THE UNIVERSITY OF
ALABAMA IN HUNTSVILLE

Report # Surface Sanitizer ESC-07-10/22/2020

90 Day Persistence Test for ESC Brands My-Shield Surface Sanitizer against a Surrogate Virus for SARS-CoV-2

10/22/2020

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1.0 Objective

The overall objective of this test was to investigate the ability of the My-Shield Surface Sanitizer provided by ESC Brands to kill a SARS-CoV- 2 surrogate virus on a stainless steel surface 90 days after treatment of the surface.

2.0 Protocol Overview

My-Shield Surface Sanitizer, Lot 03042020-SS-A, provided by ESC Brands, was tested according to ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces. The procedure was enhanced for testing the persistence of the product on the surface rather than immediate killing. To accomplish this the product was applied and dried onto the test surface according to manufacturer's directions and the virus was added to it at the time of assay. Host cells were NCTC clone 1469 cells obtained from ATCC, and the test virus was the murine hepatitis virus-S, (MHV-S), also from ATCC and recognized by the CDC as a surrogate for SARS-CoV testing (Hulkower, R.L. et al., 2011) and on a list of surrogates being reviewed by ASTM. A 96 well plate format was used with quadruplicates for each test condition. Incubation of the virus with the test product was for 10 minute at room temperature. Neutralizer was Butterfields Phosphate Buffer + Surfactants (BBP++). Sephadex G-25 columns were used to reduce cytotoxicity of the test product in the assay according to ASTM E1482-12, Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization. Test results were determined after 7 days.

3.0 Materials and Methods

3.1 Growth of stock virus

3.1.1 Cell culture

Mouse liver cell line NCTC Clone 1469 (ATCC® CCL-9.1™) was maintained in DMEM with 4500 g/l glucose plus L-gln and 1.5 g/l sodium bicarbonate, pH 7.3, supplemented with 10% Donor Horse Serum (Biotechne, Minneapolis, MN) in a humidified incubator at 37° C and 5% CO_2. Cells were passaged by scraping cells from the flask surface, centrifugation and resuspension in new growth media. 4 x 10^4 cells/well were plated in DMEM + 10% horse serum in a 96 well plate 24 hours before the assay and incubated as above.

3.1.2 Virus preparation

Murine Hepatitis virus, MHV-S (ATCC VR-766™), was used to inoculate NCTC Clone 1469 cells at a moi of about 1.0 following published procedures (Leibowitz et al., 2011). Virus was harvested after 48 hours as per Leibowitz et

al. 2011). Isolated virus was stored at -80° C in 1.0 ml aliquots. Virus titer was determined using the endpoint dilution procedure to obtain the $TCID_{50}$ on the NCTC Clone 1469 cells.

3.1.3 Sephadex Column Preparation

Columns to remove cytotoxic materials were used according to ASTM E1482-12, and Geller et al., 2009. Sephadex G-25 was obtained from Sigma-Aldrich. The gel was swelled in phosphate buffered saline (PBS) for 3 hours at room temperature (5 g resin/100 ml buffer) and then sterilized by autoclaving at 121° C for 20 minutes. Excess buffer was removed to a final volume of about 40 ml.

Five ml syringes were used as the column bodies. The plunger was removed and the "wings" at the top of the barrel were cut to allow the syringe to fit into a 50 ml conical polypropylene centrifuge tube (Fisher Scientific). A small amount of polyester fiber was placed into the bottom of the syringe to form a plug. The tubes with the syringes in them were autoclaved to sterilize them. 4.0 ml of the Sephadex slurry was pipetted into the syringes and they were centrifuged at 1000 x g for 10 min at 4°C. The eluate was removed from the tubes and the columns were kept refrigerated until use, typically 30 minutes, but no longer than 24 hours.

3.2 Surface Test Protocol

3.2.1 Disc treatment

Stainless steel alloy 304 discs, 0.5 in diameter, 16 ga thickness, with #4 grained finish from Metal Remnants, Inc., Salt lake City, UT were rinsed in 70% alcohol to remove surface oil, dried and then autoclaved. Surface Sanitizer was applied to the stainless steel discs, which were on a sterile glass plate in a BioSafety hood, by using the spray applicator provided by the manufacturer using three sprays at a 45° angle from 8 inches away as per the use instructions. The sanitizer was allowed to dry on the disc in the hood for about 60 minutes. The discs were then placed into separate wells of a sterile 12 well plate until the assay date.

3.2.2 Virucidal Assay

On 10/12/2020 NCTC Clone 1469 cells were plated at 4×10^4 cells per well in a 96 well plate in DMEM + 10% horse serum as above and incubated for 24 h at 37°C and 5% CO_2. Cells were about 70-80% confluent for the test. On

10/13/2020 50 µl of stock virus was pipetted onto one untreated disc for a virus recovery control and on one disc which had been treated with the Surface Sanitizer on 7/7/2020 according to manufacturer's directions of 3 sprays from 8 inches away at a 45 degree angle. A disc treated for 90 days that was not inoculated with virus served as the cytotoxicity control. The contact time for virus on the disc was 10 minutes at 23.6^{o} C. Each disc was then dropped into an ice cold tube to which previously had been added 1.0 ml of BBP++ neutralizer (Butterfield's buffered phosphate + surfactants) and 150 mg of sterile glass disruption beads, 0.1mm diameter, Research Products International. The tubes were vortexed at the number 1 vortex speed for 30s and placed back on ice. 750 µl from the liquid in each tube was layered onto a 2.0 ml packed Sephadex G-25 column and centrifuged for 10 min at 1000 x g and 4° C. The column eluate was recovered from the tubes and serial 10-fold dilutions in ice cold DMEM + 2% Horse serum (DMEM-2) as above but also containing 100 units/ml penicillin and 100 µg/ml streptomycin were made with the eluate of each column. A virus control that was not exposed to a disc was prepared by adding 50 µl of stock virus to a tube with 1.0 ml of BBP++ and 150 mg of glass beads. That tube was then vortexed the same as the other tubes but contents were not placed on a G-25 column. Media was removed from the NCTC clone 1469 cells in the 96 well plates and 100 µl of each dilution was added to quadruplicate wells. Control wells received only fresh medium. The plates were then incubated at 37°C and 5% CO_2 for 2 hrs. The media was then removed and 100 µl of fresh DMEM-2 with Penn-Strep was added and the plates were incubated for 7 days. Plates were scored visually for cytopathological effects (CPE) using a Zeiss inverted microscope.

4.0 Results – Test done on 10/13/2020; Plates scored on 10/20/2020

Dilution (Log_{10})	Virus control	Virus Recovery control	90-day treated disc	Cytotoxicity Control
-2	++++	++++	CT	++00
-3	++++	++++	+0+0	0000
-4	++++	++++	0000	0000
-5	+++0	0+++	0000	0000
-6	0+++	++++	0000	ND
-7	0000	0000	ND	ND
-8	0000	0000	ND	
$-Log_{10}$ $TCID_{50}$	6.33	6.5	3.0	2.0
Log_{10} Reduction			3.5	
% kill			99.95	

+ CPE (cytopathic/cytotoxic effect) present per well
0 CPE (cytopathic/cytotoxic effect) not detected
ND Not Done
CT Cytotoxicity

Neutralizer control run separately showed neutralization of the product to a $TCID_{50}$ of 10^{-5}.

A neutralizer cytotoxicity control, run separately, showed no CPE at any dilutions of the BBP++. Formaldehyde killed all cells to the 10^{-4} dilution.

5.0 Summary

The recovery of virus from the untreated disc and column was essentially 100%
The (-log 10) $TCID_{50}$ / 50µl virus on the untreated disc was determined to be 6.5
The (-log 10) $TCID_{50}$ / 50µl after 90 days was 3.0.

In summary, the My-Shield Surface Sanitizer remained active on stainless steel for 90 days and killed 99.95% of the coronavirus with a 10 minute contact time.

References:

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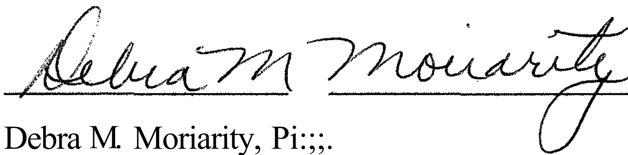

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