Technical Report for the Evaluation of the Medify Air MA-112 against Airborne Viruses

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1. APPROVALS

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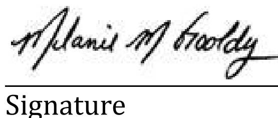
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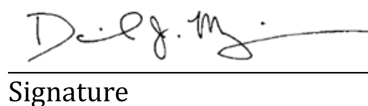
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LIST OF ACRONYMS

Acronym	Definition
ATCC	American Type Culture Collection
COVID-19	Coronavirus Disease 2019
BSL-2	Biosafety Level 2
BDL	Below Detection Limits
C	Celsius
CFM	Cubic Feet Per Minute
F	Fahrenheit
HCoV-229E	Human coronavirus 229E
HEPA	High-Efficiency Particulate Air
inHg	Inches of Mercury
L	Liter(s)
LPM	Liters Per Minute
m	Meter(s)
min	minute(s)
mL	Milliliter(s)
NIST	National Institute of Standards and Technology
OPS	Optical Particle Sizer
PFU	Plaque Forming Units
psig	Pounds per Square Inch Gauge
QM	Quality Manager
QA/QC	Quality Assurance/Quality Control
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SLPM	Standard Liters Per Minute
µL	Microliter(s)
OD	Outer Diameter
pt/cm3	particles per cubic centimeter

2. Executive Summary

CUBRC, Inc. performed a series of laboratory experiments to evaluate the performance of the Medify Air MA-112 air purification system against airborne viruses operated in an 800-cubic foot room. Two test conditions were evaluated against Human Coronavirus HCoV-229E (a surrogate for SARS-CoV-2) and Influenza A virus (H1N1). For Human Coronavirus HCoV-229E, at a fan speed of 4 (high) with the ionizer on, the MA-112 demonstrated a HCoV-229E removal efficiency of at least 99.95% within the first five minutes. No viable airborne virus was observed for any of the four samples collected over the 60-minute test duration. When operated at the lowest fan speed (speed 1) with the ionizer on, results demonstrated a removal efficiency of 97.54% within the first five minutes and by the 15-minute point, was exceeding 99.94% removal efficiency. No viable airborne virus was observed at the 15-, 35- and 55-minute time points. For Influenza A virus (H1N1), the results demonstrated that the Medify Air MA-112 air purification system operated in an 800-cubic foot room at a fan speed of 4 (high) with the ionizer on, demonstrated a removal efficiency of at least 99.94% within the first five minutes. No viable airborne virus was observed for any of the four samples collected over the 60-minute test duration. When operated at the lowest fan speed (speed 1) with the ionizer on in an 800-cubic foot room, results demonstrated a removal efficiency of 99.81% within the first five minutes and by the 15-minute point, it was exceeding 99.95% removal efficiency. No viable airborne virus was observed at the 15-, 35- and 55-minute time points.

Testing was performed within CUBRC's Biosafety Level 2 (BSL-2) facility. All work was performed in accordance with external regulatory requirements and following approved internal safety and technical protocols.

3. Introduction

This report describes the technical approach and procedures that were used to evaluate the performance of the Medify Air MA-112 air purification system against airborne viruses. The program objective was to experimentally demonstrate the reduction in airborne viral load (through filtration and/or active disinfection) over a specified time period within a room-sized test chamber. Air purification systems intended for use against SARS-CoV-2 require testing against a representative virus surrogate.[1] The experiments were performed using the Medify model MA-112 against two viruses including Human Coronavirus HCoV-229E (a surrogate for SARS-CoV-2) and Influenza A virus (H1N1).

The Medify Air MA-112[2] is a floor-standing air purification system that uses three levels of filtration to catch and remove allergens, pet dander and other airborne particles. It contains a pre-filter to remove large particles such as hair, fibers and dander followed by a high-efficiency H13 TRUE HEPA filter that has been separately tested to remove 99.9% of particles down to 0.1 microns. The system also has a carbon filter designed to remove odors, smoke and other chemical contaminants in the air. Lastly, the MA-112 comes with an optional anion generator to produce a charge on incoming particles to facilitate entrapment.[2] The MA-112 has dual air

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[1] FDA Guidance: "Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency"; Guidance for Industry and Food and Drug Administration Staff; Page 12; March 2020.

[2] https://medifyair.com/collections/air-purifiers/products/ma-112

intakes (on opposing sides) and exhausts the clean air through the top of the device. The MA-112 has four fan speeds to allow adjustment for different room sizes. The approximate volumetric flow rates for each speed are Fan Speed 1 (212 CFM), Fan Speed 2 (290 CFM), Fan Speed 3 (390 CFM) and Fan Speed 4 (518 CFM). Testing was performed using fan speeds 1 and 4 with the anion generator on.

Figure 1: Medify Air MA-112

4. TEST APPROACH

The experiments were performed using a single-chamber method where the MA-112 system was operated within a chamber containing aerosolized virus. The MA-112 efficiency was determined by measuring the rate of decay of viable airborne virus over a specified period of time and compared to the initial challenge concentration and natural decay rate of viable virus over that same time period. The MA-112 system was tested at two operational conditions for each virus as shown in Table 1 below.

Table 1 – MA-112 Test Matrix

Test Trial	Challenge Virus	Fan Speed	Anion Generator
1	Influenza A virus (H1N1)	4 (518 CFM)	ON
2		1 (212 CFM)	ON
3	Human coronavirus (229E)	4 (518 CFM)	ON
4		1 (212 CFM)	ON

The target values for the environmental conditions (temperature and relative humidity) were selected to increase viral viability during the testing based upon previous studies with SARS-

CoV and MERS-CoV.[3] The target virus challenge concentrations (PFU/L) were set to allow reproducible collection within the constraints of the sampling systems and plaque assay detection limits.

Table 2 – Test Conditions

Parameter	Condition 1 Target Value	Condition 2 Target Value
Temperature Range	20 – 25 °C	20 – 25 °C
Relative Humidity*	40 – 50%	40 – 50%
Challenge Concentration	> 10 PFU/L	> 10 PFU/L
Fan Speed / Flow Rate	Low / 212 CFM	High / 518 CFM
Air Exchanges Per Hour	15.9	38.9

*A relative humidity of 65-75% was utilized for testing due to a decrease in viability seen at lower RH's.

5. Test Chamber

Tests were conducted in CUBRC's Biosafety Level 2 (BSL-2) facilities within an 800-cubic foot aerosol test chamber having a 10-foot by 10-foot footprint and a height of 8 feet. The test chamber (Figure 2) is constructed of a powder-coated steel frame and static-dissipative PVC panels with a sealed chamber door for ingress and egress. The test chamber is fully equipped with HEPA filtration systems for both the influent and effluent airflow and is integrated into the building ventilation system to ensure test aerosols are contained and not released to the outside environment. The effluent HEPA filtration system is powered and operates at approximately 590 CFM when operated on its lowest speed. The chamber is equipped with a differential pressure gauge, a temperature and relative humidity monitor, an internal humidifier (for humidity control), lighting, multiple sampling ports, and an aerosol mixing fan.

Twin 6-jet Collison nebulizers were mounted onto a ring stand within the chamber approximately six feet above the floor and connected to a compressed filtered air supply through a bulkhead fitting and ¼ O.D. Teflon tubing. Aerosol samplers were mounted within the chamber approximately 40 inches above the floor and connected to an external vacuum manifold through ½" O.D. bulkhead fittings. The aerosol samplers were positioned at least 18 inches from the chamber wall to avoid possible static effects. Two optical particle size instruments were used for qualitative analyses during the experiments. Both were located outside the chamber and connected through ½" O.D. bulkhead fittings with ½" O.D. stainless steel sample lines extending approximately 18 inches into the chamber interior. Figure 3 below presents a general schematic of the test chamber and its major components and shows the location of the MA-112 system.

[3] P.G. da Silva, M.S.J. Nascimento, R.R.G Soares et. al., *Airborne Spread of Infectious SARS-CoV-2: Moving Forward Using Lessons from SARS-CoV and MERS-CoV*, Science of the Total Environment 764 (2021) 142802

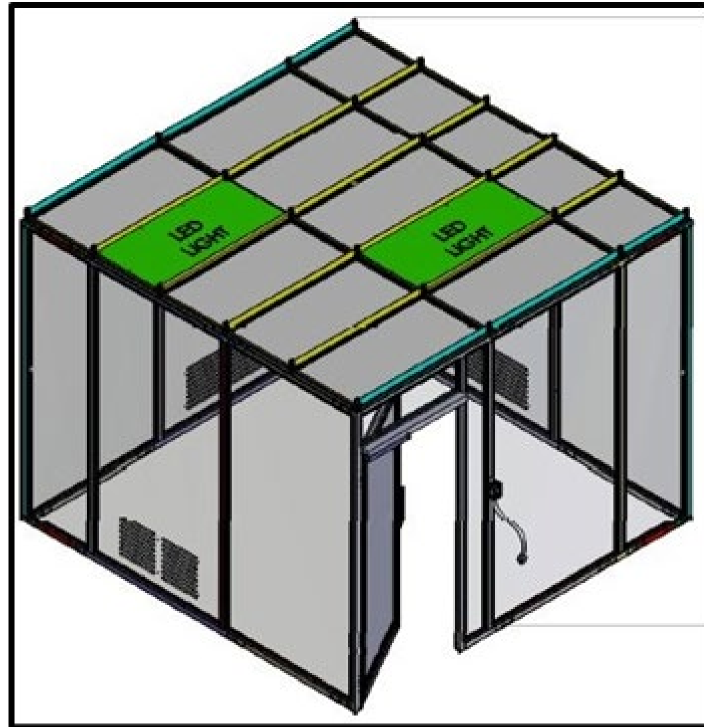


Figure 2: Aerosol Test Chamber

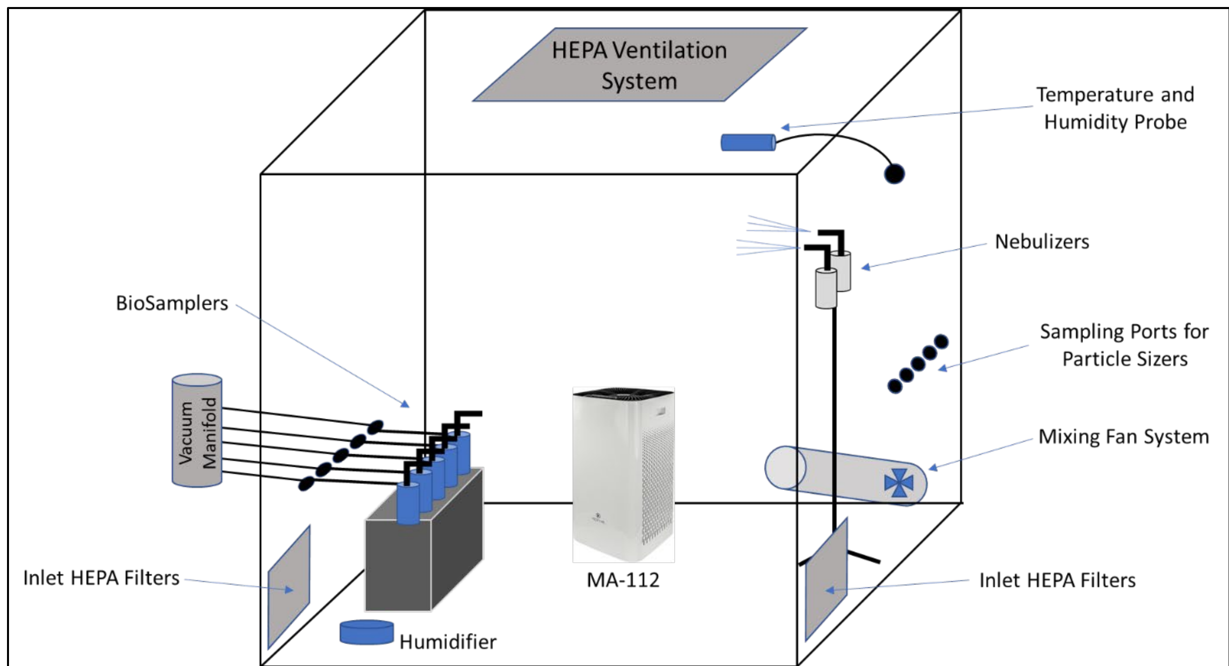


Figure 3: General Schematic of Test Chamber Set-up

6. Procedures

6.1 Pre-Test Activities

CUBRC installed the MA-112 device into the test chamber and provided electrical connections sufficient to operate test system. The MA-112 control panel was removed from the system by INTERTEK and connected to a wiring system to allow the MA-112 to be operated remotely form outside of the chamber. The control panel wiring was fed through a bulkhead fitting and sealed into place. The MA-112 was tested using the wired control panel to ensure that all operating conditions (fan speeds, anion generator) could set and controlled as prescribed in the Test Plan.

After chamber set-up, several pre-studies were performed to validate the operation of all equipment, characterize flow patterns and mixing within the test chamber, validate ventilation systems, and determine the optimal test parameters to meet the target test conditions. A series of safety studies were also conducted to monitor test chamber differential pressure throughout each experimental step. The objective was to validate that a negative ΔP could be maintained through the testing ensuring that no contents from the chamber could leak to the outside laboratory.

6.2 Virus Preparation

Human Coronavirus 229E and Influenza A virus were procured from BEI Resources (www.beiresources.org) and were prepared per BEI Resources recommendations.

Human Coronavirus 229E (HCoV-229E;NR-52726) was propagated using human lung fibroblast cells (MRC-5; ATCC® CCL-171™) in Eagle's Minimum Essential Medium (Corning™ 10010CV) supplemented with 2% fetal bovine serum. Viruses were harvested after 3 days incubation at 35°C and 5% CO_2, concentrated using centrifugal units and titrated.

Influenza A virus strain A/New York/18/2009 (H1N1; NR-15268) was propagated using canine kidney epithelial cells (MDCK; ATCC® CCL-34™) in Eagle's Minimum Essential Medium supplemented with 1 µg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin, 10 mM 4-(2- hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer and 0.125% bovine serum albumin. Viruses were harvested after 3 days incubation at 35°C and 5% CO_2 and titrated.

Once the viral stocks were prepared, daily working stock solutions were made containing a final concentration of 10% glycerol, as this was shown to increase nebulization efficiency and de-agglomeration of microorganisms.

6.3 Control Runs

Two aerosol control runs (one with each organism) were conducted to measure viable virus with the chamber and monitor its natural viability decay rate over a one-hour period. The objective was to achieve target concentrations within the chamber of > 10 PFU per liter of air over the one-hour testing period. The control runs were conducted using the same experimental procedures as discussed in the following paragraphs, except that the MA-112 was not in the chamber.

6.4 Test Runs

6.4.1 Chamber Preparation

The MA-112 was placed into the test chamber and connected to the remote wired control panel located outside of the chamber. Bioaerosol collection devices (SKC BioSamplers®) were filled with 20 mL of Eagle's Minimum Essential Medium, placed into the sample rack within the test chamber and connected to the vacuum lines. One at a time, the samplers were operated at 18 inches of vacuum (inHg) to achieve a nominal collection rate of 12.5 SLPM. The flow rate of each sampler was measured using a 0 to 20 LPM NIST-traceable, calibrated flow meter (Aalborg, Inc.) and the flow rates were recorded. Following the flow rate verifications, each SKC BioSampler® was outfitted with a clean collection reservoir containing 20 mL of Eagle's Minimum Essential Medium collection fluid.

The two 6-jet Collison nebulizers (BGI, Inc.) containing 30 mL of stock aqueous suspensions of the target virus were mounted onto the ring stand and connected to the external purified air supply. The test chamber was closed and filtered for approximately 30 minutes (to below background levels) by turning on the ventilation system and removing the inlet HEPA filter covers. Qualitative background particle measurements were made using a TSI Aerodynamic Particle Sizer (APS) Model 3320 and a TSI Optical Particle Sizer (OPS) Model 3330 until particle counts were < 5 particles (pt) per cubic centimeter (cm^3). Typical laboratory background particle counts were approximately 65 pt/cm^3.

Once the chamber was cleared down, one 10-minute air sample was collected using an SKC BioSampler® to verify the absence of viable virus prior to the test. Following completion of this sample, the mixing fan was turned on. The humidifier was turned on until the relative humidity reached 70% (about 3 to 4 minutes) as verified with a calibrated probe (Omega Model RHXL5SD).

6.4.2 Experimental Procedure

Challenge virus was introduced into the test system for 30 minutes using two 6-jet Collison nebulizers operated at an inlet air pressure of 20 psi. At this inlet pressure the output volumetric flow rate of each nebulizer is approximately 12 L/min and virus fluid dissemination rate is around 0.15 mL/min. At the completion of the aerosol dissemination period, the source air to the nebulizers was shut off and the mixing fan was turned off to allow the aerosol to settle for two to three minutes. A single aerosol sample was collected for five to ten minutes using one of the SKC BioSamplers®. This sample served as the initial challenge verification to determine the starting concentration of viable virus in units of PFU/L.

The MA-112 was turned on at the prescribed test conditions and this time was designated t=0. Over the course of the next 60 minutes, four sequential aerosol samples were collected using the SKC BioSamplers® at the following intervals: t = 0 to 10 minutes, 10 to 20 minutes, 30 to 40 minutes and 50 to 60 minutes.

During the experiments, the optical particle counters were used to qualitatively measure particle counts within the tests chamber. The systems were disconnected during the aerosol dissemination periods as the particle counts were much too high for accurate measurement.

Once the MA-112 system was turned on, the systems were reconnected to allow test personnel to observe real-time particle removal over the duration of the experiment.

After the last bioaerosol sample was collected (t=60 minutes), the MA-112 was powered off. The test chamber ventilation system was turned on to purge clean filtered air through the chamber to remove remaining aerosolized virus. This ventilation was performed for a minimum of 30 minutes and until particle counts fell below 10 pt/cm^3. The chamber and all equipment within were decontaminated with 70% ethanol. The chamber was then allowed to ventilate overnight.

Collection fluid from the SKC BioSamplers® was transferred to conical tubes. The exact volume of fluid from each sampler was measured using a graduated pipette and recorded. Conical tubes were placed on ice and transferred to a biosafety cabinet for processing.

6.4.3 Sample Processing

On the day before a test trial, 12-well assay plates (one per test sample) were prepared by seeding each well with the appropriate cell line and incubating overnight to produce host cell monolayers in each well at approximately 90% confluency. On each plate, three wells were dedicated to controls and the remaining nine wells was used for triplicate analyses of test sample (typically undiluted, 10-fold and 100-fold diluted).

Liquid aliquots were taken from the SKC BioSampler® reservoirs and appropriately diluted for analysis in the medium suitable for each virus, supplemented with antibiotic and antimycotics. Aliquots of each dilution level were transferred onto 12-well plates containing the confluent monolayers of host cells as described above. The plates were incubated at 35 °C for one hour with CO_2 and gently rocked every 15 minutes to promote virus adsorption. After the initial 1-hour incubation, the dilution aliquots were removed from each well and an overlay of microcrystalline cellulose was added to each well. The plates were incubated at 35 °C for 72 hours.

After completion of the 72-hour incubation period, the microcrystalline cellulose overlays were removed, and formalin was added to each well. The plates were incubated for 15 min to allow for cell fixation and virus inactivation. The formalin was removed, and each well was washed with water, stained with crystal violet, and incubated for 10 minutes. After incubation, the crystal violet was removed, each well was washed with water, and the plates were allowed to dry. Once the plates were dry, each well was inspected for plaques (indicating the presence of live virus) which were then counted and recorded.

7. Calculations and Data Analysis

The raw data recorded for the chamber aerosol samples includes the aerosol sampler serial number, the sample flow rate, the sample duration, the average elapsed test time (the midpoint between the start time and stop time of the sampling interval), the total volume of chamber air sampled (flow rate multiplied by the sample duration) and the volume of collection fluid removed from the SKC BioSampler® reservoir. An example data collection table for a single test trial is shown in the figure below.

Test ID	SKC Sampler	Sample Time	Average Elapsed	Flow Rate	Volume Sampled	Liquid Volume
No.	No.	[min]	[min]	[LPM]	[L]	[mL]
Chall.	2330	5	0.0	12.6	63.0	19.40
1	2350	10	5.0	12.5	125.0	18.90
2	2355	10	15.0	12.6	126.0	18.80
3	2357	10	35.0	12.6	126.0	18.20
4	2368	10	55.0	12.7	127.0	17.80

Figure 3: Example Data Collection Table

The collection fluids for each sample were diluted and processed as described in Section 6.4.3 above. The viable virus concentration within the chamber was determined by

$$C_{CF} = PFU_{well} / (DF \times V)$$

where:

C_{CF} = Concentration of viruses in the collector fluid in PFU/mL
PFU_{well} = Number of Plaque forming units per well, read from the plaque assay
DF = dilution factor
V= Volume of virus applied to the well
and

$$C_{TC} = \frac{C_{CF} \times V_{CF}}{Vc}$$

where:

C_{TC} = Concentration within the test chamber in PFU/L
V_{CF} = Volume of the collector fluid in mL
Vc = Total volume of air sampled from the test chamber in L

The performance of the MA-112 was determined by calculating the concentration of viable virus in the test chamber at each time point and comparing the values to those generated in the control experiments where the MA-112 was not operated. Removal rates were also calculated by comparing the viable virus concentration in the chamber to the baseline challenge concentration.

8. Quality Assurance and Quality Control

8.1 SKC BioSampler® Flow Rate

Before each test trial the sample flow rate of each SKC BioSampler® was measured using a calibrated flow meter. The flow rate measurements are used in all calculations where concentrations of viable aerosolized virus are determined.

8.2 SKC BioSampler® Fluid Volume

The volume of collection fluid was drawn out of each sampler reservoir using an electronic pipettor and a graduated 10-mL pipette. Prior to transfer into conical tubes, the total volume

within the pipette was measured and recorded. A new, sterile pipette was used for each sample. The volume of collection fluid was used in all calculations where concentrations of viable aerosolized virus are determined.

8.3 Test Chamber Negative Controls

At least one air sample was collected from the test chamber prior to each test to verify the absence of viable aerosolized virus at the beginning of each experiment.

8.4 Plaque Assay Negative Controls

Each 12-well plate used for the plaque assays contained three (3) wells dedicated to negative control samples. The purpose is to ensure the absence of cell contamination and the absence of virus contamination.

8.5 Challenge Concentration Measurements

Challenge verification air samples were collected from the test chamber following the completion of nebulization for each experiment. These measurements establish the baseline challenge concentration prior to operation of the MA-112. The purpose of these measurements is to verify that viable, airborne virus is present in the test chamber and to quantify the concentration to assure that the challenge levels met the target test parameters.

8.6 Viral Stock Solution Titer

The viral stock solution used for the generation of the bioaerosols was and analyzed on each day that a test was performed. Samples were collected prior to loading in into the nebulizer and again after completion of the test run(s). These analyses confirm the presence and concentration of viable virus within the challenge fluids. The purpose is to provide information to test personnel should the chamber challenge aerosol concentrations not meet target and/or expected levels. These values are not used in any calculations.

9. Test Results

9.1 H1N1 Test Results

Tables 3 and 4 below present the test results for the two H1N1 test runs. The concentration of viable airborne virus within the test chamber at each time period was calculated as described previously and compared to the starting concentration for each experiment. Removal efficiency as a percent of the starting challenge concentration was also calculated. The detection limit of the plaque assay is one PFU, which correlates to a chamber concentration of 0.05 PFU/L, therefore an assay that resulted in zero plaque forming units being observed is reported as less than 0.05 PFU/L.

As can be seen from the tables, the MA-112, when operated in an 800-cubic foot room at a fan speed of 4 (high) with the ionizer on, demonstrated a removal efficiency of at least 99.94% within the first five minutes. No viable airborne virus was observed for any of the four samples collected over the 60-minute test duration. This result is consistent with what would be expected at an hourly air exchange rate of 38.9.

The MA-112, when operated at the lowest fan speed (speed 1) with the ionizer on in an 800-cubic foot room, demonstrated a removal efficiency of 99.81% within the first five minutes and by the 15-minute point, was exceeding 99.95% removal efficiency. No viable airborne virus was observed at the 15-, 35- and 55-minute time points.

Table 3 – MA-112 Performance against H1N1 (Fan Speed 4; Anion Generator On)

Elapsed Test Time [min]	Chamber Concentration [PFU/L]	Removal Efficiency [%]
0.0	79.0	-
5.0	< 0.05	99.94
15	< 0.05	99.94
35	< 0.05	99.94
55	< 0.05	99.94

Table 4 – MA-112 Performance against H1N1 (Fan Speed 1; Anion Generator On)

Elapsed Test Time [min]	Chamber Concentration [PFU/L]	Removal Efficiency [%]
0.0	107.8	-
5.0	0.2	99.81
15	< 0.05	99.95
35	< 0.05	99.95
55	< 0.05	99.95

The data presented above are calculated in absolute terms, meaning that all calculations are based upon the starting concentration of viable virus at t=0. It is well established that airborne viruses exhibit a natural decay rate (i.e., they naturally become inactive over time), and this was confirmed through the control tests that were performed prior to the MA-112 experiments. The control experiments showed that the decrease in viability over time for airborne H1N1 followed an exponential decay function:

$$y = C_0\, e^{-0.1x}$$

where:

C_0 = the starting challenge concentration within the chamber at t=0.
x = the elapsed test time

Figures 4 and 5 below present the test data showing the MA-112 performance against the calculated virus viability natural decay rate (i.e., the expected virus viability over time if the

MA-112 were not used) for both experiments. Note that in both cases, the MA-112 demonstrates substantial improvement compared to the expected natural decay in viability.

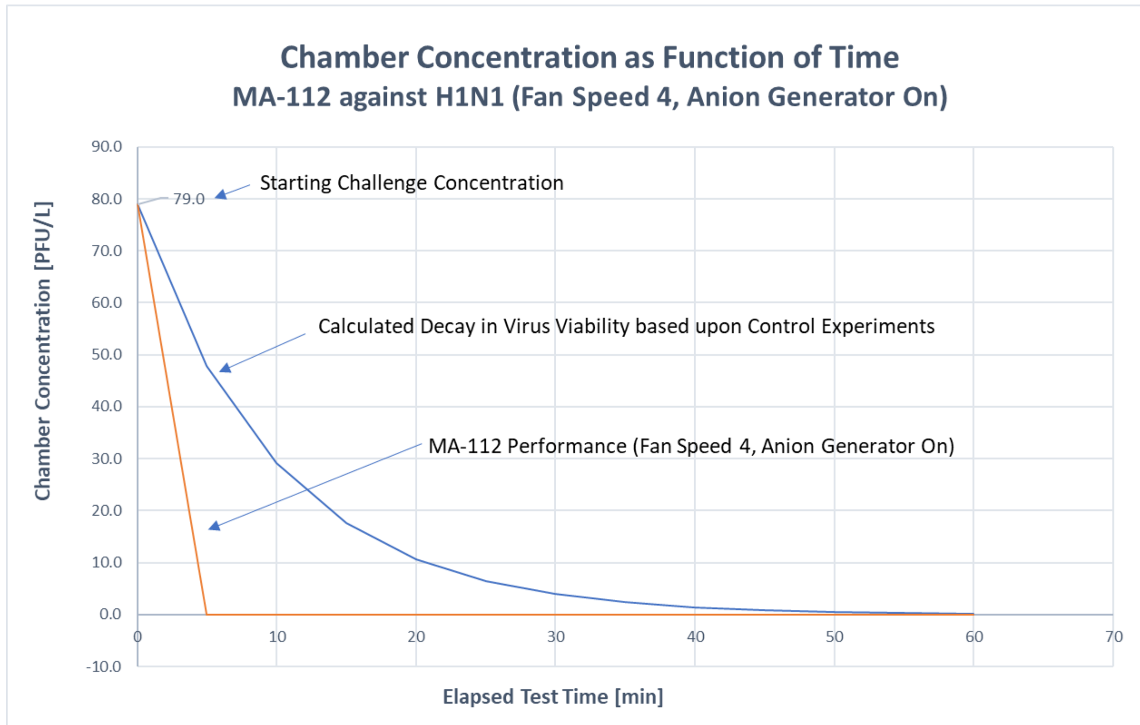


Figure 4: MA-112 Performance vs. H1N1 at Fan Speed 4 with Anion Generator On

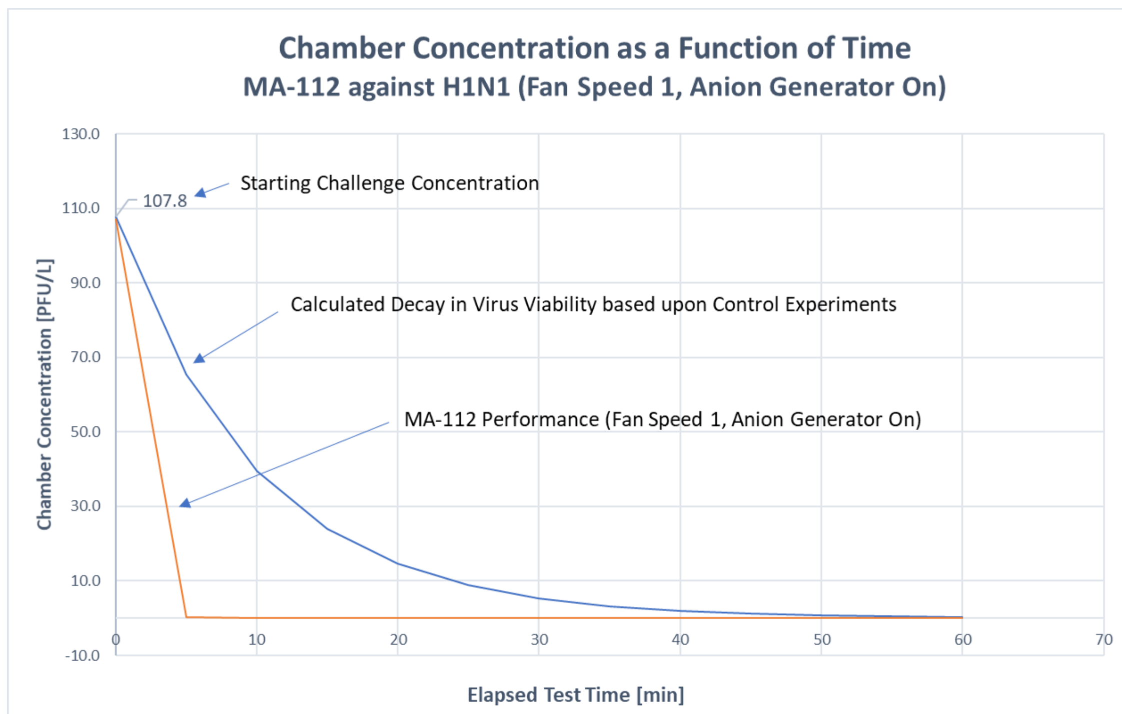


Figure 5: MA-112 Performance vs. H1N1 at Fan Speed 1 with Anion Generator On

9.2 HCoV-229 Test Results

Tables 5 and 6 below present the test results for the two HCoV-229E test runs. The concentration of viable airborne virus within the test chamber at each time period was calculated as described previously and compared to the starting concentration for each experiment. Removal efficiency as a percent of the starting challenge concentration was also calculated. The detection limit of the plaque assay is one PFU, which correlates to a chamber concentration of 0.05 PFU/L, therefore an assay that resulted in zero plaque forming units being observed is reported as less than 0.05 PFU/L.

When operated in an 800-cubic foot room at a fan speed of 4 (high) with the ionizer on, the MA-112 demonstrated a HCoV-229E removal efficiency of at least 99.95% within the first five minutes, which is consistent with the H1N1 test results. No viable airborne virus was observed for any of the four samples collected over the 60-minute test duration. This result is consistent with what would be expected at an hourly air exchange rate of 38.9.

For HCoV-229E, the MA-112, when operated at the lowest fan speed (speed 1) with the ionizer on in an 800-cubic foot room, demonstrated a removal efficiency of 97.54% within the first five minutes and by the 15-minute point, was exceeding 99.94% removal efficiency. No viable airborne virus was observed at the 15-, 35- and 55-minute time points.

Table 5 – MA-112 Performance against HCoV-229E (Fan Speed 4; Anion Generator On)

Elapsed Test Time [min]	Chamber Concentration [PFU/L]	Removal Efficiency [%]
0.0	107.8	-
5.0	< 0.05	99.95
25	< 0.05	99.95
35	< 0.05	99.95
55	< 0.05	99.95

Table 6 – MA-112 Performance against HCoV-229E (Fan Speed 1; Anion Generator On)

Elapsed Test Time [min]	Chamber Concentration [PFU/L]	Removal Efficiency [%]
0.0	86.46	-
5.0	2.13	97.54
15	< 0.05	99.94
35	< 0.05	99.94
55	< 0.05	99.94

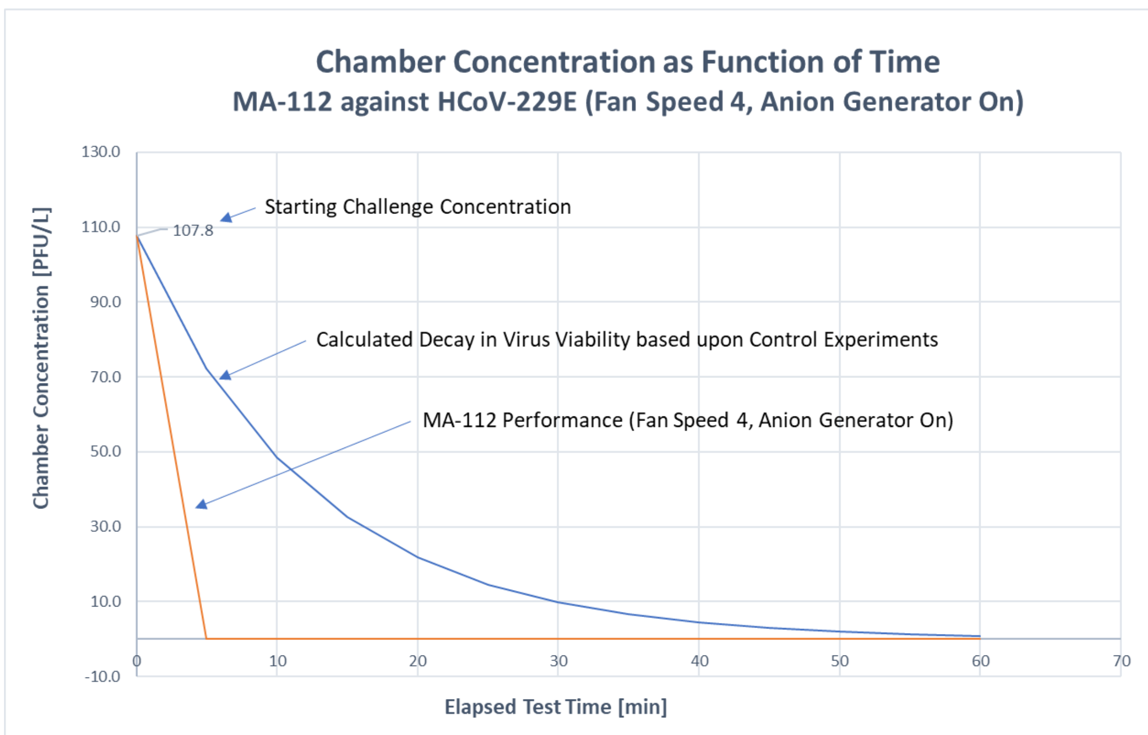
The data presented above are calculated in absolute terms, meaning that all calculations are based upon the starting concentration of viable virus at t=0. It is well established that airborne viruses exhibit a natural decay rate (i.e., they naturally become inactive over time), and this was confirmed through the control tests that were performed prior to the MA-112 experiments. The control experiments showed that the decrease in viability over time for airborne HCoV-229E followed an exponential decay function that was very consistent with what was observed with the H1N1 virus:

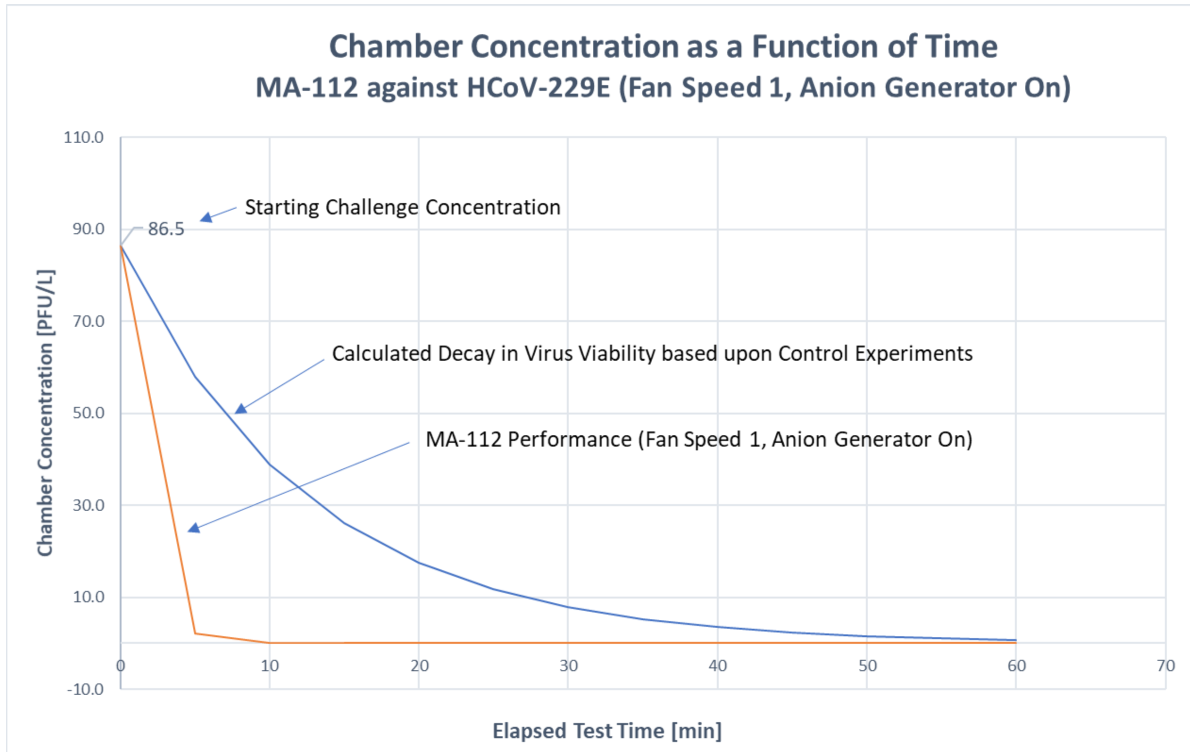
$$y = C_0\, e^{-0.08x}$$

where:

C_0 = the starting challenge concentration within the chamber at t=0.
x = the elapsed test time

Figures 6 and 7 below present the test data showing the MA-112 performance against the calculated virus viability natural decay rate (i.e., the expected virus viability over time if the MA-112 were not used) for both experiments. Note that in both cases, the MA-112 demonstrates substantial improvement compared to the expected natural decay in viability.




Chamber Concentration as a Function of Time
MA-112 against HCoV-229E (Fan Speed 1, Anion Generator On)
Starting Challenge Concentration
86.5
Calculated Decay in Virus Viability based upon Control Experiments
MA-112 Performance (Fan Speed 1, Anion Generator On)
Chamber Concentration [PFU/L]
110.0
90.0
70.0
50.0
30.0
10.0
-10.0
0
10
20
30
40
50
60
70
Elapsed Test Time [min]

APPENDIX A – RAW AND REDUCED DATA SETS

A.1 H1N1 Testing

A.1.1 Viral Stock Solution Concentration in Aerosol Nebulizer (Pre and Post Test)

Nebulizer Concentration (PFU/mL)		
Volume Plated = 1.0 mL	**Pre-Test**	**Post-Test**
Dilution Factor:	1.0E-06	1.0E-06
Plate Count 1:	26	37
Plate Count 2:	26	38
Plate Count 3:	33	35
Average:	28.3	36.7
Stock Titer:	**2.83E+07**	**3.67E+07**

A.1.2 Flu Test A: Fan Speed 4 (HIGH); Ion Generator ON

The H1N1 flu tests were run on 03 August 2021.

Table A-1: Raw Viable Virus Counts from Collector Fluid

Collector Fluid Concentration [PFU/mL]					
Volume Plated = 1.0 mL	**Challenge**	**#1**	**#2**	**#3**	**#4**
Dilution Factor:	1.0E-01	None	None	None	None
Plate Count 1:	33	0	0	0	0
Plate Count 2:	22	0	0	0	0
Plate Count 3:	22	0	0	0	0
Average:	25.7	0.3	0.3	**0.3**	0.3
Concentration:	**2.57E+02**	**3.33E-01**	**3.33E-01**	**3.33E-01**	**3.33E-01**

Table A-2: Flu Test A; Fan Speed 4 (HIGH) Results Calculation Table

Sample ID	SKC Sampler	Sample Time	Average Elapsed	Flow Rate	Volume Sampled	Liquid Volume	Sample Fluid Concentration	Total Virus Collected	Chamber Concentration	Absolute Removal
No.	No.	[min]	[min]	[LPM]	[L]	[mL]	[PFU/mL]	[PFU]	[PFU/L]	[%]
Chall.	2330	5	0.0	12.6	63.0	19.40	2.57E+02	4.98E+03	79.04	-
1	2350	10	5.0	12.5	125.0	18.80	< 3.33E-01	< 6.27E+00	< 0.05	> 99.94%
2	2355	10	15.0	12.6	126.0	18.40	< 3.33E-01	< 6.13E+00	< 0.05	> 99.94%
3	2357	10	35.0	12.6	126.0	18.70	< 3.33E-01	< 6.23E+00	< 0.05	> 99.94%
4	2368	9	55.0	12.7	114.3	18.80	< 3.33E-01	< 6.27E+00	< 0.05	> 99.94%

The virus assay detection limit is 1 PFU. The shaded cells indicate results and calculations that are based upon analyses where no viable virus was observed. Therefore the Sample Fluid Concentration, the Total Virus Collected and the Chamber Concentration are presented as “less than” the reported value. Because all calculations must use the detection limit (1 PFU), the removal efficiency is presented as “greater than” the reported value.

A.1.3 Flu Test B: Fan Speed 1 (LOW); Ion Generator ON

Table A-3: Raw Viable Virus Counts from Collector Fluid

Collector Fluid Concentration [PFU/mL]					
Volume Plated = 1.0 mL	**Challenge**	**#1**	**#2**	**#3**	**#4**
Dilution Factor:	1.0E-01	None	None	None	None
Plate Count 1:	40	1	0	0	0
Plate Count 2:	33	2	0	0	0
Plate Count 3:	32	1	0	0	0
Average:	35.0	1.3	0.0	0.0	0.0
Concentration:	**3.50E+02**	**0.00E+00**	**0.00E+00**	**0.00E+00**	**0.00E+00**

Table A-4: Flu Test B; Fan Speed 1 (LOW) Results Calculation Table

Sample ID	SKC Sampler	Sample Time	Average Elapsed	Flow Rate	Volume Sampled	Liquid Volume	Sample Fluid Concentration	Total Virus Collected	Chamber Concentration	Absolute Removal
No.	No.	[min]	[min]	[LPM]	[L]	[mL]	[PFU/mL]	[PFU]	[PFU/L]	[%]
Chall.	2330	5	0.0	12.6	63.0	19.40	3.50E+02	6.79E+03	107.78	-
1	2350	10	5.0	12.5	125.0	18.90	1.33E+00	2.52E+01	0.20	99.81%
2	2355	10	15.0	12.6	126.0	18.80	< 3.33E-01	< 6.27E+00	< 0.05	> 99.95%
3	2357	10	35.0	12.6	126.0	18.20	< 3.33E-01	< 6.07E+00	< 0.05	> 99.96%
4	2368	10	55.0	12.7	127.0	17.80	< 3.33E-01	< 5.93E+00	< 0.05	> 99.96%

The virus assay detection limit is 1 PFU. The shaded cells indicate results and calculations that are based upon analyses where no viable virus was observed. Therefore the Sample Fluid Concentration, the Total Virus Collected and the Chamber Concentration are presented as “less than” the reported value. Because all calculations must use the detection limit (1 PFU), the removal efficiency is presented as “greater than” the reported value.

A.2 HCoV-229E Testing

A.2.1 Viral Stock Solution Concentration in Aerosol Nebulizer (Pre and Post Test)

Nebulizer Concentration (PFU/mL)		
Volume Plated = 1.0 mL	**Pre-Test**	**Post-Test**
Dilution Factor:	1.0E-06	1.0E-06
Plate Count 1:	10	10
Plate Count 2:	7	13
Plate Count 3:	13	4
Average:	10.0	9.0
Stock Titer:	**1.00E+07**	**9.00E+06**

A.2.2 HCoV-229E Test A: Fan Speed 4 (HIGH); Ion Generator ON

The HCoV-229E tests were conducted on 10 August 2021.

Table A-5: Raw Viable Virus Counts from Collector Fluid

Collector Fluid Concentration [PFU/mL]					
Volume Plated = 1.0 mL	**Challenge**	**#1**	**#2**	**#3**	**#4**
Dilution Factor:	1.0E-01	None	None	None	None
Plate Count 1:	36	0	0	0	0
Plate Count 2:	40	0	0	0	0
Plate Count 3:	35	0	0	0	0
Average:	37.0	0.0	0.0	0.0	0.0
Concentration:	**3.70E+02**	**0.00E+00**	**0.00E+00**	**0.00E+00**	**0.00E+00**

Table A-6: HCoV-229E Test A; Fan Speed 4 (HIGH) Results Calculation Table

Sample ID	SKC Sampler	Sample Time	Average Elapsed	Flow Rate	Volume Sampled	Liquid Volume	Sample Fluid Concentration	Total Virus Collected	Chamber Concentration	Absolute Removal
No.	No.	[min]	[min]	[LPM]	[L]	[mL]	[PFU/mL]	[PFU]	[PFU/L]	[%]
Chall.	2330	5	0.0	12.7	63.5	18.50	3.70E+02	6.85E+03	107.80	-
1	2350	10	5.0	12.4	124.0	18.80	< 3.33E-01	< 6.27E+00	< 0.05	> 99.95%
2	2355	10	25.0	12.6	126.0	18.50	< 3.33E-01	< 6.17E+00	< 0.05	> 99.95%
3	2357	10	35.0	12.5	125.0	18.40	< 3.33E-01	< 6.13E+00	< 0.05	> 99.95%
4	2368	10	55.0	12.6	126.0	18.00	< 3.33E-01	< 6.00E+00	< 0.05	> 99.96%

The virus assay detection limit is 1 PFU. The shaded cells indicate results and calculations that are based upon analyses where no viable virus was observed. Therefore the Sample Fluid Concentration, the Total Virus Collected and the Chamber Concentration are presented as “less than” the reported value. Because all calculations must use the detection limit (1 PFU), the removal efficiency is presented as “greater than” the reported value.

A.2.3 HCoV-229E Test B: Fan Speed 1 (LOW); Ion Generator ON

Table A-7: Raw Viable Virus Counts from Collector Fluid

Collector Fluid Concentration [PFU/mL]					
Volume Plated = 1.0 mL	**Challenge**	**#1**	**#2**	**#3**	**#4**
Dilution Factor:	1.0E-01	None	None	None	None
Plate Count 1:	30	14	0	0	0
Plate Count 2:	36	18	0	0	0
Plate Count 3:	25	13	0	0	0
Average:	30.3	15.0	0.0	0.0	0.0
Concentration:	**3.03E+02**	**1.50E+01**	**0.00E+00**	**0.00E+00**	**0.00E+00**

Table A-8: HCoV-229E Test B; Fan Speed 1 (LOW) Results Calculation Table

Sample ID	SKC Sampler	Sample Time	Average Elapsed	Flow Rate	Volume Sampled	Liquid Volume	Sample Fluid Concentration	Total Virus Collected	Chamber Concentration	Absolute Removal
No.	No.	[min]	[min]	[LPM]	[L]	[mL]	[PFU/mL]	[PFU]	[PFU/L]	[%]
Chall.	2330	5	0.0	12.7	63.5	18.10	3.03E+02	5.49E+03	86.46	-
1	2350	10	5.0	12.4	124.0	17.60	1.50E+01	2.64E+02	2.13	97.54%
2	2355	10	15.0	12.6	126.0	18.50	< 3.33E-01	< 6.17E+00	< 0.05	> 99.94%
3	2357	10	35.0	12.5	125.0	18.00	< 3.33E-01	< 6.00E+00	< 0.05	> 99.94%
4	2368	10	55.0	12.6	126.0	18.00	< 3.33E-01	< 6.00E+00	< 0.05	> 99.94%

The virus assay detection limit is 1 PFU. The shaded cells indicate results and calculations that are based upon analyses where no viable virus was observed. Therefore the Sample Fluid Concentration, the Total Virus Collected and the Chamber Concentration are presented as “less than” the reported value. Because all calculations must use the detection limit (1 PFU), the removal efficiency is presented as “greater than” the reported value.