Article

Applied Biosafety, *10*(2) pp. 91-100 © ABSA 2005

Area Fumigation with Hydrogen Peroxide Vapor

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Abstract

The ability to safely and reproducibly decontaminate enclosed areas like isolators and rooms is an important consideration to many industrial, research, and healthcare facilities. Traditionally, formaldehyde has been used, but safety and efficacy concerns have highlighted the need for alternative methods. Vaporized Hydrogen Peroxide, as used in STERIS Corporation's VHP® Technology, is a dry gaseous method that has been used as a reliable alternative for aseptic processing isolators and more recently for room/facility decontamination. In this report the antimicrobial efficacy of VHP Technology has been studied under worst-case test conditions, including the presence of contaminating soils which may be present under certain applications. Geobacillus stearothermophilus spores were verified as being the most resistant organism to the fumigation process while paper was identified as the most resistant material. Efficacy was then verified in the presence of 10% and 50% whole blood, as representative soils to ensure fumigant penetration. Typical VHP fumigation processes in rigid and flexible-walled isolators showed a ≥ 6 $\log_{10}$ *reduction under these worst-case test conditions. Like other biocides, the microbiocidal activity of vaporized hydrogen peroxide is affected by the presence of soil, but these results confirm that VHP Technology can decontaminate under soiled conditions within a routine decontamination process.*

Introduction

The decontamination of enclosed environments is an important consideration for the control or remediation of pathogens and environmental contaminants in industrial, research, and healthcare facilities. The methods employed for this purpose are liquid- or gaseous-based technologies. A wide variety of liquid-based detergents and disinfectants is currently employed, including alcohol-, quaternary ammonium compound-, and phenol-based products. These formulations can vary considerably in their antimicrobial activity and are generally bactericidal, virucidal, and fungicidal, but many have limited to no activity against more resistant microorganisms, including *Mycobacterium* species and bacterial spores (McDonnell & Russell, 1999).

Alternative liquid-based formulations that demonstrate activity against these organisms include oxidizing agent and aldehyde-based formulations. The most widely used oxidizing agents include sodium hypochlorite ("bleach"), chlorine dioxide, hydrogen peroxide, peracetic acid, or combinations thereof. Oxidizing agents are recommended due to their broad spectrum, general antimicrobial activity rendering less susceptibility to resistance acquisition, and desired environmental/safety profiles; for example, hydrogen peroxide (H_2O_2) breaks down into oxygen and water. The most widely used liquid aldehyde is glutaraldehyde, but recent concerns include the development of aldehyde-resistant bacteria, stability in the environment, irritation, and recognition as a potential carcinogen.

Most liquid-based products are utilized in a spray and wipe-type application, which can have the benefit of combining cleaning with disinfection. Liquids can also be applied to larger areas using fogging methods. The main disadvantage with the use of liquid-based methods is ensuring adequate coverage over all contact surfaces for the desired time, in particular for larger areas. This is particularly true for inaccessible areas like ductwork and HVAC systems.

For this reason, gaseous-based or vapor-phase methods have been preferred. These technologies can be considered based on the type of active ingredient: formaldehyde-based or oxidizing agent-based.

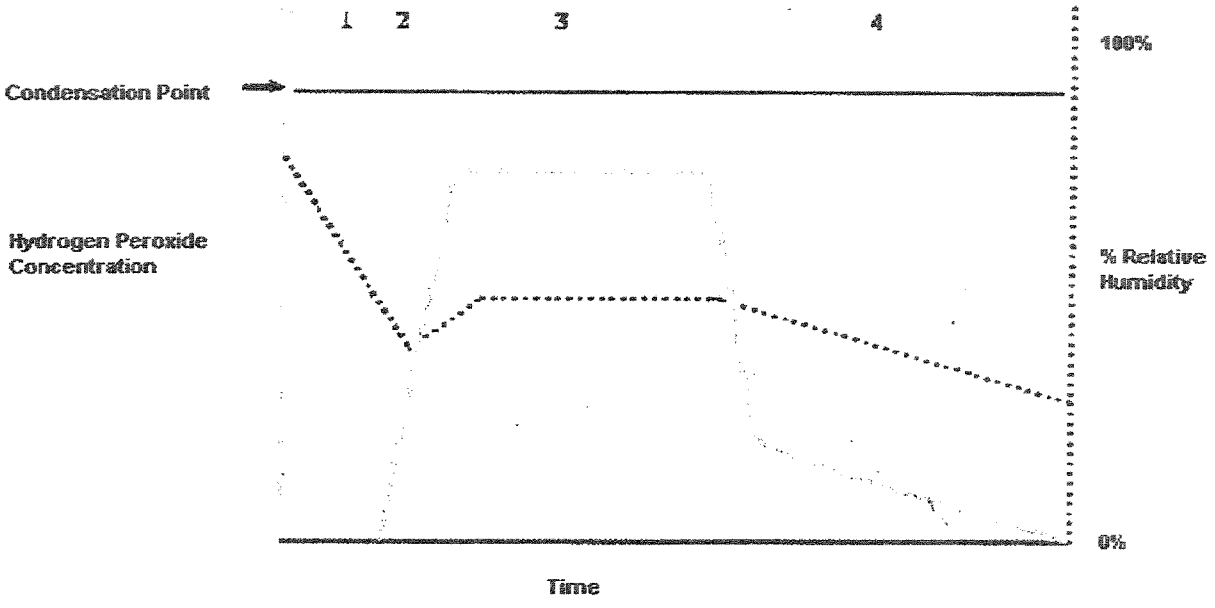
Formaldehyde is the most widely used area fumigant and has been used for over 100 years (Power, 1995). The gas may be conveniently generated either by heating a 37% formalin solution or the white, crystalline powder paraformaldehyde in water (prills and flakes may also be used). It is imperative that high humidity levels and optimal temperatures are maintained during formaldehyde fumigation in order to achieve decontamination (Cheney & Collins, 1995). Formaldehyde and residues formed following fumigation are toxic and carcinogenic, which may limit its use. Like other aldehydes, formaldehyde can be relatively stable in the environment, and it should be neutralized by ammonia or adsorption onto activated charcoal before release. Due to the safety concerns with formaldehyde, oxidizing agent-based technologies have become popular alternatives. These include ozone, peracetic acid, chlorine dioxide, and hydrogen peroxide-based gaseous systems. Hydrogen peroxide vapor systems are the most widely used and have all but replaced formaldehyde use in pharmaceutical, critical area decontamination.

Vaporized hydrogen peroxide is a broad spectrum, dry, rapid antimicrobial (Klapes & Vesley, 1990). The broad spectrum efficacy of VHP Technology has been shown against a wide range of microorganisms, including bacteria, viruses, fungi, bacterial spores, and more recently parasite eggs (Heckert et al., 1997; Kokubo et al., 1998; Krause et al., 2001). Vaporized hydrogen peroxide delivery and control systems are used for decontamination of airborne or surface contaminants, including rooms and their contents (e.g., electrical/electronic equipment), ductwork, and filters.

A typical VHP Technology decontamination cycle consists of four phases: dehumidification, conditioning, decontamination, and aeration (Figure 1). During dehumidification the relative humidity is reduced by drying, to ~ 30%-40% by circulation of the air in a closed loop. During conditioning, the decontaminant is produced by vaporization of 35% liquid hydrogen peroxide and introduced into the

Figure 1

Typical VHP Decontamination Cycle: The VHP cycle consists of four stages: (1) dehumidification, (2) conditioning, (3) decontamination, and (4) aeration.



recirculating air stream to achieve the desired hydrogen peroxide concentration. The decontamination phase proceeds identical to the conditioning phase but at a steady state injection and recirculation flow rate to maintain the concentration (generally 0.1-3.0 mg/L) for the desired exposure time. Finally, during aeration hydrogen peroxide is no longer introduced and the residual vapor is catalytically decomposed into water and oxygen by recirculation through an intrinsic platinum and palladium chemical destroyer.

In most cases, VHP Technology has been used for fumigation of relatively "clean" environments. In this report we have tested the technology under worst-case test conditions, including the presence of soil contamination, and have demonstrated that hydrogen peroxide vapor can be used to decontaminate despite the presence of interfering substances.

Materials and Methods

Bacterial Spore Preparations

Bacillus cereus (ATCC 12826), *Bacillus circulans* (ATCC 4513), *Bacillus atrophaeus* (formally known as *B. subtilis*; ATCC 19659), *Clostridium sporogenes* (ATCC 3584), and *Geobacillus stearothermophilus* (ATCC 7953) spore suspensions (Presque Isles Cultures, Presque Isle, PA) were obtained and stored at 2°-8°C until use. The spore suspensions were enumerated by serial dilutions and pour plating in trypticase soy agar (TSA; Difco, Detroit, MI) for the *Bacillus/Geobacillus* species and fluid thioglycollate agar (Difco, Detroit, MI) for *C. sporogenes*. All organisms were incubated at 37 ± 1°C, with the exception of *G. stearothermophilus* which was incubated at 56 ± 1°C, for 24-72 hours. The *Bacillus/Geobacillus* species were cultured under aerobic conditions while the *C. sporogenes* culture was incubated under anaerobic conditions within Gas-Pak incubation jars (Becton Dickinson, Sparks, MD).

Vegetative Bacteria Preparation

All original cultures were purchased from American Type Culture Collection (ATCC, Manassas, VA). Working suspensions of *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352) were prepared by inoculating -70°C stock preparations into trypticase soy broth (TSB) and incubating at 37 ± 1°C for 18 hours. A working suspension of *Mycobacterium terrae* (ATCC 15755) was prepared by inoculating Middlebrook 7H9 broth (Difco, Sparks, MD) supplemented with 0.2% glycerol and 10% ADC (8.5 g NaCl + 50 g bovine albumin + 20 g dextrose + 0.03 g catalase/liter) with a stock culture and incubating for 25 days at 37 ± 1°C. The preparation was enumerated using Middlebrook 7H11 agar (Difco, Sparks, MD) supplemented with 0.2 % glycerol and 10% OADC (ADC + 0.6 mL oleic acid/liter) by sterile filtration using 0.2 micron analytical filter apparatus (Nalge Nunc, Rochester, NY) after an incubation period of 2 weeks at 37 ± 1°C.

Fungal Spore Preparation

Aspergillus niger (ATCC 6275) and *Trichophyton mentagrophytes* (ATCC 18748) -70°C stock cultures prepared from ATCC-purchased specimens were inoculated onto potato dextrose agar and sabouraud dextrose agar respectively (Difco, Sparks, MD) and incubated at 30 ± 1°C for 2 weeks. The spores were removed from the plates using agitation and sterile inoculating loops and stored at 4°-6°C.

Working Suspension Preparation

To determine the most resistant bacterial spore species, suspensions were prepared in deionized (DI) water at 10^8 colony forming units (cfu)/mL. Subsequent evaluations conducted using the determined most resistant bacterial spore and the remaining species were prepared in 10% neonate bovine serum (Biocell, Rancho Dominguez, CA) by centrifuging an aliquot of a given suspension, decanting the supernatant, and replenishing the removed volume with the serum solution. The *M. terrae* and *A. niger* suspensions were subjected to nondestructive homogenization to ensure a consistent inoculum. Where prior enumeration data were not available, counts were verified under a Petroff Hauser counting chamber using phase contrast microscopy. Populations were adjusted to achieve 10^7 cfu/mL for the fungal spore preparations and 10^8cfu/mL for all the other species to compensate for increased size of the fungal spores.

Most Resistant Organism Identification

The most resistant organism to a decontamination method is defined as that test organism demonstrating the greatest resistance to that method. The evaluation consisted of two testing conditions; the first assessed the most resistant bacterial spore suspended in DI water (which remains viable, despite drying), and the second included the identified bacterial spore species with the remaining species to compare resistances of different microorganisms in the presence of soil, 10% neonate bovine serum.

Ten microliters of each suspension were individually inoculated onto stainless steel coupons (1cm^2 NA303; STERIS Corporation, Mentor, OH) to provide an inoculum of 10^5 CFU/coupon for the fungal species and 10^6 cfu/coupon for the rest of the microorganisms. All coupons were air-dried at ambient temperature under a biological safety cabinet.

To verify the viable population, inoculated carriers of each test organism were enumerated. Each coupon was placed into 10 mL of diluent, DI water for the spore species or TSB for the vegetative bacteria supplemented with 0.01-0.1% catalase (Sigma, St. Louis, MO), the neutralizer for hydrogen peroxide. Coupons were briefly sonicated in a water bath, vortexed, serially diluted, and quantified. *S aureus* and *K. pneumoniae* were pour plated using 45°C TSA and incubated at 37 ± 1°C for 24-72 hours while all other microorganisms were enumerated using the respective media and incubation conditions as discussed previously.

Vaporized hydrogen peroxide exposures were conducted at ambient temperature (25 ± 2°C) in a 21 ft^3 isolation chamber (Model No. 156143, La Calhene, Rush City, NY) connected to a VHP®1000 Biodecontamination System. The VHP1000 is a compact, mobile unit that generates and controls delivery of decontaminant into an enclosed environment. The unit vaporizes 35% hydrogen peroxide, known as Vaprox™ Hydrogen Peroxide Sterilant (Vaprox™ is a registered trademark of STERIS Corporation). The following VHP1000 cycle was programmed to decontaminate under the following parameters for determining the most resistant bacterial spore species:

- Dehumidification: 18 ft^3/minute air flow rate, 2.3 mg/L absolute humidity, 20 minutes
- Conditioning: 12 ft^3/minute air flow rate, 3.6 g/min injection rate, 20 minutes
- Decontamination: 12 ft^3/minute air flow rate, 1.8 g/min injection rate, 60 minutes
- Aeration: 20 ft^3/minute air flow rate, 60 minutes

The same cycle was used for determining the microbial resistance in the presence of the serum except the dehumidification air flow rate was 15 ft^3/minute and the injection rate during the conditioning phase was 2.5 g/min. For this study, all microorganisms were exposed for controlled times during the decontamination portion of the cycle. For this, the isolation chamber was constructed with an access port that allowed for the introduction and removal of test coupons from the isolator. A single coupon was exposed for each test organism at various times. Immediately after exposure, each coupon was aseptically transferred into 10 mL of DI water or TSB containing 0.01% to 0.1% catalase for neutralization of the hydrogen peroxide, sonicated, and quantified as described previously. Additional control studies indicated that this method did not affect the growth of each test organism. Agar plates exhibiting 30-300 cfu/plate were used for enumerations. D-values (the average time for a 1 $\log_{10}$ reduction of each test organism) were determined by linear regression analysis, excluding data points where populations were within 0.5 log of the initial population, for determination of the most resistant bacterial spore study. Total kill evaluations were conducted for the microbial resistance in the presence of soil evaluation. Sterility controls for each media and reagent lot were incubated at the respective temperatures with the test samples.

Most Resistant Material Identification

The resistance of a test organism to a decontamination process can vary depending on the contact surface. To identify the most resistant material to VHP, a variety of materials were chosen that represented typical environmental surfaces. These materials included stainless steel (as above), anodized aluminum (T6061, McMaster Carr, Aurora, OH), optical glass (917438,Whatman International Ltd, Clifton, NJ), ceramic tile (67132, Plaid Enterprises), lacquered beechwood (Jackson Hardwood Lumber, Newbury, OH), polyethylene (8597K52, McMaster

Carr), low-density polyethylene (LDPE) (8657K111, McMaster Carr), polypropylene (8742K111, McMaster Carr), silicone (86045K122, McMaster Carr), Viton (86075K32, McMaster Carr), ethylene propylene dimonomer (EPDM) (8610K82, McMaster Carr), polyurethane (8716K32, McMaster Carr), neoprene (N5-10, Best, Menlo, GA), polyvinyl chloride (PVC) (Model No. 156143 isolation chamber material, LaCalhene, Rush City, NY), fiberglass (N03072 GoldSeal, Becton Dickinson, Franklin Lakes, NJ), and paper (No. 2 filter paper, Whatman International Ltd., Clifton, NJ). Each material was prepared as ~ 1 cm^2 coupons.

The materials were cleaned and sterilized as follows: Optical glass, stainless steel, anodized aluminum, and ceramic tile were sonicated for 3 minutes in 0.1% Triton solution, rinsed with DI water, and air-dried. The materials were subsequently transferred to glass petri dishes lined with Whatman No. 2 filter paper and steam sterilized in an autoclave at 132°C for 15 minutes. Fiberglass and paper were purchased precleaned and steam-sterilized. Lacquered beechwood was purchased precleaned but could not be steam-sterilized. EPDM, PET, PP, LDPE, silicone, Viton, and polyurethane were washed as described above, boiled at 100°C for 15 minutes, and air-dried/cooled. Neoprene and PVC were cleaned as above but could not be presterilized. However, controls indicated the absence of microbial contamination on these coupons.

Working spore suspensions of *Geobacillus stearothermophilus* ATCC 7953 (Presque Isle Cultures, Presque Isle, PA) were prepared and enumerated as indicated previously to give a stock preparation of 10^8 cfu/mL in DI water. Ten microliters were inoculated onto each test material and allowed to air dry. Testing was again conducted in the isolation chamber attached to the VHP 1000 unit, using the same programmed cycle that was used for determining the most resistant bacterial spore. Five test carriers of each material were introduced into the isolation chamber at the same time during the decontamination phase of the cycle for each exposure time (10, 20, 30, 45, and 60 minutes). Immediately after exposure, each carrier was aseptically transferred to 10.0 mL of 0.01% catalase and mixed. An additional three inoculated, unexposed carriers for each material were enumerated to determine the average initial spore population. Recovery and incubation of all carriers were conducted as described previously.

Isolator Decontamination Studies

One centimeter square coupons were cut from Whatman No.2 filter paper and steam sterilized prior to use. A G. stearothermophilus ATCC 7953 spore suspension (Presque Isle Cultures, Presque Isle, PA) at 10^8 cfu/mL in 10% or 50% whole defibrinated sheep blood (Crane Lab., East Syracuse, NY) in sterile physiological saline. For each preparation, 10 microliters of the spore preparation were inoculated onto 13 replicate coupons and allowed to dry under ambient temperature for 1 hour. Ten coupons were dispersed at various sites in a 21ft^3 flexible isolator (Model No. 156143, La Calhene, Rush City, MN) and decontaminated with the following VHP1000 cycle:

- Dehumidification: 12 ft^3/minute air flow rate, 2.3 mg/L absolute humidity, 20 minutes
- Conditioning: 10 ft^3/minute air flow rate, 2.6 g/min injection rate, 2 minutes
- Decontamination: 10 ft^3/minute air flow rate, 2.2 g/min injection rate, 45 minutes
- Aeration: 18 ft^3/minute air flow rate, 120 minutes

Hydrogen peroxide residuals were confirmed to be below the OSHA TWA of 1.0 mg/L using a Dragger pump and hydrogen peroxide detectors (BGI Inc., Waltham, MA). All coupons were recovered by individually transferring each one into 10 mL of TSB. For each test cycle, three coupons that were not subjected to decontaminant were used as controls to verify the initial population. Recovery and incubation of all carriers were conducted as described previously at 56±°C. Triplicate decontamination cycles were evaluated.

A further series of triplicate decontamination cycles were conducted as described above in a rigid-walled laminar flow cabinet (SPACE BIO-SAFETY Cabinet, Envair Ltd., Lancashire, England) using the following cycle parameters:

- Dehumidification: 18 ft^3/minute air flow rate, 2.3 mg/L absolute humidity, 20 minutes
- Conditioning: 9 ft^3/minute air flow rate, 3.0 g/min injection rate, 6 minutes

- Decontamination: 10 ft^3/minute air flow rate, 2.6 g/min injection rate, 60 minutes
- Aeration: 18 ft^3/minute air flow rate, 60 minutes

Results

Most Resistant Organism Identification

Geobacillus stearothermophilus spores have been previously proposed as the most resistant organism to hydrogen peroxide vapor (Kokubo et al., 1998). These results have been confirmed against a wider range of test organisms (Table 1). *G. stearothermophilus* spores demonstrated the longest D-value on exposure to hydrogen peroxide vapor at 42.3 seconds. D-values for the other bacterial spore species were all <20 seconds. Further evaluations confirmed these results with other test microorganisms (Table 2). Drying effects contributed to the decreased initial populations of *S.aureus* and *K. pneumoniae* from 10^6 cfu to 10^5 and 10^4 cfu, respectively. It was interesting to note that the fungal spore species that were tested, *A. niger* and *T mentagrophytes*, appeared less resistant

Table 1

Vaporized Hydrogen Peroxide Most Resistant Bacterial Spore

Test Spore	D-value (sec)[a]
Geobacillus stearothermophilus	42.9
Bacillus atrophaeus	18.7
Clostridium sporogenes	15.6
Bacillus circulans	14.4
Bacillus cereus	<10

[a]D-value is the average time in seconds to reduce the population by 1 $\log_{10}$

Table 2

Vaporized Hydrogen Peroxide Microbial Resistance in the Presence of 10% Serum

Test Organism	Log N_o[b]	Total Kill Time (sec)	Est. D-value (sec)
Geobacillus stearothermophilus	5.8	≥ 300	150
Mycobacterium terrae	5.9	120 - 300	20 - 51
Staphylococcus aureus	5.0	120 - 300	24 - 60
Aspergillus niger spores	5.1	60 - 120	12 - 24
Klebsiella pneumoniae	4.2	60 - 120	14 - 29
Trichophyton mentagrophytes	5.4	60 - 120	11 - 22

[b] N_o = initial population

to hydrogen peroxide vapor than the vegetative bacteria *S. aureus* and *M. terrae*. Neutralization studies confirmed that microorganism growth was not affected by the recovery method used (data not shown).

Most Resistant Material Identification

The effect of the contaminated surface material type on the antimicrobial efficacy was tested by inoculating a variety of representative materials with 10^6 G. *stearothermophilus* and exposing the samples to hydrogen peroxide vapor over time (Table 3). VHP sporicidal activity of the hydrogen peroxide sterilant varied depending on the surface type, with paper demonstrating the greatest resistance over the decontamination cycle. A 6 $\log_{10}$ reduction was observed on paper at 45-60 minutes exposure. In comparison, EPDM, polyethylene, beechwood, ceramic tile, and anodized aluminum all demonstrated a 6 $\log_{10}$ reduction within the first exposure time of 10 minutes. Further, neutralization was adequate with each material exposed to VHP for 60 minutes, with no statistical differences between microbial recovery controls and neutralized tests. No visual changes were observed on any of the materials tested (data not shown).

Area Decontamination Testing

VHP decontamination cycles were tested under worst-case conditions. For this investigation 10^6 G. stearothermophilus spores, the most resistant organism, were inoculated onto paper coupons, the most resistant material, as described above in the presence of 50% whole blood, a representative organic/inorganic test soil. Ten coupons for each test cycle were distributed around the test chambers (one rigid

Table 3

Vaporized Hydrogen Peroxide Most Resistant Material

Material	Average survivors (Log_{10})[a] at VHP Exposure Times (min)				
	10	20	30	45	50
Aluminum	0	0	0	0	0
Ceramic Tile	0	0	0	0	0
EPDM	0	0	0	0	0
Polyethylene	0	0	0	0	0
Beechwood	0	0	0	0	0
Neoprene	<1	0	0	0	0
Polypropylene	<1	0	0	0	0
Silicone	<1	0	0	0	0
Stainless Steel	<1	0	0	0	0
Glass	1.2	1.1	0	0	0
LDPE	1/8	<1	0	0	0
Polyurethane	4.3	<1	0	0	0
Viton	3.6	1.9	<1	0	0
Paper	5.4	5.1	1.9	<1	0

[a] 5 coupons of each material tested at each time point

and one flexible-walled isolator) and decontamination cycles tested in triplicate. During exposure it was noted that the blood changed color from red to white, indicating reaction with the hydrogen peroxide. Following 45-60 minutes of exposure to hydrogen peroxide (decontamination" phase of the cycle as indicated in Figure 1), no growth (≥6 $\log_{10}$ spore reduction) was observed from any of the 60 exposed coupons. No physical, chemical, or functional changes were observed in the isolators tested or their contents following the decontamination cycles.

Discussion

VHP Technology has been used for over 10 years as an alternative to formaldehyde or other liquid/gaseous methods for isolator decontamination. Recently, this method has also been used for larger area decontamination, for example cleanrooms (Malborg et al., 2001), research areas (Krause et al., 2001), and, of particular note, for *B. anthracis* building decontamination (EPA, 2004). Many of these applications have emphasized the need to demonstrate the potential limitations of VHP Technology, especially its efficacy on various surface types and in the presence of contaminating soils. As for any biocide, the efficacy of hydrogen peroxide vapor could be affected by the presence of both organic (e.g., proteins, lipids) and inorganic (e.g., water hardness, heavy metals), which may reduce the penetration and activity of the agent (Block, 1991). Inclusion of these components during worst-case testing is important in order to represent a more realistic situation for area decontamination, where cleaning is often difficult (for example, in HVAC systems) or there is a desire to reduce the potential contamination before entering a given area. In the case of formaldehyde, this has not been widely tested and may be often overlooked. Formaldehyde, like other aldehydes, has a cross-linking mode of action which can fix contaminating material onto a given surface; hence a surface layer may be inactivated or fixed but viable microorganisms may remain below the surface. This may be postulated as being part of the reason that organisms exposed to a lethal dose have been proposed as being "injured" but remain viable following a disinfection process (Williams & Russell, 1993).

Hydrogen peroxide is a potent but relatively safe antimicrobial. Its mode of action is proposed to be due to direct interaction with cellular components, including proteins (McDonnell & Russell, 1999); however, the mode of action of VHP Technology appears to be somewhat distinct from liquid hydrogen peroxide, as demonstrated by differences in microbial resistance (Block, 1991). In an additional example, hydrogen peroxide vapor has been shown to break down protein, including some protein-based toxins, while liquid hydrogen peroxide (7%) may actually have a fixing reaction (McDonnell, unpublished results). Therefore, the results in this report reflect the activity observed for hydrogen peroxide only, in its 'dry' vapor phase, under conditions where the hydrogen peroxide concentration was maintained below a given condensation point (Figure 1). In this form, hydrogen peroxide was confirmed to have a broad spectrum of antimicrobial activity at relatively low concentrations (~ 1 mg/L).

In general, bacterial spores are considered the most resistant organisms to most disinfection and sterilization processes. This was also found to be the case with VHP with the confirmation of *G. stearothermophilus* spores as the most resistant organism. It should be noted that this is in contrast to studies on the most resistant organism to liquid peroxide, which is *B. atrophaeus* (previously known as *B. subtilis*). *M. terrae* and *S. aureus* actually had greater resistance than some spore-forming species (including fungi and bacterial strains). These differences were slight at the hydrogen peroxide concentrations tested and may be attributed to the presence of growth media that were not present in purified bacterial spore preparations and the production of the hydrogen peroxide degrading enzyme catalase by the *S. aureus* strain. These results have also been verified in other studies (A. Bennett, personal communication). *Mycobacterium* species are generally considered more resistant than other vegetative bacteria due to their unique lipophilic cell wall structure, which can impede the penetration and activity of biocides (McDonnell & Russell, 1999).

The activity of any biocide or physical decontamination process will also vary depending on the type of surface being decontaminated. A representative number of materials were tested that could be

found in rooms, facilities, and/or isolators, and these did vary on decontamination efficacy with hydrogen peroxide vapor. All materials showed a rapid decrease in spore concentration within the first 10 minutes of contact, with the exception of the porous materials (polyurethane, Viton, and, particularly, paper). Similar results for polyurethane and Viton have been reported for liquid chemical sterilization with an oxidizing agent (Justi et al., 2000). In these cases, it is necessary for hydrogen peroxide to penetrate into these materials to ensure adequate spore reduction. Similarly, paper is also porous and as a cellulosic material will increase the degradation rate of hydrogen peroxide in comparison to the other materials. It should also be noted that beechwood, which is also a cellulosic-based material, did not show the same resistance to hydrogen peroxide, presumably due to its processed (lacquered) nature. With prolonged incubation hydrogen peroxide demonstrated a >6 log reduction with the most resistant material being paper (45-60 minutes for complete log reduction). In general, utilization of hydrogen peroxide in these areas would be restricted and evaluated relative to type, scope, and source of contamination. Typically, areas containing large amounts of paper require a combination approach of decontamination methods, such as the coupling of liquids and gases with a removal plan.

The efficacy of hydrogen peroxide to decontaminate under worst-case conditions (defined as the presence of the most resistant organism on the most resistant material and in the presence of 50% whole blood) was also verified. In all test decontamination cycles, greater than a 6-log spore reduction was confirmed. It was clear that hydrogen peroxide reacted with the blood components, as indicated by a change in inoculum color from red to white during the test cycles; investigations of log reduction during the cycle demonstrated that little to no spore reduction was observed until the blood began to change color. These results are distinct to reports with the use of a gaseous hydrogen peroxide/plasma sterilization system, which showed that sterilization efficacy was compromised in the presence of contaminated soil (Alfa et al., 1996). As shown in this report, the efficacy of gaseous hydrogen peroxide, like any biocidal process, can be reduced in the presence of visual soil but can be improved with longer exposure times. It is also clear that the cleaner the surface the more reproducible a given decontamination method will be. A further consideration for fumigation methods is to ensure that all areas of a given enclosed area are contacted for the desired validation decontamination time.

In conclusion, VHP Technology was verified as an alternative to formaldehyde for the safe and effective decontamination of enclosed areas, even under soiled conditions. Validated processes should only be used to ensure the robustness of the fumigation process.

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