

# CONFIRMATORY VIRUCIDAL EFFECTIVENESS TEST

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## Study Title:

Evaluation of virucidal properties of materials against Murine-Hepatitis Virus (MHV) *in-vitro*

## Product Identity

C2+ Antimicrobial Coating Spray

## Test Microorganism

Murine-Hepatitis Virus, Strain A59

## Study Completion Date

26<sup>th</sup> March 2021

## Testing Facility

<sup>1</sup>Collaborative and Translational Unit for HFMD, Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A\*STAR), Singapore; <sup>2</sup>National University of Singapore

## Testing Personnel

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**Disclaimer:** Please do note that this is **not an endorsement** of the product from the researchers employed under National University of Singapore. The results are based on the scientific experiments being carried out in the stated research laboratory. The scientific data cannot be used for publicity materials or advertisement.

### **STUDY OBJECTIVE**

This test is designed to validate virucidal effectiveness claims for a product to be registered as a virucide.

It determines the potential of the test agent to disinfect hard, non-porous surfaces contaminated with

Murine-Hepatitis Virus (MHV).

## STUDY REPORT DETAILS:

### Important Dates:

Study Initiation Date: 1<sup>st</sup> February 2021

Experimental Start Date: 22<sup>nd</sup> March 2021

Experimental End Date: 26<sup>th</sup> March 2021

### Test Substance Information:

Name: C2+ Antimicrobial Coating Spray

Date Received: 22<sup>nd</sup> March 2021

Date Treated: 22<sup>nd</sup> March 2021

Active Ingredient(s): N.A

Form: Ready-to-Use

Storage Conditions: Room Temperature

### Test Parameters:

Virus: Murine-Hepatitis Virus, Strain: A59

Host Cell Line: H2.35 cells

Propagation Growth:

- a) DMEM (Sigma Aldrich) supplemented with 10% or 2% heat-inactivated FCS buffered with 2 g sodium hydrogen carbonate, maintained at 35 °C, 5% CO<sub>2</sub>.
- b) 1.2% Avicel supplemented with 2% heat-inactivated FCS buffered with 2 g sodium hydrogen carbonate, maintained at 35 °C, 5% CO<sub>2</sub>.

Number of Test Carriers: 3 (Dried C2+ Antimicrobial coated slides) and 3 (non-coated glass-slides)

Dry Temperature: 25 °C ± 1.0 °C

Dry Humidity: 75% ± 10% Relative Humidity Test

Contact Time (dry): 60 minutes

Test Temperature: Room Temperature

Exposure Method: Application by pipet

Assay Temperature: 37 ± 1.0 °C

Assay Period: 1 week including viral plaque assay

## EXPERIMENTAL DESIGN

### Test Method:

***Virus Treatment with dried-coated glass-slides*** – A coated glass-slide (treated) and non-coated glass slide (control) was prepared. 50µl of Murine-hepatitis virus (MHV) was added onto the glass-slide and allowed it to dry completely (time took: 50 minutes) before leaving it in room-temperature for 60 mins of incubation. Glass-slides for both treated and control were then rehydrated with 2% DMEM media and supernatants were collected for viral plaque assay. Experimental protocol was made reference to '*US EPA copper method and the prEN16777/ASTM 2197*' surface methodology.

***Viral Plaque assay*** – H2.35 cells were used in this experiment. H2.35 cells were seeded on 24-well plates separately. MHV-treated supernatants were serially diluted 10 times to  $10^6$  and 100µL of diluted supernatants were added to H2.35 cells. Plates were incubated for 1h for virus binding with 15 mins rocking intervals. Plates were then washed with 1X PBS twice and 1.2% avicel were added to each well. Plates were incubated 3 days. Lastly, 1.2% avicel was removed and crystal violet was added to stain for countable plaques. Plaques were then calculated using plaque forming units per mL.

## RESULTS

**Table 1:** Average total virus titre of control glass-slides and C2+ antimicrobial treated glass-slides

Non-treated Sample 1	Non-treated Sample 2	Non-treated Sample 3	Average
$6 \times 10^5$ PFU/ml	$9 \times 10^5$ PFU/ml	$9 \times 10^5$ PFU/ml	$8 \times 10^5$ PFU/ml
Treated Sample 1	Treated Sample 2	Treated Sample 3	Average
$8 \times 10^3$ PFU/ml	$7 \times 10^3$ PFU/ml	$4 \times 10^3$ PFU/ml	$6.33 \times 10^3$ PFU/ml

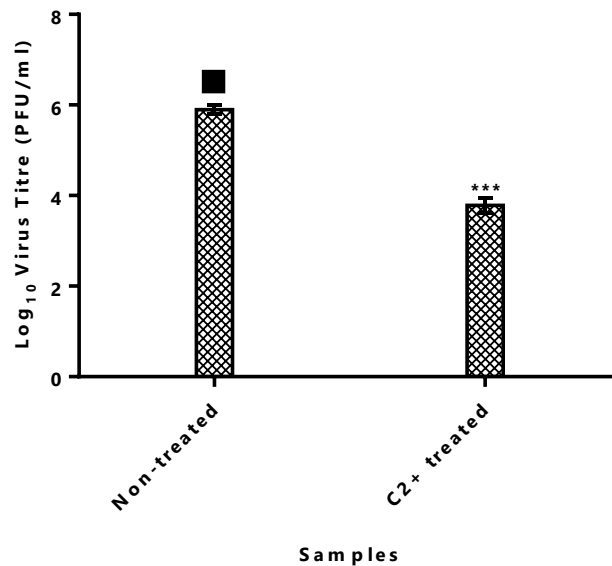
**Total log inhibition:** 2.11 PFU/ml

**Relative fold reduction:** 126.31

**Relative percentage fold reduction:** 99.21%

**Figure 1:** According to the results shown below, approximately 2.11-log inhibition was observed upon treatment comparing to the blank (control). The asterisk indicates \**p* values <0.05, \*\**p* values of <0.01 and \*\*\**p* values <0.001 by Student's *t* test using GraphPad Prism version 5.00 for Windows, GraphPad Software.

Asterisks indicate statistically significant results relative to control group (■).



### CONCLUSION:

Based on the data obtained, upon 60 minutes of contact time, 2.11-log inhibition of murine-hepatitis (MHV) was observed on upon treatment with comparing to the non-treated (control).