STUDY REPORT

Study Title
JIS Z 2801 Modified Test Method to Assess the Activity of Treated Surfaces against Viruses

Product Identity
Microshield360 Antimicrobial on Stainless

Test Microorganism
Feline calicivirus, Strain Strain F9, ATCC VR-782

Study Identification Number
NG15205

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Study Completion Date
20JUL2020

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Study Sponsor
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STUDY REPORT SUMMARY

General Study Information

Study Title:	JIS Z 2801 Modified Test Method to Assess the Activity of Treated Surfaces against Viruses
Study Identification Number:	NG15205

Test System

Test Microorganism(s):	Feline calicivirus, Strain F9, ATCC VR-782
Host Cell(s):	MRC-5, CCL-171
Test Substance:	Microshield360 Antimicrobial on Stainless
Test Substance Receipt Date:	17APR2020

Test Parameters

Test Substance Preparation:	Ready to use (UV sterilized 15 minutes per side)
Total Organic Soil Load:	No additional organic soil load incorporated into the inoculum
Number of Replicates:	Triple
Articles per Carrier:	Single
Contact Time(s):	10 minutes, 30 minutes, and 60 minutes
Inoculum Volume per Carrier:	0.100 ml
Exposure Temperature:	Room temperature (25.1 – 25.4°C) and 39 – 40% Relative Humidity (RH)
Neutralization Method(s):	Dilution method using 2% FBS EMEM

Study Dates

Experimental Start Date/Time:	09JUL2020 / 1550
Experimental Termination Date/Time:	13JUL2020 / 0859
Study Completion Date:	20JUL2020

TEST PROCEDURE

Summary

- Stock virus was thawed and was not supplemented with an organic soil load.
- The samples were UV sterilized for 15 minutes per side and placed into individual sterile plastic Petri dishes.
- A 0.100 ml inoculum of virus suspension was pipetted onto the surface of each of the test samples.
- The contact time began as soon as the inoculum came in contact with the surface.
- A plastic cover film was placed over the sample to evenly spread the inoculum over the surface, and the Petri dish was covered with the lid.
- The test carriers remained in a temperature and humidity stable environment for the duration of the contact time(s).
- At the completion of each contact time, the cover film was aseptically removed, and both the cover film and carrier were rinsed with an aliquot of test media. The surface of the carrier was scraped with a sterile cell scraper.
- The resultant volume was aseptically collected and diluted by serial 10-fold dilutions.
- The virus recovery control carrier 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. 3-4 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.

SUCCESS CRITERIA

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

- The virus titer control demonstrates 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 is conducted at a minimum of four determinations per dilution.

The product performance criteria follow:

- 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: Virus Titer and Plate Recovery Controls

		Virus Titer	Plate Recovery Control 10 minutes	Plate Recovery Control 30 minutes	Plate Recovery Control 60 minutes
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-2}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-4}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-5}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-6}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-7}	+ + + +	+ + + +	+ + + +	+ + + +
	10^{-8}	+ + + +	+ + + +	+ 0 0 +	+ + + +
	10^{-9}	0 0 0 0	0 0 0 0	0 0 0 +	0 0 0 0
$TCID_{50}$ per 0.1 ml		8.50 Log_{10}	8.50 Log_{10}	8.25 Log_{10}	8.50 Log_{10}

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

RESULTS (cont)

Table 2: Test Results for Microshield360 Antimicrobial on Stainless at 10 minutes

		Replicate #1 10 minutes	Replicate #2 10 minutes	Replicate #3 10 minutes
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	+ + + +	+ + + +
	10^{-2}	+ + + +	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +	+ + + +
	10^{-4}	+ + + +	+ + + +	+ + + +
	10^{-5}	+ + + +	+ + + +	+ + + +
	10^{-6}	+ + + +	+ + + +	+ + + +
	10^{-7}	+ + + +	+ + + +	+ + + +
	10^{-8}	+ + + 0	+ + + +	+ + + +
	10^{-9}	0 + 0 0	0 0 0 0	+ 0 0 0
$TCID_{50}$ per 0.1 ml		8.50 Log_{10}	8.50 Log_{10}	8.75 Log_{10}
Average $TCID_{50}$ per 0.1 ml		8.583 Log_{10}		
Average Log_{10} Reduction		No Reduction		
Average Percent Reduction		No Reduction		

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

RESULTS (cont.)

Table 3: Test Results for Microshield360 Antimicrobial on Stainless at 30 minutes

		Replicate #1 30 minutes	Replicate #2 30 minutes	Replicate #3 30 minutes
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	+ + + +	+ + + +
	10^{-2}	+ + + +	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +	+ + + +
	10^{-4}	+ + + +	+ + + +	+ + + +
	10^{-5}	+ + + +	+ + + +	+ + + +
	10^{-6}	+ + + +	+ + + +	+ + + +
	10^{-7}	+ + + +	+ + + +	+ + + +
	10^{-8}	+ + + +	+ + + +	0 + + +
	10^{-9}	0 0 0 0	+ 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		8.50 Log_{10}	8.75 Log_{10}	8.25 Log_{10}
Average $TCID_{50}$ per 0.1 ml		8.50 Log_{10}		
Average Log_{10} Reduction		No Reduction		
Average Percent Reduction		No Reduction		

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

RESULTS (cont.)

Table 4: Test Results for Microshield360 Antimicrobial on Stainless at 60 minutes

		Replicate #1 60 minutes	Replicate #2 60 minutes	Replicate #3 60 minutes
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	+ + + +	+ + + +
	10^{-2}	+ + + +	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +	+ + + +
	10^{-4}	+ + + +	+ + + +	+ + + +
	10^{-5}	+ + + +	+ + + +	+ + + +
	10^{-6}	+ + + +	+ + + +	+ + + +
	10^{-7}	+ + + +	+ + + +	+ + + +
	10^{-8}	0 0 0 0	+ + 0 0	0 0 0 0
	10^{-9}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		7.50 Log_{10}	8.00 Log_{10}	7.50 Log_{10}
Average $TCID_{50}$ per 0.1 ml		7.667 Log_{10}		
Average Log_{10} Reduction		0.833 Log_{10}		
Average Percent Reduction		85.32%		

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

Table 5: Cytotoxicity Control Results

		Cytotoxicity Control 60 minutes
Cell Control		0 0 0 0
Dilution	10^{-1}	0 0 0 0
	10^{-2}	0 0 0 0
	10^{-3}	0 0 0 0
$TCID_{50}$ per 0.1 ml		≤0.50 Log_{10}

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

RESULTS (cont.)

Table 6: Test Substance Neutralization Control Results

		Neutralization Control 60 minutes
Cell Control		0 0 0 0
Dilution	10^{-1}	+ + + +
	10^{-2}	+ + + +
	10^{-3}	+ + + +
Neutralized at $TCID_{50}$ per 0.1 ml		≤0.50 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 Microshield360 Antimicrobial on Stainless against Feline calicivirus Strain F9, with no additional organic soil load incorporated into the inoculum, at contact times of 10 minutes, 30 minutes, and 60 minutes, and at an exposure temperature of room temperature (25.1 – 25.4°C) and 39 – 40% RH.

The Virus Control demonstrated a viral titer of 8.50 Log_{10} $TCID_{50}$ per 0.100 ml at 10 minutes, 8.25 Log_{10} $TCID_{50}$ per 0.100 ml at 30 minutes, and 8.50 Log_{10} $TCID_{50}$ per 0.100 ml at 60 minutes.

No test substance cytotoxicity was detected in the lot of test substance assayed ($\leq$0.50 Log_{10}).

The Test Substance Neutralization Control demonstrated that the test substance was neutralized at $\leq$0.50 Log_{10}.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test substance, Microshield360 Antimicrobial on Stainless, demonstrated no Log_{10} reduction in viral titer at the 10 minutes and 30 minutes contact times, and an average 0.833 Log_{10} reduction in viral titer (85.32%) at the 60 minutes contact time.

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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