STUDY TITLE

Assessment of Healthway 950P, a Device to reduce on-site Spread of Pathogens during Patient Care: Testing with Bacteriophage MS2 as the Challenge

TEST ORGANISMS

Phage MS2 (ATCC 15597-B1)
and its host
Escherichia coli (ATCC 15597)

TEST DEVICE IDENTITY

Healthway 950P

TEST Method

Aerobiology Air Decontamination Protocol

AUTHOR

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STUDY COMPLETION DATE

June/29/20

PERFORMING LABORATORY

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

SPONSOR

HealthWay Family of Brands 3420 Maple Ave., Pulaski, New York 13142 USA

STUDY NUMBER

HWFB200505-01

STUDY PERSONNEL

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Eduardo Suarez, PhD

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title:	Assessment of Healthway 950P, a Device to reduce on-site spread of Pathogens during Patient Care: Testing with Bacteriophage MS2 as the Challenge
Study Number:	HWFB200505-01
Sponsor	HealthWay Family of Brands 3420 Maple Ave., Pulaski, New York 13142 USA
Testing Facility	CREM Co Labs Units 1-2, 3403 American Drive, Mississauga, ON, Canada

TEST SUBSTANCE IDENTITY

Test Device Name: Healthway 950P

STUDY DATES

Date Device Received: June/12/20
Study initiation date: June/15/20
Experimental Start Date: June/15/20
Experimental End Date: June/27/20
Study Completion Date: June /29/20

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 (Decraene et al., 2008; Zemouri et al., 2017), and many technologies are used to remove/inactivate such airborne pathogens in healthcare and other settings. In this study, Healthway 950P, an Electrically Enhanced Filter (EEF)-based indoor air decontamination device was tested to quantitatively assess if it could reduce contamination of the air by a variety of airborne pathogens and their surrogates in the same setting.

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 Healthway 950P (Fig. 1) for its ability to inactivate a non-enveloped virus (Bacteriophage MS2 (ATCC 15597-B1) in indoor air under ambient conditions.

Test Device:	One type of device was tested inside the aerobiology chamber according to the manufacturer's instructions. The testing was based on Electrically Enhanced Filter (EEF). For this study, the device was placed in one corner of the aerobiology chamber and remotely operated at its highest fan power settings.
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. For sampling time more than two minutes, the plate was divided to four equal sections and PFU in each section was counted.

4. **The 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. Compressed air from a cylinder was used at ~172 kPa (25 psi) to operate the nebulizer. The fluid to be nebulized consisted of a suspension of the test microbe in phosphate-buffered saline (PBS).

5. Test Organism

Phage MS2 (ATCC 15597-B1) was grown in its bacterial host *Escherichia coli* (ATCC 15597): This phage is a relatively small (about 27 nm in diam.), non-enveloped virus that is frequently used as a surrogate for human pathogenic viruses (Turgeon et al., 2014). This virus was a gift from the Bureau of Microbial Hazards, Health Canada, Ottawa, ON, 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 *E. coli,* a loopful of the stock culture was streaked on a LB agar and was incubated for 18±2 h at 36±1°C. A colony was inoculated in 25 mL of LB broth and incubated in at 36±1°C. When the optical density (OD) reached around 0.8, the bacterial suspension was used for the test.

8. Preparation of Phage Inocula for aerosolization

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

9. Device tested: Fig. 1 shows the device. The device was provided by the Sponsor. It operates using Electrically Enhanced Filter (EEF). The unit under test was placed inside the chamber in one corner and turned on remotely right after collecting the baseline sample.

Fig. 1. The Healthway 950P unit tested in its operating mode

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 device was off. 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 device efficacy, respectively.

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

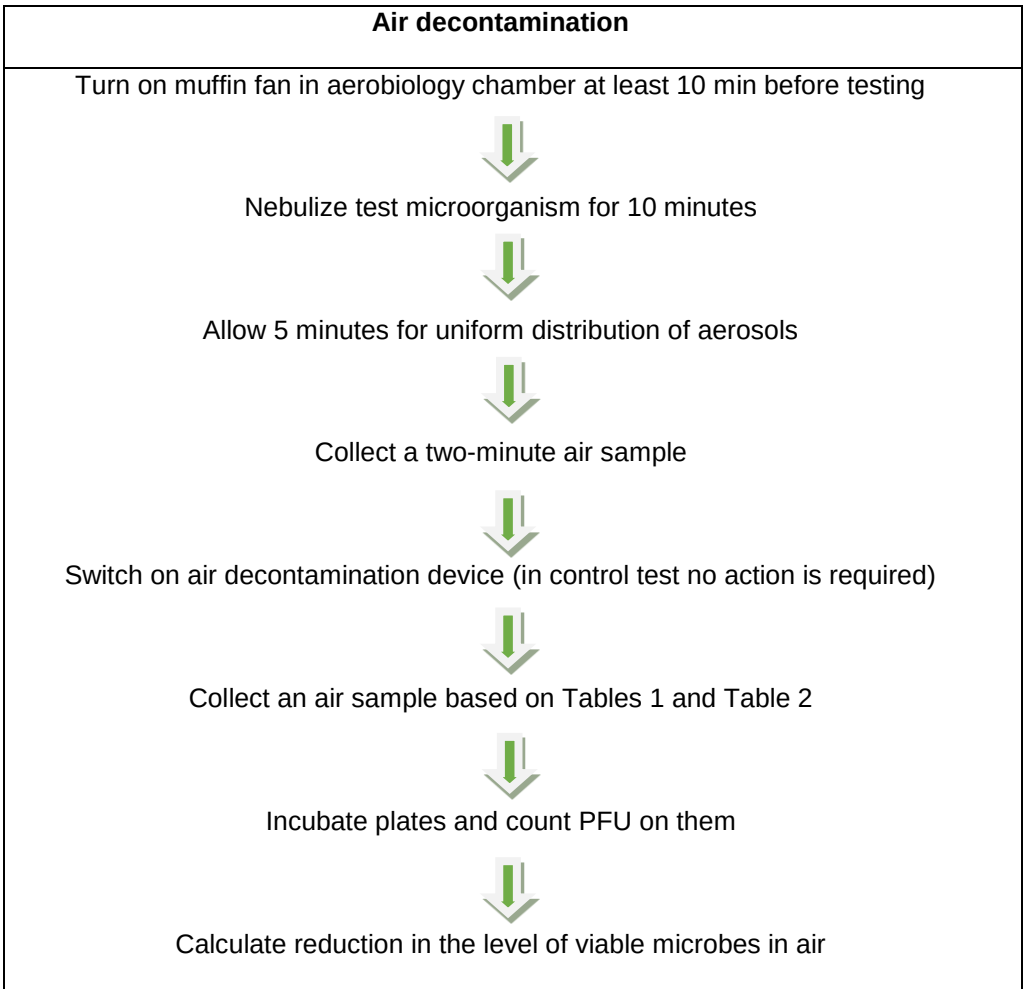


Table 1: **Time interval duration of air sampling for control tests**

Sampling point (min)	Sampling duration (min)	Sampling point (min)	Sampling duration (min)
0 (Baseline)	2	120	2
15	2	150	2
30	2	180	2
45	2	210	2
60	2	240	2
90	2		

Table 2: **Time interval and duration of air sampling for efficacy tests**

Sampling point (min)	Sampling duration (min)
0 (Baseline)	2
7.5	15
22.5	15
45	30
75	30
105	30

Experimental Design

Three control tests were performed, with the device OFF, and the muffin fan ON and three efficacy tests were performed at high speed of the fan (Turbo).

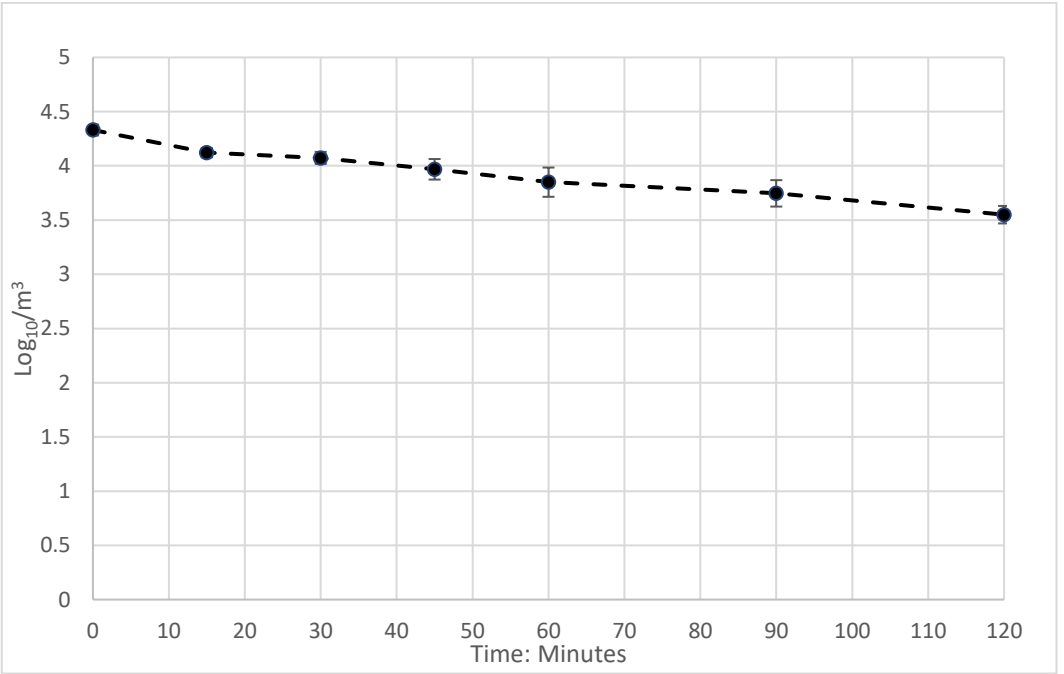
STUDY ACCEPTANCE CRITERIA

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 with device OFF and muffin fan ON. Figure 2 shows the average of three different control tests with standard deviation. The average of the three control tests was used to calculate the efficacy of the device.

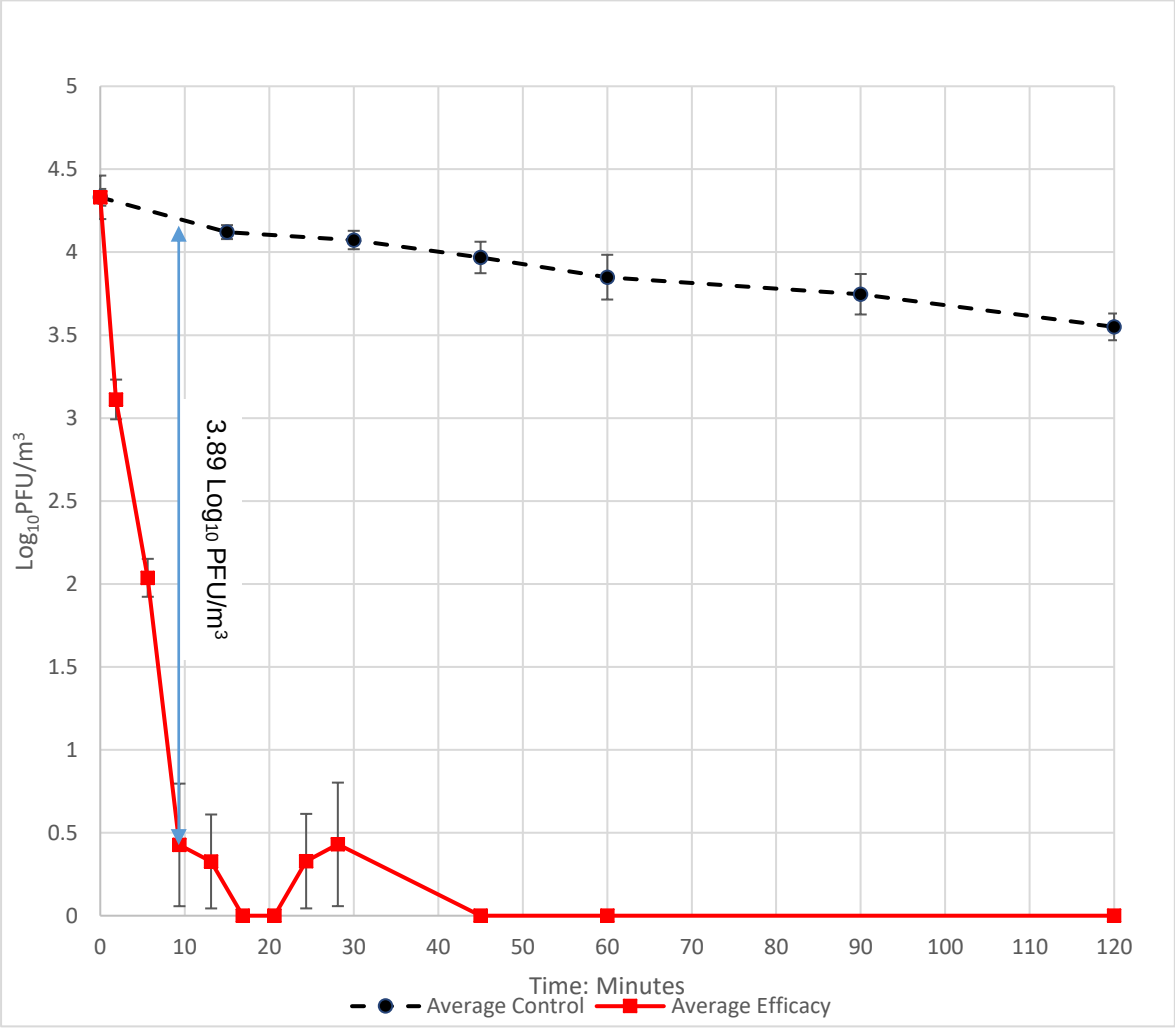
Fig. 2. The average natural decay of MS2 in three different controls: device OFF and muffin fan ON



Efficacy test of the device against MS2:

This part of the report represents data from the efficacy experiments on Healthway 950P device at high fan speed against MS2. The raw data are tabulated in Appendix A. Each Petri plate was divided into four equal sections and PFUs in each section were counted and recorded. Figure 3 shows the $\log_{10}$ PFU/m^3 recoveries. The $\log_{10}$ PFU/m^3 recoveries of the transformed control 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 demonstrates 3.89 $\log_{10}$ PFU/m^3 (99.987 percent reduction) in 10 minutes.

Fig. 3. Stability-in-air and efficacy experiments for Healthway 950P at high fan speed against MS2 phage



Appendix A:

Healthway 950P			Sampling Time Points (minutes)						
Sampling Time Points (minutes)			0	15	30	45	60	90	120
Total Colony in the room	PFU	**Control #1**	25371	14592	12354	8095	50477	4397	3333
		Control #2	38375	21251	20519	19358	17406	13514	7006
		Control #3	25936	19126	16827	13166	10344	7508	4909
Recovered on Plates	PFU	**Control #1**	1436	824	696	455	283	246	186
		Control #2	2172	2172	1200	1156	1088	976	756
		Control #3	1468	1080	948	740	580	420	274
$\log_{10}$ recovery in the room	$\log_{10}$	**Control #1**	4.40	4.16	4.09	3.91	3.70	3.64	3.52
		Control #2	4.58	4.33	4.31	4.29	4.24	4.13	3.84
		Control #3	4.41	4.28	4.23	4.12	4.01	3.87	3.69

Table 3. Natural decay of bacteria phage *MS2* 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 MS2.

Healthway 950P						Sampling Time Points (minutes)						
Sampling Time Points (minutes)			0	1.875	5.625	9.375	13.125	16.875	20.625	24.375	28.125	31.875
Total Colony in the room	PFU	**Control #1**	13569	2389	85	19	10	0	0	10	19	0
		Control #2	42473	1105	199	0	0	0	0	0	0	0
		Control #3	17032	822	76	0	0	0	0	0	0	0
Recovered on Plates	PFU	**Control #1**	768	253	9	2	1	0	0	1	2	0
		Control #2	2404	117	21	0	0	0	0	0	0	0
		Control #3	964	87	8	0	0	0	0	0	0	0
$\log_{10}$ recovery** in the room	$\log_{10}$	**Control #1**	4.13	3.37	1.93	1.28	0.98	0	0	0.99	1.29	0
		Control #2	4.63	3.04	2.30	0	0	0	0	0	0	0
		Control #3	4.23	2.91	1.88	0	0	0	0	0	0	0

Table 4. Efficacy of Healthway 950P Device, at high fan speed in reducing microbial contamination of air.

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