Study Report

Study Title

Virucidal Efficacy of a Test Substance For Use on Inanimate, Nonporous Surfaces

Test Microorganism

Human Coronavirus, Strain 229E, ATCC VR-740

Study Identification Number

NG15291

Author

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Testing Facility

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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 were dried at room temperature under laminar flow conditions.
• The test device was prepared according to the Study Sponsor's instructions as requested, and applied to the test carriers.
• The control carrier was held covered for the contact time then harvested in the same manner as the test.
• The viral suspensions were quantified to determine the levels of infectious virus using standard cell culture (e.g. TCID50) 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. The appropriate calculations were performed (e.g. Spearman-Karber) to determine viral titers.
• Log10 and percent reductions were calculated 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.

Success Criteria

The following measures are met to ensure the acceptability of virucidal efficacy data:
• A minimum of 4.80 log10 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.
• 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 ≥3.00 log10 reduction in viral titer on each surface.
• If cytotoxicity is present, the virus control titer should be increased if necessary to demonstrate a ≥3.00 log10 reduction in viral titer on each surface beyond the cytotoxicity level.

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Calculations and Statistical Analysis

The TCID50 (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 (TCD50). The TCID50, and TCD50 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 TCID50/0.1 ml (or volume of dilution inoculated) for the test, virus control, and neutralization control and TCD50/0.1 ml (or volume of dilution inoculated) for the cytotoxicity control.

Calculation of the Log Reduction

The log reduction in viral titer was calculated as follows:
Plate Recovery Control Log10 TCID50 – Virus-Test Substance Log10 TCID50

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 TCID50 of virus in control suspensions.
C = Average TCID50 of virus in virus-test suspensions.
The presence of any test substance cytotoxicity were taken into account when calculating the log and percent reductions in viral titer.
If multiple virus control and test replicates were performed, the average TCID50 of each parameter was calculated and the average result used to calculate the log reductions in viral titer.

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Results

Table 1: Virus Titer and Virus Plate Recovery Control Results

		Virus Titer	**Virus Plate Recovery Control** Time Zero	**Virus Plate Recovery Control** 30 Minutes	**Virus Plate Recovery Control** 60 Minutes	**Virus Plate Recovery Control** 120 Minutes
Dilution	Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-1}	++++	++++	++++	++++	++++
	10^{-2}	++++	++++	++++	++++	++++
	10^{-3}	++++	++++	++++	++++	++ 0 +
	10^{-4}	++++	+++ 0	0 0 0 +	0 0 0 0	0 0 0 0
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-6}	0 0 0 0	N/A	N/A	N/A	N/A
TCID 50 Per 0.1 ml		4.50	4.25	3.75 Log $_{10}$	3.50 Log $_{10}$	3.25 Log $_{10}$
TCID 50 Per Carrier		4.80	4.55	4.05 Log $_{10}$	3.80 Log $_{10}$	3.55 Log $_{10}$

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

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Results

Table 2: Test Results at 30 Minutes

		Test Results Replicate 1 30 Minutes	**Test Results Replicate 2** 30 Minutes	**Test Results Replicate 3** 30 Minutes
Dilution	Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-1}	0 0 0 +	0 0 0 +	0 0 0 0
	10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
TCID 50 per 0.1 ml		0.75 Log $_{10}$	0.75 Log $_{10}$	$\leq$ 0.50 Log $_{10}$
TCID 50 per Carrier		1.05 Log $_{10}$	1.05 Log $_{10}$	$\leq$ 0.50 Log $_{10}$
Average Log 10 Reduction		2.78 Log $_{10}$		
Average Per Reduction		99.92%		

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

Table 3: Test Results at 60 minutes

		Test Results Replicate 1 60 Minutes	**Test Results Replicate 2** 60 Minutes	**Test Results Replicate 3** 60 Minutes
Dilution	Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-1}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
TCID 50 per 0.1 ml		$\leq$ 0.50 Log $_{10}$	$\leq$ 0.50 Log $_{10}$	$\leq$ 0.50 Log $_{10}$
TCID 50 per Carrier		$\leq$ 0.80 Log $_{10}$	$\leq$ 0.80 Log $_{10}$	$\leq$ 0.80 Log $_{10}$
Average Log 10 Reduction		2.70 Log $_{10}$		
Average Per Reduction		99.90%		

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

Results

Table 4: Test Results at 120 minutes

		Test Results Replicate 1 120 Minutes	Test Results Replicate 2 120 Minutes	Test Results Replicate 3 120Minutes
Dilution	Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-1}	0 0 0 0	0 0 0 0	0 0 0 +
	10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
TCID 50 per 0.1 ml		≤ 0.50 Log $_{10}$	≤ 0.50 Log $_{10}$	≤ 0.50 Log $_{10}$
TCID 50 per Carrier		≤ 0.80 Log $_{10}$	≤ 0.80 Log $_{10}$	≤ 0.80 Log $_{10}$
Average Log 10 Reduction		2.37 Log $_{10}$		
Average Per Reduction		99.79%		

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 AtmosAir Matterhorn Series device against Human Coronavirus Strain 229E, with no additional soil load incorporated into inoculum, at contact times of 30 minutes, 60 minutes, and 120 minutes, and at an exposure temperature of 25.2 – 25.6°C, 46 – 47% RH.
At 30 minutes, the Plate Recovery Control demonstrated a viral titer of 3.75 Log10 TCID50 per 0.1 ml and 4.05 Log10 TCID50 per carrier. The evaluated test device, AtmosAir Matterhorn Series, demonstrated an average 2.78 Log10 reduction (99.92%) in viral titer.
At 60 minutes, the Plate Recovery Control demonstrated a viral titer of 3.50 Log10 TCID50 per 0.1 ml and 3.80 Log10 TCID50 per carrier. The evaluated test device, AtmosAir Matterhorn Series, demonstrated an average 2.70 Log10 reduction (99.90%) in viral titer.
At 120 minutes, the Plate Recovery Control demonstrated a viral titer of 3.25 Log10 TCID50 per 0.1 ml and 3.55 Log10 TCID50 per carrier. The evaluated test device, AtmosAir Matterhorn Series, demonstrated an average 2.37 Log10 reduction (99.79%) in viral titer.

Note:
As an enveloped virus, Human Coronavirus 229E is susceptible to inactivation during periods of prolonged drying. Drying times past 1 hour can result in decreased viral recovery due to natural inactviation.