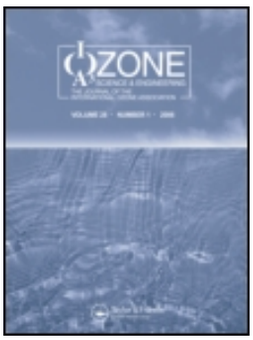

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Development of a Practical Method for Using Ozone Gas as a Virus Decontaminating Agent

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Our objective was to develop a practical method of utilizing the known anti-viral properties of ozone in a mobile apparatus that could be used to decontaminate rooms in health care facilities, hotels and other buildings. Maximum anti-viral efficacy required a short period of high humidity (>90% relative humidity) after the attainment of peak ozone gas concentration (20–25 ppm). All 12 viruses tested, on different hard and porous surfaces, and in the presence of biological fluids, could be inactivated by at least 3 $\log_{10}$ in the laboratory and in simulated field trials. The ozone was subsequently removed by a built-in catalytic converter.

Keywords Ozone, Antiviral, Decontamination, Viruses, Humidity, Ozone Generator, Catalytic Converter, Field Trials

INTRODUCTION

The anti-viral and anti-microbial properties of ozone have been well documented, although the mechanisms of action are not well understood, and several macromolecular targets could be involved (Carpendale and Freeberg, 1991; Wells et al., 1991; Khadre and Yousef, 2002; Shin and Sobsey, 2003; Cataldo, 2006; Lin and Wu, 2006; Lin et al., 2007). Aqueous solutions of ozone are in use as disinfectants in many commercial situations, including waste water treatment, laundries, and food processing (Kim et al., 1999; Shin and Sobsey, 2003; Naitou and Takahara, 2006, 2008; Cardis et al., 2007), but the use of the gas on a commercial scale as a decontamination device has not been exploited. Ozone gas however has a number of potential advantages over other decontaminating gases and liquid chemical applications (McDonnell and Russell, 1999; Barker et al., 2004).

Thus ozone is a natural compound, is easily generated in situ from oxygen or air, and breaks down to oxygen with a half-life of about 20 minutes (± 10 min depending on the environment). As a gas it can penetrate all areas within a room, including crevices, fixtures, fabrics, and the undersurfaces of furniture, much more efficiently than manually applied liquid sprays and aerosols (Barker et al., 2004; Malik et al., 2006; Hudson et al., 2007).

The only significant disadvantages are its ability to corrode certain materials, such as natural rubber, on prolonged exposure, and its potential toxicity to humans. The recognition of the risk of pathologic consequences following exposure of people and experimental animals to ozone gas has led to restrictions in its use in public areas. However the latter consideration can be offset to some extent by the potential benefits of ozone therapy in medicine and dentistry (Devlin et al., 1996; Bocci, 2004; Ciencewicki and Jaspers, 2007; Huth et al., 2007).

The health hazard can be overcome in practice by ensuring that the room to be treated is temporarily closed to people during the treatment and is sealed to prevent escape of the gas into the environment. Sensitive materials can be temporarily covered or removed if necessary. In addition the ozone gas can be removed quickly after treatment by use of a catalytic converter, which can transform the ozone back into oxygen within minutes.

We evaluated the feasibility of using ozone gas as an effective means of decontaminating various hard and porous surfaces containing dry or wet films of different viruses, in the presence and absence of cell debris and biological fluids. Following successful laboratory experiments, we then developed an efficient prototype ozone generator and catalytic converter that could be used in a room containing viral contaminants. We also examined a role for high humidity in enhancing the virus inactivation process, and incorporated this feature into the field tests.

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MATERIALS AND METHODS

Equipment

The laboratory test chamber was a molded polycarbonate box with a transparent plastic front window that could be lifted to allow access to samples. Within the test chamber was a small ozone generator (corona discharge system, from Treated Air Systems, Vancouver) fitted with a control dial that could be pre-set to determine the approximate ozone dosage in ppm, an ozone sampler tube connected to the exterior ozone measuring system (for accurately recording ozone concentration, see below), and the probe of a hygrometer for measuring relative humidity and temperature. Humidity was provided in the form of a mist of deionized sterile water by means of a spray bottle, which had been washed out with 70% ethanol

The model 1000 Viroforce ozone generator (Figure 1) was a portable module containing multiple corona discharge units, a circulating fan, and an efficient catalytic converter (scrubber) to reconvert ozone to oxygen at the termination of the ozone exposure period (further details are available in *www.viroforce.com*). In addition a portable commercial humidifier (Humidifirst Inc, Florida) was used to provide a burst of water vapor (at ambient temperature) when required.

All the components were controlled remotely from outside the test room. Ozone concentration was monitored continuously by means of an Advanced Pollution Instrumentation Inc. model 450 system (from Teledyne, San Diego), which measured samples of ozonated air passed through a UV spectrometer. This apparatus was used for all accurate ozone measurements in all test locations. The input Teflon sampling tube was taped in an appropriate location for the duration of the experiment. Relative humidity and temperature were recorded by a portable hygrometer (VWR Scientific, Ontario). The probe was taped in a convenient location inside the test room.

MATERIALS

The lids of sterile polystyrene tissue culture trays were used as plastic surfaces. Glass slides, 75×25 mm; stainless steel circular disks, 1.0 cm diameter; and pieces of fabric and cotton (typical of those used in hospital and hotel rooms) were cleaned in detergent, washed, dried, and sterilized by autoclaving. Cotton tips (Q-tips) were heated for 2 min in a microwave oven. Fetal bovine serum and PBS (phosphate buffered saline) were obtained from Invitrogen (Ontario). Sterile plastic 24-well plates and other supplies were BD-Falcon brand obtained from VWR Scientific (Ontario).

Cell Lines and Viruses

All cell lines (Vero monkey kidney cells; MDCK canine kidney cells; H-1 sub clone of HeLa cells; A549 human lung epithelial cells; feline kidney cells; all acquired originally from ATCC; mouse DBT cells, from Dr. Pierre Talbot) were passaged regularly in Dulbecco MEM, in cell culture flasks, supplemented with 5–10% fetal bovine serum, at 37°C in a 5% CO_2 atmosphere, with the exception of the H-1 cells, which were grown at 35°C. No antibiotics or antimycotic agents were used.

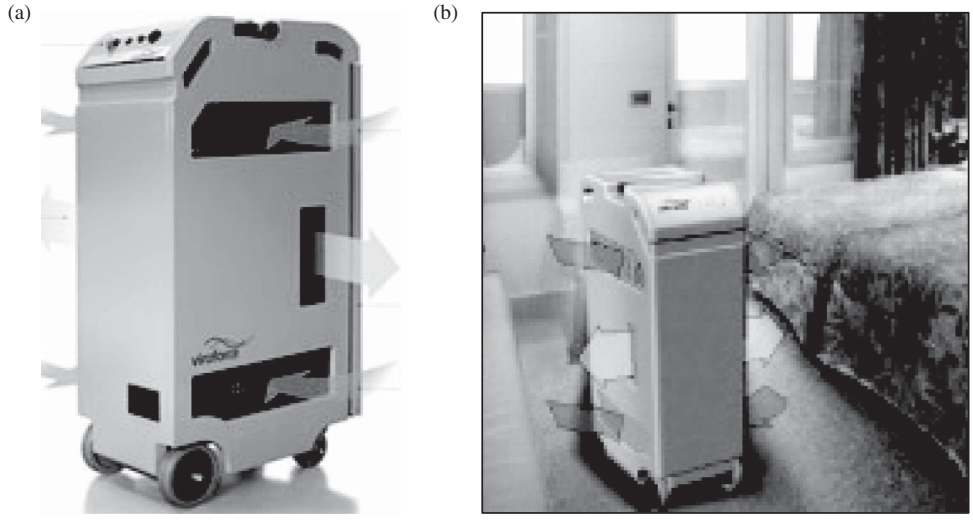

FIGURE 1. Prototype ozone generator (Viroforce 1000). The generator contains 8 corona discharge units, a powerful circulating fan, and a catalytic converter to convert ozone back to oxygen after the treatment. Part of the control panel is also visible. The unit is shown by itself (Figure 1a) and located in the test hotel room (Figure 1b). Not shown is the accessory humidifier.

The following 12 viruses were used: influenza, strain H3N2, human isolate (from BC Centre for Disease Control), propagated in MDCK cells; HSV (herpes simplex virus type 1, BC-CDC), propagated in Vero cells; rhinovirus types 1A and 14 (RV 1A and RV 14, from ATCC), propagated in H-1 cells; Adenovirus types 3 and 11 (ATCC), in A549 cells; mouse coronavirus (MCV, from Dr. Pierre Talbot) in DBT cells. Sindbis virus (SINV), yellow fever virus (YFV), vesicular stomatitis virus (VSV), poliovirus (PV, vaccine strain), vaccinia virus (VV), all ATCC strains, were grown in Vero cells. All the stock viruses were prepared as clarified cell-free supernatants, with titers ranging from 10^6 to 10^9 pfu (plaque-forming units) per mL.

Experimental Protocol

Aliquots of virus, diluted when necessary in PBS, usually 100 uL, were spotted onto the appropriate sterile surface, spread into a film by means of a sterile tip, and allowed to dry, within a biosafety cabinet (normally 30–40 min). In some experiments the spread films were left wet for the ozone treatment. The samples were then transported in sterile containers to the appropriate chamber or room for ozone treatment. Controls consisted of equivalent samples transported to the test site but not exposed to ozone, and others retained in the biosafety cabinet for the entire duration of the experiment. All control samples were contained within sealed sterile plastic boxes and kept outside the ozone-exposed room or chamber for the duration of the treatment.

Test rooms

1. The initial field trials were conducted in an unused laboratory, volume 65 m^3, in which we used 3 small ozone generators (Treated Air Systems) located in different parts of the room, together with a circulating fan. These tests were carried out at ambient humidity (40–45% RH).
2. In most of the subsequent field tests we used an office, volume 35 m^3, containing normal office furniture, which was located adjacent to the laboratory. We placed the prototype ozone generator (Viroforce model 1000) in the centre of the room, together with the humidifier. Test samples were placed in various locations of the room, and the probes for the ozone monitor and the hygrometer were taped in convenient locations. All instruments were controlled remotely from outside the test room. At the beginning of the test the air vent was covered with plastic and the door was sealed with duct tape.

   The standard program adopted for most of the tests involved increasing the ozone level over a period of 15 min to 25 ppm, maintaining this level for 10 min, at which point the humidifier was activated to produce a rapid burst of water vapor. This resulted in the RH increasing to > 95% within 5 min. Following this the humidifier and generator were switched off and the catalytic converter was switched on, which resulted in a decrease in ozone to < 1 ppm within 15 min. The door was then opened and the samples retrieved and covered for transport back to the biosafety cabinet. These samples, and equivalent control samples that had been kept in the biosafety cabinet for the duration of the test, were then reconstituted in 1.0 mL PBS and stored at −70°C until assayed by plaque formation (plaque forming units, pfu) in the appropriate cells. Unless otherwise indicated, results are presented as pfu/mL.
3. A similar protocol was employed for use in the test hotel room, a typical room with a double bed, furniture and adjacent bathroom, volume 42.5 m^3, situated in Vancouver. Dried samples of the viruses on plastic surfaces were transported in sterile containers between the laboratory and the hotel room.

RESULTS

Inactivation of Viruses by Ozone Gas on Different Surfaces

Since we wanted to evaluate the effect of ozone gas on dried samples of virus we first examined the ability of several representative viruses to retain significant infectivity following the drying process. Most of the viruses showed up to 1 $\log_{10}$ decrease in infectivity as a result of the drying process itself. After this the dried films (of HSV, influenza virus, FCV, poliovirus, and RV) showed similar decay curves, with a 50% decrease (T½) of 3–4 hours at room temperature. Thus in all cases there were more than adequate amounts of infectious virus remaining after several hours, during which experiments with ozone gas could be carried out. These decay curves were not significantly affected by the presence of 10% serum (fetal bovine serum, FBS). Similar findings on virus drying kinetics were reported recently (Terpstra et al., 2007), and these results confirm the general belief that infectious viruses can persist for long times on inanimate surfaces.

Several viruses, representing different virus families and structural features, were then treated with a single mobile ozone generator in the laboratory chamber, as described in Materials and Methods. All viruses tested, HSV, influenza, MCV, FCV, and RV, representing DNA and RNA viruses with and without membranes, showed similar kinetics of virus inactivation on three hard surfaces, plastic, glass and stainless steel. The T½ values ranged from 5–8 hours, but there were no consistent differences between the viruses or the surfaces. Examples for HSV (DNA virus with membrane), influenza (RNA virus with membrane), and RV (RNA virus without membrane)

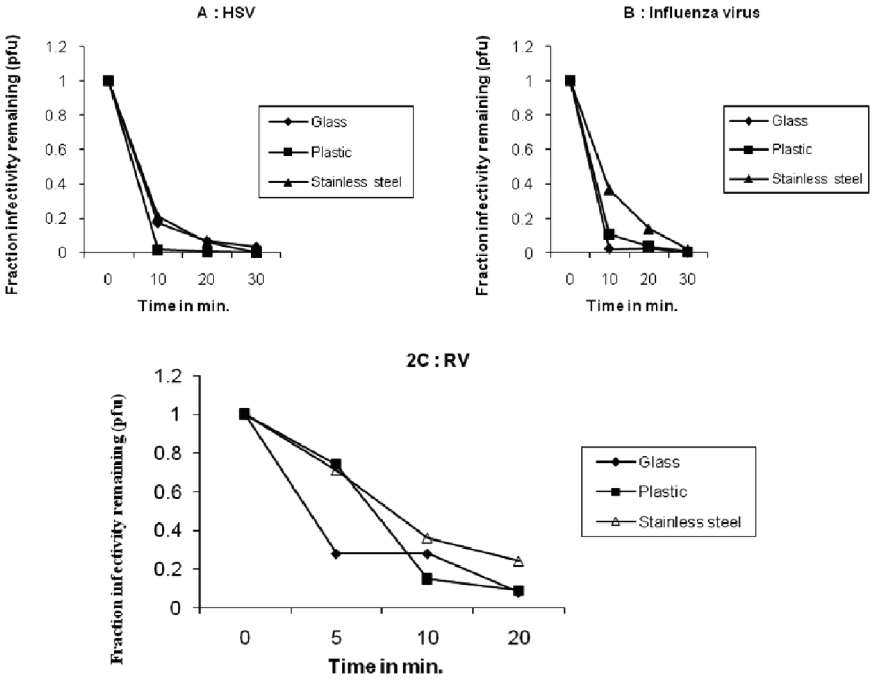

FIGURE 2. Kinetics of inactivation of viruses on different surfaces. Multiple aliquots of each virus, in separate experiments in the laboratory, were dried onto the different surfaces, and exposed to ozone gas (10 ppm) at ambient humidity (45% RH). Periodically duplicate samples were removed for reconstitution and freezing. They were subsequently thawed and assayed by plaque formation on the appropriate cell lines.

are shown in Figure 2. Rhinovirus (Figure 2c) was slightly more resistant than the other two viruses. Nevertheless these results suggest that all or most viruses should be susceptible to ozone gas.

Field Tests at Ambient Humidity

Following successful inactivation of several viruses in the laboratory experiments, we conducted tests in a large unoccupied laboratory, volume 65 m^3, with the aid of three portable ozone generators of the kind used in the previous tests. Peak ozone level attained was 28 ppm, at an ambient RH of 40%, and total time of exposure, including rise and fall periods, was 60 min. The results from 2 separate tests are combined in Table 1, and these indicate successful inactivation of 2 $\log_{10}$ or more infectious virus under these simulated field conditions. Duplicate samples showed reasonable agreement, and the results were unaffected by the position of the sample within the room. Thus prolonged exposure to a fairly high dose of ozone gas at ambient humidity can result in 2 log's inactivation of several viruses; but in practice we would prefer a system giving greater efficacy.

Enhancement of Virus Inactivation by High Humidity

We next examined the possibility of improving virus killing by treating dried samples of several viruses with

TABLE 1. Inactivation of Viruses by Ozone Gas in Large Room (Ambient Humidity)

Virus	Sample #	Fraction pfu remaining	Average $\log_{10}$ decrease
Exp #1: HSV	1a	0.0061	2.24
(herpes simplex)	1b	0.0056	
Exp #1: HSV	2a	0.021	1.96
	2b	< 0.001	
Exp #1: HSV	3a	0.018	1.66
	3b	0.025	
Exp #2: HSV	1a	0.014	1.34
	1b	0.078	
Exp #2: RV	1a	0.007	2.0
(rhinovirus 14)	1b	0.012	
Exp #2: PV	1a	< 0.01	> 2.0
(poliovirus)	1b	< 0.01	

Viruses were dried onto glass slides and transported to a large test room, volume 65 m^3, where they were treated with ozone from small generators, at ambient humidity (40% RH) and temperature (20 °C), for 1 hour; the peak level attained in both experiments was 28 ppm. Following the treatment the samples were retrieved and transported back to the laboratory for reconstitution and subsequent infectivity assays. Control slides were not exposed to ozone. Complete details are described in Materials and Methods.

TABLE 2. Effect of Humidity on Virus Inactivation in Test Room

Virus	Fraction pfu O_3 + 38% RH	Log_{10} decrease	Fraction pfu O_3 + 70% RH	Log_{10} decrease
Influenza	0.80	0.097	0.0027	2.56
FCV	0.255	0.59	< 0.0012	> 2.9
Poliovirus	1.00	0	< 0.0017	> 2.8

Viruses were dried onto plastic surfaces and placed in the test office for ozone treatment at ambient humidity (38% RH) or at elevated humidity (70% RH), in separate tests on the same day. The prototype generator was programmed to deliver up to 20 ppm for 20 min, with or without a burst of extra humidity, followed by catalytic conversion of ozone to oxygen, as described in Materials and Methods. At the end of the test, samples were retrieved for reconstitution and subsequent assays.

ozone gas in the presence of high relative humidity. Preliminary laboratory experiments indicated that the maximum enhancing effect was obtained by increasing the ozone to the maximum level first followed by a burst of water vapor to increase RH to greater than 70%, preferably >90%. However we did not have the capability of testing the enhancing effect of graded doses of humidity.

Table 2 shows the effect of RH on the degree of inactivation of 3 different viruses within a test office, 35.4 m^3 volume. Under these conditions, which involved much more restricted exposure than the conditions used for Table 1, the degree of inactivation was lower and more variable at ambient RH, but in all cases the combination of ozone gas plus high RH consistently yielded substantial inactivation. Therefore optimum efficacy of the ozone treatment requires the presence of high RH, for at least several minutes.

Composition of Virus Samples

Based on these findings, we next conducted a number of experiments with different viruses in the test office, which contained standard office furniture. For this purpose we used a newly developed prototype ozone generator, containing multiple ozone units, together with a built in catalytic converter and fan (shown in Figure 1), and an accessory humidifier capable of generating a humidity of more than 90% within 5 minutes. Details of the protocols are described in Materials and Methods. In this test system we were able to examine the effects of sample preparation and composition, organic load, and sample location within the room. Wet and dry films of viruses were found to be equally susceptible to the treatment regimen.

Also the nature of the surface on which samples were dried did not affect the result. Thus in addition to the different hard surfaces mentioned above (glass, plastic and stainless steel, Figure 2), cotton and fabric surfaces gave results similar to plastic (not shown). Inoculum size (10–1000 uL) and degree of dilution of the virus did not influence the result, nor did the presence of cellular debris in the sample. For example influenza virus and Sindbis virus in crude cell extracts and in clarified supernatants were equally susceptible (more than 3 log inactivation in dried films treated with ozone in high humidity).

We also tested the effect of serum and blood products, since samples in the field, such as tissues and corpses, and instruments used in dental and hospital clinics, might be contaminated with such materials (Cristina et al., 2008). However, as shown in Table 3, the presence of whole human blood, or human and bovine serum components, did not affect the efficacy of virus inactivation, in either dry (data shown) or wet samples of virus.

Viral Aerosols

Virus-containing aerosols, a potential problem in certain dental and medical practices (Cristina et al., 2008), were also tested by spraying known volumes of FCV suspension into the test chamber in the presence or absence of ozone gas, and collecting samples of condensate for virus assays. In comparison, similar amounts of virus were sprayed into the chamber without ozone gas,

TABLE 3. Ozone Inactivation of Virus (SINV) in the Presence or Absence of Blood Components

Treatment	No ozone (pfu)	+ ozone (pfu)	Log_{10} decrease
None	2.0×10^6	3.2×10^2	3.80
+ bovine serum albumin 1:1	4.2×10^6	3.1×10^2	4.13
+ human serum 1:1	4.2×10^6	4.3×10^2	3.99
+ whole human blood 1:1	1.0×10^7	1.3×10^2	4.89

Samples of dried SINV on plastic surfaces, containing the supplements indicated, were treated with ozone in the test office, as described in the legend for Table 2, with a burst of high humidity (90% RH).

TABLE 4. Effect of Ozone on Virus Aerosol

	Virus titer, no ozone	Virus titer + ozone	Log_{10} decrease
Exp #1	3,000	< 10	> 2.48
Exp #2	1,580	3.5	2.65

Suspensions of FCV were prepared in PBS and sprayed into the laboratory test chamber with or without 20 ppm ozone produced by a small generator. Samples of the condensate were collected in 6-well trays, and their volumes and content of infectious FCV were measured.

and measured volumes collected. This experiment was performed twice, resulting in retrieval of approximately 1% of the sprayed virus each time, and inactivation of more than 99%, as indicated in Table 4. Thus the ozone gas is also capable of efficiently killing aerosol-borne viruses.

Field Tests with High Humidity

A standard hotel room (volume 42.5 m^3) was used for the evaluation of the prototype ozone generator with accessory humidifier, using influenza- and FCV as examples of viruses with and without membranes, respectively. Known amounts of virus were dried onto glass slides, which were then transported to the room for ozone and humidity treatment, using the protocol developed in the office tests, above. Pairs of samples were placed in three different locations within the room, including an adjacent bathroom. Treated and control (unexposed) samples were then returned to the laboratory for reconstitution and assay. The results are summarized in Table 5. Both viruses were substantially inactivated, and the location of samples within the room did not affect the outcome.

Susceptible Viruses

Table 6 summarizes the viruses successfully inactivated, by 3 or more log_{10}, and their relevance. As indicated, these viruses represent many different families with a range of animal virus structures. Some of them have also been suggested to be suitable surrogates for important viruses that are difficult to cultivate in vitro or require special containment facilities (e.g., Sindbis virus and yellow fever virus for hepatitis C; These two viruses plus vesicular stomatitis viruses for HIV; human influenza virus for avian influenza; Steinman, 2004). To date we have not encountered an ozone-resistant virus.

TABLE 5. Inactivation of Viruses (Influenza, FCV) In Hotel Room

Location	Influenza, log_{10} decrease	FCV, log_{10} decrease
Bathroom	2.1	> 3.9
Bedroom	2.31	3.73
Table	2.31	> 3.9

Dried samples of the viruses were transported to the hotel, duplicate pairs were placed in different locations within the test hotel room, and ozone treatment conducted as described in Table 2, with an accessory humidifier, which gave a maximum RH of 95%. Following the treatment, samples were returned to the laboratory for reconstitution and assay, together with unexposed controls.

DISCUSSION

The objective of this study was to develop a practical and efficient apparatus for decontamination of confined spaces containing infectious viruses. Such an apparatus could be very useful in hospitals and health care facilities, and other locations where outbreaks are relatively common, such as cruise liners (Lawrence, 2004). In addition there are many other public and private buildings that could benefit from an appropriate antiviral decontamination apparatus. Existing technologies are clearly inadequate (McDonnell and Russell, 1999; Barker et al., 2004; Sattar, 2004).

Previous studies with ozone in water have proven its usefulness in commercial laundries and food processing facilities (Kim et al., 1999; Shin and Sobsey, 2003; Naitou and Takahara, 2006; 2008; Cardis et al., 2007). However, in order to decrease or eradicate virus contaminants in inaccessible locations, such as crevices, fixtures, undersides of furniture, etc. it is necessary to utilize the efficient penetrating ability of a gas. Since ethylene oxide is not considered an acceptable alternative (McDonnell and Russell, 1999; Tseng and Li, 2008), then gaseous ozone should be the best choice available.

A few studies have indicated the feasibility of ozone gas as an antiviral agent (Carpendale and Freeberg, 1991; Wells et al., 1991; Khadre and Yousef, 2002; Shin and Sobsey, 2003; Cataldo, 2006; Lin and Wu, 2006; Tseng and Li, 2008). We verified this by means of laboratory studies and several field trials in a large room. We then discovered that the addition of a burst of high humidity, following the attainment of peak ozone level, resulted in substantially greater reductions in virus infectivity, under a variety of conditions. The precise mechanisms of action against virus are not understood; however the broad oxidizing activity against many macromolecules (Cataldo, 2006) suggests that viral membranes, protein coats and nucleic acids could all be vulnerable.

Nevertheless the requirement of humidity for optimal efficacy indicates that hydroxyl ions and possibly additional water-derived radicals could be involved, as suggested for the aqueous environments (Lin and Wu, 2006; Tseng and Li, 2008). We developed the prototype apparatus to take advantage of the desired features based on these experimental results (Figure 1, Viroforce 1000). The key features are: a battery of ozone generators enclosed within the machine; a powerful catalytic converter to convert ozone back to oxygen within minutes, allowing immediate entry to the decontaminated premises; a

TABLE 6. Viruses Susceptible to Ozone Gas + High Humidity[1] (>3 Log $_{10}$ Inactivation)

Virus	Significance	Membrane (+ or −)
Herpes simplex virus (HSV)	Representative herpes virus	yes
Adenovirus types 3 and 11 (Ad 3,11)	Representative adenoviruses	no
Vaccinia virus (VV)	Representative pox virus	yes
Influenza virus (human strain H3N2)	Representative of human and avian influenza viruses	yes
Murine coronavirus (MCV)	Surrogate for SARS virus	yes
Sindbis virus (SINV)	Surrogate for Hepatitis C virus[2]	yes
Yellow fever virus (YFV)	Surrogate for Hepatitis C virus[2]	yes
Vesicular stomatitis virus (VSV)	Rhabdovirus (ubiquitous in vertebrates, invertebrates, plants)	yes
Poliovirus (PV)	Enteric virus	no
Rhinovirus types 1A & 14 (RV 1A, 14)	Common cold viruses	no
Feline calicivirus (FCV)	Surrogate for Norovirus	no

[1]No ozone-resistant viruses have been found.

[2]Sindbis virus and Yellow fever virus are in the same virus family as Hepatitis C virus, and have been promoted as suitable substitutes in antiviral testing.

Influenza virus, murine coronavirus, Sindbis virus, Yellow Fever virus, and vesicular stomatitis virus are all RNA viruses with membranes, with structures similar to HIV. Therefore this combination could be considered as a suitable substitute for HIV in antiviral testing.

circulating fan; built in remote control and programmable functions. In addition we employ an accessory humidifier, which produces an immediate cloud or mist of microscopic water droplets, without heating. We demonstrated that this apparatus was capable of inactivating 3 logs or more of many different infectious viruses in rooms such as an office and a hotel room. We also reported recently that the same apparatus worked efficiently in a cruise liner cabin to inactivate norovirus (Hudson et al., 2007).

To date we have successfully tested the apparatus in laboratory and field conditions against 12 representative viruses, mostly human pathogens. Some of these viruses (Table 6 legend) have also been promoted as valid surrogates for viruses that are difficult or dangerous to cultivate and test by conventional techniques, such as hepatitis C virus, HIV, avian influenza (Steinman, 2004).

The location of the test virus in the room was not a factor, a result that might be expected considering the penetrability of the ozone gas, nor was the presence of blood and serum products. The latter was an important result since the possibility of microbe protection against ozone by organic films has been suggested (Serra et al., 2003). In addition, the presence of such contaminated materials has been suggested as a risk for spread of infections in medical and dental practices (Cristina et al., 2008). Another possible factor, which has been shown to play a role in other liquid anti-microbial applications (Sattar, 2004; Malik et al., 2006), is the presence of a porous surface such as fabric or carpet in which the virus or other organism is embedded. This limitation was not seen however in our experience with ozone gas against viruses or bacteria (Hudson et al., 2007; Sharma and Hudson, 2008). As a result of these studies, we believe that the apparatus we have developed, based on the use of ozone gas and high humidity, has many potential applications wherever efficient decontamination of rooms is required.

ABBREVIATIONS

DMEM	Dulbecco minimum Eagle medium
PBS	phosphate-buffered saline
pfu	plaque-forming unit (1 pfu = 1 infectious virus particle)
RH	relative humidity

Viruses:

Ad 3/11	adenovirus type 3/11; FCV, feline calicivirus
HSV	herpes simplex virus type 1
MCV	mouse coronavirus
PV	poliovirus type 1 vaccine strain
RV1A/14	rhinovirus type 1A/14; SINV, Sindbis virus
VSV	vesicular stomatitis virus
VV	vaccinia virus
YFV	yellow fever virus, vaccine strain.

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