STUDY REPORT

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

Product Identity
Hexagon Wound Dressing

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

Study Identification Number
NG14786

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Study Completion Date
07APR2020

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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:	NG14786

Test System

Test Microorganism(s):	Human Coronavirus, Strain 229-E, ATCC VR-740
Host Cell(s):	MRC-5, CCL-171 for Human Coronavirus
Test Substance:	Hexagon Wound Dressing
Lot Number(s):	Single Replicate
Test Substance Receipt Date:	03MAR2020

Test Parameters

Test Substance Dilution:	Ready to Use
Organic Soil Load:	No additional supplementation of organic soil load other than what the virus was propagated with
Number of Replicates Per Lot:	Single Replicate
Contact Time(s):	30 seconds, 2 minutes, and 10 minutes
Exposure Temperature:	25.4°C and 46% Relative Humidity (RH)
Neutralization Method(s):	Dilution media: 2% fetal bovine serum (FBS) EMEM

Study Dates

Experimental Start Date/Time:	27MAR2020 / 1218
Experimental Termination Date/Time:	07APR2020 / 0851
Study Completion Date:	07APR2020

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 30 seconds, 2 minutes, and 10 minutes, 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

The test substance was weighed out into a 50 ml conical tube, centrifuged for 10 minutes at 3000 rpm, and then placed into a water bath that ranged from 36.0°C to 37.5°C. The test substance was held in the water bath prior to and during testing, for a total time of 25 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: Preliminary Reading Results for Virus Control and Test Results for 30MAR2020 – 3 Days of Incubation

		Virus Control 10 minutes	30 seconds	2 minutes	10 minutes
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-2}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-3}	$^{+}/_{0}$ $^{+}/_{0}$ + $^{+}/_{0}$	$^{+}/_{0}$ $^{+}/_{0}$ $^{+}/_{0}$ $^{+}/_{0}$	$^{+}/_{0}$ $^{+}/_{0}$ $^{+}/_{0}$ $^{+}/_{0}$	$^{+}/_{0}$ $^{+}/_{0}$ $^{+}/_{0}$ $^{+}/_{0}$
	10^{-4}	0 0 0 0	$^{+}/_{0}$ $^{+}/_{0}$ 0 0	0 $^{+}/_{0}$ 0 0	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	0 0 0 0	0 0 0 0	0 0 0 0

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

Table 2: Preliminary Reading Results Cytotoxicity and Neutralization Control Results for 30MAR2020

		Test Samples	
		Cytotoxicity	Neutralization
Cell Control		0 0 0 0	0 0 0 0
Dilution	10^{-2}	0 0 0 0	+ + + +
	10^{-3}	0 0 0 0	+ + + +
	10^{-4}	0 0 0 0	+ + + +
TCD_{50} per 0.1 ml		≤1.50 Log_{10}	≤1.50 Log_{10}

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

Table 3: Virus Control and Test Results for 01APR2020 – 5 Days of Incubation

		Virus Control 10 minutes	30 seconds	2 minutes	10 minutes
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-2}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +	+ + + +	+ + + +
	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	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		4.25 Log_{10}	4.25 Log_{10}	4.00 Log_{10}	3.50 Log_{10}
Log_{10} Reduction		N/A	No Reduction	0.25 Log_{10}	0.75 Log_{10}

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

Table 4: Virus Control and Test Results for 03APR2020 – 7 Days of Incubation

		Virus Control 10 minutes	30 seconds	2 minutes	10 minutes[1]
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-2}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-4}	+ 0 + +	+ 0 0 0	+ + 0 0	+ + + +
	10^{-5}	0 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	0 0 0 0
$TCID_{50}$ per 0.1 ml		4.25 Log_{10}	3.75 Log_{10}	4.00 Log_{10}	5.25 Log_{10}
Log_{10} Reduction		N/A	0.50 Log_{10}	0.25 Log_{10}	No Reduction

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

[1]Note: Test suspension aliquots from the 10 minute contact time were plated to a subset of permissive host cells that were grown to a different cell confluency level than the other contact times evaluated. Because of suspected cell overgrowth after 5 days of incubation, observations made after 5 days of incubation for the 10 minute contact time data were thought to not be as reliable as the 5 day observation for this datapoint. 5 day observations were made by consulting personnel, Nicholas Garcia, B.S. who was contacted and authorized to make these observations on the behalf of the Study Sponsor.

Table 5: Cytotoxicity and Neutralization Control Results for 03APR2020

		Test Samples	
		Cytotoxicity	Neutralization
Cell Control		0 0 0 0	0 0 0 0
Dilution	10^{-2}	0 0 0 0	+ + + +
	10^{-3}	0 0 0 0	+ + + +
	10^{-4}	0 0 0 0	+ + + +
TCD_{50} per 0.1 ml		≤1.50 Log_{10}	≤1.50 Log_{10}

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

Table 6: Cytotoxicity and Neutralization Control Results for 07APR2020

		Test Samples	
		Cytotoxicity	Neutralization
Cell Control		0 0 0 0	0 0 0 0
Dilution	10^{-2}	0 0 0 0	+ + + +
	10^{-3}	0 0 0 0	+ + + +
	10^{-4}	0 0 0 0	+ + + +
TCD_{50} per 0.1 ml		≤1.50 Log_{10}	≤1.50 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 Hexagon Wound Dressing, Single Replicate, against Human Coronavirus, Strain 229-E, ATCC VR-740 at contact times of 30 seconds, 2 minutes, and 10 minutes and at an exposure temperature of 25.4°C and 46% Relative Humidity (RH).

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

For the results evaluated on day 5 of incubation (01APR2020) and taking the cytotoxicity and neutralization control results into consideration, the evaluated test substance, Hexagon Wound Dressing, demonstrated no reduction in viral titer at the 30 second contact time, 0.25 log_{10} reduction in viral titer at the 2 minute contact time, and 0.75 log_{10} reduction reduction in viral titer at the 10 minute contact time.

Neutralization Control for the test substance demonstrated that the test substance was neutralized at ≤1.50 Log_{10} at the longest contact time assayed. No test substance cytotoxicity was detected in test substance assayed (≤1.50 Log_{10}).

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.