STUDY REPORT

Study Title
Modified ASTM E1053
Virucidal Efficacy of a UV Test Device on an N95 Respirator

Product Identity
Lumin

Test Microorganism
Human Coronavirus 229E, ATCC VR-740

Study Identification Number
NG14983

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Study Completion Date
01JUN2020

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Study Sponsor
3B Medical
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STUDY REPORT SUMMARY

General Study Information

Study Title: Modified ASTM E1053, Virucidal Efficacy of a UV Test Device on an N95 Respirator

Study Identification Number: NG14983

Test System

Test Microorganism: Human Coronavirus 229E, ATCC VR-740

Host Cell: MRC-5

Test Substance: Lumin Device

Test Substance Receipt Date: 24MAR2020

Test Parameters

Test Substance Dilution: Ready to Use Device

Test Substance Application: Preset Device settings

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

Number of Replicates Per Lot: Double; inside and outside of mask

Contact Time: 5 minutes

Exposure Temperature: Ambient room temperature (25.5°C to 25.8°C and 45.7% to 46.2% Relative Humidity (RH))

Neutralization Method: Extraction/neutralization with test media

Study Dates

Experimental Start Date:	20MAY2020
Experimental Termination Date:	27MAY2020
Study Completion Date:	01JUN2020

TEST PROCEDURE

Summary

- Stock virus was thawed and was supplemented with an organic soil load.
- The inside and outside of the mask were inoculated with 0.200 ml of virus suspension containing 5% FBS soil load on designated 1in x 1in squares. An equivalent volume of virus suspension was inoculated on an appropriate amount of control carriers.
- The inoculated carriers were placed inside the device and treated for the predetermined contact time(s), and then neutralized using test media and 10-fold serial dilutions.
- The control carrier was held 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 ≥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 ≥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 Plate Recovery Controls and Test Results

Dilution	Virus Plate Recovery Control Replicate #1	Virus Plate Recovery Control Replicate #2
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	+ + + +	+ + + +
10^{-2}	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +
10^{-4}	0 0 0 0	0 + 0 +
10^{-5}	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml	3.50 Log_{10}	4.00 Log_{10}
$TCID_{50}$ per Carrier	3.80 Log_{10}	4.30 Log_{10}
Average $TCID_{50}$ per Carrier	4.05 Log_{10}	

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

Table 2: Test Results: Lumin

Dilution	Test Results Inside	Test Results Outside
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	N/A	N/A
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
$TCID_{50}$ 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}	
Log Reduction Per Carrier	≥ 3.25 Log_{10}	
Percent Reduction	$\geq 99.94\%$	

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

Table 3: Cytotoxicity Control Results

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

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

Table 4: Test Substance Neutralization Control Results

Dilution	Neutralization Control
Cell Control	0 0 0 0
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 the Lumin device against Human Coronavirus 229E, ATCC VR-740 supplemented with a 5% FBS soil load, at a contact time of 5 minutes at an exposure temperature of room temperature (25.5°C to 25.8°C and 45.7% to 46.2% RH).

The Plate Recovery Control demonstrated a viral titer of 3.75 $\log_{10}$ $TCID_{50}$ per 0.1 ml and 4.05 $\log_{10}$ $TCID_{50}$ per carrier.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test device demonstrated an average $\geq$3.25 Log_{10} reduction in viral titer (99.94% reduction).

No test substance cytotoxicity was detected in either 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} for the lot assayed.

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.