STUDY REPORT

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

Product Identity
Hexagen Wound Dressing
Lot: 19H30B1

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

Study Identification Number
NG15237-A1

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Study Completion Date
19MAY2020

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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:	NG15237

Test System

Test Microorganism:	Human Coronavirus, Strain 229E, ATCC VR-740
Host Cell:	MRC-5, CCL-171
Test Substance:	Hexagen Wound Dressing Lot: 19H30B1
Test Substance Receipt Date:	03MAR2020

Test Parameters

Test Substance Dilution:	Ready to use liquid test substance
Total Organic Soil Load:	No additional soil load was incorporated into test inoculum
Number of Replicates Per Lot:	Single
Contact Time(s):	1 hour and 2 hours
Exposure Temperature:	37.5 - 37.8°C 36 – 37% Relative Humidity (RH)
Neutralization Method:	2% fetal bovine serum (FBS) EMEM

Study Dates

Experimental Start Date/Time:	11MAY2020 / 1347
Experimental Termination Date/Time:	18MAY2020 / 1015
Study Completion Date:	19MAY2020

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 1 hour and 2 hours 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.

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: Final Test Results – 7 Days of Incubation

		Hexagen Wound Dressing Lot: 19H30B1 **1 hour**	Hexagen Wound Dressing Lot: 19H30B1 **2 hours**
Cell Control		0 0 0 0	0 0 0 0
Dilution	10^{-1}	N/A	N/A
	10^{-2}	+ + + +	+ + + +
	10^{-3}	+ + + +	+ 0 0 0
	10^{-4}	+ 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
$TCID_{50}$ per 0.1 ml		4.00 Log_{10}	2.75 Log_{10}
Log_{10} Reduction per 0.1 ml		1.00 Log_{10}	1.75 Log_{10}
Percent Reduction		90.0%	98.22%

Table 2: Final Results of Virus Control Results – 7 Days of Incubation

		Virus Titer	Virus Control 1 hour	Virus Control 2 hours
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	N/A	N/A
	10^{-2}	+ + + +	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +	+ + + +
	10^{-4}	+ + + +	+ + + +	0 0 + +
	10^{-5}	0 0 + +	0 + + 0	0 0 + +
	10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		5.00 Log_{10}	5.00 Log_{10}	4.50 Log_{10}

Table 3: Test Substance Neutralization Control and Cytotoxicity Control Results

		Neutralization	Cytoxicity
Cell Control		0 0 0 0	0 0 0 0
Dilution	10^{-1}	N/A	N/A
	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}

STUDY CONCLUSION

The purpose of the study was to determine the virucidal efficacy of Hexagen Wound Dressing (Lot: 19H30B1) against Human Coronavirus, Strain 229E, ATCC VR-740, at contact times of 10 minutes, 30 minutes, 1 hour and 2 hours and at an exposure temperature of 37.5 - 37.8°C and 36 – 37% RH.

The Virus Control demonstrated an average viral titer of 5.00 Log_{10} $TCID_{50}$ per 0.1 ml.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test substance, Hexagen Wound Dressing (Lot: 19H30B1), demonstrated a 1.00 Log_{10} reduction (90.0%) in viral titer for Lot 19H30B1 at 1 hour and a 1.75 Log_{10} reduction (98.22%) in viral titer for Lot 19H30B1 at 2 hours.

No test substance cytotoxicity was detected in either lot of test substance assayed (≤1.50 Log_{10}).

Neutralization Control for all test substances demonstrated that the test substance was neutralized at ≤1.50 Log_{10} for lot 19H30B1.

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.