
MICROCHEM
LABORATORY

Study report

Study Title

Non-GLP Custom Virucidal Efficacy of a Device

Product Identity

Neat™ Steam Cleaner

Test Microorganism

Human coronavirus, 229E strain, ATCC VR-740

Study Identification Number

NG18600

Author

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Study Completion Date

13DEC2021

Report Amended

17DEC2021

Testing Facility

Microchem Laboratory
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Study Sponsor

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Study report summary

General Study Information

Study Title:	Non-GLP Custom Virucidal Efficacy of a Device
Study Identification Number:	NG18600

Test System

Test Microorganism:	Human coronavirus, 229E strain, ATCC VR-740
Host Cell:	MRC-5 cells (ATCC CCL-171)

Test Device:	Neat™ Steam Cleaner
Test Device Receipt Date:	17SEP2021

Test Parameters

Test Device Preparation:	• Prior to testing, the device was filled with 1 L sterile deionize (DI) water. • After the device was filled with DI water, it was preheated until the power button on the machine turned green, indicating it was ready for use. • The steam was released from the hose for 15 seconds. • After installing the triangular accessory plus bonnet, the steam was released for 5 seconds until the bonnet was warmed up. • The side of the bonnet to be in contact with the test virus was UV sterilized for ≥15 minutes prior to testing.
Test Device Application:	Direct contact with Virus
Total Organic Soil Load:	No additional supplementation of organic soil load incorporated into the test inoculum*
Number of Replicates Per Lot:	Single replicate
Contact Time:	30 seconds
Exposure Temperature:	Room temperature (20.1 °C) and 56.7–57.6% Relative Humidity (RH)
Neutralization Method:	Not applicable

Study Dates

Experimental Start Date/Time:	01DEC2021 / 1127
Experimental Termination Date/Time:	08DEC2021/ 1128
Study Completion Date:	13DEC2021

*Note: Virus was propagated with 2% FBS and was used in testing as propagated.

Summary of the test procedure

- Stock virus thawed and was not supplemented with an organic soil load.
- A sterile glass glass slide (1" x 3") was used as the test carrier. For the device assayed, one carrier was inoculated with a 0.100 ml volume of virus suspension. One plate recovery control carrier was also prepared.
- The inoculated carriers were dried at the appropriate temperature and relative humidity to lessen the level of virus inactivation due to drying.
- The test device was prepared according to the Study Sponsor's instructions as requested and applied to the test carriers.
- The treated carrier was held for the Study Sponsor specified contact time at the Study Sponsor specified exposure temperature.
- The plate recovery 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 techniques (e.g. $TCID_{50}$).
- The inoculated cell culture plates were incubated for the period most suitable for the virus-host cell system (e.g. 7 days).
- Following the incubation period, the assay was microscopically scored for the presence/absence of test virus. The appropriate calculations were performed (e.g. Spearman-Kärber) to determine viral titers.
- The log_{10} and percent reductions in viral titer were calculated for viral films exposed tothe test product relative to the titer obtained for the study control carrier(s).

Success criteria

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

- A minimum of 4.00 log_{10} to infective units/control carrier is recovered from each plate recovery control film(s).
- The virus titer control demonstrates obvious and/or typical cytopathic effects on the monolayers unless a detection method other than cytopathic effect is used.
- Comparable levels of infective units must be recovered from the neutralized test substance and neutralization control substance.
- Quantification of the test and control parameters are conducted at a minimum of four determinations per dilution.

Note: The log and percent reduction of the test virus following exposure to the test device are calculated however, there is no minimum reduction level to qualify as "passing" or an "efficacious" product.

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} were 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)

The result of this calculation is expressed as $TCID_{50}$/0.1 ml for the test, virus control

Calculation of the Log_{10} Reduction

The log_{10} 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 and percent
reductions in viral titer.

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

Statistical Analysis

Not applicable.

Methods for the Control of Bias

Not applicable.

Virus titer, plate recovery control, and test results

		Virus Titer	Recovery Control	Neat™ Steam Cleaner
Cell Control		0 0 0 0	0 0 0 0	N/A
Dilution	10^{-1}	+ + + +	+ + + +	0 0 0 0
	10^{-2}	+ + + +	+ + + +	0 0 0 0
	10^{-3}	+ + + +	+ + + +	0 0 0 0
	10^{-4}	+ + + +	+ + + +	0 0 0 0
	10^{-5}	+ + 0 +	+ 0 + 0	0 0 0 0
	10^{-6}	0 0 0 0	+ 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		≤ 0.50 log_{10}	5.25 log_{10}	≤ 0.50 log_{10}
Log_{10} Reduction		N/A	N/A	≥ 4.75 log_{10}
Percent Reduction				≥ 99.998 %

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

Study conclusion

The purpose of the study was to determine the virucidal efficacy of Neat™ Steam Cleaner against Human coronavirus, 229E strain, ATCC VR-740, with no additional supplementation of organic soil load incorporated into the test inoculum, at a contact time of 30 seconds and exposure temperature of room temperature (20.1 °C and 56.7–57.6% RH).

The Virus Control demonstrated a viral titer of 5.25 $\log_{10}$ $TCID_{50}$ per 0.1 ml.

The evaluated test substance, Neat™ Steam Cleaner, demonstrated a ≥ 4.75 $\log_{10}$ reduction (≥ 99.998 %) in viral titer.

The test substance(s) 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 substance(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.