# STUDY REPORT

**Study Title**
Modified ASTM E1053 Method
Virucidal Efficacy of a UV Device

**Product Identity**
Lumin Device

**Test Microorganism**
Influenza A (H1N1), Strain A/PR/8/34, ATCC VR-1469

**Study Identification Number**
NG15294

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**Study Completion Date**
17JUL2020

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**Study Sponsor**
3B Medical, Inc.
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## STUDY REPORT SUMMARY

General Study Information

| | |
|---|---|
| Study Title: | Modified ASTM E1053 Method<br>Virucidal Efficacy of a UV Device |
| Study Identification Number: | NG15294 |

Test System

| | |
|---|---|
| Test Microorganism: | Influenza A (H1N1), Strain A/PR/8/34, ATCC VR-1469 |
| Host Cell: | MDCK (CCL-34) |
| Test Substance: | Lumin Device treatment of N95 mask (3M 8511) |
| Test Substance Receipt Date: | 24MAR2020 |

Test Parameters

| | |
|---|---|
| Test Substance Preparation: | Ready to use custom UV device |
| Test Substance Application: | Ready to use custom UV device |
| Organic Soil Load: | No additional soil load incorporated into the inoculum |
| Number of Replicates: | Single; inside of the N95 mask facing upwards towards light source |
| Contact Time(s): | 5 minutes |
| Exposure Temperature: | Ambient room temperature (25.3°C) and 39% Relative Humidity (RH) |
| Neutralization Method(s): | Extraction/neutralization with test media |

Study Dates

| | |
|---|---|
| Experimental Start Date/Time: | 02JUL2020 / 1742 |
| Experimental Termination Date/Time: | 09JUL2020 / 1148 |
| Study Completion Date: | 17JUL2020 |

## TEST PROCEDURE

Summary

- Stock virus was thawed and was not supplemented with an organic soil load.
- The inside of the mast was inoculated with 0.100 ml of virus suspension containing no additional 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, with the inside of the mask facing up, and treated for the predetermined contact time, 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 $\geq$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 $\geq$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 Control and Test Results

| | | Virus Plate Recovery Control | Lumin Device Treatment of N95 Mask |
|---|---|---|---|
| Cell Control | | 0 0 0 0 | 0 0 0 0 |
| Dilution | $10^{-1}$ | N/A | N/A |
| | $10^{-2}$ | + + + + | 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 | N/A |
| $TCID_{50}$ per 0.1 ml | | 6.80 $Log_{10}$ | 2.55 $Log_{10}$ |
| $Log_{10}$ Reduction | | N/A | 4.25 $Log_{10}$ |
| Percent Reduction | | | 99.994% |

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

### Table 2: Cytotoxicity Control Results

| | | Cytoxicity |
|---|---|---|
| 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 | | ≤0.80 $Log_{10}$ |

**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 Lumin Device on an N95 mask (3M 8511) against Influenza A (H1N1) Strain A/PR/8/34, with no additional soil load incorporated into the inoculum, at a contact time of 5 minutes, and at an exposure temperature of room temperature (25.3°C) and 39% RH.

The Plate Recovery Control demonstrated a viral titer of 6.80 $Log_{10}$ $TCID_{50}$ per 0.1 ml.

No test substance cytotoxicity was detected ($\leq$0.80 $Log_{10}$).

Taking the cytotoxicity results into consideration, the evaluated test device, Lumin Device, demonstrated a 4.25 $Log_{10}$ reduction in viral titer (99.994%) when treating the inside of the N95 mask.

*The test device 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 device only. Extrapolation of findings to related materials is the responsibility of the Sponsor.*