



## STUDY REPORT

### Study Title

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

### Product Identity

Nanolava - Nanoblaster

(BIO-1001)

### Test Microorganism

Human coronavirus, Strain 229E, ATCC VR-740

### Study Identification Number

NG15491

### Author

Emily Cox, B.S.

### Study Completion Date

05AUG2020

### Testing Facility

Microchem Laboratory  
1304 W. Industrial Blvd.  
Round Rock, Texas 78681

### Study Sponsor

Nanolava - Nanoblaster



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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: Nanolava - Nanoblaster (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



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## 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<sub>50</sub>) 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-Kärber) to determine viral titers and levels of test substance cytotoxicity, where applicable.
- Log<sub>10</sub> 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.



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## SUCCESS CRITERIA

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

- A minimum of 4.80 log<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> reduction in viral titer on each surface beyond the cytotoxicity level.



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## CALCULATIONS AND STATISTICAL ANALYSIS

The TCID<sub>50</sub> (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<sub>50</sub>). The TCID<sub>50</sub>, and TCD<sub>50</sub> was determined using the Spearman-Kärber method and calculated as follows:

Negative logarithm of endpoint titer =

$[-\text{Log of first dilution inoculated}] - [((\text{sum of \% mortality at each dilution}/100) - 0.5) \times \text{Logarithm of dilution}]$

The result of this calculation is expressed as TCID<sub>50</sub>/0.1 ml (or volume of dilution inoculated) for the test, virus control, and neutralization control and TCD<sub>50</sub>/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<sub>10</sub> TCID<sub>50</sub> – Virus-Test Substance Log<sub>10</sub> TCID<sub>50</sub>

### Calculation of the Percent Reduction

The percent reduction in viral titer was calculated as follows:

Percent Reduction =  $1 - (C/B) \times 100$ , where:

B = Average TCID<sub>50</sub> of virus in control suspensions.

C = Average TCID<sub>50</sub> 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<sub>50</sub> 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 <sup>-1</sup>	+ + + +	+ + + +	+ + + +
	10 <sup>-2</sup>	+ + + +	+ + + +	+ + + +
	10 <sup>-3</sup>	+ + + +	+ + + +	+ + + +
	10 <sup>-4</sup>	+ + + +	+ + + +	+ 0 + +
	10 <sup>-5</sup>	0 0 + +	0 0 + 0	0 0 0 0
	10 <sup>-6</sup>	0 0 0 0	0 0 0 0	0 0 0 0
	10 <sup>-7</sup>	0 0 0 0	0 0 0 0	0 0 0 0
TCID <sub>50</sub> per 0.1 ml		5.00 Log <sub>10</sub>	4.75 Log <sub>10</sub>	4.25 Log <sub>10</sub>
TCID <sub>50</sub> per Carrier		N/A	5.05 Log <sub>10</sub>	4.55 Log <sub>10</sub>
Average TCID <sub>50</sub> per Carrier		4.80		

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



**Table 2: Test Results**

		(BIO-1001) Replicate #1	(BIO-1001) Replicate #2
<b>Cell Control</b>		0 0 0 0	0 0 0 0
<b>Dilution</b>	10 <sup>-1</sup>	0 0 0 0	0 0 0 0
	10 <sup>-2</sup>	0 0 0 0	0 0 0 0
	10 <sup>-3</sup>	0 0 0 0	0 0 0 0
	10 <sup>-4</sup>	0 0 0 0	0 0 0 0
	10 <sup>-5</sup>	0 0 0 0	0 0 0 0
	10 <sup>-6</sup>	0 0 0 0	0 0 0 0
	10 <sup>-7</sup>	0 0 0 0	0 0 0 0
<b>TCID<sub>50</sub> per 0.1 ml</b>		≤0.50 Log <sub>10</sub>	≤0.50 Log <sub>10</sub>
<b>TCID<sub>50</sub> per Carrier</b>		≤0.80 Log <sub>10</sub>	≤0.80 Log <sub>10</sub>
<b>Average TCID<sub>50</sub> per Carrier</b>		≤0.80 Log <sub>10</sub>	
<b>Average Log<sub>10</sub> Reduction</b>		≥3.00*	
<b>Average Percent Reduction</b>		≥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**

		<b>Cytotoxicity</b>	<b>Neutralization</b>
<b>Cell Control</b>		0 0 0 0	0 0 0 0
<b>Dilution</b>	10 <sup>-1</sup>	T T T T	T T T T
	10 <sup>-2</sup>	0 0 0 0	+ + + +
	10 <sup>-3</sup>	0 0 0 0	+ + + +
<b>TCID<sub>50</sub> per 0.1 ml</b>		1.50 Log <sub>10</sub>	1.50 Log <sub>10</sub>

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

T = Cytotoxicity observed



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## STUDY CONCLUSION

The purpose of the study was to determine the virucidal efficacy of Nanolava - Nanoblaster (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<sub>10</sub> TCID<sub>50</sub> per carrier.

Test Substance cytotoxicity was detected in the lot of test substance assayed at 1.50 Log<sub>10</sub>.

The Test Substance Neutralization Control demonstrated that the test substance was neutralized at 1.50 Log<sub>10</sub> for the lot assayed.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test substance, Nanolava - Nanoblaster (BIO-1001), demonstrated an average  $\geq 3.00$  Log<sub>10</sub> reduction in viral titer ( $\geq 99.90\%$ ) 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.*

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