramic coupons were laid out in the BSC, inoculated with 100 µL of viral preparation and allowed to dry for 45 minutes (Figure 2). The viral preparation had a titer of approximately 1×10^6 plaque forming units (PFU) per milliliter, independently confirmed through the analysis of control samples where 100 µL of the viral preparation was placed directly into prefilled extraction tubes. Viral plaques form when a virus infects a cell within the cell monolayer. This resulted in approximately 1×10^5 PFU being placed onto each test sample. Following the drying period, the positive control samples were removed from the BSC to protect them from exposure to the UV-C disinfection.

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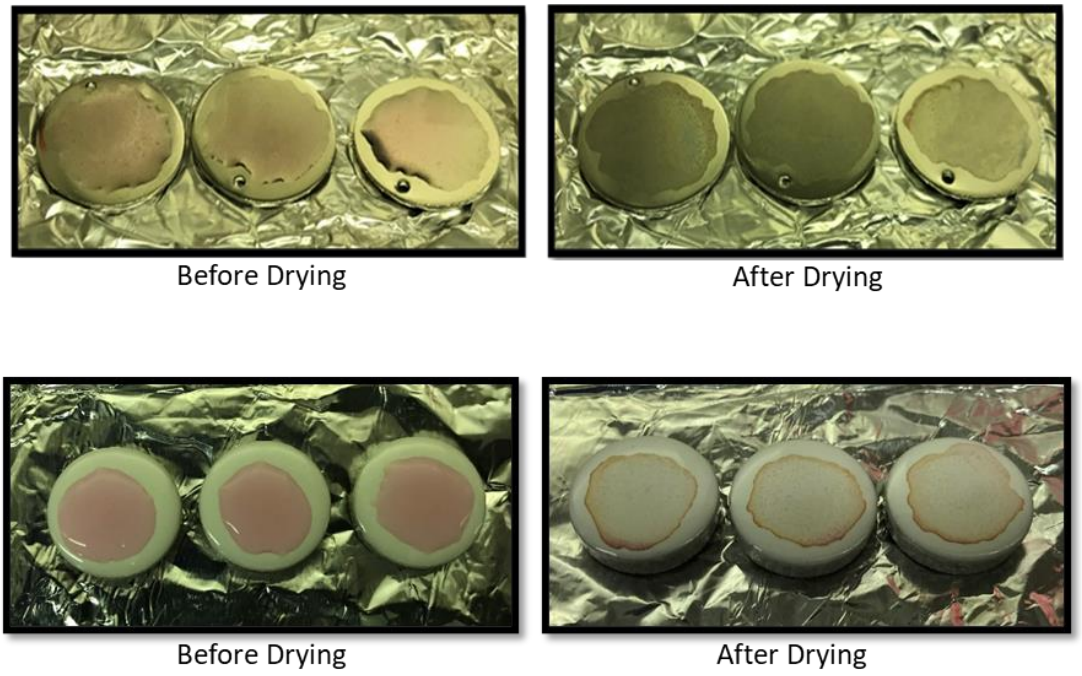

Figure 2: Inoculated Test Samples Before and After Drying (stainless steel coupon (top) and ceramic coupon (bottom))

The SteriLumen® SLR-1 Disinfecting Bar was placed within the biosafety cabinet (BSC) in preparation for the disinfection treatment as shown in Figure 3. The device was positioned to achieve a target distance of 7 inches between the test sample surface and the center of the LED. A test jig was constructed by SteriLumen (Figure 3) and was used in the test to mimic the real-world mounting height which will result in a distance of 7" from the surface to be disinfected to the emitter window on the bottom of the device.

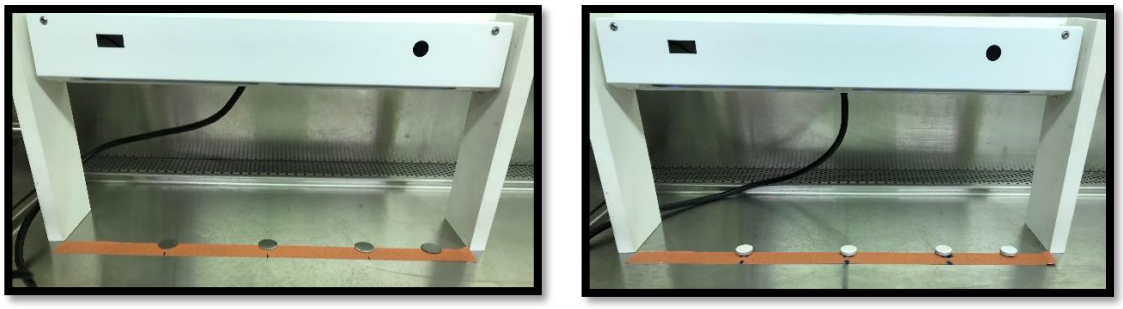

Figure 3: SteriLumen® SLR-1 Disinfecting Bar with Test Samples (stainless steel coupon (left) and ceramic coupon (right))

The test samples and negative control sample were placed under the SteriLumen® SLR-1 Disinfecting Bar and disinfected by activating the device for 5 minutes or 20 minutes. Following disinfection, each test coupon and negative control coupon was placed into separate tubes containing prepared media consisting of Eagle's Minimal Essential Media (EMEM) with Fetal Bovine Serum (FBS). Antibiotic/antimycotic solution was added for ceramic testing only to mitigate contamination. The tubes were vortexed, coupons were rinsed four times, and appropriate dilutions were performed for analysis. The positive control coupons were also placed into pre-prepared tubes for processing and analysis. Triplicate aliquots of each dilution from all samples

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(test samples and positive controls) were transferred onto the 12-well plates containing the confluent monolayers of host cells as described in Section 3.1. The plates were incubated at 37 °C for one hour with CO_2 and gently rocked every 15 minutes to promote virus adsorption. After the initial 1-hour incubation, the dilution aliquots were removed from each well and an overlay of microcrystalline cellulose was added to each well. The plates were incubated at 37 °C for 96 hours.

After completion of the 96-hour incubation period, the microcrystalline cellulose overlays were removed, and formalin was added to each well. The plates were incubated for one hour to allow for cell fixation and virus inactivation. The formalin was removed, and each well was washed with water, stained with crystal violet, and incubated for 10 minutes. After incubation, the crystal violet was removed, each well was washed with water, and the plates were allowed to dry. Once the plates were dry, the plaques (indicating the presence of live virus) were counted in each well.

3.3 Calculations of Disinfection Efficacy

The PFUs counted were used to perform calculations of disinfection efficacy of the SteriLumen® SLR-1 Disinfecting Bar as shown in the equations below. Calculations were made for each individual test sample using the average value of the positive control samples as the value of *Vc* and *Vs* set to < 1 PFU when no plaque formation is observed.

$$Log\ Reduction = \log_{10}(Vc/Vs)$$

$$Percent\ Reduction = (1 - 10^{-LR}) \times 100\%$$

where:

Vs = number of viable organisms remaining on the test sample
Vc = average number of viable organisms on the positive control samples
LR = Log Reduction

4. Results and Discussion

The test results are presented in the tables below for each test trial. Table 1 presents the results of the control samples including the cell control, positive controls, laboratory control, and negative control. Table 1 also shows the disinfection efficacy results for each test trial and test condition exposure time.

The results of the inoculation control samples demonstrate that the average number of viable SARS-CoV-2 particles placed onto each sample ranges from 3.10 x 10^4 (stainless steel coupons) to 9.94 x 10^4 (ceramic coupons). The drying process results in some degree of viral inactivation and this is an expected phenomenon. After drying, the average amount of viable virus remaining on the stainless-steel test samples was 6.19 x 10^3 PFU, corresponding to 3.79-log and the average amount of viable virus remaining on the ceramic test samples was 5.32 x 10^4 PFU, corresponding to 4.73-log. The negative control samples showed no observable plaque formation.

The results demonstrate that the SteriLumen® SLR-1 Disinfecting Bar achieved viral inactivation (i.e., viral particles rendered non-infectious) of >3.04-log reduction (>99.908%) at the 5-minute exposure time and >3.66-log reduction (>99.978%) at the 20-minute exposure time on stainless steel, at a distance of 7 inches. On ceramic, the viral inactivation was >3.60-log reduction (> 99.975%) at the 5-minute exposure time and >4.43-log reduction (>99.996%) at the 20-minute exposure time at a distance of 7 inches.

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Table 1 – SteriLumen® SLR-1 Disinfecting Bar Test Results Summary

SteriLumen® SLR-1 Disinfecting Bar – Stainless Steel - 5 minutes				
Control Sample Results (PFU per Sample)				
	Replicate 1	**Replicate 2**	**Replicate 3**	
Cell Control	0	0	0	
Negative Control	0	0	0	
Laboratory Control	0	0	0	
Test Sample Results				
	Positive	**Sample 1**	**Sample 2**	**Sample 3**
PFU per sample	6.9×10^3	< 9.52	< 3.57	< 5.95
Average Log Reduction	-	> 3.04		
Percent Reduction	-	> 99.908%		

SteriLumen® SLR-1 Disinfecting Bar – Stainless Steel - 20 minutes				
Control Sample Results (PFU per Sample)				
	Replicate 1	**Replicate 2**	**Replicate 3**	
Cell Control	0	0	0	
Negative Control	0	0	0	
Laboratory Control	0	0	0	
Test Sample Results				
	Positive	**Sample 1**	**Sample 2**	**Sample 3**
PFU per sample	5.48×10^3	< 1	< 1.19	< 2.38
Average Log Reduction	-	> 3.66		
Percent Reduction	-	> 99.978%		
*If no plaque growth is observed for a given Test Sample, the value is reported as < 1 plaque.				

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SteriLumen® SLR-1 Disinfecting Bar – Ceramic - 5 minutes				
Control Sample Results (PFU per Sample)				
	Replicate 1	**Replicate 2**	**Replicate 3**	
Cell Control	0	0	0	
Negative Control	0	0	0	
Laboratory Control	0	0	0	
Test Sample Results				
	Positive	**Sample 1**	**Sample 2**	**Sample 3**
PFU per sample	5.32×10^4	< 23.8	< 7.14	< 9.52
Average Log Reduction	-	> 3.60		
Percent Reduction	-	> 99.975%		

SteriLumen® SLR-1 Disinfecting Bar – Ceramic - 20 minutes				
Control Sample Results (PFU per Sample)				
	Replicate 1	**Replicate 2**	**Replicate 3**	
Cell Control	0	0	0	
Negative Control	0	0	0	
Laboratory Control	0	0	0	
Test Sample Results				
	Positive	**Sample 1**	**Sample 2**	**Sample 3**
PFU per sample	5.32×10^4	< 1	< 1	< 5.95
Average Log Reduction	-	> 4.43		
Percent Reduction	-	> 99.996%		
*If no plaque growth is observed for a given Test Sample, the value is reported as < 1 plaque.				

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