STUDY TITLE

Assessment of MedicAir 2.0 Device to Reduce Airborne Pathogens: Testing with *Cystovirus Phi6* as the Challenge

TEST ORGANISM

Cystovirus Phi6 (ATCC 21781-B1):
Host:
Pseudomonas syringae (ATCC 19310).

TEST PRODUCT IDENTITY

MedicAir 2.0 device

TEST Method

Air Decontamination Protocol based on US EPA Guidelines OCSPP 810.2500 for Efficacy Test Recommendations on Air Sanitizers

AUTHOR

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STUDY COMPLETION DATE

Aug/09/21

PERFORMING LABORATORY

CREM Co. Labs. Units 1-2, 3403 American Dr., Mississauga, Ontario, Canada L4V 1T4

SPONSOR

MedicAir DentAir Ltd,
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STUDY NUMBER

MADA210408-01-Phi6

STUDY PERSONNEL

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STUDY REPORT

GENERAL STUDY INFORMATION

Study Title:	Assessment of MedicAir 2.0 device to reduce airborne pathogens: Testing with Cystovirus Phi6 as the challenge
Study Number:	MADA210408-01-Phi6
Sponsor	Medic Air Ltd,
Testing Facility	CREM Co Labs Units 1-2, 3403 American Drive, Mississauga, ON, Canada

TEST SUBSTANCE IDENTITY

Test Substance Name: MedicAir 2.0 device

STUDY DATES

Date Device Received: July/01/21
Study initiation date: July/01/21
Experimental Start Date: July/15/21
Experimental End Date: July/30/21
Study Completion Date: Aug/09/21

I. BACKGROUND AND INTRODUCTION

Indoor air is well-recognized as a vehicle for the direct and indirect spread of a wide variety of human pathogens, and many technologies are used to remove/inactivate such airborne pathogens in healthcare and other settings. In this study, MedicAir 2.0 device was tested to quantitatively assess if it could reduce the contamination of the air by an enveloped bacteriophage (Phi6) as a surrogate for enveloped viruses such as influenza- and coronaviruses. The technology tested is based on the UV and HEPA filter. The device itself is a stand-alone system with four fan speeds. The device was tested at the highest fan speed (max).

II. RATIONALE

Indoor air can be an important vehicle for a variety of human pathogens and airborne pathogens can contaminate other parts of the environment to give rise to secondary vehicles leading to an air-surface-air nexus with possible transmission to susceptible hosts. Various groups of human pathogens with potential airborne spread include: vegetative bacteria (staphylococci and legionellae), fungi (*Aspergillus, Penicillium*, and *Cladosporium* spp. and *Stachybotrys chartarum*), enteric viruses (noro- and rotaviruses), respiratory viruses (influenza and coronaviruses), mycobacteria (tuberculous and nontuberculous), and bacterial spore-formers (*Clostrioides difficile* and *Bacillus anthracis*). Many technologies have been developed to decontaminate indoor air under field-relevant conditions. Furthermore, air decontamination may play a role in reducing the contamination of environmental surfaces and have an impact on interrupting the risk of pathogen spread.

OBJECTIVE

To assess the efficacy of MedicAir 2.0 device for its ability to inactivate enveloped virus (*Cystovirus Phi6 (ATCC 21781-B1)*) in indoor air under ambient conditions.

Test Device: MedicAir 2.0 device

Room Temperature Ambient temperature (22±2ºC)
Relative Humidity (RH): 50±10%

MATERIAL AND METHODS

1. The aerobiology chamber

The details of our aerobiology chamber have been published before (Sattar et al., 2016). Briefly, the chamber (25 m^3) was built to comply with the guidelines from the U.S. Environmental Agency (U.S. EPA 2012). A PVC pipe connected to a nebulizer introduced microbial aerosols into the center of the chamber and another PVC pipe connected to an air sampler collected the airborne microbes directly onto nutrient agar plates inside the sampler. The nebulizer was operated for the desired length of time with air pressure (25 psi) from a compressed air cylinder. A glove-box on one side of the chamber permitted the handling of the required items without breaching the containment barrier. A muffin fan (Nidec Alpha V, TA300, Model AF31022-20; 80 mm X 80 mm, with an output of 0.17 cubic meters/minute) inside the chamber enabled the uniform mixing of the air inside it. Between uses, fresh air was used to flush out the chamber of any residual airborne microbes.

2. **Environmental monitoring:** The air temperature (22±2°C) and RH (50±10%) inside the chamber were measured and recorded using a remote-sensing device (RTR-500 Datalogger).

3. The air sampler

A programmable slit-to-agar (STA) sampler (Particle Measuring Systems, Boulder, CO; hiip://www.pmeasuring.com/home) was used to collect air samples from the aerobiology chamber at the rate of 28.3 L (1 ft^3)/min. The sampler was placed outside the chamber and the sampler's inlet was connected via a PVC pipe to withdraw air from the aerobiology chamber. A fresh plate (150 mm diameter) with a suitable nutrient agar was used to collect an air sample and the plates incubated for the development of PFU of the test microbes. When collecting the airborne phage, the recovery plate was first inoculated with a suspension of its bacterial host and placed in the sampler. The air sample collection time varied from 2 to 60 minutes depending on the nature of the experiment.

4. Collison nebulizer

A six-jet Collison nebulizer (CH Tech., Westwood, NJ; www.inhalation.org) was used to generate the aerosols of the test microbe for ten minutes. Air from a compressed air cylinder at ~172 kPa (25 psi) was used to operate the nebulizer. The fluid to be nebulized consisted of a suspension of the test microbe in normal saline.

5. Test Pathogen

Phage Cystovirus Phi6 (ATCC 21781-B1) was grown in its bacterial host *P. syringae* (ATCC 19310). This phage is a relatively large (about 100 nm in diam.), enveloped virus that is frequently used as a surrogate for human pathogenic viruses. This virus was a gift

from the Laval University, Laval, Quebec, Canada.

6. Test Medium

The vegetative microbial growth and recovery media in this study were Luria Broth (LB) and Luria Broth Agar (LBA).

7. Preparation of Test Pathogen Suspension

To prepare a broth culture of *P. syringae,* a loopful of the stock culture was streaked on a LB agar and was incubated for 18±2 h at 28±1°C. A colony was inoculated in 25 mL of LB broth and incubated in at 28±1°C. When the optical density (OD) reached around 0.7, the bacterial suspension was used for the test.

8. The soil load:

The soil load used in this study is based on an earlier publication (Springthorpe and Sattar, 2007) and now also incorporated in a standard of ASTM International (2017) as well as a guidance document from the Organization of Economic Cooperation & Development (OECD; 2013). It consisted of the following three stock solutions:

0.5 g of yeast extract in 10 mL of phosphate buffered saline (Dulbecco's PBS, pH 7.2±0.2).
0.5 g of bovine serum albumin (BSA) in 10 mL of PBS.
0.04 g of bovine mucin in 10 mL of PBS.

All three stock solutions were sterilized by passage through a syringe-mounted (25 mm diameter) polyethersulfone (PES) membrane (0.22 μm pore diam.), and then aliquoted as 1.5 mL volumes and stored at -20±2°C with a shelf-life of at least one year. For short-term storage, the vials can be kept at 4±2°C for no longer than 90±5 days.

9. Preparation of Phage Inocula for aerosolization

The test phage suspended in soil load and nebulized into the aerobiology chamber (Sattar et al., 2016) using a six-jet Collison nebulizer.

To prepare the fluid for each aerosolization into the aerobiology chamber, the nebulizer reservoir received 0.75 mL BSA, 1.05 mL yeast extract, 3.0 mL mucin, and 50 uL of the test bacteriophage suspension to 10.15 mL of Dulbecco's PBS. The fluid to be nebulized was assayed for PFU before aerosolization by making five 10-fold dilutions (e.g., add 100 µL to 900 µL of PBS). Appropriate dilutions were tested in duplicate by the pour plating technique by placing five 100-µL droplets from each the last three dilutions on a 100 mm plate of LBA with a predried agar surface containing the host bacteria. The plates were incubated for 18±2 h at 36±1ºC, and the CFU recorded.

TEST METHOD

1. Experimental setup

Flowchart 1 provides the sequence of steps in a typical experiment for testing the air-decontamination device. As control, the study included testing the natural decay of the test organism over time while the fan of the device was on without turning on the device. Table 1 and Table 2 list the times at which the air samples from the chamber were collected and the duration of sampling for each in control and efficacy test, respectively.

Flowchart 1. **Sequence of steps in a typical experiment.**

Air decontamination

Turn on muffin fan in aerobiology chamber at least 10 min before testing and placing 10-150 mm plates on the floors in five different locations

↓

Nebulize test microorganism for 10 minutes

↓

Allow 5 minutes for uniform distribution of aerosols

↓

Collect a two-minute air sample

↓

Turning on the device (in control test no action is required)

↓

Collect an air sample based on Tables 1 and Table 2

↓

Count PFU on plates after 24 hour of incubation

↓

Calculate reduction in the level of viable microbes in air and surface

Table 1: **Time interval of air sampling for control test**

Sampling point (min)	Sampling duration (min)
0 (Baseline)	2
15	2
30	2
45	2
60	2

Table 2: Time interval of air sampling for efficacy test

Sampling point (min)	Sampling duration (min)
0 (Baseline)	2
0-15	15
15-30	15
30-45	15
45-60	15

In efficacy, all plates were divided to the sections with 3.75 min sampling period and the PFU in each area was counted and used for calculating the concentration of the bacteriophage in the chamber at the median of that interval.

Experimental Design

Three control tests were performed, with the device OFF, and the muffin fan ON. 150 mm plates with agar and host bacteria were placed in in the STA machine to sample the air. Two multi-challenge efficacy tests were performed. In efficacy test after sampling the baseline, the device turned ON and kept ON until the end of the test.

STUDY ACCEPTANCE CRITERIA

No product acceptance criterion was specified for this range-finding study.

RESULTS

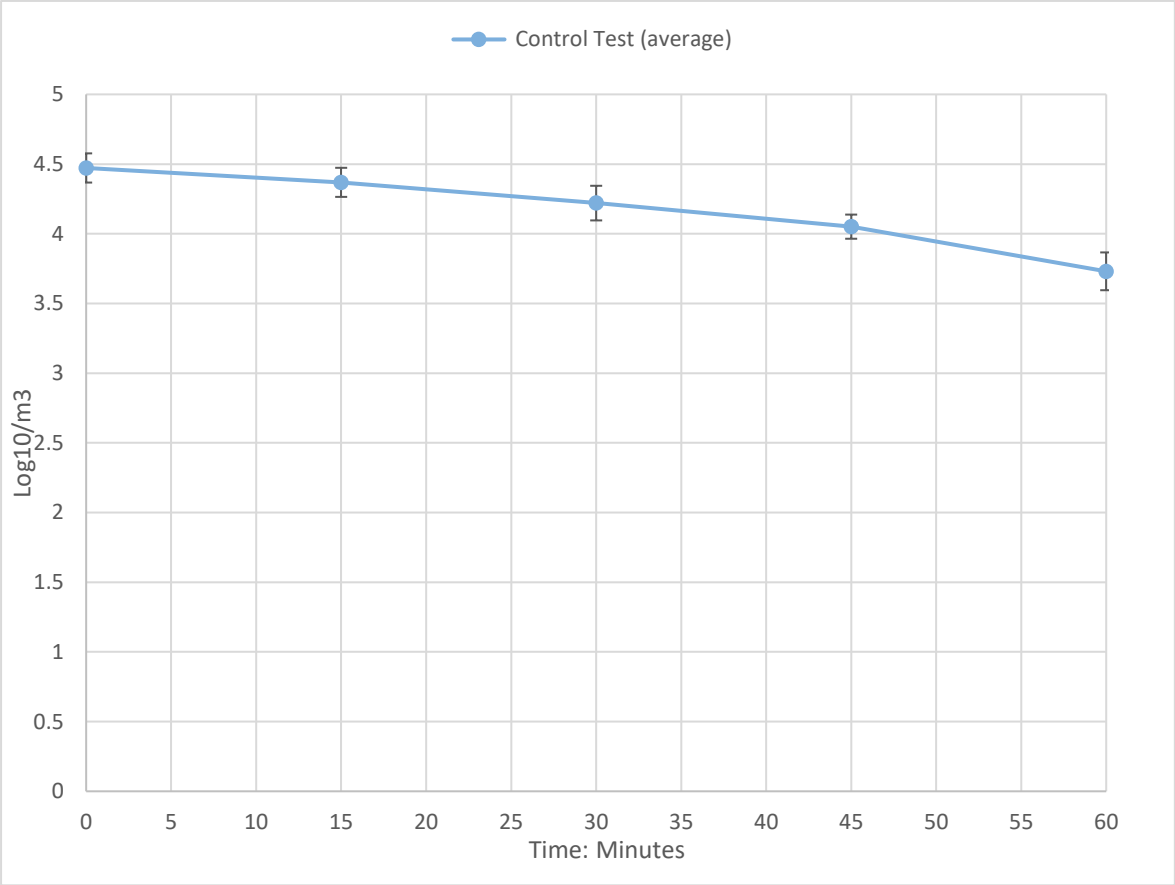
Testing phage survival: Any meaningful assessment of air decontamination requires that the aerosolized challenge microorganisms remain viable in the experimentally-contaminated air long enough to allow for proper differentiation between biological decay and inactivation/removal by the technology being tested. Such airborne viability of the microorganism used in this study was tested in the aerobiology chamber with three control tests without turning on the device while muffin fan was ON. The average of the three control tests was used to calculate the efficacy of MedicAir 2.0 device.

Efficacy test of the MedicAir 2.0 device against *Cystovirus* Phi6:

This part of the report represents data from the efficacy experiments on the MedicAir 2.0 device against Phi6 at RH 50±10%. The raw data are tabulated in Appendix A.

Figure 1 shows the average $\log_{10}$ PFU/m^3 recoveries for the three control tests (biological decay) with the corresponding standard deviation at each sampling interval.

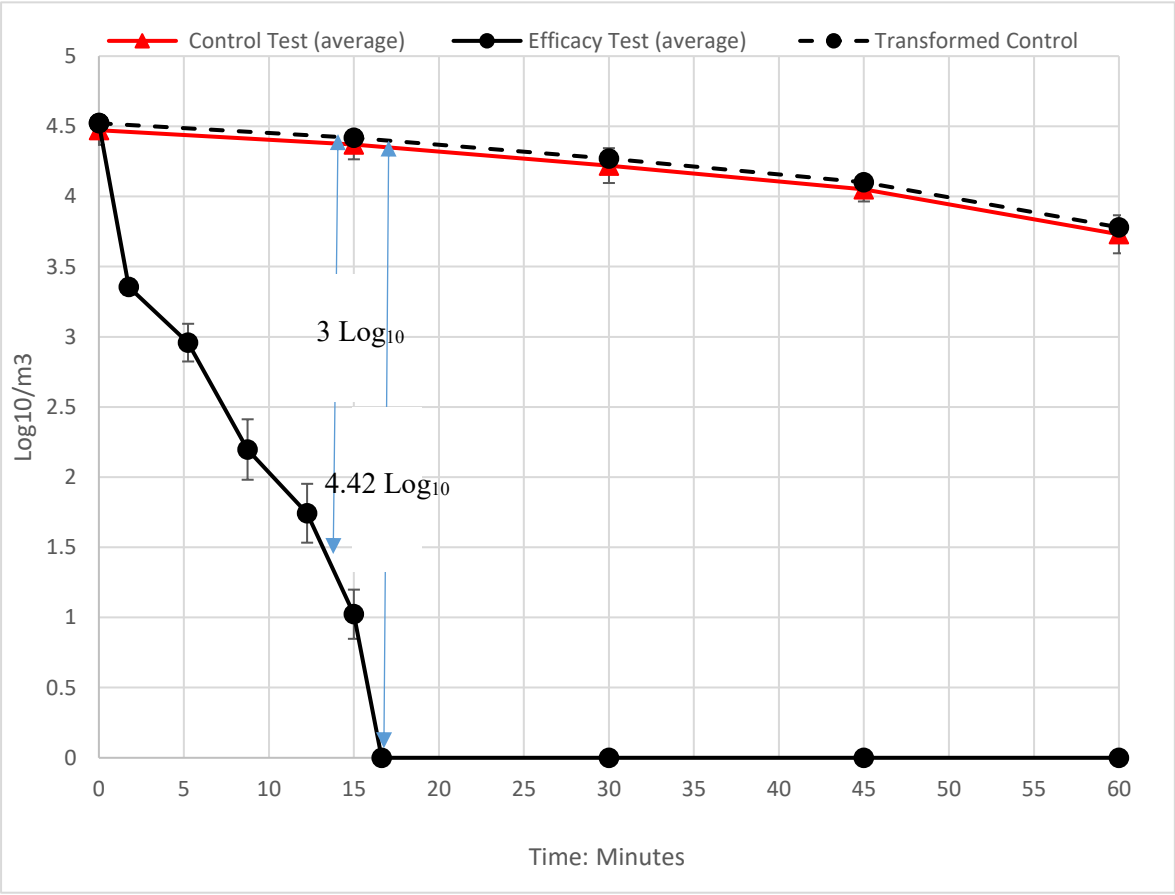
Fig. 1. The average of three Stability-in-air tests (natural decay) against Phi6 phage with the standard deviation at each sampling point.



Three efficacy tests were performed on the device. Figure 2 shows the average log_{10} PFU/m^3 recoveries for the three control and efficacy tests. The average of log_{10} PFU/m^3 recoveries of the transformed control of the three control tests are also shown. 'Transformed control' is the curve generated when the log_{10} PFU data for biological decay were transformed to be compared to the data for the efficacy experiment.

The device demonstrated a 3 Log_{10} reduction (99.9% reduction) after 13.27±1.72 minutes of turning on the device. No phi6 was recovered from the chamber after 16 minutes in the three tests and the device demonstrates 4.42 Log_{10} reduction (99.996% reduction) after 16 minutes.

Fig 2. Efficacy of MedicAir 2.0 device in reducing microbial contamination of air. The average of three control and three efficacy tests. Reductions were calculated using the % recovery formula for the determination of the biological decay with log_{10} and % reductions at each time point for Phi6.



Appendix A:

Table 3. Natural decay of bacteriophage *Phi6* with soil load, Reductions were calculated using the % recovery formula for the determination of the biological decay with log_{10} and % reductions at each time point for *Phi6*.

			Sampling Time Points (minutes)				
Sampling Time Points (minutes)			0	15	30	45	60
Sampling Period (minutes)			2	2	2	2	2
Total Colony in the room	PFU	**Control#1**	27562	19692	16117	12810	6937
		Control #2	19081	16151	9514	7188	2639
		Control#3	49611	40235	29821	15443	8489
Recovered on Plates	PFU	**Control #1**	1560	1112	908	720	389
		Control #2	1080	912	536	404	148
		Control#3	2808	2272	1680	868	476
log_{10} reduction**	log_{10}	**Control #1**	4.44	4.29	4.21	4.11	3.84
		Control #2	4.28	4.21	3.98	3.85	3.42
		Control#3	4.70	4.60	4.47	4.19	3.93

Table 4. Efficacy of MedicAir 2.0 in reducing microbial contamination of air. Reductions were calculated using the % recovery formula for the determination of the biological decay with $\log_{10}$ and % reductions at each time point for *Phi6*.

MedicAir 2.0			Sampling Time Points (minutes)									
Sampling Time Points (minutes)			0	1.75	5.25	8.75	12.25	15	16.625	30	45	60
Sampling Period (minutes)			2	3.75	3.75	3.75	3.75	2	3.75	2	15	15
Total PFU in the room	PFU	**Test #1**	34276	2115	766	91	44	10	0	0	0	0
		Test #2	26360	1851	543	86	24	5	0	0	0	0
		Test #3	40707	2951	1804	494	160	24	0	0	0	0
Recovered on Plates	PFU	**Test #1**	1940	448	161	19	9	2	0	0	0	0
		Test #2	1492	392	114	18	5	1	0	0	0	0
		Test #3	2304	625	379	103	33	5	0	0	0	0
$\log_{10}$ reduction**	$\log_{10}$	**Test #1**	4.53	3.32	2.88	1.96	1.64	0.99	0	0	0	0
		Test #2	4.42	3.27	2.73	1.94	1.38	0.69	0	0	0	0
		Test #3	4.61	3.47	3.26	2.69	2.20	1.39	0	0	0	0

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