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SARS-CoV-2 USA-CA1/2020

CLIENT: AIRQUALITY TECHNOLOGY (SHANGHAI) CO. LTD

PROJECT: SARS-CoV-2 MESP® AIR STERILIZATION TECHNOLOGY

PRODUCT: (FAH01M-A) MESP® AIR STERILIZING PURIFIER

CAP LIC NO: 886029801

CLIA LIC NO: O5D0955926

STATE ID: CLF 00324630

CHALLENGE VIRUS: SARS-CoV-2 USA-CA1/2020

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ABSTRACT: EFFICACY OF THE AIRQUALITY AHU-MESP AIR PURIFIER AGAINST SARS-CoV-2

Background: This in vitro study was designed to determine the efficacy of an MESP technology electrostatic air filter designed for HVAC systems. The product was provided for testing by AirQuality and is a commercially available in-duct mechanical electrostatic filtration system. The AirQuality AHU-MESP® HVAC sterilizing purifier is designed to be placed inside the HVAC ductwork or air handling unit (AHU) of a facility to decrease the spread of pathogens throughout the HVAC system while it is operating. For this challenge, the SARS-CoV-2 USA-CA1/2020 pathogen was used. Coronavirus can be spread through the air and by touching contaminated surfaces. There is a demand for disinfectant devices that have a proven ability to reduce infectious pathogens in the air thereby reducing the risk of human infection and transmission. AirQuality supplied a pre-packaged AHU-MESP® air sterilizing purifier. For the testing, power was supplied through a step-up ITU-3000 regulator for 220v with surge protector and backup battery system. Test procedures were followed using internal SOPs for aerosolized viral pathogen challenges and subsequent decontamination. All internal SOPs and processes follow GCLP guidelines and recommendations.

Results: When tested against SARS-CoV-2 USA-CA1/2020 virus, the presence of the electrostatic filter inside the modified HVAC ducting showed a reduction of detectable pathogen at the downstream collection point. Under optimal conditions with a pre-defined CFM the system was able to achieve a 99.99% reduction of recoverable viral media in the airstream of the testing system.

EQUIPMENT PROVIDED:

MANUFACTURER: AIRQUALITY TECHNOLOGY (SHANGHAI) CO., LTD.

MODEL: FAH01M-A

SERIAL #: 0401005320C08010016

AIRQUALITY EQUIPMENT:

The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. The electrostatic filter came pre-wired with the 220v plug and needed no adjustment or alteration from lab staff. Functionality was confirmed visually with the light activation over the power symbol when the device was turned on.

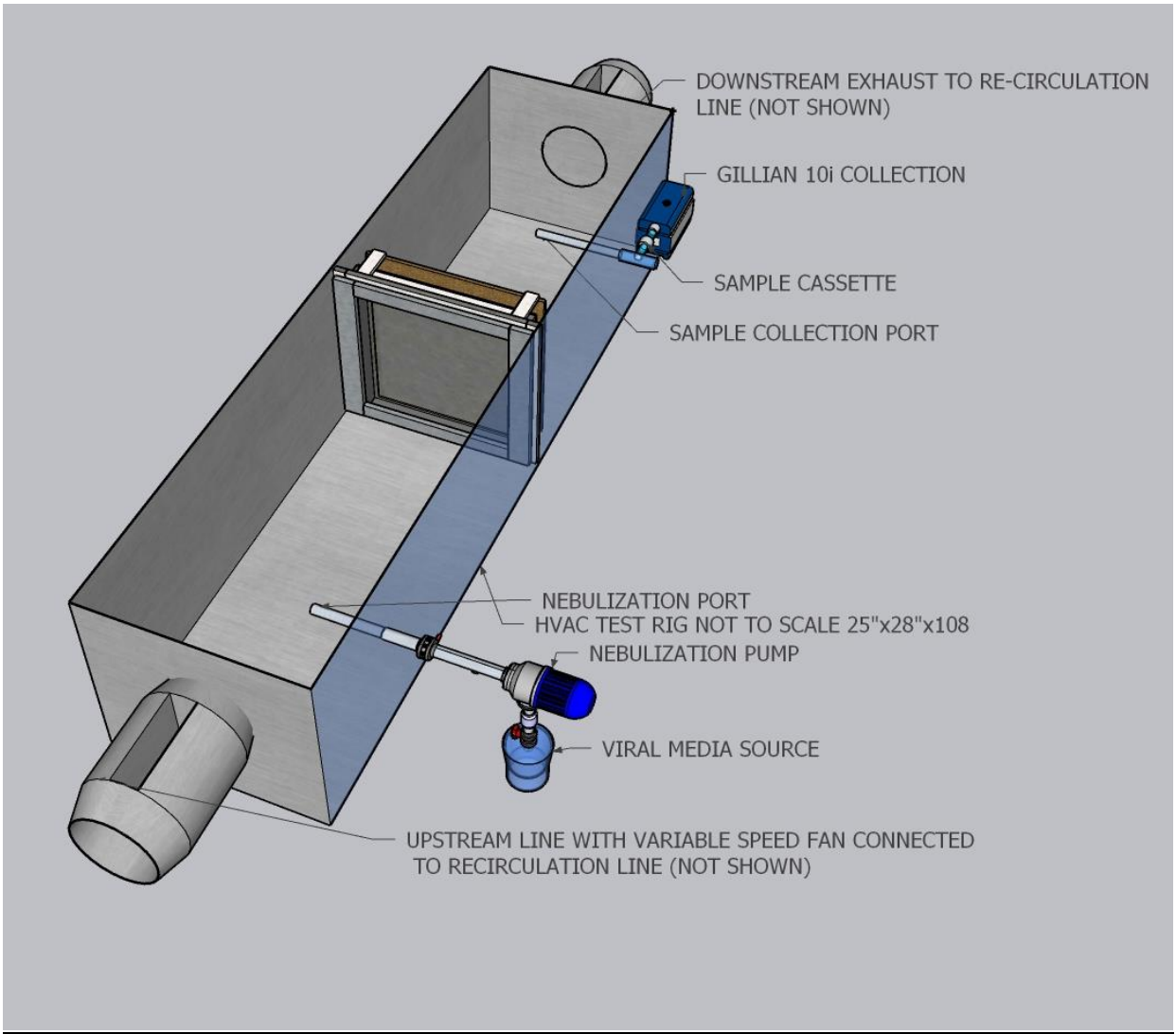
VIRAL CHALLENGE TESTING CHAMBER: MODIFIED ASHRAE 185.1

The test was conducted inside a sealed controlled BSL3 chamber pod using a standard sized galvanized metal HVAC duct which was modified for testing purposes. The custom HVAC testing rig consisted of three sections which each measured approximately 25”x28”x36”. The airflow was looped from the end of the test chamber on the downstream side back to the upstream side through a 6” circular flexible duct. Airflow in the ducting system was controlled using a 6” variable speed fan connected to the 6” ducting prior to the expansion to the 24”x28”x36” upstream section of the test rig. HVAC testing system was constructed to meet internal SOP requirements and all seams were sealed. During testing the ambient temperature was 71°F with a humidity of 46%. Prior to testing, the HVAC rig was pressure tested using an air compressor and analog PSI meter. HVAC system was pressurized and maintain internal pressure for 20 minutes confirming no leaks were present.

The BSL3 environment the HVAC rig was placed inside was a large negative pressure lab with the pressure continuously monitored. The lab designed to meet current BSL3 standards and had a redundant dedicated HEPA HVAC extraction system. The testing chamber was equipped with 2 sealed viewing windows, anti-chamber with key card accessible and lockable chamber door for entry and exit.

Prior to testing, the chamber was pressure tested to confirm negative pressure was maintained and visual inspections were made using a colored smoking device. All seals for the chamber were confirmed and all equipment used had a function tests to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status. The testing environment was based on a modified ISO 15714 and ASHRAE 185.1 standard.

TEST ENVIRONMENT:



EXPERIMENTAL SUMMARY:

- Prior to the initial control test and following each trial run the testing area was decontaminated and prepped per internal procedures.
- Testing rig was for viral efficacy and not to measure penetration correlation testing.
- Temperature during all test runs was approximately 71F +/- 2F with a relative humidity of 46%.
- Air samplers were calibrated by the manufacturer on September 3, 2020 and set at a standard flow of 5.02L/min. Calibration records indicate a 0.20% tolerance.
- All sample collection volumes were set to 15-minute continual draws.
- Nebulization for control and viral test challenges were performed in the same manner.
- Prior to control runs and challenge run the electrostatic filter was turned on and a dry run with a fine smoke was pushed through the testing rig to confirm the filter was powered on.
- For each test run a known quantity of viral media was nebulized into the air stream on the upstream side of the filtration unit.
- Viral media was nebulized at a constant rate for 10 minutes.
- During each pathogen challenge air samples were continuously taken from the airflow on the downstream.
- Bioaerosol sampling was not collected on the upstream side as two control tests were conducted without the filter present to confirm 0 filtration values.
- Air sampling collection occurred for the 10 minutes during the nebulization period and 5 minutes after nebulization ceased for a total of 15 minutes per challenge.
- Test condition had the AirQuality filter positioned in the center of the test rig.
- The velocity of airflow on the upstream side was approximately a consistent 500FT/min and was confirmed using a vane anemometer.
- Sample cassettes were manually removed from the collection system after each control run and each air pass challenge.
- Upon cassette removal after each challenge, cassette sets were taken to an adjacent bio safety cabinet for extraction and placement into viral suspension media.
- 2 controls were completed, and 3 viral challenges were completed using the same methodology.

BIOAEROSOL GENERATION:

For the control and the viral challenges, the nebulizer was filled with the same amount of viral stock 6.02 x 10^6 TCID50 per mL and nebulized at a flow rate of 1ml/min. Nebulizer was driven by untreated local atmospheric air. The nebulizer's remaining viral stock volume was weighed after each completion to confirm the same amount of viral stock that was nebulized. Bioaerosol charge neutralizer was not used due to the alternate nebulization system chosen over the six-jet variant. Nebulizer used an adjustable nozzle; the average particle size was measured prior to testing at .8-2.0µm.

BIOAEROSOL SAMPLING:

For air sampling 1 Gillian 10i programmable vacuum device was used. Air samplers were calibrated by the manufacturer in September 2020 and certificates were inspected prior to use. Air sample volume collections were confirmed prior to use with a Gilian Gilibrator 2 SN- 200700-12 and a high flow bubble generator SN-2009012-H. Air sampler was operated in conjunction with removable sealed cassettes, which were manually removed after each challenge. Cassettes had a delicate internal filtration disc to collect viral samples.

VIRUS STRAIN BACKGROUND:

The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through the BEI Resources, BIAID, NIH SARS-Related Coronavirus 2, Isolate USA-CA1/2020, NR-52382.

POST DECONTAMINATION:

At the conclusion of each viral challenge test the UV system inside the testing chamber was activated for 30 minutes. After 30 minutes of UV exposure the chamber was fogged with a Hydrogen Peroxide gas mixture followed by a 30-minute air purge. All test equipment was cleaned at the end of each day with a 70% alcohol solution. Collection lines were soaked in a bleach bath mixture for 30 minutes then rinsed repeatedly with DI water. Nebulizer and Vacuum collection pumps were decontaminated with Hydrogen Peroxide mixtures.

TCID50 PROCEDURE:

Materials and Equipment:

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips – 20uL, 200uL, 1000uL.
- Inverted Microscope
- Tubes for dilution
- Hemocytometer with cover slip
- Cell Media for infection
- Growth Media appropriate for cell line
- 0.4 % Trypan Blue Solution
- Lint Free Wipes saturated with 70% isopropyl alcohol
- CO_2 Incubator set at 37°C or 34°C or other temperature indicated.

Procedure:

1. One day prior to infection, prepare 96 well dishes by seeding each well with Vero E6 cells in DMEM plus 7.5 % fetal bovine serum, 4mM Glutamine, and antibiotics.
2. On the day of infection, make dilutions of virus sample in PBS.
3. Make a series of dilutions at 1:10 of the original virus sample. First tube with 2.0 mL PBS and subsequent tubes with 1.8mL
4. Vortex Viral samples, transfer 20 uL of virus to first tube, vortex, discard tip.
5. With new tip, serial dilute subsequent tips transferring 200 uL.

Additions of virus dilutions to cells

1. Label lid of 96 well dish by drawing grid lines to delineate quadruplicates and number each grid to correspond to the virus sample and label the rows of the plate for the dilution which will be plated.
2. Include 4 Negative wells on each plate which will not be infected.
3. Remove all but 0.1 mL of media from each well by vacuum aspiration.
4. Starting from the most dilute sample, add 0.1 mL of virus dilution to each of the quadruplicate wells for that dilution
5. Infect 4 wells per dilution, working backward.
6. Allow the virus to absorb to cells at 37°C for 2 hours.
7. After absorption, remove virus inoculum. Start with the most dilute and work backwards
8. Add 0.5 mL infection medium to each well being careful to not touch the wells with the pipette.
9. Place plates at 37°C and monitor CPE using the inverted microscope over a period of 1 to 4 weeks.
10. Record the number of positive and negative wells.

CONTROL:

Two control tests were conducted without the AirQuality filter installed in the testing chamber. Nebulization of viral media and collection methods were the same for the control as the viral challenge. Control testing was used for the comparative baseline to assess the viral reduction when the electrostatic air filter was operated in the challenge trials, to enable net reduction calculations to be made. Prior to running the viral challenges temperature and humidity were confirmed to be in relative range to the control +/- 5%.

VIRAL CHALLENGE:

The challenge pathogen, SARS-CoV-2 USA-CA1/2020, was used for testing the efficacy of the in-duct UV-C lamps. During the challenge tests the pressure in the challenge chamber was monitored to confirm no portion of the chamber was leaking. The bioaerosol efficacy challenge was completed in three distinct trials with the live pathogen to create a baseline of data for each test air pass. Prior to nebulizing the pathogen for testing the electrostatic filter was engaged to allow them to reach optimal operating conditions which was replicated in all test scenarios. Collections were made via the vacuum equipment utilizing viral media coated filters for maximum pathogen trapping and stability. Collection samples were provided to lab staff for pooling after each collection.

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VIRAL STOCK: SARS-CoV-2 USA-CA1/2020 (BEI NR-52382)

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero 6 cells	Cell Rounding and Detachment	Cell Rounding and Detachment
Next Generation Sequencing (NGS) of complete genome using Illumina® iSeq™ 100 Platform (Approx. 940 Nucleotides)	≥ 98% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1 ≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1	99.9% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1 100% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1
Titer by TCID50 in Vero E6 Cells by Cytopathic effect	Report Results	2.8 X 10^5 TCID50 per mL in 5 days at 37°C and 5% CO2
Sterility (21-Day Incubation) Harpos HTYE Broth, aerobic Trypticase Soy Broth, aerobic Sabourad Broth, aerobic Sheep Blood Agar, aerobic Sheep Blood Agar, anaerobic Thioglycollate Broth, anaerobic DMEM with 10% FBS	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth
Sterility (21-Day Incubation) Harpos HTYE Broth, aerobic Trypticase Soy Broth, aerobic Sabourad Broth, aerobic Sheep Blood Agar, aerobic Sheep Blood Agar, anaerobic Thioglycollate Broth, anaerobic DMEM with 10% FBS	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth	 No Growth No Growth No Growth No Growth No Growth No Growth No Growth
Mycoplasma Contamination Agar and Broth Culture DNA Detection by PCR of extracted Test Article nucleic acid.	 None Detected None Detected	 None Detected None Detected

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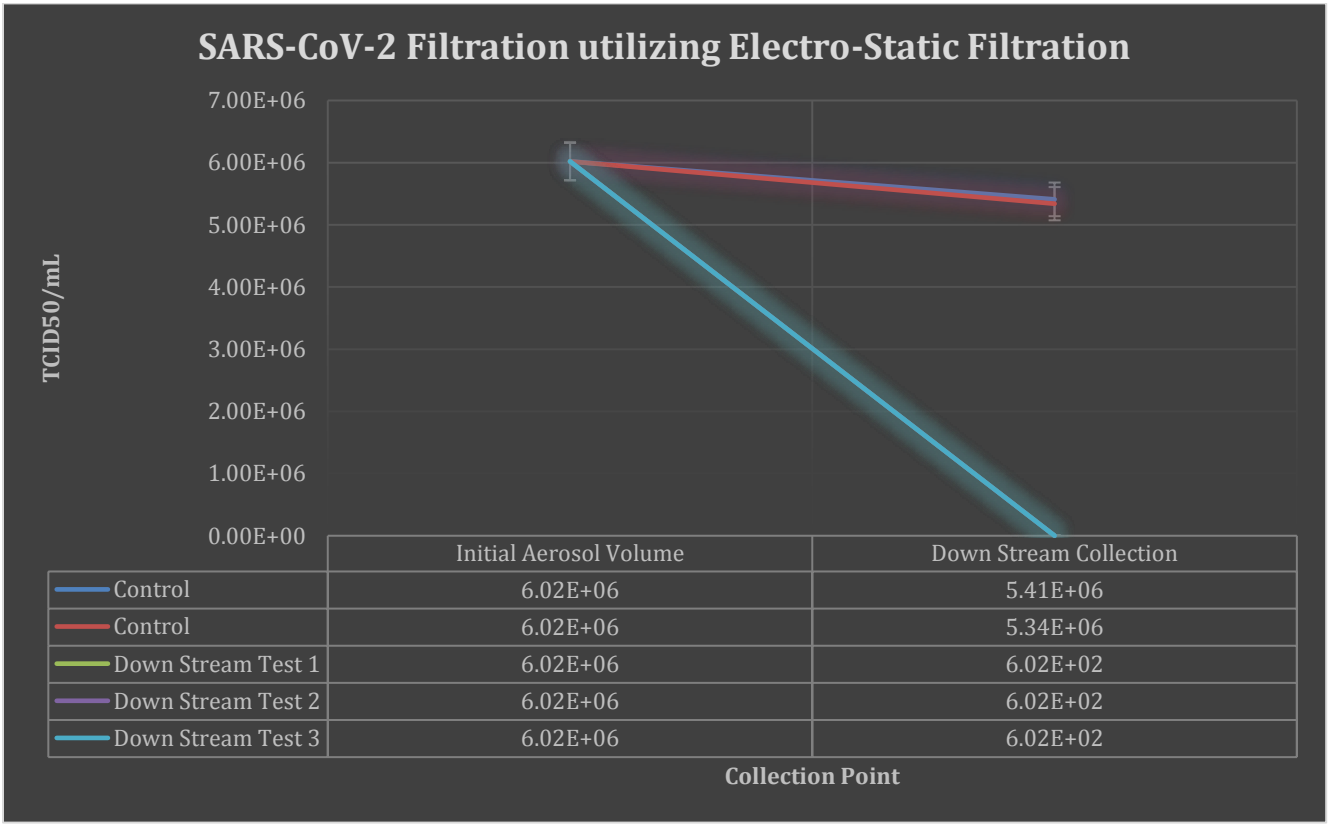
Aerosolization of Viral Media:

Controls samples were performed in the same manner as the viral test at the time-points and rate of collection. A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 6.02 X 10^6 TCID50/mL was used for this experiment.

RESULTS:



	Initial Aerosol Volume	Down Stream Collection
Control	6.02E+06	5.41E+06
Control	6.02E+06	5.34E+06
Down Stream Test 1	6.02E+06	6.02E+02
Down Stream Test 2	6.02E+06	6.02E+02
Down Stream Test 3	6.02E+06	6.02E+02

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CONCLUSIONS:

In aerosol there was an observed 99.99% reduction of collectable viral media from the downstream collection port with the filtration system installed. Collection samples were compared to control value collections to obtain the average % reduction.

When aerosolizing pathogens and collecting said pathogens, there are variables that cannot be fully accounted for, namely, placement of pathogen, collection volume, collection points, surface saturation, viral destruction on collection, viral destruction on nebulization, and possibly others. Every effort was made to address these constraints with the design and execution of the trials. And these efforts are reflected in the meaningful recovery of virus in the control test.

Taking these variables into account, there was a high level of inactivation efficacy achieved by in-duct electrostatic filtration system created by AirQuality.

DISCLAIMER:

The Innovative Bioanalysis, Inc. ("Innovative Bioanalysis") laboratory is not certified or licensed by the United States Environmental Protection Agency and makes no equipment emissions claims pertaining to ozone or byproduct of any AIRQUALITY device. Innovative Bioanalysis makes no claims to the overall efficacy of any AIRQUALITY FAH filter. The experiment results are solely applicable to the device used in the trial. The results are only representative of the experiment design described in this report. Innovative Bioanalysis makes no claims as to the reproducibility of the experiment results given the possible variation of experiment results even with an identical test environment, viral strain, collection method, inoculation, nebulization, viral media, cell type, and culture procedure. Innovative Bioanalysis makes no claims to third parties and takes no responsibility for any consequences arising out of the use of, or reliance on, the experiment results by third parties.

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