Report# Body Spray-ESC-005-10/9/2020

Suspension Test for ESC Brands My-Shield Body Spray using a Surrogate Virus for SARS-CoV-2

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1.0 Objective

The overall objective of this test was to investigate the ability of the My-Shield Surface Body Spray product provided by ESC Brands to kill a CDC recognized SARS-CoV- 2 surrogate virus in a quantitative suspension test.

2.0 Protocol Overview

My-Shield Body Spray, Lot 03282020-BS-A, provided by ESC Brands, was tested using a quantitative suspension assay according to EN 14476:2013 standard. 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-2 testing (Hulkower, R.L. et al., 2011). A 96 well plate format was used with quadruplicates for each test condition. Incubation of the virus with the test product was for 1 minute at 23.8^{o}C. Neutralizer was Butterfields Phosphate Buffer + Surfactants (BBP++). Sephadex G-25 columns were used to reduce cytotoxicity of the test product in the assay. Test results were determined 72 hours after inoculation.

3.0 Materials and Methods

3.1 Growth of cell line and stock virus

3.1.1 Cell culture

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, (Sigma-Aldrich), 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. Cells were typically seeded at 2 x 10^6 cells per T-75 flask. Media was changed 3-4 times per week. For testing 5 x 10^4 cells/well were plated in DMEM + 10% horse serum in 96 well plates 24 hours before the $TCID_{50}$ assay and incubated as above. Cells were approximately 75-80% confluent at time of testing.

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 and harvested 48 hours later following published procedures (Leibowitz et al., 2011). Isolated virus was stored at -80^{o}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. Titer was calculated using the Reed-Muench Method, (Reed, L.J.; Muench, H.,1938) as described by Leibovitz (Leibovitz et al., 2011).

3.2 Sephadex Column Preparation

Columns to remove cytotoxic materials were used according to ASTME 1482-12, 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^{o} 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^{o}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.

3.3 Test Procedure

The test was performed on 9/29/2020.

Sterile 5ml plastic tubes with caps were used for the suspension test. Tubes contained materials as shown in the table below. Upon addition of the virus the tube was briefly vortexed and then incubated for 1 minute, at which time 1.0 ml of BBP++ was added to the tube and it was vortexed and placed on ice. Virus addition was staggered to tubes every 5 minutes to allow time to process each tube at the end of the incubation.

Tube	0.3% BSA	Body Spray	PBS	Virus	G-25Column (Y/N)
A	0.1 ml	0	0.8 ml	0.1 ml	N
B	0.1 ml	0	0.8 ml	0.1 ml	Y
C	0.1 ml	0.8 ml	0	0.1 ml	Y
D	0.1 ml	0.8 ml	0	0	Y

After BBP++ addition 750 µl from each tube was applied to a G-25 column and the columns were centrifuged again at 1000 x g for 10 min at 4^{o}C. The eluate from each column was transferred to 1.5ml microfuge tube and 10 fold dilutions were made in DMEM + 2% horse serum on ice. Media was removed from the 96 well plates and 100 µl of each dilution was added to each of 4 wells. DMEM + 2% horse serum was added to a set of 4 wells on each plate as

a control. 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 100 µl of fresh DMEM with 2% horse serum was added to each well and they were placed back into the incubator. After 72 hours visual inspection of the cells was made using a Zeiss inverted microscope and evidence of cytopathic effects (CPE) were noted.

4.0 Results

Visual inspection of the wells gave the following results after 72 hours.

Dilution (Log	Virus control	Virus Recovery	Test Product	Cytotoxicity	Cell Control
-2	++++	++++	CT	++++	0000
-3	++++	++++	0000	0000	NA
-4	++++	++++	000	0000	
-5	++++	++++	0000	0000	
-6	0000	0000	0000	ND	
-7	0000	0000	ND	ND	
$-Log_{10}TCID_{50}$	5.5	5.5	2.5	3.5	
Log_{10} Reduction		0	3.0		
% kill			≥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^{++}.

A Neutralizer control run separately indicated virus growth in the presence of the product after neutralization up to the 10^{-5} dilution.

7.5% Formaldehyde killed all cells up to the 10^{-4} dilution.

The virus recovery control indicated 100% virus titer recovery after the column passage.

5.0 Conclusion

After just 1 min exposure to the ESC Brands My-Shield Body Spray at typical room temperature, there was a log 3 reduction in viral activity, indicating a 99.9% kill of the virus. Since there was cytotoxicity from just the product and neutralizer alone, the kill rate is **≥ 99.9%.**

6.0 References:

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Testing certified by

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