Biodecontamination of Animal Rooms and Heat-Sensitive Equipment with Vaporized Hydrogen Peroxide

JUERGEN KRAUSE BSC,[1] GERALD MCDONNELL BSC, PHD,[2] AND HERMANN RIEDESEL DVM, PHD[1*]

Common methods used to decontaminate and disinfect laboratory animal areas are difficult to standardize, labor-intensive, and potentially hazardous for staff members and the environment. As an alternative to traditional methods, we tested fumigation with vaporized hydrogen peroxide by using the VHP 1000 Biodecontamination System. The design of our air-conditioning system allowed the connection of the generator to any animal room by using the ventilation piping, thus forming a closed circuit. A 3-h cycle consisting of dehumidification, conditioning, sterilization, and aeration was developed and shown to be effective. The biodecontamination process was monitored during five independent trials using chemical and biological (*Bacillus stearothermophilus* spores) contaminants. Contact plates for testing surfaces and room air for environmental bacteria, yeasts, and fungi consistently showed fewer than 10 colony-forming units per 100 cm^2 or per 1 liter air. In addition, this method proved successful with heat-sensitive equipment like the blower units of individually ventilated caging systems. Overall, the system was easy to use and very effective in biodecontaminating animal rooms and equipment in a reproducible manner. There were no signs of corrosion or functional damage after more than 10 fumigation cycles. Work load and potential health risk for staff members and the environment was negligible.

A variety of methods and antimicrobial agents have been used for the periodic decontamination of critical environments. These methods generally are difficult to standardize, labor-intensive, and potentially hazardous for staff members and the environment. Conventional wipe-down techniques with antimicrobial products are very time-consuming and difficult to standardize. For disinfecting laboratory animal rooms and temperature-sensitive (nonautoclavable) materials, it is general practice to use spraying, fogging, or fumigation techniques, in particular with formaldehyde-based agents. Traditional fumigation with formaldehyde may be effective (1), but it is slow, difficult to standardize, and disruptive, but more importantly, toxic and carcinogenic. For these reasons, the use of formaldehyde has been strictly regulated by authorities for some years.

Safe, automated decontamination methods are being more widely used as an alternative to formaldehyde because of their ease of use, higher levels of sterility assurance, and overall cost savings to a facility. Vaporized hydrogen peroxide (VHP) has been used widely for sterilization of pharmaceutical applications, including production filling lines, sterility testing environments, sealable enclosures, production rooms, and lyophilizers (2, 3). This method has more recently been applied for the decontamination of animal rooms, as an alternative to formaldehyde. Compared to formaldehyde (Table 1), hydrogen peroxide vapor is a broad-spectrum antimicrobial with virucidal, bactericidal, fungicidal, and sporicidal activity (4-6). In addition, because the vapor readily breaks down into water and oxygen, the process has none of the environmental concerns associated with formaldehyde. The VHP decontamination process is based on the production and maintenance of hydrogen peroxide vapor in an enclosed environment. The process maintains the hydrogen peroxide vapor concentration below the condensation (or dew) point; therefore, VHP decontamination is essentially 'dry' and demonstrates excellent material compatibility. The aim of this study was to develop, evaluate, and validate the VHP process for the decontamination of animal rooms.

Animal Unit and Transgenic Control Facility, Max Planck Institute for Experimental Medicine, Hermann Rein Strasse 3, 37075 Goettingen, Germany,[1] and STERIS Corporation, 5960 Heisley Road, Mentor, Ohio 44060[2]

**Corresponding author*

Materials and Methods

VHP 1000 Biodecontamination System. The VHP 1000 Biodecontamination System (STERIS Corporation, Mentor, Ohio) is a compact, mobile unit that generates and controls VHP delivery into an enclosed environment. The cycle consists of four phases: dehumidification, conditioning, decontamination, and aeration. During dehumidification, the relative humidity is reduced to 10% to 30% by circulation of the air in a closed loop. During conditioning, VHP is produced by vaporization of 31% or 35% liquid hydrogen peroxide and is introduced into the recirculating air stream to achieve the desired VHP concentration rapidly. The decontamination phase proceeds identically to the conditioning phase but at a steady-state injection and recirculation flow rate to maintain the VHP concentration for the desired exposure time. In contrast to the liquid, the vapor is sporicidal at low concentrations (typically, 1 to 2 mg/L in vapor at 25°C) (4), and the concentration is maintained at a constant level by continually introducing VHP in the incoming air and catalytically degrading VHP present in the returning air line over the programmed exposure cycle. Finally, during aeration, VHP is no longer introduced, and the residual vapor is catalytically decomposed into water and oxygen by recirculation through the destroyer or by using the room ventilation system after decontamination. The VHP 1000 microprocessor automatically monitors and/or controls the process parameters during each cycle.

Room decontamination. The animal room had a volume of 63 m^3 (2230 ft^3) and included 10 movable stainless-steel racks, each with seven shelves, yielding a maximum capacity of 350 Type-II cages (Type 1284 L, 365 mm × 207 mm × 140 mm, Tecniplast S. A. R. L., Buguggiate, Italy). The air-conditioning system allowed the direct integration of the VHP 1000 unit into the room air supply, by using the ventilation piping to form a closed circuit (Fig. 1). Vaporized hydrogen peroxide was generated from a 31% hydrogen peroxide solution (Vaprox, STERIS Corporation). To ensure equal distribution of the vapor, four oscillating warm-air fan heaters (Atlantis Electronic, Berlin, Germany) were placed in the room to raise the temperature from 22 to 35°C and to reduce the relative humidity from 55% to 22%. The decontamination cycle (total time, 3 h) was developed according to manufacturer's instructions and in light of the room

Table 1. Comparison of formaldehyde and VHP 1000 biodecontamination processes

Fumigant	Description	Advantages	Disadvantages
Formaldehyde	Liquid or aerosolized formalin or formaldehyde gas formed by heating paraformaldehyde or formalin added to potassium permanganate crystals	Inexpensive Claimed broad-spectrum efficacy	Slow-acting, long exposure times Residues can accumulate on surfaces and may be difficult to remove Cross-linking mode of action Extremely toxic, carcinogenic, strong irritant Surfaces need to be precleaned Requires high humidity for efficacy Not automated
Vaporized Hydrogen Peroxide ('Dry')	STERIS VHP 1000 System generates and delivers vaporized hydrogen peroxide at pre-set control cycles, preventing condensation onto a target surface	Rapid Validated Published broad-spectrum efficacy Material compatibility Rapidly degrades into water and oxygen Automated, controlled process	May cause minor cosmetic material surface changes[a] Surfaces should be precleaned Does not work well with highly absorptive materials (e.g., cellulosics)

[a]For example, slight discolorization of colored anodized surfaces over repeated cycles.

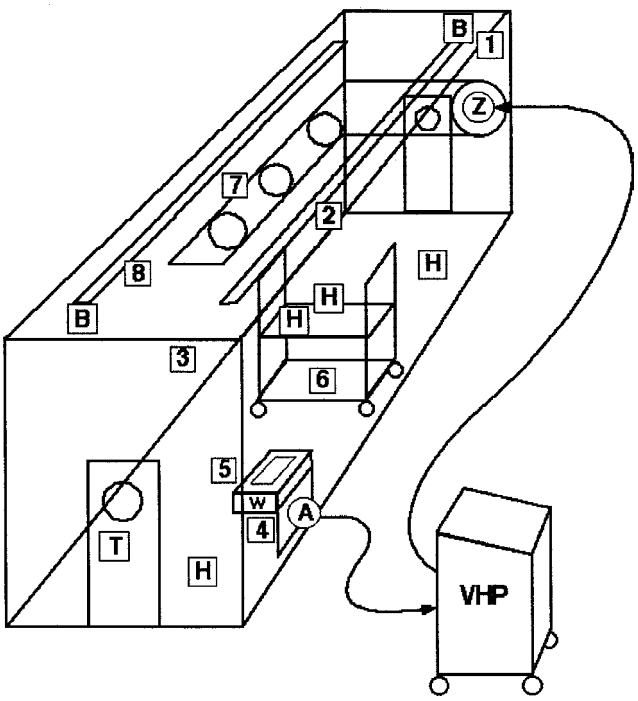

Figure 1. Animal room set-up for decontamination. (A) exhaust air; (B) light fixtures; (H) air fan heaters; (T) thermohydrograph; (VHP) VHP 1000 unit (outside room); (W) hand basin; (Z) air inlet; (1 through 8) locations of biological and chemical indicator strips.

volume, air temperature, and humidity:

Dehumidification	Airflow: 32 m^3/hour Time: 10 min Humidity: 6.9 mg/L (30% relative humidity at 25°C)
Conditioning	Airflow: 32 m^3/hour H_2O_2 injection: 8.2 g/min Time: 15 min
Decontamination	Airflow: 32 m^3/hour H_2O_2 injection: 11 g/min Time: 75 min
Aeration	Air-handling system runs normally for 30 min

Decontamination validation and monitoring. Air temperature and humidity were monitored by using a thermohydrometer (Model CT 485, Newport Electronics, Deckenpfronn, Germany). Draeger glass vials (No. 8101041, Draeger, Luebeck, Germany) were used to monitor hydrogen peroxide concentrations in the room and adjacent areas.

Decontamination validation of five biodecontamination cycles was performed with chemical and biological indicators (STERIS Chemido-VHP-Short Strip and STERIS Spordex-VHP-Bioindicators, 5×10^5 spores of *Bacillus stearothermophilus* in glassine envelopes; STERIS Corporation) placed throughout the room. After decontamination cycles, the chemical indicators were examined for color changes indicative of the presence of VHP. The biological indicators were recovered and incubated in growth media (CASO medium, Merck, Darmstadt, Germany) at 55°C for 7 days, to indicate the presence or absence of growth. Microbiological air quality was analyzed by using an MAS-100 air sampler (Merck), and surface contamination was monitored by using standardized Envirocheck Rodac contact plates (GKZ- and H+S- plates, Merck). 500 L of room air was sampled before and after each decontamination cycle. All plates were incubated in an incubator at 25°C for up to 7 days and examined for growth. Results were recorded as total colony-forming units or yeast and fungal counts per cm^2 contact plate surface or per 1 L sampled air.

Decontamination of blower units. Biodecontamination of the room was repeated, as described above, but in the presence of five used blower units of an individually ventilated caging (IVC) system. The units were operating but not connected to the cage racks.

Results

Development of the room decontamination cycle. The design of the air conditioning system allowed for the direct connection of the VHP 1000 system to any separate animal room or section of the facility. This property allowed for the decontamination of a room while work continued in adjacent rooms or areas. The room described in this report included a cage-rack system, the contents of which were cleaned prior to decontamination. Preheating the room to 35°C allowed for a more rapid cycle to be developed at higher allowable hydrogen peroxide concentrations, but below the dew point to avoid condensation. Condensation of hydrogen peroxide needed to be avoided to prevent material incompatibility and variable vapor distribution. During cycle development and subsequent validation, no corrosion, cosmetic changes, or residues were observed in the room or on components. The cycle time was further decreased by using the room ventilation system to flush the residual vapor from the room by the introduction of fresh, HEPA-filtered air. A total cycle time of 3 h is much less than that previously required for formaldehyde-based decontamination (> 12 h).

Validation of room decontamination. To validate the efficiency of biodecontamination, chemical and biological indicators were placed in various locations in the rooms (Fig. 1). The results following five biodecontamination cycles are shown in Table 2. With the exception of the variable light-fixture results, the

Table 2. Chemical and biological results following biodecontamination cycles

Sample no.	Location[a]	No. chemical indicators that passed[b]	No. biological indicators that showed growth[b]
1	Top of wall	5	0
2	Bottom of wall	5	0
3	Middle of wall	5	0
4	Hand basin (bottom)	5	0
5	Floor	5	0
6	Trolley	5	0
7	Air-conditioning vent	5	0
8	Light fixture[c]	3	3
	Total	38 of 40	3 of 40

[a]See Fig. 1 for location of indicators in room.
[b]Each site was tested by using a single chemical and biological indicator for each of five biodecontamination cycles.
[c]These indicators were placed in a barely accessible gap between the neon bulbs and light fixture.

biodecontamination cycles were successful. Only two chemical and three biological indicators demonstrated growth, and these had been placed in a barely accessible gap between the neon tubes and the light fixture in the room. This location was not deemed to be a critical area, but positioning additional fans could ensure contact in this area. To provide further information on vapor distribution in the room, additional chemical indicators were placed on the door windows and in miscellaneous key areas, including the floor drain, closed wall sockets, and a hose rack. The presence of hydrogen peroxide was verified in all locations tested. During room fumigation, hydrogen peroxide concentrations on the external corridor adjacent to the room were monitored and never exceeded 0.02 ppm, well below the acceptable range of < 1ppm. After room aeration for 30 min., the hydrogen peroxide concentrations were always below 0.2 ppm, which allowed access to the room.

Environmental monitoring. The results from microbiological monitoring of the room air and contact surfaces are summarized in Table 3. After biodecontamination, no noteworthy contaminants were observed, confirming the efficacy of the process.

Biodecontamination of IVC blower units. Biodecontamination cycles were repeated in the presence of five IVC blower units, which contain heat-sensitive equipment. During these cycles, chemical and biological indicators were placed in the air supply and exhaust to test for vapor penetration (Table 4). All indicators repeatedly confirmed efficacy. In addition, prior to and directly after fumigation, microbial contamination was monitored (Table 4). After fumigation, no contamination was observed, even in extremely contaminated and dusty areas of the exhaust pipes, thereby confirming efficacy of the process. Further, no residues or material or physical damage was observed after repeated decontamination cycles.

Discussion

Biodecontamination with vapor phase hydrogen peroxide by using the VHP 1000 system was found to be a very effective method of decontaminating animal rooms, laboratory equipment, and heat-sensitive caging equipment. The process is documented, is reproducible, and fully complies with GLP/GMP regulations. The facility was designed in such a way that every animal room could be used as a pass-through room, so that animals can be transferred to a clean corridor during decontamination. Decontamination was performed conveniently by linking the VHP 1000 system external to the target room and directly to the air-conditioning ductwork to allow the introduction, circulation, and removal of hydrogen peroxide vapor. This capability was an important consideration during the design of the facility described. In other typical applications, the system can be directly linked to any room via wall-mounted vapor inlet and output ports and ensuring that the rooms are reasonably airtight. Because rooms may vary in size and shape, fumigation cycles are developed to assure that an effective dose of the vapor is distributed evenly in the room. The room decontamination protocol described in this report was validated by using biological strips with *B. stearothermophilus* spores, the organism most resistant to vaporized hydrogen peroxide (4, 7, 8); these indicators showed no growth after exposure to vaporized hydrogen peroxide. In addition, total colony counts for environmental bacteria, yeasts, and fungi showed fewer than 10 colony-forming units per 100 cm^2 or per 1 L room air. Similar cycles have been developed and validated for other rooms and common areas of the facility.

Table 3. Microbiological monitoring results before and after fumigation cycles

Sample location	Total CFU[a] before fumigation	Total CFU[a] after fumigation	Yeast + fungal colonies[b] before fumigation	Yeast + fungal colonies[b] after fumigation
Floor surface	330 ± 107	0	290 ± 76	0
Room air	426 ± 151	2 ± 1	337 ± 83	0

[a]Average number of colony-forming units (CFU) per 100 cm^2 of floor contact surface (Rodac plates) or per 1 L of air sampled. Average of five runs tested are given as mean ± 1 standard deviation. CFU ≥ 5 are considered significant.
[b]Average total yeast and fungal colony counts per 100 cm^2 of floor contact surface (Rodac plates) or per 1 L of air sampled. Average of five runs tested are given as mean ± standard deviation.

Table 4. Biodecontamination of five used blower units of an individually ventilated caging system

Location	No. chemical indicators that passed[a]	No. biological indicators that showed growth[a]	Total CFU[b] before fumigation	Total CFU[b] after fumigation	Yeast + fungal colonies[c] before fumigation	Yeast + fungal colonies[c] after fumigation
Air supply (prefilter)	5	0	51 ± 5	0	15 ± 3	0
Air supply (pipe)	5	0	9 ± 2	0	2 ± 2	0
Exhaust air (pipe)	5	0	> 1000	0	> 1000	0
Exhaust air (prefilter)	5	0	168 ± 59	0	50 ± 21	0

[a]Each site was tested by using a single chemical and biological indicator for each of five biodecontamination cycles.
[b]Average number of colony-forming units (CFU) per 100 cm^2 of floor contact surface (Rodac plates) or per 1 L of air sampled. Average of five runs tested are given as mean ± 1 standard deviation. CFU ≥ 5 are considered significant.
[c]Average total yeast and fungal colony counts per 100 cm^2 of floor contact surface (Rodac plates) or per 1 L of air sampled. Average of five runs tested are given as mean ± standard deviation.

The VHP method also can be used for decontaminating ventilation pipes, HEPA filters, heat-sensitive equipment like IVC blower units, cage-changing stations, and a variety of lab equipment (e.g., scales, warming plates, stereomicroscopes, and centrifuges). In addition to the example given in this report, we have demonstrated successful biodecontamination of fully equipped transgenic and embryo-handling laboratories, with no physical, chemical, functional, or electronic incompatibilities observed to date.

Compared to other decontamination methods like ethylene oxide or formaldehyde fumigation, which are toxic and harmful for personnel and the environment, the VHP biodecontamination is a safe alternative. Hydrogen peroxide breaks down into oxygen and water, both of which can be safely released into the atmosphere. After formaldehyde fumigation, residues have to be neutralized with ammonia. In addition, the remaining hexamethylene-tetramine salt has to be cleaned by hand from all room surfaces. This compound also can clog HEPA filters and reduce their life span. Further, formaldehyde fumigation is disruptive to a facility's operation and often requires evacuation during long cycle times.

VHP fumigation is performed automatically from the dirty corridor of our facility via the ventilation pipes and can be isolated to individual rooms. Staff were only potentially exposed to hydrogen peroxide liquid when inserting a fresh hydrogen peroxide bottle into the VHP generator. During fumigation, the VHP concentration on the floor close to the room or in the generator amounts to 0.02 ppm or less, even though the animal rooms did not have specifically gas-tight doors. However, as for any fumigation process, it was important that all surfaces to be decontaminated had access to the vapor. Narrow gaps may not be fully penetrated by the decontaminant and may require special consideration. It is recommended that these locations (e.g., floor drains) should be manually disinfected by using a broad-spectrum disinfectant/sterilant.

In conclusion, vaporized hydrogen peroxide is a highly effective and safe method for decontamination of animal rooms as well as heat-sensitive caging and various types of laboratory equipment. This method could be validated, was reproducible, and significantly reduced the time necessary for fumigation (3 h). The VHP method entailed a minimum workload, with no risk to personnel and the environment. No signs of corrosion or functional damage were detected after multiple decontamination cycles.

Acknowledgments

We thank Monika Schindler and Simone Schmidt for their skillful technical assistance and microbiological monitoring.

References

1. **Peters, J., and G. Spicher.** 1981. Room disinfection by formaldehyde vaporization. Hyg. Med. **6:**337-344.
2. **Jahnke, M., and G. Lauth.** 1997. Biodecontamination of a large volume filling room with hydrogen peroxide. Pharm. Eng. **17:**96-108.
3. **Suen, J., L. Alderman, and M. P. Kiley.** 1990. Use of vapor phase hydrogen peroxide (VPHP) as a space decontaminant, abstract 33, p. 21. *In* Program and abstracts of the ASM International Symposium on Chemical Germicides. American Society for Microbiology, Washington, D.C.
4. **Block, S. S.** 1991. Peroxygen compounds, p. 167-181. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation. Lea & Febiger, Philadelphia.
5. **Klapes, N. A., and D. Vesley.** 1990. Vapor-phase hydrogen peroxide as a surface decontaminant and sterilant. Appl. Environ. Microbiol. **56:**503-506.
6. **Heckert, R. A., M. Best, L. T. Jordan, G. C. Dulac, D. L. Eddington, and W. G. Sterritt.** 1997. Efficacy of vaporized hydrogen peroxide against exotic animal viruses. Appl. Environ. Microbiol. **63:**3916-3918.
7. **Rickloff, J., and P. Osrelski.** 1989. Resistance of various microorganisms to vaporized hydrogen peroxide in a prototype table top sterilizer, abstract Q-59, p. 339. *In* Abstracts of the 89th Annual Meeting of the American Society for Microbiology. American Society for Microbiology, Washington, D.C.
8. **Kokubo, M., T. Inoue, and J. Akers.** 1998. Resistance of common environmental spores of the genus *Bacillus* to vapor hydrogen peroxide vapor. J. Pharm. Sci. Technol. **52:**228-231.