

STUDY TITLE

Assessment of IONaer 7000 Ion Generator to reduce airborne pathogens: Testing with
Cystovirus Phi6 (ATCC 21781-B1) as the challenge

TEST ORGANISM

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

TEST PRODUCT IDENTITY

IONaer 7000 Ion Generator Carrier

TEST Method

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

AUTHOR

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STUDY COMPLETION DATE

April/14/20

PERFORMING LABORATORY

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SPONSOR

Clean Air EXP

STUDY NUMBER

CAE200306-01

Study No.: CAE200306-01

Assessment of IONaer 7000 Ion Generator to
reduce airborne pathogens: Testing with Cystovirus
Phi6 as the challenge



STUDY PERSONNEL

STUDY DIRECTOR: Bahram. Zargar, PhD

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STUDY REPORT

GENERAL STUDY INFORMATION

Study Title:	Assessment of IONaer 7000 Ion Generator to reduce airborne pathogens: Testing with Cystovirus Phi6 (ATCC 21781-B1) as the challenge
Study Number:	CAE200306-01
Sponsor	Clean Air EXP
Testing Facility	CREM Co Labs Units 1-2, 3403 American Drive, Mississauga, ON, Canada

TEST SUBSTANCE IDENTITY

Test Substance Name: IONaer 7000 Ion Generator

STUDY DATES

Date Device Received:	
Study initiation date:	March/03/06
Experimental Start Date:	March/03/20
Experimental End Date:	April/02/20
Study Completion Date:	April/14/20

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, IONaer 7000 Ion Generator 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 generation of cold plasma to charge indoor air. The device itself is mounted on the HVAC system to take advantage of the air movements in it.

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 (*Clostridioides 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 IONaer 7000 Ion Generator for its ability to inactivate enveloped virus (*Cystovirus Phi6* (ATCC 21781-B1)) in indoor air under ambient conditions.

Test Device: IONaer 7000 Ion Generator

Room Temperature: Ambient temperature (22±2°C)

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 (26 m³) 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; <http://www.pmeasuring.com/home>) was used to collect air samples from the aerobiology chamber at the rate of 28.3 L (1 ft³)/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 airborne phages, the recovery plate was first inoculated with a suspension of their respective 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 PBS.

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. Preparation of Phage Inocula for aerosolization

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

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.

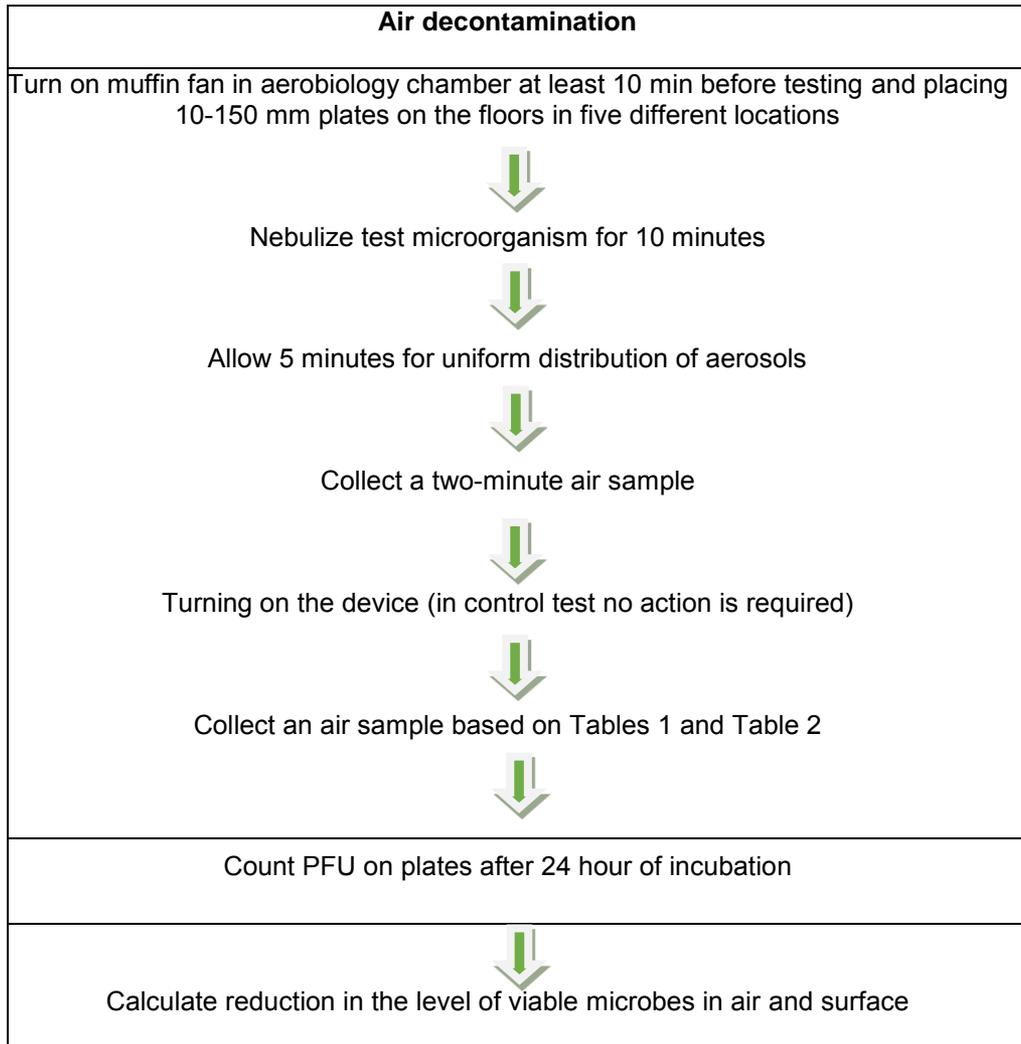


Table 1: Time interval of air sampling for control test

Sampling point (min)	Sampling duration (min)
0 (Baseline)	2
15	2
30	6
45	10
60	20
70-100	30
100-160	60
160-220	60

Table 2: Time interval of air sampling for efficacy test

Sampling point (min)	Sampling duration (min)
0 (Baseline)	2
15 (0-30)	30
45 (30-60)	30
67.5 (60-75)	15
82.5 (75-90)	15
105 (90-120)	30

In efficacy, all plates were divided to the sections with 7.5 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

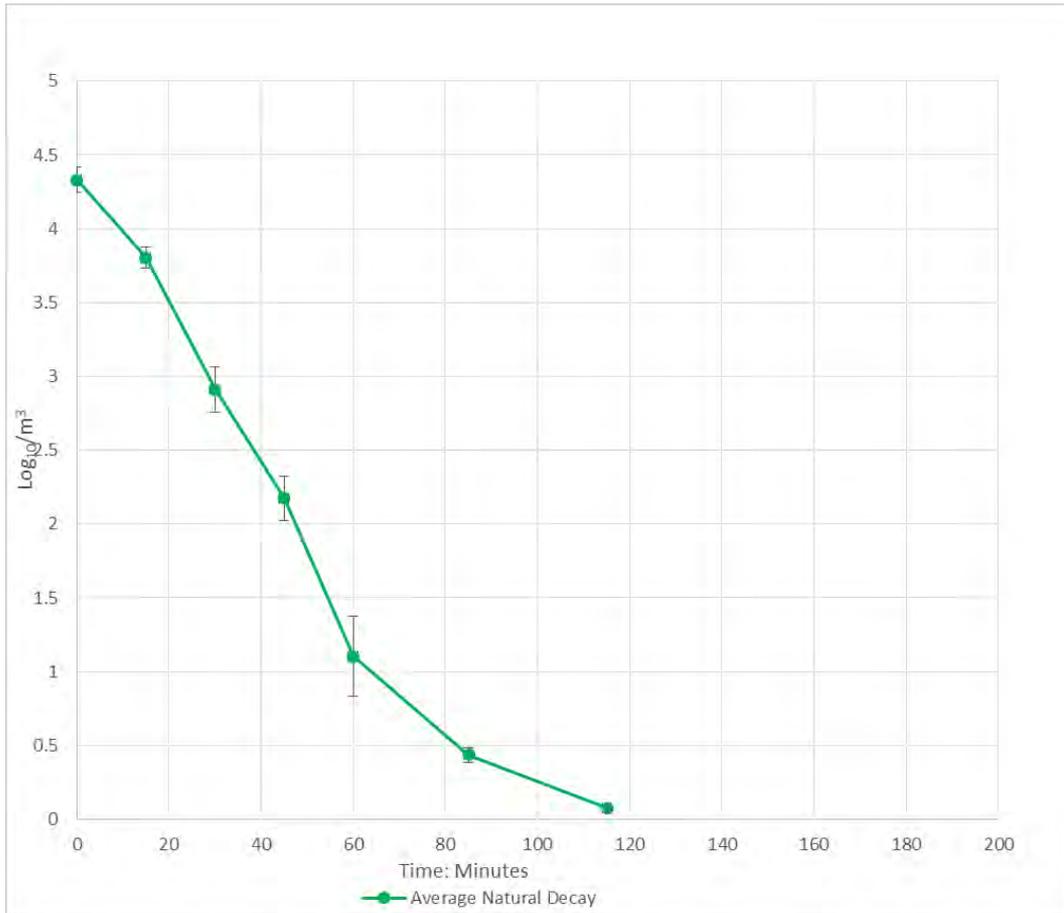
Testing phage survival: Any meaningful assessment of air decontamination requires that the aerosolized challenge microorganisms remain viable in the experimentally-contaminated air long enough to allow for proper differentiation between biological decay and inactivation/removal by the technology being 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 IONaer 7000 Ion Generator Carrier.

Efficacy test of the IONaer 7000 Ion Generator against *Cystovirus* Phi6:

This part of the report represents data from the efficacy experiments on the IONaer 7000 Ion Generator against Phi6 at two different RH: 45% and 55%. The raw data are tabulated in Appendix A.

Figure 1 shows the average \log_{10} PFU/m³ recoveries for the three control tests (biological decay) with the corresponding standard deviation at each sampling interval. The concentration of Phage becomes undetectable after 2 hours.

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



Two multi-challenge efficacy tests were performed on the device at two different relative humidity levels. Figure 2 shows the humidity of the chamber during the test. The average relative humidity in Test #1 was 53.5 % and in test #2 was 44%.

Fig. 2. The relative humidity in the two efficacy tests against Phi6

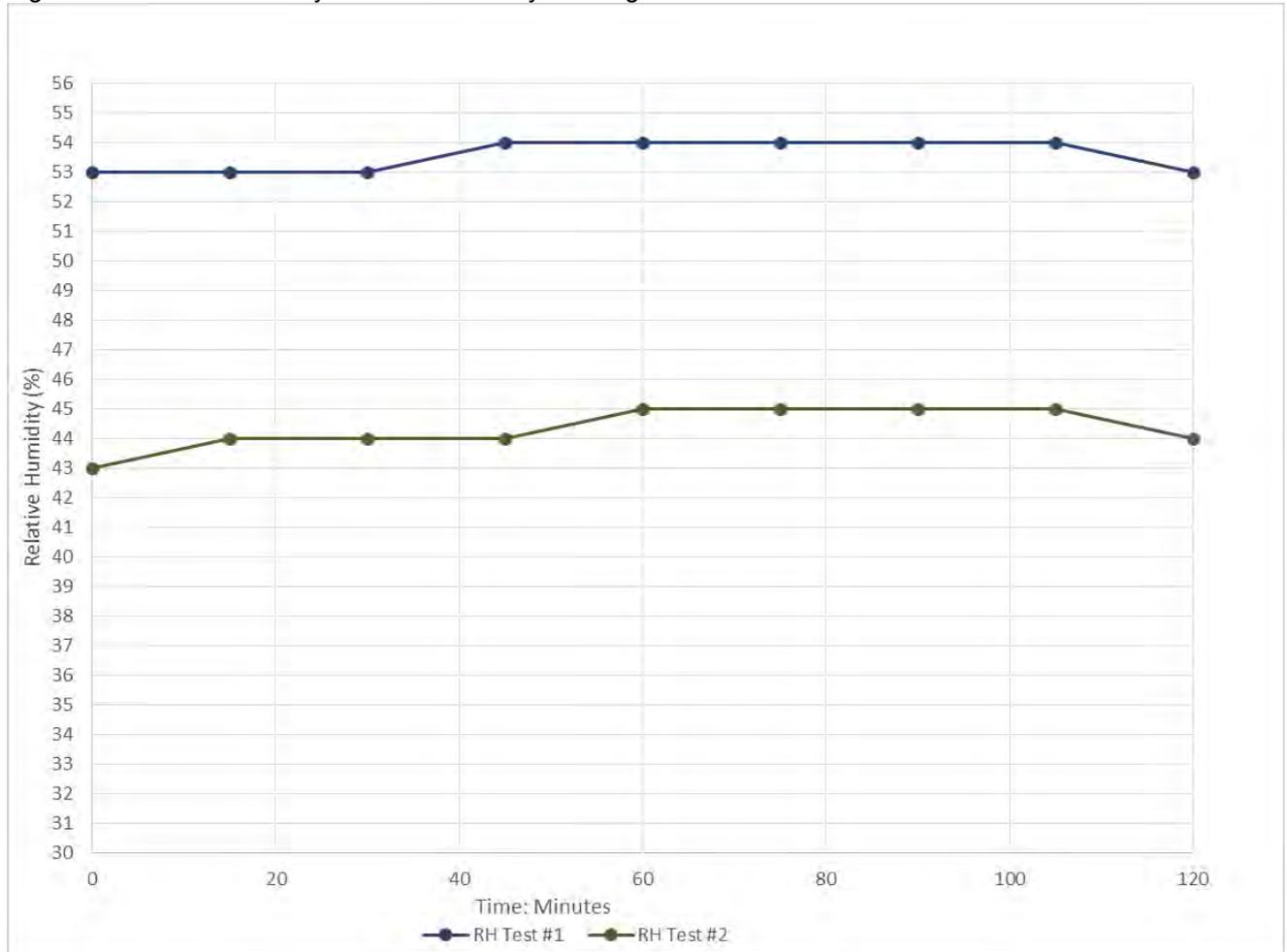


Figure 3 and 4 compares the average \log_{10} PFU/m³ recoveries for the two tests. The average of \log_{10} PFU/m³ recoveries of the transformed control of the three control tests are also shown. 'Transformed control' is the curve generated when the \log_{10} PFU data for biological decay were transformed to be compared to the data for the efficacy experiment.

In test #1(Average RH of 53.5%) , the device demonstrate 2.75 \log_{10} reduction (99.82% reduction) after 33 minutes of introducing the first challenge and demonstrate 4.2 \log_{10} (99.994% reduction) reduction in 10 minutes after introducing of the second challenge. In the second test (RH of 44%) the device demonstrate 2.6 \log_{10} reduction (99.75% reduction) after 33 minutes of introducing the first challenge and demonstrate 3 \log_{10} reduction (99.90% reduction) in 30 minutes after introducing of the second challenge.

Fig. 3. The average of three Stability-in-air tests and the first multi-challenge efficacy experiment on IONaer 7000 Ion Generator device against Phi6 phage with the average RH of 53.5 %

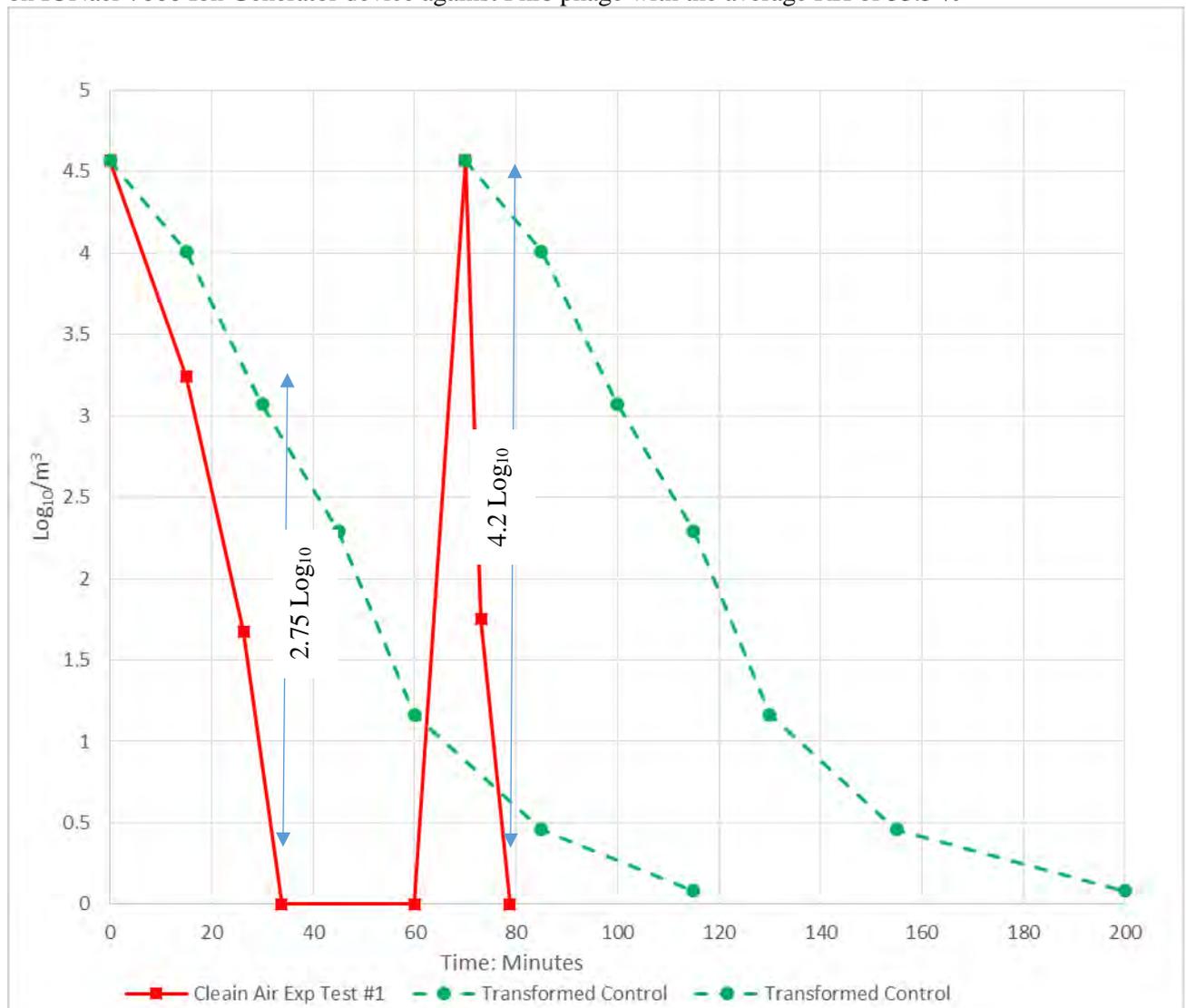
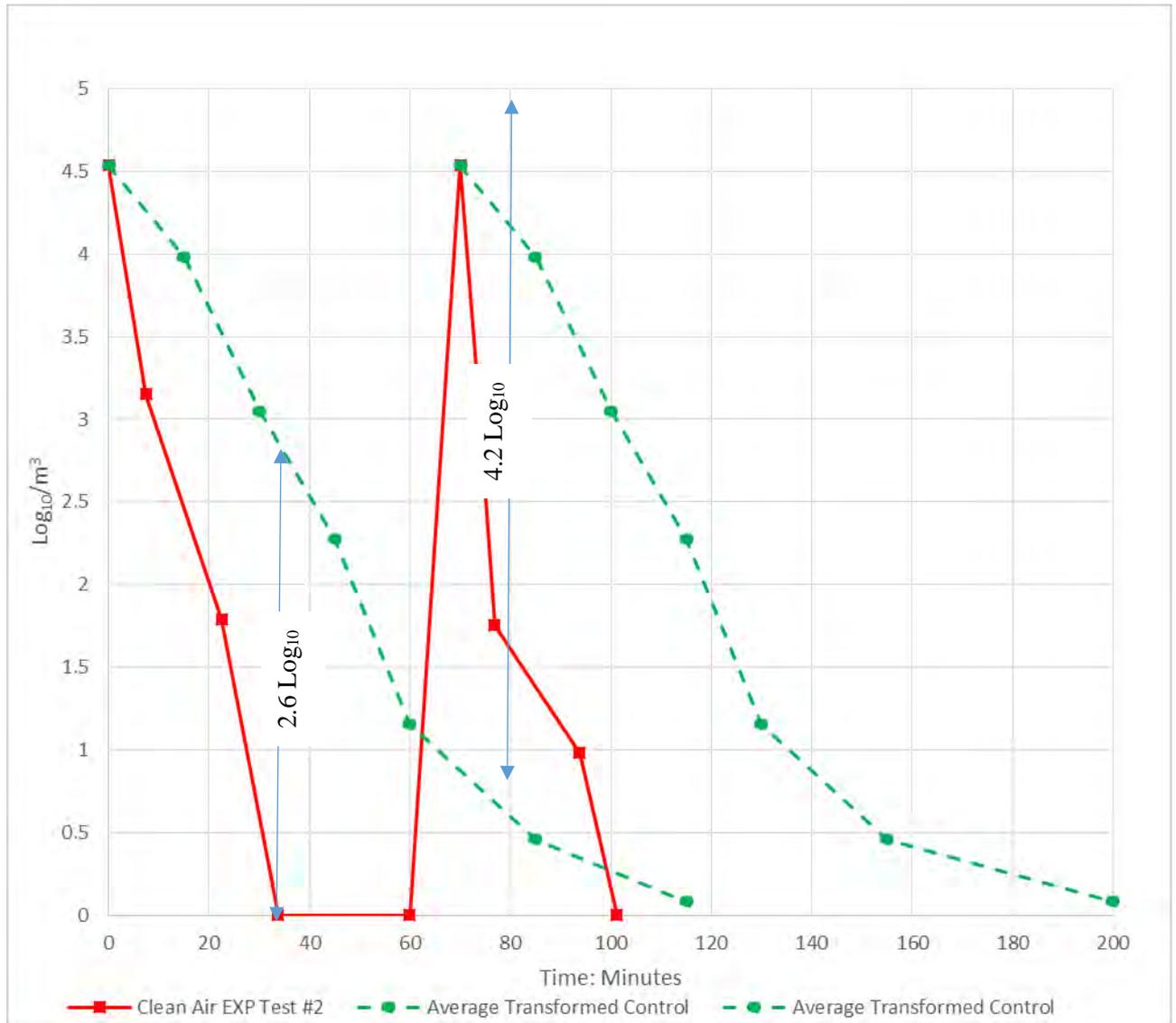


Fig. 3. The average of three Stability-in-air tests and the second multi-challenge efficacy experiment on IONaer 7000 Ion Generator device against Phi6 phage with the average RH of 44 %



Appendix A:

Table 4. Natural decay of bacteriophage *Phi6* without 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	85	115
Sampling Period (minutes)			2	2	6	10	20	30	60
Total Colony in the room	PFU	Control #1	21431	6357	716	89	5	2	1
		Control #2	32067	8819	1727	327	53	2	1
		Control #3	14417	4622	438	114	7	4	1
Recovered on Plates	PFU	Control #1	1213	359	121	25	3	2	1
		Control #2	1815	498	292	92	30	2	1
		Control #3	816	261	74	32	4	3	1
\log_{10} reduction**	\log_{10}	Control #1	4.33	3.80	2.85	1.95	0.73	0.38	0.077
		Control #2	4.51	3.94 992	3.24	2.52	1.73	0.38	0.077
		Control #3	4.16	3.66	2.64	2.06	0.85	0.55	0.0771

Table 5. Efficacy of IONaer 7000 Ion Generator when used with a fogger 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*.

IONaer 7000 Ion Generator			Sampling Time Points (minutes)										
Sampling Time Points (minutes)			0	7.5	22.5	33.75	60	71	73.125	76.875	93.75	101.25	110
Sampling Period (minutes)			2	15	15	7.5	20	2	3.75	3.75	7.5	3.75	17.5
Total Colony in the room	PFU	Test #1	36961	1759	47	0	0	37308	56	0	0	0	36961
		Test #2	34399	1421	62	0	0	34722	2002	56	10	0	34399
Recovered on Plates	PFU	Test #1	2092	745	10	0	0	2092	11	0	0	0	2092
		Test #2	1947	602	26	0	0	1947	210	11	2	0	1947
\log_{10} reduction**	\log_{10}	Test #1	4.57	3.25	1.68	0	0	4.57	1.75	0	0	0	0
		Test #2	4.54	3.15	1.79	0	0	4.54	3.30	1.75	0.98	0	4.53

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