STUDY REPORT

Study Title
Virucidal Efficacy of a Test Substance For Use on Inanimate, Nonporous Surfaces

Product Identity
Bioneat
(BIO-1001)

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

Study Identification Number
NG15491

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Study Completion Date
05AUG2020

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Study Sponsor
United Food Products
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STUDY REPORT SUMMARY

General Study Information

Study Title:	ASTM E1053 Method Virucidal Efficacy of a Test Substance For Use on Inanimate, Nonporous Surfaces
Study Identification Number:	NG15491

Test System

Test Microorganism:	Human coronavirus, Strain 229E, ATCC VR-740
Host Cell:	MRC-5 (CCL-171)
Test Substance:	Bioneat (BIO-1001)
Test Substance Receipt Date:	22MAY2020

Test Parameters

Test Substance Dilution:	Ready to use liquid test substance
Test Substance Application:	2.0 ml delivered by serological pipette
Organic Soil Load:	No additional soil load incorporated into the inoculum
Number of Replicates Per Lot:	Double
Contact Time:	10 minutes
Exposure Temperature:	Ambient room temperature (23.4 – 23.5°C) and 42% Relative Humidity (RH)
Neutralization Method:	Sephadex LH-20 gel filtration columns

Study Dates

Experimental Start Date/Time:	17JUL2020 / 1135
Experimental Termination Date/Time:	24JUL2020 / 1545
Study Completion Date:	05AUG2020

TEST PROCEDURE

Summary

- Stock virus was thawed and was not supplemented with an organic soil load.
- Sterile glass Petri dish carriers (100 x 15 mm) were inoculated with a volume of virus suspension. A sufficient number of test and control carriers were prepared.
- Inoculated carriers were dried at room temperature under laminar flow conditions.
- The test substance was prepared according to the Study Sponsor's instructions as requested, and applied to the test carriers using a serological pipette.
- The treated carriers were held for the predetermined contact time(s), and then neutralized in a manner appropriate for the test substance (e.g. dilution and/or gel filtration).
- The 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 (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 calculated for viral films exposed to the test product relative to the titer obtained for the study control carrier(s), and reported to the Study Sponsor.

SUCCESS CRITERIA

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

- A minimum of 4.80 $\log_{10}$ 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.
- 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:

- In the presence or absence of cytotoxicity, the product should demonstrate a $\geq$3.00 $\log_{10}$ reduction in viral titer on each surface.
- If cytotoxicity is present, the virus control titer should be increased if necessary to demonstrate a $\geq$3.00 $\log_{10}$ reduction in viral titer on each surface beyond the cytotoxicity level.

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 Plate Recovery Control Results

		Virus Titer	Virus Plate Recovery Control Replicate #1	Virus Plate Recovery Control Replicate #2
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	+ + + +	+ + + +
	10^{-2}	+ + + +	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +	+ + + +
	10^{-4}	+ + + +	+ + + +	+ 0 + +
	10^{-5}	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		5.00 Log_{10}	4.75 Log_{10}	4.25 Log_{10}
$TCID_{50}$ per Carrier		N/A	5.05 Log_{10}	4.55 Log_{10}
Average $TCID_{50}$ per Carrier		4.80		

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

Table 2: Test Results

		Bioneat (BIO-1001) Replicate #1	Bioneat (BIO-1001) Replicate #2
Cell Control		0 0 0 0	0 0 0 0
Dilution	10^{-1}	0 0 0 0	0 0 0 0
	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
	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		≤ 0.50 Log_{10}	≤ 0.50 Log_{10}
$TCID_{50}$ per Carrier		≤ 0.80 Log_{10}	≤ 0.80 Log_{10}
Average $TCID_{50}$ per Carrier		≤ 0.80 Log_{10}	
Average Log_{10} Reduction		≥ 3.00*	
Average Percent Reduction		$\geq 99.90\%$	

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Cytotoxicity observed
*Log Reduction calculations performed taking cytotoxicity into account

Table 3: Cytotoxicity and Neutralization Control Results

		Cytotoxicity	Neutralization
Cell Control		0 0 0 0	0 0 0 0
Dilution	10^{-1}	T T T T	T T T T
	10^{-2}	0 0 0 0	+ + + +
	10^{-3}	0 0 0 0	+ + + +
$TCID_{50}$ 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 Bioneat (BIO-1001) against Human coronavirus Strain 229E, with no additional soil load incorporated into the inoculum, at a contact time of 10 minutes, and at an exposure temperature of room temperature (23.4 – 23.5°C) and 42% RH.

The Plate Recovery Control demonstrated an average viral titer of 4.80 Log_{10} $TCID_{50}$ per carrier.

Test Substance cytotoxicity was detected in the lot of test substance assayed at 1.50 Log_{10}.

The Test Substance Neutralization Control demonstrated that the test substance was neutralized at 1.50 Log_{10} for the lot assayed.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test substance, Bioneat (BIO-1001), demonstrated an average ≥3.00 Log_{10} reduction in viral titer (≥99.90%) at a contact time of 10 minutes.

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.