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Vaporized Hydrogen Peroxide-based Biodecontamination of a High-Containment Laboratory Under Negative Pressure

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Abstract

The authors evaluated vaporized hydrogen peroxide as an alternative to formaldehyde for space biodecontamination in a containment level 3 laboratory suite. The laboratory air pressure during the biodecontamination process was maintained at a slightly negative pressure. This was done as a preventive measure to ensure that hazardous vaporized hydrogen peroxide would not escape during the process. Parameters such as temperature, relative humidity, vaporized hydrogen peroxide concentration, and pressure within the laboratory suite were monitored during the biodecontamination. The success of the decontamination process was validated using spores of G. stearothermophilus, the most resistant microorganism to vaporized hydrogen peroxide (Kokubo et al., 1998; Meszaros, 2005; Rickloff & Orelski, 1989). This research demonstrates the usefulness of vaporized hydrogen peroxide as a space biodecontaminant.

Introduction

The Canadian Science Centre for Human and Animal Health houses 16 animal cubicles, five containment level 3 (CL3) suites, and seven CL4 suites, each containing individual laboratories. Since its opening in 1997, formaldehyde gas has been used to decontaminate the high-containment laboratories, animal cubicles, and biosafety cabinets. This process has often been slow, disruptive, and difficult to standardize (Krause et al., 2001; Spiner & Hoffmann, 1971). Additionally, formaldehyde gas upon neutralization polymerizes to paraformaldehyde and settles on the surfaces, warranting thorough postdecontamination clean-up. Porous materials such as wood, paper, and clothing absorb, retain, and release formaldehyde gas over time (Braswell et al., 1970). In addition to being a health hazard (Cogliano et al., 2004; *Lancet*, 1983; Rutala, 1990, 1996), a mixture of formaldehyde gas or paraformaldehyde dust in air has the potential to explode (WHO, 1994a; WHO, 1994b). Therefore, the authors have been exploring safer and automated alternative technologies for space decontamination.

Vaporized hydrogen peroxide (VHP)-based biodecontamination technology was developed in the 1980s and commercialized in the early 1990s (Graham & Rickloff, 1992; Heckert et al., 1997a; Rickloff & Graham, 1989). This technology has since been gaining popularity, now used for the decontamination of clean rooms, animal rooms, ambulances, large volume filling rooms, and hospital wards contaminated with antibiotic-resistant bacteria (French et al., 2004; Jahnke & Gerhard, 1997; Krause et al., 2001; Malmborg, 2001; Mitchell, 2005). VHP is known to be a powerful oxidizer and it inactivates viruses, fungi, bacteria, bacterial spores, nematode eggs, and even prions (Fichet et al., 2004; Heckert et al., 1997b; Kokubo et al., 1998; Krause & Riedesel, 2004; Meszaros, 2005). The VHP process is rapid, dry, mobile, compatible with electronics, and effective at low concentrations and temperatures. Unlike formaldehyde, VHP produces nontoxic by-products (water and oxygen) and, therefore, is ecologically safer and requires no postprocess neutralization and cleaning. However, a VHP concentration of over 75ppm is considered an immediate risk to human health; the accepted personal exposure level is under 1ppm (American Industrial Hygiene Association, 1957; National Institute for Occupational Safety and Health, 1996). In this study the authors have evaluated VHP biodecontamination in one CL3 laboratory suite. To address the safety concern, they maintained the laboratory suite at a slightly negative pressure to prevent VHP from escaping to the neighboring areas. Biological and chemical indicators were placed within the lab to assess the success of the VHP decontamination processes.

Materials and Methods

Laboratory Suite

The laboratory suite was built as a CL3 lab and has adjacent dirty change, shower-out, and clean change rooms. It had a volume of 3,000 cubic feet and contained biosafety cabinets (Class II Type A2 & Class III), incubators, refrigerator, freezer, centrifuge, telephone, fax ma-

chine, computer, security camera, microscope, and other routine laboratory equipment. The laboratory was supplied with conditioned air and exhausted through double HEPA filters directly to the outside.

Preparation for Biodecontamination

For thorough circulation of VHP within the labspace, six oscillating fans were positioned inside the laboratory suite (Figure 1). Their locations and directions of oscillation were determined by a smoke test using Drager air current tubes. Additionally, the Class II BSC was left running to further enhance VHP distribution. To decontaminate the dirty change and shower-out rooms, the doors between the laboratory and dirty change room, and between the dirty change and shower-out rooms were held open. All the electronics and laboratory equipment were left *in-situ* to determine their compatibility to VHP. The VHP generator (STERIS® VHP 1000ED) was situated in the mechanical space above the laboratory. VHP was piped-in and the return air piped-out of the lab using two 1-1/2-inch stainless steel pipe penetrations in the concrete floor slab.

Biodecontamination Program Cycle

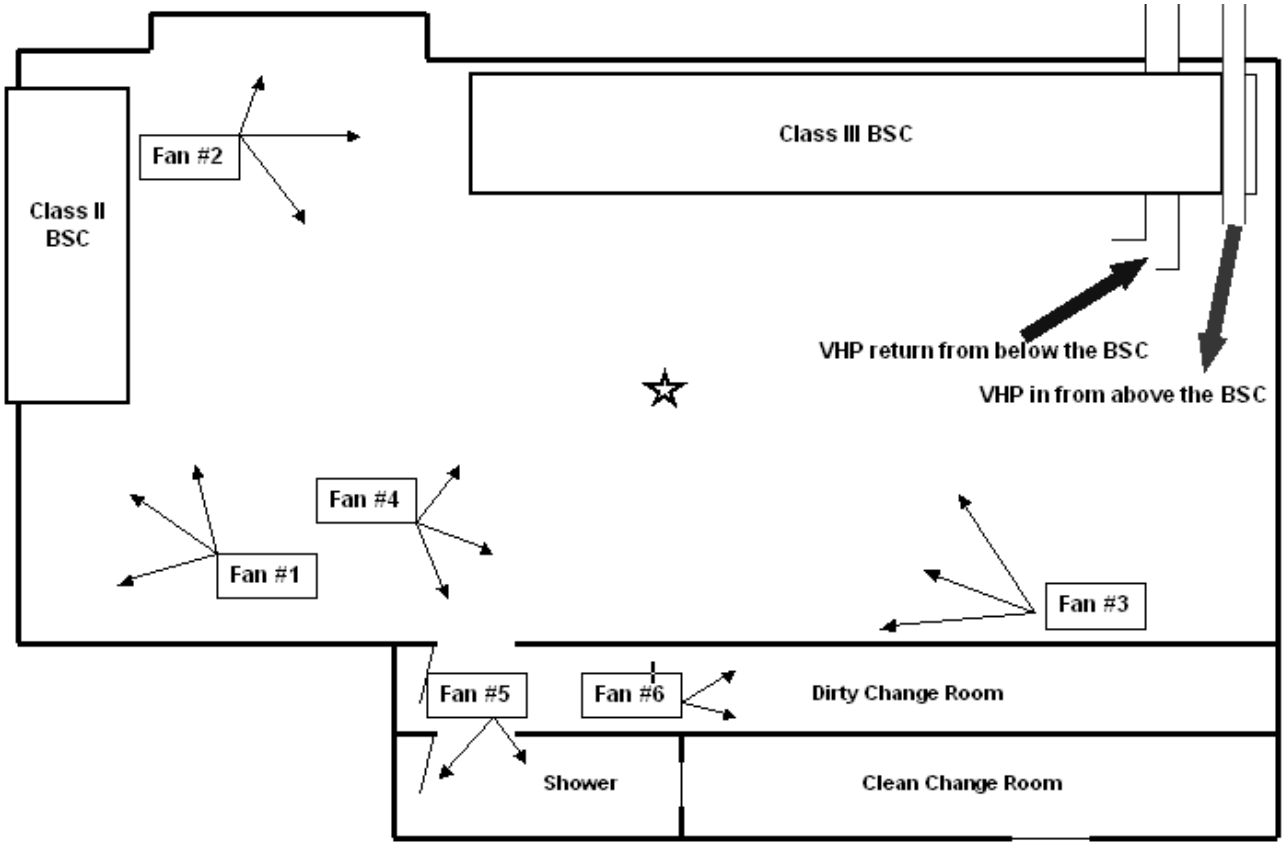
The following parameters were programmed into the VHP generator: *Dehumidification* to 30% relative humidity (30 minutes), *Conditioning* sterilant injection rate 11g of 35% H_2O_2/min (20 minutes), *Decontamination* injection rate 8g H_2O_2/min (90 minutes), *Aeration* 60 minutes and the *Flow rate* was set at 20cfm (STERIS, 2002).

Safety Assurance

The laboratory suite was not completely airtight. To prevent hazardous levels of VHP from leaking out, the lab pressure was set at approximately minus 10 Pascals. This was accomplished by shutting down the HVAC system and opening a manual bioseal damper on the laboratory's exhaust duct to vent out a small volume of airflow. During the process, air was sampled in the neighboring labs, rooms, and the penthouse mechanical space for the presence of VHP using Dräger H_2O_2 detection tubes capable of measuring as low as 0.1 ppm VHP (Drager Safety, 2005). No VHP was detected. On the following day, the HVAC system was started to aerate the lab and to decrease the VHP concentration to safe levels. To determine

Figure 1

Laboratory suite set up for VHP decontamination. The laboratory suite is approximately 3000 cubic feet in volume. Thin arrows indicate fans' directions of oscillation and the star represents the location of the VHP sensor suspended from the ceiling.



the concentration of residual VHP inside the lab, exhaust duct air was sampled using Dräger H_2O_2 tubes. After about 24 hours of HVAC-assisted aeration, the VHP concentration fell below 0.3ppm, which is well below the personal exposure level. Therefore, normal access to the lab was permitted for the retrieval of biological and chemical indicators.

Process Control and Monitoring

An ATI series B12 two-wire gas transmitter fitted with an H_2O_2 electrochemical sensor (0-2000ppm) was suspended from the ceiling in the middle of the laboratory to monitor real-time VHP concentration. A second VHP sensor was mounted in the exhaust duct to determine the amount of VHP being lost during the decontamination process and the post process residual VHP concentration. The lab pressure during the process was monitored using a digital manometer (ATE-100, Ashcroft Instruments Canada Inc. Mississauga, Ontario). The data from these monitors were logged using OM-CP process input data loggers (±25mA) (OM-CP-PROCESS110-25MA, Omega Technologies, Laval, Quebec). The temperature and relative humidity in the lab were also monitored and logged (OM-CP RFRHTEMP101A, Omega Technologies). VHP chemical indicators (NB305, Steris®, Mentor, Ohio) were placed at different locations (N=50) in the lab to visualize the extent of VHP distribution.

Sterility Validation

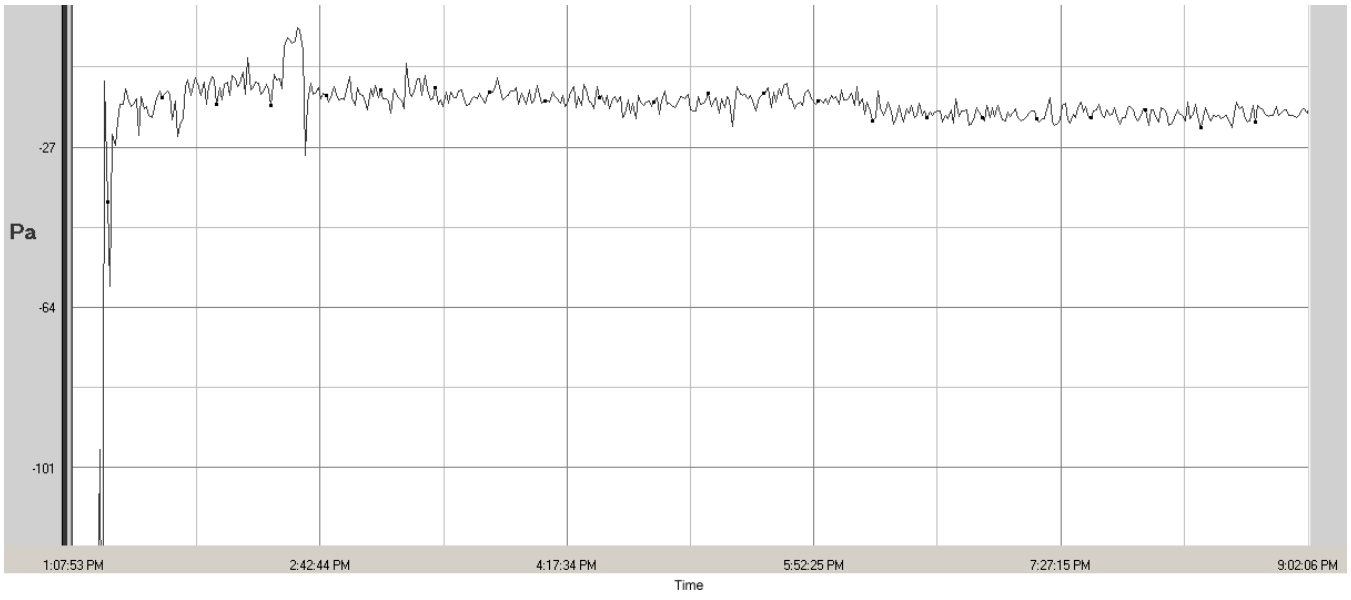
To validate the process' extent and efficiency of microbial sterilization within the lab-space, biological indicator pouches containing >106 spores of *Geobacillus stearothermophilus* dried on stainless steel metal discs sealed in Tyvek pouches (Apex Laboratories, Inc. Apex, NC) were placed, in pairs with chemical indicators, at different locations (ceilings, walls, floors, corners, and behind, under, and inside of cabinets and various equipment) within the laboratory suite (N=50). Three batches of the biological indicators were used and their lot numbers and D values were H1535 (1.6 min), H0635 (1.6 min), and H0035 (1.4 min). Upon completion of the biodecontamination program, the pouches were retrieved and opened and the discs were transferred aseptically into Tryptic Soy Broth and incubated at 56°C. An unexposed biological indicator was also included as a positive growth control. The cultures were observed for bacterial growth for up to 7 days. All cultures remained negative for growth except for the positive control, which became positive after overnight incubation.

Results and Discussion

The authors believe that this study is the first published work describing the VHP-based biodecontamination of a laboratory suite under negative pressure while the rest of the building was occupied. VHP has been in use as an alternative to formaldehyde for space biodecontamination. Following the post 9/11 anthrax letter campaign in the United States, the State Department mail-processing facility SA32 (1.4 million cubic feet of volume) was decontaminated with VHP (National Homeland Security Research Center, 2005). The mail-processing facil-

Figure 2

Laboratory's negative pressure. The laboratory suite was kept at about minus 10 Pascals to prevent VHP from leaking out into the surrounding area. This was monitored and maintained throughout the VHP decontamination process.



ity was kept at negative pressure during the decontamination to prevent VHP from escaping into the surroundings. However, this was done only after complete evacuation of the facility. An average of minus 11.28 Pascals of negative pressure (Figure 2) was maintained throughout the decontamination process by venting out 2.97 (average) cubic feet of air per minute from the lab (Figure 3). By doing so, it was anticipated that a significant amount of VHP would be lost via the exhaust air. However, VHP was not detected in the exhaust duct until 6 hours after the end of H_2O_2 injection. Possible explanations for this lag in detection of VHP by the sensor mounted on the exhaust duct include the following:

1. The volume of air preexisting in the exhaust duct (about 60 cubic feet) had to be vented out at a rate of 2.97 cubic feet per minute before the VHP-containing air reaches the sensor.
2. The sensor was placed upstream to two 24 X 24 HEPA filters and they are known to absorb and retain VHP (Jones et al., 2004).
3. The galvanized metal exhaust ductwork is capable of decomposing H_2O_2 into water and oxygen and thus diminishing small quantities of VHP from reaching the sensor.

Spores of *G. stearothermophilus* have been identified as the hardiest bacterial spores to VHP inactivation (Kokubo et al., 1998; Meszaros, 2005; Rickloff & Orelski, 1989). Therefore, we have chosen to validate our biodecontamination processes using *G. stearothermophilus* spore-discs as biological indicators. Furthermore, the biological indicators were strategically placed on locations (behind, under, and inside cabinets and equipment) that are harder for the VHP to reach. The peak VHP concentration measured at the central location of the laboratory suite was 517ppm (Figure 4), slightly higher than the concentration reported in a recent hospital ward decontamination study (French et al., 2004). However, cultures of all the biological indicators failed to grow upon incubation up to a week indicating the thoroughness of microbial decontamination achieved within the laboratory suite (data not shown). This was not surprising because a VHP concentration of less than 100 ppm was shown to be cidal to *G. stearothermophilus* spores (National Homeland Security Research Center, 2005). The authors were unable to do total colony counts of the environmental microbes before and after the decontamination processes because the laboratory was supplied with nonsterile un-HEPA-filtered air.

The authors were able to visualize most of the chemical indicators placed within the laboratory in real-time using the security camera. A few minutes after the sterilant injection, these underwent a color change from blue to beige indicating their contact with VHP. All 50 chemical indicators retrieved at the end underwent color changes indicating that VHP reached virtually everywhere within the laboratory suite.

The VHP concentration in the laboratory continued rising until the beginning of the aeration phase; then it started to decline slowly. The longest phase of the exercise was the postprocess aeration. It took over 24 hours of HVAC-assisted aeration for the VHP concentration to fall below 1ppm, the permissible personal exposure limit, whereas the actual decontamination program cycle required only 3 hours and 20 minutes. The design of the laboratory suite with its exhaust air vented out directly

Figure 3

Exhaust air flow. A slight flow of air from the lab was exhausted to maintain negative pressure in the laboratory suite. This was monitored throughout the VHP decontamination process.

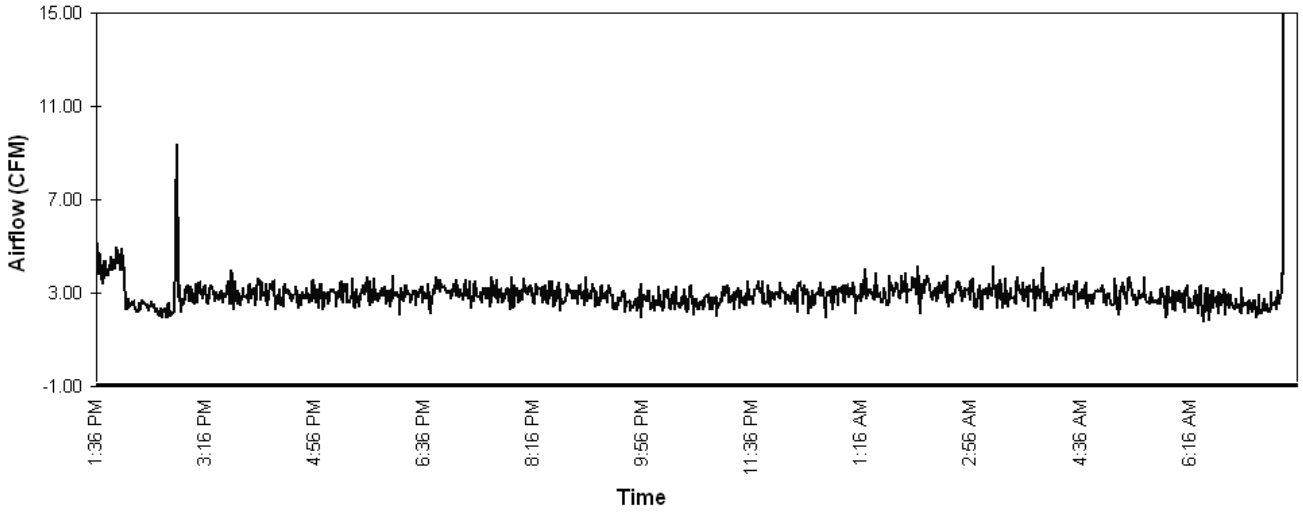


Figure 4

VHP concentration recorded in the lab (thin line) and in the exhaust duct (thick line). Note that the actual decontamination program cycle lasted only 200 minutes (boxed) and no VHP was detected in the exhaust air during this period.

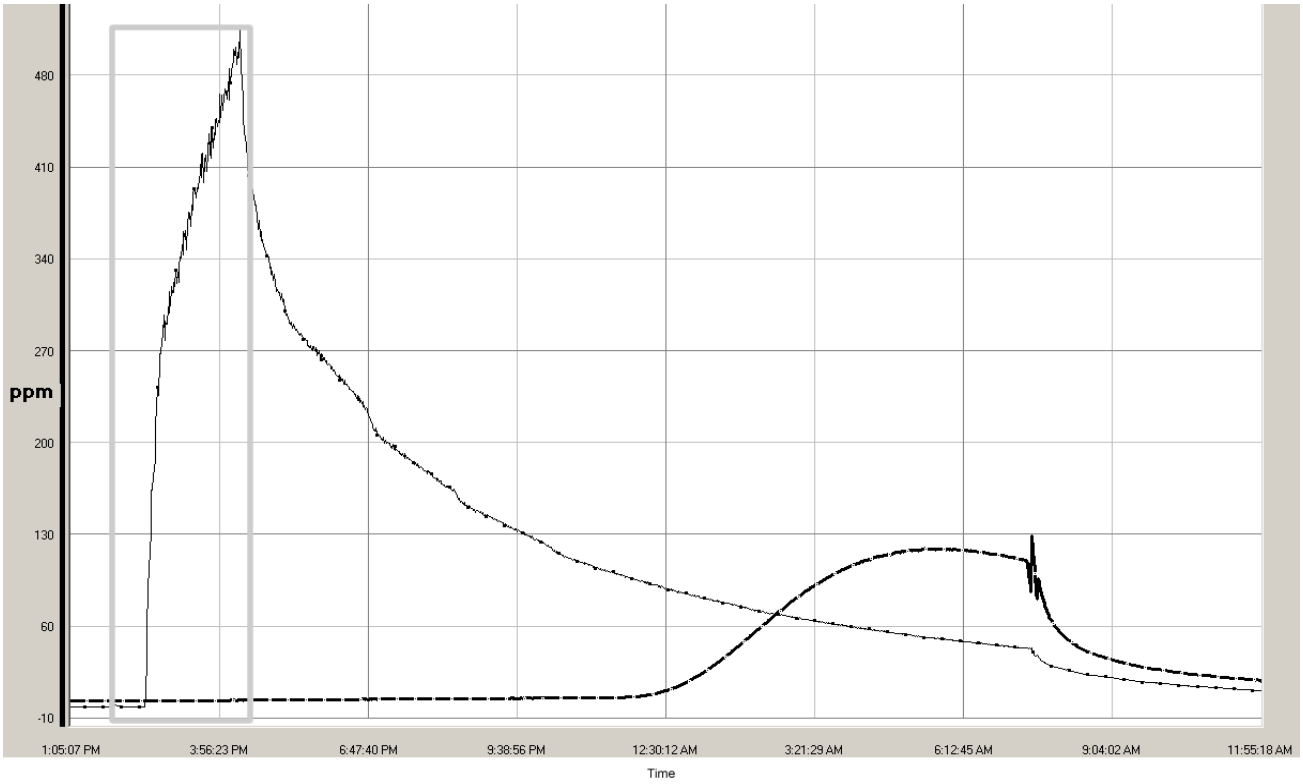
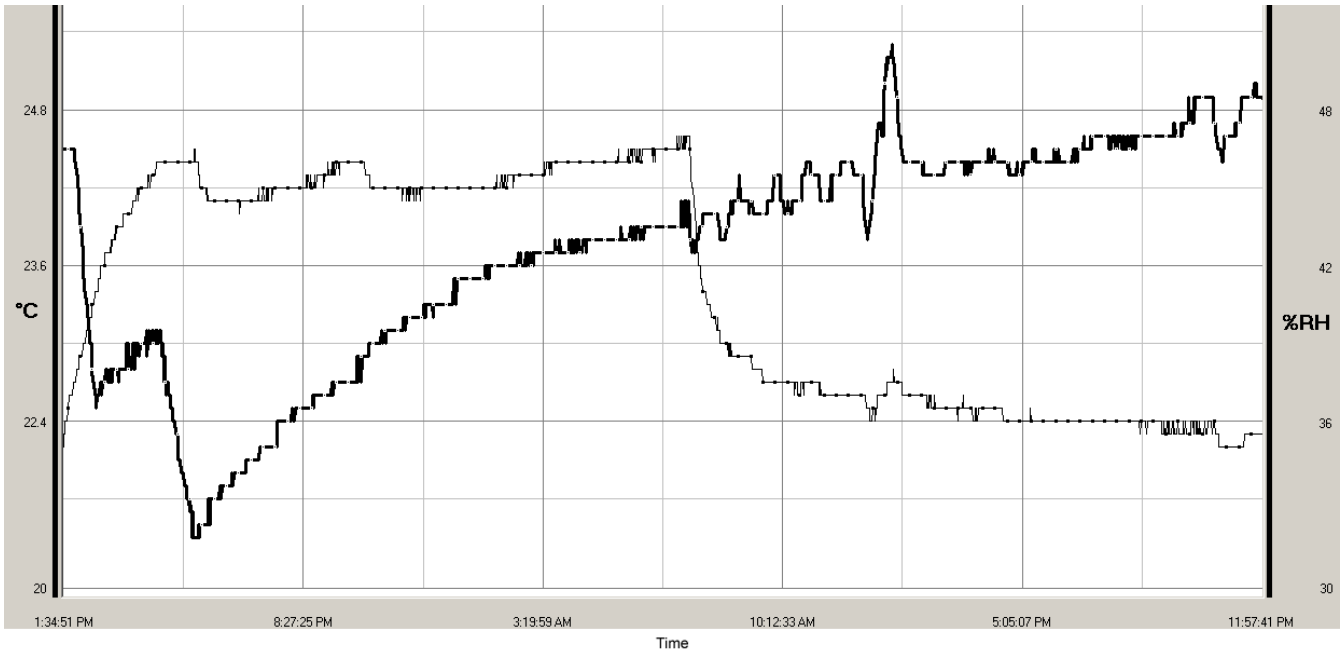


Figure 5

Temperature and relative humidity recorded in the lab. The temperature (thin line) rose from 21.3°C to 24.5°C by the end of the aeration phase whereas the relative humidity (thick line) fell from the initial 47% to 36.5% during the dehumidification phase, rebound back to 39.5% by the end of decontamination phase before falling further to 32%.



facilitated the aeration and removal of residual VHP from the laboratory. All the equipment and electronics in the laboratory remained fully functional after the biodecontamination processes (N=5).

Relative humidity directly affects the condensation of VHP; therefore, it was important to reduce the relative humidity to approximately 40% to prevent the VHP from condensing to liquid H_2O_2, which would otherwise lead to a wet decontamination process. Even though relative humidity was programmed at 30%, the authors were able to achieve only 36.5% from the initial 47% by the end of the dehumidification phase. This is not unusual when dehumidifying such a large volume. During the conditioning phase, the relative humidity started to increase and peaked at 39.5% by the end of the decontamination phase and further fell to 32% by the end of the aeration phase (Figure 5). The initial temperature in the lab was 21.3°C, which continued rising throughout the program cycle and peaked at 24.5°C by the end of the aeration phase (Figure 5). Even though the temperature was not controlled, the above-noted range of temperatures proves that the VHP-based biodecontamination can be performed under ambient conditions.

Conclusion

VHP can be used safely to biodecontaminate a laboratory under negative pressure. The negative pressure virtually isolated the lab from the rest of the facility which was occupied by hundreds of people without interruption. The VHP-based biodecontamination was auditable, reproducible, and compatible with a variety of routine laboratory equipment and electronics. The decontamination process was fast, just over 3 hours, but the postprocess aeration required more than 24 hours. In conclusion, this study shows that VHP is an effective alternative to formaldehyde for volume decontamination.

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a. Controls & HVAC Systems division
b. Containment Services Division
c. Safety & Environmental Services Division

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