LRI™ Environmental Solutions
A Division of Life's Resources Inc.

Effect of the Healthway® EMF Air Cleaner on the Survival of Microorganisms

August 25, 2000
Updated April 29, 2005

Prepared for

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Report No. 7674-00-2

by

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1.0 Introduction

1.1 Purpose

The purpose of these tests was to determine the effect of the Healthway® EMF Air Cleaner on the survival of bacteria, molds, and viruses collected on its internal filter.

1.2 Background

Air is an important vehicle for transmitting infectious and allergenic microorganisms. One important method of reducing the adverse effects of these microorganisms is to reduce the level of exposure to which susceptible persons are subjected. This can be accomplished by either eliminating contaminating sources or by reducing the densities of infection- or allergy-causing microorganisms within the air. Use of efficient air cleaners can be an important method for removing microorganisms from the air. However, if microorganisms collecting on air cleaner filters continue to survive or grow, as may occur under some conditions of operation, then the filters can become a source of further contamination in indoor environments. In addition, when collected microorganisms survive on the filter the routine maintenance that includes filter change can subject users to an additional likelihood of becoming exposed to those microorganisms. The Healthway® EMF Air Cleaner was reported by the manufacturer to be designed to significantly reduce the survival of airborne microorganisms collected on the internal filter surface.

1.4 Tests Performed

We performed tests on four different microorganisms to determine the effect of the operating Healthway® EMF Air Cleaner on their survival. The organisms included two bacteria, a gram negative rod and a gram positive spore-forming rod, a bacteriophage virus, and a fungus that was representative of common molds. Each of the microorganisms used in these tests has been used extensively in studies of factors affecting the effects of specific environmental conditions on survival.

Gram staining is an important and widely used method for differentiating bacteria. When the bacteria are subjected to a staining procedure, some are stained and some are not stained. The stained bacteria develop a deep violet color and are called “gram-positive bacteria.” The unstained bacteria are counterstained and develop a red color and are called “gram-negative bacteria.” The differences between the bacteria that take the stain and those that do not take the stain lie in the chemical make up of their cell walls.

Serratia marcescens, a gram negative rod-shaped bacterium, has been used extensively in studies to determine the airborne survival of microorganisms.[1] Other gram negative rod-shaped bacteria include *Escherichia coli* and *Haemophilus influenzae*. *Bacillus subtilis*, a gram-positive rod-shaped bacterium, has also been used in studies on the survival of bacteria in the air. Other gram-positive rod-shaped bacteria include *Corynebacterium diptheriae* and *Bacillus anthracis* (Anthrax).

Penicillium funiculosum has been used, for example, as a model organism to evaluate the effects of aerial disinfection of libraries.[2] A wide range of molds have similar characteristics in indoor environments. Other molds that grow under high moisture conditions include *Aspergillus* sp. and *Stachybotrys* sp.

Coliphages have been used and studied extensively as indicators of the survival of animal and human viruses.[3] The MS-2 coliphage has been used to model the airborne stability of viruses.[4] Viruses with similar physical characteristics include the enteric viruses, such as those that can cause both gastrointestinal and respiratory illnesses. Such virus include Coxsackieviruses, Echoviruses, Adenoviruses, and the Norwalk agent.

Tests

1.1 Bacteria Survival Tests

1.1.1 Apparatus Tested

The test apparatus was identified as a Healthway® EMF Air Cleaner and was supplied by Healthway Corporation. The test unit, shown in Figure 1.

Figure 1. Healthway® EMF Air Cleaner

The replaceable internal filter used in the test unit is shown in Figure 2.

Figure 2. Internal Filter

1.1.2 Test Setup and Procedures

Test Conditions

Tests were performed under ambient indoor air conditions. The test unit was operated at medium speed during the tests.

In-Duct Microorganism-Suspending Holders

The tests were performed using sterile membrane filter (Gelman Sciences) test surfaces inoculated with the test microorganisms. The inoculated filters were placed onto the upstream surface, the bottom side, of the internal air filter as shown in Figure 3. Filter edges were secured with masking tape.

Inoculated Test Filter

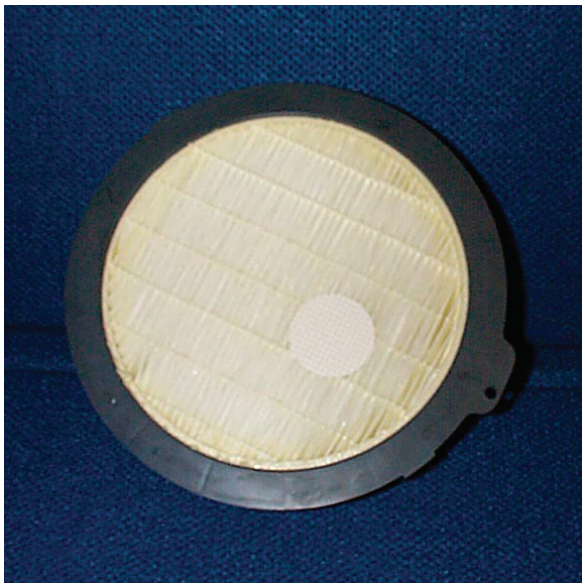

Figure 3. Inoculated Test Filter

Test Organisms

The test organisms were *Serratia marcescens* (American Type Culture Collection (ATCC) No. 14756), Bacillus subtilis (ATCC No. 9372), *Escherichia coli* phage MS-2 (ATCC 15597-B1), and *Penicillium funiculosum* (ATCC 11797).

Test Procedure

Bacteria

Prior to each series of tests, the organisms were grown overnight at 95° F in nutrient broth (Difco). Ten-fold dilutions of the organism culture were made in phosphate buffered water. Selected dilutions of the test organisms were inoculated onto the test filters using a membrane filtration procedure and rinsed with phosphate buffered water. Inoculated filters were aseptically placed onto the surface of the test filter and secured on the edges with two pieces of masking tape. The filters inoculated with the test organisms were then placed into the test air cleaner. Tests were performed with the air cleaner energized and operating at medium speed.

Following exposure to specified conditions, the inoculated membrane filters were aseptically removed from the surface of the internal filter using sterile forceps. The inoculated membrane filters were then placed onto the

surface of nutrient agar-containing petri dishes for assay. Following incubation at 95° F for 24 hrs, the *Serratia marcescens* cultures were placed at room temperature until red pigmentation developed. Colony counts following appropriate incubation were used to determine the viability of the organisms.

Viruses

Escherichia coli phage MS-2 (ATCC 15597-B1), a bacterial virus, was grown in a host culture of *Escherichia coli* C3000 (ATCC 15597) bacterial cells at 35° C. Tests on the survival of the virus were performed by inoculating 0.5 mL of an appropriate dilution of the virus onto a sterile membrane filter placed on an impervious filter liner. The inoculated filter and liner were then placed onto the inlet side of the filter in the test air cleaner. The air cleaner was then operated at medium speed for approximately 24 hr.

Base agar plates were prepared by using nutrient agar (Difco), containing 15 g/L agar and with added 5 g/L NaCl, 0.2 g/L $MgSO_4 \cdot 7H_2O$, and 0.15 g/L $CaCl_2$. The viruses were assayed on *Escherichia coli* C3000 bacterial cells. The virus-inoculated test filters and liners were removed from the test air cleaner and placed onto the surface of the base agar plates. The filters were then overlaid with a lawn of the host bacteria using a soft agar overlay held at 45° C. The soft agar overlay contained nutrient agar medium prepared from nutrient broth (Difco), with added 7.0 g/L agar, 5 g/L NaCl, 0.2 g/L $MgSO_4 \cdot 7H_2O$, and 0.15 g/L $CaCl_2$. The overlay was inoculated with 0.5 mL of a culture of the host bacteria and mixed just prior to overlay. The plates were then incubated at 35° C and examined for cell lysis.

Mold/Fungi

Penicillium funiculosum (ATCC 11797) was grown on potato dextrose agar (Difco) at room temperature. Ten-fold dilutions of the organism culture were made in phosphate buffered water. Selected dilutions of the test organisms were inoculated onto the test filters using a membrane filtration procedure and rinsed with phosphate buffered water. Inoculated filters were aseptically placed onto the surface of the test filter and secured on the edges with two pieces of masking tape. The filters inoculated with the test organisms were then placed into the test air cleaner. Tests were performed with the air cleaner energized and operating at medium speed.

Following exposure to specified conditions, the inoculated membrane filters were aseptically removed from the surface of the internal filter using sterile forceps. The inoculated membrane filters were then placed onto the surface of nutrient agar-containing petri dishes for assay. Colony counts following appropriate incubation were used to determine the viability of the organisms.

Relative Humidity and Temperature

Relative humidity and temperature were measured using a wet/dry bulb sling psychrometer.

2.0 Results

3.1 Effect of the Test Air Cleaner on *Serratia marcescens* Bacteria

The tests on the effect of the test unit on the survival of *Serratia marcescens* bacteria were performed at room temperature levels ranging from 70 to 75° F and at room relative humidity levels ranging from 51% to 60%. The effect of the test unit on the survival of the bacteria is clearly illustrated by comparing the results shown in Figure 4 to those shown in Figure 5. Figure 4 shows a bacteria culture not subjected to exposure to the test air cleaner. Figure 5 shows the a similar culture that was exposed in the test air cleaner for approximately 4 hrs. The results showed a loss in bacteria viability by 100 percent by exposure to the conditions within the test air cleaner.

Figure 4. *Serratia marcescens* Culture Not Exposed to Test Unit

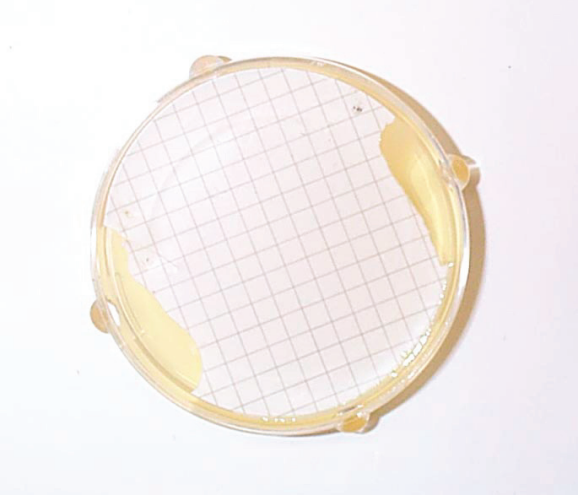

Figure 5. *Serratia marcescens* Culture Exposed in Test Unit for Approximately 4 hr

A *Serratia marcescens* culture containing 4.1 x 10^8 colony forming units (cfu) per mL was diluted each test filter was inoculated with approximately 408 cfu of the bacteria. Following exposure in the test air cleaner for approximately 4 hr, the number of bacteria were reduced by 100 percent.

3.2 Effect of the Test Air Cleaner on *Bacillus subtilis* Bacteria

A *Bacillus subtilis* culture containing 6.0 x 10^7 colony forming units (cfu) per mL was diluted each test filter was inoculated with approximately 60 cfu of the bacteria. Figure 6 shows the bacteria culture without exposure in the test air cleaner. Following exposure in the test air cleaner for approximately 4 hr, the number of cfu was reduced to one. The number of *Bacillus subtilis* cfu was reduced by 98 percent after exposure on the test air cleaner filter. Figure 7 shows the bacteria culture with exposure in the test air cleaner.

Figure 6. *Bacillis subtilus* Culture Not Exposed in Test Unit

Figure 7. *Bacillus subtilis* Culture Exposed in Test Unit for Approximately 4 hr

3.2 Effect of the Test Air Cleaner on MS-2 Coliphage Virus

A MS-2 coliphage virus culture containing 2.2 x 10^7 plaque forming units (pfu) per mL was diluted and the test filter was inoculated with approximately 1.1 x 10^6 (pfu). Figure 8 shows the coliphage plaque growth without exposure in the test air cleaner. Following exposure in the test air cleaner for approximately 24 hr, no pfu were detectable. The number of MS-2 coliphage pfu was reduced by 100 percent after exposure in the test air cleaner filter. Figure 9 shows the coliphage culture with exposure in the test air cleaner for approximately 24 hr.

Plaques (lysis zones) showing presence of the virus.

Figure 8. MS-2 Phage Culture Not Exposed in Test Unit

No plaques observed showing absence of the virus.

Figure 9. MS-2 Phage Culture Exposed in Test Unit for Approximately 24 hr

3.3 Effect of the Test Air Cleaner on *Penicillium funiculosum*

Penicillium funiculosum

A *Penicillium funiculosum* fungus containing 2.8 x 10^4 colony forming units (cfu) was diluted and the test filter was inoculated with approximately 28 colony forming units (cfu) . Figure 10 shows the *Penicillium funiculosum* growth without exposure in the test air cleaner. Following exposure in the test air cleaner for approximately 24 hr and incubation at room temperature for six days, 18 cfu were detectable. The number of fungus pfu was reduced by 36 percent after exposure in the test air cleaner filter. Figure 11 shows the *Penicillium funiculosum* fungus culture with exposure in the test air cleaner for approximately 24 hr.

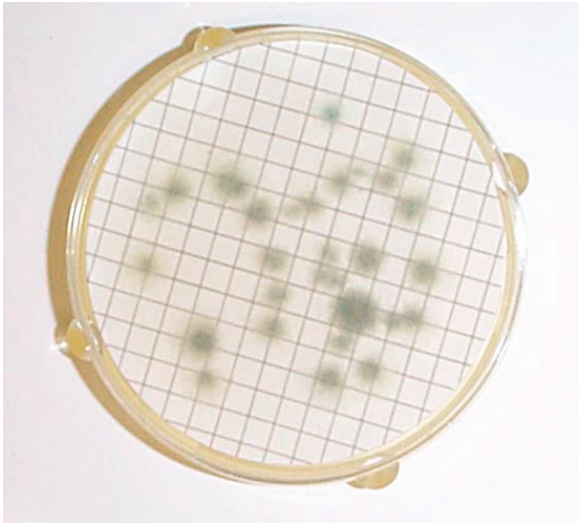

Figure 10. *Penicillium funiculosum* Fungus Culture Not Exposed in Test Unit

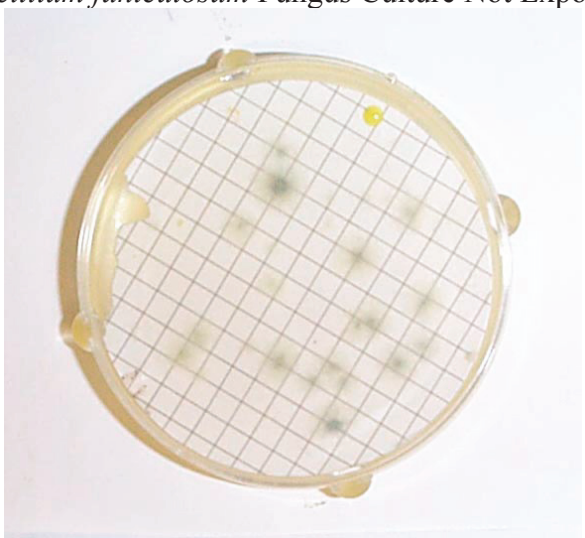

Figure 11. *Penicillium funiculosum* Fungus Culture Exposed in Test Unit for Approximately 24 hr

4.0 Conclusions

Whether the microorganisms were classified as living was determined by their ability to grow and produce observable evidence of that growth. For the bacteria and the fungus that observable growth was determined by colony formation in cultures. Observed colonies were expressed as colony-forming units (cfu). For the virus the evidence of growth was a clear zone indicating the destruction (lysis) of bacterial cells that were used as a host for the virus. These clear zones in a layer of host bacterial cells were referred to as plaques and were expressed as plaque-forming units (pfu).

In these tests, the bacteria were each exposed in the air cleaner for approximately four hours. The virus was exposed for approximately twenty-four hours. The fungus was exposed for approximately seventy-two hours. The percent reduction in either cfu or pfu for each microorganism was determined by comparing the number of detectable cfu or pfu in cultures following exposure in the test air cleaner compared to the cfu or pfu observed in cultures not subject to exposure in the test air cleaner.

The exposure of *Serratia marcescens* bacteria, *Bacillus subtilis* bacteria, *Escherichia coli* phage MS-2, and *Penicillium funiculosum* cultures on the internal filter of the Healthway® EMF Air Cleaner reduced the concentration of the viable microorganisms by up to 100 percent. The number of *Serratia marcescens* colony forming units was reduced by 100 percent within approximately 4 hrs while the number of colony forming units for *Bacillus subtilis* bacteria was reduced by 98 percent within approximately 4 hr. The number of MS-2 plaque forming units was reduced by 100 percent within approximately 24 hr. The number of *Penicillium funiculosum* colony forming units was reduced by up to 100 percent within approximately 24 hr.

There are many factors that affect the survival of airborne bacteria in indoor environments. These include relative humidity, temperature, and the presence of other air contaminants. The findings of these tests show that exposure of bacteria on the surface of the internal filter in the Healthway® EMF Air Cleaner can significantly reduce their survival.

(Footnotes)

[1] F.M. Collins, "Relative susceptibility of acid-fast and non-acid fast bacteria to ultraviolet light." Applied Microbiology, 1971, 21: 411-413.

[2] Malalanirina W. Rakotonirainy et al., "Research on Fungicides for Aerial Disinfection by Thermal Fogging in Libraries and Archives," International Biodeterioration & Biodegradation, 1999, 44, pp. 133-139.

[3] K.F. Fannin et al., "Field Studies on Coliphages and Coliforms as Indicators of Airborne Animal Viral Contamination from Wastewater Treatment Facilities," Water Research, 1977, 11, pp. 181-188.

[4] Edward J. Dubovi and Thomas G. Akers, "Airborne Stability of Tailless Bacterial Viruses S-13 and MS-2," Applied Microbiology, 1970, pp. 674-628.