



STUDY CONCLUSION

The purpose of the study was to determine the virucidal efficacy of Goodtech Nano Sanitizing Spray against Human coronavirus Strain 229E with no supplementation of organic soil load incorporated into the test inoculum, at a contact time of 1 minute, at room temperature (23.1°C and 48% RH).

The Virus Control demonstrated a viral titer of 5.25 Log₁₀ TCID₅₀ per 0.1 ml.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test substance, Goodtech Nano Sanitizing Spray, demonstrated a 3.25 Log₁₀ reduction (99.94%) in viral titer, compared to the corresponding Plate Recovery Control.

The Test Substance Neutralization Control demonstrated that the test substance was neutralized at ≤ 1.50 Log₁₀ for the lot assayed.

No test substance cytotoxicity was detected in the lot of test substance assayed (≤ 1.50 Log₁₀).

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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RESULTS (cont.)

Table 3: Cytotoxicity Control Results

		Cytotoxicity Control
Cell Control		0 0 0 0
Dilution	10 ⁻¹	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

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

Table 4: Test Substance Neutralization Control Results

		Neutralization Control
Cell Control		0 0 0 0
Dilution	10 ⁻¹	N/A
	10 ⁻²	+ + + +
	10 ⁻³	+ + + +
	10 ⁻⁴	+ + + +
Neutralized at TCID ₅₀ per 0.1 ml		≤ 1.50 Log ₁₀

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



RESULTS

Table 1: Virus Controls

		Virus Titer	Virus Control
Cell Control		0 0 0 0	0 0 0 0
Dilution	10 ⁻¹	N/A	N/A
	10 ⁻²	N/A	+ + + +
	10 ⁻³	N/A	+ + + +
	10 ⁻⁴	+ + + +	+ + + +
	10 ⁻⁵	+ + + +	+ 0 + +
	10 ⁻⁶	0 + 0 +	0 0 0 0
	10 ⁻⁷	0 0 0 0	0 0 0 0
	TCID ₅₀ per 0.1 ml		6.00 Log ₁₀

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

Table 2: Test Results for Goodtech Nano Sanitizing Spray

		Test Results
Cell Control		0 0 0 0
Dilution	10 ⁻¹	N/A
	10 ⁻²	+ 0 0 +
	10 ⁻³	0 0 0 0
	10 ⁻⁴	0 0 0 0
	10 ⁻⁵	0 0 0 0
	10 ⁻⁶	0 0 0 0
	10 ⁻⁷	0 0 0 0
	TCID ₅₀ per 0.1 ml	
Log Reduction		3.25 Log ₁₀
Percent Reduction		99.94%

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



CALCULATIONS AND STATISTICAL ANALYSIS

The TCID₅₀ (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 (TCD₅₀). The TCID₅₀, and TCD₅₀ was determined using the Spearman-Kärber method and calculated as follows:

$$\begin{array}{l} \text{Negative} \\ \text{logarithm of} \\ \text{endpoint} \\ \text{titer} \end{array} = [- \text{Log of first dilution inoculated}] - [((\text{sum of \% mortality at each} \\ \text{dilution}/100) - 0.5) \times \text{Logarithm of dilution}]$$

The result of this calculation is expressed as TCID₅₀/0.1 ml (or volume of dilution inoculated) for the test, virus control, and neutralization control and TCD₅₀/0.1 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:

$$\text{Plate Recovery Control Log}_{10} \text{TCID}_{50} - \text{Virus-Test Substance Log}_{10} \text{TCID}_{50}$$

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 TCID₅₀ of virus in control suspensions.

C = Average TCID₅₀ 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 TCID₅₀ of each parameter was calculated and the average result used to calculate the log reductions in viral titer.



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) 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. TCID₅₀) assay techniques.
- The cell culture 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.
- Log₁₀ 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.



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: NG15874

Test System

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

Host Cell: MRC-5 (ATCC CCL-171)

Test Substance: Goodtech Nano Sanitizing Spray

Test Substance Receipt Date: 02JUL2020

Test Parameters

Test Substance Dilution: Ready to use liquid test substance

Total Organic Soil Load: No supplementation of organic soil load incorporated
into the test inoculum

Number of Replicates Per Lot: Single

Contact Time: 1 minute

Exposure Temperature: Room temperature (23.1°C) and 48% Relative Humidity
(RH)

Neutralization Method: Dilution method using fetal bovine serum (FBS)

Study Dates

Experimental Start Date/Time: 26SEP2020 / 1226

Experimental Termination Date/Time: 03OCT2020 / 1229

Study Completion Date: 09OCT2020



STUDY REPORT

Study Title

ASTM E1052

Standard Test Method to Assess the Activity of Microbicides Against Viruses in Suspension

Product Identity

Goodtech Nano Sanitizing Spray

Test Microorganism

Human coronavirus, Strain 229E, ATCC VR-740

Study Identification Number

NG15874

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Study Completion Date

09OCT2020

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