STUDY REPORT

Study Title
Non-GLP ASTM E1053: Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Non-porous Environmental Surfaces

Product Identity
AGent+ Hard Surface Cleaner & Protectant (Lot: HSLS-006)

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

Study Identification Number
NG15946

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Study Completion Date
08SEP2020

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Microchem Laboratory
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STUDY REPORT SUMMARY

General Study Information

Study Title:	ASTM E1053: Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Non-porous Environmental Surface
Study Identification Number:	NG15946

Test System

Test Microorganism:	Human coronavirus, Strain 229E, ATCC VR-740
Host Cell:	MRC-5 (ATCC CCL-171)
Test Substance:	AGent+ Hard Surface Cleaner & Protectant
Lot Number(s):	HSLS-006
Test Substance Receipt Date:	10JUL2020

Test Parameters

Test Substance Dilution:	Ready to use liquid test substance
Test Substance Application:	Six sprays/ distance 4 – 6 inches
Organic Soil Load:	No additional supplementation of organic soil load incorporated into the test inoculum, virus used as propagated
Number of Replicates Per Lot:	Single replicate per lot and contact time
Contact Time(s):	3 minutes and 10 minutes (HSLS-006) 10 minutes (Cytotoxicity/Neutralization Controls)
Exposure Temperature:	Ambient room temperature (24.2 – 24.4°C) and 41% Relative Humidity (RH)
Neutralization Method:	Sephadex LH-20 gel filtration column

Study Dates

Experimental Start Date/Time:	11AUG2020 / 1510
Experimental Termination Date/Time:	25AUG2020 / 1537
Study Completion Date:	08SEP2020

SUMMARY OF THE TEST PROCEDURE

- The stock virus was thawed and was not supplemented with an organic soil load.
- Sterile glass Petri dishes (100 x 15 mm) were used as the test carrier. For each lot of substance and contact time assayed, one carrier was inoculated with a 0.200 ml volume of virus suspension. The appropriate number of plate recovery control carriers were 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 substance was prepared according to the Study Sponsor's instructions as requested, and applied to the test carriers using a spray device or pipette. For spray products, the distance, angle, and number of sprays applied were performed as requested by the Study Sponsor. For pipette delivery products, ta 2.0 ml volume was applied per carrier.
- The treated carriers were held for the Study Sponsor specified contact time(s) at the Study Sponsor specified exposure temperature, and then neutralized in a manner appropriate for the test substance (e.g. dilution and/or gel filtration).
- The plate recovery control carrier was held covered for the contact time then harvested and neutralized in the same manner as the test.
- Following neutralization of test and control carriers, 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 and cytotoxic effects. The appropriate calculations were performed (e.g. Spearman-Karber) to determine viral titers and levels of test substance cytotoxicity, where applicable.
- The log_{10} and percent reductions in viral titer were calculated for viral films exposed to the test product relative to the titer obtained for the study control carrier(s).

Study Notes

Trigger nozzles were UV sterilized for 15 minutes prior to assembling bottles.

SUCCESS CRITERIA

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

- A minimum of 4.80 $\log_{10}$ to 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.
- Comparable levels of infective units must be recoverd 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: Although the test method does not specify a product performance criteria, for registration as a hard surface disinfectant, the U.S. EPA requires a ≥ 3 $\log_{10}$ reduction in viral titer as compared to the corresponding plate recovery control.

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, virus control, and neutralization control and TCD_{50}/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 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 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 $TCID_{50}$ 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 (10 minutes)
Cell Control		0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	+ + + +
	10^{-2}	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +
	10^{-4}	+ + + +	+ + + +
	10^{-5}	+ + + +	+ + + +
	10^{-6}	0 0 0 +	+ 0 + 0
	10^{-7}	0 0 0 0	0 0 0 0
	10^{-8}	0 0 0 0	N/A
$TCID_{50}$ per 0.1 ml		5.75 Log_{10}	6.00 Log_{10}
$TCID_{50}$ per Carrier		Not applicable	6.30 Log_{10}

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

Table 2: Test Results for AGent+ Hard Surface Cleaner & Protectant (Lot: HSLS-006)

		Test Results (3 minutes)	Test Results (10 minutes)
Cell Control		0 0 0 0	0 0 0 0
Dilution	10^{-1}	T T T T	T T T T
	10^{-2}	T T T T	0 T 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
	10^{-6}	0 0 0 0	0 0 0 0
	10^{-7}	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		≤2.50 Log_{10}	≤1.75 Log_{10}
$TCID_{50}$ per Carrier		≤2.80 Log_{10}	≤2.05 Log_{10}
Log_{10} Reduction		≥3.50 Log_{10}	≥4.25 Log_{10}
Percent Reduction		≥99.97%	≥99.994%

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

RESULTS (cont.)

Table 3: Cytotoxicity Control Results

		Cytotoxicity Control Lot: HSLS-006
Cell Control		0 0 0 0
Dilution	10^{-1}	T T T T
	10^{-2}	T T T T
	10^{-3}	0 0 0 0
TCD_{50} per 0.1 ml		2.50 Log_{10}

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

Table 4: Neutralization Control Results

		Neutralization Control Lot: HSLS-006
Cell Control		0 0 0 0
Dilution	10^{-1}	T T T T
	10^{-2}	T T T T
	10^{-3}	+ + + +
$TCID_{50}$ per 0.1 ml		2.50 Log_{10}

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 AGent+ Hard Surface Cleaner & Protectant (Lot: HSLS-006) against Human coronavirus Strain 229E, with no additional supplementation of organic soil load incorporated into the test inoculum, virus used as propagated, at contact times of 3 minutes and 10 minutes (HSLS-006), and at an exposure temperature of 24.2 – 24.4°C and 41% RH.

The Plate Recovery Control demonstrated a viral titer of 6.00 Log_{10} $TCID_{50}$per 0.1 ml and 6.30 Log_{10} $TCID_{50}$ per carrier.

Taking the cytotoxicity and neutralization control results into consideration:

- The evaluated test substance AGent+ Hard Surface Cleaner & Protectant (Lot: HSLS-006) demonstrated a ≥3.50 Log_{10} reduction (≥99.97%) in viral titer at 3 minutes and a ≥4.25 Log_{10} reduction (≥99.994%) in viral titer at 10 minutes.

Test substance cytotoxic effects to the host monolayer were observed at 2.50 Log_{10} TCD_{50} per 0.1 ml for test substances assayed.

The Test Substance Neutralization Control demonstrated that the test substance was neutralized at 2.50 Log_{10} for the all lots 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.