# STUDY REPORT

**Study Title**
Custom Virucidal Efficacy of a Device

**Product Identity**
AtmosAir Matterhorn

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

**Study Identification Number**
NG16149

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**Study Completion Date**
11SEP2020

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## STUDY REPORT SUMMARY

General Study Information

| | |
|---|---|
| Study Title: | Custom Virucidal Efficacy of a Device |
| Study Identification Number: | NG16149 |

Test System

| | |
|---|---|
| Test Microorganism(s): | Human coronavirus, Strain 229E, ATCC VR-740 |
| Host Cell(s): | MRC-5 (ATCC CCL-171) |
| Test Substance: | AtmosAir Matterhorn |

Test Parameters

| | |
|---|---|
| Test Device Preparation: | Arrived ready to use and was run for 30 minutes prior to use in testing |
| Test Device Application: | Fogging (The Matterhorn device for this study was calibrated to an ion saturation of 1,500 ions per $cm^3$) |
| Test Articles Tested: | Mask type: Grey Fabric |
| Volume of Inoculum: | 0.200 ml total; (Front and back: 0.05 µl over seam and ~1/2 inch toward mask center) |
| Total Organic Soil Load: | 5% (v/v) fetal bovine serum (FBS) |
| Number of Replicates Per Lot: | Single |
| Contact Time(s): | 15 minutes and 30 minutes |
| Exposure Temperature: | Ambient room temperature |
| Neutralization Method(s): | Dilution method using 2% FBS EMEM (4 ml) |

Study Dates

| | |
|---|---|
| Experimental Start Date: | 06AUG2020 |
| Experimental Termination Date: | 17AUG2020 |
| Study Completion Date: | 11SEP2020 |

## STUDY PHOTOS

*Note: The image depicts the distance of treatment of sample.*

## TEST PROCEDURE

Summary

- Prior to testing, the Sponsor provided samples were UV-Sterilized for 15 minutes.
- The appropriate volume (0.200 ml total) of each virus was inoculated into each of the sample surfaces. Both samples types were inoculated on the front and back. Each inoculated section received 0.05 $\mu$l over the seam and ~1/2 inch toward the center of the mask. Inoculated masks were dried for ~7 minutes.
- Device was placed in a 1m$^3$ aerosol chamber, near the door of chamber and ~2 ft from the masks.
- Inoculated masks were aseptically placed ~2ft from the device on test tube racks approximately mid-height of the device such that the inoculated sites were free from contact with the racks for each contact time.
- At the completion of each contact time, each inoculated areas of the mask were aseptically cut and harvested into an appropriate volume of test media (4.0 ml) vortexed mixed.
- After vortexing, a 0.1 ml aliquot was removed and a series of 10-fold dilutions was performed in 0.9 ml aliquots of appropriate test medium. Each dilution was inoculated into the appropriate host cells in quadruplicate.
- For the recovery controls, each of the untreated mask control surfaces were prepared and inoculated in the same manner as the test surfaces.
- Following the contact time, the same recovery procedure as the test was performed.
- 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-Karber) to determine viral titers and levels of test substance cytotoxicity, where applicable.
- The $\log_{10}$ and percent reductions in viral titer were calculated for test sample exposed to the test device relative to the titer obtained for the study control surface(s).

## 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 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 sample 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:

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 Controls- Grey Fabric

| | | Control Sample for Grey Fabric - 15 minutes | Control Sample for Grey Fabric - 30 minutes |
|---|---|---|---|
| Cell Control | | 0 0 0 0 | 0 0 0 0 |
| Dilution | $10^{-1.30}$ | + + + + | + + + + |
| | $10^{-2.30}$ | + + 0 + | + + + 0 |
| | $10^{-3.30}$ | 0 0 + + | 0 0 0 0 |
| | $10^{-4.30}$ | 0 0 0 0 | 0 0 0 0 |
| | $10^{-5.30}$ | 0 0 0 0 | 0 0 0 0 |
| | $10^{-6.30}$ | 0 0 0 0 | 0 0 0 0 |
| $TCID_{50}$ per 0.1 ml | | 3.05 $Log_{10}$ | 2.55 $Log_{10}$ |

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

### Table 2: Test Results- Grey Fabric

| | | Test Sample for Grey Fabric - 15 minutes | Test Sample for Grey Fabric - 30 minutes |
|---|---|---|---|
| Cell Control | | 0 0 0 0 | 0 0 0 0 |
| Dilution | $10^{-1.30}$ | 0 0 0 0 | 0 0 0 0 |
| | $10^{-2.30}$ | 0 0 0 0 | 0 0 0 0 |
| | $10^{-3.30}$ | 0 0 0 0 | 0 0 0 0 |
| | $10^{-4.30}$ | 0 0 0 0 | 0 0 0 0 |
| | $10^{-5.30}$ | 0 0 0 0 | 0 0 0 0 |
| | $10^{-6.30}$ | 0 0 0 0 | 0 0 0 0 |
| $TCID_{50}$ per 0.1 ml | | ≤ 0.80 $Log_{10}$ | ≤ 0.80 $Log_{10}$ |
| Log Reduction | | ≥2.25 $Log_{10}$ | ≥2.25 $Log_{10}$ |
| Percent Reduction | | ≥99.44% | ≥99.44% |

**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 AtmosAir Matterhorn Device, tested against one mask type, against Human coronavirus Strain 229E, supplemented with a 5% (v/v) fetal bovine serum (FBS) organic soil load, at contact times of 15 minutesand 30 minutes, and at an exposure temperature of ambient room temperature.

The virus recovery control for the grey fabric mask demonstrated a viral titer of 3.05 $Log_{10}$ $TCID_{50}$ per 0.1 ml at a 15 minute contact time and a viral titer of 2.55 $Log_{10}$ $TCID_{50}$ per 0.1 ml at a 30 minute contact time.

The evaluated test device, demonstrated a $\geq$2.25 $Log_{10}$ reduction ($\geq$99.44%) viral titer for the grey fabric mask at 15 minutes and $\geq$1.75 $Log_{10}$ reduction ($\geq$98.22%) in viral titer for the grey fabric mask at 30 minutes.

*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.*