STUDY REPORT

Study Title
Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces Modified for a Custom Device

Product Identity
The Air Reactor

Test Microorganism
Human Coronavirus, Strain 229E, ATCC VR-740

Study Identification Number
NG14823

Author
Victoria Zarate, B.S.

Study Completion Date
24MAR2020

Testing Facility
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, Texas 78681

Study Sponsor
Hi Tech Air Solutions
5400 SE 134th ST
Oklahoma City, OK 73165

STUDY REPORT SUMMARY

General Study Information

Study Title: Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces Modified for a Custom Device

Study Identification Number: NG14823

Test System

Test Microorganism: Human Coronavirus, Strain 229E, ATCC VR-740

Host Cell: MRC-5, ATCC CCL-171

Test Substance: The Air Reactor

Test Substance Receipt Date: 10MAR2020

Test Parameters

Test Substance: Ready to Custom Device

Organic Soil Load: No supplementation of an organic soil load was incorporated into inoculum

Number of Replicates Per Lot: 1

Contact Time: 1 hour contact time

Test Distances Assayed: 10 ft from the device and 1 ft from the device

Exposure Temperature: Ambient room temperature
(22.8-24.3°C and 63-64% Relative Humidity (RH))

Neutralization Method: Dilution Method using 2% FBS EMEM Media

Study Dates

Experimental Start Date/Time: 10MAR2020 / 0957
Experimental Termination Date/Time: 17MAR2020 / 1143
Study Completion Date: 24MAR2020

TEST PROCEDURE

Summary

- Stock virus was thawed and was not supplemented with an organic soil load.
- Sterile glass Petri dish carriers (100 x 15 mm) were inoculated with a volume of virus suspension. A sufficient number of test and control carriers were prepared.
- Inoculated carriers used in for testing were not dried and were placed in contact with the test device.
- One inoculated carrier was dried at room temperature under laminar flow conditions, but was not used for comparison for log reduction for this study.
- The test substance was prepared according to the Study Sponsor's instructions as requested.
- The treated carriers were held for the predetermined contact time(s), and then neutralized in a manner appropriate for the test substance (e.g. dilution and/or gel filtration).
- The control carrier was harvested using an equivalent volume cell culture medium or other suitable buffer.
- Following neutralization of test and control carriers, the viral suspensions were quantified to determine the levels of infectious virus using standard cell culture (e.g. $TCID_{50}$) or plaque assay techniques.
- Assay trays/plates were incubated for the period most suitable for the virus-host cell system (e.g. 7 days).
- After the incubation period, the assay was scored for the presence/absence of test virus and cytotoxic effects. The appropriate calculations were performed (e.g. Spearman-Karber) to determine viral titers and levels of test substance cytotoxicity, where applicable.
- Log_{10} and percent reductions were computed for viral films exposed to the test product relative to the titer obtained for the study control carrier(s), and reported to the Study Sponsor.

Study Notes

Per the Study Sponsor's request, two distances from the device were evaluated by measuring 1 ft away from the device and 10 ft from the device. Two plate recovery controls were inoculated with 0.200 ml of test virus, along with two test carriers. The carriers remained wet and were not dried. The plate recovery control carriers were held open for the duration of the contact time in a Biological Safety Cabinet, while the test carriers were placed in a lifted orientation (to ensure direct contact with the device) in front of the device at measured points. Following the contact time for both the plate recovery controls and treated test carriers, 2.0 ml of test media was added to the carriers to recover any remaining virus.

SUCCESS CRITERIA

The following measures are met to ensure the acceptability of virucidal efficacy data:

- A minimum of 4.80 $\log_{10}$ infective units/control carrier is recovered from each plate recovery control film(s).
- The virus titer control demonstrate obvious and or typical cytopathic effects on the monolayers unless a detection method other than cytopathic effect is used.
- Neutralization of the test substance with a low titer (e.g. 1000-5000 infective units) of the test virus is demonstrated.
- Quantification of the test and control parameters are conducted at a minimum of four determinations per dilution.

The product performance criteria follows:

- In the presence or absence of cytotoxicity, the product should demonstrate a $\geq$3.00 $\log_{10}$ reduction in viral titer on each surface.
- If cytotoxicity is present, the virus control titer should be increased if necessary to demonstrate a $\geq$3.00 $\log_{10}$ reduction in viral titer on each surface beyond the cytotoxicity level.

CALCULATIONS AND STATISTICAL ANALYSIS

The $TCID_{50}$ (Tissue Culture Infectivity Dose) represents the endpoint dilution where 50% of the cell cultures exhibit cytopathic effects due to infection by the test virus. The endpoint dilution at which 50% of the host cell monolayers exhibit cytotoxicity is termed the Tissue Culture Dose (TCD_{50}). The $TCID_{50}$, and TCD_{50} was determined using the Spearman-Kärber method and calculated as follows:

Negative logarithm of endpoint titer =

[– Log of first dilution inoculated] – [((sum of % mortality at each dilution/100) – 0.5) x Logarithm of dilution]

The result of this calculation is expressed as $TCID_{50}$/0.1 ml (or volume of dilution inoculated) for the test and plate recovery control. Determination of viral titer per carrier is established by accounting for the volume of viral inoculum per carrier.

To calculate $TCID_{50}$/carrier the following equation is used:

The antilog of the $TCID_{50}$/volume inoculated x (volume of inoculum on carrier/volume of dilution inoculated)

The log_{10} of this result is performed to achieve the $TCID_{50}$/carrier

Calculation of the Log Reduction

The log reduction in viral titer was calculated as follows:

Plate Recovery Control Log_{10} $TCID_{50}$ – Virus-Test Substance Log_{10} $TCID_{50}$

Calculation of the Percent Reduction

The percent reduction in viral titer was calculated as follows:

Percent Reduction = 1- (C/B) x 100, where:
B = Average $TCID_{50}$ of virus in control suspensions.
C = Average $TCID_{50}$ of virus in virus-test suspensions.

The presence of any test substance cytotoxicity was taken into account when calculating the log reduction in viral titer.

If multiple plate recovery control and test replicates were performed, the average $TCID_{50}$ of each parameter was calculated and the average result used to calculate the log reduction in viral titer.

RESULTS

Table 1: Virus Plate Recovery Control, Test Results, and Virus Titer

Dilution	Virus Plate Recovery Control	Test Result for 1 ft away from device	Test Result for 10 ft away from device	Virus Titer
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	N/A	N/A	N/A	N/A
10^{-2}	+ + + +	+ + + +	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +	+ + + +	+ + + +
10^{-4}	+ + + +	+ 0 + +	0 + + +	+ + + +
10^{-5}	+ 0 + +	0 0 0 0	0 + 0 0	+ + + 0
10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml	4.25 Log_{10}	3.25 Log_{10}	3.50 Log_{10}	4.25 Log_{10}
$TCID_{50}$ per Carrier	4.55 Log_{10}	3.55 Log_{10}	3.80 Log_{10}	N/A
Log Reduction	N/A	1.00 Log_{10}	0.75 Log_{10}	N/A
Percent Reduction	N/A	90.0%	82.21%	N/A

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed;
T = Cytotoxicity observed

Table 2: Cytotoxicity Control Results

Dilution	Cytotoxicity Control
Cell Control	0 0 0 0
10^{-1}	0 0 0 0
10^{-2}	0 0 0 0
10^{-3}	0 0 0 0
TCD_{50} per 0.1 ml	≤0.50 Log_{10}

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed;
T = Cytotoxicity observed

Table 3: Test Substance Neutralization Control Results

Dilution	Neutralization Control
Cell Control	0 0 0 0
10^{-1}	+ + + +
10^{-2}	+ + + +
10^{-3}	+ + + +
Neutralized at $TCID_{50}$ per 0.1 ml	≤ 0.50 Log_{10}

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed;
T = Cytotoxicity observed

STUDY CONCLUSION

The purpose of the study was to determine the virucidal efficacy of The Air Reactor Device against Human Coronavirus, Strain 229E, ATCC VR-740 at a distance of 1 ft and 10 ft and a contact time of 1 hr, with an exposure temperature of room temperature (22.8-24.3°C and 63-64% RH).

The Virus Plate Recovery Control demonstrated a viral titer of 4.00 $\log_{10}$ $TCID_{50}$ per 0.1 ml and a 4.55 $\log_{10}$ $TCID_{50}$ per carrier.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test device demonstrated a 1.00 Log_{10} reduction in viral titer (90.0%) at the 1 ft distance assayed and 0.75 Log_{10} reduction in viral titer (82.21%) at the 10 ft distance assayed.

No test device cytotoxicity was detected in the shortest distance assayed ($\leq$0.50 Log_{10}).

The test device Neutralization Control demonstrated that the test device was neutralized at $\leq$0.50 Log_{10} for the shortest distance assayed.

The test substance will be disposed of 30 days after the completion of this study, unless otherwise requested by the Study Sponsor.

The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.