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Assessment of Varionix® technology to reduce airborne pathogens

Test Summary

Test summary of the test report: Testing with Cystovirus Phi6 (ATCC 21781-B1) as the challenge.

Test organism: Cystovirus Phi6 (ATCC 21781-B1); host: Pseudomonas syringae (ATCC 19310)

Virus compatibility: Surrogate for infectious enveloped viruses like coronavirus and influenza virus.

Test product indentity: VARIONIX KK1-F

Test method: Air Decontamination Protocol based on United States Environmental Protection Agency (U.S. EPA) Guidelines OCSPP 810.2500 for Efficacy Test Recommendations on Air Sanitizers

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Study Completion Date: Aug/18/20

THE EFFECT OF DBD BIPOLAR IONIZATION ON THE CONCENTRATION OF BACTERIOPHAGE PHI 6 IN THE AIR AS AN INFLUENZA AND CORONAVIRUS SURROGATE

The effect of DBD bipolar ionization on the concentration of bacteriophage phi 6 in the air as an influenza and coronavirus surrogate

Test Summary

In this study, the summary results of a laboratory test “Assessment of Varionix® KK1-F to reduce airborne pathogens: Testing with Cystovirus Phi 6 (ATCC 21781-B1) as the challenge” [1] are examined.

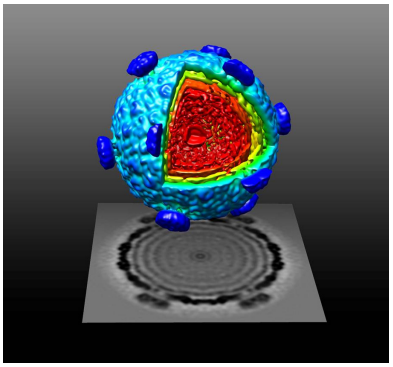
TEST SETUP The details of the aerobiology chamber have been published before (Sattar et al., 2016) [2]. Briefly, the chamber (918 ft3) was built to comply with the guidelines from the U.S. Environmental Agency (U.S. EPA 2012) [3]. The air temperature (72±3°F) and RH (50±10%) inside the chamber were measured and recorded using a remote-sensing device.

THE VIRUS USED IN STUDY Phage Cystovirus Phi 6 (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 nonpathogenic surrogate for human pathogenic viruses, such as the influenza and coronaviruses.

MEASUREMENTS AND FINDINGS In the study a certain amount of virus aerosols were injected into to the test chamber. After the aerosols were left to spread around the space, the concentration of the virus in the chamber was observed by collecting a series of air samples. At the first stage of the study, the natural decay of the virus in the air was analyzed for 120 minutes by collecting three series of control samples.

In the second stage of the study, the test was repeated such that the Varionix® bipolar ionizer was turned on after the virus aerosols had been injected and the first air sample collected. A series of air samples were collected to observe the concentration of the virus in the chamber when the bipolar ionizator was on. After 70 minutes, more virus aerosols were injected in the chamber while the ionizator was still on. The test set was repeated three times and the ion concentration was observed throughout the study.

The concentration of the virus in the air was studied as a function of time. The measurements showed that the concentration of the virus in the air decayed noticeably faster when the bipolar ionizator was turned on, as compared to the natural decay. Moreover, the virus concentration decayed even faster if the virus was injected when the ionizator was already turned on. The concentration of negative ions in the air was at highest 1,200 pcs/cm3 throughout.



Φ6 (Phi 6) is the best-studied bacteriophage of the virus family Cystoviridae. It infects Pseudomonas bacteria (typically plant-pathogenic P. syringae). It has a three-part, segmented, double-stranded RNA genome, totalling ~13.5 kb in length.

Φ6 (Phi 6) is an enveloped bacteriophage and was chosen for this study because it has been used as a surrogate for the persistence of other enveloped viruses such as the influenza virus, coronavirusess (including SARS-CoV-2), and Venezuelan equine encephalitis virus. Using a nonpathogenic surrogate removes the need for resources associated with a BSL-3 or BSL-4 agent. The persistence of phi 6 is similar to the published reports of EBOV, human respiratory viruses, and coronavirus in that the phage persisted longer in colder temperatures and at lower relative and absolute humidity.

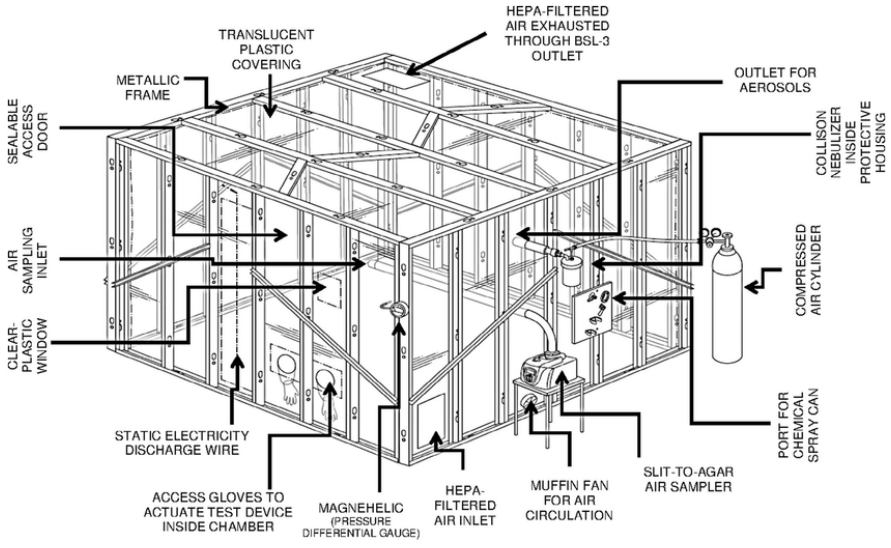
The effect of DBD bipolar ionization on the concentration of bacteriophage phi 6 in the air as an influenza and coronavirus surrogate

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 facilities. In this study, Varionix® KK1-F was tested to quantitatively assess if it could reduce the air contamination of an envelopoed bacteriophage (Phi6) as a surrogate for enveloped viruses such as influenza and coronaviruses. The technology tested is based on the DBD (Dielectric Barrier Discharge) bipolar ionization generated cold plasma to charge indoor air. The device itself is mounted on the HVAC system to take advantage of the air delivery into the occupied space.

RATIONALE Indoor air is an important vehicle for a variety of human pathogens as 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). Numerous 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 to mitigate the risk of pathogen spread.The virus used in the study, Phage Cystovirus Phi 6 (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 nonpathogenic surrogate for human pathogenic viruses, such as influenza and coronaviruses [2].

THE AEROBIOLOGY CHAMBER The details of the aerobiology chamber have been published before (Sattar et al., 2016) [3]. Briefly, the chamber (26 m^3/918 ft^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 circulated the air uniformly inside it. Between uses, fresh air was used to flush out the chamber of any residual airborne microbes.



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TEST METHOD Flowchart 1 provides the sequence of steps in a typical experiment for testing the air decontamination device. As a control, the study included testing the natural decay of the test organism over time while the muffin fan was on while leaving the Varionix® KK1-F bipolar ionization device off.

SEQUENCE OF STEPS IN THE EXPERIMENT

Air Decontamination

1. Turn on the muffin fan in the aerobiology chamber at least 10 min before
2. Nebulize the test microorganism for 10 minutes
3. Allow 5 minutes for uniform distribution of aerosols
4. Collect a two-minute air sample
5. Turn on the device (in the control test no action is required)
6. Collect an air sample based on Tables 1 and Table 2
7. Count PFU on the plates after 24 hours of incubation

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 the efficacy test after sampling the baseline, the device was turned ON and kept ON until the end of the test.

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 the 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 becomes undetectable after 2 hours.

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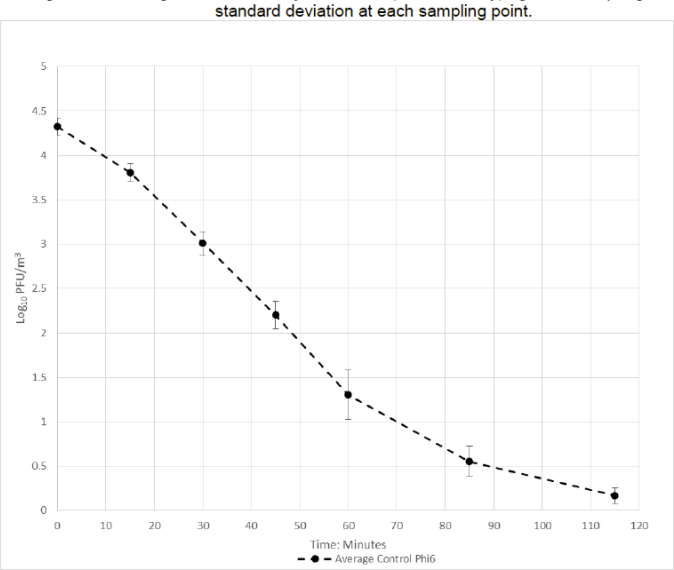
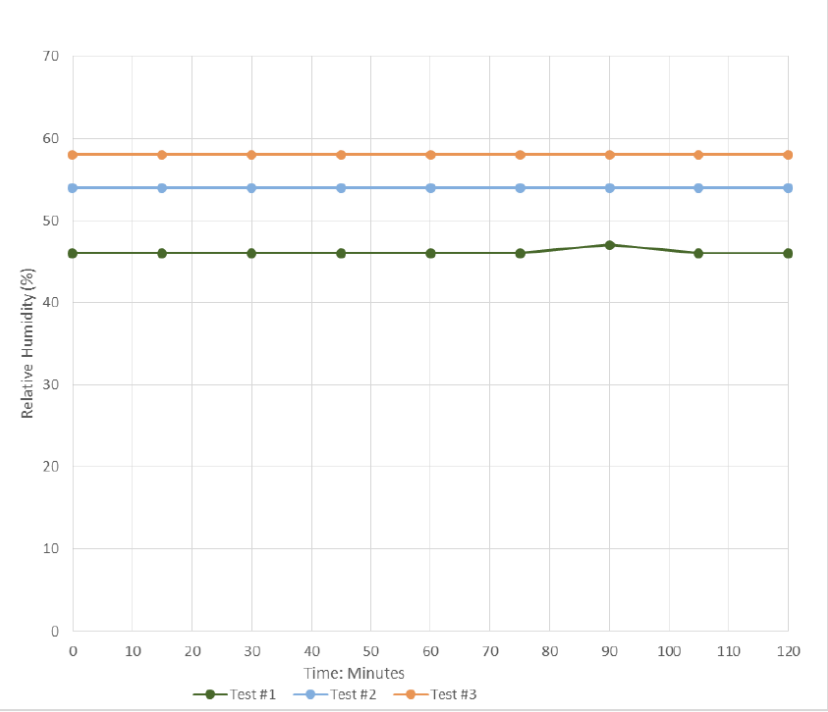
Fig. 1. The average of three stability-in-air tests (natural decay) against Phi6 phage with the standard deviation at each sampling point.



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



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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 log10 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 demonstrated a 3.4 log10 reduction (99.96% reduction) after 21 minutes of introducing the first challenge and demonstrated a 4.2 log10 (99.993% reduction) reduction in 5.5 minutes after introducing the second challenge. In **test #2** (RH of 48%), the device demonstrated a 3.25 log10 reduction (99.94% reduction) after 21 minutes of introducing the first challenge and a 4.2 log10 reduction (99.993% reduction) in 5.5 minutes after introducing the second challenge. In **test #3** (RH of 41%), the device demonstrated a 3.5 Log10 reduction (99.97% reduction) after 21 minutes of introducing the first challenge and a 4.2 Log10 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

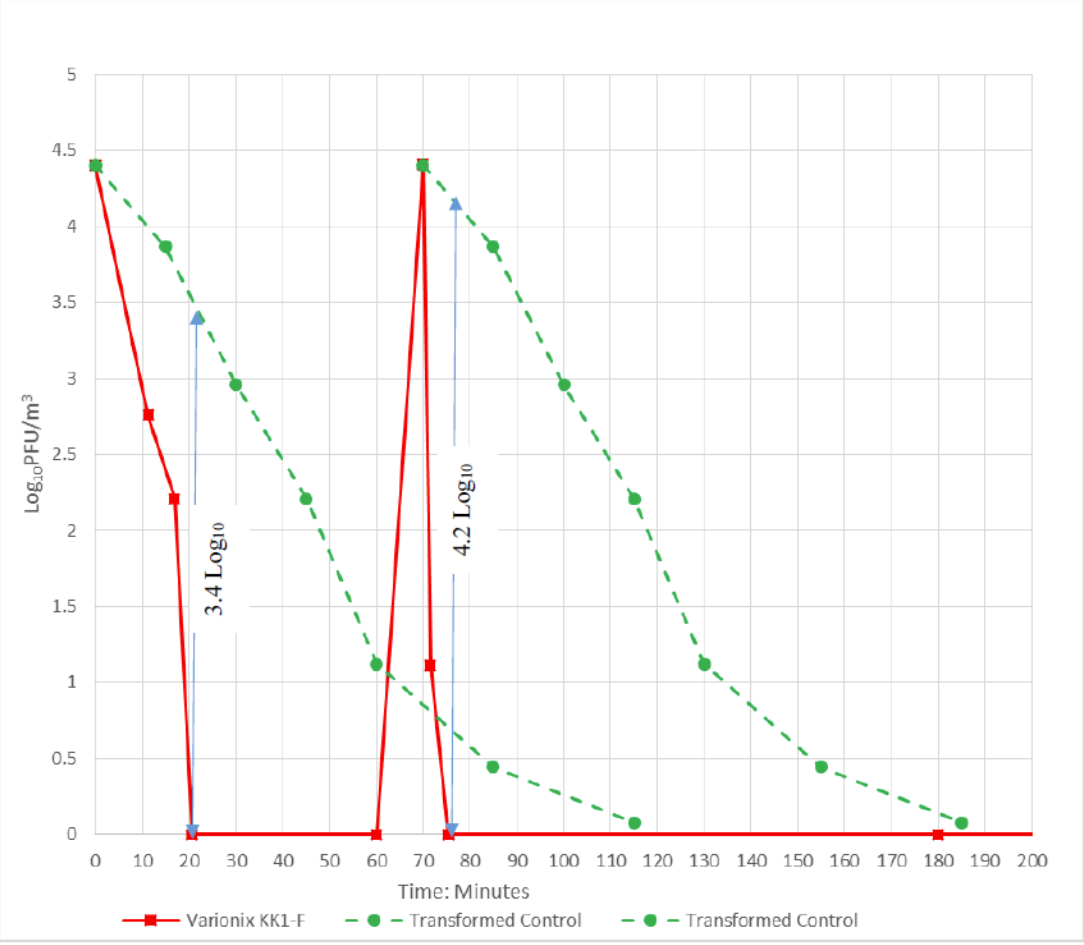

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Fig. 4. Stability-in-air and the second efficacy experiment on Varionix® KK1-F against Phi6 phage

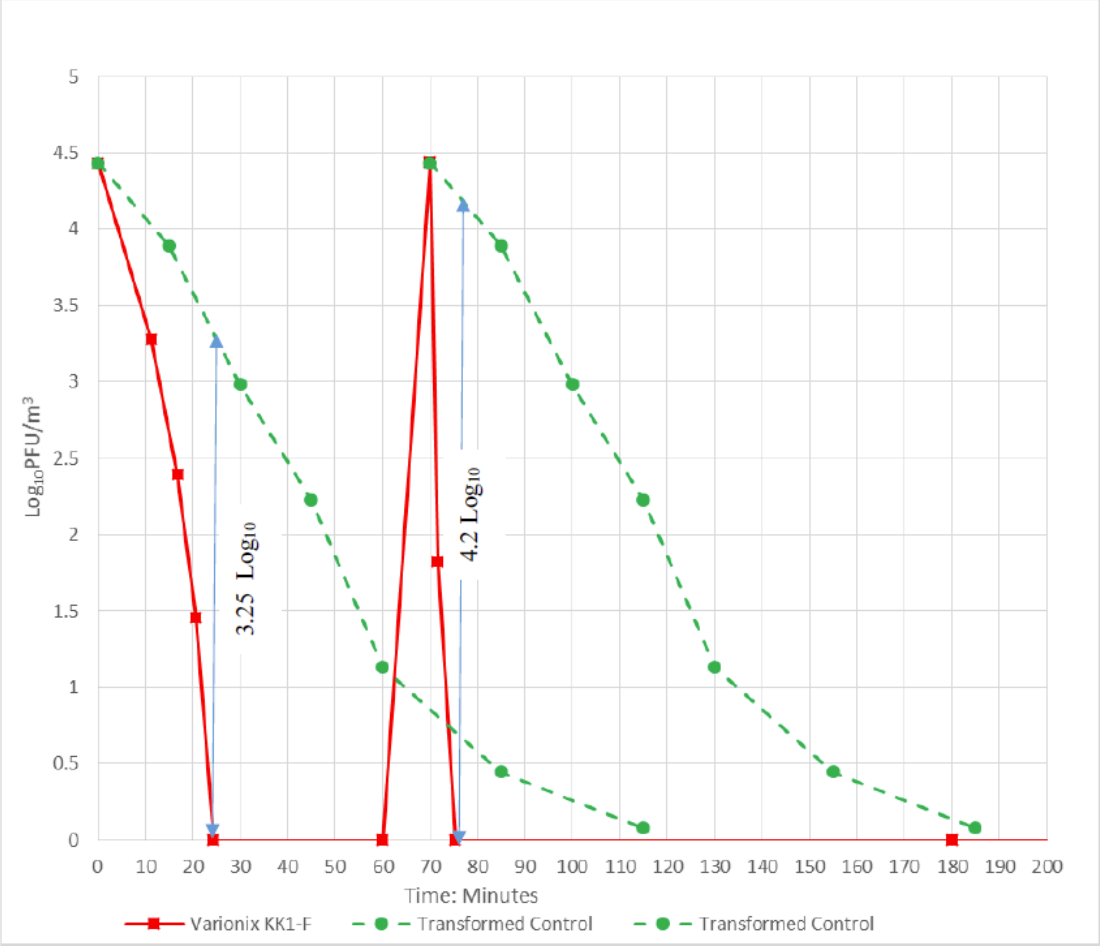

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Fig. 5. Stability-in-air and the third efficacy experiment on Varionix® KK1-F against Phi6 phage

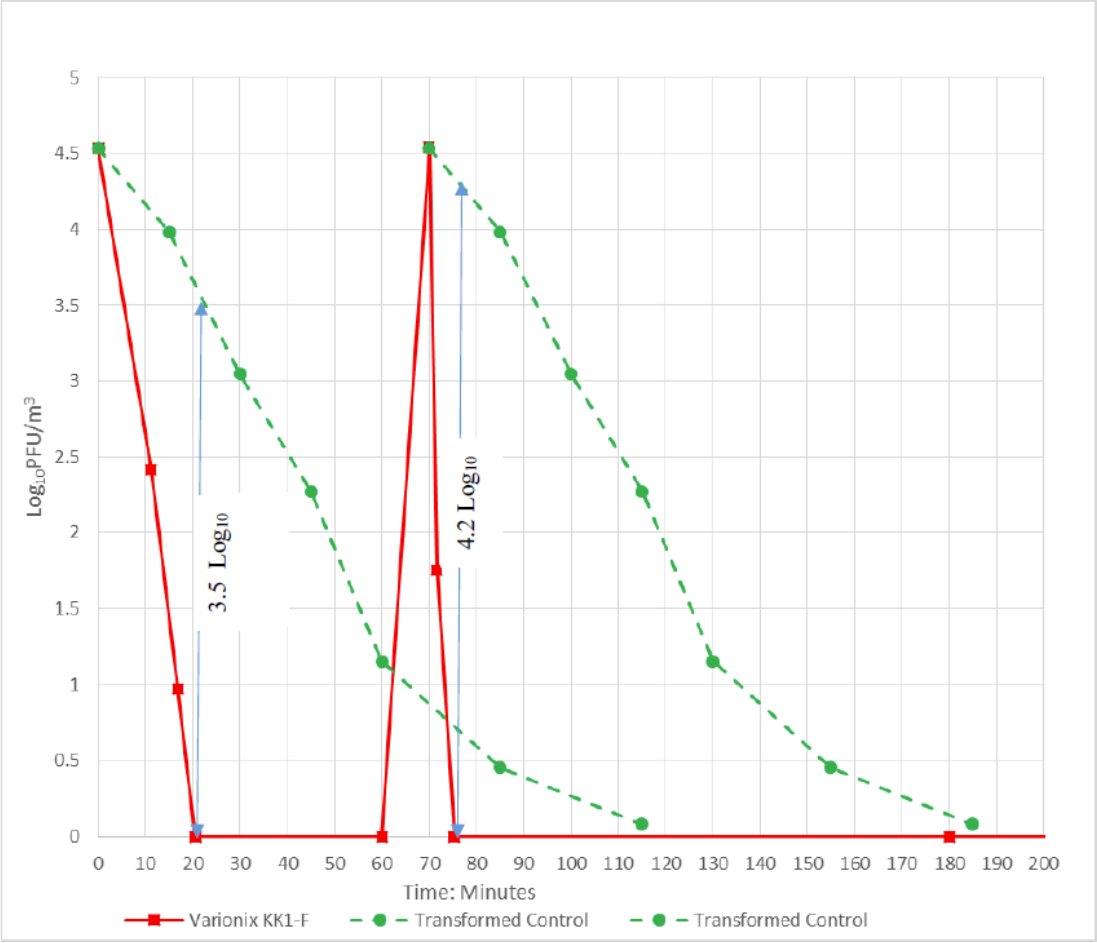

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Table 3 summarizes the efficacy of the Varionix® KK1-F device in three different efficacy tests. On average, the device demonstrates a 3.38.87 log_{10} reduction (99.96%) after 21 minutes of introducing the first challenge and a 4.2 log_{10} reduction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

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. Varionix® DBD bipolar ionization technology generates equal amount of negative and positive ions. The maximum level of detected ions did not exceed 1200 ions/cm^3 during the test.

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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

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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.

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CONCLUSIONS

Based on this study carried out in laboratory conditions, it can be noted that the concentration of the Phi6 virus in the air can be decreased by applying DBD bipolar ionization. Phi6 is an enveloped virus that is frequently used as a nonpathogenic surrogate for human pathogenic viruses, such as influenza and coronaviruses*. The physical conditions (temperature and relative humidity) correspond relatively well to typical conditions in an office. Additionally, the ion concentration is not exceptionally high, but corresponds to the typical ion concentration in a room where bipolar ionizator is occupied [4].

* SARS-CoV-2, a virus causing the COVID-19 pandemic, is a beta coronavirus. The genetic material of the virus is protected by a capsid, which is surrounded by an envelope of lipids. Diameter of the virus is around 50-200 nm.

REFERENCES

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