

Report no. 933322

Inactivation of aerosolized vira: MS2 bacteriophages

**Jimco MAC500**



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# Inactivation of aerosolized vira: MS2 bacteriophages

Jimco MAC500

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September 2020

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## Summary

The purpose of the test is to determine the efficiency of the air purifier to reduce the concentration active of aerosolized MS2 bacteriophages using a modified ISO 16000-36:2018 method. The tested air purifier is a Jimco MAC500.

The significant and consistent difference between the Natural decay test and the Product test clearly shows a reduction of the concentration of active and airborne MS2 caused by the air purifier.

The measured decay of the concentration of active MS2 during the tests is attributed to a natural decay of the aerosol and an attribution of the air purifier. The determined attribution of the air purifier is 0.73-1.2 log-reduction (base 10) per hour in the 20 m<sup>3</sup> room.

According to Kowalski\* and Walkert† the UV-susceptibility for bacteriophage MS2 is lower than the UV-susceptibility for the enveloped virus, vaccinia virus. Hence, the indicated efficacy of the tested MAC500 UV-C device to degrade the bacteriophage MS2 will be at least similar to the efficacy against enveloped vaccinia virus.

Efficacy against vaccinia virus allows for a claim for efficacy against all enveloped viruses (e.g. MERS-CoV, SARS-CoV-1 and SARS-CoV-2) according to DS/EN 14885:2018.

\* Kowalski W. Ultraviolet Germicidal irradiation Handbook. Springer 2009

† Walker and Ko, ENVIRONMENTAL SCIENCE & TECHNOLOGY / VOL. 41, NO. 15, 2007



## Method and Materials

The purpose of this test is to determine the inactivation effect of the air purifier on MS2 bacteriophages aerosolized in a test chamber. The natural decay rate of the concentration of active aerosolized MS2 is determined by sampling the air in the chamber over a 2-hour period and the enhanced decay rate due to the air purifier is determined in a similar manner.

The volume of the used chamber is 20 m<sup>3</sup> and it has an inert FEP lining for chemical resistance and easy cleaning. The room is airtight, and a fan is in the room to mix the air and secure a homogenous concentration of aerosols. The aerosol is generated within the room using a nebulizer (Palas AGK 2000) and the air purifier is placed on a stainless-steel table in the middle of the room with a height of about 100cm. See the setup in Figure 1.

The room is cleaned using a 10 ppm ozone system and it is heavily ventilated using clean air for more than 48 hours before the test. The air purifier is turned on more than 24 before the test and a slight overpressure is applied to keep the room clean and reduce build-up of ozone from the device. The ozone concentration before and during the test was below the health exposure limit of 0.1 ppm.

The relative humidity adjusted to 60 +/- 5 %RH and temperature is 22.5 °C +/- 0.5 °C

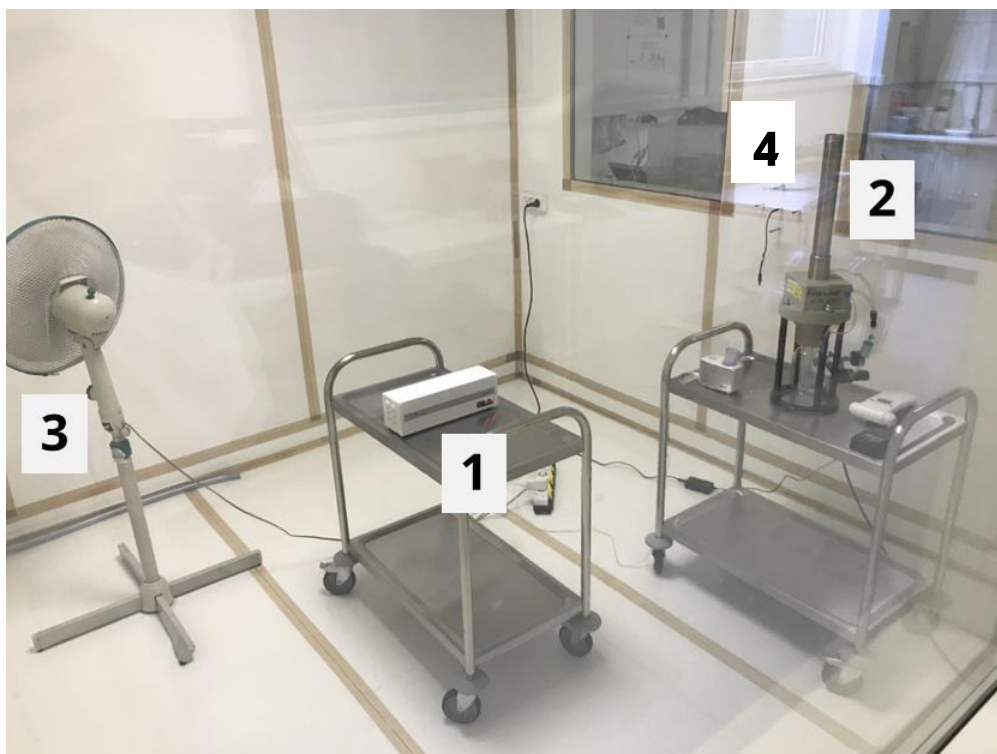


Figure 1: Test chamber. 1: Air Purifier MAC500. 2: Nebulizer (PALAS AGK 2000). 3: Mixing Fan, 4: Sampling port



The sampling of the air is done through a 6mm stainless steel tube in the sidewall of the room using GilAir plus pump at 4 L/min. A total of 20L is extracted per sample into an impinger with 60mL SM-buffer. The timing of sampling is: 0, 15, 30, 60, 120 minutes after finishing aerosolization. The start of the first sample (t = 0 minutes) is less than a minute after the nebulizer is stopped.

The procedure is the following:

1. A suspension of MS2 in SM-buffer is prepared and the concentration is determined.
2. A background sample is taken before the test and injection of aerosol.
3. The air purifier is running during injection of the MS2 containing aerosol based on a suspension of  $8 \cdot 10^9$  PFU\*/ml. The Palas nebulizer is working at 3.2 bar pressure for a total time of 15 minutes.
4. The sampling is carried out according to the timing plan.
5. After the 2 hours test with the air purifier on, the device is turned off and the room is flushed with clean air for 40 minutes. The particle count is checked to ensure that it is reduced to background level.
6. A reference test of the natural decay is carried out by the same procedure as the above described test but without the air purifier turned on.
7. The sampling is carried out according to the timing plan.
8. The concentration of active MS2 is evaluated for each sample by mixing dilutions series with a fresh culture of the host bacteria, cultivation, and enumeration of PFU following incubation.

The test date is the 23/9 2020 and the plates are counted 24/9 and 25/9 2020.

\*PFU is Plaque forming units

#### Microbiological Test Parameters:

Test organism:	MS2 bacteriophage, ATCC 15597-B1
Host organism:	<i>Escherichia coli</i> , ATCC 15597
Growth conditions:	Coliform top agar at $37 \pm 1^\circ\text{C}$ for 48 hours
Sampling and dilution solution:	SM-buffer



## Results

The concentration of active MS2 expressed as PFU/m<sup>3</sup> is shown in Table 1 and in graph in Figure 2. The room background is measured before the first injection of aerosols.

Time	Natural decay	Product test
Minutes	PFU/m <sup>3</sup>	PFU/m <sup>3</sup>
Background		0
0	6.85E+06	3.86E+06
15	4.22E+06	1.21E+06
30	5.46E+05	1.77E+05
60	9.64E+04	3.22E+03
120	6.07E+03	*

Table 1: The concentration of active MS2 (PFU/m<sup>3</sup>) for the Natural decay and the Product test. \*The Product test sample at 120 minutes is below the detection limit which is determined to be 1.5E+03 PFU/m<sup>3</sup>.

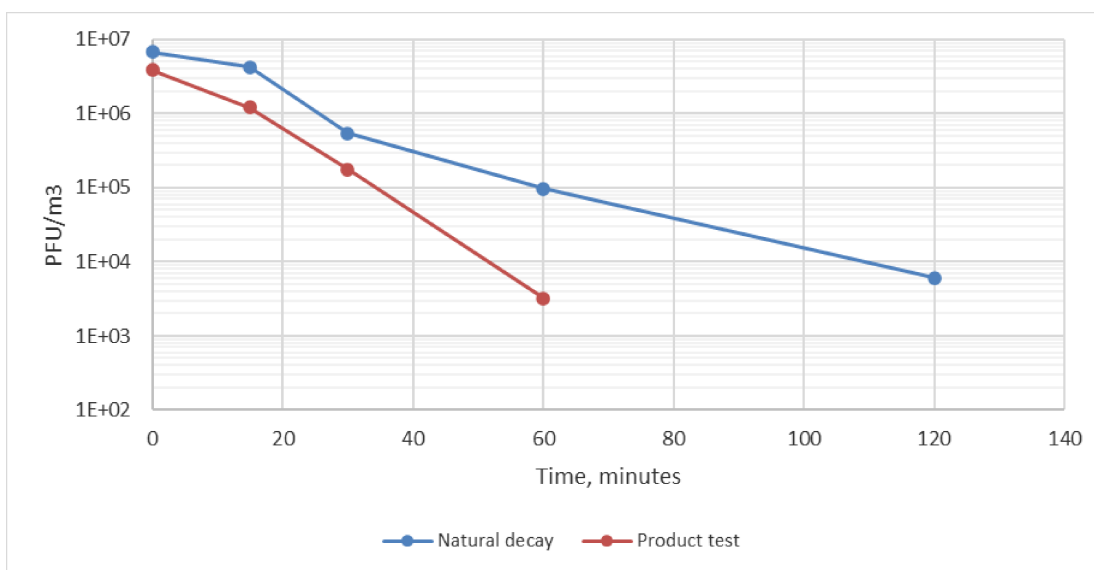


Figure 2: The concentration of active MS2 for the Natural decay and for the Product test



The air purifier's attribution to the overall decay of concentration of MS2 is calculated by the difference in decay constant ( $k$ ) from the exponential fit to both the Natural decay and the Product test decay:

$$\text{Active MS2 [PFU/m}^3\text{]} = a \cdot \exp[-k \cdot \text{time}]$$

The decay constants are shown in the fits in Figure 3 and summarized in Table 2. The points at 120 minutes have been removed because of larger uncertainties close to the detection limit. The Product attribution is calculated by subtracting the decay constant of the Product test and the Natural decay.

Table 2: Decay constant and corresponding half time and Log-reduction (base 10) per hour.

	Decay constant, min <sup>-1</sup>	Half time, min	Log-reduction per hour
Natural decay	0.075	9.24	1.95
Product test	0.121	5.73	3.15
Product attribution	0.046	15.07	1.20

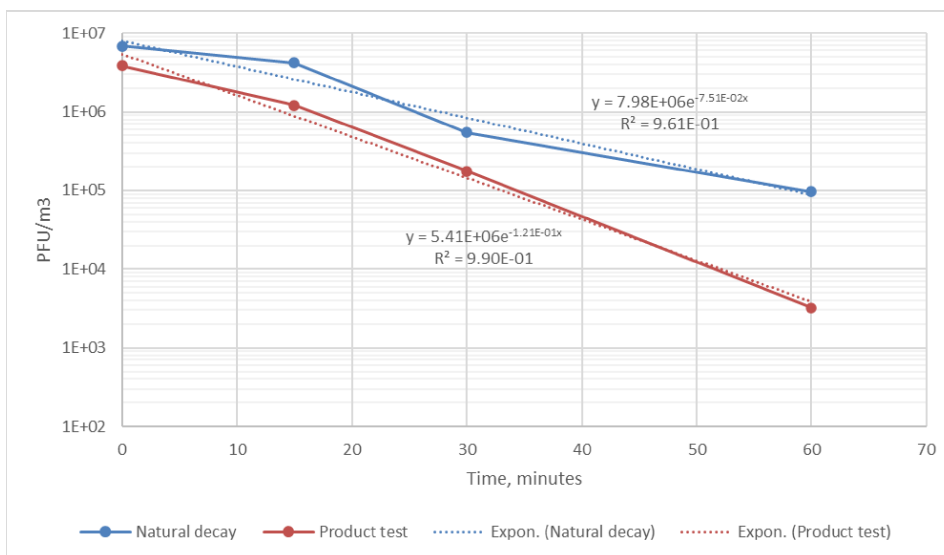


Figure 3: Fit to decay of the concentration of active MS2



## Discussion

The performed test is designed to allow for direct evaluation of the effect of the air purifier on the concentration of aerosolized and active MS2 bacteriophages. The significant and consistent difference between the Natural decay test and the Product test clearly shows a reduction of the concentration of active MS2 caused by the air purifier.

A single test was performed so the uncertainty cannot be calculated. However, the differently timed sampling point allows for an evaluation of the variability. If the sampling point at 60 minutes is removed from the dataset for the Product test (which is closest to the detection limit and thus more uncertain), the product attribution yields a 0.73 log-reduction per hour. Therefore, the products attribution to the inactivation of MS2 likely falls in the interval of 0.73-1.2 log-reduction per hour.

It is worth mentioning that the product attribution to the reduction is due to inactivation of MS2 whereas the natural decay is mainly due to fallout of the MS2-containing aerosol to surfaces in the chamber.





## The exponential reduction model and virus UV-susceptibility

The reduction rate of the concentration of aerosolized and active MS2 is found to be 0.73-1.2 log-reductions per hour and the mean value of these points yield of 0.97 log-reductions per hour (in the 20 m<sup>3</sup> test chamber). According to Kowalski W. (Ultraviolet Germicidal irradiation Handbook, Springer 2009), the UV-susceptibility of different type of viruses span about an order of magnitude and MS2 is among the lowest of the tested. The theoretical reduction rates of the air purifier are calculated for increasing UV-susceptibilities in Table 3 and shown in Figure 4.

Time, minutes	15	30	45	60	75	90	105	120
MS2 susceptibility: 0.97 log/hour								
Reduction, %	42.6	67.1	81.1	89.2	93.8	96.4	98.0	98.8
Log-reduction	0.24	0.48	0.72	0.97	1.21	1.45	1.69	1.93
3 times more susceptible than MS2: 2.9 log/hour								
Reduction, %	81.1	96.4	99.3	99.9	99.976	99.995	99.999	99.9998
Log-reduction	0.72	1.45	2.17	2.90	3.62	4.34	5.07	5.79
5 times more susceptible than MS2: 4.8 log/hour								
Reduction, %	93.8	99.6	99.976	99.999	99.9999	100	100	100
Log-reduction	1.21	2.41	3.62	4.83	6.03	7.24	8.44	9.65

Table 3: Reduction rates over time and for different UV-susceptibilities.

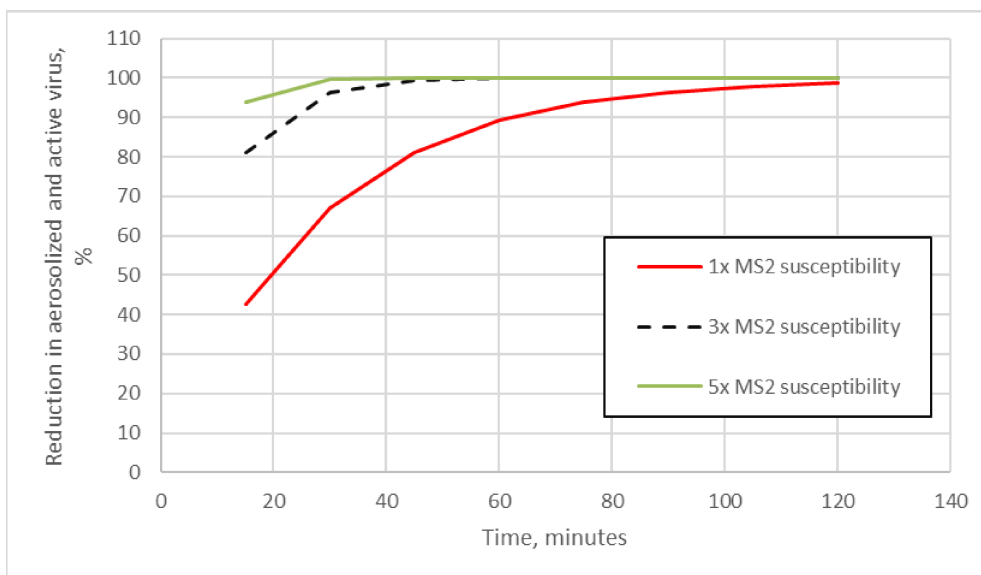


Figure 4: Reduction rate over time and for different UV-susceptibilities.



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2 October 2020

## **Declaration of test and assessment**

Danish Technological Institute has performed tests of the efficiency for inactivation virus of the Jimco MAC500 air purifier.

The test was conducted with the unit installed in a 20 m<sup>3</sup> sealed room. The efficiency of the air purifier was tested using MS2 bacteriophages (ATCC 15597-B1) on host *Escherichia coli* (ATCC 15597) as a virus surrogate. The rate of inactivation of the aerosolized MS2 was determined as the difference between the natural inactivation rate and the inactivation rate measured during the use of the Jimco MAC500 air purifier. These inactivation rates were determined by sampling of the air in the chamber over a 2-hour period. The significant and consistent difference between the Natural decay test and the Product test clearly shows a reduction of the concentration of airborne and active MS2 caused by the air purifier.

Based on the measured inactivation efficiency of the MAC500, the reductions in % and in log-reductions are calculated and are found in the table below:

<b>Product attribution</b>	<b>1 hour</b>	<b>2 hours</b>	<b>3 hours</b>
Reduction, %	89% ± 8%	99% ± 2.3%	99.9 ± 0.5%
Log-reduction (base 10)	0.97 ± 0.24	1.93 ± 0.47	2.9 ± 0.71

According to Kowalski\* and Walkert† the UV-susceptibility for bacteriophage MS2 is lower than the UV-susceptibility for the enveloped virus, vaccinia virus. Hence, the indicated efficacy of the tested MAC500 UV-C device to degrade the bacteriophage MS2 will be at least similar to the efficacy against enveloped vaccinia virus. Efficacy against vaccinia virus allows for a claim for efficacy against all enveloped viruses (e.g. MERS-CoV, SARS-CoV-1 and SARS-CoV-2) according to DS/EN 14885:2018.

\* Kowalski W. Ultraviolet Germicidal irradiation Handbook. Springer 2009

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The full testing procedures are presented in report no. 933322.

Best regards,

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# Effect of Ultraviolet Germicidal Irradiation on Viral Aerosols

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Ultraviolet (UV) germicidal air disinfection is an engineering method used to control the airborne transmission of pathogenic microorganisms in high-risk settings. Despite the recent emergence of respiratory viral pathogens such as SARS and avian influenza viruses, UV disinfection of pathogenic viral aerosols has not been examined. Hence, we characterized the UV disinfection of viral aerosols using the bacteriophage MS2, adenovirus, and coronavirus. Our objectives were to characterize the effect of nebulization and air sampling on the survival of important viral pathogens, quantitatively characterize and estimate the UV susceptibility of pathogenic viral aerosols, and evaluate the effect of relative humidity (RH) on the susceptibility of viral aerosols, to 254 nm UV—C. The viruses were aerosolized into an experimental chamber using a six-jet Collison nebulizer, exposed to 254 nm UV, and sampled using an AGI-30 liquid impinger. Both the MS2 and adenovirus aerosols were very resistant to UV air disinfection, with a reduction of less than 1 logarithm in viable viral aerosols at a UV dose of 2608  $\mu\text{W s}/\text{cm}^2$ . The susceptibility of coronavirus aerosols was 7–10 times that of the MS2 and adenovirus aerosols. Unlike bacterial aerosols, there was no significant protective effect of high RH on UV susceptibility of the tested viral aerosols. We confirmed that the UV disinfection rate differs greatly between viral aerosols and viruses suspended in liquid.

## Introduction

Bioterrorism threats and the potential airborne spread of new pathogens such as severe acute respiratory syndrome (SARS) coronavirus and influenza virus (1–4) have stimulated engineering control measures to prevent airborne transmission of infectious microorganisms indoors. One currently recommended engineering control method for high-risk settings is the use of 254 nm ultraviolet germicidal irradiation (UVGI) (5–7). UVGI is effective for inactivating infectious microorganisms in various settings (5, 8, 9). UV air disinfection is applied as either duct irradiation or upper room UVGI (UV irradiation above people's heads in a room) to inactivate airborne infectious agents and microbial toxins. Until recently, air disinfection by UVGI was mainly focused on preventing the transmission of tuberculosis (TB) in high-risk settings such as hospitals (10, 11). The Centers for Disease Control and Prevention (CDC) has recommended UVGI as

a supplemental control for TB transmission in hospital isolation rooms (11). A number of previous studies have indicated that UV air disinfection protects humans from adverse health effects caused by airborne microorganisms indoors (12–14).

The efficacy of UV air disinfection depends on the UV susceptibility of the airborne microorganism, the level of UV irradiance, and the UV-irradiated air volume (15). Characterization of the UV susceptibility of viral aerosols is the first step in determining the potential usefulness of UV irradiation for preventing the transmission of airborne infectious viruses in the indoor environment. UV inactivation rates of bacteria suspended in liquid (16) and in air (17–20) vary greatly among species.

Most studies of viral UV inactivation have examined UV disinfection on surfaces or in water (12, 21–23). Although UVGI has potential as an effective infection control measure for bacterial aerosols, limited data exist regarding UVGI inactivation of viral aerosols (9, 16, 24). There are currently no data that evaluate the usefulness of UV air disinfection in preventing the airborne transmission of respiratory animal viral aerosols such as the SARS coronavirus.

Relative humidity (RH) is an important parameter determining the UV inactivation rate of bacterial aerosols (20, 25, 26). Various bacteria such as *Serratia marcescens*, mycobacteria, and *Escherichia coli* display increased UV resistance with increased RH. The mechanism underlying the effect of RH on the UV inactivation rate of airborne microorganisms is not well characterized. Increased UV resistance is most likely associated with hygroscopic characteristics of bioaerosols because increased particle size is observed in bacterial aerosols at high RH (20). However, a different study using *E. coli* found no specific relationship between the UV inactivation rate and RH (27). Furthermore, the inactivation rate of bacterial aerosols by sunlight increases with increases in RH (28). The effect of RH on the UV susceptibility of pathogenic viral aerosols is unknown.

We examined the effects of nebulization and sampling on respiratory viral aerosols. We also determined the UV susceptibility of three viral aerosols, i.e., respiratory adenovirus, coronavirus (a surrogate for the SARS coronavirus), and the bacteriophage MS2, as well as the effect of RH on their UV susceptibility. Finally, the UV inactivation rates for the viral aerosols were compared with those for viruses suspended in liquid to determine the usefulness of extrapolating from previous studies of the UV susceptibility of bioaerosols.

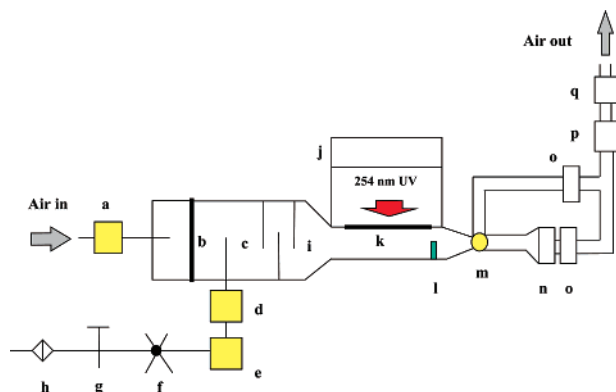
## Materials and Methods

**Cultivation and Assay of Tested Viruses.** Bacteriophage MS2. Both the MS2 (ATCC 15597-B1) bacteriophage and *E. coli* C3000 host strain (ATCC 15597) were obtained from the American Type Culture Collection (ATCC). MS2 was assayed using the single agar overlay method (EPA Method 1601). The preparation of coliphage stocks was performed by inoculating 100 mL of TSB (50 mM  $\text{MgCl}_2$ ) with 100  $\mu\text{L}$  of stock coliphage and 300  $\mu\text{L}$  of log-phase *E. coli* C3000. The culture was incubated overnight at 37 °C with shaking. Chloroform was added to the culture (0.4 volume), which vortexed for 1 min and centrifuged for 20 min at 4000g. The supernatant was decanted and stored at –70 °C until use. For the MS2 assay, log-phase *E. coli* C3000 was prepared by adding 300  $\mu\text{L}$  of overnight culture to 30 mL of TSB and incubating for 4 h at 37 °C. The optical density (OD) of the culture was determined as the absorbance at 520 nm, using

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**FIGURE 1. Schematic diagram of the experimental chamber; (a) humidifier; (b) HEPA filter paper; (c) manifold; (d) dehumidifier; (e) Collison nebulizer; (f) pressure regulator; (g) air valve; (h) high efficiency filter; (i) baffles; (j) UV fixture; (k) UV exposure window; (l) real-time temperature and RH sensor; (m) two-way air valves; (n) AGI-30 liquid impinger; (o) HEPA filters; (p) vacuum pump; (q) rotameter.**

a spectrophotometer. Cultures with ODs between 0.1 and 0.5 were used in the assay.

**Respiratory Adenovirus (Serotype 2) and MHV Coronavirus.** Adenovirus serotype 2 (ATCC: VR-846) was obtained from the ATCC and cultured in minimal essential medium (MEM) containing 10% fetal bovine serum (FBS), using A549 cells. The murine hepatitis virus (MHV) coronavirus and the murine astrocytoma (DBT) cell line were donated by Shinki Makino of the University of Texas Medical Branch Sealy Center for Vaccine Development. MHV was cultured in DBT cells grown in MEM with 10% FBS and 1% Nystatin (Sigma-Aldrich). The adenovirus and MHV coronavirus were assayed by plaque assay using the A549 and DBT cell lines, respectively. Briefly, 300  $\mu$ L of the virus inoculum was added to a 60 mm tissue culture dish containing a near monolayer of A549 or DBT cells after the maintenance medium had been removed. The cells and inoculum were incubated for 1 h at 37 °C in 5% CO<sub>2</sub>, with gentle rocking every 15 min. After incubation, the cells and inoculum were overlain with 5 mL of overlay medium, which consisted of one part 2  $\times$  MEM with 200 mM L glutamine and nonessential amino acids, 1.5 M HEPES buffer, 4 M MgCl<sub>2</sub>, 7.5% NaHCO<sub>3</sub>, FBS, 1.5 mg/mL Nystatin, 1.5% neutral red, and one part 1.5% noble agar. The plaques were counted after 5–7 days, at which time they became clearly visible for adenovirus, or after 2–3 days for MHV coronavirus.

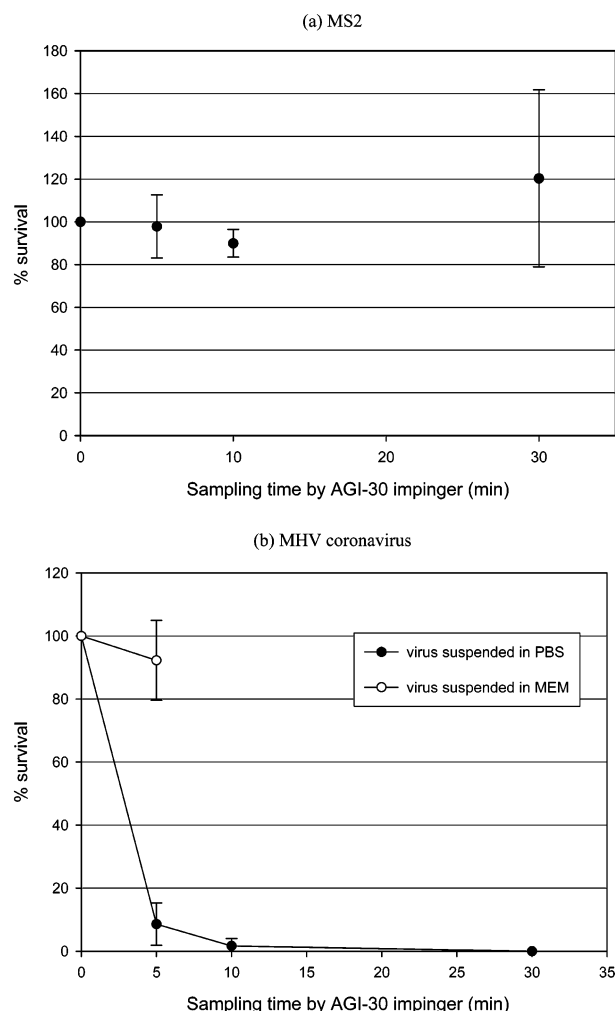
**Description of Experimental Chamber.** An experimental apparatus was designed and built to measure the UV susceptibility of viral aerosols exposed to various UV doses at predetermined RH levels (Figure 1). The chamber consisted of three parts: the intake plenum (165 mm high  $\times$  165 mm wide  $\times$  244 mm long), the main body (50 mm high  $\times$  260 mm wide  $\times$  455 mm long), and the exhaust plenum (49 mm high  $\times$  49 mm wide  $\times$  100 mm long). A 254 nm UV fixture containing six 36 W UV lamps (Lumalier, Memphis, TN) was installed above a fused quartz UV exposure window (260 mm wide  $\times$  260 mm long) in the main body. Screens were placed beneath the UV fixture to adjust the amount of UV irradiance. The adjusted UV light passed through the fused quartz UV exposure window and irradiated flowing viral aerosols inside the chamber. The UV irradiance to which viral aerosols were exposed was measured directly during the experiments through a single 29 mm diameter window on the bottom of the main body. A vacuum pressure pump, located at the extreme downstream end of the apparatus, provided the desired airflow rate, which was monitored continuously by a rotameter at the discharge (room pressure

side) during the experiments. Airflow through the system was maintained at 12.5 L/min throughout the experiments. The airflow pattern was checked using smoke tubes, and it approximated laminar flow. The time required for a particle to pass through the exposure window was calculated as 16.2 s at 12.5 L/min airflow and was checked using smoke tubes. This measurement was considered the UV exposure time. Air passed through a humidifier before entering the inlet to the experimental chamber to achieve the desired RH for each experiment. The humidified air entered a 165  $\times$  165 mm square duct through a HEPA filter paper fitted into the intake plenum. The HEPA filter prevented the entry of stray microorganisms and prevented backflow of aerosolized microorganisms, should an upset condition occur. It also prevented the entry of droplets leaving the air humidifier into the exposure chamber section. The aerosols were generated by a six-jet Collison nebulizer (model CN-38, BGI, Waltham, MA), with the air pressure maintained at 138 kPa (20 psi). A manifold, located 25 mm from the end of the intake plenum, was attached to the nebulizer and had three downstream-facing 4.9 mm diameter ports for the introduction of uniformly distributed aerosols into the chamber. The exhaust plenum had two outflow ports. One of the ports led to the outlet from which the samples were taken; the other was a bypass path that was connected to a filter cassette loaded with a HEPA filter. Viral aerosols were collected during the experiments using an AGI-30 liquid impinger and were routed to the bypass filter during the transition periods. The various sections of the chamber had gaskets to prevent leaks and were held together by 12 countersunk machine screws and wing-nuts. To provide additional protection, the experimental chamber was housed within a Class II, Type A, certified biological safety cabinet.

A radiometer (SEL240 D3660, International Light Inc., Newburyport, MA) was used to precisely map the UV radiation inside the chamber. UV measurements were taken at transverse intervals at three vertical distances from the lamp across the UV exposure window, resulting in 90 discrete UV measurements. The center of the UV exposure window had the highest UV irradiance, and it gradually decreased from the center. The difference between the highest and lowest irradiance across the exposure window was less than 25%. The distribution of UV irradiance across the exposure window was the same for all UV irradiance levels, and the UV measurements remained constant, regardless of RH. The difference between the average UV irradiance across the entire UV exposure chamber and a UV measurement through the bottom window of the chamber was evaluated and used to adjust the offset. Both RH and temperature were monitored continuously using a Vaisala Humitter sensor (Vaisala Oyj, Helsinki, Finland) and data logging software, using a laptop computer.

**Effects of Nebulization and Impingement on the Survival of Tested Viruses.** We tested the effects of nebulization and air sampling with the AGI-30 impinger on the survival of both MS2 and MHV coronavirus. To test the effects of nebulization on the survival, we used the following procedure. Either MS2 or MHV coronavirus was suspended in 40 mL of either PBS with 0.01% Tween or MEM with 10% FBS, respectively. After the viral suspension was added to the Collison nebulizer, the nebulizer was operated at 20 psi. A 100  $\mu$ L aliquot of sample was taken from the nebulizer at 0, 5, 10, 30, and 60 min after the onset of nebulization. The collected samples were serially diluted and then assayed by the methods described above.

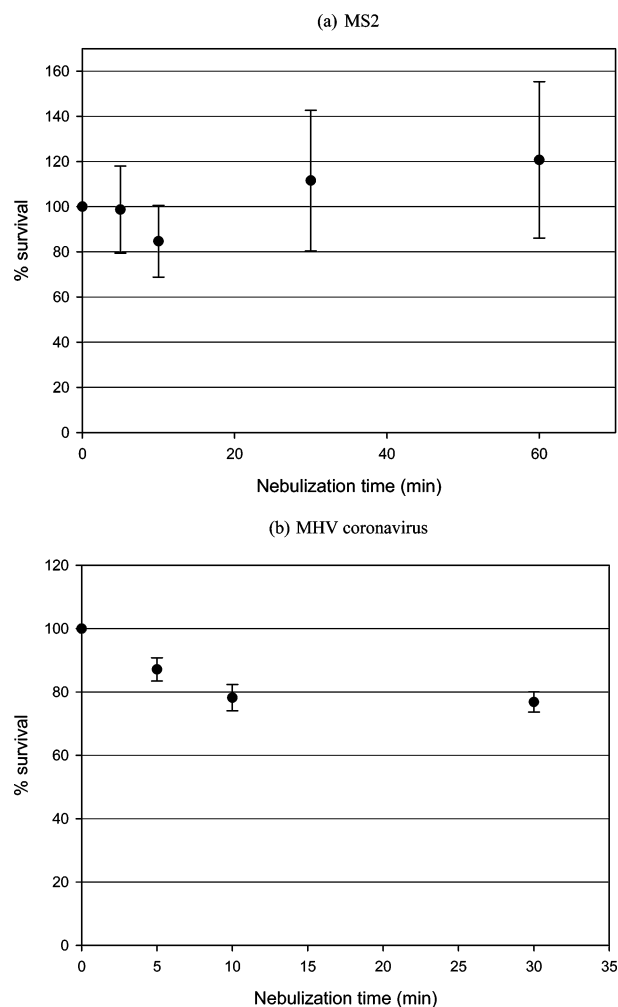
To characterize the effect of air sampling with the AGI-30 liquid impinger on viral survival, the tested virus was suspended in 20 mL of PBS with 0.01% Tween 80 or MEM media with 10% FBS. Antifoam A was added to the viral suspension at a final concentration of 0.005%. The viral



**FIGURE 2.** Effect of sampling using an AGI-30 liquid impinger on the survival (percent of infectious viral units recovered) of (a) the MS2 bacteriophage ( $N = 5$ ) and (b) murine hepatitis virus (MHV) coronavirus ( $N = 3$ ) suspended in either phosphate-buffered saline or minimum essential medium with 10% fetal bovine serum. (a) MS2 (b) MHV coronavirus.

suspension was added into the AGI-30 liquid impinger, which was operated at 12.5 L/min airflow. Samples were taken from the impinger at 0, 5, 10, and 30 min after the onset of airflow, serially diluted, and assayed as described above. Each experiment was repeated at least three times.

**Measurement Of UV Susceptibility of Viral Aerosols.** Viral aerosols were generated in a Collison nebulizer at 20 psi and passed into the chamber at a flow rate of 12.5 L/min. Appropriate dilutions for aerosolization were determined from the concentrations of the sampled aliquots. One-cell aliquots were suspended in PBS (pH = 7.4), and the viral concentration was adjusted to  $10^4$ – $10^5$  PFU/mL. A total of 30 mL of prepared viral suspension was loaded into the Collison nebulizer for aerosolization. Initial experiments were performed to ensure that the aerosols dried completely during passage through the chamber. A filter cassette loaded with flat HEPA filter paper treated with methylene blue powder was fitted to the adapter. Viral aerosols were generated and collected onto the treated filters. Visible blue color on the filter indicated inadequate drying of the aerosol droplets. The duration of sample collection was 15 min, with the UV on (UV dose = 2608 or 599  $\mu\text{W s/cm}^2$ ) or off. The virus was collected in an AGI-30 liquid impinger, after which samples were taken for serial dilution and analysis by plaque assay. The  $Z$  value for each viral aerosol was estimated



**FIGURE 3.** Effect of aerosolization using a Collison nebulizer on the survival (percent of infectious viral units recovered) of (a) MS2 bacteriophage suspended in phosphate-buffered saline ( $N = 3$ ) and (b) murine hepatitis virus (MHV) coronavirus suspended in minimum essential medium with 10% fetal bovine serum ( $N = 3$ ). (a) MS2 (b) MHV coronavirus.

as  $Z = \ln(N_0/N_{UV})/D$ , where  $N_0$  is the number of plaques in the absence of UV exposure,  $N_{UV}$  is the number of plaques in the presence of UV exposure, and  $D$  is the UV dose in  $\mu\text{W s/cm}^2$ . Each experiment was repeated at least three times.

## Results

Air sampling with the AGI-30 liquid impinger (Figure 2) and aerosolization by the Collison nebulizer (Figure 3) had similar effects on the recovery of infectious viruses after different time intervals. The MS2 bacteriophage was resistant to stress during both aerosolization and sampling. A slight decrease in the number of MS2 plaques was observed for the first 10 min, followed by an increase in the number of plaques after 30 min, but these changes were not statistically significant ( $p > 0.05$ ;  $t$  test). The suspension medium was important in determining the survival of MHV coronavirus (Figure 2). Virus suspended in PBS with 0.01% Tween was inactivated much faster than that suspended in MEM with 10% FBS. In addition, the MHV coronavirus was easily inactivated by mechanical forces during aerosolization and sampling (Figures 2 and 3) in comparison with MS2. After 10 min of aerosolization, a high rate of inactivation of the MHV coronavirus was observed. This change was statistically significant ( $p < 0.05$ ;  $t$  test).

For the UV susceptibility of viral aerosols at 50% RH, the number of plaques ranged from too numerous to count



**TABLE 1. Ultraviolet Germicidal Irradiation Susceptibility (Z Value) of the MS2 Bacteriophage, Respiratory Adenovirus Serotype 2, and Murine Hepatitis Virus Coronavirus, at 50% Relative Humidity**

	UV dose ( $\mu\text{W s/cm}^2$ )	percent survival <sup>a</sup>	Z value ( $\times 10^4$ ) <sup>b</sup>
MS2 (N = 5)	2608	31.1 $\pm$ 2.9	3.8 $\pm$ 0.3
adenovirus (N = 4)	2608	32.9 $\pm$ 2.3	3.9 $\pm$ 0.3
coronavirus (N = 3)	599	12.2 $\pm$ 7.2	37.7 $\pm$ 11.9

<sup>a</sup> Percent survival =  $100 \times (\text{number of plaques in the presence of UV exposure})/(\text{number of plaques in the absence of UV exposure})$ . <sup>b</sup> Z values ( $\times 10^4$ ) were calculated as  $-10^4 \times \log(\% \text{ survival})/\text{UV dose } (\mu\text{W s/cm}^2)$ .

**TABLE 2. Effect of Relative Humidity on the UV Susceptibility of the MS2 Bacteriophage and Respiratory Adenovirus Serotype 2**

microorganism	UV dose ( $\mu\text{W s/cm}^2$ )	relative humidity	percent survival	Z value ( $\times 10^4$ )
MS2 (N = 5)	2608	32–50%	31.1 $\pm$ 2.9	3.8 $\pm$ 0.3
	2608	74–82%	24.6 $\pm$ 3.5	4.8 $\pm$ 0.5
adenovirus (N = 4)	2608	27–40%	32.9 $\pm$ 2.3	3.9 $\pm$ 0.3
	2608	50–55%	20.6 $\pm$ 2.5	5.2 $\pm$ 0.4
	2608	76–80%	13.6 $\pm$ 0.5	6.8 $\pm$ 0.2

(TNTC) to less than 10 (Table 1). Between 5 and 100 plaques were used to calculate the Z values for each viral aerosol. MS2 was the most resistant to 254 nm UV–C. Approximately 31% of MS2 aerosols survived after exposure to 2608  $\mu\text{W s/cm}^2$  UV–C. The adenovirus showed an inactivation rate similar to that of MS2: approximately 33% of the adenovirus survived after exposure to 2608  $\mu\text{W s/cm}^2$  UV–C. However, the coronavirus was much more sensitive to 254 nm UV–C, with only 12% of the aerosolized virus surviving exposure to 599  $\mu\text{W s/cm}^2$  UV–C.

To determine the effect of RH on UV susceptibility, the Z values of the aerosolized MS2 and adenovirus were measured at different RHs (Table 2). Experiments with methylene blue-treated filters indicated that no residual water droplets remained in the generated viral aerosols at any RH. Unlike with many bacterial aerosols, no protective effect was observed at high RH. Instead, the Z values at high RH were even higher than those at low RH for both MS2 and the adenovirus. The  $Z \times 10^4$  values for MS2 were 3.8 at low RH (32–50% RH) and 4.8 at high RH (74–82%). The  $Z \times 10^4$  values for the adenovirus were 3.9 at low RH (27–40%), 5.2 at medium RH (50–55%), and 6.8 at high RH (76–80%). These results suggest higher UV susceptibility at higher RH.

Finally, the Z values for the viral aerosols were much higher than those for viruses suspended in liquid (Table 3). The Z values for viral aerosols were 7–86 times those for viruses suspended in liquid, indicating higher UV susceptibility of

the viral aerosols tested compared with those in liquid suspension.

## Discussion

Prior to characterizing the UV susceptibility of the tested viral aerosols, we determined the effects of nebulization and air sampling to optimize the experimental conditions. The MS2 bacteriophage was more resistant to biological stresses during aerosolization and sampling than was the coronavirus. We observed no significant decrease in MS2 recovery. The slight upward trend in the percent recovery of MS2 after a lengthy period of aerosolization or sampling was likely caused by either the evaporation of liquid or breakage of viral clumps by mechanical shearing and impaction.

The MHV coronavirus was highly susceptible to inactivation by both aerosolization and sampling. Unlike MS2, MHV coronavirus is an enveloped virus and is thus more likely to be inactivated by mechanical stress. Additionally, MHV coronavirus was easily inactivated in PBS with 0.01% Tween but was relatively stable when suspended in MEM with 10% FBS. The protein concentration of MEM with 10% FBS was very close to that of saliva (28), and the higher protein concentration probably protected the coronavirus. Any effects of aerosolization or sampling on viral survival were normalized because each UV-on sample was immediately followed by a UV-off control sample, or vice versa. The UV-on and UV-off samples were used to determine UV susceptibility (Z value).

We demonstrated that UVGI may be an effective engineering control measure to prevent the transmission of respiratory viral diseases. Our results suggest that the dose required for significant inactivation of certain viruses such as adenovirus may be higher than that required for bacterial aerosols. Adenovirus is one of the microorganisms most resistant to 254 nm UV–C; the high UV resistance of adenovirus suspended in liquid is well documented (21, 22, 29, 30). This resistance is likely a result of its double-stranded DNA genome and its refractory nature to irreparable damage by UV light in comparison with other types of nucleic acids (22). It is noteworthy that the MS2 aerosols were resistant to 254 nm UV at similar levels as the adenovirus. In liquid suspension, the UV susceptibility of MS2 was generally higher than that of adenovirus. In addition, the coronavirus, which is an enveloped virus, was inactivated by a relatively low dose of 254 nm UV. The high UV susceptibility of coronavirus aerosols suggests that UV air disinfection may be an effective tool for preventing important respiratory viral diseases such as SARS.

The effect of RH on the UV susceptibility of viral aerosols was markedly different from that previously reported for bacterial aerosols (20, 25, 26). In interpreting these results, the change in the particle size of bioaerosols in response to changes in RH must be considered. A number of studies reported that higher RH increased the aerodynamic diameter

**TABLE 3. Comparison of the UV Susceptibility (Z Value [ $\times 10^4$ ]) of the MS2 Bacteriophage, Adenovirus, and Coronavirus in Aerosol at 50% RH and in Liquid Suspension**

microorganism	viral aerosol (Z value [ $\times 10^4$ ])	liquid suspension (Z value [ $\times 10^4$ ])	Z value ratio, (aerosol)/ (liquid)
MS2	3.8 $\pm$ 0.3	0.55 (refs 29, 33, 35, 36) 0.24 (serotype 2: 15, 40, 41) (refs 21, 22, 29, 30)	6.9 16.3
adenovirus	3.9 $\pm$ 0.3	0.18 (serotype 40: 41) (refs 22, 23)	21.7
coronavirus	37.7 $\pm$ 11.9	0.44 (ref 37)	85.7

of bacterial aerosols (20, 31). A greater aerodynamic diameter may in turn result in higher entrainment efficiency, potentially creating an artifactual finding that high RH is protective (32). Additionally, the change in particle size with changes in RH depends on both the hygroscopic characteristics of bioaerosols and the chemical composition of the suspension medium. However, further research is required because another study found no change in aerodynamic diameter with changes in RH (26). We used an aerosol dryer to ensure uniformity of the particles before they entered the experimental chamber, and the complete dryness of the generated aerosols was checked using methylene blue powder. Viral particles are generally much smaller than bacteria, and this also may be a factor in the eventual size of the droplets when they enter the UV exposure chamber. Thus, if indeed a greater particle size is protective from UVGI because of the attenuation of the UV dose by the sorption of water onto the surface of the microbe, bacteria have a higher potential to be protected from UV by high RH. However, both the MS2 and adenovirus aerosols were less resistant to 254 nm UV at higher RHs. These results suggest that the effects of RH on UVGI effectiveness against bacteria and viruses could be contrasting. Elucidating the UV dose needed to inactivate microbes, as well as other environmental factors related to microbe inactivation by UVGI, is critical to the design of UVGI systems used in occupational and environmental settings. Our results may be useful for determining the UV dosage levels required under various environmental conditions.

Two of the most important factors to be considered for a better understanding of virus inactivation by UVGI are the exact dose response curves of various organisms and differences in the inactivation of viruses on surfaces versus those in suspension. We exposed viral aerosols to a single dose of UV and, therefore, could not determine a dose-response relationship. By definition, *Z* values assume a linear relationship between the UV dose and the logarithm of the percent survival. Many previous studies have suggested that there is a linear relationship between UV dose and the logarithm of percent survival of microorganisms (5, 20, 33, 34).

Data on the UV inactivation of viral aerosols are very limited (24). The assumptions underlying engineering control designs for bacterial aerosols by UVGI or the UV inactivation rate of viruses suspended in liquid may be inappropriate for designing and optimizing UV air disinfection for respiratory viral aerosols. To our knowledge, we present the first measurements of the UV susceptibility of airborne infectious animal viruses, including a coronavirus and an adenovirus. Our results suggest that UV susceptibility is higher in viral aerosols than in viral liquid suspensions (Table 3). This finding was consistent regardless of the size of the virus (90–100 nm or 30–40 nm), the type of nucleic acid (DNA or RNA), and the viral structure (naked or enveloped). In addition to UV irradiation, other physical and biological factors that occur during nebulization and aerial transport might affect the UV susceptibility of viral aerosols.

To our knowledge, this is the first investigation of the effect of nebulization and liquid impingement on the survival of animal viruses and the first report of the UV susceptibility of pathogenic animal viruses. One limitation is that we only examined one UV dose in determining the UV susceptibility of airborne viral aerosols. We determined *Z* values, which assume a linear relationship between UV dose and percent survival. A linear relationship between UV dose and percent survival assumes that the tested virus has equal UV susceptibility, that a single hit by UV can inactivate the tested virus, and that the number of hits by UV is proportional to the UV dose. This is the simplest type of UV dose-inactivation model and explains the results of most previous studies reasonably well (2, 20, 34). Another limitation is that the

particle size of viral aerosols was not measured directly. Even though the generated viral aerosols dried completely, the viral particle size could have varied owing to both viral clumping and associations with suspended proteins. However, both viral clumping and associations with other proteins also occur in natural settings.

In conclusion, air disinfection using 254 nm UV-C may be an effective tool for inactivating viral aerosols. Of the three viruses examined, adenovirus was the most resistant to 254 nm UV-C and should be exposed to high UV doses for complete inactivation. Further laboratory and epidemiological studies are needed to elucidate the effectiveness of UV air disinfection in the reduction of respiratory viral diseases.

## Acknowledgments

This study was supported in part by the Sloan Foundation and Woongjin Coway Inc. We thank Lumalier Inc. for providing the UV lamp.

## Literature Cited

- Koopmans, M.; Wilbrink, B.; Conyn, M.; Natrop, G.; van der Nat, H.; Vennema, H.; Meijer, A.; van Steenbergen, J.; Fouchier, R.; Osterhaus, A.; Bosman, A. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet* **2004**, 363 (9409), 587–93.
- Peiris, J. S.; Yu, W. C.; Leung, C. W.; Cheung, C. Y.; Ng, W. F.; Nicholls, J. M.; Ng, T. K.; Chan, K. H.; Lai, S. T.; Lim, W. L.; Yuen, K. Y.; Guan, Y., Re-emergence of fatal human influenza A subtype H5N1 disease. *Lancet* **2004**, 363 (9409), 617–9.
- Drosten, C.; Gunther, S.; Preiser, W.; van der Werf, S.; Brodt, H. R.; Becker, S.; Rabenau, H.; Panning, M.; Kolesnikova, L.; Fouchier, R. A.; Berger, A.; Burguiere, A. M.; Cinatl, J.; Eickmann, M.; Escriviou, N.; Grywna, K.; Kramme, S.; Manuguerra, J. C.; Muller, S.; Rickerts, V.; Sturmer, M.; Vieth, S.; Klenk, H. D.; Osterhaus, A. D.; Schmitz, H.; Doerr, H. W. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N. Engl. J. Med.* **2003**, 348 (20), 1967–76.
- Kaiser, J. Bioterrorism. BioShield is slow to build U.S. defenses against bioweapons. *Science* **2006**, 313 (5783), 28–9.
- Green, C. F.; Scarpino, P. V.; Jensen, P.; Jensen, N. J.; Gibbs, S. G., Disinfection of selected *Aspergillus* spp. using ultraviolet germicidal irradiation. *Can. J. Microbiol.* **2004**, 50 (3), 221–4.
- Xu, P.; Kujundzic, E.; Peccia, J.; Schafer, M. P.; Moss, G.; Hernandez, M.; Miller, S. L. Impact of environmental factors on efficacy of upper-room air ultraviolet germicidal irradiation for inactivating airborne mycobacteria. *Environ. Sci. Technol.* **2005**, 39 (24), 9656–64.
- Xu, P.; Peccia, J.; Fabian, P.; Martyny, J. W.; Fennelly, K.; Hernandez, M.; Miller, S. L. Efficacy of ultraviolet germicidal irradiation of upper-room air in inactivating bacterial spores and mycobacteria in full-scale studies. *Atmos. Environ.* **2003**, 37, 405–19.
- Ko, G.; First, M. W.; Burge, H. A. The characterization of upper room ultraviolet germicidal irradiation in inactivating airborne microorganisms. *Environ. Health Perspect.* **2001**, 110 (1), pp. 95–101.
- Perkins, J. E.; Bahlke, A. M.; Silverman, H. F. Effect of ultraviolet irradiation of classrooms on spread of measles in large rural central schools. *Am. J. Public Health Nations Health* **1947**, 37, 529–37.
- Dumyahn, T.; First, M. Characterization of ultraviolet room air disinfection devices. *Am. Ind. Hyg. Assoc. J.* **1999**, 60, 219–27.
- CDC, Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care facility. *MMWR* **1994**, 43 (RR-13), 1–132.
- Menzies, D.; Popa, J.; Hanley, J. A.; Rand, T.; Milton, D. K. Effect of ultraviolet germicidal lights installed in office ventilation systems on workers' health and wellbeing: double-blind multiple crossover trial. *Lancet* **2003**, 362 (9398), 1785–91.
- Nardell, E. A. Use and misuse of germicidal UV air disinfection for TB in high-prevalence settings. *Int. J. Tuberc. Lung. Dis.* **2002**, 6 (8), 647–8.
- Medical Research Council, *Air Disinfection with Ultraviolet Irradiation: Its Effect on Illness Among Schoolchildren*; Her Majesty's Stationery Office: London, UK, 1954.



- (15) Nardell, E. A.; Brickner, P. W. Tuberculosis in New York City: focal transmission of an often fatal disease. *JAMA* **1996**, *276* (15), 1259–60.
- (16) Jensen, M. M. Inactivation of airborne viruses by ultraviolet irradiation. *Appl. Microbiol.* **1964**, *12*, 418–20.
- (17) Chang, J. C.; Ossoff, S. F.; Lobe, D. C.; Dorfman, M. H.; Dumais, C. M.; Qualls, R. G.; Johnson, J. D. UV inactivation of pathogenic and indicator microorganisms. *Appl. Environ. Microbiol.* **1985**, *49* (6), 1361–5.
- (18) Riley, R. L.; Knight, M.; Middlebrook, G. Ultraviolet susceptibility of BCG and virulent tubercle bacilli. *Am. Rev. Respir. Dis.* **1976**, *113* (4), 413–8.
- (19) Abshire, R. L.; Dunton, H., Resistance of selected strains of *Pseudomonas aeruginosa* to low-intensity ultraviolet radiation. *Appl. Environ. Microbiol.* **1981**, *41* (6), 1419–23.
- (20) Ko, G.; First, M. W.; Burge, H. A., Influence of relative humidity on particle size and UV sensitivity of *Serratia marcescens* and BCG aerosols. *Tuberc. Lung Dis.* **2000**, *80* (4/5), 217–28.
- (21) Gerba, C. P.; Gramos, D. M.; Nwachuku, N. Comparative inactivation of enteroviruses and adenovirus 2 by UV light. *Appl. Environ. Microbiol.* **2002**, *68* (10), 5167–9.
- (22) Thurston-Enriquez, J. A.; Haas, C. N.; Jacangelo, J.; Riley, K.; Gerba, C. P. Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. *Applied. Environ. Microbiol.* **2003**, *69* (1), 577–82.
- (23) Ko, G.; Cromeans, T. L.; Sobsey, M. D. UV inactivation of adenovirus type 41 measured by cell culture mRNA RT-PCR. *Water Res.* **2005**, *39* (15), 3643–9.
- (24) Tseng, C. C.; Li, C. S. Inactivation of virus-containing aerosols by ultraviolet germicidal irradiation. *Aerosol Sci. Technol.* **2005**, *39* (12), 1136–42.
- (25) Riley, R. L.; Kaufman, J. E., Effect of relative humidity on the inactivation of airborne *Serratia marcescens* by ultraviolet radiation. *Appl. Microbiol.* **1972**, *23* (6), 1113–20.
- (26) Peccia, J.; Werth, H. M.; Miller, S.; Hernandez, M. Effects of relative humidity on the ultraviolet induced inactivation of airborne bacteria. *Aerosol Sci. Technol.* **2001**, *35*, 728–40.
- (27) Wells, W. F.; Fair, G. M., Viability of *E.coli* exposed to ultraviolet radiation in air. *Science* **1935**, *82*, 280–1.
- (28) Theunissen, H. J.; Lemmens-den Toom, N. A.; Burggraaf, A.; Stolz, E.; Michel, M. F., Influence of temperature and relative humidity on the survival of *Chlamydia pneumoniae* in aerosols. *Appl. Environ. Microbiol.* **1993**, *59* (8), 2589–93.
- (29) Meng, Q. S.; Gerba, C. P. Comparative inactivation of enteric adenoviruses, polioviruses and coliphages by ultraviolet irradiation. *Water Res.* **1996**, *30* (11), 2665–8.
- (30) Thompson, S. S.; Jackson, J. L.; Suva-Castillo, M.; Yanko, W. A.; Eljack, Z.; Kuo, J.; Chen, C.; Williams, F. P.; Schnurr, D. P. Detection of infectious human adenoviruses in tertiary-treated and ultraviolet-disinfected wastewater. *Water Environ. Res.* **2003**, *75* (2), 163–70.
- (31) Kethley, T. W.; Branch, K.; Ultraviolet lamps for room air disinfection. Effect of sampling location and particle size of bacterial aerosols. *Arch. Environ. Health* **1972**, *25* (3), 205–14.
- (32) Lin, X.; Wileke, K.; Ulevicius, V.; Grinshpun, S. Effect of sampling time on the efficiency of all-glass impingers. *Am. Ind. Hyg. J.* **1997**, *58*, 480–8.
- (33) Hijnen, W. A.; Beerendonk, E. F.; Medema, G. J. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)-cysts in water: a review. *Water Res.* **2006**, *40* (1), 3–22.
- (34) Peccia, J.; Hernandez, M., UV-induced inactivation rates for airborne *Mycobacterium bovis* BCG. *J. Occup. Environ. Hyg.* **2004**, *1* (7), 430–5.
- (35) Mamane-Gravetz, H.; Linden, K. G.; Cabaj, A.; Sommer, R., Spectral sensitivity of *Bacillus subtilis* spores and MS2 coliphage for validation testing of ultraviolet reactors for water disinfection. *Environ. Sci. Technol.* **2005**, *39* (20), 7845–52.
- (36) Sommer, R.; Pribil, W.; Appelt, S.; Gehringer, P.; Eschweiler, H.; Leth, H.; Cabaj, A.; Haider, T. Inactivation of bacteriophages in water by means of non-ionizing (UV-253.7 nm) and ionizing (gamma) radiation: a comparative approach. *Water Res.* **2001**, *35* (13), 3109–16.
- (37) Kariwa, H.; Fujii, N.; Takashima, I., Inactivation of SARS coronavirus by means of povidone-iodine, physical conditions and chemical reagents. *Dermatology* **2006**, *212* Suppl 1, 119–23.

Received for review January 9, 2007. Revised manuscript received April 22, 2007. Accepted May 7, 2007.

ES070056U