HVAC Considerations for Gaseous Decontamination of Laboratory Spaces using Hydrogen Peroxide Vapor
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Introduction

Some critical environments, such as animal rooms in research facilities, require periodic biodecontamination. Maintaining biological cleanliness in these facilities has many benefits but the primary reasons are:

• To protect the integrity of research by decontaminating between and during experiments.
• To eliminate pathogens introduced into the facility with contaminated animals.
• To eradicate outbreaks of disease or other infectious outbreaks within the facility.

A wide range of biological decontaminants are available commercially, and all have distinct benefits and risks. This paper does not discuss the merits of those different technologies; instead, it focuses on the use of hydrogen peroxide vapor (HPV) as a biodecontamination agent and the effects of the ventilation system design on the efficacy of HPV as a decontaminant.

Phoenix Controls and STERIS Corporation recently conducted some tests using HPV to better understand its behavior as a decontaminant under varying HVAC conditions. The results of these tests are presented in this white paper. We selected HPV because of its growing popularity as a decontaminant.

Overview of the Hydrogen Peroxide Vapor Decontamination Process

Hydrogen peroxide, in its vapor phase form, is becoming increasingly popular for the gaseous decontamination of facilities. The vapor readily breaks down into water and oxygen, leaves no residue, is environmentally friendly and has been used successfully as a nondestructive sterilant for decontaminating electronic laboratory equipment. HPV is efficacious against various microorganisms, including bacteria, yeast, fungal and bacterial spores, as well as viruses. Heckert, et al. has demonstrated that hydrogen peroxide vapor is an effective, safe method to inactivate exotic animal viruses, which may contaminate temperature-sensitive equipment in a biocontainment level III (BSL-3) laboratory. Companies either offer equipment specially designed for hydrogen peroxide gaseous decontamination or provide decontamination services. The technologies used to generate the gas and control the gassing cycles vary slightly, but most have been proven to be effective alternatives to paraformaldehyde, which leaves a residue and is considered a carcinogen by the World Health Organization (WHO).

These HPV generators are generally mobile units or, less commonly, integrated systems. The mobile generator used in this study circulates room air in a closed-loop pattern in which it produces gaseous hydrogen peroxide vapor from a hydrogen peroxide solution at a programmed injection rate. The vapor returned from the room to the unit is decomposed into water and oxygen by a catalytic converter.

Phases of the Decontamination Process

The decontamination process consists of three main phases: conditioning, decontamination and aeration (see Figure 1). Before the cycle is initiated, the ventilation system serving the room is isolated to prevent gas dilution and unwanted room pressurization. During the conditioning phase, room air is recirculated through the HPV generator until the vapor concentration levels within the room reach the required level. In the decontamination phase, the vapor concentration levels are maintained at the required level for a sufficient time to achieve biodecontamination. The duration of this period is based on the room size and conditions. Finally, the room is aerated by reopening the room ventilation system until the gas concentration has fallen to a safe level, normally less than one part per million (ppm) (the Occupational Safety and Health Administration’s eight-hour time-weighted average, or TWA) before re-entering the space. Typically, special hand-held gas detectors are used to determine this level.

Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Methods</td>
<td>3</td>
</tr>
<tr>
<td>Results</td>
<td>5</td>
</tr>
<tr>
<td>Discussion and Conclusions</td>
<td>5</td>
</tr>
<tr>
<td>Footnotes</td>
<td>6</td>
</tr>
<tr>
<td>References</td>
<td>7</td>
</tr>
<tr>
<td>Appendix A: Shut-off Leakage Performance</td>
<td>8</td>
</tr>
<tr>
<td>Appendix B: Room Testing Specifications</td>
<td>8</td>
</tr>
</tbody>
</table>
Validation Methods

To achieve an effective kill, the required sporicidal concentrations with HPV range from 140 to 1400 ppm (0.2 to 2 mg/L) at room temperature. The amount of time required to kill 90% of a microbe population at a specific concentration is defined as the D-value and is equivalent to one log of spore reduction.

HPV produces shorter D-values for *Bacillus subtilis* than paraformaldehyde and ethylene oxide at optimum sterilization conditions. However, the most resistant bacterial spore to HPV is *Geobacillus stearothermophilus*. For validation purposes, most HPV decontamination applications require six logs (one million spores) of *Geobacillus stearothermophilus* spore reduction, depending on the application.

The biological indicator (BI) typically consists of a stainless steel disc inoculated with approximately one million *Geobacillus stearothermophilus* spores and packaged in a slightly porous pouch. The BI is exposed to hydrogen peroxide vapor, and then the disc is removed from the pouch after aeration. It is incubated in a tryptic soy broth growth media to determine whether a microbial kill was achieved. Exposed BIs are incubated for seven days at 140°F (60°C). Live spores will grow and turn growth media turbid, normally within 24 hours.

**HVAC Issues Related to HPV Decontamination**

Currently, little is understood about the impact of HPV on the design and operation of the ventilation system. The only area of consensus is that the supply and exhaust ventilation connected to the space must be shut off during the decontamination process. No standards and guidelines currently exist on how tightly the room should be sealed while it is contaminated. Clearly, any leaks in the ventilation serving the space during decontamination process creates two risks:

- Gas dilution, which may impact the efficacy of the decontamination process.
- Accidentally pressurizing the space during decontamination. Positively pressurizing the space is undesirable because gas will be forced into adjacent spaces. Negative pressurization could introduce cross-contamination from adjacent spaces.

Designs generally err on the side of caution. As a consequence, they sometimes specify expensive bubble-tight dampers, which can significantly increase project first costs by as much as $5000-10,000 per room.

Field experience has indicated that successful decontamination can be achieved with small amounts of ventilation leakage through the ventilation control device. As a precaution, cracks around the doors to the space are sealed with a suitable tape to prevent gas from leaking into the adjacent space(s).

**Room Preparation Issues**

Getting the room to the state of preparation described above can be time consuming and sometimes challenging.

First, shutting off the ventilation to the space in a controlled sequence can present challenges. If the supply and exhaust are not closed in a controlled fashion, the...
space could become pressurized, which potentially introduces unwanted airflows into or out of a critical environment. Additionally, structural damage could occur if the space is over- or under-pressurized.

Second, if local controls are not available, then communication is required between the decontamination staff at the room level and the people with access to the controls to operate the ventilation for the different phases of the decontamination process.

**Methods**

In February 2006, Phoenix Controls and STERIS Corporation conducted several tests using HPV to decontaminate laboratory spaces to better understand its behavior as a decontaminant and the impact of changing airflows on the efficacy of the decontamination process. The testing was conducted at Phoenix Controls' demonstration laboratories in Acton, Massachusetts.

**Hypotheses Tested**

Three hypotheses were tested during this project.

**Hypothesis 1:**
Maintaining neutral room pressurization with small amounts of airflow leakage will have a minimum impact on the efficacy of the process.
- Bubble-tight dampers are not necessary.
- Gas dilution will not impact the efficacy of the decontamination process.

**Hypothesis 2:**
Maintaining negative room pressurization with an increased amount of exhaust airflow will contain the gas and have a minimum impact on the efficacy of the process.
- Sealing the cracks around the doors with tape may be avoided.
- The gas will be contained without impacting the efficacy of the process.

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**Figure 2. Room Setup**

![Diagram of room setup with labels for exhaust duct, shut-off valve, fan, hydrogen peroxide sensor, biological indicators, user interface, corridor, and supply duct.]

HVC Considerations for Gaseous Decontamination of Laboratory Spaces using Hydrogen Peroxide Vapor (HPV)
Hypothesis 3: Local HVAC control is achievable and safe.

- One person can operate the process locally and maintain correct pressurization at all times.
- The gas will stay contained in the room.

Room Setup

The room setup is shown in Figure 2. For more details, see Appendix B.

Room Ventilation Modes

Three room ventilation modes were pre-programmed into the HVAC controls:
- Standby
- Decontamination
- Purge (see Table 1)

Each mode was initiated at the push of a button directly outside the room through a local user interface panel.

Test Procedures

Three types of tests were conducted:
- Control
- Neutral pressurization
- Negative pressurization

Each test is described in more detail below.

Control Test

A control test to establish whether a successful decontamination could be accomplished in the space was completed first. For this test, the HVAC system fans were switched off to ensure that there was no airflow into or out of the room. Cracks around the door were sealed with tape to contain any diffusion of the gas.

Neutral Pressurization Tests

The second and third tests were conducted with the supply and exhaust devices in the shut-off position, and the HVAC system fans on. Five inches of static pressure were maintained in the supply and exhaust duct, creating known leakage into and out of the room (see Appendix A for leakage performance table). Cycle time for the decontamination period was set at 55 minutes. Cracks around the door were sealed with tape to contain any diffusion of the gas.

Negative Pressurization Tests

The remaining four tests were conducted with the exhaust valve opened to 50 CFM, the supply valve closed and the sealing tape removed from the door. This negative volumetric offset created a room pressure of -0.015 inch WC. Because air was introduced from the corridor, it was assumed that some gas dilution would occur. It was anticipated that such dilution may impact the efficacy. To compensate for this, gassing time was extended by 15 minutes for the second test in the series. Overnight test results after the first two tests of this series verified this; therefore, the extended gassing time was maintained for the third and fourth test of the series (see Table 2).

For the final test, the distribution fans inside the room were switched off.

Validation

The biological indicators were incubated for 24 hours and for full verification of a 100% kill, incubation continued for another seven days. To validate the quality of the biological indicator in use, one unexposed BI for each test run served as a control.

<table>
<thead>
<tr>
<th>Mode</th>
<th>Supply</th>
<th>Exhaust</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Standby</td>
<td>Shut-off</td>
<td>50 CFM</td>
<td>Reduces ventilation in a controlled manner by maintaining negative pressurization. Allows operators to prepare the room and tape the doors.</td>
</tr>
<tr>
<td>Decontamination</td>
<td>Shut-off</td>
<td>Shut-off</td>
<td>Isolates the ventilation during decontamination and maintains neutral pressure.</td>
</tr>
<tr>
<td>Purge</td>
<td>700 CFM</td>
<td>850 CFM</td>
<td>Purge cycle</td>
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<table>
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<tr>
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<td>—</td>
<td>—</td>
<td>55</td>
<td>Control test—HVAC fans off</td>
</tr>
<tr>
<td>2</td>
<td>5 CFM</td>
<td>5 CFM</td>
<td>55</td>
<td>Neutral pressurization test</td>
</tr>
<tr>
<td>3</td>
<td>5 CFM</td>
<td>5 CFM</td>
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</tr>
<tr>
<td>4</td>
<td>5 CFM</td>
<td>50 CFM</td>
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<tr>
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Results

Neutral Pressurization Test

Neutral pressurization test findings indicated that gas concentrations were slightly lower than the control test (see Figure 3). A 100% kill was achieved in all three neutral pressurization tests (see Table 3).

Negative Pressurization Test

In test number four, the decontamination, or “gassing” phase, was 55 minutes and there was a single biological indicator that exhibited some growth. The gassing time was extended by 15 minutes for tests five and six, and a 100% kill was achieved for both tests (see Figure 4 and Table 4).

Finally, in test seven, the distribution fans inside the room were switched off and three biological indicators exhibited some growth (see Table 4).

Discussion and Conclusions

Hypothesis 1:
Maintaining neutral room pressurization with small amounts of airflow leakage will have a minimum impact on the efficacy of the process.

By selecting devices with very similar leakage performance characteristic curves and low leakage, the volumetric offset will be kept to an absolute minimum. Consequently, the room pressure will be negligible, thereby minimizing the possibility of gas escaping from the room during the decontamination process. [For example, if the supply and exhaust devices have a leakage of less than 5 CFM each, the variance between the two devices (the volumetric offset) will be much less than 5 CFM. As a result, the room pressure is negligible.]

The testing showed that very small amounts of HVAC airflow (≤ 5 CFM) through a space will have a minimum impact on the efficacy of the decontamination process while using HPV. Therefore, bubble-tight dampers are not necessary.*

* Field conditions may vary.

Hypothesis 2:
Maintaining negative room pressurization with an increased amount of exhaust airflow will contain the gas and have a minimum impact on the efficacy of the process.

The testing showed that negative pressurization contained the gas and with slightly extended gassing times, successful decontamination could be accomplished. Slight negative pressurization will contain the gas but care must be taken with disrupting the flow of high velocity, fresh air leakage into the room. The door undercut was 0.75 inch high. By assuming that only 70% of the leakage came through the undercut, the incoming air velocity was approximately 186 fpm. Unless this incoming air is made turbulent by using strategically placed fans or other air circulation devices, gas distribution may be affected. Consideration should also be given to research activities in adjoining spaces and the possibility of contamination through infiltration from those spaces. If it is impractical to study airflow patterns before decontamination or there are concerns about cross-contamination from adjacent spaces, then room pressurization should be neutral throughout the gassing process.

Table 3. Biological Indicator Kill Success Rate for Tests 1-3

<table>
<thead>
<tr>
<th>Test Number</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<td>Y</td>
<td>Y</td>
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Hypothesis 3:
Local HVAC control is achievable and safe.

The operator was able to switch the ventilation between the pre-configured modes safely. The room pressurization remained negative and gas levels in the hallway remained below the prescribed limit of 1 ppm.

Other Considerations

Failsafe modes of ventilation shut-off devices should be fail-to-last position. Once closed, they will continue to maintain neutral pressurization of the room in the event of a power failure, which will prevent gas from escaping accidentally or cross-contamination from adjacent rooms.

Fail-open ventilation shut-off devices should be avoided because once closed, a power failure could cause one or more of the devices to open, resulting in accidental negative or positive pressurization of the space.

Clearly more research and sharing of knowledge on the operation of laboratories that use hydrogen peroxide as a decontaminant will benefit the laboratory design community. As the technology matures and improves, knowledge and experience will undoubtedly raise yet more questions that need to be answered.

Footnotes


References


Appendix A: Shut-off Leakage Performance

The room volume for the test was 1500 cubic feet (42.47 cubic meters). Supply and exhaust ventilation ducts serving the space were 10 inches (250 mm) in diameter and the airflow volume regulating devices were factory characterized, pressure-independent venturi valves with shut-off capabilities (see Appendix A for shut-off leakage).

Under normal operating conditions, the room was negatively pressurized to -0.03 inch WC (-7.5 Pa), which was achieved by using a volumetric-offset pressurization control strategy. Room pressure was monitored using a true differential pressure monitor with an accuracy of 0.5% FS.

Hydrogen peroxide levels inside the room were measured with an electronic sensor (ATI Series B-12) and recorded using a data logger (Dataq DI-158U). Gas levels in the hallway were monitored throughout the testing using a Dräger Pac III with a range of 0-20 ppm and a resolution of 0.1 ppm. The alarm set point of this device was set at one ppm. Room temperature and humidity were monitored and recorded throughout the testing. Circulation fans were placed in the room to aid circulation of the gas during decontamination. Eight biological indicators were used for each test run, one in each corner of the room at the floor and ceiling levels.

Appendix B: Room Testing Specifications

The room volume for the test was 1500 cubic feet (42.47 cubic meters). Supply and exhaust ventilation ducts serving the space were 10 inches (250 mm) in diameter and the airflow volume regulating devices were factory characterized, pressure-independent venturi valves with shut-off capabilities (see Appendix A for shut-off leakage).

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