STUDY REPORT

Study Title
ASTM E1052
Standard Test Method to Assess the Activity of Microbicides Against Viruses in Suspension

Product Identity
NCCO IG

Lot Number:
MFO-14179

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

Study Identification Number
NG16164-1

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Study Completion Date
07OCT2020

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STUDY REPORT SUMMARY

General Study Information

Study Title:	ASTM E1052 Standard Test Method to Assess the Activity of Microbicides Against Viruses in Suspension
Study Identification Number:	NG16164-1

Test System

Test Microorganism:	Human coronavirus, Strain 229E, ATCC VR-740
Host Cell:	MRC-5 (ATCC CCL-171)
Test Substance:	NCCO IG, Lot Number: MFO-14179
Test Substance Receipt Date:	05AUG2020

Test Parameters

Test Substance Dilution:	Ready to use liquid test substance
Total Organic Soil Load:	No supplementation of organic soil load incorporated into the test inoculum
Number of Replicates Per Lot:	Triple
Contact Time:	1 minute
Exposure Temperature:	Room temperature (20.8 – 21.0°C) and 45 – 46% Relative Humidity (RH)
Neutralization Method:	Dilution method using fetal bovine serum (FBS)

Study Dates

Experimental Start Date/Time:	19SEP2020 / 1413
Experimental Termination Date/Time:	26SEP2020 / 1148
Study Completion Date:	07OCT2020

TEST PROCEDURE

Summary

- Stock virus was thawed and was not supplemented with an organic soil load.
- Test and virus control substances were dispensed in 9-part equivalent volumes into sterile vessels.
- Test and virus control substances were each inoculated with 1-part equivalent volumes of the test virus.
- The test suspensions were held for the contact time(s) specified by the Study Sponsor, and then neutralized by ten-fold serial dilutions into the appropriate solution.
- The virus control suspension was neutralized in the same manner as the test suspensions.
- Following neutralization, the viral suspensions were quantified to determine the levels of infectious virus using standard cell culture (e.g. $TCID_{50}$) assay techniques.
- The cell culture 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 test suspensions relative to the control suspensions, and reported to the Study Sponsor.
- Unless otherwise noted, no modifications to the method were made for this study.

SUCCESS CRITERIA

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

- 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:

- The log and percent reduction of the test virus following exposure to the test substance 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} 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 Controls

		Virus Titer	Virus Control Replicate #1	Virus Control Replicate #2	Virus Control Replicate #3
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	N/A	N/A	N/A	N/A
	10^{-2}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-4}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-5}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-6}	+ + + +	+ 0 0 0	+ 0 + +	+ 0 0 +
	10^{-7}	0 0 0 +	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		6.75 Log_{10}	5.75 Log_{10}	6.25 Log_{10}	6.00 Log_{10}
Average $TCID_{50}$ per 0.1 ml		N/A	6.05 Log_{10}		

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

RESULTS (cont.)

Table 2: Test Results for NCCO IG — 1 minute

		Test Results Replicate #1	Test Results Replicate #2	Test Results Replicate #3
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	N/A	N/A	N/A
	10^{-2}	T T T T	T T T T	T T T T
	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
	10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		≤2.50 Log_{10}	≤2.50 Log_{10}	≤2.50 Log_{10}
Average $TCID_{50}$ per 0.1 ml		≤2.50 Log_{10}		
Average Log Reduction		≥3.55 Log_{10}		
Average Percent Reduction		≥99.97%		

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

Table 3: Cytotoxicity Control Results

		Cytotoxicity Control
Cell Control		0 0 0 0
Dilution	10^{-1}	N/A
	10^{-2}	T T T T
	10^{-3}	0 0 0 0
	10^{-4}	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; N/A = not applicable

RESULTS (cont.)

Table 4: Test Substance Neutralization Control Results

		Neutralization Control
Cell Control		0 0 0 0
Dilution	10^{-1}	N/A
	10^{-2}	T T T T
	10^{-3}	+ + + +
	10^{-4}	+ + + +
Neutralized at $TCID_{50}$ 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 NCCO IG (Lot: MFO-14179) against Human coronavirus Strain 229E, with no supplementation of organic soil load incorporated into the test inoculum, at contact times of 1 minute, at room temperature (20.8 – 21.0°C and 45 – 46% RH).

The Virus Control demonstrated an average viral titer of 6.05 Log_{10} $TCID_{50}$ per 0.1 ml.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test substance, NCCO IG, demonstrated a ≥3.55 Log_{10} reduction (≥99.97%) in viral titer at a contact time of 1 minute

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

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