

Evaluation of a Dry Fogging System for Laboratory Decontamination

Jay Krishnan*, Greg Fey, Carol Stansfield, Laura Landry, Hung Nguy, Stan Klassen, and Catherine Robertson

Public Health Agency of Canada, Winnipeg, Manitoba, Canada

Abstract

Dry fogging is a relatively new decontamination technology that uses liquid disinfectant and compressed air as consumables. The ultrafine droplet size of the dry fog prevents it from easily falling onto surfaces, a desirable quality for space/area decontaminations. Liquid peracetic acid (PAA) has been shown to have excellent microbicidal activity; however, it has compatibility issues with a variety of materials. The objectives of this study were to determine the microbicidal activity, compatibility to electronic equipment, decontamination potential for laboratories, and mold remediation potential for a walk-in cooler of the dry fogging system (DFS) using PAA. Stainless steel coupons spiked with a select number of microbial agents (Escherichia coli, Staphylococcus aureus, Bacillus atrophaeus spores, Vesicular stomatitis virus, and Human adenovirus 5) were exposed to the dry fog to determine its microbicidal activity. Decontaminations of a simulated laboratory and high-containment laboratories were validated using commercially available biological indicators placed at several locations within the area. Compatibility to electronic equipment was assessed by repeatedly exposing personal computers to the dry fog over a 6-month period. All test microbial agents were inactivated by the dry fog; laboratory decontamination and mold remediation validations were successful. No functional impairment was detected in the personal computers following six rounds of exposure. Results show that the DFS is an effective decontamination technology for laboratories as an alternative to formaldehyde, vaporous hydrogen peroxide, or gaseous chlorine dioxide (GCD).

Introduction

In 1968 Portner and Hoffman atomized PAA in a chamber at relative humidity levels lower than 80% to inactivate *B. atrophaeus* spores seeded onto paper and glass surfaces (Portner & Hoffman, 1968). This was done to eliminate the corrosiveness of PAA if used as a wet fog. In 2001, an automatic fogging disinfection system was manufactured by Ikeuchi Co., Ltd. (Osaka, Japan) (Nakata et al., 2001) for decontaminating hospital rooms and operation theaters. The system generated fine fog particles, which were almost uniform at 10 microns in size. They used a number of disinfectants—acidic electrolyte water, alkyldiaminoethylglycine, benzalkonium chloride and chlorhexidine gluconate, glutaral, povidone iodine, and sodium hypochlorite. Since that time, fogging technology has improved and is currently known as the dry fogging system (DFS). It creates uniform fog particles at size 7.5 microns or less (Ikeuchi & Co., available at www.kirinoikeuchi.co.jp/eng/products/akimiste_dry_fog_humidifier.html#01),

and the nozzles used for dry fogging are available commercially from several sources. Unlike a wet fog, the ultrafine dry fog particles do not settle onto surfaces right away, behaving more like a gas/vapor to fill the space being decontaminated. Additionally, the dry fog diffuses widely and does not wet the surfaces with which it comes in contact (Fog Master Corporation, Deerfield Beach, FL. Available at www.fogmaster.com/info.html), hence the name “dry fog.”

Even though it is possible to fog any liquid disinfectant, the authors have chosen to use peracetic acid (PAA). Liquid PAA is widely used for sterilization-in-place by the food processing, dairy, and beverage industries because of its effectiveness at low temperatures and non-toxic by-products, namely water, oxygen, and carbon dioxide (Orth, 1998). Peracetic acid is a clear, colorless solution with a piercing odor. It is usually produced by a reaction of hydrogen peroxide with acetic acid in the presence of a catalyst, such as sulfuric acid (Greenspan, 1946). To prevent the reverse reaction from occurring, the solution is fortified with excess acetic acid and hydrogen peroxide. Depending on the initial concentration of hydrogen peroxide used, the PAA concentration could be as high as 46%. To minimize container failure and spill, PAA should be stored in original containers, preferably at cool temperatures. Pure aluminum, stainless steel, and tin-plated iron are resistant to PAA; however, plain steel, galvanized iron, copper, brass, and bronze are susceptible to corrosion (Schroder, 1984).

While PAA is a product of a chemical reaction between hydrogen peroxide and acetic acid, it is superior to hydrogen peroxide because of its lipid solubility, potent microbicidal activity at low temperatures (Hussaini & Ruby, 1976; Jones et al., 1967), and tolerance to organic soiling (Baldry & French, 1989; Sagripanti & Bonifacino, 1997). “The excellent disinfecting and cold sterilization action of PAA” was reported as early as 1902 (Freer & Novy, 1902). A 1949 study showed PAA to be the most active of 23 disinfectants tested against bacterial spores (Hutchings & Xezones, 1949). The bactericidal, fungicidal, and sporicidal concentrations of PAA have been demonstrated as low as 0.001%, 0.003%, and 0.3%, respectively (Greenspan & MacKellar, 1951). Virucidal concentrations have been determined in the range of 0.002%–0.225% against a variety of enveloped and non-enveloped viruses (Block, 2001). PAA has been widely used as a cold disinfectant in the health care industry, especially for heat-labile medical equipment (Carter & Barry, 2011; Dettenkofer & Block, 2005). PAA has also been evaluated as a sterilant for bone and skin allografts (Lomas et al., 2003; Pruss et

al., 1999; Pruss et al., 2001) and heart valves (Aidulis et al., 2002; Farrington et al., 2002). Currently, 11 peroxyacetic acid-containing pesticides are registered with the U.S. Environmental Protection Agency (EPA) and 17 with Health Canada.

Materials and Methods

Institutional biosafety procedures and protocols were observed while performing all the experiments described here, and risk assessments were conducted as appropriate.

Dry Fogging System Equipment

Two different types of dry fogging equipment were used for this study, the Mini Dry Fog[®] system (Mar Cor Purification, Skippack, PA) and a portable dry fog (Ikeuchi USA, Inc., West Chester, OH). The volume of the space being decontaminated determined which unit would be used. The 0.5 L reservoir capacity of the Mini Dry Fog[®] system is sufficient for volumes up to 1,100 ft³; whereas the portable dry fog system's reservoir capacity of 19 L is capable of decontaminating a much larger space. The Mini Dry Fog[®] system is marketed as a disinfection system, and the Ikeuchi system is sold primarily as a space humidification system. Two different fogging heads are available for use on the Ikeuchi system—AKIMist[®] D and AKIMist[®] E. AKIMist[®] E is the preferred fogger head as it consumes 20% less compressed air than the AKIMist[®] D and its spray volume ranges from 2.4-9.6 L/hour depending on the number of nozzles (1-4) used at 43.5 psi. The ball adaptor that can be purchased from Ikeuchi connects the nozzles to the AKIMist[®] heads, which enables the user to adjust the spray direction within 10-50 degrees. A portable air compressor (Model #2807CE72, Gardner Denver Thomas, Sheboygan, WI) was used with both fogging systems.

Disinfectant

A commercially available liquid PAA, Minncare[®] Cold Sterilant (Mar Cor Purification, Skippack, PA) was used for this study. It is a registered disinfectant, both with Health Canada and the U.S. EPA, and contains 4%-6% PAA, 20%-24% hydrogen peroxide, and 8%-10% acetic acid. The Minncare[®] was diluted in deionised water just before use and the required dilution was determined using the Dry Fog Calculation Software provided by Mar Cor Purification. The calculation was dependent on the initial relative humidity (RH) and temperature in the space being decontaminated; higher RH would result in a lower dilution (less water) and vice versa.

No electro-chemical sensor was available to measure the concentration of PAA; hence, RH was used as an indirect indicator to monitor and control the decontamination process. The fogging cycle was terminated when the RH reached 75%-80%; higher than 80% RH could result in condensation and subsequent material compatibility issues.

Determination of Microbicidal Activity

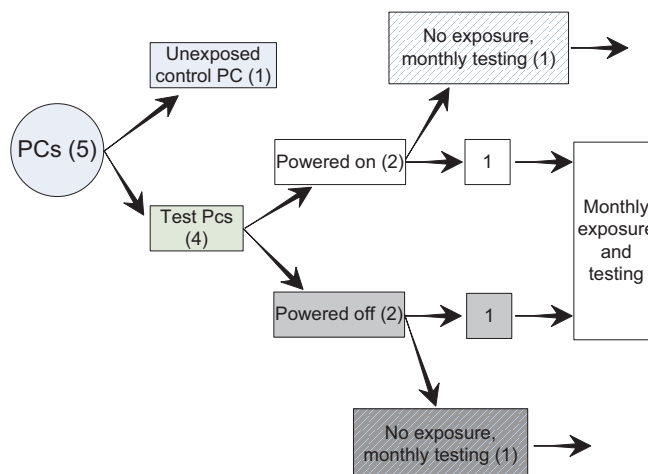
Even though data on the microbicidal activity of liquid peracetic acid are abundant, scant data are published when it is applied as a dry fog, especially using a standardized testing methodology (Gregersen & Roth, 2012). This experiment was undertaken to determine the microbicidal activity of the dry fog on a select number of candidate microbial agents using a standardized testing protocol. A total of five microbial agents were used as test agents for this validation. They included Gram negative (*Escherichia coli* ATCC 25922) and Gram positive (*Staphylococcus aureus* ATCC 25923), non-spore forming bacteria, bacterial spores (*Bacillus atrophaeus* ATCC 51189), and enveloped (Vesicular stomatitis virus Indiana serotype), and non-enveloped (Human adenovirus 5) viruses. The microbicidal activity of the DFS was determined using the Quantitative Carrier Test (Springthorpe & Sattar, 2005), a widely adopted disinfectant testing protocol. A standard tripartite protein (BSA, tryptone, mucin) soil load was mixed with the test microbial agent, deposited on stainless steel coupons (pre-sterilized by autoclaving), and dried inside a Class II biosafety cabinet (BSC) for 45-60 minutes. The test coupons (N=7) were then placed inside a glove box and exposed to dry fogging. After exposure, the bacterial-coupons were aseptically transferred to Trypticase soy broth (TSB) and incubated for growth (turbidity) at 37°C for 2 days. The material from virus-coupons was eluted off in 1 mL Dulbecco's Modified Eagle's Medium (DMEM) with 2% FBS and inoculated onto six well plates seeded with VeroE6 cells and incubated for growth (cytopathic effect) at 37°C for 1 week for Vesicular stomatitis virus and 2 weeks for Human adenovirus. The material eluted off the coupons did not require neutralization as no residual chemical was detected by PAA strip (Minntech, Minneapolis, MN) testing. To account for the reduction in microbial viability due to drying of the coupons, material from 1-hour dried bacterial-coupons (N=3) was eluted off in saline, serially diluted, and plated to determine the number of colony-forming units. Similarly, material from 1-hour dried viral-coupons was eluted off in DMEM with 2% FBS and its titer determined by TCID₅₀ (viral dose to infect 50% of cells in culture) on VeroE6 cells in 96 well plates. All experiments were performed in duplicate.

Determination of Compatibility to Electronic Equipment

Five Dell personal computers (Inspiron 560) were used to examine the effect of repeated exposure to DFS on electronic equipment. Personal computers were chosen as the test vehicles as they contain typical components and materials found in any electronic equipment. As shown in Figure 1, one computer served as a negative control and received no DFS exposure. Two computers were exposed to a single round of DFS; of these, one was powered off during exposure and the other powered on. The final two computers were subjected to monthly DFS fumigation (one

Figure 1

Monthly exposure protocol of personal computer (PC) to the dry fog for 6 months, number of PCs in parenthesis.



powered on and the other powered off during all exposures) for 6 months. Following each round of DFS, all five computers were photographed and visually inspected for physical changes, and subsequently assessed for functional as well as hardware impairment using the software PC-Doctor Service Center™ 7.5 (Microsoft Corp., Redmond, WA) which diagnoses performance failure of all the computer's key hardware subsystems. To eliminate bias, a blind analysis technique was used, where the IT technician who performed monthly analysis of the PCs using the software was unaware which PCs were exposed or unexposed. To validate the decontamination of PCs, a biological indicator (Apex biological indicator, Mesa Labs, Lakewood, CO) was placed inside each of the PCs before the side cover was replaced and secured.

Validation of Laboratory Decontamination

For assessing the decontamination potential of the DFS in laboratory spaces, a simulated laboratory (1,024 ft³) with 10 air changes/hour was built using metal stud framing and 1/4-inch thick polypropylene walls (Figure 2). The inward airflow was via a 1-inch gap at the door's bottom and the exhaust via an 8-inch duct on top of the structure, which was connected to the building exhaust system. No laboratory equipment or furniture was present in the simulated space; 13 commercially available biological indicators (BIs) were placed at various locations (Figure 3). The Mini fogger, filled with typically 400 mL of freshly diluted MinnCare®, was used for the decontaminations of this simulated laboratory.

Subsequently, decontamination validations were performed in two BSL-4 laboratory suites, which were initially decontaminated using formaldehyde fumigation (as had always been the practice since commissioning of the laboratories) for the annual recertification. A small BSL-4 laboratory and adjacent autoclave room (volume 3,700 ft³) were validated first. A total of 20 BIs were placed at various

locations in both rooms (N=15), including inside the BSCs and drawers (N=5). Both BSCs in the lab were turned off, but the refrigerator (N=1), freezers (N=3), and incubators (N=3) remained on. The door between the main laboratory and autoclave room was left open, allowing the dry fog to permeate. Approximately 2 L of diluted MinnCare® was used in the portable dry fogger that had been fitted with two AKIMist® D nozzles. This unit was placed at the center of the laboratory with the nozzles approximately 7.5 feet high above the floor (Figure 4).

Two portable foggers, each fitted with three AKIMist® E nozzles were used to validate the decontamination of a larger BSL-4 laboratory suite (11,500 ft³). This suite contained five BSCs and a laminar flow hood, all of which were turned off, while the refrigerators (N=3), freezers (N=5), and incubators (N=8) were left running. Twenty-nine BIs were placed throughout, at various locations within the laboratory and BSCs. Five litres (2.5 L/fogger) of diluted MinnCare® were used. The door between the laboratory and an adjacent small animal room was left open, and a small floor fan (12 inches) was placed at the entrance to direct some fog into the small animal room (Figure 5).

For all laboratory validations, fogging was initiated and terminated remotely by using an indoor/outdoor wireless remote outlet (#49568, Woods Industries, Markham, Ontario, Canada) that controlled the portable compressor. The process termination point of 80% RH was determined using a digital RH sensor (TSI VelociCalc Plus #8386-E-GB Multi Parameter Ventilation Meter, TSI Incorporated, Shoreview, MN) that was set up in the area being fumigated; a typical chart is shown (Figure 6). A contact period of approximately 18 hours (overnight) was allowed for the decontamination to occur. Prior to retrieving the BIs on the following day, the space was aerated and a hydrogen peroxide vapour concentration of less than 1 ppm (Dräger Pac III [Dräger Safety, Inc., Pittsburgh, PA] with hydrogen peroxide sensor) was confirmed.

Figure 2

The simulated laboratory (1024 ft³) that was built (metal stud framing and transparent polypropylene sheet walls) for decontamination validations using the dry fog.

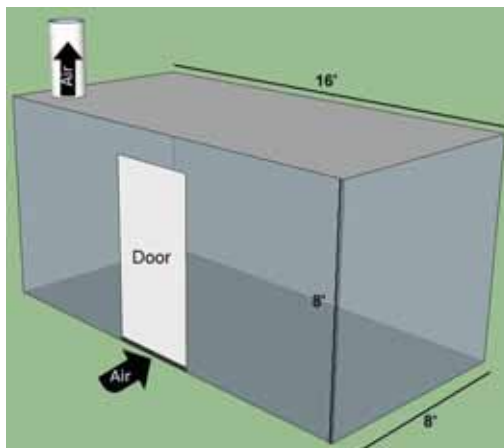


Figure 3

Preparation of simulated laboratory for decontamination using the dry fog. Locations of biological indicators and the mini fogger are shown. Arrow indicates the direction of the fog delivery.

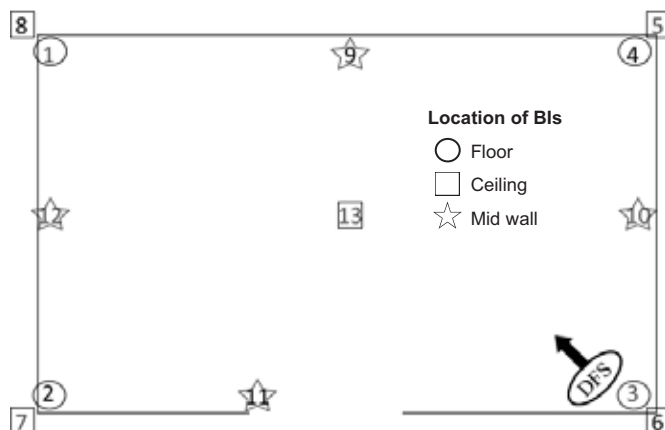
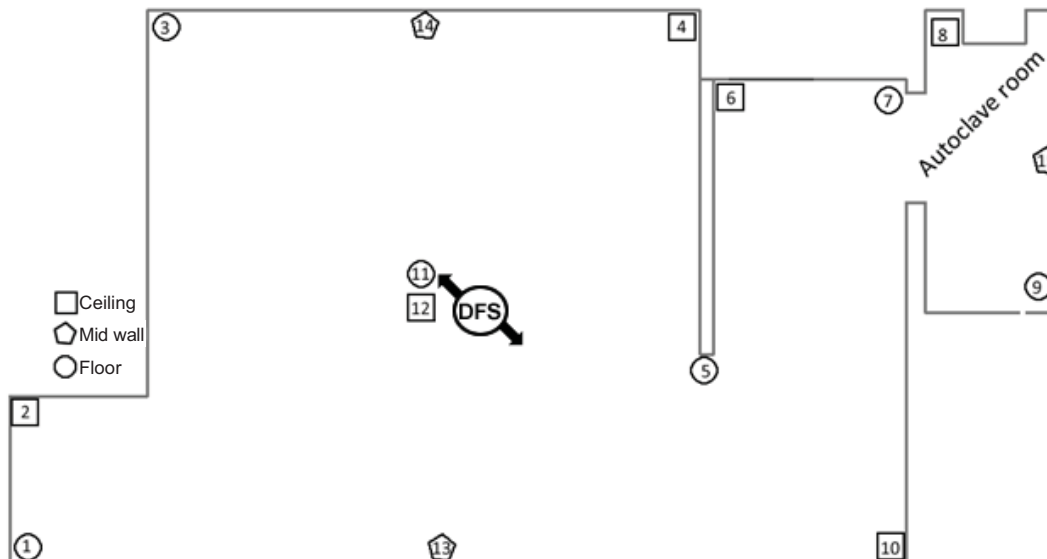


Figure 4

Preparation of small BSL-4 laboratory for decontamination using the dry fog. Locations of biological indicators and the portable fogger are shown. Arrows indicate the orientation of the nozzles and fog delivery directions.



Each biological indicator contained $\geq 10^6$ *Geobacillus stearothermophilus* spores dried on a stainless steel coupon in a Tyvek pouch. Following fumigation, the BIs were processed aseptically inside a BSC; each of the spore coupons was removed from its pouch and transferred to a TSB tube containing 0.5 mg/100 mL phenol red as an indicator. All the tubes, including positive (unexposed BI coupon in

TSB) and negative (TSB without BI coupon) controls were incubated at 56°C. The positive control tubes yielded bacterial growth (color change from red to yellow and development of turbidity) upon overnight incubation. This study's criterion for a successful decontamination was inactivation of all the BIs that were placed in the area being fumigated.

Figure 5

Preparation of large BSL-4 laboratory for decontamination using the dry fog. Locations of biological indicators, floor fan, and the portable foggers are shown. Arrows indicate the orientation of nozzles and fog delivery directions.

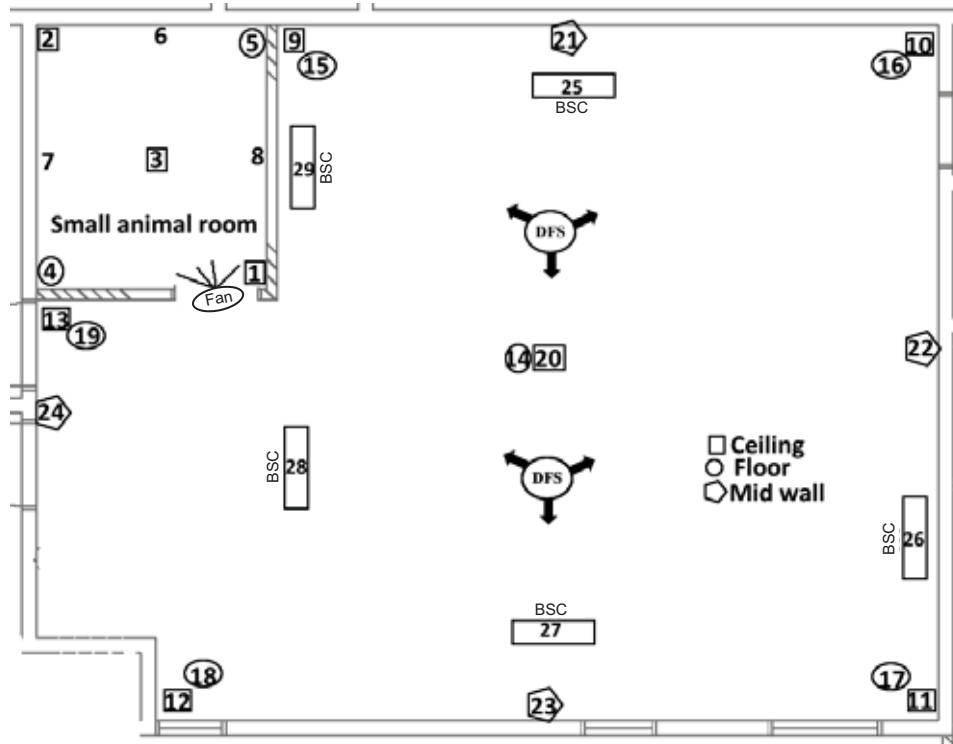
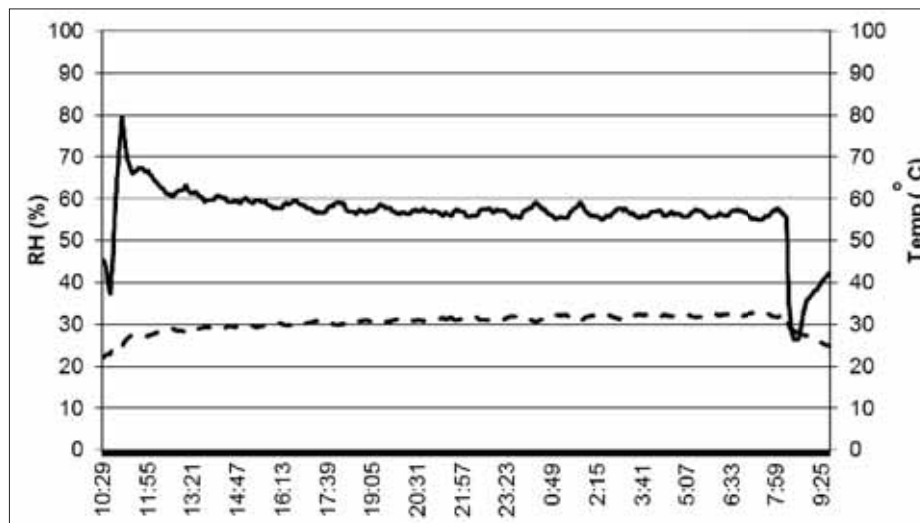


Figure 6

Relative humidity (solid line) and temperature (dashed line) recorded during decontamination of the small BSL-4 laboratory.



Validation of Mold Remediation in a Walk-in Cooler

A walk-in cooler was showing signs of mold contamination, evidenced by visible growth on the shelving and cardboard storage boxes. Prior to fumigation, 500 L of air samples (MAS 100 Eco[®] Air Sampler, VWR International, Radnor, PA) were collected on Sabouraud Dextrose Agar plates (BD Difco[™], Becton Dickinson, Franklin Lakes, NJ) both from inside the cooler and outside the cooler door. All materials stored in the cooler were left in place, with the storage boxes opened for the fumigant's permeation. The Mini Dry Fog[®] unit, filled with 100 mL of diluted MinnCare[®], was used for the decontamination of this 600-ft³ cooler. The cooler fan was turned off briefly prior to fogging for approximately 15 minutes. After the fogging process, the cooler fan was turned on again and an overnight contact period was allowed for the remediation to occur. On the following day, the cooler was sampled for residual hydrogen peroxide vapor prior to collecting post-decontamination air samples. Since the cooler had no ventilation system, aerating out the residual PAA was not possible. However, a charcoal-based scrubber (ClorDiSys Solutions, Lebanon, NJ) was available to remove the residual PAA in the cooler. Surprisingly, this was not required as there was no detectable hydrogen peroxide vapor (Dräger Pac III with hydrogen peroxide sensor) in the cooler on the following morning. After the first round of fogging, all the paper storage boxes were removed for destruction and their contents transferred to plastic storage boxes. The shelves were transferred to an alternate space to pressure wash and dry before being placed back in the cooler, which had also been completely wiped down. A second round of fogging was done exactly as described above after the entire contents of the cooler were put back in place.

Results

Determination of Microbicidal Activity

Loss of microbial titer due to drying of the coupons ranged from 0.2-1.6 logs (Table 1). Vesicular stomatitis virus and *E. coli* had the highest loss while *S. aureus* and *B. atrophaeus* had the least reduction in titer from an hour

of drying. All BIs (*G. stearothersophilus* spores, no protein soil load) were inactivated upon 30 minutes of exposure to dry fog (Table 2). However, inactivation of the test microbial agents suspended in a standard protein soil load required longer exposure times. Non-spore forming bacteria and viruses required an hour of exposure (no shorter time points were investigated), while *Bacillus atrophaeus* spores required overnight (approximately 18 hours) exposure for complete inactivation.

Determination of Compatibility to Electronic Equipment

Monthly computer diagnostics performed using the PC-Doctor[™] software showed no decline in performance or failure of any of the key subsystems. Also, no visible damages or changes were found on any of the computers' critical parts, such as the motherboard, the memory, the data cables connecting the drives (no discoloration/degradation of the plastic), and the hard disk drives. However, the metal captive screws, which were initially shiny and smooth, became dull and rough upon exposure to the dry fog. Interestingly, even though the screws on the outside were affected on both PCs that were powered on and off during exposure, the inside screws on the motherboard were affected only on the PC that was powered off during exposure (Figure 7). The BIs placed inside the PC that was powered on were inactivated all six times, while the BIs in the powered off PC were inactivated only five times (data not shown).

Validation of Laboratory Decontamination

The laboratory decontamination validation processes were straightforward and fairly easy. The time required for aeration was relatively short—about 1 hour for the simulated lab and 2-3 hours for the BSL-4 laboratories. Vaporous hydrogen peroxide (VHP) and formaldehyde fumigation, on the other hand, require aeration times of 12-24 hours (Krishnan et al., 2006a). All BIs placed throughout the simulated laboratory and the large BSL-4 laboratory showed no growth when incubated, indicating successful decontamination processes. However, decontamination of the small BSL-4 laboratory was not successful; four BIs (6, 8, 10, 12; Figure 4) grew upon overnight incubation.

Table 1

Effect of drying on microbial viability. Numbers represent means and standard deviations of counts from six coupons.

Microbial Agent	Titre ($\log_{10} \pm \text{SD}$)	
	Pre-drying	Post-drying
Adenovirus*	6.3 \pm 0.28	6.0 \pm 0.09
Vesicular stomatitis virus*	7.0 \pm 0.17	5.4 \pm 0.41
<i>S. aureus</i> **	6.2 \pm 0.03	6.0 \pm 0.07
<i>E. coli</i> **	6.9 \pm 0.04	5.7 \pm 0.11
<i>B. atrophaeus</i> spores**	5.5 \pm 0.05	5.2 \pm 0.03

*TCID₅₀: 50% tissue culture infectious dose; **CFU: Colony forming units

Table 2

Inactivation by the dry fog of microbial agents that are suspended in protein soil load and dried on stainless steel coupons.

Microbial Agent	Exposure Time (hrs)	Positive/Total*
Adenovirus	1.0	0/14
Vesicular stomatitis virus	1.0	0/14
<i>S. aureus</i>	1.0	0/14
<i>E. coli</i>	1.0	0/14
<i>G. stearothermophilus</i> spores**	0.5	0/24
<i>B. atrophaeus</i> spores	1.0	3/3
<i>B. atrophaeus</i> spores	2.5	1/3
<i>B. atrophaeus</i> spores	5.0	2/3
<i>B. atrophaeus</i> spores	7.0	1/3
<i>B. atrophaeus</i> spores	Overnight	0/6
<i>B. atrophaeus</i> spores	Overnight	0/6

*Number of coupons showing growth over the total number of exposed coupons

**Commercial biological indicators, contain no protein soil load

Validation of Mold Remediation in a Walk-in Cooler

Although there was visible growth on shelving and other surfaces prior to fogging, the number of airborne fungal spores inside the cooler remained low—25 in 500 L of air in comparison to 7 from outside the cooler (Table 3). However, the fogging was effective in reducing the airborne fungal spores to undetectable levels.

Discussion

Loss of microbial viability due to drying is well documented (Gerba & Kennedy, 2007; Lai et al., 2005; Stowell et al., 2012; Xie et al., 2006); generally, enveloped viruses and Gram negative bacteria tend to lose viability at a faster rate than non-enveloped viruses, Gram positive bacteria, and bacterial spores. Data show minimal loss of viability for *S. aureus*, Adenovirus, and *B. atrophaeus* spores while *E. coli* and Vesicular stomatitis virus lost over 1 log₁₀ after an hour of drying under ambient laboratory conditions.

As elution efficiency would never be 100%, a qualitative growth/no growth assessment was used to better determine the complete inactivation of test microbial agents as opposed to eluting the material and subsequent dilution plating (Rogers & Choi, 2008). Dry fogging inactivated all five test microbial agents in the presence of a standard protein soil load. This is consistent with a recent publication by Gregersen and Roth (2012), where inactivation of three non-enveloped and stable viruses was easily accomplished using the dry fog. Portner and Hoffman (1968) showed inactivation of *B. atrophaeus* spores in 20 minutes using atomized PAA. Similarly, this study showed inactivation of *G. stearothermophilus* spores in 30 minutes in the absence

of protein soiling; no shorter time points were tested. However, inactivation of *B. atrophaeus* spores required overnight exposure in the presence of soil load. Other laboratory fumigation agents such as VHP, formaldehyde, and gaseous chlorine dioxide (GCD) have varying degrees of soil load tolerance (Casella & Schmidt-Lorenz, 1989; Julie et al., 2011; Koen & Frank, 2011; Meszaros, 2005; Pottage et al., 2010). Among them, GCD appears to tolerate soil load best (Krishnan et al., 2006b).

Repeated exposures of personal computers to DFS over a period of 6 months showed no evidence of functional impairment or damage/change to any of the critical parts. While VHP is compatible to sensitive materials and electronic equipment (Pottage, 2011), a report published by the U.S. EPA showed GCD having some compatibility issues (EPA, 2010). The PC compatibility testing in this study was not as exhaustive as those mentioned above; however, six rounds of exposure over a period of 6 months showed no critical functional or physical damages by DFS. Interestingly, the captive screws on the inside of the computer were not affected when it was left powered on, presumably because the computer fan prevented condensation occurring there. A similar observation was reported in the U.S. EPA study where the computer that was powered off during GCD exposure was more adversely affected.

Decontamination of the simulated lab was easily accomplished and repeated many times. This was done without using any fans to circulate the fog. However, this success without air circulation could not be repeated in the small BSL-4 laboratory. Even though BIs placed horizontally far from the fogger were inactivated, four BIs placed on the ceiling, including the one placed just above the fog-

ger (the closest one to the fogger), were not inactivated. This clearly indicates that the fog didn't reach the ceiling level in the absence of air turbulence. Heat generated by the freezers, refrigerators, and the steam-jacketed barrier autoclave may have created a static hot air layer at the top of the space. Higher temperature leads to lower RH, which

could effectively prevent microcondensation formation that is often required for microbial inactivation (Watling et al., 2002). This could have been easily overcome by employing a couple of fans in the lab to create turbulence. However, the authors could not revalidate the decontamination process using fans in the small BSL-4 laboratory as the lab

Figure 7

Effect of dry fogging on personal computers. Arrows indicate the affected (dull and rough) and unaffected (shiny and smooth) captive screws. **A)** Unexposed; **B and C)** Exposed monthly while powered on; and **D)** Exposed monthly while powered off. Arrows indicate some of the affected screws.



Table 3

Mold colony counts obtained from 500 L air samples collected from inside and outside the walk-in cooler before and after fumigation using the dry fog.

Location	Number of Mold Colonies		
	Pre-decontamination	Post-decontamination 1	Post-decontamination 2
Inside cooler	25	1	0
Outside cooler	7	12	10

was no longer available for further testing. Fumigation of the large BSL-4 laboratory was straightforward. However, better fog nozzles (AKIMist® E) and ball adaptors in combination with a small floor fan were used to accomplish decontamination.

The dry fogging process in the laboratories was simple and straightforward; however, unlike VHP and GCD, the decontamination equipment (fogger, compressor, remote power outlet, etc.) had to be taken into the high-containment laboratories. Thus, decontaminating a hot high-containment laboratory would require carrying the equipment into the laboratory while wearing appropriate PPE, which could be cumbersome. It is also important to ensure the nozzles are not clogged while setting up the fogger. In this study, nozzles were cleaned after use by fogging a small amount of water followed by drying them using compressed air.

The mold remediation of the walk-in cooler was easily accomplished. This should not be surprising as PAA has been shown to have excellent fungicidal activity at lower temperatures (Baldry, 1983). However, the authors were surprised to find the absence of residual hydrogen peroxide vapor in the cooler after overnight contact time, which would have otherwise necessitated air scrubbing. Cellulose-containing materials such as cardboard boxes are known to absorb hydrogen peroxide vapor, which may have played a role here.

Conclusions

This study shows the dry fog system's ability to completely inactivate all the microbial agents tested in the presence of soil load. The technology was compatible to electronics and can be used to decontamination laboratory spaces and walk-in coolers. Additionally, it is inexpensive to acquire, maintain, and operate and is extremely portable for field deployment. This technology has the potential as an alternative to formaldehyde, VHP, and GCD to decontaminate laboratories and health care facilities.

Acknowledgment

*Correspondence should be addressed to Jay Krishnan at jay.krishnan@phac-aspc.gc.ca.

References

Aidulis, D., Pegg, D. E., Hunt, C. J., Goffin, Y. A., Vanderkelen, A., Van Hoeck, B., et al. (2002). Processing of ovine cardiac valve allografts: 1. Effects of preservation method on structure and mechanical properties. *Cell Tissue Bank*, 3(2), 79-89.

Baldry, M. G. C. (1983). The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid. *Journal of Applied Microbiology*, 54(3), 417-423.

Baldry, M. G. C., & French, M. S. (1989). Activity of peracetic acid against sewage indicator organisms. *Water Science & Technology*, 21(12), 1747-1749.

Block, S. S. (2001). Peroxygen compounds. In S. S. Block (Ed.), *Disinfection, sterilization and preservation* (5th ed.). Philadelphia: Lippincott Williams & Wilkins.

Carter, Y., & Barry, D. (2011). Tackling *C difficile* with environmental cleaning. *Nursing Times*, 107(36), 22-25.

Casella, M. L., & Schmidt-Lorenz, W. (1989). Disinfection with gaseous formaldehyde. Fifth Part: Influence of albumin, mucin and blood on the bactericidal and sporicidal effectiveness. *Zentralbl Hyg Umweltmed*, 189(1), 37-49.

Dettenkofer, M., & Block, C. (2005). Hospital disinfection: Efficacy and safety issues. *Current Opinion in Infectious Diseases*, 18(4), 320-325.

Farrington, M., Wreghitt, T., Matthews, I., Scarr, D., Sutehall, G., Hunt, C. J., et al. (2002). Processing of cardiac valve allografts: 2. Effects of antimicrobial treatment on sterility, structure and mechanical properties. *Cell Tissue Bank*, 3(2), 91-103.

Freer, P. C., & Novy, F. G. (1902). On the formation, decomposition, and germicidal action of benzoyl acetyl and diacetyl peroxides. *Journal of the American Chemical Society*, 27, 161-193.

Fogmaster Corporation. *General info on fog*. Available at: www.fogmaster.com/info.html. Accessed online 2012.

Gerba, C. P., & Kennedy, D. (2007). Enteric virus survival during household laundering and impact of disinfection with sodium hypochlorite. *Applied Environmental Microbiology*, 73(14), 4425-4428.

Greenspan, F. P. (1946). The convenient preparation of per-acids. *Journal of the American Chemical Society*, 68(5), 907.

Greenspan, F. P., & MacKellar, D. G. (1951). The application of peracetic acid germicidal washes to mold control of tomatoes. *Food Technology*, 5, 95-97.

Gregersen, J. P., & Roth, B. (2012). Inactivation of stable viruses in cell culture facilities by peracetic acid fogging. *Biologicals*, 40(4), 282-287.

Hussaini, S. N., & Ruby, K. R. (1976). Sporicidal activity of peracetic acid against B anthracis spores. *Veterinary Record*, 98(13), 257-259.

Hutchings, I. J., & Xezones, H. (1949). Comparative evaluation of the bactericidal efficiency of peracetic acid, quaternaries and chlorine containing compounds. *Proceedings of the 49th Annual Meeting of the Society of American Bacteriology*, Cincinnati, OH.

Ikeuchi & Co., Ltd., AKIMist® "E" Dry Fog Humidifier. Available at: www.kirinoikeuchi.co.jp/eng/products/akimiste_dry_fog_humidifier.html#01. Accessed online 2012.

Jones, L. A., Jr., Hoffman, R. K., & Phillips, C. R. (1967). Sporicidal activity of peracetic acid and beta-propiolactone at subzero temperatures. *Journal of Applied Microbiology*, 15(2), 357-362.

Julie, F., Yongjie, H., & Uwe, M. (2011). Experience and studies on formaldehyde fumigation at the Institute for Animal Health. *Epizone Workshop on Formaldehyde Replacement*, Lelystad, Netherlands.

- Koen, Q., & Frank, K. (2011). Vaporised hydrogen peroxide: A promising alternative for formaldehyde fumigation. *Epizone Workshop on Formaldehyde Replacement*, Lelystad, Netherlands.
- Krishnan, J., Berry, J., Fey, G., & Wagener, S. (2006a). Vaporized hydrogen peroxide-based biodecontamination of a high-containment laboratory under negative pressure. *Applied Biosafety: Journal of the American Biological Safety Association*, 11(2), 74-80.
- Krishnan, J., Fey, G., Berry, J., & Wagener, S. (2006b). Biodecontamination studies using vaporous hydrogen peroxide and gaseous chlorine dioxide. *ABSA Conference*, Boston, MA.
- Lai, M. Y., Cheng, P. K., & Lim, W. W. (2005). Survival of severe acute respiratory syndrome coronavirus. *Clinical Infectious Diseases*, 41(7), e67-71.
- Lomas, R. J., Cruse-Sawyer, J. E., Simpson, C., Ingham, E., Bojar, R., & Kearney, J. N. (2003). Assessment of the biological properties of human split skin allografts disinfected with peracetic acid and preserved in glycerol. *Burns*, 29(6), 515-525.
- Meszaros, J. E., Antloga, K., Justi, C., Plesnicher, C., & McDonnell, G. (2005). Area fumigation with hydrogen peroxide vapor. *Applied Biosafety: Journal of the American Biological Safety Association*, 10(2), 91-100.
- Nakata, S., Ikeda, T., Nakatani, H., Sakamoto, M., Higashidutsumi, M., Honda, T., et al. (2001). Evaluation of an automatic fogging disinfection unit. *Environmental Health and Prevention Medicine*, 6(3), 160-164.
- Orth, R. (1998). The importance of disinfection for the hygiene in the dairy and beverage production. *International Biodeterioration and Biodegradation*, 41, 201-208.
- Portner, D. M., & Hoffman, R. K. (1968). Sporicidal effect of peracetic acid vapor. *Journal of Applied Microbiology*, 16(11), 1782-1785.
- Pottage, T. (2011). Assessment of gaseous decontamination technologies for use on spacecraft. *ABSA Conference*, Anaheim, CA.
- Pottage, T., Richardson, C., Parks, S., Walker, J. T., & Bennett, A. M. (2010). Evaluation of hydrogen peroxide gaseous disinfection systems to decontaminate viruses. *Journal of Hospital Infection*, 74(1), 55-61.
- Pruss, A., Baumann, B., Seibold, M., Kao, M., Tintelnot, K., von Versen, R., et al. (2001). Validation of the sterilization procedure of allogeneic avital bone transplants using peracetic acid-ethanol. *Biologicals*, 29(2), 59-66.
- Pruss, A., Kao, M., Kieseletter, H., von Versen, R., & Pauli, G. (1999). Virus safety of avital bone tissue transplants: Evaluation of sterilization steps of spongiosa cuboids using a peracetic acid-methanol mixture. *Biologicals*, 27(3), 195-201.
- Rogers, J. V., & Choi, Y. W. (2008). Inactivation of *Francisella tularensis* Schu S4 in a biological safety cabinet using hydrogen peroxide fumigation. *Applied Biosafety: Journal of the American Biological Safety Association*, 13(1), 15-20.
- Sagripani, J. L., & Bonifacino, A. (1997). Effects of salt and serum on the sporicidal activity of liquid disinfectants. *Journal of AOAC International*, 80(6), 1198-1207.
- Schroder, W. (1984). Peracetic acid: Disinfectant for the food industry. *Nürnberg: Brauwelt International*, 1, 118-120.
- Springthorpe, V. S., & Sattar, S. A. (2005). Carrier tests to assess microbicidal activities of chemical disinfectants for use on medical devices and environmental surfaces. *Journal of AOAC International*, 88(1), 182-201.
- Stowell, J. D., Forlin-Passoni, D., Din, E., Radford, K., Brown, D., White, A., et al. (2012). Cytomegalovirus survival on common environmental surfaces: Opportunities for viral transmission. *Journal of Infectious Diseases*, 205(2), 211-214.
- U.S. Environmental Protection Agency (2010). *Compatibility of material and electronic equipment with chlorine dioxide fumigation*. Available at http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsr/&dirEntryId=219487. Accessed online 2012.
- Watling, D., Ryle, C., Parks, M., & Christopher, M. (2002). Theoretical analysis of the condensation of hydrogen peroxide gas and water vapour as used in surface decontamination. *PDA Journal of Pharmaceutical Science and Technology*, 56(6), 291-299.
- Xie, X., Li, Y., Zhang, T., & Fang, H. H. (2006). Bacterial survival in evaporating deposited droplets on a teflon-coated surface. *Applied Microbiology and Biotechnology*, 73(3), 703-712.

EPA Releases New Resource Directory on Safely Controlling and Preventing Mosquitoes

The EPA has reorganized its web site to provide information on protecting oneself from mosquitoes in response to recent increases in mosquito-borne diseases across the United States, especially the West Nile virus. Available at: www.epa.gov/mosquitocontrol