

vessels does not significantly change the system protocols or introduce any complications. Direct steam injection provides several benefits to the overall process and is equal in efficacy to the currently used method.

## Acknowledgments

The authors would like to thank staff members of the Real Property Safety and Security Division (RPSSD) of the Canadian Science Centre for Human and Animal Health, specifically Brian Adlem for his technical assistance.

## References

- Edwards, S., Lamb, B., & Maurer, D. (2002). Design and operation of a high-containment sewage treatment facility. In J. Y. Richmond (Ed.), *Anthology of biosafety V: BSL-4 laboratories*. Mundelein, IL: American Biological Safety Association (ABSA).
- Mani, P., & Langevin, P. (2006). *Veterinary containment facilities design and construction handbook*. International Veterinary Biosafety Working Group. [www.vetbiosafety.org/history.html](http://www.vetbiosafety.org/history.html)
- Palani, M., & Duthu, D. (2006). Steam effluent decontamination system. In J. Y. Richmond (Ed.), *Anthology of biosafety IX: Exploring the performance envelope for BSL-3 and BSL-4 laboratories*. Mundelein, IL: American Biological Safety Association (ABSA).
- Public Health Agency of Canada. (2004). *The laboratory biosafety guidelines* (3rd ed.). Ottawa, Ontario, Canada: Minister of Public Works and Government Services.
- 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, 182-201.
- Wittmeier, L., & Wagner, S. (2004). Treatment of solid and liquid biowaste and ISO registration of the process. In J. Y. Richmond (Ed.), *Anthology of biosafety VII: Biosafety level 3*. Mundelein, IL: American Biological Safety Association (ABSA).
- U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, & National Institutes of Health. (2007). In L. C. Chosewood & D. E. Wilson (Eds.), *Biosafety in microbiological and biomedical laboratories* (5th ed.). Washington, DC: U.S. Government Printing Office.

# A Novel Approach for Conducting Room-scale Vaporous Hydrogen Peroxide Decontamination of Virulent *Bacillus anthracis* Spores

William R. Richter, Morgan Q. S. Wendling, and James V. Rogers

Battelle Memorial Institute, Columbus, Ohio

## Abstract

*Studies have been conducted to determine the efficacy of various decontamination technologies against virulent B. anthracis and surrogate spores within small, bench-scale chambers. This study assessed an approach for evaluating room-scale (~2,700 ft<sup>3</sup>) decontamination efficacy of vaporous hydrogen peroxide fumigation against B. anthracis Ames and B. subtilis spores deposited onto porous and non-porous indoor surface materials. Approximately 1x10<sup>8</sup> colony-forming units (CFU) of B. anthracis and B. subtilis spores were dried onto galvanized metal and ceiling tile coupons and then exposed to vaporous hydrogen peroxide. The materials contaminated with B. anthracis spores were placed inside a Class III biosafety cabinet (BSC III) that circulated vaporous hydrogen peroxide from within the decontaminated room, into and out of the BSC III. Identical materials inoculated in the same manner and at the same density with B. subtilis were placed both inside and outside of the BSC III to compare decontamination efficacy. Three fumigations were conducted using two sets of cycle parameters. The first set of cycle*

*parameters for vaporous hydrogen peroxide exposure (10 minutes of conditioning at 12 g/min; 75 minutes of decontamination at 11 g/min) yielded log reductions in viable B. anthracis and B. subtilis spores ranging from 6.1 to 7.0 on all materials, while only 76% of the commercial biological indicators (1x10<sup>6</sup> CFU) evaluated in parallel were completely inactivated. The second set of cycle parameters (12 minutes of conditioning at 12 g/min; 104 minutes of decontamination at 8 g/min) yielded log reductions in viable B. anthracis and B. subtilis spores ranging from 6.7 to 7.4 on all materials and complete inactivation of biological indicators. These results demonstrate this method as a viable approach to assess room-scale fumigant decontamination efficacy against B. anthracis Ames spores.*

## Introduction

Since the intentional release of *Bacillus anthracis* spores in 2001 in the United States, studies have been conducted to determine the efficacy of various decontamination technologies against *B. anthracis* spores, which have included liquids, gels, and fumigants. Of

these, fumigants (gases and vapors) are the preferred method for decontaminating large areas due to advantages in distribution and the ability to interact with large, complex surfaces. An industry standard for years has been formaldehyde fumigation. This decontamination approach is efficacious against *B. anthracis* spores on porous and non-porous materials (Rogers et al., 2007); however, when the formaldehyde is neutralized, it leaves behind a residue (hexamethylene tetramine) that requires additional clean-up efforts. Vaporous hydrogen peroxide, which was used to remediate the Hart Senate Suite in 2001, offers a cleaner, less toxic alternative to formaldehyde and is an accepted method for decontamination in the scientific, medical, and pharmaceutical communities (Anderson et al., 2006; Boyce et al., 2008; Dryden et al., 2008; Fichet et al., 2004; French et al., 2004; Heckert et al., 1997; Hillman, 2004; Johnston et al., 2005; Klapes & Vesley, 1990; Krause et al., 2001; Krishna et al., 2000; Otter & Budde-Niekie, 2009; Otter & French, 2009; Otter et al., 2009; Rastogi et al., 2009; Rogers et al., 2009; Verce et al., 2008; Wagenaar & Snijders, 2004). These studies using vaporous hydrogen peroxide provide efficacy data over a wide range of operational and environmental conditions. However, there is a need to test and challenge the efficacy of fumigant decontamination technologies in larger room-scale environments against various biological agents since most studies conducted to date have been on smaller-scale environments.

Laboratory-controlled decontamination testing of vaporous hydrogen peroxide at room-scale has been conducted using surrogates for *B. anthracis* spores or other biological select agents (Hall et al., 2007; Kahnert et al., 2005; Otter & French, 2009). Using a 317 L chamber, vaporous hydrogen peroxide was shown in previous studies to be efficacious against *B. anthracis*, *B. subtilis*, and *G. stearothermophilus* spores in which statistical differences were observed between *B. anthracis* and surrogate spores on porous and non-porous materials (Rogers et al., 2005). The efficacy of vaporous hydrogen peroxide has also been demonstrated against two virulent strains and one avirulent strain of *B. anthracis* within a 15,000 L chamber (Rogers et al., 2009). However, potential challenges are associated with switching from a bench-scale approach to a room scale, such as controlling environmental conditions, material compatibility, and achieving sufficient fumigant concentrations necessary to inactivate microorganisms. Such complexities could potentially confound the utilization of efficacy data derived from bench-scale tests for making informed decisions regarding the decontamination of rooms or buildings. This prompts a growing need to test and generate more efficacy data using fumigant decontamination technologies in large room-scale environments. Therefore, the purpose of this study was to demonstrate a safe approach to conducting controlled room-scale fumigant decontamination studies to assess the efficacy

of vaporous hydrogen peroxide against *B. anthracis* Ames spores.

## Materials and Methods

### Test Organisms

All phases of testing were performed under biosafety level 3 (BSL-3) conditions. Virulent *B. anthracis* Ames spores were prepared in a fermentor as previously described (Rogers et al., 2005). The *B. subtilis* (ATCC 19659) spores were prepared using a shaker flask method as previously described (Rogers et al., 2006). The purified spore preparations were resuspended in sterile water and evaluated by phase-contrast microscopy. Preparations having >95% refractile spores with <5% cellular debris were enumerated, diluted to approximately  $1 \times 10^9$  CFU/ml and stored at 2°-8°C.

Stainless steel biological indicators (BI) (Raven Labs, Omaha, NE) inoculated with  $\sim 1 \times 10^6$  *B. atrophaeus* spores packaged in Tyvek were used to qualify the fumigant distribution and efficacy of the decontamination cycle parameters.

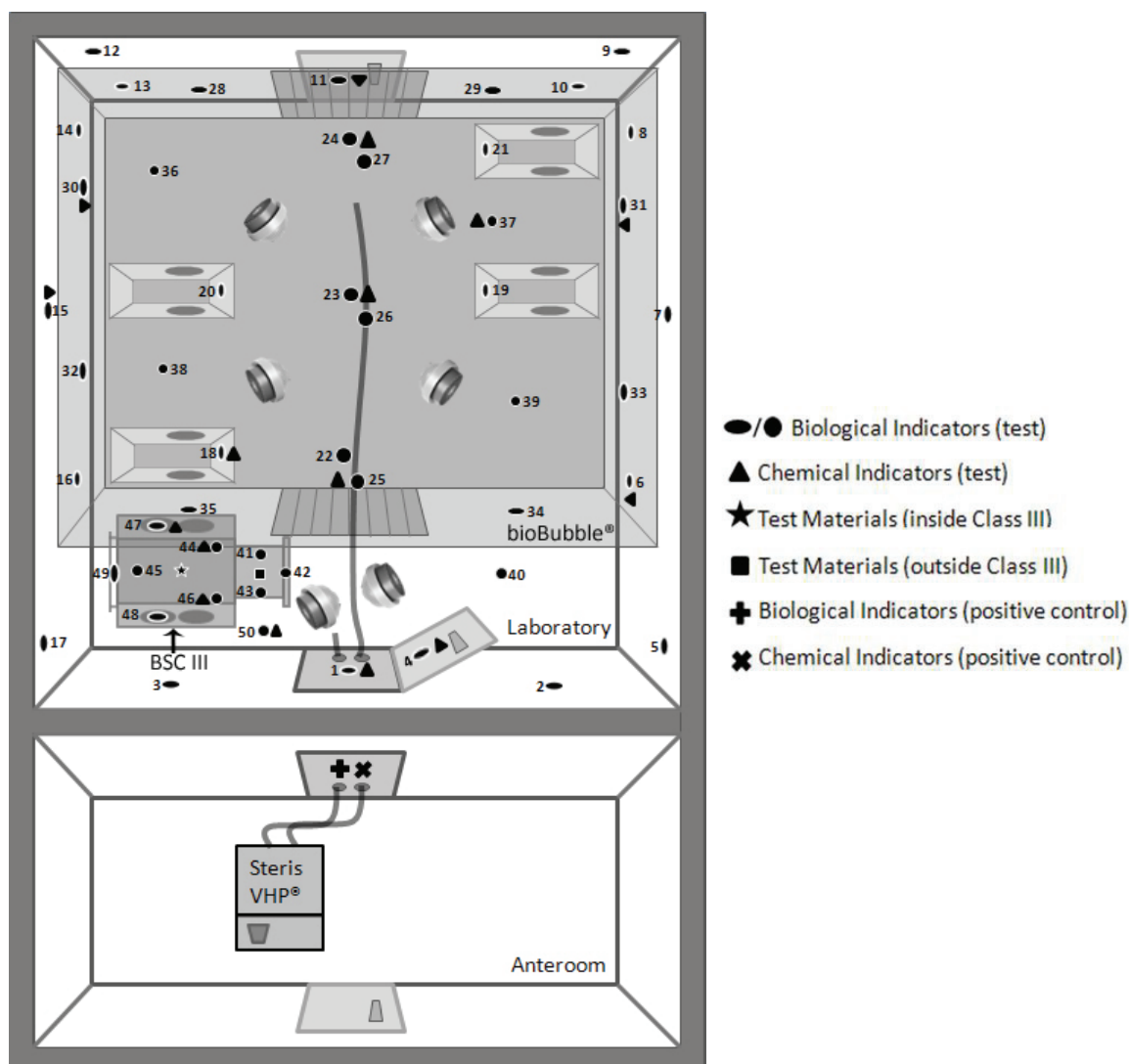
### Test Materials and Chamber

Galvanized metal and ceiling tile test materials (1.9 cm x 7.5 cm) were selected to represent non-porous and porous surfaces, respectively. Each test material was inspected for defects and appearance before and after decontamination to aid in determining material compatibility with the fumigant. Additionally, 16 hydrogen peroxide chemical indicator (CI) strips NB305 (STERIS Corporation, Mentor, OH) were located throughout the room, including three located within the BSC III (Figure 1). These indicators were used to qualify a decontamination cycle within the enclosure by indicating exposure to hydrogen peroxide.

The experiments were conducted in a 2,727 ft<sup>3</sup> (77.2 m<sup>3</sup>) BSL-3 laboratory. The room contained a 1,680 ft<sup>3</sup> (47.6 m<sup>3</sup>) bioBubble® (The Colorado Clean Room Company, Ft. Collins, CO) possessing four HEPA-filtered blower units that maintained negative pressure with approximately 100 air exchanges per hour. The bioBubble® is a soft-walled enclosure constructed of 16 mil clear vinyl walls stretched over a 3.18 cm tubular aluminum frame. The vinyl walls connect using hook and loop seams. A 27 ft<sup>3</sup> Class III biosafety cabinet (BSC III) (The Baker Company, Sanford, MA) was placed within the room and outside the bioBubble® which circulates vaporous hydrogen peroxide from the laboratory air into and out of the enclosure through HEPA filters at the rate of 100 cubic feet per minute (CFM). In addition to the four bioBubble® blowers, six circulation fans were placed throughout the room to aid in distribution of the vapor (Figure 1). Injection and return ports to the laboratory were not available; therefore, a Plexiglas "false door" was constructed to cover the doorway opening that was modified with ports to accommodate the inlet and return

**Figure 1**

Test material, biological and chemical indicator placement.



lines to the Steris VHP® 1000 (Figure 1).

Test materials (N=3 per material) were inoculated with 100  $\mu\text{L}$  of a  $1 \times 10^9$  CFU/ml suspension of *B. anthracis* or *B. subtilis*, resulting in  $\sim 1 \times 10^8$  CFU/test material. This was accomplished using a multichannel pipette that dispensed two rows of five droplets (10  $\mu\text{L}$  per droplet). These materials were allowed to dry within a BSC III for approximately 8 hours before initiating the decontamination cycle. Prior to decontamination, three test materials of each type were inoculated with *B. anthracis* and *B. subtilis* and corresponding un-inoculated blanks (N=1) were transferred into the test chamber BSC III. Three additional galvanized metal coupons inoculated with *B. subtilis* (and one blank) were placed adjacent to and outside the BSC III to compare a potential difference in decontamination efficacy between the laboratory room and the BSC III. A total of 50 BI were placed within the laboratory of which three were placed inside the BSC III (Figure 1). In parallel, three control coupons

(inoculated, not decontaminated) and one blank (not inoculated, not decontaminated) of each material type and organism were transferred into a Plaslabs model 830-ABC compact glove box control chamber (Plaslabs Inc., Lansing, MI) that was maintained at ambient temperature and relative humidity for the duration of the decontamination cycle.

### Decontamination Procedure

A Steris® series 1000 VHP® generating unit, which uses 35% hydrogen peroxide (Vaprox®; STERIS® Corporation) to generate the vapor within the target enclosure, was used to decontaminate the laboratory. This generator performs and controls four phases during the sterilization cycle: dehumidification, conditioning, decontamination, and aeration. During the dehumidification phase, the unit removes moisture from the enclosure to a defined set point prior to the injection of 35% hydrogen peroxide. The conditioning phase promotes a rapid in-

crease in hydrogen peroxide levels within the enclosure over a relatively short amount of time (e.g., 10 minutes). The decontamination phase has a reduced injection rate that maintains the vaporous hydrogen peroxide concentration (achieved during conditioning) over an extended period of time (e.g., 75 minutes). Aeration, in which the vaporous hydrogen peroxide is circulated through HEPA filters and a catalytic converter for neutralization, is initiated once the programmed decontamination time has concluded.

Three fumigations were conducted using two separate sets of cycle parameters (Table 1). The first set of cycle parameters was established by following the Cycle Development Guide for VHP® Biodecontamination System Products (STERIS Corporation). Cycle parameters for subsequent runs were adjusted based on data obtained from the first set of cycle parameters. Prior to the initiation of the decontamination cycle, the HVAC to the room was shut down to prevent the vaporous hydrogen peroxide from exhausting from the laboratory. Each run was initiated at the end of the workday and cycle parameters run in order as outlined in Table 1 and allowed to run overnight. The following morning the HVAC was reopened and the room was allowed to conclude the aeration process to below 1 ppm hydrogen peroxide. These concentrations were measured using a low level Dräger monitor (Dräger Safety, Inc., Pittsburgh, PA). The temperature and relative humidity (RH) were monitored using a HOBO data logger (Onset Computer Corporation, Pocasset, MA).

### Sample Processing

Following the aeration phase of the decontamination cycle, the test coupons, biological indicators, and chemical indicators were removed from the laboratory. The galvanized metal and ceiling tile test coupons were placed in 50 ml conical tubes containing 10 ml of sterile phosphate buffered saline (Sigma, St. Louis, MO) containing 0.1% Triton X-100 (Sigma). The spores were extracted via agitation at 200 rpm on an orbital shaker for 15 minutes at room temperature. Following extraction, 1.0 ml of

each extract was removed and tenfold serial dilutions were prepared in sterile water. A quantitative assessment of each sample was determined by dilution plating where 100 µl of the undiluted extract and each serial dilution were plated onto tryptic soy agar (TSA) (Hardy Diagnostics, Santa Maria, CA) plates in triplicate, allowed to dry, and incubated for 18-24 hours at 37°C. Following incubation, the plates were enumerated and CFU/ml was determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Data were then expressed as the mean ± SD of the total CFU.

All biological indicators were aseptically transferred into 15 ml tubes containing 10 ml of tryptic soy broth (Remel, Lenexa, KS), gently agitated, and incubated at 37°C. Samples were assessed for growth (turbid culture) or no growth (clear culture) at 1 day and 7 days. Chemical indicator strips were visually assessed for a color change, indicating the presence of hydrogen peroxide.

## Results

Three decontamination runs were completed with vaporous hydrogen peroxide in which the decontamination phases included 85 minutes (run 1) or 116 minutes (runs 2 and 3) delivering approximately 945 g and 976 g of hydrogen peroxide, respectively. The total cycle time for each replicate run, including dehumidification, conditioning, decontamination, and aeration phases, totaled approximately 17 hours. From the initiation of the run, temperature and RH levels increased from 18.6°C and 38.5% RH to 33.9°C and 95.8% RH at the end of the decontamination phase. This increase in temperature may have resulted from the approximate 60°C delivery temperature of the vaporous hydrogen peroxide into the room in which the environmental controls for air flow and conditioning had been turned off. Temperature and relative humidity levels returned to normal conditions (~20°C and ~42% RH) following the aeration phase. Figure 2 provides a representative graph of temperature

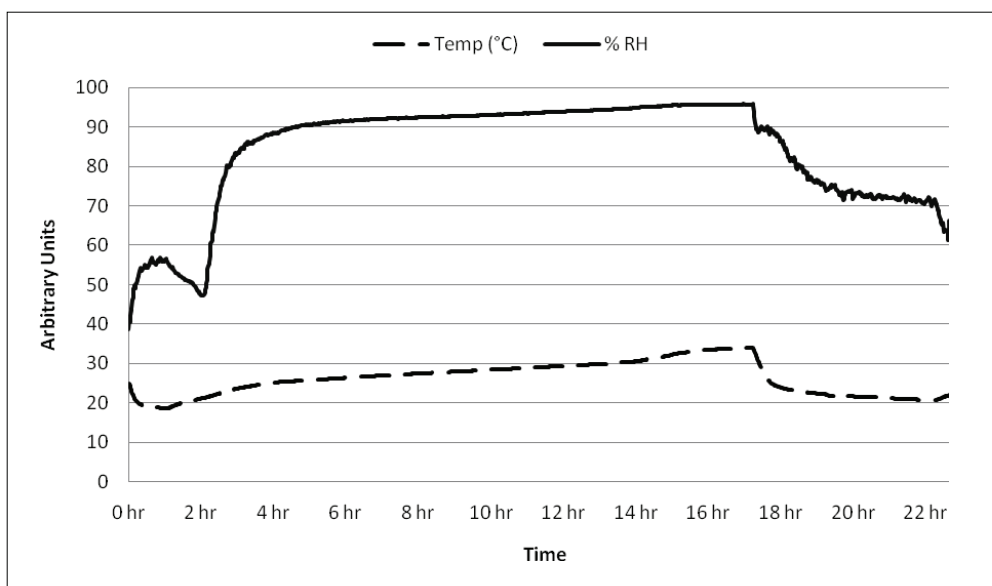
**Table 1**  
Vaporous Hydrogen Peroxide Decontamination Parameters

	Dehumidification			Conditioning			Decontamination			Aeration
	Flow Rate (CFM <sup>a</sup> )	H <sub>2</sub> O <sub>2</sub> mg/L (%RH)	Duration (min)	Flow Rate (CFM)	Injection Rate (g/min)	Duration (min)	Flow Rate (CFM)	Injection Rate (g/min)	Duration (min)	Flow Rate (CFM)
<b>Test Run #1</b>	20	6.9 (30)	15	20	12	10	20	11	75	20
<b>Test Run #2</b>	20	6.9 (30)	15	20	12	12	20	8	104	20
<b>Test Run #3</b>	20	6.9 (30)	15	20	12	12	20	8	104	20

<sup>a</sup>CFM = cubic feet per minute

**Figure 2**

Laboratory representative temperature and relative humidity.



and relative humidity within the laboratory for the duration of each experimental run.

For all three decontamination runs, visual inspection of the CIs revealed that all 16 were positive for color change, indicating exposure to the vaporous hydrogen peroxide. The unexposed control CIs located in the ante-room exhibited no color change. For test 1, 12 out of 50 (24%) BIs were positive for growth at 1 day and 7 days (Table 2). Replicate tests 2 and 3 resulted in 0 out of 50 BIs exhibiting growth at 1 day and 7 days (Table 2). For all three decontamination runs, all positive control BIs exhibited growth; the negative control tubes exhibited no bacterial growth at 1 day and 7 days (Table 2). A sample of each positive test carrier was plated onto TSA to confirm the identity and homogeneity of the test organism.

Test materials contaminated with *B. anthracis* Ames and exposed to vaporous hydrogen peroxide resulted in complete inactivation and log reductions ranging from 6.7 to 7.0 on ceiling tile and 7.0 to 7.1 on galvanized metal (Tables 3 and 4). Test materials contaminated with *B. subtilis* and exposed to vaporous hydrogen peroxide exhibited log reductions ranging from 6.1 to 7.4 on galvanized metal (inside BSC III), 6.6 to 7.4 on galvanized metal (outside BSC III), and 6.8 on ceiling tile (Tables 3 and 4). Viable test organisms were not recovered from any of the blank (uninoculated) test materials. Upon visual inspection, no physical damage was observed for the hydrogen peroxide-exposed coupons.

## Discussion

This study provides a new approach for room-scale decontamination of biological select agents using vaporous or gaseous decontaminants. The inactivation data

for *B. anthracis* Ames spores presented here resulted from an iterative process of establishing vaporous hydrogen peroxide decontamination cycle parameters for an approximately 2,700 ft<sup>3</sup> laboratory. The method outlined in this study provides a safe and controlled approach to test whether or not established decontamination parameters are effective for inactivating biological agents on different types of material surfaces. Previously, room-scale or larger decontamination studies have targeted non-select agents, such as methicillin-resistant *Staphylococcus aureus*, *Acinetobacter* spp., *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, *Clostridium difficile*, bacteriophages, and fungi (Andersen et al., 2006; Boyce et al., 2008; Dryden et al., 2008; French et al., 2004; Grare et al., 2008; Hall et al., 2007; Hall et al., 2008; Kahnert et al., 2005; Luftman et al., 2006; Otter & Budde-Niekietl, 2009; Otter & French, 2009; Otter et al., 2009). In response to the 2001 anthrax attacks, the inactivation of *B. anthracis* spores by fumigants was performed by decontaminating spaces having volumes ranging from approximately 8,300 ft<sup>3</sup> to 14 million ft<sup>3</sup> (Canter et al., 2005). However, most of the laboratory testing of fumigant decontaminants against biological select agents (e.g., *B. anthracis*, *Francisella tularensis*, *Yersinia pestis*) has been conducted using bench-scale chambers ranging from approximately 300 L to 1,275 L (Rogers et al., 2005; Rogers et al., 2007; Rogers & Choi, 2008; Rogers et al., 2008).

In this study, the efficacy data from decontamination run 1 using the first set of cycle parameters (Table 1) show inactivation of both *B. anthracis* Ames and *B. subtilis* on all test materials inside the BSC III as well as *B. subtilis* on galvanized metal in the room (outside the BSC III) to a level of  $\geq 6.1$  logs. All of the CIs within

**Table 2**  
Biological and Chemical Indicator Results

	<b>Biological Indicators (No. positive/No. tested)</b>				<b>Chemical Indicators (No. positive/No. tested)</b>	
	Day 1	Day 7	Negative Control	Positive Control	Vaporous Hydrogen Peroxide Exposed	Negative Control
<b>Test Run #1</b>	12/50	12/50	0/3	1/1	16/16	0/1
<b>Test Run #2</b>	0/50	0/50	0/3	1/1	16/16	0/1
<b>Test Run #3</b>	0/50	0/50	0/3	1/1	16/16	0/1

**Table 3**  
Vaporous Hydrogen Peroxide Decontamination of *B. anthracis* and *B. subtilis* on Porous and Non-porous Surfaces Test Run 1

<b>Treatment</b>	<b>Organism</b>	<b>Mean Total Recovered Spores <math>\pm</math>SD (N=3)</b>	<b>Mean Log Reduction</b>
Ceiling Tile Control	<i>B. anthracis</i>	1.07 $\pm$ 0.22 x 10 <sup>7</sup>	-
Galvanized Metal Control	<i>B. anthracis</i>	9.78 $\pm$ 0.33 x 10 <sup>6</sup>	-
Ceiling Tile Decon	<i>B. anthracis</i>	0	$\geq$ 7.03
Galvanized Metal Decon	<i>B. anthracis</i>	0	$\geq$ 6.99
Ceiling Tile Control	<i>B. subtilis</i>	6.24 $\pm$ 3.57 x 10 <sup>6</sup>	-
Galvanized Metal Control	<i>B. subtilis</i>	4.04 $\pm$ 0.32 x 10 <sup>6</sup>	-
Ceiling Tile Decon	<i>B. subtilis</i>	0	$\geq$ 6.80
Galvanized Metal Decon	<i>B. subtilis</i>	0	$\geq$ 6.10
Galvanized Metal Decon (outside BSC III)	<i>B. subtilis</i>	0	$\geq$ 6.61

**Table 4**  
Vaporous Hydrogen Peroxide Decontamination of *B. anthracis* and *B. subtilis* on Porous and Non-porous Surfaces Test Runs 2 and 3

<b>Treatment</b>	<b>Organism</b>	<b>Mean Total Recovered Spores <math>\pm</math>SD (N=6)</b>	<b>Mean Log Reduction</b>
Ceiling Tile Control	<i>B. anthracis</i>	6.51 $\pm$ 3.93 x 10 <sup>6</sup>	-
Galvanized Metal Control	<i>B. anthracis</i>	1.75 $\pm$ 1.36 x 10 <sup>7</sup>	-
Ceiling Tile Decon	<i>B. anthracis</i>	0	$\geq$ 6.74
Galvanized Metal Decon	<i>B. anthracis</i>	0	$\geq$ 7.10
Ceiling Tile Control	<i>B. subtilis</i>	5.68 $\pm$ 1.21 x 10 <sup>6</sup>	-
Galvanized Metal Control	<i>B. subtilis</i>	2.95 $\pm$ 1.75 x 10 <sup>7</sup>	-
Ceiling Tile Decon	<i>B. subtilis</i>	0	$\geq$ 6.75
Galvanized Metal Decon	<i>B. subtilis</i>	0	$\geq$ 7.39
Galvanized Metal Decon (outside BSC III)	<i>B. subtilis</i>	0	$\geq$ 7.39

the BSC III changed color, indicating positive exposure to vaporous hydrogen peroxide. These results suggest: (1) the vaporous hydrogen peroxide introduced into the room is being circulated from the room into the BSC III as intended; and (2) the relative concentration of vaporous hydrogen peroxide in the room and BSC III yielded comparable levels of spore inactivation. However, for this first run complete inactivation of all BIs (Table 2) in which the BIs exhibiting positive growth were located at positions within the room that were expected to possess reduced or minimal air circulation (see locations in Figure 1), such as on the wall behind the laboratory door (BIs #2, 5), in between the bioBubble® and the wall/ceiling (BIs 15, 26), on the inside wall of the bioBubble® (BIs #28, 31, 34, 35), inside a bioBubble® exhaust flue (BI #18), and in and around the BSC III (BIs #44, 46, 49), was not achieved. Since most of the BIs yielding positive growth were in close proximity to the bioBubble®, possibly an additional factor contributing to this lack of BI inactivation could be due to absorption of vaporous hydrogen peroxide by the large plastic covering of the bioBubble®. This could potentially lead to a decrease in the local and total room concentration of vaporous hydrogen peroxide, thus affecting decontamination efficacy. When switching from laboratory-scale to room-scale testing, factors that could impact the final efficacy results must be considered. These can include controlling temperature, relative humidity, ability to seal the room, achieving/maintaining target decontaminant concentration, and the complexity of the structural materials. Material compatibility issues need to be considered as vaporous/gaseous decontaminants can partition into or react with different materials within the containment space, thereby reducing the available decontaminant concentration and ultimately affecting microbial inactivation. Such partitioning or potential reaction with materials by vaporous hydrogen peroxide has been described previously for Plexiglas® (Baron et al., 2007) and metal ductwork (Verge et al., 2008). Therefore, based on the findings from decontamination run 1, adjustments to the cycle parameters were made to extend the initial injection time of hydrogen peroxide from 10 minutes to 12 minutes, lower the decontamination injection rate from 11 g/min to 8 g/min, and extend the overall contact time (conditioning plus decontamination) from 85 minutes to 116 minutes. The changed parameters were confined to the limits of the VHP® 1000 capacity of approximately 1,000 g of total hydrogen peroxide delivery. The subsequent two runs implementing these adjustments resulted in the inactivation of spores inoculated on test materials and complete inactivation of all BIs throughout the room and inside the BSC III, indicating the second set of decontamination parameters were the most efficacious.

Fumigation technologies often rely on theoretical

calculations for determining the final decontaminant concentration within the targeted fumigation space without directly measuring the decontaminant to verify or confirm these values. This can be problematic when conducting decontamination runs in large spaces filled with different material types (e.g., plastics), spaces that cannot be completely sealed (e.g., HVAC exhaust), or chemicals that break down in the presence of various environmental conditions (e.g., chlorine dioxide degradation in light). The VHP® 1000 used in this study does not monitor vaporous hydrogen peroxide concentrations in real time; this decontamination system injects a known quantity of hydrogen peroxide over a specified length of time. The ability to monitor and maintain fumigant concentrations throughout a decontamination cycle seems to be a critical component to achieving consistent biological inactivation. This is especially important when potential material compatibility issues reduce actual concentrations compared to theoretical calculations. One important factor in implementing the decontamination approach, described in this study using a recirculating BSC III inside a laboratory undergoing fumigation, is determining whether the decontaminant concentration in the BSC III is similar to or equivalent to that of the fumigated laboratory. Therefore, further investigation applying the approach described in this paper could utilize real-time hydrogen peroxide sensors both inside the laboratory and BSC III that would allow for a direct comparison of hydrogen peroxide concentrations, enabling a better understanding of the decontamination profile and chemical stability throughout the cycle.

This method will provide end-users, technology developers, and regulatory agencies the opportunity to investigate the efficacy of fumigant decontamination technologies against various select agents while challenging them with all the complexities of room- or building-scale decontamination. Previous bench-scale decontamination studies have established benchmarks for efficacy of decontamination for many fumigation technologies; however, more studies are needed to generate larger-scale decontamination efficacy data. Building size, material complexity, as well as other factors, may directly impact the technology selected or the manner in which that technology is implemented to successfully conduct the decontamination. Confidence in the ability of fumigants to inactivate spores and other persistent threat agents requires the establishment of efficacy data for these targeted select agents in room- and building-scale environments.

## Acknowledgments

This work was funded by Battelle's Biomedical Research Center, West Jefferson, OH. We thank Jennifer Price for her review of this manuscript.

## References

- Andersen, B. M., Rasch, M., Hochlin, K., Jensen, F. H., Wismar, P., & Fredriksen, J. E. (2006). Decontamination of rooms, medical equipment and ambulances using an aerosol of hydrogen peroxide disinfectant. *Journal of Hospital Infection*, 62, 149-155.
- Baron, P. A., Estill, C. F., Beard, J. K., Hein, M. J., & Larsen, L. (2007). Bacterial endospore inactivation caused by outgassing of vaporous hydrogen peroxide from polymethyl methacrylate (Plexiglas®). *Letters in Applied Microbiology*, 45, 485-490.
- Boyce, J. M., Havill, N. L., Otter, J. A., McDonald, L. C., Adams, N. M., Cooper, T., et al. (2008). Impact of hydrogen peroxide vapor room decontamination on *Clostridium difficile* environmental contamination and transmission in a healthcare setting. *Infection Control and Hospital Epidemiology*, 29, 723-729.
- Canter, D. A., Gunning, D., Rodgers, P., O'Connor, L., Traunero, C., & Kempter, C. J. (2005). Remediation of *Bacillus anthracis* contamination in the U.S. Department of Justice mail facility. *Biosecurity and Bioterrorism*, 3, 119-127.
- Dryden, M., Parnaby, R., Dailly, S., Davis-Blues, K., Otter, J. A., & Kearns, A. M. (2008). Hydrogen peroxide vapour decontamination in the control of a polyclonal methicillin-resistant *Staphylococcus aureus* outbreak on a surgical ward. *Journal of Hospital Infection*, 68, 190-192.
- Fichet, G., Comoy, E., Duval, C., Antloga, K., Dehen, C., Charbonnier, A., et al. (2004). Novel methods for disinfection of prion-contaminated medical devices. *Lancet*, 364, 521-526.
- French, G. L., Otter, J. A., Shannon, K. P., Adams, N. M. T., Watling, D., & Parks, M. J. (2004). Tackling contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus* (MRSA): A comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination. *Journal of Hospital Infection*, 57, 31-37.
- Grare, M., Dailloux, M., Simon, L., Dimajo, P., & Laurain, C. (2008). Efficacy of dry mist of hydrogen peroxide (DMHP) against *Mycobacterium tuberculosis* and use of DMHP for routine decontamination of biosafety level 3 laboratories. *Journal of Clinical Microbiology*, 46, 2955-2958.
- Hall, L., Otter, J. A., Chewins, J., & Wengenack, N. L. (2007). Use of hydrogen peroxide vapor for deactivation of *Mycobacterium tuberculosis* in a biological safety cabinet and a room. *Journal of Clinical Microbiology*, 45, 810-815.
- Hall, L., Otter, J. A., Chewins, J., & Wengenack, N. L. (2008). Deactivation of the dimorphic fungi *Histoplasma capsulatum*, *Blastomyces dermatitidis* and *Coccidioides immitis* using hydrogen peroxide vapor. *Medical Mycology*, 46, 189-191.
- Heckert, R. A., Best, M., Jordan, L. T., Dulac, G. C., Eddington, D. L., & Sterritt, W. G. (1997). Efficacy of vaporized hydrogen peroxide against exotic animal viruses. *Applied and Environmental Microbiology*, 63, 3916-3918.
- Hillman, D. (2004). Vapor phase hydrogen peroxide gas decontamination of a BSC. *Performance Review*, 10, 10-11.
- Johnston, M. D., Lawson, S., & Otter, J. A. (2005). Evaluation of hydrogen peroxide vapour as a method for the decontamination of surfaces contaminated with *Clostridium botulinum* spores. *Journal of Microbiological Methods*, 60, 403-411.
- Kahnert, A., Seiler, P., Stein, M., Aze, B., McDonnell, G., & Kaufmann, S. H. E. (2005). Decontamination with vaporized hydrogen peroxide is effective against *Mycobacterium tuberculosis*. *Letters in Applied Microbiology*, 40, 448-452.
- Klapes, N. A., & Vesley, D. (1990). Vapor-phase hydrogen peroxide as a surface decontaminant and sterilant. *Applied and Environmental Microbiology*, 56, 503-506.
- Krause, J., McDonnell, G., & Riedesel, H. (2001). Bio-decontamination of animal rooms and heat-sensitive equipment with vaporized hydrogen peroxide. *Contemporary Topics in Laboratory Animal Science*, 40, 18-21.
- Krishna, A. K., Lodhi, S. A., & Harris, M. R. (2000). Isolation technology for research and development applications: From concept to production. *Pharmaceutical Development and Technology*, 5, 507-520.
- Luftman, H. S., Regits, M. A., Lorcheim, P., Czarneski, M. A., Boyle, T., Aceto, H., et al. (2006). Chlorine dioxide gas decontamination of large animal hospital intensive and neonatal care units. *Applied Biosafety: Journal of the American Biological Safety Association*, 11(3), 144-154.
- Otter, J. A., & Budde-Niekkel, A. (2009). Hydrogen peroxide vapor: A novel method for the environmental control of lactococcal bacteriophages. *Journal of Food Protection*, 72, 412-414.
- Otter, J. A., & French, G. L. (2009). Survival of nosocomial bacteria and spores on surfaces and inactivation by hydrogen peroxide vapor. *Journal of Clinical Microbiology*, 47, 205-207.
- Otter, J. A., Puchowicz, M., Ryan, D., Salkeld, J. A., Cooper, T. A., Havill, N. L., et al. (2009). Feasibility of routinely using hydrogen peroxide vapor to decontaminate rooms in a busy United States hospital. *Infection Control and Hospital Epidemiology*, 30, 574-577.
- Rastogi, V. K., Wallace, L., Smith, L. S., Ryan, S. P., & Martin, B. (2009). Sporocidal decontamination of building surfaces by gaseous fumigants—I. Quantitative test method and issues addressed related to laboratory-scale studies. *Applied and Environmental Microbiology*, 75, 3688-3694.
- 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.
- Rogers, J. V., Choi, Y. W., Richter, W. R., Rudnicki, D. C., Joseph, D. W., Sabourin, C. L. K., et al. (2007). Formaldehyde gas inactivation of *Bacillus anthracis*, *Bacillus subtilis*, and *Geobacillus stearothermophilus* spores on indoor surface materials. *Journal of Applied Microbiology*, 103, 1104-1112.
- Rogers, J. V., Ducatte, G. R., Choi, Y. W., & Early, P. C. (2006). A preliminary assessment of *Bacillus anthracis* spore inactivation using an electrochemically-activated solution (ECASOL™). *Letters in Applied Microbiology*, 43, 482-488.
- Rogers, J. V., Richter, W. R., Shaw, M. Q., & Choi, Y. W. (2008). Vapour-phase hydrogen peroxide inactivates *Yersinia pestis* dried on polymers, steel, and glass surfaces. *Letters in Applied Microbiology*, 47, 279-285.
- Rogers, J. V., Richter, W. R., Shaw, M. Q., & Shesky, A. M. (2009). Large-scale inactivation of *Bacillus anthracis* Ames, Vollum, and Sterne spores using vaporous hydrogen peroxide. *Applied Biosafety: Journal of the American Biological Safety Association*, 14(3), 127-134.
- Rogers, J. V., Sabourin, C. L. K., Choi, Y. W., Richter, W. R., Rudnicki, D. C., Riggs, K. B., Taylor, M. L., & Chang, J. (2005). Decontamination assessment of *Bacillus anthracis*, *Bacillus subtilis*, and *Geobacillus stearothermophilus* spores on indoor surfaces using a hydrogen peroxide gas generator. *Journal of Applied Microbiology*, 99, 739-748.
- Verce, M. F., Jayaraman, B., Ford, T. D., Fisher, S. E., Gadgil, A. J., & Carlsen, T. M. (2008). Minimizing decomposition of vaporized hydrogen peroxide for biological decontamination of galvanized steel ducting. *Environmental Science and Technology*, 42, 5765-5771.
- Wagenaar, C. L., & Snijders, J. M. A. (2004). Decontamination of broilers with hydrogen peroxide stabilized with glycerol during processing. *International Journal of Food Microbiology*, 91, 205-208.