APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1997, p. 3916–3918 Vol. 63, No. 10
0099-2240/97/$04.00+0

Efficacy of Vaporized Hydrogen Peroxide against Exotic Animal Viruses

R. A. HECKERT,[1]* M. BEST,[2] L. T. JORDAN,[3] G. C. DULAC,[4]
D. L. EDDINGTON,[5] AND W. G. STERRITT[2]

Animal Diseases Research Institute[1] and Animal and Plant Health Directorate,[2] Canadian Food Inspection Agency, Nepean, Ontario, Canada K2H 8P9; Animal Diseases Research Institute, Canadian Food Inspection Agency, Lethbridge, Alberta, Canada T1J 3Z4[3]; Animal and Plant Health Directorate, Canadian Food Inspection Agency, Nepean, Ontario, Canada K1A 0Y9[4]; and Steris Corporation, Mentor, Ohio 44060-1834[5]

Received 15 April 1997/Accepted 30 July 1997

The efficacy of vapor-phase hydrogen peroxide in a pass-through box for the decontamination of equipment and inanimate materials potentially contaminated with exotic animal viruses was evaluated. Tests were conducted with a variety of viral agents, which included representatives of several virus families (*Orthomyxoviridae*, *Reoviridae*, *Flaviviridae*, *Paramyxoviridae*, *Herpesviridae*, *Picornaviridae*, *Caliciviridae*, and *Rhabdoviridae*) from both avian and mammalian species, with particular emphasis on animal viruses exotic to Canada. The effects of the gas on a variety of laboratory equipment were also studied. Virus suspensions in cell culture media, egg fluid, or blood were dried onto glass and stainless steel. Virus viability was assessed after exposure to vapor-phase hydrogen peroxide for 30 min. For all viruses tested and under all conditions (except one), the decontamination process reduced the virus titer to 0 embryo-lethal doses for the avian viruses (avian influenza and Newcastle disease viruses) or less than 10 tissue culture infective doses for the mammalian viruses (African swine fever, bluetongue, hog cholera, pseudorabies, swine vesicular disease, vesicular exanthema, and vesicular stomatitis viruses). The laboratory equipment exposed to the gas appeared to suffer no adverse effects. Vapor-phase hydrogen peroxide decontamination can be recommended as a safe and efficacious way of removing potentially virus-contaminated objects from biocontainment level III laboratories in which exotic animal disease virus agents are handled.

While aqueous hydrogen peroxide (H_2O_2) has been successfully used for the disinfection of inanimate surfaces, the use of vapor-phase H_2O_2 (VPHP) as a gaseous disinfectant is a relatively new development. In the past, equipment and heat-sensitive materials that could not otherwise be treated were decontaminated with formaldehyde or ethylene oxide. While these gases are highly effective, they are toxic, carcinogenic, and potentially explosive; they require careful handling procedures; and ethylene oxide requires long ventilation periods. VPHP represents a safe alternative to the use of these toxic gases and the need for their neutralization prior to release into the atmosphere. VPHP breaks down into oxygen and water, both of which are environmentally benign.

Aqueous H_2O_2 is active against a wide range of organisms: bacteria, yeasts, fungi, viruses, and spores (1). There is, however, a paucity of information available on the efficacy of VPHP, and its activity against exotic animal viruses is not known. Klapes and Vesley (4) demonstrated sporicidal capability in the experimental use of VPHP for the decontamination of an ultracentrifuge. Rickloff and Orelski reported on the use of VPHP for the sterilization of dental instruments (6). In another paper, Suen et al. (7) used VPHP for decontamination of rooms and biological safety cabinets. They demonstrated that coupons contaminated with bacterial spores and suspended in different parts of the room were successfully decontaminated (7). VPHP has also been successfully used for the sterilization of freeze dryers (2).

VPHP has potential for use in laboratories and research institutions for the decontamination of laboratory equipment susceptible to heat. In a biocontainment level III laboratory setting, all materials exiting the facility must be effectively decontaminated. This can present a problem for items that cannot be autoclaved or passed through a liquid-disinfectant tank. VPHP represents a potentially useful fumigant in pass-through boxes used to decontaminate materials exiting the laboratory.

This study was initiated to validate the efficacy of VPHP in a pass-through box for the decontamination of equipment and inanimate materials potentially contaminated with exotic animal viruses. Tests were conducted with viral agents derived from lists A and B of the Office International des Epizooties (5). These included representatives of several virus families, from both avian and mammalian species.

MATERIALS AND METHODS

Equipment. VPHP was generated by a biodecontamination system (VHP 1000; Steris Corporation, Mentor, Ohio) which controlled all phases (dehumidification, peroxide generation, and aeration) of the decontamination process. This machine was connected to an 18-ft^3 stainless-steel pass-through box via 1.5-in. Tygon tubing. The pass-through box was equipped with two internal circulation fans to ensure even distribution of VPHP within the box. The pressure within the box was monitored by a manometer during each decontamination run. The temperature was monitored at three different locations within the box by temperature probes during all decontamination runs. The pass box was leak tested by pressurizing the enclosure to an equivalent of a 2.0-in. water column, and a decay rate of 0.08 in./h was calculated over an observation period of 18 h.

Cycle parameters. The biodecontamination system was programed to perform the following phases. Dehumidification was carried out for 10 min with an airflow rate of 10 ft^3/min (cfm) to a target relative humidity of <5%. VPHP was then generated from a 30% (wt/wt) solution of aqueous H_2O_2 at a rate of 2 g/min for 30 min at an airflow rate of 10 cfm, calculated to maintain a steady state of hydrogen peroxide of approximately 1.73 mg/liter or 1,211 ppm. Following decontamination, aeration occurred for 210 min at an airflow rate of 10 cfm. At all phases of the decontamination cycle, a negative pressure equivalent to that of a 2.0- to 3.0-in. water column was maintained in the enclosure.

* Corresponding author. Mailing address: Canadian Food Inspection Agency, Animal Diseases Research Institute, 3851 Fallowfield Rd., P.O. Box 11300, Station H, Nepean, Ontario, Canada K2H 8P9. Phone: (819) 997-3303. Fax: (819) 953-6399.

TABLE 1. Virus types and original titers subject to VPHP treatment

Common virus name	Virus family	Strain	Source	Titer (log_{10}/ml)
Avian influenza virus	*Orthomyxoviridae*	A/H5N2/Chick/Penn/83	Allantoic fluid	8.5
African swine fever virus	Unclassified	Lisbon 61	Cell culture	7.5
Bluetonge virus	*Reoviridae*	Type 2	Cell culture	6.5
Hog cholera virus	*Flaviviridae*	Baker-Endpoint	Cell culture	7.1
		Standard	Blood	6.6
Newcastle disease virus	*Paramyxoviridae*	Texas GB	Allantoic fluid	9.6
Pseudorabies virus	*Herpesviridae*	Shope	Cell culture	8.0
Swine vesicular disease virus	*Picornaviridae*	UK/72	Cell culture	8.1
Vesicular exanthema virus	*Caliciviridae*		Cell culture	9.0
Vesicular stomatitis virus	*Rhabdoviridae*	New Jersey	Cell culture	7.8
			Allantoic fluid	7.8

Biological indicators. Spore strips impregnated with *Bacillus stearothermophilus* (Spordex; Steris Corporation) were placed in the pass-through box during each of the decontamination runs. After the decontamination run, the indicators were suspended in Trypticase soy broth and incubated at 56°C for 7 days. Turbidity of the medium was an indication of bacterial growth. Decontamination runs were required to destroy 3.4×10^5 spores in order for the process to be considered acceptable.

Eight different viruses, representing eight different families of virus, were used in these studies (Table 1). All virus stocks and recovered viruses were titrated by standard methods. Viruses were suspended in either (i) minimal essential medium with 5% fetal bovine serum (a standard growth medium for virus propagation in cells), (ii) allantoic fluid (for viruses grown in eggs), or (iii) porcine blood (for viruses presented in animal blood). Virus inactivation by VPHP was evaluated with the viruses in two conditions: suspended in liquid or dried onto a solid support.

For the liquid studies, 100 μl of each viral suspension was placed onto the bottom of one glass vial or onto one stainless-steel coupon (2.2 by 2.2 mm) and then subjected to the decontamination process (glass only). After decontamination, virus recovery and titration were performed by standard techniques (5). To obtain dried virus, the viruses were dispensed as above, air dried for 16 h (time for complete drying) in a biological safety cabinet, and then subjected to decontamination. After decontamination the dried viruses were resuspended in 1 ml of minimal essential medium for virus recovery and titration.

As a control for the liquid-virus decontamination process, virus was also inoculated into one glass vial or one stainless-steel coupon and kept at −70°C or put in the pass-through box during the decontamination process, but in a sealed container impervious to VPHP gas (glass only). As a control for the dried-virus decontamination process, virus was again inoculated onto glass or stainless steel, dried, and kept either at room temperature (20 ± 2°C) or in the pass-through box during the decontamination process, but not exposed to VPHP gas.

In most cases, each decontamination run (containing one coupon or glass vial for each virus) was carried out three times. The arithmetic mean and standard deviation of titers from the three runs for each of the viruses tested were calculated and are presented.

Compatibility testing. To ensure that VPHP gas could successfully be used as a nondestructive sterilant, several different types of equipment were also fumigated and evaluated for damage. These included a telephone, a camera lens, undeveloped film, X-ray film, developed Polaroid pictures, computer disks (five runs), a laptop computer (five runs), an electric drill, a watch, an electronic timer, and a pipette aid.

RESULTS

During all phases of each run, the pressure inside the enclosure remained at 2 to 3 in. of water. Any deviation from this range would have been an indication of a mechanical failure and reason to abort the cycle. During the dehumidification phase, the temperatures within the pass-through box increased

TABLE 2. Titers of several different exotic animal viruses, suspended in liquid or dried onto a glass or steel surface, before and after exposure to VPHP gas

Virus	Titer (mean ± SD, log_{10}/ml) of virus[a] in:									
	Liquid suspension				Dried state					
	No VPHP			VPHP, in box, glass	No VPHP				VPHP, in box	
	Out of box		In box, glass		Out of box		In box			
	Glass	Steel			Glass	Steel	Glass	Steel	Glass	Steel
AIV	ND	ND	4.5[b]	0	5.68 ± 0.14	5.68 ± 0.14	2.60 ± 0.14	2.91 ± 0.63	0 ± 0.0	0 ± 0.0
ASFV	6.73 ± 0.8	6.35 ± 0.58	5.96 ± 1.06	<1[c]	5.89 ± 0.29	6.05 ± 0.25	0.06 ± 0.04	0.06 ± 0.01	<1	<1
BTV	4.43 ± 0.14	4.35 ± 0.14	4.39 ± 0.18	<1	4.43 ± 0.14	4.55 ± 0.25	1.32 ± 0.72	1.31 ± 0.8	<1	<1
HCV-CC	6.55 ± 0.25	6.85 ± 0.14	6.0[b]	<1	58.5 ± 0.14	5.74 ± 0.29	0 ± 0.0	0 ± 0.0	<1	<1
HCV-WB	6.99 ± 0.29	6.74 ± 0.29	5.64 ± 0.18	5.5 ± 0.0	6.8 ± 0.25	6.86 ± 0.43	4.3 ± 0.25	3.81 ± 0.8	4.18 ± 0.14	4.35 ± 0.14
NDV	ND	ND	8.25[b]	0 ± 0.0	9.14 ± 0.29	8.5 ± 0.0	6.5 ± 0.0	6.1 ± 0.14	0 ± 0.0	0 ± 0.0
PRV	6.95 ± 0.38	7.24 ± 0.29	6.75 ± 0.0	<1	6.1 ± 0.14	6.1 ± 0.14	4.35 ± 0.14	4.43 ± 0.14	<1	<1
SVDV	7.8 ± 0.25	8.18 ± 0.14	7.75[b]	<1	7.7 ± 0.38	8.01 ± 0.38	0 ± 0.0	0 ± 0.0	<1	<1
VEV	7.7 ± 0.38	8.0 ± 0.0	2.0 ± 0.0	<1	2.26 ± 0.38	2.55 ± 0.25	0.1 ± 0.0	0.1 ± 0.0	<1	<1
VSV-CC	5.04 ± 1.15	5.99 ± 0.29	4.75[b]	<1	3.04 ± 1.5	4.55 ± 0.25	0 ± 0.0	0 ± 0.0	<1	<1
VSV-AF	6.86 ± 0.43	7.01 ± 0.38	7.75[b]	<1	5.24 ± 0.52	4.34 ± 0.66	3.68 ± 0.14	3.89 ± 0.29	<1	<1

[a] AIV, avian influenza virus; ASFV, African swine fever virus; BTV, bluetongue virus; HCV-CC, hog cholera virus in cell culture medium; HCV-WB, hog cholera virus in whole blood; NDV, Newcastle disease virus; PRV, pseudoroabies virus; SVDV, swine vesicular disease virus; VEV, vesicular exanthema virus; VSV-CC, vesicular stomatitis virus in cell culture medium; VSV-AF, vesicular stomatitis virus in allantoic fluid. Out of box, samples not placed in decontamination chamber; in box, samples placed in decontamination chamber; ND, not done.

[b] Not replicated.

[c] Sample could not be assayed at a dilution of less than 1/10 because of toxicity in the assay system at lower dilutions.

considerably from ambient temperatures (20 to 24°C) to a range of 30 to 40°C depending on the location within the enclosure. The temperature continued to be within this range during the sterilization phase but then decreased to room temperature during aeration.

Culturing of the biological indicators after each decontamination run indicated a complete inactivation of all the *Bacillus* spores due to a lack of spore growth after incubation in growth media, and the run was considered successful.

The effects of the decontamination process on the titers of the various viruses are shown in Table 2. In all cases (except for hog cholera virus suspended in blood) the decontamination process reduced the virus titer from 8.5 or 9.6 50% embryo lethal doses (ELD_{50}) to 0 ELD_{50} for the avian viruses or from 6.5 to 9.0 50% tissue culture infective doses ($TCID_{50}$) to less than 10 $TCID_{50}$ for the mammalian viruses. This observation was true when the viruses were tested both in liquid and in dried states. In the case of swine vesicular exanthema virus, heating alone reduced the amount of recoverable virus by several log units. In the case of vesicular disease virus, hog cholera virus in cell culture medium, and vesicular stomatitis virus in cell culture medium, drying alone reduced the recoverable virus titer to less than 10 $TCID_{50}$. Only avian influenza virus, hog cholera virus in blood, Newcastle disease virus, pseudorabies virus, and vesicular stomatitis virus in allantoic fluid retained a titer of greater than 1 log unit after being dried and then heated in the pass-through box but not exposed to VPHP (Table 2). In most cases, there were no apparent differences between the virus titers recovered from glass and those recovered from steel. Nor was there any apparent difference in the killing ability of the VPHP gas when the virus was present in cell culture fluid or allantoic fluid, as shown for vesicular stomatitis virus.

No adverse effects of the VPHP gas were seen on the various types of equipment that were fumigated for compatibility testing. All the pieces of equipment were used in their respective applications after exposure to VPHP and appeared to operate successfully.

DISCUSSION

The results of this study show that VPHP gas can be safely generated to high concentrations within an enclosure. The concentration of hydrogen peroxide gas generated and/or the process of decontamination was shown to reduce the titers of eight different viruses to 0 ELD_{50} for avian viruses or less than 10 $TCID_{50}$ for mammalian viruses. Previous studies have shown that *B. stearothermophilus* spores are the most resistant to VPHP (6). It would thus be expected that VPHP would also be effective against less resistant classes of microorganisms, such as viruses. This is consistent with the findings of Klapes, who noted the rapid inactivation of pathogenic bacteria, yeast, fungal spores, viruses, and bacterial spores (3). The only clear exception to this was cases when the virus was present in blood. It is possible that blood, being a protein-rich substance, protected the virus in some way from the oxidizing properties of the gas, although the other viruses were either in 5% fetal calf serum or in egg fluid (also very protein rich). It is also possible, since cells naturally contain peroxidase and catalase, that these endogenous enzymes in the erythrocytes neutralized some or all of the hydrogen peroxide, rendering the decontamination process ineffective.

Animal disease virology laboratories around the world that are working at level III or IV biocontainment with exotic viruses have traditionally used formaldehyde gas as the fumigant for decontamination of objects being removed from the laboratory. This study shows that VPHP gas would be an excellent substitute for this purpose. The VPHP fumigation process, although very effective at destroying any potential virus contaminants (in a liquid or a dried state) on objects, did not appear to damage any of the laboratory equipment tested. This makes VPHP gas a suitable substance for decontamination of objects needing to be removed from a biocontainment level III or IV laboratory. Since this was a preliminary study, not all potentially contaminated types of material (rubber, cloth, plastic, etc.) were tested. It is hoped that further studies might address these needs.

ACKNOWLEDGMENTS

We thank A. Tremblay and J. Riva for the virus titration work.

REFERENCES

1. **Block, S. S.** 1991. Peroxygen compounds, p. 167–181. *In* S. S. Block (ed.), Disinfection, sterilization and preservation, 4th ed. Lea and Febiger, Philadelphia, Pa.
2. **Johnson, J. W., J. F. Arnold, S. F. Nail, and E. Renzi.** 1992. Vaporized hydrogen peroxide sterilization of freeze dryers. J. Parenter. Sci. Technol. **46:**215–225.
3. **Klapes, N. A.** 1990. New applications of chemical germicides: hydrogen peroxide, abstr. 20, p. 14–15. *In* Program and abstracts of the ASM International Symposium on Chemical Germicides. American Society for Microbiology, Washington, D.C.
4. **Klapes, N. A., and D. Vesley.** 1990. Vapor-phase hydrogen peroxide as a surface decontaminant and sterilant. Appl. Environ. Microbiol. **56:**503–506.
5. **Office International des Epizooties.** 1992. Manual of standards for diagnostic tests and vaccines. Office International des Epizooties, Paris, France.
6. **Rickloff, J., and P. Orelski.** 1989. Resistance of various microorganisms to vapor phase hydrogen peroxide in a prototype dental hand piece/general instrument sterilizer, abstr. Q-59, p. 339. *In* Abstracts of the 89th Annual Meeting of the American Society for Microbiology 1989. American Society for Microbiology, Washington, D.C.
7. **Suen, J., L. Alderman, and M. P. Kiley.** 1990. Use of vapor phase hydrogen peroxide (VPHP) as a space decontaminant, abstr. 33, p. 21. *In* Program and abstracts of the ASM International Symposium on Chemical Germicides. American Society for Microbiology, Washington, D.C.