

Study No.: RADIC200318-02

Assessment of Radic8 VK102 device to prevent/reduce contamination of environmental surfaces from airborne pathogens: Testing with Cystovirus Phi6 (ATCC21781-B1) as the challenge



STUDY TITLE

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TEST ORGANISM

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

TEST PRODUCT IDENTITY

Radic8 VK102

TEST Method

Air Decontamination Protocol and Surface 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

May/17/20

PERFORMING LABORATORY

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

SPONSOR

Radic8

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STUDY PERSONNEL

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STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Assessment of Radic8 VK102 to reduce on-site Spread of Pathogens: Testing with Cystovirus Phi6 (ATCC 21781-B1) as the challenge
Study Number: RADIC200318-02
Sponsor: RADIC8
Testing Facility: CREM Co Labs
Units 1-2, 3403 American Drive, Mississauga, ON, Canada

TEST SUBSTANCE IDENTITY

Test Substance Name: Radic8 VK102

STUDY DATES

Date Device Received:
Study initiation date: March/03/18
Experimental Start Date: April/19/20
Experimental End Date: May/12/20
Study Completion Date: May/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, Radic8 VK102 was tested to quantitatively assess if it could reduce the surface contamination 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 (*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 Radic8 VK102 to prevent/reduce contamination of environmental surfaces from airborne pathogens (*Cystovirus Phi6* (ATCC 21781-B1))

Test Device:	Radic8 VK102
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 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

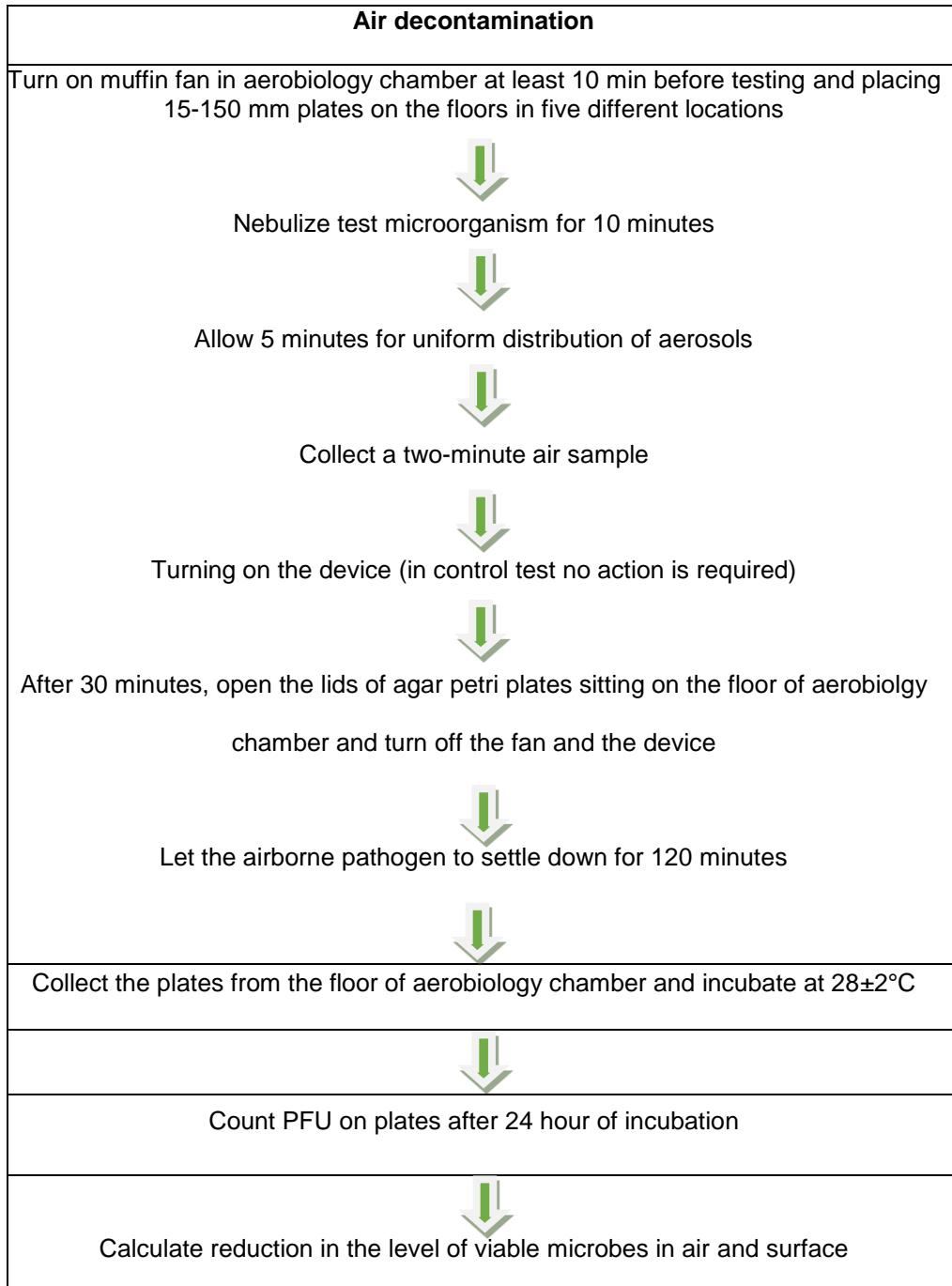
The test phage suspended in saline and nebulized into the aerobiology chamber (Sattar et al., 2016) using a six-jet Collison nebulizer.

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.

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



Experimental Design

Two control tests were performed, with the device OFF, and the muffin fan ON for 30 minutes. 15x150 mm plates with agar and host bacteria were placed in set of three in five different locations on the floor of the aerobiology chamber. After 30 minutes of sampling the baseline the fan and device turned off and the lids of the plates removed for 120 minutes to settle down the airborne pathogen. Two efficacy tests were performed. In efficacy test after sampling the baseline, the device turned ON for 30 minutes and the fan and device were turned off after 30 minutes.

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.

Table 1 in Appendix summarizes the PFU on 150 mm petri plates located on the floor. The percent reduction of contamination on the floor has been calculated using the average of two control and two efficacy tests. The contamination on the floor reduced 99.81 % in efficacy tests compare to Control tests.

Appendix A:

Table 1. PFU in Petri plates after 24 hours of incubation from test for inactivation of *Phi6 bacteriophage* on the Petri plates using Radic8 VK102. LB=Left Bottom, LT= Left Top, RB=Right Bottom, RT=Right Top, MC=Center.

Sample Name	PFU				Total PFU/ m ²	
	Control #1	Control #1	Test #1	Test #2	Average Control	Average Test
LB1	90	20	0	0	3056	0
LB2	9	23	0	0	889	0
LB3	41	93	0	0	3722	0
RB1	26	20	0	0	1278	0
RB2	29	52	0	0	2250	0
RB3	21	86	0	0	2972	0
LT1	23	64	0	0	2417	0
LT2	28	39	0	0	1861	0
LT3	25	16	0	1	1139	27.78
C1	5	55	0	0	1667	0
C2	28	103	0	1	7278	27.78
C3	9	34	0	0	2389	0
RT1	15	26	0	0	1139	0
RT2	38	110	0	1	4111	27.78
RT3	3	18	0	0	583	0
Percent Reduction					99.81%	

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.

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