THE UNIVERSITY OF
ALABAMA IN HUNTSVILLE

Persistence Test
for
My Shield Laundry Complete
against a
Surrogate virus for
SARS-C0V-2
using fabric treated over 200 days
before testing.

Test performed on 8/4/2020 by Dr. Debra M. Moriarity, Professor Emerita, Dept. of Biological Sciences, The University of Alabama in Huntsville, Huntsville, AL in the Shelby Center for Science and Technology.

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Test Objective

The overall objective of this test was to investigate the persistence of antiviral activity of fabric treated with the ESC Brands My Shield Laundry Complete product over 7 months ago.

1.0 Protocol Overview

The murine hepatitis virus, MHV-S, a CDC recognized surrogate virus for SARS-CoV testing, was grown in a mouse liver cell line, NCTC clone 1469. Isolated virus was incubated with a piece of fabric treated with My Shield Laundry Complete on December 16, 2019 and kept in a sterile container since then. Controls were untreated fabric pieces of the same size. After incubation with the virus for 15, 30 or 60 minutes the samples were neutralized with D/E broth, vortexed to remove the virus from the fabric and passed over a Sephadex LH-20 column to remove potential cytotoxic substances. Serial dilutions of the column eluates were used to inoculate NCTC clone 1469 cells in a 96 well plate to determine the $TCID_{50}$ for the virus at each time. After 14 days the wells were examined for cytopathological effects (CPE) in the cells and scored accordingly.

2.0 Materials and Methods

2.1 Growth of stock virus

2.1.1 Cell culture

NCTC Clone 1469 (ATCC@CCL-9.F^{M}) was maintained in DMEM with 4500 g/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 37^{o}C 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 assay and incubated as above.

2.1.2 Virus preparation

MHV-S (ATCC VR-766^{TM}) 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 -80^{0}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.

2.2 Product Test

My Shield Laundry Complete, Lot 05062020-LC-A, was diluted at a ratio of 4.69 ml to 250 ml with ultrapure water, which is the dilution that would occur in the rinse cycle of a standard washing machine. Sterile tubes were set up as follows with a 20 mm x 20 mm square of either treated or non-treated fabric placed in the bottom of the tube. The virus was added for the 60 minute time point. 30 minutes later virus was added for that time point and 15 minutes later the final virus was added to that tube. Incubation was at 22.4°C. At the end of the total time 1.0 ml of neutralizer (50% PBS, 50% D/E broth) was added and the tube was vortexed for 30 s. Tubes were then placed on ice. 0.5 ml from each tube was loaded onto a prepared Sephadex LH-20 column in a 5 ml syringe in a sterile 50 ml tube. The tubes with the columns in them were centrifuged for 10 minutes at 4°C at the highest setting of an IEC tabletop clinical centrifuge. After centrifugation the columns were discarded and the eluate was transferred to microfuge tubes and centrifuged for 15s at 12,000 rcf to pellet any column resin that was in the sample. The supernatants were then serially diluted in DMEM with 4500 g/l glucose, L-gln, sodium bicarbonate and 2% horse serum, pH 7.3.

Tube	Cloth	Virus	Incubation Time (min)
A	None	0.1 ml	N/A
B	Untreated	0.1ml	15
C	Treated	0.1ml	15
D	Untreated	0.1ml	30
E	Treated	0.1ml	30
F	Untreated	0.1ml	60
G	Treated	0.1ml	60
H	Treated	None	N/A

Media was removed from cells in the 96-well plate and 100 µl samples of each dilution were added to 8 replicate wells. The plates were incubated at 37°C and 5% CO_2 for 2 hrs and the media was replaced with just DMEM + 2% horse serum. Incubation continued for 14 days, at which time they were examined for CPE and scored.

3.0 Results

Dilution (-Log $_{10}$)	Virus, no cloth	15 minute**		30 minute**		60 minute**		Cytotoxicity
		Control	Treated	Control	Treated	Control	treated	
2	++++ ++++	++++ ++++	++++ ++++	++++ ++++	++++ ++++	++++ ++++	++++ ++++	++++ ++++
3	++++ ++++	++++ ++++	++++ ++++	++++ ++++	0+++ ++++	++++ ++++	++++ ++++	0000 0000
4	++++ ++++	++++ ++0+	++00 0000	+0++ +0++	0000 +++0	++++ ++++	+00+ 0+0+	0000 0000
5	000+ 00+0	0+00 000+	00+0 0000	++00 ++0+	0000 00+0	+0++ ++0+	000+ 0000	0000 0000
6	0000 0000	0000 0000	0000 00+0	0000 0000	0000 0000	0++0 +0+0	0000 0000	ND
7	0000 0000	0+00 0000	0000 0000	0000 00+0	0000 0000	0+00 0000	0000 0000	ND
-Log $_{10}$ $TCID_{50}$/ml	4.67	4.44	3.67	5.20	3.75	6.00	4.00	2.50
Log difference*			≥0.77		≥1.45		≥2.0	NA
% kill			≥83.2%		≥96.5		≥99%	

+ = CPE
0 = live cells still visible
ND = not determined
*between control and treated fabric coupon for each time point
** time virus was exposed to the fabric coupon

4.0 Summary

1. The virus $TCID_{50}$ (log 10) was determined to be 4.67
2. Cytotoxicity was only observed at 10^{-2} dilution.
3. A neutralizer cytotoxicity control, run separately, showed no CPE at any dilutions of the D/E broth.
4. Formaldehyde killed all cells to the 10-4 dilution.
5. Most significantly, the persistence of the virucidal activity of the My Shield Laundry Complete was evident even after **more than 200 days** with at least **99% kill** of virus after only an hour of contact with the fabric, and nearly 97% kill after only 30 min.

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.