## STUDY TITLE

Assessment of Radic8 VK102 device 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

Radic8 VK102

## TEST Method

Air Decontamination Protocol based on U.S. EPA Guidelines OCSPP 810.2500 for Efficacy Test Recommendations on Air Sanitizers

## AUTHOR

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## STUDY COMPLETION DATE

April/19/20

## PERFORMING LABORATORY

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

## SPONSOR

Radic8

## STUDY NUMBER

RADIC200318-01

## STUDY PERSONNEL

STUDY DIRECTOR: Bahram. Zargar, PhD

PROFESSIONAL PERSONNEL INVOLVED: Sepideh Khoshnevis, MSc

**STUDY REPORT**

## GENERAL STUDY INFORMATION

| | |
|---|---|
| **Study Title:** | Assessment of Radic8 VK102 to reduce airborne pathogens: Testing with Cystovirus Phi6 (ATCC 21781-B1) as the challenge |
| **Study Number:** | RADIC200318-01 |
| **Sponsor** | RADIC8 |
| **Testing Facility** | CREM Co Labs<br>Units 1-2, 3403 American Drive, Mississauga, ON, Canada |

## TEST SUBSTANCE IDENTITY

**Test Substance Name:** RADIC8 Device

## STUDY DATES

**Date Device Received:**
**Study initiation date:** March/03/18
**Experimental Start Date:** March/03/20
**Experimental End Date:** April/18/20
**Study Completion Date:** April/19/20

## 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, Radic8 VK102 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 light, titanium dioxide and HEPA filtration. The device itself is a stand-alone system with three fan speeds. The device was tested at the highest fan speed (#3).

## 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 Radic8 VK102 for its ability to inactivate enveloped virus (*Cystovirus Phi6 (ATCC 21781-B1)*) in indoor air under ambient conditions.

| | |
|---|---|
| **Test Device:** | Radic8 VK102 |
| **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 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. 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.**

**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 | 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 |
| 75 (60-90) | 30 |
| 115 (90-120) | 30 |

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**

Two 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**

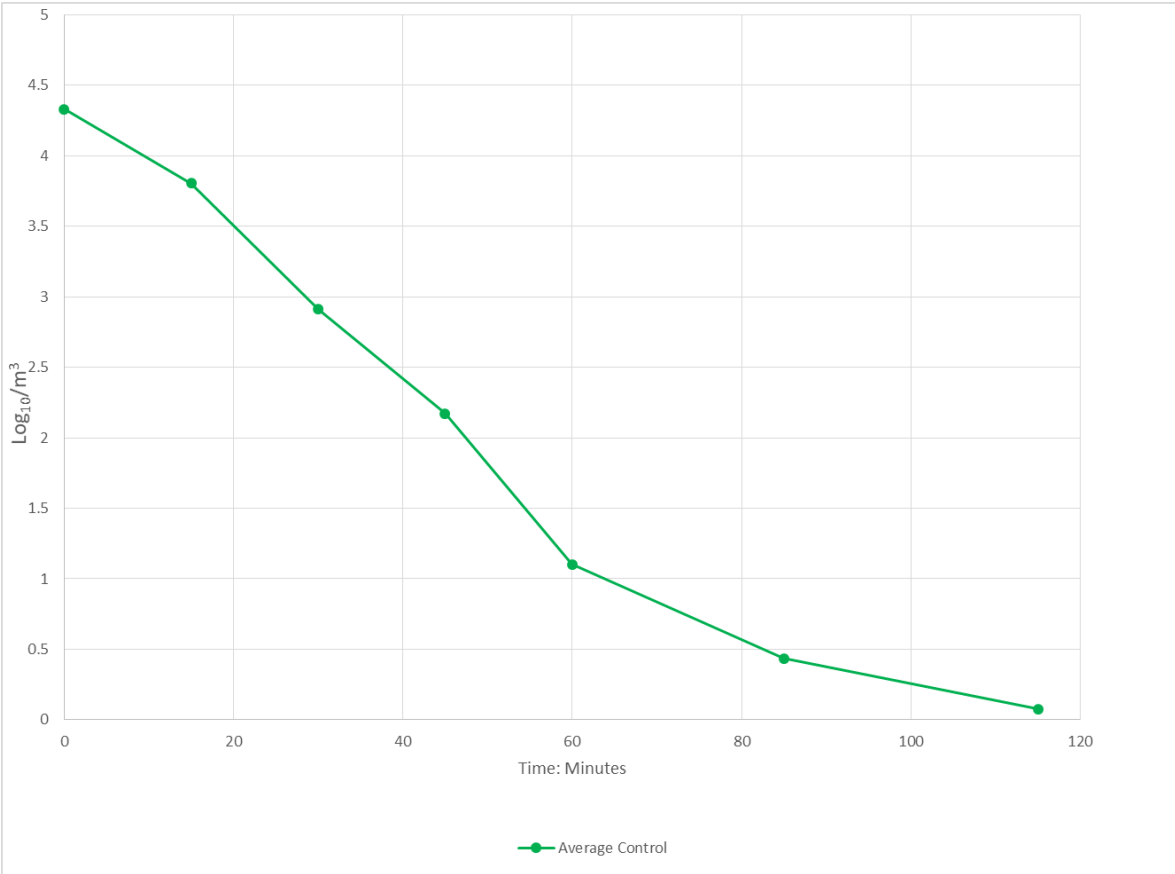
**Testing phage survival:** Any meaningful assessment of air decontamination requires that the aerosolized challenge microorganisms remain viable in the experimentally-contaminated air long enough to allow for proper differentiation between biological decay and inactivation/removal by the technology being tested. Such airborne viability of the microorganism used in this study was tested in the aerobiology chamber with two control tests without turning on the device while muffin fan was ON. The average of the two control tests was used to calculate the efficacy of Radic8 VK102.

**Efficacy test of the Radic8 VK102 against *Cystovirus* Phi6:**

This part of the report represents data from the efficacy experiments on the Radic8 VK102 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 two control tests (biological decay) with the corresponding standard deviation at each sampling interval. The concentration of Phage becomes undetectable after 2 hours.

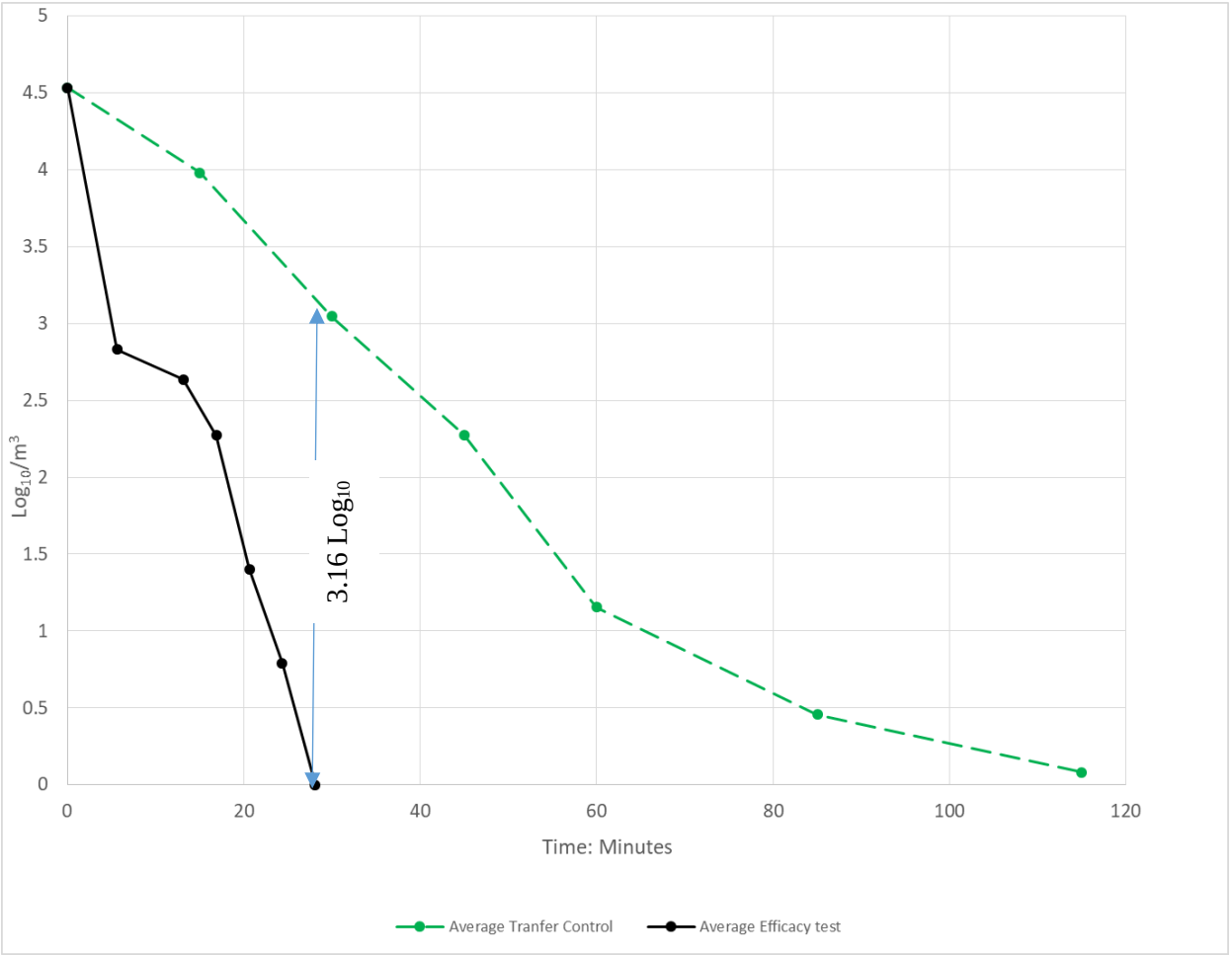
Fig. 1. The average of three Stability-in-air tests (natural decay) against Phi6 phage with the standard deviation at each sampling point.




Two efficacy tests were performed on the device. Figure 2 shows the average $log_{10}$ PFU/$m^3$ recoveries for the two tests. The average of $log_{10}$ PFU/$m^3$ recoveries of the transformed control of the two 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 demonstrate 3.16 $Log_{10}$ reduction (99.93% reduction) after 28 minutes of turning on the device.

Fig 2. Efficacy of Radic8 VK102 in reducing microbial contamination of air. The average of two control and two 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 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 |
| | | | | | | | | | |
| Recovered on Plates | PFU | **Control #1** | 1213 | 359 | 121 | 25 | 3 | 2 | 1 |
| | | **Control #2** | 1815 | 498 | 292 | 92 | 30 | 2 | 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 |
| | | | | | | | | | |

Table 5. Efficacy of Radic8 VK102 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*.

| Radic8 VK102 | | | Sampling Time Points (minutes) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sampling Time Points (minutes) | | | 0 | 5.625 | 13.125 | 16.875 | 20.625 | 24.375 | 28.125 | 31.875 |
| Sampling Period (minutes) | | | 2 | 3.75 | 3.75 | 3.75 | 3.75 | 3.75 | 3.75 | 3.75 |
| Total PFU in the room | PFU | **Test #1** | 32650 | 671 | 549 | 218 | 10 | 0 | 0 | 0 |
| | | **Test #2** | 35830 | 689 | 341 | 161 | 67 | 38 | 0 | 0 |
| Recovered on Plates | PFU | **Test #1** | 1848 | 71 | 58 | 23 | 1 | 0 | 0 | 0 |
| | | **Test #2** | 2028 | 73 | 36 | 17 | 7 | 4 | 0 | 0 |
| $log_{10}$ reduction** | $log_{10}$ | **Test #1** | 4.55 | 2.84 | 2.53 | 2.21 | 1.82 | 1.58 | 0 | 0 |
| | | **Test #2** | 4.51 | 2.83 | 2.74 | 2.34 | 0.98 | 0 | 0 | 0 |

**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.