THE UNIVERSITY OF
ALABAMA IN HUNTSVILLE

My-Shield Surface Sanitizer Test on Stainless Steel Discs under Simulated Use Conditions against a Surrogate Virus for SARS-CoV-2

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Tested by Dr. Debra M. Moriarity, Professor Emerita, at
The University of Alabama in Huntsville

Debra M Moriarity 9/3/2020

1.0 Objective

The overall objective of this test was to investigate the ability of the My-Shield Surface Sanitizer product provided by ESC Brands to kill a CDC recognized SARS-CoV- 2 surrogate virus on stainless steel surfaces that were touched for 14 and 4 days post treatment with the product to simulate use of the surface.

2.0 Protocol Overview

My Shield Surface Sanitizer, Lot 10222019-SS-BB provided by ESC Brands, was applied to stainless steel discs according to instructions by the supplier. The discs were allowed to dry in a Biosafety hood and then placed into wells of a 24 well plate and held for 3 weeks. At that time touching of discs began and continued for 4 days and 14 days by two volunteers. Virucidal activity was tested by applying virus to the discs in the plate wells for 10 minute. Discs were then transferred to tubes containing neutralizer and glass beads and vortexed for 30s to recover virus. A sample from each tube was pipetted onto a Sephadex LH-20 column and the eluate was used to make dilutions for a $TCID_{50}$ determination in 96 well plates. Murine hepatitis virus, MHV-S, a CDC recognized surrogate virus for SARS-CoV-2 testing, was grown in a mouse liver cell line, NCTC Clone 1469. The same cell line was used for virucidal assays.

The procedure was consistent with ASTM E1053 – 20 publication, except that the surface was first treated with the product and then the virus was added for the test.

3.0 Materials and Methods

3.1 Growth of stock virus

3.1.1 Cell culture

NCTC Clone 1469 (ATCC® CCL-9.1™) was maintained in DMEM with 4500g/l glucose plus L-gln and 1.5g/l sodium bicarbonate, pH 7.3, supplemented with 10% Donor Horse Serum (Biotechne, Minneapolis, MN) in a humidified incubator at 37ºC and 5% CO_2. Cells were passaged by scraping cells from the flask surface, centrifuging and resuspending in new growth media. 5 x 10^4 cells/well were plated in DMEM + 10% horse serum in a 96 well plate 24 hours before the $TCID_{50}$ assay and incubated as above.

3.1.2 Virus preparation

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). Isolated virus was stored at -80ºC 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.

3.2 Non-porous surface sanitizer assay

3.2 Test Materials

3.2.1 Stainless Steel Discs

Stainless steel discs (Metal Remnants, Inc) were stainless alloy 304, #4-grained polish finish. The diameter was 0.5 inches and it was 16ga thickness. The surface area was 1.27cm^2.

3.2.2 Neutralizer

Neutralizer was Butterfield's Buffered Phosphate + surfactants (BBP^{++}).

3.2.3 Columns to remove cytotoxicity

Columns were prepared with Sephadex LH-20 resin as per ASTM E1482-12 to reduce the cytotoxicity of the surface sanitizer.

3.2.4 Glass beads

Glass beads (Research Products International Disruption Beads) were 0.1mm diameter and 2.5g/cc. The beads were prepared by washing in a mild detergent, extensive rinsing in deionized water, soaking in 70% ethanol, and then spread out and dried. They were then autoclaved to be sterile.

3.3 Methodology

Stainless steel discs were sprayed with the My-Shield Surface Sanitizer with 3 sprays using the applicator supplied by ESC at a distance of 8 inches and about a 45^o angle. The discs were allowed to dry in a biosafety hood (about 40 minutes). The discs used in this test were sprayed on 7/29/2020 and tested on 8/18/2020. A single finger touch per day to two discs (two separate sets) began on 8/3/2020 for the 14 day treatment and 8/13 for the 4 day treatment.

On 8/18/2020 the test was performed using 96 well plates of NCTC clone 1469 cells prepared on 8/17/2020 as described above. 50µl of the stock virus solution was place in the middle of the stainless steel discs in the wells of the 24-well plate. After 10 minutes at 23.3ºC, 47% relative humidity, each disc was carefully transferred into culture tubes on ice which already contained 1.0 ml of BBP++ neutralizer and 150mg of glass beads. Care was taken to be sure that no virus suspension dripped off the disc. Any liquid that had run off into the wells was also transferred into the tube with the discs. The tubes were vortexed for 30s and placed on ice. 0.5ml of the solution was placed onto cold Sephadex LH-20 columns containing a volume of 2.0ml of packed resin that had been swelled in phosphate buffered saline and centrifuged to remove the buffer. The columns with

the samples were centrifuged at the top speed of an IEC clinical centrifuge in 50ml polypropylene tubes for 10min at 4°C. Ten-fold serial dilutions of the eluate were made in DMEM containing 2% horse serum. Media was removed from the 96 well plates and 100 µl of each dilution were placed in quadruplicate wells. The plates were incubated for 2hrs at 37°C and 5% CO_2 in a humidified incubator. At that time the media was removed and 100µl of fresh DMEM with 2% horse serum was added to each well and they were placed back into the incubator. Test samples were as follows:

Tube	Disc	Virus (µl)	Days touched	
A	None	50	na	
B	Untreated	50	na	Virus control
C	Treated	50	14	
D	Treated	50	14	
E	Treated	50	4	
F	Treated	50	4	
G	Treated	-	na	Cytotoxicity control
H	Treated	50 – added after neutralizer	na	Neutralizer control

4.0 Results

Dilution -(Log $_{10}$)	**Virus**	**Virus on disc**	**14 day (1)**	**14 day (2)**	**4 day (1)**	**4 day (2)**	**Cytotoxicity**	**Neutralizer Control**	**Cell Control**
2	++++	++++	++++	++++	++++	++++	++++	++++	0000
3	++++	++++	0000	0000	0000	0000	0000	++++	NA
4	++++	++++	0000	0000	0000	0000	0000	++++	
5	++++	0+++	0000	0000	0000	0000	0000	0000	
6	0+0+	+000	0000	0000	0000	0000	ND	ND	
7	0000	0000	0000	0000	0000	0000	ND	ND	
Log $_{10}$ $TCID_{50}$	6.0	5.5	2.5	2.5	2.5	2.5	2.5	4.5	
Log_{10} Reduction			≥3.0	≥3.0	≥3.0	≥3.0			
% kill			**≥99.9**	**≥99.9**	**≥99.9**	**≥99.9**			

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

A neutralizer cytotoxicity control, run separately, showed CPE only at the 10^{-2} dilution of the BBP^{++}.
7.5% Formaldehyde killed all cells up to the 10^{-4} dilution.

5.0 Summary

My-Shield Hand Sanitizer demonstrated at least a log 3 difference from untreated virus on the discs, which represents **≥ 99.9% kill** of the surrogate SARS-Cov-2 virus after 4 and 14 days of simulated "use".

Cytotoxicity was observed at the 10^{-2} dilution of the product so no greater than a log 3 difference of the product could be determined.

Only a 0.5 log difference was seen between the virus suspension and the virus recovered from untreated discs, indicating that the procedure effectively recovered the virus within the acceptable range (ASTM

The neutralizer control demonstrated that the virucidal activity was in fact neutralized upon addition of the BBP++.

References:

Leibowitz, J., Kaufman, G and Liu, P. *Coronaviruses: Propagation, Quantification, Storage and Construction of Recombinant Mouse Hepatitis Virus.* Current Protocols in Microbiology; John Wiley and Sons, Wiley Online Library; May, 2011, Supplement 21, CH 15.