DISINFECT™ & SHIELD

Suspension Test for Laundry Complete Product against a Surrogate Virus for SARS-CoV-2

Tested by Dr. Debra M. Moriarity, Professor Emerita, at

The University of Alabama in Huntsville

Debra M Moriarity 7/13/2020

College of Science
Department of Biological Sciences

Shelby Center for Science & Technology 369A Huntsville, Alabama 35899

Objective

The overall objective of this test was to investigate the ability of the Laundry Complete product provided by Disinfect & Shield to kill a SARS-CoV- 2 surrogate virus when used under typical laundry conditions.

1 Protocol Overview

The 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. Isolated virus was incubated with a dilution of the Laundry Complete based on its use instructions, at room temperature for 15 minutes. The samples were then neutralized and passed over a dextran bead column to remove potential cytotoxic substances. Column eluates were used to inoculate NCTC clone 1469 cells in a 96 well plate. After 7 days the wells were examined for cytopathological effects (CPE) in the cells and scored accordingly. The protocol followed DIN EN-14476 European Standard for Quantitative Suspension Test of Chemical Disinfectants and Antiseptics for Virucidal Activity, Dec. 2015.

2 Materials and Methods

2.0 Growth of stock virus

2.0.1 Cell culture

NCTC Clone 1469 (ATCC® CCL-9.l™) was maintained in DMEM with 4500 g/1 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°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.

2.0.2 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 -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.

2.1 Suspension Test Protocol

Laundry Complete, Lot 05062020-LC-A, was diluted at a ratio of 4.69 ml to 250 ml with ultrapure water. All tests contained 0.03% bovine serum albumin (BSA) as a clean interfering substance. Sterile tubes were set up as follows:

Tube A: Virus titer: 0.1ml stock virus 0.1ml 0.3% BSA
0.8 ml PBS (phosphate buffered saline)

Tube B: Product test: 0.1 ml stock virus
0.1ml 0.3% BSA
0.8 ml diluted Laundry Complete

Tube C: Cytotoxicity Control
0.1 ml DMEM +2% horse serum(DMEM-2)
0.1 ml 0.3% BSA
0.8 ml diluted Laundry Complete

Tube D: Virus killing control 0.1 ml stock virus
0.1 ml 0.3% BSA
0.2 ml 1.4% Formaldehyde

All tubes were incubated for 15 minutes at 21.8°C, 52% relative humidity. At the end of that incubation 1.0 ml of Dey/Engly Broth (D/E broth) neutralizer was added to tubes A, B and C and 0.4 ml ofD/E broth was added to tube D. All tubes were place on ice. A 500 µl sample from each tube was placed on a 4.0 ml column of Sephadex LH-20 and centrifuged at 100 x g at 4°C for 10 min. The eluant was collected and serial 10-fold dilutions were made in ice-cold DMEM-2. Media was removed from cells in the 96-well plate and 100 µ1 samples from each test condition were added to quadruplicate wells. The plates were incubated at 37°C and 5% CO_2 for 7 days, at which time they were examined for CPE.

3 Results -Test done on 7/3/2020; plate scored on 7/10/2020

Dilution (Log 10)	**Virus control**	**Test Product**	**Cytotoxicity**	**Cell Control**
-2	++++	CT	++++	**0000**
-3	++++	0++0	0000	**NA**
-4	++++	0000	0000	
-5	++++	0000	0000	
-6	++++	0000	0000	
-7	+00+	0000	0000	
Log $_{10}$ TCIDso	-7.0	-3.0	-2.5	
Lo g_{10} Reduction		4.0		
%kill		>99.99%		

\+ CPE (cytopathic/cytotoxic effect) present
0 CPE (cytopathic/cytotoxic effect) not detected
NIA Not applicable
CT Cytotoxicity

A neutralizer cytotoxicity control, run separately, showed no CPE at any dilutions of the *DIE*
broth.Formaldehyde killed all cells to the 10^4 dilution.

4 Summary

The virus $TCID_{50}$ (log 10) was determined to be 7.0
The virus $TCID_{50}$ (log 10) after treatment with Laundry Complete was 3.0.

The log difference between them was 4.0 indicating **99.99%** of the virus was killed.

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.