STUDY REPORT

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

Product Identity
Pure & Clean Wound Solutions, Wound Cleanser Clinical Strength
Lot: 01020-1

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

Study Identification Number
NG14797

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Study Completion Date
08APR2020

Testing Facility
Microchem Laboratory
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Study Sponsor
Pure & Clean, LLC
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STUDY REPORT SUMMARY

General Study Information

Study Title:	ASTM E1052 Method Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension
Study Identification Number:	NG14797

Test System

Test Microorganism:	Human Coronavirus, Strain 229-E, ATCC VR-740
Host Cell(s):	MRC-5, CCL-171
Test Substance:	Pure & Clean Wound Solutions, Wound Cleanser Clinical Strength
Lot Number:	01020-1
Test Substance Receipt Date:	04MAR2020

Test Parameters

Test Substance Dilution:	N/A - Ready to Use
Organic Soil Load:	No supplementation of organic soil load incorporated into inoculum (at level used to propagate virus)
Number of Replicates Per Lot:	One
Contact Time(s):	15 seconds and 30 seconds
Exposure Temperature:	26.3°C and 46% Relative Humidity (RH)
Neutralization Method(s):	Dilution Method using 2% fetal bovine serum (FBS) EMEM

Study Dates

Experimental Start Date/Time:	28MAR2020 / 1323
Experimental Termination Date/Time:	04APR2020 / 0938
Study Completion Date:	08APR2020

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) of 15 seconds and 30 seconds, as 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. $TCID_{50}$) 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 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.

Study Notes

Total Incubation Time: 6 days 19 hours 45 minutes

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 Control and Test Results

		Virus Control	Lot: 01020-1 15 seconds	Lot: 01020-1 30 seconds
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	N/A	N/A
	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 0
	10^{-5}	0 0 0 0	0 0 0 0	N/A
$TCID_{50}$ per 0.1 ml		4.25 Log_{10}	≤1.50 Log_{10}	≤1.50 Log_{10}
Log_{10} Reduction per 0.1 ml		N/A	≥2.75 Log_{10}	≥2.75 Log_{10}
Percent Reduction		N/A	≥99.82%	≥99.82%

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

Table 2: Cytotoxicity and Neutralization Control Results

		Test Sample 01020-1	
		Neutralization	Cytotoxicity
Cell Control		0 0 0 0	0000
Dilution	10^{-1}	+ + + +	T T T T
	10^{-2}	+ + + +	0 0 0 0
	10^{-3}	+ + + +	0 0 0 0
TCD_{50} per 0.1 ml		≤0.50 Log_{10}	≤0.50 Log_{10}

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 Pure & Clean Wound Solutions, Wound Cleanser Clinical Strength (Lot: 01020-1) against Human Coronavirus, Strain 229-E, ATCC VR-740, at contact times of 15 seconds and 30 seconds and at an exposure temperature of 26.3°C and 46% Relative Humidity (RH).

The Virus Control demonstrated an average viral titer of 4.25 Log_{10} $TCID_{50}$ per 0.1 ml.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test substance, Pure & Clean Wound Solutions, Wound Cleanser Clinical Strength, demonstrated a ≥2.75 Log_{10} reduction (≥99.82%) in viral titer at 15 seconds; a ≥2.75 Log_{10} reduction (≥99.82%) at 30 seconds.

Neutralization Control for all test substances demonstrated that the test substance was neutralized at ≤0.50 Log_{10}.

Test substance cytotoxic effects to the host monolayer were observed at ≤0.50 Log_{10} TCD_{50} per carrier for Lot 01020-1.

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.