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Assessment of UV-C LED Sterilization Bar Against SARS-CoV-2

Final Report

FOR

Nitride Solutions, Inc.

3333 West Pawnee Street
Wichita, Kansas 67217

MRIGlobal Project No. 311625.01.001

June 26, 2020

Preface

This final report was prepared at MRIGlobal (MRIGlobal) for the work performed under MRIGlobal Task No. 311625.01.001, “Assessment of UV-C LED Sterilize Bar against SARS-CoV-2”

Test devices were supplied to MRIGlobal by Nitride Solutions, Inc. for the conduct of the program. The experimental phase of this task was initiated by MRIGlobal on May 19, 2020 and ended on June 15, 2020.

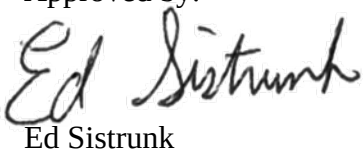
The Study Director of the program was Rick Tuttle. Execution of the study was assisted by Carl Gelhaus, Ph.D., Luca Popescu, Ph.D., Kristen Solocinski, Ph.D., Sam Humphries, and managed by William Sosna.

Although this study did not require compliance with the FDA Good Laboratory Practice Regulations (21 *CFR* 58); the studies were performed in compliance with MRIGlobal QA procedures. All operations pertaining to this study, unless specifically defined in this protocol, were performed according to the Standard Operating Procedures of MRIGlobal or approved laboratory procedures, and any deviations were documented.

MRIGlobal

Rick Tuttle
Study Director

Approved by:



Ed Sistrunk
Division Director
Medical Countermeasures

June 26, 2020

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Section 1.
Objective

The emergent threat of COVID-19 infection originating from SARS-CoV-2 and the high rate of transmission associated severe illness and fatalities, has created a needed response for rapid development and evaluation of effective countermeasures. In response to Nitride Solutions, Inc. MRIGobal evaluated a novel UV-C LED Sterilization platform developed by Nitride Solutions, Inc. The UV-C LED sterilizer is a 20 mW High-Intensity sterilization bar designed for portability and ease of use for sterilization of surfaces contaminated with bacteria, molds, virus's, and spores. With the emergent threat of COVID-19 infection, high rate of transmission, severe illness and fatalities created by the pandemic, MRIGlobal was contracted to perform a study to assess the efficacy of the UV-C LED sterilizer's efficacy in deactivating the SARS-CoV-2 strain USA-WA1/2020 virus on surfaces. The study evaluated the UV-C sterilizer for viable reduction of SARS-CoV-2 virus inoculated on stainless steel, and N95 mask filter materials.

Section 2.
Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor

Nitride Solutions, Inc.
3333 West Pawnee Street
Witchita, KS 67217

2.2 Sponsor's Representative

Jeremy Jones
President and CEO
Nitride Solutions, Inc.

2.3 Testing Laboratories

MRIGlobal
425 Volker Boulevard
Kansas City, MO 64110
Phone: (816) 753-7600
Fax: (816) 753-8823

2.4 Personnel Responsibilities

Study Director—MRIGlobal

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Email: rtuttle@mriglobal.org

Section 3.
Test Systems and Methods

3.1 Equipment

Test Equipment

Testing was conducted with a single UV-C sterilizer bar provided to MRIGlobal by Nitride Solutions, Inc. The systems operate using a rechargeable hand held digital proprietary UV-C LED Sterilization platform creating high intensity UV-C. The sterilizer bar tested is a rechargeable handheld unit with an integrated timer that can be activated for 30 second UV-C operation, and can be operated in increments of 10 second increases for longer duration operation. UV-C is ultraviolet light with wavelengths between 200-280 nm.

3.2 Methods

Testing Description

MRIGlobal conducted testing characterization of the UV-C sterilizer bar in surface decontamination trials to evaluate the log reduction destructive kill effectiveness against an envelope virus (SARS-CoV-2) strain USA-WA1/2020. USA-WA1/2020 was obtained from The University of Texas Medical Branch (UTMB) from an isolate of a patient who traveled to an infected region of China and developed the clinical disease (COVID-19) January 2020 in Washington, USA. The complete genome of USA-WA1/2020 has been sequenced. The Isolate-GenBank: MN985325 and after one passage in in Vero cells GenBank: MT020880. The complete genome of SARS-CoV-2 strain USA-WA1/2020 has been sequenced after four passages in collaboration with Database for Reference Grade Microbial Sequence (FDA-ARGOS; GenBank: MT246667). Each vial used on study contains approximately 0.5 mL of cell lysate and supernatant from Cercopithecus aethiops kidney cells infected with SARS-CoV-2 isolate USA-WA1/2020.

All tests were conducted in a biological class 3 facility at MRIGlobal, Kansas City, MO. The study characterized the efficacy of the UV-C LED sterilizer bar device at three (3) distances from inoculated substrates and at three (3) exposure time points with in triplicate testing for each variable. SARS-CoV-2 virus deactivation tests consisted of 54 total UV exposures with 6 positive control samples. Positive control samples were used for baseline substrate viral concentration determination and calculation of the UV-C LED sterilizer viral deactivation reduction efficacy. Viral reduction efficacy was measured using a TCID50 (50% tissue culture infectious dose of a virus) assay and Reed Muench formula to calculate viral reduction on SARS-CoV-2 inoculated test materials.

Testing was conducted at MRIGlobals' Kansas City, Biological Level 3 facility. Test coupons were fabricated from two materials including 1mm thick 304 stainless steel, and N95 mask material. The test coupons were fabricated from both materials using a circular mechanical die press with a diameter 5.08 cm or 2 inches. A total of sixty (60) individual test coupons consisting of thirty (30) stainless steel and thirty (30) N95 mask coupons were used on study. Test coupons were packaged in autoclave sterilization bags and autoclaved at 121°C (250°F) with the addition of biological indicators to assure sterility. Coupon preparation including SARS-CoV-2

inoculation, drying, UV exposure testing, extractions, and cell assay plating were conducted in a sterile class 2 biological safety cabinet. Individual test coupons were placed in test identification labeled sterile petri dishes and inoculated from a standard stock viral suspension with 200 µL of SARS-CoV-2 virus using a calibrated micropipette. The viral suspension was then evenly coated over the test coupons using sterile cell spreaders. Coated test coupons were air dried in the biological level 2 safety cabinet prior to UV exposure tests. Additional positive control coupons were similarly prepared and were subjected to the same environmental conditions and time course as test coupons without being subjected to UV exposure. The positive control coupons served as viral concentration standards to define the efficacy of the UV-C LED sterilizer in deactivating the SARS-CoV-2 virus from test coupons.

Testing was conducted in a biological class 2 safety cabinet in a BL3 laboratory dedicated to SARS-CoV-2 research. The UV-C LED sterilizer wand viral surface deactivation tests were conducted at three distances of 3, 5, and 7 cm from viral coated test coupons. The UV-C sterilization wand was tested for viral inactivation efficacy at three time points of 30, 60, and 90 seconds for each distance with in triplicate testing at each time point and distance. This testing was conducted for both the 304 stainless steel and N95 test coupons resulting in a total of 54 UV exposure tests for the study. Three additional non-exposed positive control coupons were also prepared for each coupon material UV characterization trial. A test matrix is shown in Table 1.

Table 1. UV-C Device Test Matrix

Coupon Material	Time of Coupon UV-C Exposure	Distance of UV wand to Coupon (cm)	Number Of Test Coupons	Coupon Material	Time of Coupon UV-C Exposure	Distance of UV wand to Coupon (cm)	Number Of Test Coupons
304 Stainless Steel	30sec	3	3	N95 Mask	30sec	3	3
		5	3			5	3
		7	3			7	3
	60sec	3	3		60sec	3	3
		5	3			5	3
		7	3			7	3
	90sec	3	3		90sec	3	3
		5	3			5	3
		7	3			7	3
SS Positive Control Coupons			3	N95 Mask Positive Control Coupons			3

The UV-C LED sterilizer wand was mounted on a ring stand in the class 2 biological safety cabinet using a ring stand clamp. The ring stand base was marked for central alignment of the UV source to the center of the circular fabricated test coupons. The ring stand bar was distance graduate marked at 3, 5, and 7 cm for proper height adjustment of the UV wand light source to the SARS-CoV-2 inoculated coupons. Following the drying process of inoculated coupons, test coupons contained in labeled bottom petri dishes (top removed) were transferred and center aligned with the UV wand for exposure. Coupons were exposed in sequential group order at the 3, 5, and 7cm distances respectively to reduce potential error in height and centering readjustment of the ring stand apparatus. Following UV exposure to each coupon, a second operator working in the same class 2 cabinet performed viral surface extractions in sample corresponding labeled sterile collection dishes. The coupons were extracted using a sterile cell scraper for viral removal and serial dilution on 96 well host cell assay plates.

Section 4.
Sample Analysis and Results

Stock virus used for test and control coupon inoculation (SARS-CoV-2, strain USA-WA1/2020) were concentration titered by serial dilution to obtain the 50% tissue culture infectious dose (TCID50). This was conducted to ensure that sufficient quantities of virus were available for testing. Untreated virus control concentrations were assessed to ensure that titers remained consistent. For cell and virus cultures, sterile DMEM (Mediatech) supplemented with 7% fetal bovine serum (HyClone), GlutaMax (Gibco), and penicillin-streptomycin-neomycin antibiotic mixture (Gibco) were utilized. Vero E6 cells (monkey kidney cells) that were originally obtained from ATCC (CRL-1586) were used for assays with ASFV. All cells were maintained at 36°-38°C and 5% CO_2 in a humidified atmosphere, and cells were seeded into flasks for propagation and expanded into 96 well plates for titration of SARS-CoV-2 virus. Cells were infected with viral coupon sample extractions at 70% confluence and observed for the presence of cytopathic effect (CPE) for four (4) to five (5) days post-infection. A 10x serial dilution of coupon sample viral extractions were applied to cell assay plates at up to an 8 log dilution factor for the presence of viral growth into the plate host cells. Plates were inoculated with 5 replicate samples at each dilution level, with each row of replicates 10x more dilute than that used in the preceding row for viral cell infectivity detection. Viral propagation plate readings were conducted under high intensity magnification of each plate cell for viral host cell infectivity and recorded on a sample test log for positive (+) or negative (-) viral propagation. Data was entered into a Reed Muench calculation for sample concentration measurement and determination of the $TCID_{50}$ (50% tissue culture infectious dose of a virus).

Test Results:

Coupon preparation including SARS-CoV-2 inoculation, drying, exposure testing, extractions, and cell assay plating were conducted in a sterile class 2 biological safety cabinet. Following a 4 day plate assay viral incubation period, plates were read for viral infectivity and data recorded on TCID50 test logs. Results were entered into a Reed Muench data analysis program for results and comparison of positive test control sample viral titer concentrations to UV-C exposed test coupon results. A plot showing the log reduction efficacy of the UV-C sterilizer wand in deactivating SARS-CoV-2 infected stainless steel coupons is shown in Figure 1.

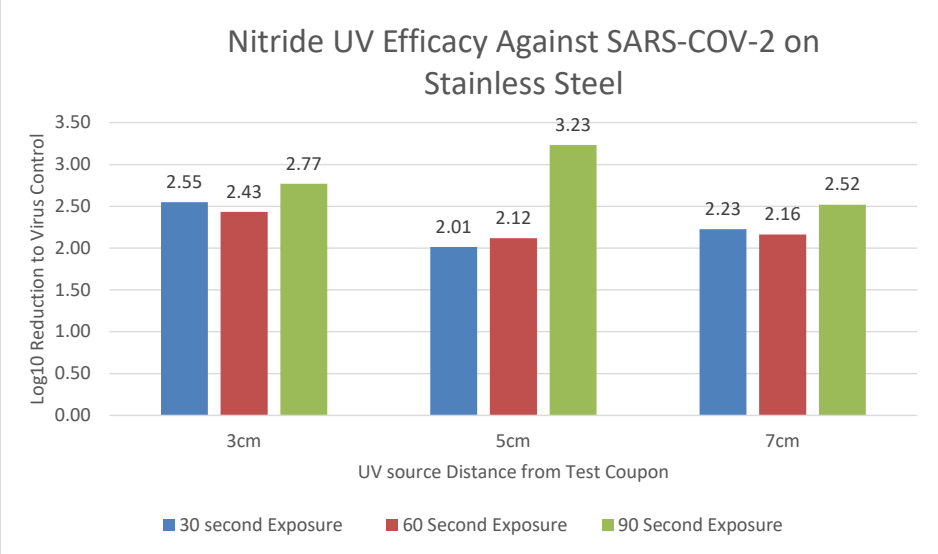

Figure 1. Test Results for UV-C Wand Viral Deactivation Efficacy on SS Coupons

N95 coupons were mask test coupons inoculated with the SARS-CoV-2 virus were UV-C exposed under the same conditions as Stainless Steel coupons. Data analysis of the log viral deactivation from Reed Muench data analysis results with comparison to positive test control N95 samples are shown in Figure 2.

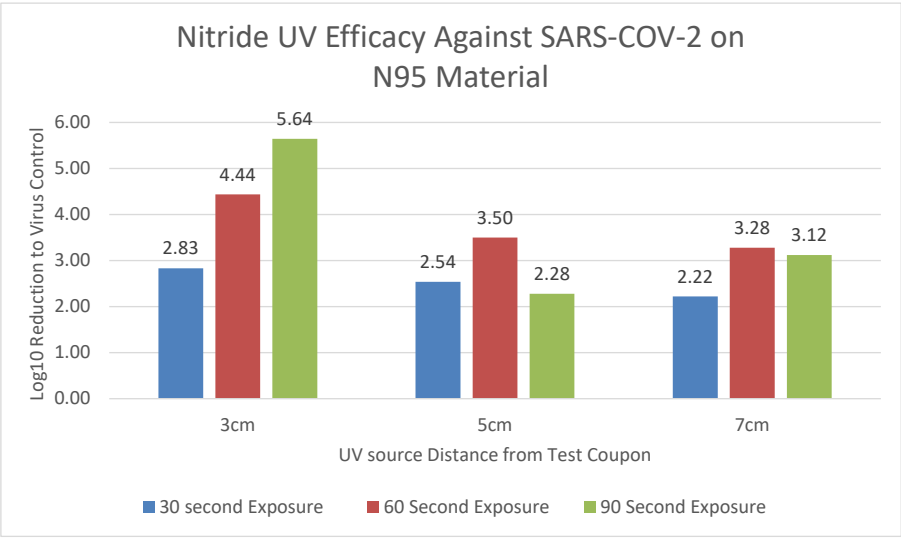

Figure 2. Test Results for UV-C Wand Viral Deactivation Efficacy on N95 Mask Material

Testing results shown in Figures 1, and 2 show the averaged log reduction of all in triplicate test results ontained for the 30, 60, and 90 second coupon UV-C exposure trials in relation to the positive control coupons from each material test. Test results with individual test run coupon log reduction, the average of distance and time in triplicate testing, and the percent viral destruction efficiency of testing is shown in Table 2.

Table 2. Tabulated Test Results

COV - 2 Cell Assay Averaged Data In Triplicate Testing						
Material	Time (s)	Distance (cm)	Average TCID50	Average Log10 TCID50	Log10 Reduction to Virus Control	Percent Log Reduction (% Efficacy)
Stainless Steel	30	3	71.39	1.85	2.55	99.72%
		5	245.55	2.39	2.01	99.03%
		7	150.44	2.18	2.23	99.40%
	60	3	93.07	1.97	2.43	99.63%
		5	191.82	2.28	2.12	99.24%
		7	173.85	2.24	2.16	99.31%
	90	3	42.84	1.63	2.77	99.83%
		5	14.74	1.17	3.23	99.94%
		7	76.35	1.88	2.52	99.70%
	Control		25278.27	4.40	NA	NA
N95	30	3	645.27	2.81	2.83	99.85%
		5	1274.66	3.11	2.54	99.71%
		7	2643.77	3.42	2.22	99.40%
	60	3	16.06	1.21	4.44	>99.99%
		5	138.91	2.14	3.50	99.97%
		7	231.88	2.37	3.28	99.95%
	90	3	<10*	0.00	5.64	>99.99%
		5	2299.47	3.36	2.28	99.47%
		7	334.65	2.52	3.12	99.92%
	Control		437482.73	5.64	NA	NA

*The Log10 value is below a reportable ReedMuench calculated threshold value. The calculation assumes the Log10 value to be below 10. For log reduction calculations, this value is assumed to be 0.

Testing of the four UV-C LED sterilization wand showed viral destruction efficacy, and a high active reduction of SARS-CoV-2 surface viability in conducted tests at all distances and exposure times. The log reductions data shown in Figure 1, and 2, and tabulated in Table 2 were also converted to percent viral reduction for each coupon type and exposure condition.

Section 5.
Quality Assurance

5.1 Type of Study

This non-GLP study was executed according to the protocol (as amended) and using established SOPs, at MRIGlobal in Kansas City, MO that is fully qualified to conduct GLP studies; and all procedures utilized were technically valid. This study was not audited by the MRIGlobal Quality Assurance Department. Portions of the study conducted at MRIGlobal were performed according to MRIGlobal Standard Operating Procedures and/or laboratory procedures.

5.2 Standard Operating Procedures

The study was performed according to the relevant standard operating procedures and/or laboratory procedures of MRIGlobal.

Section 6.
Location of Study Data

Exact copies of all raw data, correspondence, records, final protocol, amendments, and deviations, and any other study documentation necessary for reconstruction of the study will be archived at MRIGlobal. All raw data (including original study records, data sheets, work sheets, and computer printouts) will be archived by MRIGlobal.