Decontamination of a Worst-case Scenario Class II Biosafety Cabinet Using Vaporous Hydrogen Peroxide

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Abstract

The objective of this study was to evaluate both condensing (wet) and non-condensing (dry) vaporous hydrogen peroxide (VHP) technologies for decontaminating a worst-case scenario Class II biosafety cabinet. A 23-year-old Class II, type A2 biosafety cabinet equipped with loaded HEPA filters and an inoperable blower was used for this study. Biological indicators were placed at various locations within the cabinet, including between the pleats of supply and exhaust HEPA filters, to assess the success of the decontamination processes. A variety of decontamination program cycle parameters in combination with two different routes of VHP introduction and minor biosafety cabinet preparations were assessed. Initial decontamination attempts using routine program cycles failed (at least one biological indicator grew upon incubation); however, program cycles consisting of modified parameters and BSC preparations resulted in successful (all the biological indicators were inactivated) and repeatable decontaminations. This study concludes that VHP, either wet or dry, could be used to decontaminate an entire biosafety cabinet only if appropriate and thoroughly validated decontamination processes are employed.

Introduction

Biosafety cabinets (BSC) have been widely used in clinical and research laboratories for many years as containment devices to protect the product, environment, and personnel (Coriell & McGarrity, 1968; McDade et al., 1968). Biosafety cabinets, just like any other standard equipment, require regular maintenance, repair, calibration, and certification. Before accessing or repairing internal surfaces or parts, a BSC needs to be thoroughly decontaminated to prevent the release of microbial contaminants, contamination of certification equipment, and exposure to personnel. BSCs are complex devices and because of their intricate geometry and convoluted internal surfaces, it is impossible to decontaminate an entire BSC using a liquid disinfectant. Therefore, gaseous and vaporous decontamination agents that could fill up the entire space and contact all the surfaces within a BSC are used for its decontamination.

Formaldehyde gas fumigation is the most widely used method for BSC decontamination (Fink et al., 1988; Munro et al., 1999; National Sanitation Foundation, 2007). However, formaldehyde is carcinogenic (Cogliano et al., 2005) and requires neutralization with ammonia (Luftman, 2005) and subsequent post-process clean up (Cheney & Collins, 1995). Currently, technologies such as gaseous chlorine dioxide (Luftman et al., 2008) and vaporous hydrogen peroxide (VHP) are increasingly being used as an alternative to formaldehyde for space and equipment decontamination.

VHP is an area/space decontamination technology that has been used for the decontamination of ambulances (Andersen et al., 2006), laboratories (Krishnan et al., 2006), isolators (Meszaros et al., 2005), hospital rooms (Dryden et al., 2008; French et al., 2004; Hardy et al., 2007), and clean rooms (Malmborg et al., 2001). Since the technology is compatible with electronics (Hall et al., 2007), it is also being used for the decontamination of computers and medical and laboratory equipment.

Proper decontamination of a biosafety cabinet using VHP has never been a straightforward undertaking because of the aforementioned complex nature of the Class II BSC. A limited number of studies have been published on the decontamination of Class II BSCs using VHP. However, each of those studies suffers from noticeable limitations:

1. No biological indicators (BI) were placed between the HEPA pleats (Hillman, 2004; Jones et al., 1993a) or in the HEPA plenum, so it was difficult to validate or confirm the decontamination of HEPA filters.
2. Turning the BSC blower on briefly mid-cycle was considered to force the vapour through the filters and to dead-end plenums (Jones et al., 1993b), which is not possible on a BSC with a non-functional blower.
3. An external channel was installed between the supply plenum and the plenum above the exhaust filter for better distribution of VHP (Jones et al., 1993a), which is not feasible without breaching the containment of a BSC. Moreover, such VHP circumvention around the exhaust filter could also result in incomplete decontamination of the exhaust filter.
4. It appears that brand new BSCs were used for the decontamination studies (Lin & Atmadi, 2009; Lin et al., 2009) which may not compare well to a used BSC with loaded filters. Furthermore, there are also dead-end spaces inside a BSC where air/vapour would not be able to circulate freely.

In light of these difficulties associated with the VHP decontamination of Class II BSC, Jones et al. recommended new cabinet designs that would facilitate VHP decontamination (Jones et al., 1993b). This study was undertaken to determine if an existing old and used Class II BSC with loaded filters could be thoroughly decontaminated using VHP without invasive modifications to the BSC.

Materials and Methods

Biosafety Cabinet

One possible worst-case scenario BSC in a microbiology laboratory was chosen for this study: a used, 23-year-old, 6-foot, Class II-type A2 BSC (SG600, the Baker Company, Sanford, ME); equipped with original, wood-framed (particle board), loaded HEPA filters, and an inoperable blower. To validate decontamination of virtually every part of the BSC, 38 biological indicators (BI) were placed at different locations within the BSC including the work area, under the tray, between the pleats of the supply (both sides) and the exhaust HEPA filters, and also the positive and negative pressure plenums (Table 1, Figure 1). Thin plastic applicators were used to attach BIs and insert them halfway into the pleats of the exhaust filter (Figure 2). The common plenum access panel was removed to place the BIs in the plenums and on the dirty side of the supply filter. Control panel and sliding sash were removed prior to covering the front access of the BSC with a polycarbonate sheet. The polycarbonate sheet had two ports—one to introduce/retrieve VHP and the other to place a VHP sensor (ATI, Collegeville, PA) to determine VHP concentration in the BSC's work area. The exhaust filter was covered with a custom-made plastic cap (24" x 30" rectangle-base pyramid transitioned to 1.5" circular port for a cam lock coupling), which was designed to be placed on the threaded studs on the front of the exhaust filter well and fit around the well on the other three sides. Both the polycarbonate sheet and the plastic cap were sealed with vapour-barrier tape (Figures 2 and 3).

Biological Indicators

The biological indicators were purchased from Apex Laboratories (Apex, NC) and each of them contained $\geq 10^6$ *Geobacillus stearothermophilus* spores dried on a stainless steel coupon in a Tyvek (DuPont, Wilmington, DE) pouch. Following decontamination, all the BIs were retrieved and processed aseptically inside a BSC, where each of the spore coupons was removed from its pouch and transferred to a tube of trypticase soy broth (TSB) containing 0.5 mg% phenol red indicator. All the tubes, including positive (unexposed BI coupon in TSB) and negative (TSB without BI coupon) controls were incubated at 56°C. The cultures with no evidence of bacterial growth were incubated up to 7 days. The positive control tubes yielded bacterial growth (color change from red to yellow and development of turbidity) upon overnight incubation, and the negative control tubes remained clear and red (lack of bacterial growth) throughout the length of the incubation period. This study's criterion for a successful decontamination was inactivation of all the BIs that were placed within the BSC, and if any one of the BIs turned up positive for bacterial growth, the decontamination was considered incomplete and hence unsuccessful.

VHP Generators

VHP 100p HO (Steris Corporation, Mentor, OH) and Clarus C (Bioquell, Hampshire, UK) VHP generators were used for this study, and they delivered dry and wet VHP respectively. The four phases in a typical decontamination program cycle of the VHP 100p HO are dehumidification, conditioning, decontamination, and aeration. During the dehumidification phase, the relative humidity inside an enclosure being decontaminated is reduced (10%-40%) to prevent VHP from condensing out. The conditioning phase involves introduction of higher amounts of hydrogen peroxide vapour into the enclosure for a shorter period of time to elevate the VHP concentration rapidly and is followed by the decontamination phase, where the VHP is introduced at a lower rate to maintain the concentration. Aeration is the last and the longest phase of the program cycle, where the residual VHP is removed from the enclosure.

The Clarus C generator also has four corresponding phases that are named differently: conditioning, gassing, gassing dwell, and aeration.

Decontamination Program Cycles

Initially, typical decontamination program cycles consisting of dehumidification, conditioning, decontamination, and aeration were used. However, for subsequent runs, a variety of VHP decontamination program cycles were evaluated, which consisted of varying dehumidification/conditioning phases, decontamination/dwelling phases, injection rates, and air flow rates. Decontamination attempts were also evaluated by introducing the VHP into either the work area or the exhaust filter side of the BSC (Tables 2 and 3). The decontamination program cycles that were utilized for this study were developed around our ambient temperatures (18°C-22°C) and hence the temperatures during the decon runs were not monitored.

Results

Decontamination Using Dry VHP

On average, the highest levels of VHP concentrations, 675 ppm and 1200 ppm, were recorded in the work area when the VHP was introduced into the exhaust filter well and work area side respectively. No correlation could be established between these VHP con-

Table 1

Biological Indicator Placements

BI #	Location
1	Top of exhaust HEPA, right-hand side back corner
2	Top of exhaust HEPA well, middle front
3	Middle of exhaust HEPA in the pleats
4	Left-hand side, upper negative plenum between exhaust well and side wall
5	Upper negative plenum back wall, right-hand side middle
6	Upper negative plenum, right-hand wall middle
7	Upper negative plenum, center back wall middle
8	Upper negative plenum, front on blower housing
9	Upper negative plenum back wall, center behind exhaust HEPA well
10	Upper negative plenum, center of front cover of exhaust HEPA well
11	Common plenum, back right-hand side of supply HEPA
12	Common plenum, back left-hand side of supply HEPA
13	Common plenum, middle left-hand side of supply HEPA
14	Common plenum, middle right-hand side of supply HEPA
15	Common plenum on front right-hand side of blower discharge diffuser
16	Common plenum, front left-hand side on supply HEPA frame
17	Common plenum, front middle on supply HEPA frame
18	Common plenum, front right-hand side on supply HEPA frame
19	On common plenum cover plate middle
20	Left-hand side middle of supply diffuser in work area
21	Right-hand side middle of supply diffuser in work area
22	Middle of left-hand side wall in work area
23	Middle of right-hand side wall in work area
24	Middle of back wall in work area
25	Inside left-hand side electrical receptacle knock out in work area
26	Middle/front of the supply HEPA in the pleats (clean side)
27	Below work tray, left-hand side middle
28	Below work tray, center middle
29	Below work tray, right-hand side middle
30	Center of the work tray middle
31	Right of center front of the supply HEPA in the pleats (dirty side)
32	Left-hand side back of supply HEPA in the pleats (dirty side)
33	Left of center front of supply HEPA in the pleats (dirty side)
34	Right-hand side back of supply HEPA in the pleats (dirty side)
35	Front right-hand corner of exhaust HEPA in the pleats (clean side)
36	Back left-hand corner of exhaust HEPA in the pleats (clean side)
37	Front left-hand side of supply HEPA in the pleats (clean side)
38	Front right-hand side of supply HEPA in the pleats (clean side)

Figure 1

Preparation of BSC for decontamination: A few locations where the biological indicators were placed are shown. Inset shows a biological indicator inserted into the pleats of supply HEPA filter.

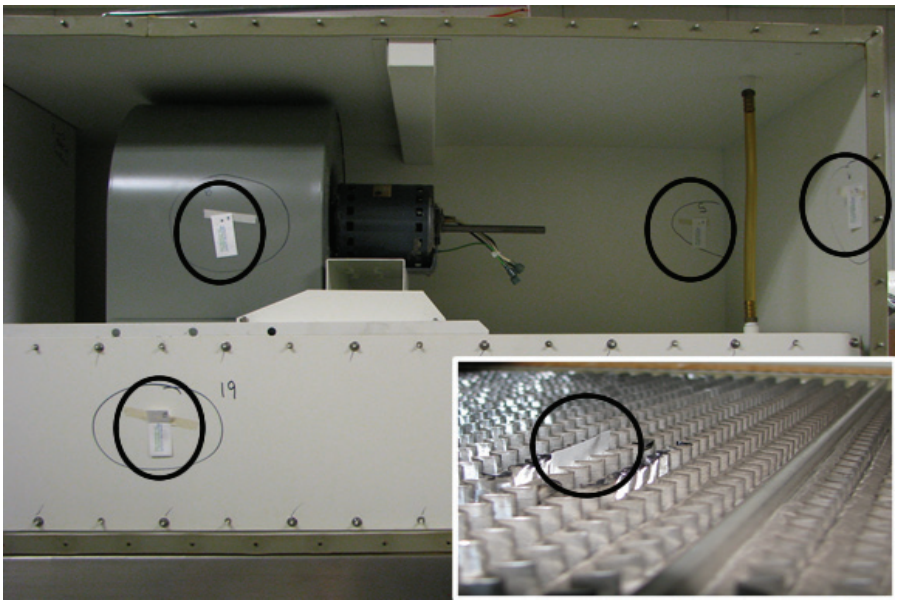


Figure 2

Placement of biological indicators into the exhaust HEPA filter pleats. Biological indicators were attached to plastic pieces (inset) to introduce them deeper into the middle part of the exhaust filter. The cap used to cover the exhaust filter well is also shown.

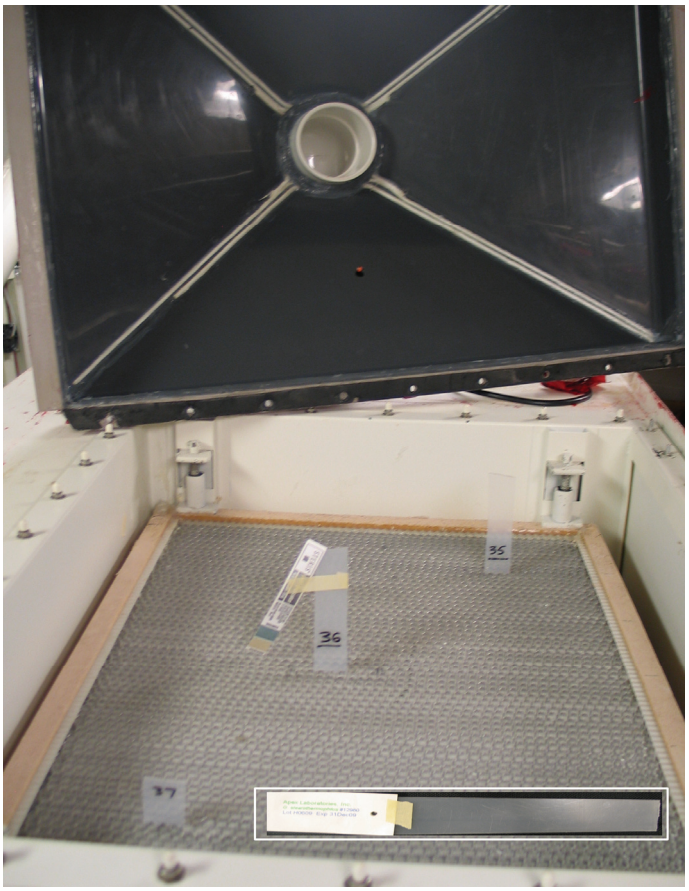
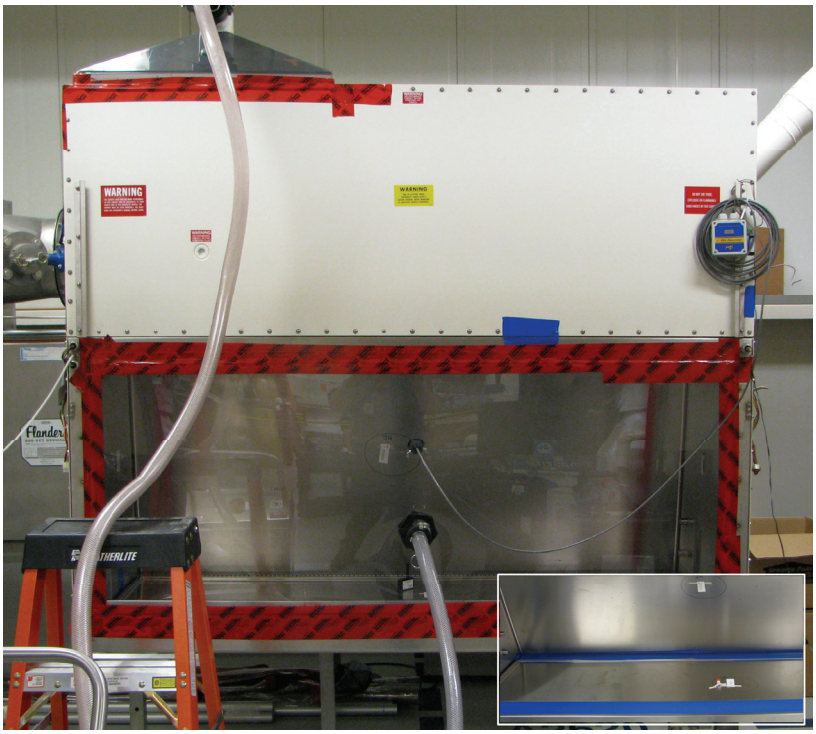


Figure 3

Preparation of BSC for VHP decontamination. Front access and exhaust filter opening were closed with Polycarbonate sheet and plastic cap respectively, and sealed with vapour barrier tape. The work area grills, on the front and rear were also sealed with vinyl tape (inset) for subsequent decon runs to force VHP through the supply filter.



centrations in the work area and the success or failure of the decontamination efforts. Four out of 4 of the initial decontamination attempts using typical program cycles failed to inactivate all the BIs, indicating inadequate decontamination (Table 2). On average, 11% ± 3% (mean ± standard deviation) of the BIs grew upon incubation. These failures were largely due to the lack of inactivation of BIs placed in the pleats of the supply filter (5 out of 13 failed biological indicators) and supply plenum (7 out of 13 failed biological indicators). This led us to believe that the VHP was circumventing the supply filter. Instead of passing through the supply filter, the vapour was flowing through the less resistant path, i.e., work area → down grill → up negative plenum → common plenum via the blower opening and out to the VHP generator via exhaust filter when VHP was injected into the work area (Figure 4), and in reverse order when the sterilant was injected into the exhaust filter well. To force the VHP through the supply filter, the front and back grills in the work area were sealed with tape (Figure 3), which resulted in the mitigation of BI failures on supply filter pleats.

Subsequent decontamination attempts were also not successful because of the failure of BIs placed in "dead space" locations such as inside plenum on the front outside frame of the supply filter (BI location #17). This was attributed to the failure of VHP to reach the dead space locations that are away from the uninterrupted, continuous flow path of the vapour. This was rectified by turning off the VHP generator just prior to the aeration phase. This pause disrupted the constant circular flow of the VHP and may have allowed the residual vapour to dissipate throughout the BSC overnight and result in successful inactivation of all BIs. The BSC was aerated for about 3 hours on the following morning to achieve safe vapour levels for the retrieval of BIs. Three consecutive successful decontaminations were obtained when a program cycle consisting of 15 minutes of dehumidification (20% RH), 5 minutes of conditioning (6 gm/min), and 90 minutes of decontamination (3.3 gm/min) followed by an overnight pause was used (Table 2).

Decontamination Using Wet VHP

Initially, 6 out of 6 of the decontamination attempts using the wet VHP technology were also unsuccessful (Table 3). On average, 6% ± 3.9% of the biological indicators yielded growth upon incubation. The locations and percentage of the failed BIs were supply filter (43%), common plenum (29%), supply plenum (21%), and top of the exhaust filter (7%). Unlike the initial dry VHP decontamination trials, the work area grills were taped off for all the wet VHP decontamination attempts. As a result, the locations of the failed BIs were random. Also,

Table 2

Trial Decontamination Attempts Using Dry VHP Technology

Different program cycles consisting of varying dehumidification, conditioning, decontamination, and air flow rate parameters along with two different VHP introduction routes were used. Some runs also included a pause prior to the aeration phase. A program cycle that resulted in three consecutive successful decontaminations was chosen as the suitable program cycle.

Runs	Dehumidification*	Conditioning†	Decontamination‡	Pause§	VHP Route¶	Result#
1	10', 10%, 18cfm	5', 5g/m, 18cfm	60', 2g/m, 14cfm		work area	unsuccessful
2	10', 10%, 18cfm	5', 5g/m, 18cfm	60', 2g/m, 14cfm		exhaust filter	unsuccessful
3	10', 10%, 20cfm	5', 6g/m, 20cfm	60', 3g/m, 20cfm		exhaust filter	unsuccessful
4	10', 10%, 20cfm	5', 6g/m, 20cfm	60', 3.3g/m, 20cfm		exhaust filter	unsuccessful
5	10', 10%, 20cfm	5', 6g/m, 20cfm	60', 3.3g/m, 20cfm		exhaust filter	unsuccessful
6	10', 10%, 20cfm	5', 6g/m, 20cfm	60', 3.3g/m, 20cfm	5 hours	work area	**successful**
7	10', 10%, 20cfm	5', 6g/m, 20cfm	60', 3.3g/m, 20cfm	5 hours	work area	unsuccessful
8	10', 10%, 20cfm	15', 3g/m, 20cfm	60', 1.5g/m, 8cfm		exhaust filter	unsuccessful
9	10', 10%, 20cfm	15', 3g/m, 20cfm	60', 1.5g/m, 8cfm		work area	unsuccessful
10	20', 10%, 20cfm	0', 0g/m, 20cfm	20', 5g/m, 20cfm	5 hours	exhaust filter	unsuccessful
11	20', 10%, 20cfm	84', 1.5g/m, 20cfm	84', 1.5g/m, 8cfm		work area	**successful**
12	20', 10%, 20cfm	84', 1.5g/m, 20cfm	84', 1.5g/m, 8cfm		work area	unsuccessful
13	30', 10%, 20cfm	15', 5g/m, 18cfm	15', 5g/m, 15cfm	overnight	exhaust filter	**successful**
14	30', 10%, 20cfm	15', 5g/m, 18cfm	15', 5g/m, 15cfm	overnight	exhaust filter	unsuccessful
15	20', 10%, 20cfm	84', 1.5g/m, 20cfm	84', 1.5g/m, 8cfm		work area	unsuccessful
16	20', 10%, 20cfm	6', 5g/m, 20cfm	148', 1.5g/m, 16cfm		work area	unsuccessful
17	20', 10%, 20cfm	10', 3g/m, 20cfm	220', 1g/m, 16cfm	VHP generator malfunctioned.		
18	15', 10%, 20cfm	0', 0g/m, 0cfm	75', 2g/m, 18cfm	overnight	exhaust filter	unsuccessful
19	15', 10%, 20cfm	0', 0g/m, 0cfm	100', 2g/m, 15cfm	overnight	exhaust filter	unsuccessful
20	15', 10%, 20cfm	5', 6g/m, 20cfm	90', 3.3g/m, 20cfm	overnight	work area	**successful**
21	15', 10%, 20cfm	5', 6g/m, 20cfm	90', 3.3g/m, 20cfm	overnight	work area	**successful**
22	15', 10%, 20cfm	5', 6g/m, 20cfm	90', 3.3g/m, 20cfm	overnight	work area	**successful**

* Dehumidification parameters: time in minutes ('), relative humidity (%), airflow rate in cubic feet per minute (cfm)

† Conditioning parameters: time in minutes ('), rate of liquid hydrogen peroxide being vaporized in grams per minute (g/m), airflow rate in cubic feet per minute (cfm)

‡ Decontamination parameters: time in minutes ('), rate of liquid hydrogen peroxide being vaporized in grams per minute (g/m), airflow rate in cubic feet per minute (cfm)

§ Pause: The VHP generator was turned off immediately after the decontamination phase and prior to the aeration phase for 5 hours or overnight.

¶ VHP route: VHP was introduced into either the work area or the exhaust filter well.

\# Result: Outcome of the decontamination: Unsuccessful: At least one of the biological indicators grew upon incubation in culture. Successful: All the 38 biological indicators were inactivated.

Table 3

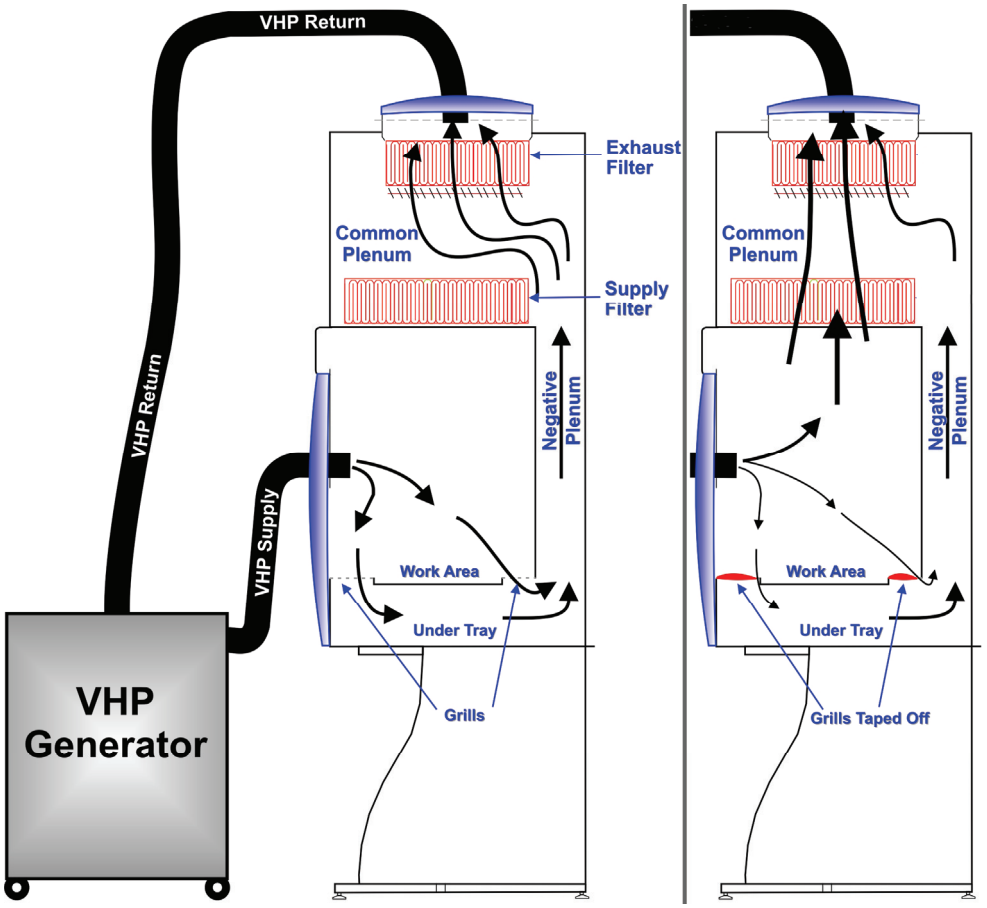
Trial Decontamination Attempts Using Wet VHP Technology

An air flow rate of 30m^3/hr was used for conditioning, gassing, and gassing dwell phases of the decontamination program.

Runs	Conditioning	Gassing	Gassing Dwell	VHP Routes	Result
1	20 min	5 min, 5 g/min	37 min, 2 g/min	exhaust filter	unsuccessful
2	20 min	50 min, 2 g/min	480 min, 0 g/min	exhaust filter	unsuccessful
3	20 min	50 min, 2 g/min	480 min, 0.1 g/min	work area	unsuccessful
4	20 min	50 min, 2 g/min	480 min, 0.1 g/min	work area	unsuccessful
5	20 min	20 min, 6 g/min	20 min, 1.5 g/min	work area	unsuccessful
6	20 min	25 min, 3 g/min	25 min, 3 g/min	work area	unsuccessful
7	20 min	25 min, 3 g/min	480 min, 0.3 g/min	work area	**successful**
8	20 min	25 min, 3 g/min	480 min, 0.3 g/min	work area	**successful**
9	20 min	25 min, 3 g/min	480 min, 0.3 g/min	work area	**successful**

Figure 4

Flow of VHP during routine decontamination runs (left), where the vapour circumvents the supply filter and flows through the least resistant path. Upon sealing the work area grills (right), the vapour was forced to pass through the supply filter, and thus decontaminating it.



the tactic of leaving the dry VHP overnight to dissipate throughout the BSC did not work with the wet VHP. This could be ascribed to the lack of vapour in wet VHP technology, where the vapour condenses (micro condensation) immediately upon introduction into the BSC. However, successful decontamination was achieved when low amounts of hydrogen peroxide were injected over an extended period of 8 hours (Table 3). The unit then went straight into the aeration phase to remove the residual vapour overnight. Three consecutive successful decontaminations were obtained when a program cycle consisting of 20 minutes of conditioning, 25 minutes of gassing (3 gm/min), and 8 hours of gassing dwell (0.3 gm/min) was used (Table 3).

Discussion

The information presented here demonstrates the difficulties associated with the decontamination of complex equipment and devices using VHP. Unlike rooms or laboratories, a Class II BSC is intricate in design and has convoluted surfaces and dead spaces that are out of reach for the VHP. Although other studies have reported decontamination of biosafety cabinets using VHP, most of those studies focused primarily on the inactivation of a few biological indicators and failed to demonstrate thorough decontamination of the entire cabinet including the HEPA filters. Some of the studies have even gone as far as making invasive modifications to the BSC so as to achieve inactivation of biological indicators placed on the other side of the filters. Such decontamination approaches are often impractical and unsafe and may result in incomplete decontamination of the equipment.

A "worst-case scenario BSC" could mean a variety of things and is primarily dependent on the history of the cabinet's usage. As a result, it could mean a severely damaged BSC contaminated with chemical carcinogens, potent biological toxins, or extremely resistant microbial agents such as *Bacillus anthracis* spores. However, one possible worst-case scenario in a microbiology laboratory could be an old, properly functioning BSC (hence loaded and contaminated filters) suddenly becoming inoperable

because of blower failure. From a decontamination perspective, this creates difficulties as the loaded filters wouldn't let the vapour sterilant cross easily nor could the non-functional blower be used to push it across the filters. In our experience at the Canadian Science Centre for human and animal health, most often decontamination of biosafety cabinets was done to replace their inoperable blowers.

To validate the thoroughness of the decontamination, 38 biological indicators were placed at different locations within the BSC. A decontamination process was considered incomplete if any of the indicators failed, and appropriate changes were made to the decontamination process to get the vapour where the failed BIs were located. Such measures included sealing of grills to force the VHP across the supply filter and turning off the VHP generator just prior to the aeration phase to let the residual VHP permeate to the dead space locations within the BSC. Interestingly, sealing the grills did not result in failure of BIs placed under the work tray. In fact, the area below the work tray becomes a continuous part of the negative plenum, which gets filled with VHP passing through the supply filter.

Even though polycarbonate sheet and plastic cap were used in this study for sealing the work area and exhaust filter well, one could easily adapt and use other means such as polyethylene sheeting to accomplish the same. However, it would be difficult to add a cam lock coupling to the low-density polyethylene sheet to introduce/retrieve VHP.

Conclusions

Decontamination program cycles, especially for complex equipment, need to be developed after carefully designed validation studies. The lack of such studies would lead to increased reliance on incomplete decontamination protocols and practices that could have potential consequences from a safety perspective.

This study's primary focus was thorough decontamination of the entire Class II BSC cabinet including the supply and exhaust filters. A total of 38 biological indicators were used to cover virtually every part of the BSC and to map locations where the VHP failed to reach at sporicidal concentrations. Also from a practical standpoint, only safely achievable minor BSC preparations were sufficient to force the VHP to reach those inaccessible areas to result in successful decontamination. These include sealing the front access, exhaust filter openings, and the work area grills; this can be performed safely after a routine surface decon and without breaching containment of a BSC or affecting the NSF (National Sanitation Foundation) listing of the BSC. Thus, the combination of cabinet preparations and decontamination program parameters ensured that the VHP reached all parts of the BSC including the filters.

The BSC was disassembled to place the biological indicators at various locations for this study. However, such extensive placement of biological indicators is neither required nor safe when decontaminating a BSC that is in use. This study recommends placing at least four biological indicators, one each on top of the exhaust filter, middle of the supply diffuser, left side on the work surface, and right side under the work surface while performing a BSC decontamination using VHP. Both wet and dry VHP technologies can be used to successfully decontaminate a Class II biosafety cabinet entirely. However, carefully designed decontamination studies need to be conducted thoroughly prior to implementing new technologies for the complete decontamination of complex devices such as biosafety cabinets.

Disclaimer

Please observe caution when placing biological indicators on top of the HEPA filters as they are delicate.

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2010 Report—Public Health Preparedness: Strengthening the Nation's Emergency Response State by State

A report on CDC-funded preparedness and response activities in 50 states, 4 cities, and 8 U.S. insular areas
Tuesday, September 21, 2010

Public Health Preparedness: Strengthening the Nation's Emergency Response State by State highlights progress in preparedness and presents data on a broad range of preparedness and response activities occurring at state and local levels across the nation. The report features national data and individual fact sheets for the 50 states and four directly funded localities (Chicago, the District of Columbia, Los Angeles County, and New York City) supported by CDC's Public Health Emergency Preparedness (PHEP) Cooperative Agreement. An overview of the preparedness activities and challenges in the U.S. territories, commonwealths, and freely associated states funded by PHEP are also included. Fact sheet data expand and update those presented in CDC's first state preparedness report (2008), and cover activities conducted in 2008 and 2009. The report also highlights state and local preparedness and response activities occurring during the 2009 H1N1 influenza pandemic. All CDC preparedness reports are an important part of CDC's overall focus on demonstrating results, driving program improvements, and increasing accountability for the nation's investment in public health preparedness. www.bt.cdc.gov/publications/2010phprep/