



STUDY REPORT

Study Title

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

Product Identity

AtmosAir Matterhorn Series

Test Microorganism

Human Coronavirus, Strain 229E, ATCC VR-740

Study Identification Number

NG15291

Author

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Study Completion Date

04JUN2020

Testing Facility

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

Study Sponsor

AtmosAir Solutions
Tony Abate
418 Meadow Street, Suite 204
Fairfield, CT 06824



STUDY REPORT SUMMARY

General Study Information

Study Title: ASTM E1053 Method (Modified)
Virucidal Efficacy of a Test Substance For Use on
Inanimate, Nonporous Surfaces

Study Identification Number: NG15291

Test System

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

Host Cell(s): MRC-5, CCL-171

Test Substance: AtmosAir Matterhorn Series

Test Substance Receipt Date: 09APR2020

Test Parameters

Test Substance Dilution: Ready to use

Test Substance Application: Fogging (The Matterhorn device for this study was calibrated to an ion saturation of 1,500 ions per cm³)

Organic Soil Load: No additional soil load incorporated into inoculum

Number of Replicates Per Contact Time: 3

Contact Time(s): 30 minutes, 60 minutes, and 120 minutes

Exposure Temperature: Ambient room temperature
(25.2 – 25.6°C, 46 – 47% Relative Humidity (RH))

Neutralization Method(s): N/A

Study Dates

Experimental Start Date/Time: 21MAY2020 / 1615

Experimental Termination Date/Time: 29MAY2020 / 0938

Study Completion Date: 04JUN2020



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-Kärber) 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.



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 =

$[- \text{Log of first dilution inoculated}] - [(\text{sum of \% mortality at each dilution}/100) - 0.5] \times \text{Logarithm of dilution}$

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 = $1 - (C/B) \times 100$, 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.



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
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10 ⁻¹	+ + + +	+ + + +	+ + + +	+ + + +
	10 ⁻²	+ + + +	+ + + +	+ + + +	+ + + +
	10 ⁻³	+ + + +	+ + + +	+ + + +	+ + 0 +
	10 ⁻⁴	+ + + +	+ + + 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
	10 ⁻⁶	0 0 0 0	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

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
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10 ⁻¹	0 0 0 +	0 0 0 +
	10 ⁻²	0 0 0 0	0 0 0 0
	10 ⁻³	0 0 0 0	0 0 0 0
	10 ⁻⁴	0 0 0 0	0 0 0 0
	10 ⁻⁵	0 0 0 0	0 0 0 0
TCID ₅₀ per 0.1 ml	0.75 Log ₁₀	0.75 Log ₁₀	≤0.50 Log ₁₀
TCID ₅₀ per Carrier	1.05 Log ₁₀	1.05 Log ₁₀	≤0.80 Log ₁₀
Average Log ₁₀ Reduction	2.78 Log ₁₀		
Average Percent Reduction	99.92%		

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed;
T = Cytotoxicity observed; [†]Taking cytotoxicity and neutralization controls into account.



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
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	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		≤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		2.70 Log ₁₀		
Average Percent Reduction		99.90%		

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed;
 T = Cytotoxicity observed; † Taking cytotoxicity and neutralization controls into account.

Table 4: Test Results at 120 minutes

		Test Results Replicate 1 120 minutes	Test Results Replicate 2 120 minutes	Test Results Replicate 3 120 minutes
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	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	0.75 Log ₁₀
TCID ₅₀ per Carrier		≤0.80 Log ₁₀	≤0.80 Log ₁₀	1.05 Log ₁₀
Average Log ₁₀ Reduction		2.37 Log ₁₀		
Average Percent Reduction		99.79%		

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed;
 T = Cytotoxicity observed; † Taking cytotoxicity and neutralization controls into account.



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 inactivation.

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.

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