STUDY TITLE

Assessment of Varionix® KK1-F 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

Varionix® KK1-F

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/14/20

PERFORMING LABORATORY

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

SPONSOR

Airionex LLC

STUDY NUMBER

ARNX200713-01

STUDY PERSONNEL

STUDY DIRECTOR: Bahram. Zargar, PhD

PROFESSIONAL PERSONNEL INVOLVED: Eduardo Raul Saurez Pestana, PhD

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title:	Assessment of Varionix® KK1-F to reduce airborne pathogens: Testing with Cystovirus Phi6 as the challenge
Study Number:	ARNX200713-01
Sponsor	Airionex LLC
Testing Facility	CREM Co Labs Units 1-2, 3403 American Drive, Mississauga, ON, Canada

TEST SUBSTANCE IDENTITY

Test Device Name: Varionix® KK1-F

STUDY DATES

Date Device Received:
Study initiation date: July/06/20
Experimental Start Date: July/13/20
Experimental End Date: Aug/05/20
Study Completion Date: Aug/18/20

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, Varionix® KK1-F 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 (*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 Varionix® KK1-F for its ability to inactivate enveloped virus (*Cystovirus Phi6 (ATCC 21781-B1)*) in indoor air under ambient conditions.

Test Device:	Varionix® KK1-F
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 (26 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; http://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 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 muffin fan 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

↓

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	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
7.5 (0-15)	15
22.5 (15-30)	15
37.5 (30-45)	15
52.5 (45-60)	15
67.5 (60-75)	15
82.5 (75-90)	15
105 (90-120)	30
135 (120-150)	30

In efficacy tests, all plates were divided into four equal sections and the PFU in each area were 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. Three multi-challenge efficacy tests were performed. In efficacy test after sampling the baseline, the device was 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

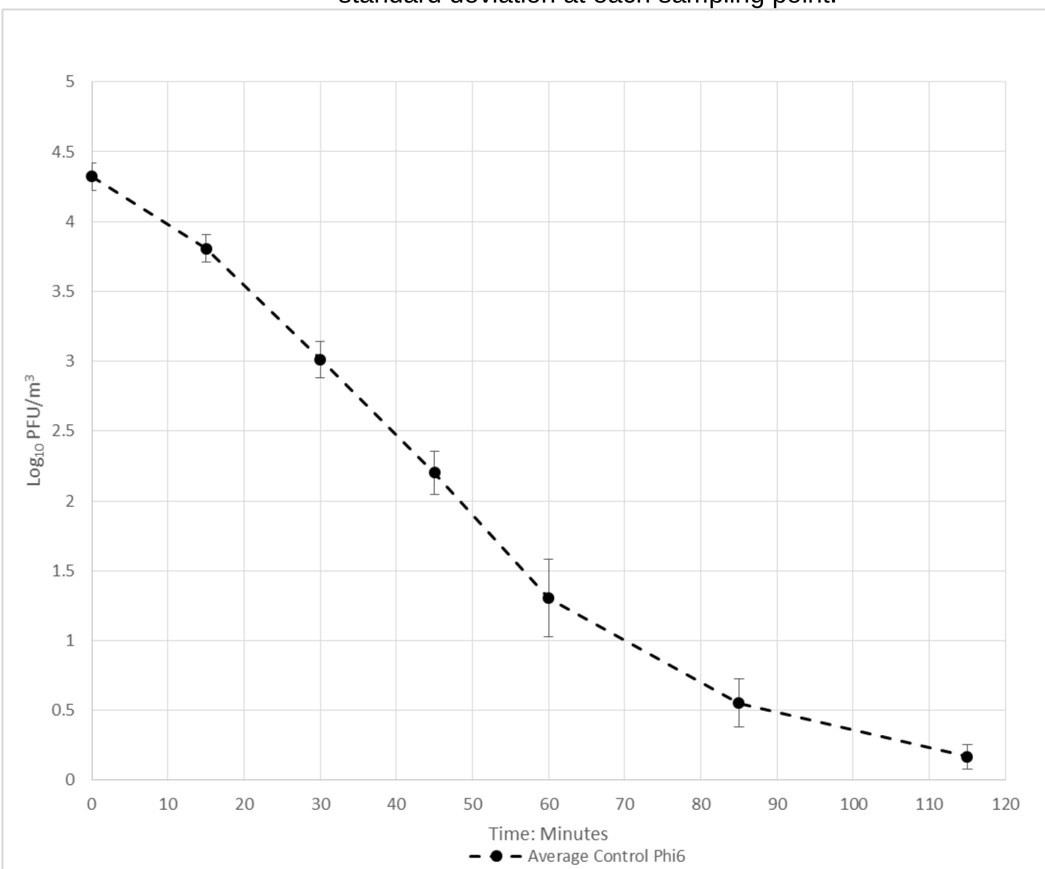
Testing phage survival: Any meaningful assessment of air decontamination requires that the aerosolized challenge microorganisms remain viable in the experimentally-contaminated air long enough to allow for proper differentiation between biological decay and inactivation/removal by the technology being 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 Varionix® KK1-F.

Efficacy test of the Varionix® KK1-F against *Cystovirus* Phi6:

This part of the report represents data from the efficacy experiments on the Varionix® KK1-F against Phi6. The raw data are tabulated in Appendix A.

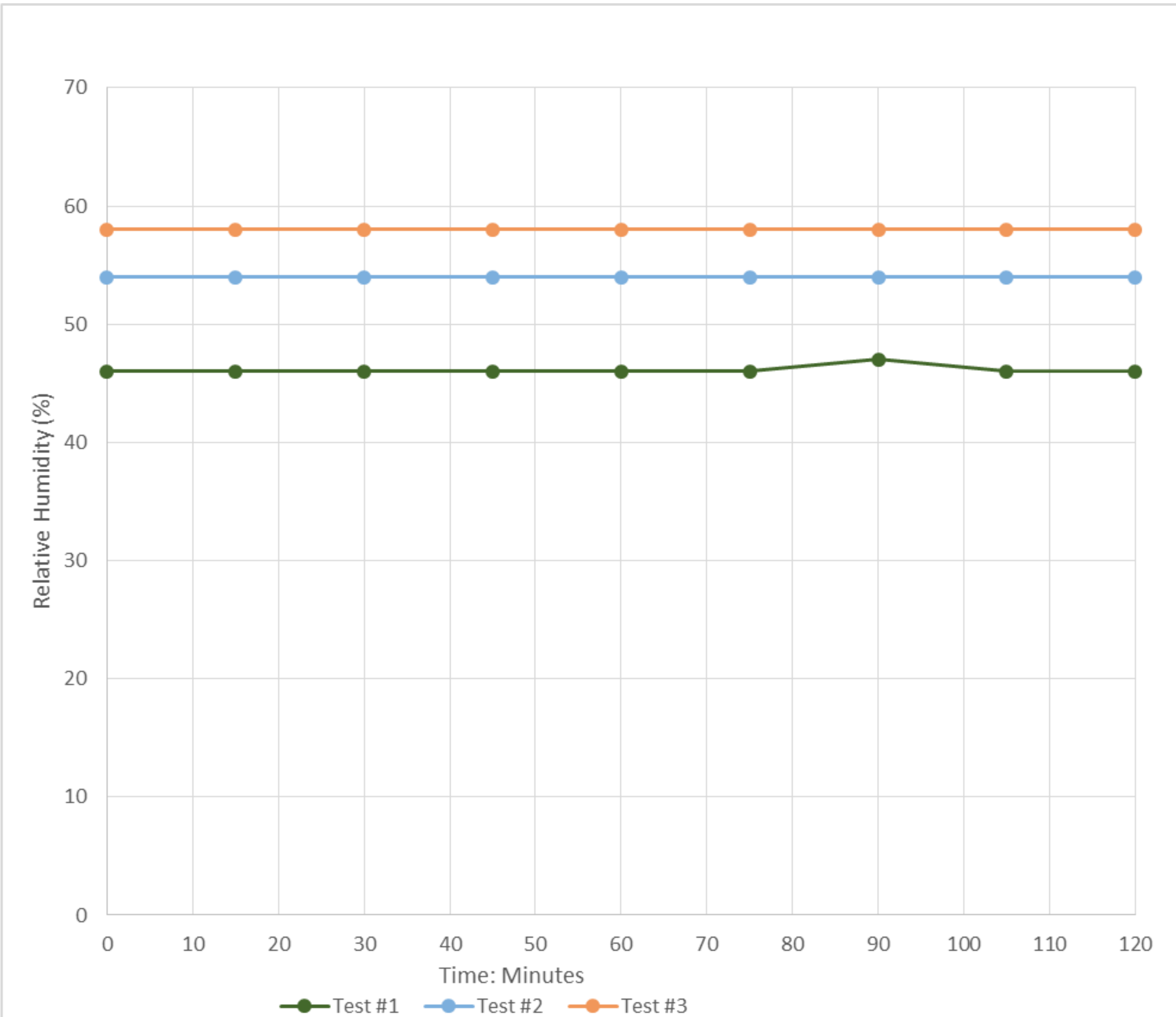
Figure 1 shows the average $\log_{10}$ PFU/m^3 recoveries for the three control tests (biological decay) with the corresponding standard deviation at each sampling interval. The concentration of Phage become undetectable after 2 hours.

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



Three multi-challenge efficacy tests were performed on the device and RH was recorded in each test. Figure 2 shows the RH levels in the chamber during the tests. The average RH values were 46% in Test #1, 54% in Test #2 and 58% in Test #3.

Fig. 2. The Relative humidity (RH) values during the three tests



Figures 3, 4 and 5 compare the average $\log_{10}$ PFU/m^3 recoveries in each efficacy test with that of the controls. 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 are transformed to be compared to the data for the efficacy experiment.

In test #1 (Average RH of 47%), the device demonstrate 3.4 $\log_{10}$ reduction (99.96% reduction) after 21 minutes of introducing the first challenge and demonstrated a 4.2 $\log_{10}$ (99.993% reduction) reduction in 5.5 minutes after introduction of the second challenge. In the second test (RH of 48%), the device demonstrated a 3.25 $\log_{10}$ reduction (99.94% reduction) after 21 minutes of introducing the first challenge and a 4.2 $\log_{10}$ reduction (99.993% reduction) in 5.5 minutes after introducing the second challenge. In the third test (RH of 41%), the device demonstrated a 3.5 Log_{10} reduction

(99.97% reduction) after 21 minutes of introducing the first challenge and a 4.2 Log_{10} reduction (99.993% reduction) in 5.5 minutes after introducing the second challenge.

Fig. 3. Stability-in-air and the first efficacy experiment on Varionix® KK1-F against Phi6 phage

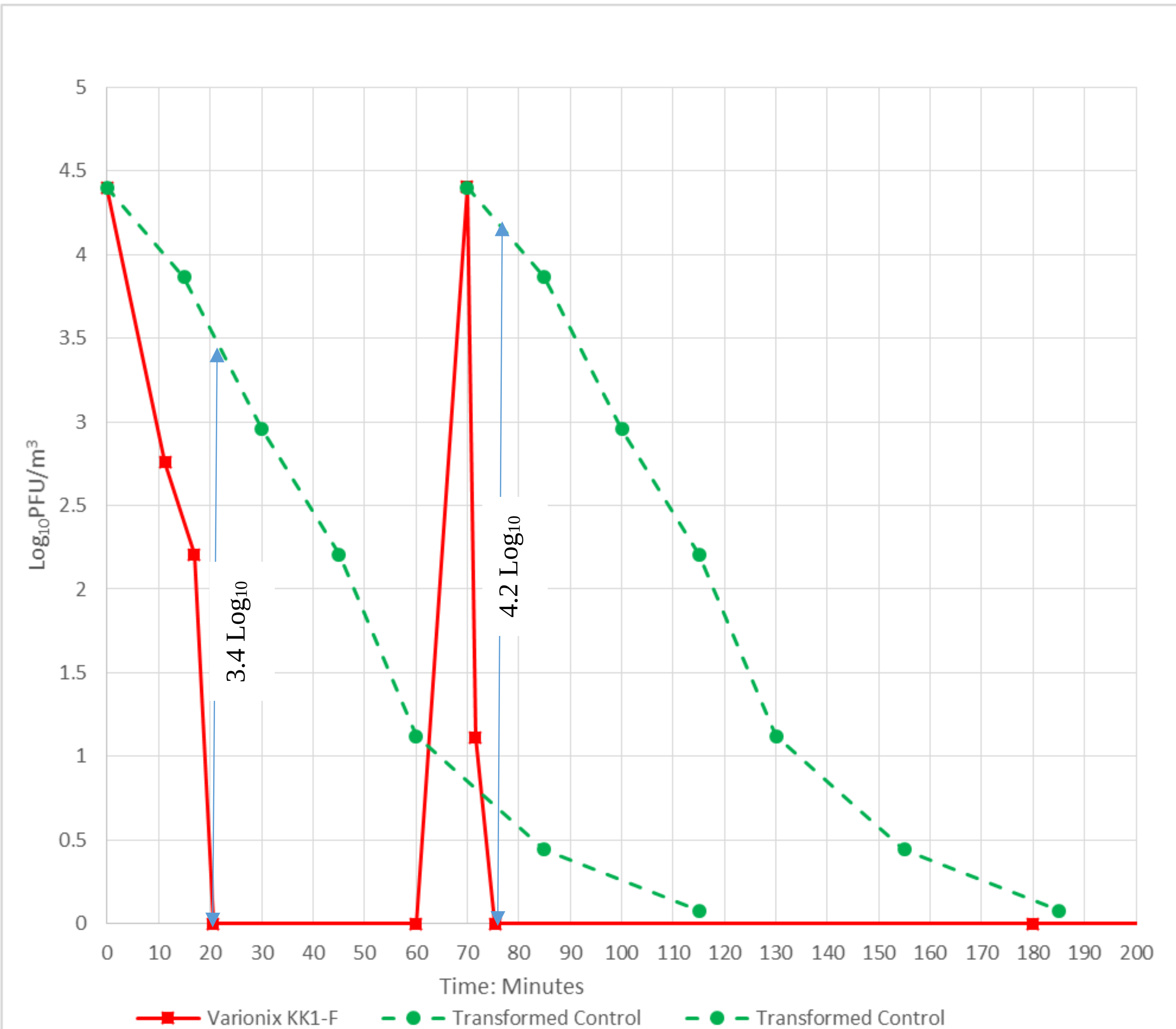


Fig. 4. Stability-in-air and the second efficacy experiment on Varionix® KK1-F against Phi6 phage

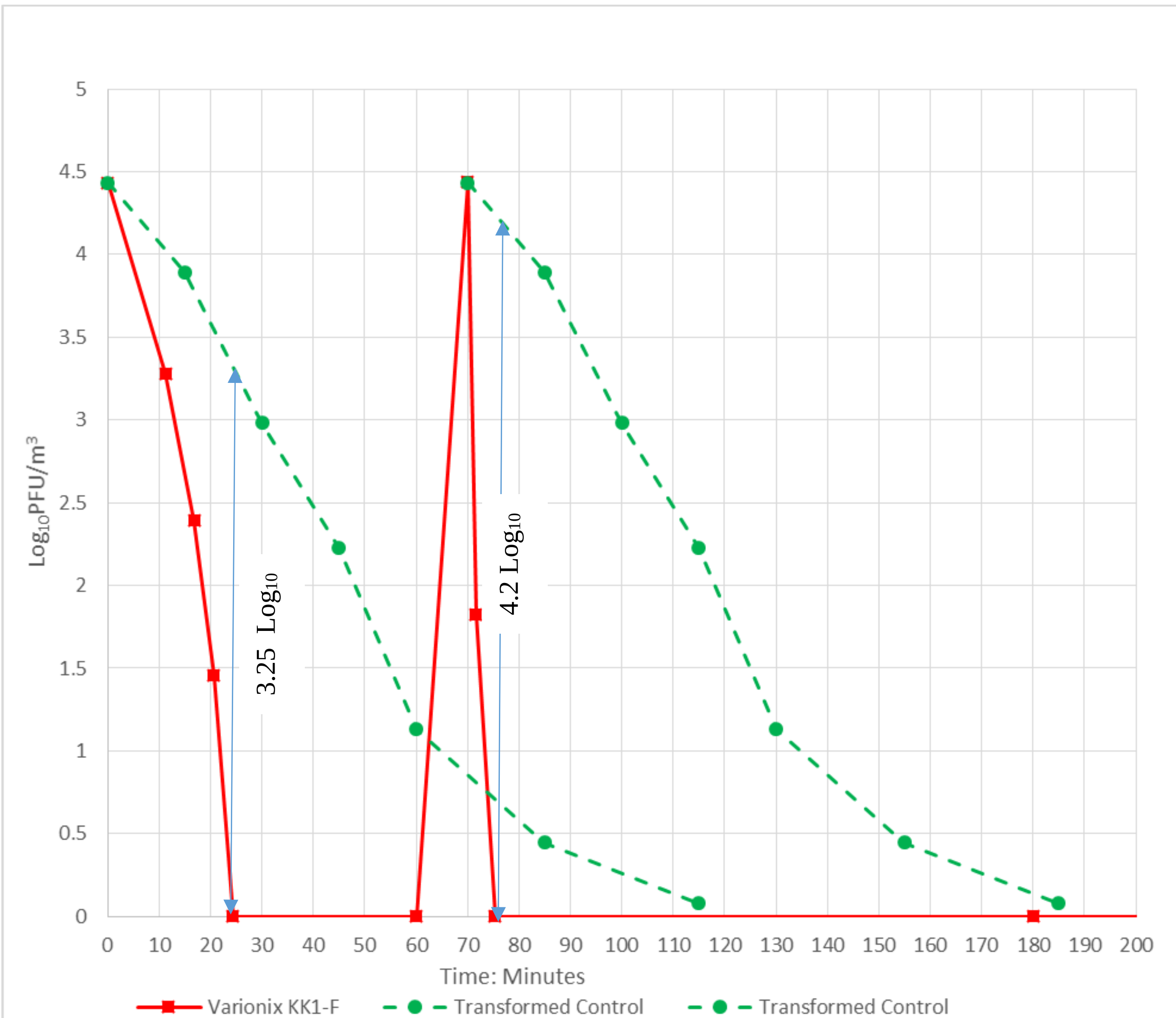


Fig. 5. Stability-in-air and the third efficacy experiment on Varionix® KK1-F against Phi6 phage

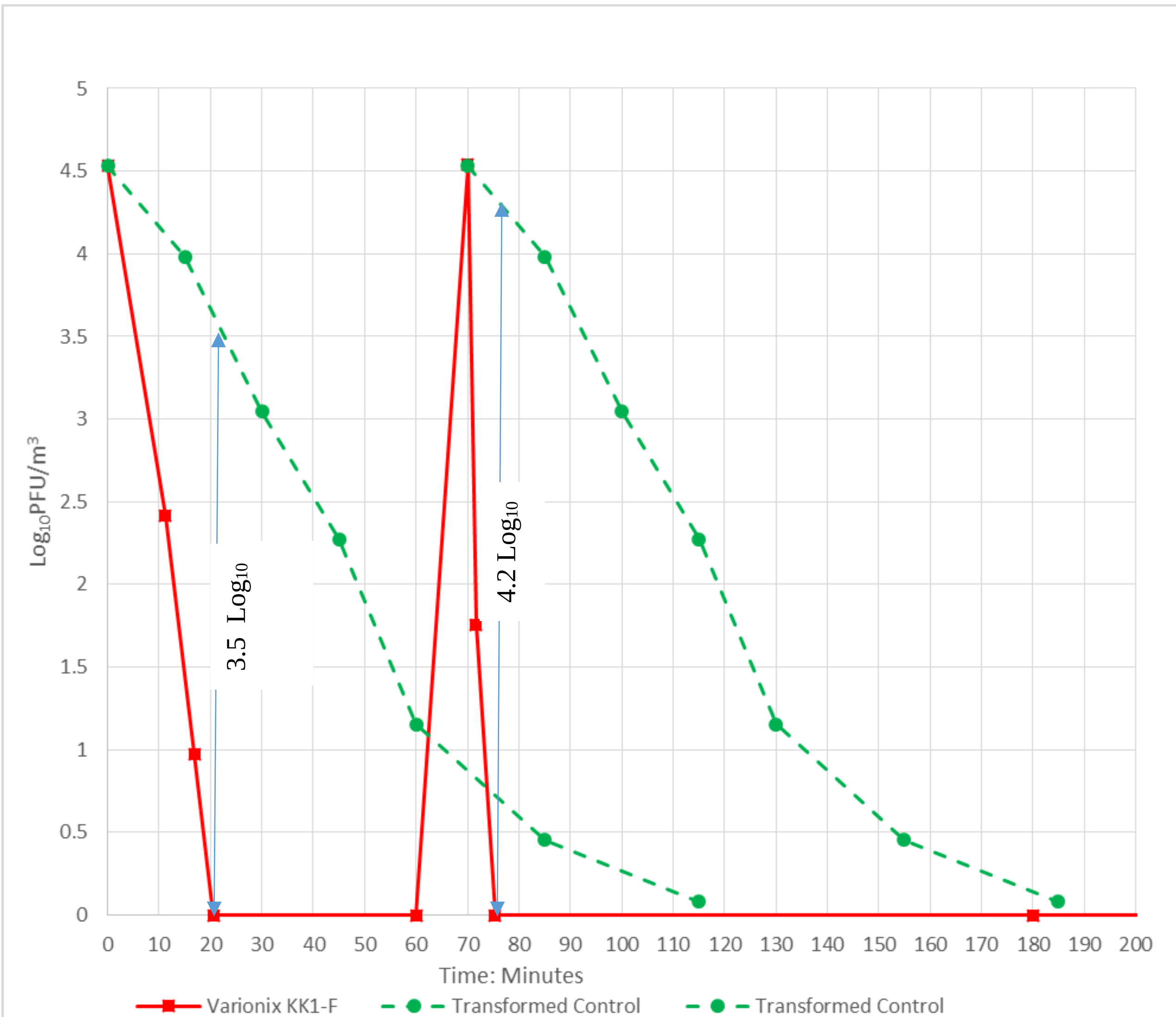


Table 3 summarizes the efficacy of Varionix® KK1-F device in three different efficacy tests. On average, the device demonstrates 3.38.87 $\log_{10}$ reduction (99.96%) after 21 minutes of introducing the first challenge and 4.2 $\log_{10}$ reduction (99.98%) after 5.5 minutes of introducing the second challenge on average

Table 3. Log_{10} reduction for each test and average log_{10} reductions and percent reductions.

Sample ID	Test #1	Test #2	Test #3	Average
Log_{10} Reduction after first challenge	3.4 in 21 min	3.25 in 21 min	3.5 in 21 min	3.38 (99.96%) in 21 min
Log_{10} Reduction after second challenge	4.2 in 5.5 min	4.2 in 5.5 min	4.2 in 5.5 min	**4.2 (99.993%) in 5.5 min**

Control and efficacy curves after each challenge were estimated by straight lines and the time at which the device demonstrate 3 log_{10} PFU/m^3 reduction was calculated. The device demonstrated 3 log_{10} PFU/m^3 in 19.92 minutes and 3.07 minutes after the first and second challenge, correspondingly.

Table 4. The time device demonstrate 3 Log_{10} after each challenge

Sample ID	Test #1	Test #2	Test #3	Average
Time reaching 3 Log_{10} Reduction after first challenge	20.5 min	20.5 min	18.75 min	19.92 min
Time reaching 3 Log_{10} Reduction after second challenge	3.04 min	3.11 min	3.06 min	**3.07 min**

The levels of negative ion were monitored in each test using an ion counter (Ion Counter NT-C101A), which was located in the middle of one side of the chamber. The maximum ion concentration was 300 pcs/cc. A separate test was performed to measure the concentration of the ions closer to the device. Figure 6 shows the concentrations of the ions detected around 1.5 meters away from the device and at the same height as the phage injection port. The maximum level of detected ions did not exceed 1200 ions/cc during the test. These investigations confirm that negative ions do not accumulate in the chamber.

Fig. 6. Location of the ion counter in the chamber during the test which measured the level of negative ions in the chamber

Appendix A:

Table 5. 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*.

Varionix® KK1-F			Sampling Time Points (minutes)						
Sampling Time Points (minutes)			0	15	30	45	60	90	120
Total Plaques in the room	PFU	Control #1	21431	6357	716	89	5	2	1
		Control #2	52155	15460	2260	274	43	9	2
		Control #3	32067	8819	1728	327	53	2	1
Recovered on Plates	PFU	Control #1	1213	359	121	25	3	2	1
		Control #2	2952	873	382	77	24	8	2
		Control #3	1815	498	292	92	30	2	1
log_{10} recovery in the room	log_{10}	Control #1	4.33	3.80	2.85	1.95	0.73	0.38	0.08
		Control #2	4.72	4.19	3.35	2.44	1.63	0.98	0.38
		Control #3	4.51	3.95	3.24	2.51	1.73	0.38	0.08

Table 6. Efficacy of Varionix® KK1-F when used 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*.

Varionix® KK1-F			Sampling Time Points (minutes)										
Sampling Time Points (minutes)*			0	11.25	16.88	20.63	24.37	55	70	71.63	75.38	105	135
Sampling Period (minutes)*			2	7.5	3.75	3.75	3.75	10	2	3.75	3.75	30	30
Total Plaque in the room	PFU	**Efficacy #1**	25124	571	160	0	0	0	25360	13	0	0	0
		Efficacy #2	27208	1903	246	28	0	0	27208	67	0	0	0
		Efficacy #3	34134	260	9	0	0	0	34134	57	0	0	0
Recovered on Plates	PFU	**Efficacy #1**	1422	121	17	0	0	0	1422	1	0	0	0
		Efficacy #2	1540	403	26	3	0	0	1540	7	0	0	0
		Efficacy #3	1932	55	1	0	0	0	1932	6	0	0	0
log_{10} recovery** in the room	log_{10}	**Efficacy #1**	4.40	2.76	2.21	0	0	0	4.40	1.11	0	0	0
		Efficacy #2	4.43	3.28	2.39	1.45	0	0	4.43	1.82	0	0	0
		Efficacy #3	4.53	2.41	0.98	0	0	0	4.53	1.76	0	0	0

* All plates were divided to four equal sections and the PFU in each area were counted and used for calculating the concentration of the bacteriophage in the chamber at the median of that interval.

References

Environ. Protection Agency (Dec. 2012). Air Sanitizers – Efficacy Data Recommendations. OCSPP 810.2500.

Sattar, S.A., Kibbee, R.J., Zargar, Z., Wright, K.E., Rubino, J.R., Khalid, M.K. (2016). Decontamination of indoor air to reduce the risk of airborne infections: Studies on survival and inactivation of airborne pathogens using an aerobiology chamber. Am. J. Infect. Control. 44: e177-e182.