EVALUATION OF THE VIRUCIDAL PROPERTIES OF A TREATED NON-POROUS MATERIAL VERSUS CORONAVIRUS

Prepared for SPONSOR:
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PURPOSE OF STUDY:

The purpose of this study was to evaluate the virucidal activity of an antimicrobial-coated surface when challenged with Coronavirus.

SCOPE:

This study was designed to evaluate the virucidal property of one surface. The virucidal efficacy of the test surface was compared with that of the control surface. Ten-fold serial dilutions of the test virus were inoculated onto the test and control surfaces, and incubated at 25 °C. Following the timed exposure, the samples were retrieved from the surfaces, and inoculated onto susceptible cells. Replicates of test and control samples were evaluated. Inoculation of cells was also performed in replicates.

JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM:

The Sponsor has requested an antimicrobial surface label claim for Coronavirus. Mouse Coronavirus strain MHV-A59 was used for testing.

TEST MATERIAL:

The evaluated test and control materials were provided to the Testing Facility by the Study Sponsor, complete with appropriate documentation. Certificates of Analysis were not provided to the Testing Facility. Responsibility for the determination of the identity, strength, purity, composition, and stability of the test and control materials, as well as the retention of the test and control materials, rests with the Sponsor.

Test Material: Treated Film; Monovalent Copper.
Lot Number: March 2020.
Manufacture Date: March 2020.
Expiration Date: Not Provided.

Control Material: Untreated Film.
Lot Number: March 2020.
Manufacture Date: March 2020.
Expiration Date: Not Provided.

TEST CONDITIONS:

Exposure Time: 10 minutes.
Exposure Temperature: 25 °C.

CHALLENGE VIRAL STRAIN:

Mouse Coronavirus (Betacoronavirus), strain MHV-A59.

HOST CELL:

H2.35 (ATCC #CRL-1995; mouse liver, epithelial).
ATCC : American Type Culture Collection.

HOST CELL PREPARATION:

Cells were maintained as monolayers in disposable cell culture labware. Prior to testing, host cell cultures were seeded into multi-well cell culture plates. Cell monolayers were ~80% confluent, and less than 48 hours old before inoculation with the virus. The culture medium (CM) consisted of DMEM supplemented with fetal bovine serum.

TEST VIRUS PREPARATION:

Coronavirus propagated and stored was used for this study. On the day of use, aliquots of a stock virus suspension were removed from a -80 °C freezer, and thawed in a water bath. The stock virus was diluted to obtain the different titers of virus inoculum starting from 2×6.00 $\log_{10}$ PFU per ml.
(Reference: Chiow KH, Phoon MC, Putti T, Tan BK, Chow VT. Evaluation of antiviral activities of *Houttuynia cordata Thunb*. extract, quercetin, quercetrin and cinanserin on murine coronavirus and dengue virus infection. *Asian Pacific Journal of Tropical Medicine* 2016; 9:1-7).

TEST VIRUS IDENTIFICATION:

Virus-specific cytopathic effect or CPE (such as cell rounding and sloughing, attached and floating syncytial debris) in H2.35 cells susceptible to the virus infection.

PREPARATION OF TEST MATERIAL:

Test Materials were plastic films treated (Test) and untreated (Control) with antimicrobial substance. Test and Control Materials were provided by the Sponsor, and were cut into smaller film pieces for evaluation.

SIMULATED CONTAMINATION OF TEST AND CONTROL MATERIALS:

The virus from the laboratory's high-titer virus collection was used in this study to simulate viral contamination. Ten-fold serial dilutions of virus were made in culture medium (CM), i.e. ranging from 2×6.00 $\log_{10}$ to zero plaque-forming units (PFU) per mL.

Test and control film pieces were placed in a Petri or similar dish. A 20-microliter aliquot of each inoculum was transferred to the surface of the test and control film pieces. The exposure time commenced following film application.

TEST PROCEDURE:

Test Samples. The test film pieces were inoculated with each virus dilution, and subjected to 10-minute exposure time at 25 °C. After the exposure time elapsed, each virus dilution sample was completely pipetted from the surface, and was then plated onto host cells in replicates.

Control Samples. The control film pieces were inoculated with each virus dilution, and subjected to 10-minute exposure time at 25 °C. After the exposure time elapsed, each virus dilution sample was completely pipetted from the surfaces, and was then plated onto host cells in replicates.

Initial Virus Population. The test virus was diluted in CM, and dilutions were plated in replicates.

Cell Culture Control. Intact cell culture monolayers served as the control of cell culture viability.

The plates were incubated for 3 to 4 days at 37 °C in a CO_2 incubator.

Evaluation of Virus Recovery. Cytopathic effect (CPE) or cytotoxic effect was monitored using an inverted compound microscope. Mouse coronavirus causes cytopathic effect in H2.35 cells such as cell rounding and sloughing, attached and floating syncytial debris.

CALCULATIONS:

Viral titers were expressed as $-\log_{10}$ of the 50% titration end-point for infectivity. The viral titer was calculated based on the unit of 50% tissue culture infectious dose ($TCID_{50}$).
Virus titers were converted from PFU/mL to $TCID_{50}$/mL using the equation described in www.atcc.org (PFU/mL = $TCID_{50}$/mL ÷ 0.7).
The average of virus $TCID_{50}$ recoveries for test and control replicates, and virus reductions were calculated and presented. No control of bias was performed.

The reduction of virus population (antiviral activity) was calculated as follows:
The $\log_{10}$ reduction was applied to express the relative number of viable or infectious virus particles that are eliminated by disinfection. For example, a 1 $\log_{10}$ reduction corresponds to inactivating 90 percent of the target virus, with the virus count being reduced by a factor of 10.

TEST ACCEPTANCE CRITERIA:

The test was considered to be valid based on the following factors: (a) 6.00 $\log_{10}$ of virus per mL was recovered from Initial Population; (b) at least 3.0 $\log_{10}$ of virus per ml recovered from Control film pieces; (c) cells in the Cell Control wells were viable and attached to the bottom of the well; (d) the culture medium was free of “non-viral” contamination in all wells of the plate.

LIABILITY AND INDEMNIFICATION:

The Testing Facility's liability to the Study Sponsor under this Protocol shall be limited to the price of this evaluation. The Study Sponsor shall be responsible to Study Participants (when applicable) and to other third parties for the fitness and use of the product.

FINAL RESULTS:

Below is the overall summary of the testing results.

The testing was considered to be valid, based on fulfilment of the test acceptance criteria outlined above.

The treated test film could inhibit coronavirus at or below a viral concentration of 285.7 $TCID_{50}$ per mL (or equivalent to 200 PFU per mL), as shown by absence of CPE.

The untreated control test film could inhibit coronavirus at or below a viral concentration of 28.57 $TCID_{50}$ per mL (or equivalent to 20 PFU per mL), as shown by absence of CPE.

Hence, there was a 1 $\log_{10}$ reduction in the relative number of viable or infectious coronavirus particles inhibited by the test film compared with the control film. This reduction corresponds to the test film inactivating 90% of coronavirus, with the coronavirus titer being reduced by a factor of 10.
