

Evaluation of Vaporized Hydrogen Peroxide Fumigation as a Method for the Bio-decontamination of the High Efficiency Particulate Air Filter Unit*

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Abstract

Objective To evaluate the performance of vaporized hydrogen peroxide (VHP) for the bio-decontamination of the high efficiency particulate air (HEPA) filter unit.

Methods Self-made or commercially available bioindicators were placed at designated locations in the HEPA filter unit under VHP fumigation. The spores on coupons were then extracted by 0.5 h submergence in eluent followed by 200- time violent knocks.

Results Due to the presence of HEPA filter in the box, spore recovery from coupons placed at the bottom of the filter downstream was significantly higher than that from coupons placed at the other locations. The gap of decontamination efficiency between the top and the bottom of the filter downstream became narrower with the exposure time extended. The decontamination efficiency of the bottom of the filter downstream only improved gently with the injection rate of H₂O₂ increased and the decontamination efficiency decreased instead when the injection rate exceeded 2.5 g/min. The commercially available bioindicators were competent to indicate the disinfection efficiency of VHP for the HEPA filter unit.

Conclusion The HEPA filter unit is more difficult than common enclosure to decontaminate using VHP fumigation. Complete decontamination can be achieved by extending fumigation time. VHP fumigation can be applied for in-situ biodecontamination of the HEPA filter unit as an alternative method to formaldehyde fumigation.

Key words: Vaporized hydrogen peroxide; Fumigation; Bio-decontamination; High efficiency particulate air filter unit; Spore killing

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INTRODUCTION

High efficiency particulate air (HEPA) filtration is an effective approach for the prevention of hazardous

bioaerosol released from the biosafety laboratory into the environment. The HEPA filter is mainly made of multi-layer corrugated glass fiber paper which can trap airborne microorganisms effectively and it needs to be replaced at regular intervals

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because the increasing accumulation of dust and debris in the filter restricts air flow through the filter^[1]. The HEPA filter must be bio-decontaminated thoroughly before being detached and replaced as the microorganisms held by the exhaust HEPA filter of high-level biosafety laboratory are highly pathogenic.

Since fumigants are easily dispersed and can potentially penetrate the filter, it is generally practical to use gas or vapor fumigation technique for the decontamination of the HEPA filter unit. Traditionally, formaldehyde has been a fumigant used most extensively for space decontamination. In spite of the bio-decontaminating effectiveness of formaldehyde, the method is tedious as it needs neutralization with ammonia prior to its release to atmosphere and the neutralization product-hexamethylenetetramine attached on the decontaminated surface needs cleaning with water^[2]. More importantly, it is toxic, carcinogenic^[3] and difficult to standardize. This method increasingly becomes undesirable with the availability of effective alternative fumigants such as chlorine dioxide gas and vaporized hydrogen peroxide (VHP). Compared with chlorine dioxide gas, VHP represents a safer and more convenient alternative to formaldehyde. VHP can be generated by heating a solution of 30%-35% hydrogen peroxide in water and it breaks down into oxygen and water eventually, both of which are environmentally benign. At the same time, VHP belongs to a kind of broad-spectrum antimicrobial with virucidal, bactericidal, fungicidal and sporicidal activity and even can inactivate nematode eggs, exotoxin and prion^[4-6]. In addition, unlike gas fumigation with chlorine dioxide or formaldehyde which need humid environment, the decontamination process with VHP is essentially 'dry' and therefore demonstrates excellent material compatibility^[7]. In most of the cases, VHP technology has been used for fumigation of air or surface of the sealed enclosures, such as isolators, glove boxes, incubators, laboratory animal rooms and pharmaceutical aseptic manufacturing areas^[8-9].

However, since VHP is in vaporized state whereas it is not a real gas after all, its penetrating power is weaker than gaseous fumigants (such as chlorine dioxide gas). In addition, the HEPA filter unit which contains a thick HEPA filter made of multi-layer corrugated glass fiber paper media is distinguished from common enclosures. Up to the present, the information on the application of VHP fumigation for in-site disinfection of the HEPA filter

unit is scarce. Firstly, can upstream VHP penetrate the thick HEPA filter and bio-decontaminate the whole box enclosure completely? Secondly, at which location should bioindicators be placed to indicate appropriately the performance of VHP for the whole HEPA filter container decontamination? Thirdly, what cycle parameters are fitted for the decontamination of the HEPA filter unit with VHP? Finally, which kind of porous material can be used as microorganism carrier of bioindicator for the decontamination of the HEPA filter unit? The objective of this study was therefore, (1) to investigate the efficiency and characteristics of VHP fumigation as a method for the bio-decontamination of the HEPA filter unit, (2) to evaluate the effect of parameters of decontamination program cycles on the decontamination consequence of the HEPA filter unit, (3) to investigate what bioindicator is fit to be used to evaluate the decontamination result of VHP for the HEPA filter unit.

MATERIALS AND METHODS

Biological Indicators Preparation

Spores of *Bacillus atrophaeus* (ATCC9372) and *Geobacillus stearothermophilus* (ATCC7953) were used to evaluate the bio-decontamination efficiency of VHP fumigation for the HEPA filter unit in this study. Spores preparation and coupons inoculation were carried out in accordance with slightly modified standard method^[10]. Cotton cloth coupons (2×10^7 cfu spores of *Bacillus atrophaeus*) were prepared for quantitative evaluation of bio-decontamination efficiency. Cotton cloth coupons (3×10^6 cfu spores of *Bacillus atrophaeus*), glass fiber paper coupons (3×10^6 cfu spores of *Bacillus atrophaeus*) and the commercially available common filter paper coupons (3×10^6 cfu spores of *Bacillus atrophaeus* or *Geobacillus stearothermophilus*) were prepared for qualitative evaluation of bio-decontamination efficiency.

Decontamination Procedure

For each decontamination run, coupons were placed at the designated locations in the box (6 replicate coupons for each location). Then the VHP decontamination system (Steris Corporation) was connected with the HEPA filter unit via gas-tight piping and was initiated to perform dehumidification. Conditioning, decontamination and aeration phases automatically in accordance with the predetermined

parameter value (Figure 1). At the completion of the decontamination cycle, coupons were taken out from the box aseptically. Blank controls (coupons not inoculated with spores) and positive controls

(coupons inoculated with spores, not decontaminated by VHP fumigation) were left at room temperature when the test coupons were decontaminated.

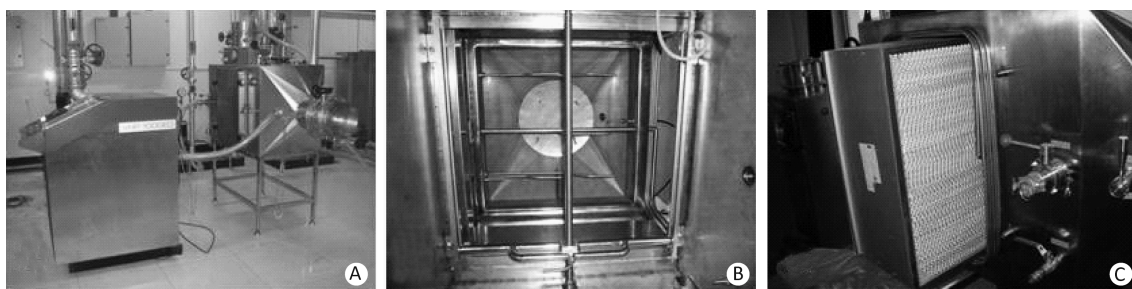


Figure 1. VHP fumigation for the HEPA filter unit. A, VHP decontamination system was connected with the HEPA filter unit via gas-tight piping. B, Inner no-porous surface of the HEPA filter unit. C, The HEPA filter made of multi-layer corrugated glass fiber paper.

Spore Extraction from Coupons

All quantitatively tested coupons were transferred into 5 mL eluant (0.05% Tween 80, and 0.01% catalase) in a 30 mL glass sterile tube. The coupons were submerged in eluant for 30 min and then tubes were knocked violently to dislodge the survival spores from coupons. Serial dilutions of extract at 1:10 were performed as needed and 1.0 mL aliquots of undiluted extract or dilutions were added in triplicate to plates. 15 to 20 mL volume of liquefied nutrient agar (45-50 °C) was added to each plate and was mixed with the extract solution by gentle rotational swirling. The plates were incubated at 37 °C for 24 h. All qualitative coupons were put into 8ml nutrient broth (for *Bacillus atrophaeus*) or glucose peptone water medium (for *Geobacillus stearothermophilus*) directly and incubated for 7 d at 37 °C (for *Bacillus atrophaeus*) or at 56 °C (for *Geobacillus stearothermophilus*). The turbidity and the color change of the culture medium were the indications of bacterial growth.

Data Collection and Statistical Analysis

Plates were enumerated and the number of CFUs per coupon was determined by multiplying the average number of colonies per plate by the dilution factor and the coefficient of eluant volume. The sporicidal efficiency was expressed in terms of a Log reduction that was computed by subtracting the log survival spores CFUs of fumigated coupons from the log viable spores CFUs of the positive control coupons. The mean ($\pm$ SD) log reduction was calculated from three independent experiments. The

two-way ANOVA and *t*-tests (SPSS version 11.5) were used for statistical analysis of data. $P < 0.05$ was used as the level for significance.

RESULTS

Decontamination Efficiencies of VHP Fumigation at Different Locations in the HEPA Filter Unit

In order to compare the decontamination efficiencies between the upstream and the downstream, the top and the bottom, and the front and the back of the HEPA filter unit, respective four corners and center point of the upstream and the downstream of the filter were selected as locations where bio-indicators were placed (Figure 2). Since the volume of the HEPA filter unit was small, the injection rate of H_2O_2 was set slightly higher than the minimal value of the VHP generator. The spore log reduction values of the coupons placed in the two bottom corners of the filter downstream were found significantly less than those placed at the other locations (Figure 3). In order to define the contribution of the filter to the diversity of VHP disinfecting efficiency in the filter unit we compared the bio-decontamination efficiencies of VHP at the same positions in the filter unit at the presence or the absence of the HEPA filter. It was observed that the difference in decontamination efficiency between the top and the bottom of the filter downstream disappeared with the absence of the HEPA filter in the box (Figure 4). Except for the bottom of the filter downstream there were no significant differences in VHP decontamination

efficiencies among the other positions in the filter box (Figure 3). Consequently, the top and the bottom of the downstream of the filter were selected as two representing positions for evaluating the effect of the

decontamination parameters including circulation air flow rate, decontamination time and the injection rate of H_2O_2 on the diversity of VHP decontamination efficiency in the HEPA filter unit.

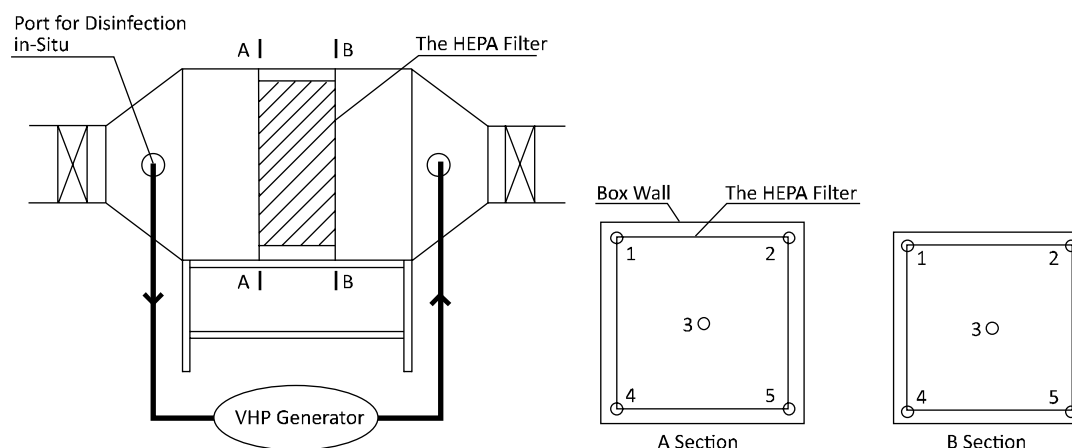


Figure 2. A diagram of the HEPA filter unit connected with VHP generator for decontamination. A section: downstream of the filter, B section: upstream of the filter. Sample locations indicated by numbers were as follows: 1: top front, 2: top back, 3: center, 4: bottom front, 5: bottom back.

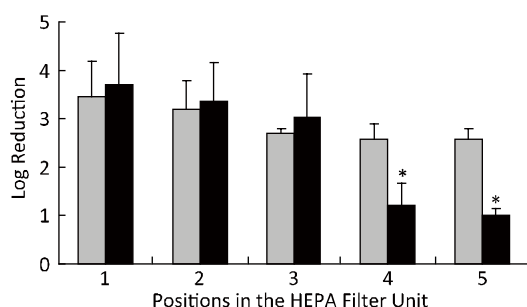


Figure 3. Sporicidal efficacy of VHP (at 1.5 g/min injection rate for 30 min) for samples placed at the downstream (black bar) or the upstream (grey bar) of the HEPA filter. Log reduction of spores was calculated as described in section "MATERIALS AND METHODS". The results were expressed as the mean $\pm$ SD of three independent experiments. Asterisks denote statistical significance compared with the center of the filter upstream.

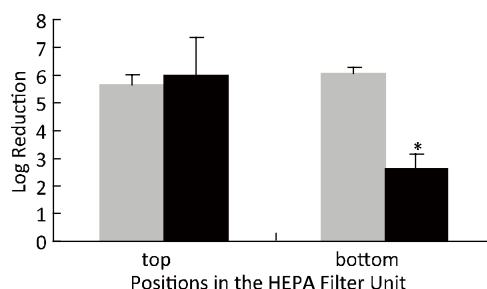


Figure 4. Sporicidal efficacy of VHP (at 2.0 g/min injection rate for 30 min) for samples placed at the top or bottom of the HEPA filter downstream in the presence (black bar) or absence (grey bar) of the HEPA filter in the box. Log reduction of spores was calculated as described in section "MATERIALS AND METHODS". The results were expressed as the mean $\pm$ SD of three independent experiments. Asterisks denote statistical significance in comparison with the top of the filter downstream.

The Effect of Circulation Air Flow Rate on the Gap of Decontamination Efficiency of VHP between the Bottom and the Top of the Filter Downstream

Now that the HEPA filter with airflow resistance affected VHP even dispersion in the box, It seemed reasonable that increasing the circulation air flow rate could improve the homogeneity of VHP distribution

in the HEPA filter unit and reduce the gap of decontamination efficiency of VHP between the bottom and the top of the filter downstream. The injection rate of H_2O_2 and the exposure time were set as 2 g/min and 30 min respectively. The results indicated that there was no significant alteration in the discrepancy of VHP decontamination efficiency between the top and the bottom of the filter

downstream as the circulation airflow rate was adjusted from minimal 14 m³/h to maximal 30 m³/h (Figure 5).

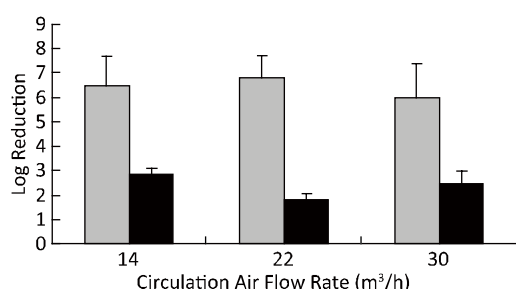


Figure 5. Sporidical efficacy of VHP (at 2.0 g/min injection rate for 30 min) for samples placed at the top (grey bar) or bottom (black bar) of the HEPA filter downstream at different volumes of circulating air. Log reduction of spores was calculated as described in section “MATERIALS AND METHODS”. The results were expressed as the mean±SD of three independent experiments.

The Effect of VHP Exposure Time on the Decontamination Efficiency of VHP for the HEPA Filter Unit

Due to the gap in decontamination efficiency between the bottom of the filter downstream and the other locations, it is more difficult to disinfect the HEPA filter unit than to disinfect common enclosures by using VHP. Only if the decontamination of the most difficult location meets the sterilization demand can successful decontamination consequence of the HEPA filter unit be achieved. So we observed whether complete bio-decontamination could be achieved by extending fumigation time or not. Time-dependent results for spore killing were observed and the gap in decontamination efficiency between the top and the bottom of the filter downstream became narrower with VHP exposure time extended. The difference in log reduction of spore between the top and the bottom was up to 2.8 when the coupons were exposed to VHP fumigation for 30 min and decreased to 1.3 when the exposure time was increased one time. When the VHP exposure time was increased to 90min, the spores inoculated on the most resistant material placed in the most difficult location to decontaminate were killed completely (the log reduction of spores over 7.3), which met the sterilization requirement (Figure 6).

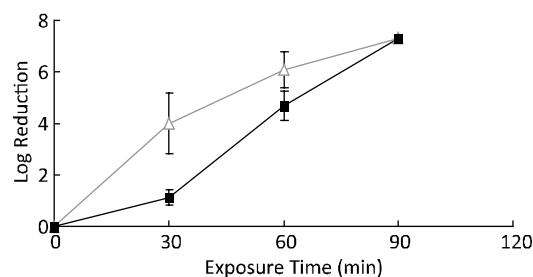


Figure 6. Time dependent sporicidal efficacy of VHP (at 2.0 g/min injection rate of hydrogen peroxide) for samples placed at the top (open triangle) or bottom (closed squares) of the HEPA filter downstream. Log reduction of spores was calculated as described in section “MATERIALS AND METHODS”. The results were expressed as the mean±SD of three independent experiments.

The Effect of the Injection Rate of Hydrogen Peroxide on the Decontamination Efficiency of VHP for the HEPA Filter Unit

We also observed whether complete decontamination could be achieved by increasing H₂O₂ injection rate or not. However, H₂O₂ injection rate dependent decontamination efficiencies were observed just when injection rate was below 2.5 g/min. The decontamination efficiency decreased instead when the injection rate was increased from 2.5 g/min to 3 g/min. In addition, not only did the decontamination efficiency at the bottom of the filter downstream increase very slowly but also the gap of decontamination efficiency between the top and the bottom became wider with the increasing of H₂O₂ injection rate (<2.5 g/min) (Figure 7).

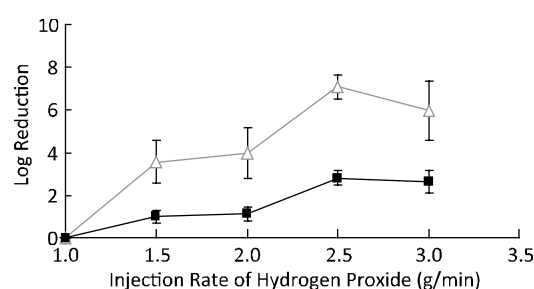


Figure 7. Sporidical efficacy of VHP (for 30 min) for samples placed at the top (open triangle) or bottom (closed squares) of the HEPA filter downstream at different injection rate of hydrogen peroxide. Log reduction of spores was calculated as described in section “MATERIALS AND METHODS”. The results were expressed as the mean±SD of three independent experiments.

The Qualitative Evaluation of the VHP Decontamination for the HEPA Filter Unit

In addition, glass fiber paper, common filter paper and cotton cloth coupons containing about 3×10^6 spores of *Bacillus atrophaeus* or *Geobacillus stearothermophilus* were used for the qualitative evaluation of decontamination efficiency. The results likewise displayed the difference in decontamination efficiency of VHP between the top and the bottom of

the filter downstream. At the same time, we found that spores of *Geobacillus stearothermophilus* were more resistant than spores of *Bacillus atrophaeus* to VHP fumigation, and common filter paper was more resistant than glass filter paper but less resistant than cotton cloth to VHP fumigation. On the whole, the cotton cloth coupons inoculated with spores of *Bacillus atrophaeus* was the most difficult to disinfect among all four types of coupons used in this study (Table 1).

Table 1. Sterilizing Results of VHP for Coupons Made of Four Materials and Inoculated with Two Types of Spores in the HEPA Filter Unit

locations	Spore Types	Material Types	H ₂ O ₂ Injection Rate (g/min)/VHP Exposure Time (min)					
			1.5/30	2/30	2.5/30	3/30	2/60	2/90
Top	BA	GFP	-	-	-	-	-	-
		CFP	-	-	-	-	-	-
		CC	+	+	+	+	+	-
	GS	CFP	+	+	-	-	-	-
Tottom	BA	GFP	+(-)	-	-	-	-	-
		CFP	+(-)	+(-)	-	-	-	-
		CC	+	+	+	+	+	-
	GS	CFP	+	+	+	+	-	-

Note. BA: *Bacillus atrophaeus*; GS: *Geobacillus stearothermophilus*; GFP: Glass fiber paper; CFP: Common filter paper; CC: cotton cloth; +: bacterial growth of all coupons; -: no bacterial growth of all coupons; +(-): bacterial growth of some coupons. These results were based on three independent experiments, each kind of coupons with six replicate samples.

DISCUSSION

Due to the advantages of VHP fumigation in safety, automatic performance, material compatibility and decontamination efficiency, this decontamination technique has now been widely applied in enclosure bio-decontamination. However, the study involved in the application of VHP for the HEPA filter unit decontamination has not been reported. Therefore, this study focused on assessing the feasibility of this desirable fumigation method for the decontamination of a peculiar enclosure-the HEPA filter unit. The data presented here demonstrated the characteristics associated with the decontamination of the HEPA filter unit using VHP. The thick HEPA filter had a strong impact on the decontamination efficiency of VHP for the filter unit and resulted in the bottom of the filter downstream being difficult location to disinfect. Multi-layer

corrugated glass fiber paper of which the HEPA filter is made belongs to thermal insulation material so that the temperature of hot air containing VHP would decrease after airflow went through the HEPA filter. In addition, hot air was apt to accumulate in the top of the box. Hence, the temperature of the air at the bottom of the downstream of the filter was lower than that at the other positions. VHP in air was apt to condense and the real concentration of VHP would become lower when air temperature dropped. The extent of color change of the chemical indicator (the data were not showed) indicated that the concentration of VHP at the bottom of the HEPA filter downstream was lower in troth than that at other locations. In addition, weak penetration power of VHP also may be one factor that led to lower decontamination efficiency of VHP for the bottom of the downstream of the filter.

Alteration of circulation air flow rate did not modify the heterogeneity of VHP decontamination

for the HEPA filter unit. In fact, as for the 0.6 m³ volume common enclosure, the minimal circulation air flow rate (14 m³/h) of the VHP 1000 ED decontamination system should be sufficient to create even distribution of VHP; as for the HEPA filter unit containing thick HEPA filter, the maximal air flow rate (30 m³/h) of the machine was of no avail to even VHP distribution. moreover, increasing the circulation airflow rate would cause the concentration of VHP to get lower if the injection rate of H₂O₂ remains unchanged.

Although the concentration of VHP at the bottom of the HEPA filter unit downstream was always lower than that at other locations of this peculiar enclosure, the inferior infection efficiency at the bottom of the HEPA filter unit downstream got weakened gradually and the difficult location could reach sterilization level along with fumigation time extended. However, increasing of injection rate of H₂O₂ did not significantly contribute to the decontamination efficiency at the difficult location of the HEPA filter unit. Furthermore, VHP concentration must be limited below the condensation point^[11]. Hence, it is more advisable to extend the exposure time than to increase the injection rate of H₂O₂ for this kind of difficult decontamination enclosure.

Other than exposure time and concentration of fumigants, the nature of the contaminated material and the type of microorganisms were also associated with the effectiveness of VHP decontamination process. Inner surface of The HEPA filter unit mainly consists of stainless steel and glass fiber paper media. Compared with the glass fiber media, the no-porous stainless steel surface is less resistant to VHP decontamination^[12]. Therefore, we prepared coupons with glass fiber paper in order to indicate the decontamination efficiency of the HEPA filter unit in a more realistic way. However, it was extremely difficult to extract spores dried on the coupons made of that kind of material. Therefore, we selected another porous material-cotton cloth as carrier material of spores in the quantitative assessment. We found that it was more difficult to kill the spores on the common filter paper than to kill the spores on the glass filter paper but was easier than to kill the spores on the cotton cloth. In addition to the material property, more even distribution of spore suspension with less clumping on the glass fiber paper or common filter paper may be a factor to facilitate effective decontamination. Just like the observations in other reports^[13], spores of *Geobacillus stearothermophilus* were more

resistant to VHP than spores of *Bacillus atrophaeus* on the same carrier material. Altogether, the cotton cloth coupons inoculated with spores of *Bacillus atrophaeus* were the most resistant coupons to VHP fumigation. Hence, cotton cloth coupons were more than sufficient to indicate the decontamination efficiency of VHP for the HEPA filter unit. In fact, the commercially available common filter paper inoculated with spores of *Geobacillus stearothermophilus* was absolutely competent to indicate the decontamination efficiency of VHP for the HEPA filter unit.

The generally acknowledged strategy to decide whether the decontamination of the HEPA filter unit meets the demand or not is to observe the disinfection outcomes of bio-indicators placed at the downstream of the filter. However, we found that decontamination performances of VHP at different locations of the HEPA filter unit were largely varied in this study. It is suggested that the bio-indicator should be placed at the most difficult position to decontaminate to avoid overestimating the decontamination performance.

In summary, the study provided the information related to VHP fumigation for the bio-decontamination of the HEPA filter unit. We also validated the feasibility of VHP fumigation for the HEPA filter unit decontamination through bio-indicators and established the optimal operating parameters.

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