



STUDY REPORT

Study Title

Non-GLP ASTM E1053: Standard Practice to Assess Virucidal Activity of Chemicals
Intended for Disinfection of Inanimate, Non-porous Environmental Surfaces

Product Identity

TK60 One-Step Disinfectant

Test Microorganism

Human coronavirus, Strain 229E, ATCC VR-740

Study Identification Number

NG16554

Author

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Study Completion Date

19OCT2020

Testing Facility

Microchem Laboratory
1304 W. Industrial Blvd.
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Study Sponsor

R-Water, LLC
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STUDY REPORT SUMMARY

General Study Information

Study Title: Non-GLP ASTM E1053: Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Non-porous Environmental Surfaces

Study Identification Number: NG16554

Test System

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

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

Test Substance: TK60 One-Step Disinfectant

Test Substance Receipt Date: 08OCT2020

Test Parameters

Test Substance Dilution: Ready to use spray substance

Test Substance Application: Five sprays / distance 6 – 8 inches

Organic Soil Load: 5% fetal bovine serum (FBS)

Number of Replicates Per Lot: Single

Contact Time(s): 30 seconds and 60 seconds

Exposure Temperature: Ambient room temperature (24.8°C) and 38% Relative Humidity (RH)

Neutralization Method(s): Sephadex LH-20 gel filtration column

Study Dates

Experimental Start Date/Time: 08OCT2020 / 1822

Experimental Termination Date/Time: 15OCT2020 / 1315

Study Completion Date: 19OCT2020



SUMMARY OF THE TEST PROCEDURE

- Stock virus was thawed and was supplemented with an organic soil load.
- Sterile glass Petri dishes (100 x 15 mm) were used as the test carrier . For each lot of substance assayed, one carrier was inoculated with a 0.200 ml volume of virus suspension. The appropriate number of plate recovery control carriers were also prepared.
- The inoculated carriers were dried at the appropriate temperature and relative humidity to lessen the level of virus inactivation due to drying.
- The test substance was prepared according to the Study Sponsor's instructions as requested and applied to the test carriers using a spray device. The distance and number of sprays were performed as requested by the Study Sponsor.
- The treated carriers were held for the Study Sponsor specified contact time(s) at the Study Sponsor specified exposure temperature, and then neutralized in a manner appropriate for the test substance (e.g. dilution and/or gel filtration).
- The plate recovery 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 techniques (e.g. TCID₅₀).
- The inoculated cell culture plates were incubated for the period most suitable for the virus-host cell system (e.g. ~7 days).
- Following the incubation period, the assay was microscopically 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.
- The log₁₀ and percent reductions in viral titer were calculated for viral films exposed to the test product relative to the titer obtained for the study control carrier(s).

SUCCESS CRITERIA

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

- A minimum of 4.80 log₁₀ to 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.
- Comparable levels of infective units must be recovered from the neutralized test substance and neutralization control substance.
- Quantification of the test and control parameters are conducted at a minimum of four determinations per dilution.

Note: Although the test method does not specify a product performance criteria, for registration as a hard surface disinfectant, the U.S. EPA requires a ≥ 3 log₁₀ reduction in viral titer as compared to the corresponding plate recovery control.



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:

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

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:

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

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.



RESULTS

Table 1: Virus Titer and Virus Plate Recovery Control Results

		Virus Titer	Virus Plate Recovery Control
Cell Control		0 0 0 0	0 0 0 0
Dilution	10⁻¹	N/A	+ + + +
	10⁻²	+ + + +	+ + + +
	10⁻³	+ + + +	+ + + +
	10⁻⁴	+ + + +	+ + + +
	10⁻⁵	+ + + +	+ + + +
	10⁻⁶	0 0 + 0	+ + 0 +
	10⁻⁷	0 0 0 0	0 0 0 0
TCID₅₀ per 0.1 ml		5.75 Log ₁₀	6.25 Log ₁₀
TCID₅₀ per Carrier		N/A	6.55 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 TK60 One-Step Disinfectant

		Test Results (30 seconds)	Test Results (60 seconds)
Cell Control		0 0 0 0	0 0 0 0
Dilution	10⁻¹	+ + + +	+ + + +
	10⁻²	+ 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
TCID₅₀ per 0.1 ml		1.75 Log ₁₀	2.25 Log ₁₀
TCID₅₀ per Carrier		2.05 Log ₁₀	2.55 Log ₁₀
Log₁₀ Reduction		4.50 Log ₁₀	4.00 Log ₁₀
Percent Reduction		99.997%	99.990%

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



RESULTS (cont.)

Table 3: Cytotoxicity and Neutralization Control Results

		Cytotoxicity Control	Neutralization Control – Test Substance	Neutralization Control – Control Substance
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10 ⁻¹	0 0 0 0	+ + + +	+ + + +
	10 ⁻²	0 0 0 0	+ 0 0 +	0 + 0 0
	10 ⁻³	0 0 0 0	0 0 0 0	0 0 0 0
	10 ⁻⁴	N/A	0 0 0 0	0 0 0 0
	10 ⁻⁵	N/A	0 0 0 0	0 0 0 0
	10 ⁻⁶	N/A	0 0 0 0	0 0 0 0
TCID ₅₀ per 0.1 ml		≤0.50 Log ₁₀ *	2.00 Log ₁₀	1.75 Log ₁₀

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

*Cytotoxicity control reported as TCD₅₀ per 0.1 ml

The test substance and control substance demonstrated comparable levels of infective units recovered in the Neutralization Control.



STUDY CONCLUSION

The purpose of the study was to determine the virucidal efficacy of TK60 One-Step Disinfectant against Human coronavirus Strain 229E, supplemented with a 5% FBS organic soil load at contact times of 30 seconds and 60 seconds at room temperature (24.8°C and 38% RH).

The Plate Recovery Control demonstrated a viral titer of 6.25 Log₁₀ TCID₅₀ per 0.1 ml and 6.55 Log₁₀ TCID₅₀ per carrier.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test substance, TK60 One-Step Disinfectant, demonstrated a 4.50 Log₁₀ reduction in viral titer (99.997%) at a contact time of 30 seconds and a 4.00 Log₁₀ reduction in viral titer (99.990%) at a contact time of 60 seconds, as compared to the titer of the corresponding Plate Recovery Control.

No test substance cytotoxicity was detected for the test substance assayed (≤ 0.50 Log₁₀ TCD₅₀ per 0.1 ml).

The Test Substance Neutralization Control demonstrated that the test substance was neutralized at 2.00 Log₁₀. The control substance was neutralized at 1.75 Log₁₀. The test substance and control substance demonstrated comparable levels of infective units recovered in the Neutralization Control.

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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