RESEARCH, DISCOVERY & INNOVATION

Water & Energy Sustainable Technology Center

NON-GLP STUDY REPORT

STUDY TITLE

Test for Continuous Viral Reduction on Coated Surfaces;

EPA-approved protocol granted to Corning Incorporated

PRODUCT IDENTITY

Commercial Paint 1

STUDY SCIENTISTS

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PERFORMING LABORATORY

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SPONSOR

Corning Incorporated

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STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Test for Continuous Viral Reduction on Coated Surfaces; EPA-approved protocol granted to Corning Incorporated

TEST SUBSTANCE IDENTITY

Test Sample: Commercial Paint 1

Control Samples: Commercial Paint 1 Control

WEST Center Control Film (1" x 1")

REPORT DATE: 09 November 2020

TEST PARAMETERS

Virus: SARS-CoV-2, Isolate USA-WA1/2020 (BEI Resources NR-52281)

Inoculum Volume Tested: 60 uL

Exposure Times: 0 hours (i.e. upon inoculation), 2 hours (from inoculation)

Organic Soil Load: 5% Fetal Bovine Serum (FBS) soil load

Neutralization: 5% FBS Minimal Essential Medium (MEM), followed by Sephadex G-10 gel filtration

Test Medium: 5% FBS MEM

Indicator Cell Cultures: Vero E6 Cells (ATCC® CRL-1586)

Temperature and Humidity: 22 - 23 degrees C, 40 - 50% RH

Conducted in triplicate per Contact Time (except for WEST Control which was performed in singlet)

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EXPERIMENT DESIGN

Wear Testing

The Sponsor conducted a wear procedure on one set of control and test coupons that simulated six years of washing and abrasion (as detailed in the Sponsor's EPA-approved test method). The Sponsor then provided the coupons to the Performing Laboratory. Efficacy testing was conducted concurrently for non-wear tested coupons and wear-tested coupons as described below within five days of the wear procedure.

Efficacy Test Procedure and Infectivity Assay

1" x 1" test and control coupons were each inoculated with 60 uL of the viral inoculum. The exposure time began as soon as the samples were inoculated. The inoculum was not covered with a film, and the Petri dish lids were left on for the duration of exposure period (2 hrs). The conditions of exposure were maintained at 22 - 23 degrees C and 40 - 50% RH. Upon closure of the exposure time(s), 1.00 mL aliquots of neutralizer solution were individually added to the carriers. The surface of each carrier was individually scraped with a sterile plastic cell scraper to remove remaining infectious viruses. The test suspensions were collected and then passed through individual Sephadex G-10 columns.

Each filtrate was titered by 10-fold serial dilutions, and the dilutions were assayed in replicates of six for infectivity using Vero E6 host cells prepared in 96-well trays. Cultures were scored periodically over 10 days for the absence/presence of cytopathic effects (CPE), cytotoxicity and viability. The CPE associated with SARS-CoV-2 was visually evidenced under the microscope by the presence of cell rounding, aggregation, detachment from the monolayer.

Plate Recovery Control

An inoculum of the virus (60 µL) was inoculated onto a 1" x 1" clean glass slide and incubated for 2 hours concurrently with control and test carriers. Media was added (1 mL) to the slide and the virus was recovered using the same methodology employed for coated test and control coupons. The suspension was passed through a Sephadex G-10 filtration column and the filtrate was assayed for infectivity of host cells. This control allows for determination of the relative loss in virus infectivity resulting from drying and neutralization alone. The results from the plate recovery control were compared with coated control

results to compare recovery of virus on a non-porous surface vs. porous coated surface and to confirm recovery of at least 4.8 $\log_{10}$ of infectious viruses following drying and neutralization.

Cell Viability Control

A minimum of six wells with monolayers of Vero E6 host cells were inoculated with cell culture media only and incubate in a humidified atmosphere of 5-7% CO2 at 36-38°C in for 10 days. This control demonstrated that cells remained viable throughout the course of assay period.

Virus Stock Titer Control

SARS-CoV-2 was titered at the time of the test to determine the relative infectivity of the virus and to demonstrate the susceptibility of Vero E6 host cells to support infection. Serial tenfold dilutions were prepared in cell culture media. Select dilutions were inoculated into six wells per dilution and incubated under the same conditions as the test.

Cytotoxicity and Neutralization Effectiveness Controls

One coupon, contained in a sterile petri dish, was inoculated with a 60µL aliquot of 5% FBS MEM in lieu of the virus. The exposure conditions and procedure were as detailed above. Following the 2-hour exposure time, a 1.00 mL aliquot of neutralizing solution was added to the cytotoxicity control carrier. The surface of the carrier was scraped with a sterile plastic cell scraper. The test suspension was collected and then passed through a Sephadex G-10 column. The filtrate was titered by 10-fold serial dilutions and the dilutions were assayed for cytotoxicity on Vero E6 host cell monolayers prepared in 96-well trays. For the neutralization control, an aliquot of the filtrate underwent a separate set of 10-fold dilutions. A low titer inoculum (2.0 – 2.50 $\log_{10}$) of SARS-CoV-2 was added to each dilution tube and held for 10 minutes prior to plating in replicates of six onto Vero E6 host cells prepared in 96-well trays.

RESULTS

Table 1. Efficacy Data for Commercial Paint 1 Control and Commercial Paint 1 Test Paints (Non-Wear Tested Samples)*

Sample ID	Contact Time	Viral Titer (Log_{10}/Carrier)	Mean Viral Titer (Log_{10}/Carrier)	Log_{10} Reduction
Commercial Paint 1 Control – 1	Time Zero (0 Hour)	6.67	6.56 ± 0.098	N.A.
Commercial Paint 1 Control – 2		6.50		
Commercial Paint 1 Control – 3		6.50		
Commercial Paint 1 Control – 1	2 Hours	5.83	5.44 ± 0.42	N.A.
Commercial Paint 1 Control – 2		5.50		
Commercial Paint 1 Control – 3		5.00		
Commercial Paint 1 Test – 1	2 Hours	< 1.50	< 1.50 ± 0.00	> 3.94
Commercial Paint 1 Test – 2		< 1.50		
Commercial Paint 1 Test – 3		< 1.50		

*N.A.: Not applicable.

Table 2. Efficacy Data for Commercial Paint 1 Control and Commercial Paint 1 Test Paints (Wear Tested Samples)*

Sample ID	Contact Time	Viral Titer (Log_{10}/Carrier)	Mean Viral Titer (Log_{10}/Carrier)	Log_{10} Reduction
Commercial Paint 1 Control – 1	Time Zero (0 Hour)	6.67	6.06 ± 0.54	N.A.
Commercial Paint 1 Control – 2		5.83		
Commercial Paint 1 Control – 3		5.67		
Commercial Paint 1 Control – 1	2 Hours	5.50	5.50 ± 0.50	N.A.
Commercial Paint 1 Control – 2		5.00		
Commercial Paint 1 Control – 3		6.00		
Commercial Paint 1 Test – 1	2 Hours	< 1.50	< 1.50 ± 0.00	> 4.00
Commercial Paint 1 Test – 2		< 1.50		
Commercial Paint 1 Test – 3		< 1.50		

*N.A.: Not applicable.

RESULTS

Table 3. Plate Recovery Control*

Sample ID	Contact Time	Viral Titer (Log_{10}/Carrier)	Log_{10} Reduction
Glass Slide Carrier (1' x 1")	0 Hour	6.33	N.A.
Glass Slide Carrier (1" x 1")	2 Hours	6.17	0.16

*N.A.: Not applicable.

Table 4. WEST Center Film Control*

Sample ID	Contact Time	Viral Titer (Log_{10}/Carrier)	Log_{10} Reduction
WEST Center Film (1" x 1")	0 Hour	6.67	N.A.
WEST Center Film (1" x 1")	2 Hours	6.33	0.34

*N.A.: Not applicable.

Table 5. Virus Stock Titer Control

Test Virus Stock	Viral Titer (Log_{10}/Carrier)
SARS-CoV-2	8.33

Table 6. Cytotoxicity Control Data*

Sample ID	Cytotoxicity Titer (Log_{10}/Carrier)
Commercial Paint 1 Control (Non-Wear Tested)	< 0.50
Commercial Paint 1 Test (Non-Wear Tested)	1.50
Commercial Paint 1 Control (Wear Tested)	< 0.50
Commercial Paint 1 Test (Wear Tested)	1.50

*No cytotoxicity observed for non-wear tested and wear-tested Commercial Paint 1 Controls; cytotoxicity observed in the 0 dilution only for non-wear tested and wear-tested Commercial Paint 1 Test carriers.

CONCLUSIONS

Non-wear tested Commercial Paint 1 reduces SARS-CoV-2 to levels below the limit of detection (< 1.50 log_{10}) within 2 hours of exposure (> 3.94 log_{10}). Wear tested Commercial Paint 1 samples performed similarly, reducing SARS-CoV-2 to non-detectable levels (< 1.50 log_{10}) within a 2-hour contact time (>4.00). No contamination was observed during the study, and Vero E6 host cells remained viable over the 10-day incubation period as evident by the Cell Viability Control. The method of neutralization was validated as viral cytopathology was observed beyond the level of toxicity (1.50 log_{10}) for Commercial Paint 1 (non-wear tested and wear tested samples) by way of the Neutralization Effectiveness Control.

Study Scientists:

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Date: 09 November 2020

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