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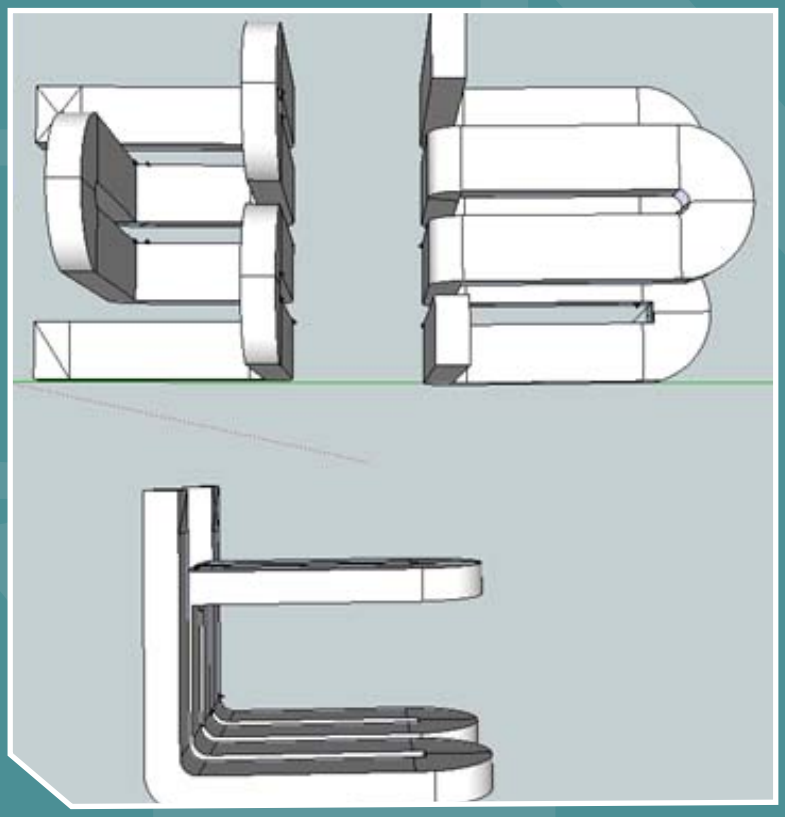
Evaluation of Hydrogen Peroxide Fumigation for HVAC Decontamination



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Evaluation of Hydrogen Peroxide Fumigation for HVAC Decontamination

Assessment and Evaluation Report

National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Disclaimer

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Appendix A Miscellaneous Operating Procedures (MOPs)

List of Acronyms and Abbreviations

APPCD	Air Pollution Prevention and Control Division
ATCC	American Type Culture Collection
B.	*Bacillus*
BSC	Biological Safety Cabinet
ClO_2	Chlorine dioxide
CBR	Chemical, Biological, or Radiological
CBRN	Chemical, Biological, Radiological, and Nuclear
CFU	Colony Forming Units(s)
CM	Critical Measurements
CMAT	Consequence Management Advisory Team
COC	Chain of custody
CT	Concentration x Time
DCMD	Decontamination and Consequence Management Division
DHS	Department of Homeland Security
DI	Deionized
DF	Decimal Factor
DQI	Data Quality Indicator
DQO	Data Quality Objective
ECBC	Edgewood Chemical Biological Center
EPA	U. S. Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GMP	A product name, rather than an acronym
HEPA	High-Efficiency Particulate Air
H_2O_2	Hydrogen peroxide
hp	horse power
HSRP	Homeland Security Research Program
HVAC	Heating, Ventilation, and Air Conditioning
LR	Log reduction
MDI	Metered Dose Inhaler
MOP	Miscellaneous Operating Procedure
NDT	National Decontamination Team
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OCSPP	Office of Chemical Safety and Pollution Prevention
ORLS	On-Site Research Laboratory Support
ORD	Office of Research and Development
OSWER	Office of Solid Waste and Emergency Response
PBST	Phosphate Buffered Saline Tween20

PPE	Person Protective Equipment
ppm	parts per million
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RH	Relative Humidity
SEM	Scanning Electron Microscopy
SOP	Standard Operating Procedure
TBD	To Be Determined
TRIO	Taskforce on Research to Inform and Optimize CBR (chemical, biological, and radiological) Response/Readiness
TSA	Tryptic Soy Agar
VHP	Vaporized Hydrogen Peroxide
WAM	Work Assignment Manager

Executive Summary

This project supports the mission of the U.S. Environmental Protection Agency's Office of Research and Development's Homeland Security Research Program (HSRP) by providing information relevant to the decontamination of areas contaminated as a result of an act of terrorism. The primary objective of this project was to determine the efficacy of fumigation with hydrogen peroxide (H_2O_2) vapor on deactivating spores inside a Heating, Ventilation and Air Conditioning (HVAC) duct. For these tests a STERIS 1000ED VHP® mobile biodecontamination system was used to generate and inject H_2O_2 vapor. Secondary objectives were to determine the effect that flow rate, distance from injection point, flow and pressure points at turns such as elbows, inlet concentration of fumigant, and fumigant residual effects may have on the decontamination efficacy. Two types of duct were tested: galvanized metal and galvanized metal lined internally with fiberglass duct insulation.

The efficacy of H_2O_2 for the decontamination of an unlined duct varied based on the location in the duct. For a single fumigation condition, the average log reduction (LR) per location ranged from 0.6 LR to full decontamination (≥7.4 LR, no recoverable viable spores). These results suggest that flow patterns can be very complex in ductwork, and those complexities can make gaseous decontamination more difficult in certain locations within the ductwork. Flow separation, eddying, and flow reversal occurred at certain locations in the duct immediately following elbows. These locations were very difficult to decontaminate in an unlined, metal duct. Increasing the flow rate through the duct seemed to exacerbate these effects, though additional research is needed to confirm this result.

Lined duct proved easier to decontaminate than unlined metal duct. The lining absorbed H_2O_2, and desorbed it over a period of over 48 hours. This desorption contributed significantly to VHP levels within the duct following the initial fumigation, and resulted in higher efficacies than observed in unlined ductwork. The results demonstrate that fumigation with H_2O_2can be an effective decontaminant on lined duct even at low concentrations for a prolonged period of time (24 hours). Fumigations with a concentration-time product (CT) of 550 ppm-hours exposure to H_2O_2 provided more than a 6 log reduction.

1 Introduction

This project supports the mission of the U.S. Environmental Protection Agency's Office of Research and Development's HSRP by providing information relevant to the decontamination of areas contaminated as a result of an act of terrorism. Under Homeland Security Presidential Directive -10, the U.S. Department of Homeland Security (DHS) is tasked to coordinate with other appropriate Federal departments and agencies, to develop comprehensive plans that, "provide for seamless, coordinated Federal, state, local, and international responses to a biological attack." As part of these plans, the EPA, in a coordinated effort with DHS, is responsible for "developing strategies, guidelines, and plans for decontamination of persons, equipment, and facilities" to mitigate the risks of contamination following a biological weapons attack. The EPA's National Homeland Security Research Center (NHSRC) provides expertise and products, through implementation of the HSRP, that can be widely used to prevent, prepare for, and recover from public health and environmental emergencies arising from terrorist threats and incidents. The goal of NHSRC"s decontamination research is to provide products and expertise that guide the selection and implementation of decontamination methods and provide the scientific basis for a significant reduction in the time and cost of decontamination events. This research supports the Office of Solid Waste and Emergency Response (OSWER) and the Office of Chemical Safety and Pollution Prevention (OCSPP). OSWER, through its Special Teams that includes the CBRN Consequence Management Advisory Team (CMAT), supports the emergency response functions carried out by the Regional Offices. OCSPP supports the decontamination effort by providing expertise on biological agent inactivation and ensuring that the use of pesticides in such efforts is done in accordance with the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

Close collaboration among the different program offices having homeland security responsibilities is sought to rapidly increase EPA's capabilities to help the Nation recover from a terrorist event involving the intentional release of chemical, biological, or radiological (CBR) materials.

In 2001, the introduction of a few letters containing anthrax spores into the U.S. Postal Service system resulted in the contamination of several facilities. Although most of the facilities where these letters were processed or received in 2001 were heavily-contaminated, they were successfully remediated with approaches such as fumigation with vaporized hydrogen peroxide (VHP®) or chlorine dioxide (ClO_2) , including the HVAC ducts.[1,2] While these decontamination methods have been studied extensively for decontamination of surfaces found in the open spaces of a building (walls, floors, windows, etc.), this research will help to determine the efficacy of the decontamination method within the confined spaces of an HVAC system and on the materials found within these systems.

1.1 Process

The general process being investigated in this project is decontamination of surfaces contaminated with *Bacillus* spores (i.e., surrogates of *B. anthracis*). Decontamination can be defined as the process of inactivating or reducing a contaminant in or on humans, animals, plants, food, water, soil, air, areas, or items through physical, chemical, or other methods to meet a cleanup goal. In terms of the surface of a material, decontamination can be accomplished by physical removal of the contamination or via inactivation of the contaminant with antimicrobial chemicals, heat, ultraviolet light, etc. Physical removal could be accomplished via *in situ* removal of the contamination from the material or physical removal of the material itself (i.e., disposal). Similarly, inactivation of the contaminant can be conducted *in situ* or after removal of the material for ultimate disposal. During the decontamination activities following the

results of the 2001 anthrax incidents, a combination of removal and *in situ* decontamination was used.[3] The balance between the two was facility-dependent and factored in many issues (e.g., physical state of the facility). One factor was that such remediation was unprecedented for the United States Government and no technologies had been proven for such use at the time. The cost of disposal proved to be very significant and was complicated by the nature of the waste (e.g., finding an ultimate disposal site).[3,4] Since 2001, a primary focus for facility remediation has been improving the effectiveness and practical application of *in situ* decontamination methods and evaluating waste treatment options to be able to provide the information necessary to optimize the decontamination/disposal paradigm. This optimization has a significant impact on reducing the cost of and time for the remediation effort.

In this study, the decontamination efficacy was evaluated for H_2O_2 vapor when used to inactivate *Bacillus* spores inside a lab-scale HVAC system. Coupons of HVAC duct material were loaded with spores using a deposition device. Test materials were 18 mm diameter coupons prepared from the same materials as the duct. Test and procedural blank coupons were placed in the test duct and decontaminated as described using H_2O_2 as the fumigant of choice for this project. After fumigation, the test coupon holders were removed from each testing section of the duct, and the coupons were then removed from the coupon holders for spore extraction and quantification. Positive control coupons (i.e., contaminated with spores but untreated) were used to determine the pre-treatment (i.e., inoculum) loading on each coupon type. Spores were extracted and quantified from the test coupons, positive control coupons, and QC samples. Quality control (QC) samples included procedural blank coupons (coupons that underwent the fumigation process but which were not inoculated) and negative controls (which did not undergo the fumigation process)

1.2 Project Objectives

The primary objective of this project was to determine the efficacy of the H_2O_2 fumigation method on deactivating spores inside an HVAC duct. For this project a STERIS 1000ED VHP® mobile biodecontamination system was used to generate H_2O_2 vapor and inject it into the HVAC duct. Secondary objectives were to determine the effect that flow rate, distance from injection point, flow and pressure points at turns such as elbows, inlet concentration of fumigant, and fumigant residual effects may have on the decontamination efficacy. The latter was determined based upon the comparison of the number of spores (measured as colony forming units (CFUs)) recovered from positive control coupons versus the recovery from test coupons. The static pressure inside the duct and the concentration of the fumigant were measured at several locations along the duct. These parameters were used to characterize the behavior of the fumigant inside the duct.

1.3 Experimental Approach

A closed loop duct was constructed and subjected to fumigation with H_2O_2 vapor under different operating conditions. Inoculated coupons of the duct material, whether lined or unlined, were placed at different points along the duct, flush with the duct surface, and exposed to the decontamination technique. The efficacy of the decontamination method was measured by comparing the number of colony-forming *Bacillus* spores recovered from these test coupons as compared to positive control coupons.

1.3.1 Testing Sequence

Testing was conducted in test ductwork fabricated in High-Bay Room 122-A at EPA's Research Triangle Park facility. A test matrix was developed at the start of the testing campaign, and this matrix was

sequentially modified as the results of completed tests were analyzed. In general, the testing sequence was conducted as follows:

1. Sterilization of all coupons and materials needed for the test. The sterility of the coupons was verified through the use of laboratory blank control samples.

2. Inoculation of test and positive control coupons with spores of *B. subtilis* using a metered dose inhaler (MDI).

3. Insertion of the test coupon holders loaded with a set of five coupons each (four test coupons and one negative coupon) at eight defined testing locations along the length of the ductwork. These locations were chosen specifically to determine a) the potential effects of temporal degradation of fumigant in the duct, and b) the effect on efficacy due to differing flow patterns within the duct including low pressure points at either angular or curved turns.

4. Application of a prescribed fumigation sequence with H_2O_2 vapor using a STERIS VHP® 1000ED generator. The target test condition (fumigation concentration, duct flow rate, and exposure time) was set and controlled at the inlet of the ductwork. Relative Humidity (RH) and temperature during testing were monitored, but not controlled. The fumigant concentration was monitored continuously at three locations (inlet, mid-, and at the end of the duct closed loop) to determine the concentration profile as a function of length and time in the duct. After the exposure time was reached, the ductwork was immediately aerated until fumigant concentrations were low enough to allow safe removal of the test coupons for analysis.

5. Transfer of the test coupons, procedural blanks, and positive controls to the NHSRC Biocontaminant Laboratory in sterile primary independent packaging within sterile secondary containment containing logical groups of samples for analysis. All samples were accompanied by a completed chain of custody (COC) form.

6. Quantitative assessment of initial viable spore loading by sampling and analysis of positive control coupons.

7. Quantitative assessment of remaining viable spores on test coupons following treatment, and quantitative assessment of spores on negative control coupons.

8. Determination of surface decontamination efficacy (comparison of viable spore concentrations from positive controls and test coupons).

For the lined duct, a series of tests was added to the above testing sequence to determine sporicidal efficacy of off-gassing H_2O_2 following the decontamination phase (which is defined in Section 2.2). After removing the first series of test coupons subjected to the prescribed fumigation conditions, a second series of inoculated coupons and blank coupons was loaded in the duct at one or two locations for a quantitative assessment of residual decontaminant off-gassing (low decontaminant concentration) to remove/inactivate the viable spores during an extended aeration phase.

In addition to the steps outlined above, all test activities were documented during the activity via narratives in laboratory journals, real-time data acquisition, and the use of digital photography. The documentation included, but was not limited to, a record of time required for each decontamination step or procedure, any deviations from the test plans, and physical impacts on the materials.

All tests were conducted in accordance with developed miscellaneous operating procedures (MOPs), listed in Appendix A, to ensure repeatability and adherence to the data quality validation criteria set for this project.

1.3.2 Definitions of Effectiveness

The sporicidal effectiveness (efficacy) of the decontamination technique is a measure of the ability of the method to inactivate the spores on a contaminated material surface (i.e., represented by coupons in this study). Efficacy is evaluated by measuring the difference in the logarithm (Log_{10}) of the measured CFU before decontamination (determined from sampling the positive control coupons) and after decontamination (determined from sampling the test coupons) for the same type of material. The number of viable spores was measured as CFU. This value is reported as a $\log_{10}$ reduction on the specific material surface as defined in Equation 1-1.

$$\eta_i = \frac{\sum_{k=1}^{N_C} \log(CFU_{C,k})}{N_C} - \frac{\sum_{k=1}^{N_t} \log(CFU_{S,k})}{N_s} \qquad (1\text{-}1)$$

where:

η_i = Surface decontamination effectiveness; the average log reduction of spores on a specific material surface (surface material designated by *i*)

$\frac{\sum_{k=1}^{N_C} \log(CFU_{C,k})}{N_C}$ = The average of the logarithm (or geometric mean) of the number of viable spores (determined by CFU) recovered on the positive control coupons (C indicates control and N_C is the number of control coupons)

$\frac{\sum_{k=1}^{N_t} \log(CFU_{S,k})}{N_s}$ = The average of the logarithm (or geometric mean) of the number of viable spores (determined by CFU) remaining on the surface of a decontaminated coupon (S indicates a test coupon and N_s is the number of coupons tested).

When no viable spores were detected, a value of 0.5 CFU was assigned to the maximum plated volume to determine the detection limit for $CFU_{S,k}$ and the efficacy was reported as greater than or equal to the value calculated by Eqn. 1-1. The choice of 0.5 CFU as the detection limit allowed differentiation between detect (1 CFU) and non-detect, a vital distinction in a field event.

The standard deviation of the average log reduction of spores on a specific material (η_i) is calculated by Eqn. 1-2:

$$SD_{\eta_i} = \sqrt{\frac{\sum_{k=1}^{N_s}(x_k - \eta_i)^2}{N_S - 1}} \quad (1\text{-}2)$$

where:

SD_{η_i} = Standard deviation of η_i, the average log reduction of spores on a specific material surface

η_i = The average log reduction of spores on a specific material surface (surface material designated by *i*)

x_k = The average of the log reduction from the surface of a test coupon (Equation 1-3)

N_S = Number of test coupons of a material surface type.

and

$$x_k = \frac{\sum_{k=1}^{N_s}((\overline{\log(CFU_c)} - \log(CFU_{s,k}))}{N_s} \quad (1\text{-}3)$$

where:

$\overline{\log(CFU_C)} = \frac{\sum_{k=1}^{N_C}\log(CFU_{C,k})}{N_C}$ = Represents the "mean of the logs" (geometric mean), the average of the logarithm-transformed number of viable spores (determined by CFU) recovered on the control coupons (C = positive control coupons, N_c = number of positive control coupons, k = test coupon number and N_s is the number of test coupons)

$CFU_{s,k}$ = Number of CFU on the surface of the k^{th} test coupon

N_s = Total number (1,k) of test coupons of a material type.

2 Materials and Methods

2.1 Facility Design

Testing was conducted in a test ductwork that was fabricated in High-Bay Room 122-A at EPA's Research Triangle Park facility. Figures 2-1 and 2-2 show a diagram of the test duct, and the actual testing facility, respectively. The test duct consisted of 16-inch high by 8-inch wide, 18 gauge galvanized steel duct work within secondary containment (a spray booth, containing an exhaust ventilation system independent of the High Bay Building). The design was chosen to maximize duct length, provide complex flow regions including elbows, and fit inside the spray booth chamber (secondary containment). The test duct included both the square ell 90° turns typical of many HVAC systems and radial ells included to reduce the total pressure drop. A blower (Model 7C651. modified with ½ horsepower (HP) inverter duty motor, Dayton, Electric Manufacturing, Niles, IL) provided recirculation of fumigant within the ductwork, when desirable. Due to the higher than normal pressure drop of this duct design, a larger ½ HP motor was required on the blower to provide a full dynamic range of flow rates. The ductwork was made to disassemble easily and be fabricated in both lined and un-lined forms. Sample ports were fashioned at various points along the length of the duct to allow coupons to be inserted into the duct flush with the inside surface of the duct. For the lined test condition, the duct was internally coated with Knauf Sonic XP 1.5# 1" fiberglass duct liner (Knauf Insulation, Shelbyville, IN).

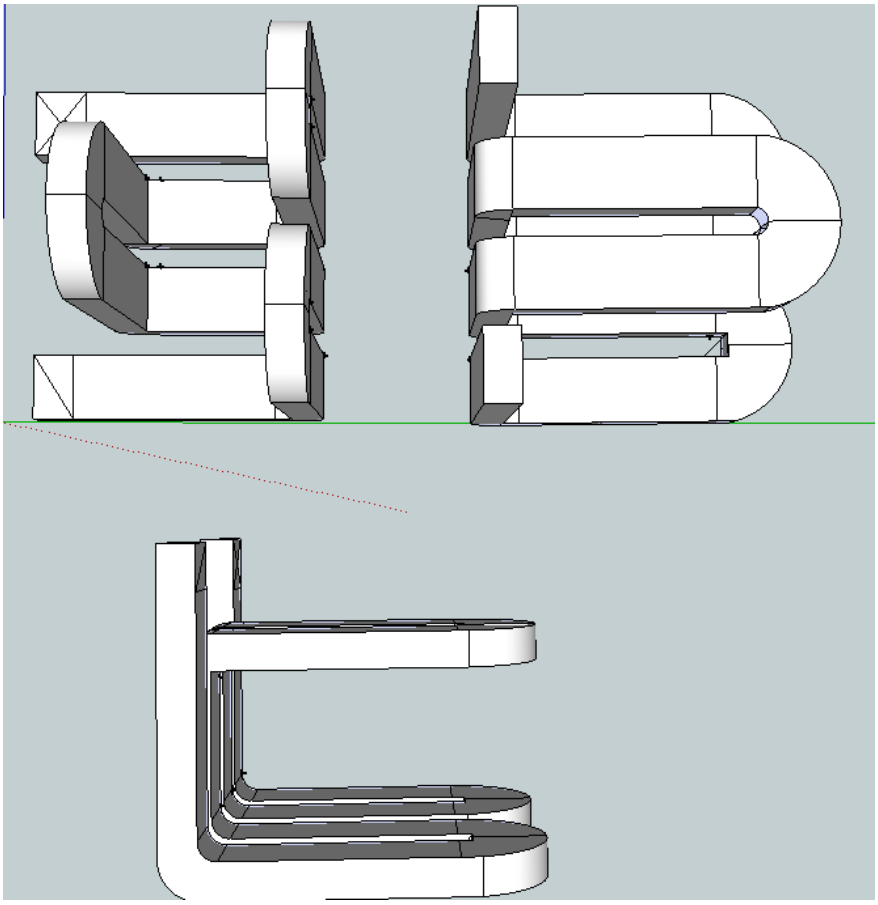

Figure 2-1. Front (top left), Side (top right), and Top views (bottom) of Duct Design (motor and round duct connections not shown in this diagram, see next figure)

Figure 2-2. Photo of Actual Testing Facility

2.2 Hydrogen Peroxide Cycle

The H_2O_2 vapor in this study was generated using a STERIS VHP® 1000ED generator (referred to as Vaporized Hydrogen Peroxide, or VHP®) loaded with a 35% H_2O_2 Vaprox®, cartridge. The STERIS hydrogen peroxide product has been registered by EPA under FIFRA (Reg.# 58779-4). The STERIS generator was operated with a closed control loop in-line with the duct testing facility (See Figure 2-3). To control and monitor the concentration of H_2O_2 in the duct, three Analytical Technology Corp. (Collegeville, PA) H_2O_2 electrochemical sensors (model B12-34-6-1000-1) were used to provide real-time concentration readings. The H_2O_2 was injected at the duct blower outlet, and the first sensor (located downstream) was used to control a solenoid valves (V1 and V2 in Figure 2-3) on the control loop. When the sensors indicated the concentration was above the setpoint, V1 and V2 were switched to the bypass loop. Sensors located at the duct mid- and end-points were used for monitoring purposes only.

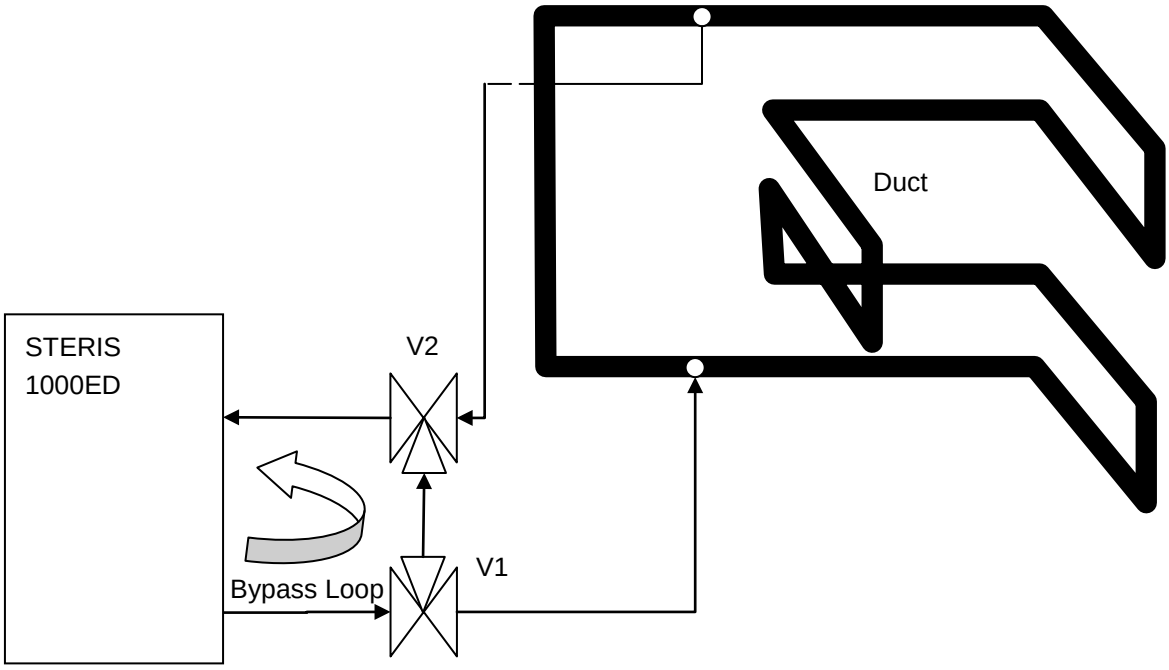

Figure 2-3. Control Loop Schematic

Two controllers of the STERIS VHP® 1000ED store the target operating conditions including the desired time for each fumigation phase, operating pressure, H_2O_2 injection rate, airflow rates, and target RH. The controllers also monitor the amount of hydrogen peroxide available in the reservoir and the dryer capacity.

After the hydrogen peroxide solution reservoir is filled, a VHP® fumigation cycle was programmed to include three operational phases: Conditioning, Decontamination, and Aeration. To initiate the cycle, hydrogen peroxide is first pumped from the cartridge to a reservoir.

- Conditioning Phase: The STERIS VHP® 1000ED pulls 17 acfm of air from the duct, pushes it through a desiccator and a High-Efficiency Particulate Air (HEPA) filter. This dry filtered air is then returned to the duct, with H_2O_2 vapor injected into the air stream just before it leaves the STERIS VHP® 1000ED with a controllable (1-12 g/min) injection rate. The condition phase facilitates reaching the desired decontamination concentration more quickly in larger sealed enclosures. The condition time is affected by sterilant injection rate and enclosure volume. The conditions were selected for the purpose of reducing the total cycle time. Use of the condition phase does not reduce the time of exposure during the Decontamination Phase.

- Decontamination Phase. A constant flow of the H_2O_2 vapor/HEPA-filtered air mixture is maintained at the selected H_2O_2 injection rate, within the controllable range. The Decontamination time was set for the length of the test (90 or 240 minutes) with the injection rate adequate to maintain the H_2O_2

concentration. The control loop helped improve precision and prevent overshoot with regard to H_2O_2 vapor concentration.

- Aeration Phase. There are two stages of the Aeration Phase, one provided by the STERIS VHP® 1000ED, and one provided by the PDAQ control system. For the STERIS stage, H_2O_2 vapor injection is stopped and the recirculation flow of dry HEPA-filtered air through a catalyst at 17 acfm continues for 4 hours to reduce the H_2O_2 concentration within the enclosure. In addition to the STERIS aeration, a pressure relief blower was used to remove air from the duct and pass it through activated carbon before release. Laboratory air was used to replace air removed from the duct.

2.3 Coupon Preparation

2.3.1 Test Coupons

Test materials were 18 mm diameter coupons prepared from the same materials as the duct: 18 gauge galvanized steel (P/N 01170, Eastcoast Metal Distributors, Durham, NC) and liner (Knauf 1.5# 1" fiberglass. Shelbyville, IN). The liner coupon consisted of a 1 mm-thick slice of the liner (including the inner, intended surface of exposure) affixed to a galvanized stub using double-sided adhesive tape (P/N 16073-2, Ted Pella, Inc., Redding, CA). The coupons were fastened to 18 mm aluminum stubs (P/N 16119, Ted Pella, Inc., Redding, CA) using an adhesive-backed magnet (P/N 5775K8, McMaster Carr., Atlanta, GA). The galvanized coupons were sterilized prior to use by steam autoclave consistent with NHSRC Biocontaminant Laboratory MOP 6570 (Appendix A). Liner coupons were sterilized using ethylene oxide (Anderson EOGas Sterilizer, Haw River, NC). Appendix A lists all of the associated MOPs, which can be found in the project *Quality Assurance Project Plan (QAPP) for the Evaluation of Medium and High Tech Methods for HVAC Decontamination.*[5]

A set of five coupons (four test coupons, and one negative coupon) was collocated on a test coupon holder (Figure 2-4) and inserted at each testing location immediately before the start of the test. Magnetic seals were used to ensure that the coupons were aligned with the corresponding holes in the duct. The test and procedural blank coupon holders were designed so that the surface of the coupon would be planar with the inner surface of the duct, thereby minimizing flow disruptions.

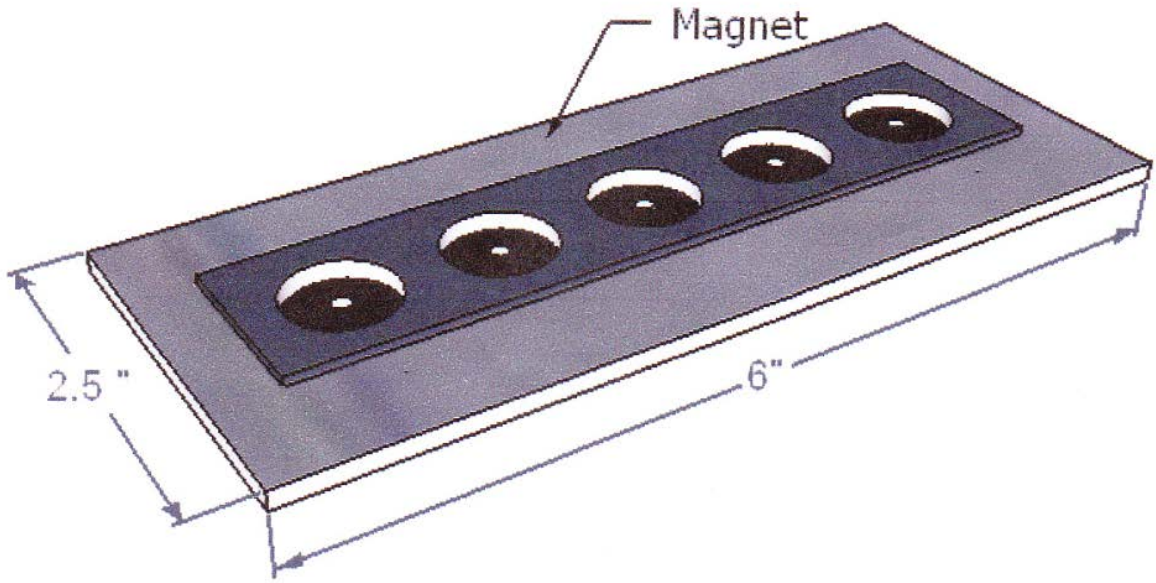

Figure 2-4. Test Coupons Holder Setup

2.3.2 Positive Control Coupons

The positive control coupon holders are slightly different from the test coupon holders, as shown in Figure 2-5. Two to three holders were utilized for each test. Positive controls were inoculated at the beginning, middle, and end of the test coupon inoculation sequence to ensure that inoculations were equal across all test coupons.

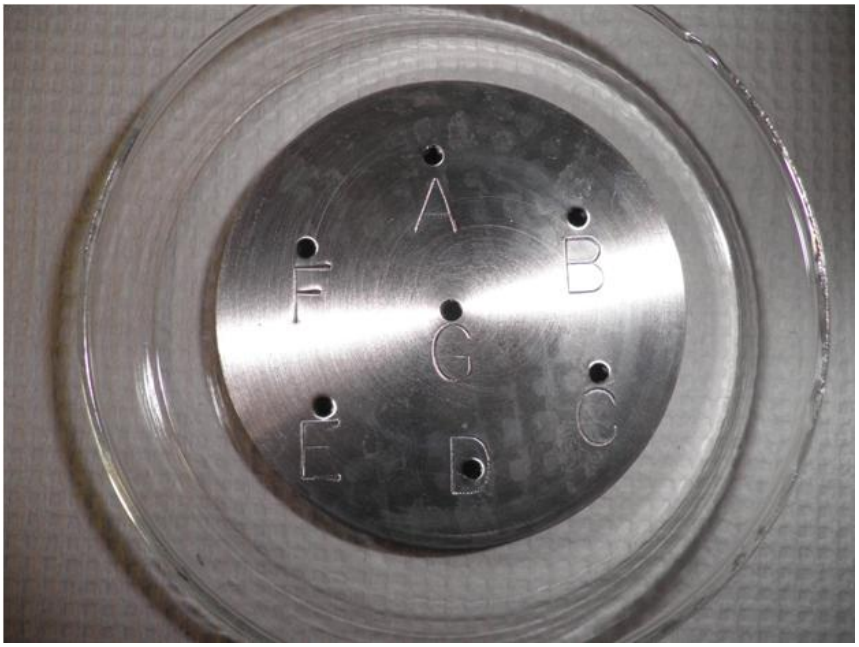

Figure 2-5. Positive Control Coupon Holder

2.3.3 Spore Preparation

The test organism for this work was a powdered spore preparation of *B. subtilis* (American Type Culture Collection (ATCC) 19659; Manassa, VA) and silicon dioxide particles. A preparation resulting in a powdered matrix containing approximately 1 x 10^{11} viable spores per gram was prepared by dry blending and jet milling the dried spores with fumed silica particles (Deguss, Frankfurt am Main, Germany). The powdered preparation was loaded into metered dose inhalers (MDIs)[6] by the U.S. Army Edgewood Chemical Biological Center (ECBC) according to a proprietary protocol.[7,8] Quality assurance documentation is provided by ECBC with each batch of MDIs. Control checks for each MDI were included in the batches of coupons contaminated with a single MDI.

2.3.4 Coupon Inoculation and Test Preparation

Coupons of different types of HVAC materials were inoculated (loaded) with spores of *B. subtilis* using an MDI. The deposition of spores onto the coupons is conducted in accordance with a procedure detailed in MOP 3157 included in Appendix A. In brief, the inoculation procedure involves placing the coupon (18 mm-diameter galvanized HVAC material with or without duct liner attached) on a sterile stub (18 mm-diameter SEM pin stub, Ted Pella, Redding, CA) used for inoculation, and placing it at a precise distance from an MDI during actuation. Following inoculation, the coupon is transferred to a new sterile stub, and the original inoculated stub is discarded. This process is repeated for each coupon. To avoid biases among the positive controls and the test coupons, the following spore loading sequence was adopted:

1. Inoculate the first set of four positive control coupons (4 total)
2. Inoculate the first four sets of four test coupons (16 total)
3. Inoculate the second set of four positive control coupons (when present, 4 total)
4. Inoculate the second four sets of four test coupons (16 total)
5. Inoculate the last set of four positive control coupons (4 total)

The MDIs are set to provide up to 200 discharges before degradation of spore concentration. The number of discharges per MDI was tracked so that use did not exceed this value. Additionally, in accordance with MOP 3157, the weight of each MDI was determined after completion of the contamination of each coupon. If an MDI weighed less than 10.5 g at the start of the contamination procedure described in MOP 3157, it was retired and a new MDI was used.

A log was maintained for each set of coupons that were dosed via the method of MOP 3157. Each record in this log contained the unique coupon identifier, the MDI unique identifier, the date, the operator, the weight of the MDI before dissemination into the coupon dosing device, the weight of the MDI after dissemination, and the difference between these two weights. After inoculation, the coupons were aseptically transferred to sterilized coupon holders. Each test required the inoculation of 32 test coupons and 12 positive control coupons.

2.4 Test Matrix

This work was accomplished in several tests for lined and unlined HVAC ducts, using H_2O_2 generated by the STERIS VHP® 1000ED as the fumigant of choice. The test matrix shown in Table 2-1 represents the overall work performed under this project and reflects the modifications to the operating parameters such

as fumigation concentration, exposure time, and flow rate being necessitated as each test's results were reviewed and evaluated. Note that the numbering for this series of tests starts at 13 since it is part of a larger matrix outlined in the QAPP entitled "Quality Assurance Project Plan for the Evaluation of Medium and High Tech Methods for HVAC Decontamination"[5], and includes testing of other volumetric decontaminants such as chlorine dioxide gas and fogging technologies. The numbering scheme for these tests was kept consistent with the QAPP in order to avoid confusion upon completion of the other phases of the test plan.

Table 2-1. Test Matrix

Test #	Fumigant	Concentration (ppm)	Exposure time (min)	Blower speed	Lined
13	H_2O_2	250	240	15 Hz	No
14	H_2O_2	250	90	15 Hz	No
15	H_2O_2	250	240	15 Hz	No
13b	H_2O_2	250	240	15 Hz	No
16x[a]	H_2O_2	250	240	60 Hz	No
14b	H_2O_2	250	90	15 Hz	No
01	H_2O_2	250	240	15 Hz	Yes
01p	H_2O_2	residual	1440	0 Hz	Yes
02	H_2O_2	250	90	15 Hz	Yes
02p	H_2O_2	residual	1440	0 Hz	Yes
03	None	0	90	15 Hz	Yes
04x[a]	H_2O_2	50	90	15 Hz	Yes
04p	H_2O_2	residual	1440	0 Hz	Yes

a. Test added during the course of the testing program

2.5 Sampling and Analytical Procedures

2.5.1 Test Facility Sampling Procedures

2.5.1.1 Sampling/Monitoring Points

Coupon locations along the test duct were chosen to capture a wide range of in-duct variability in the dynamic HVAC duct environment. The parameters of interest included the following:

- Distance from the injection/monitoring point. This measurement potentially provided information about the degradation of the fumigant as it traveled through the duct.

- Height inside the duct. The flow of the air through the duct was expected to be turbulent; however the highly convoluted flow pattern could produce a stratified flow. Efficacy at each location was measured in quadruplicate (i.e., four replicate coupons per sample location, each at spatially distinct positions with regards to height within the duct). A stratified flow was expected to manifest itself as a trend in efficacy as a function of height inside the duct.

- Pressure points at turns. The flow pattern was expected to have high pressure points on the outside of 90° turns and low pressure points at the inside of the turns. Sampling locations were chosen at both points at the same turn (hence the same distance from injection point). Boundary layers could be thicker at the low pressure points, with lower fumigant concentration reaching the spores.

Other measurements include fumigant concentration, differential pressure (related to flow), RH, and temperature. Figure 2-6 shows all sampling and monitoring locations in the duct. The frequency of sampling and monitoring events is presented in Table 2-2. Table 2-3 lists the critical and non-critical measurements for each sample.

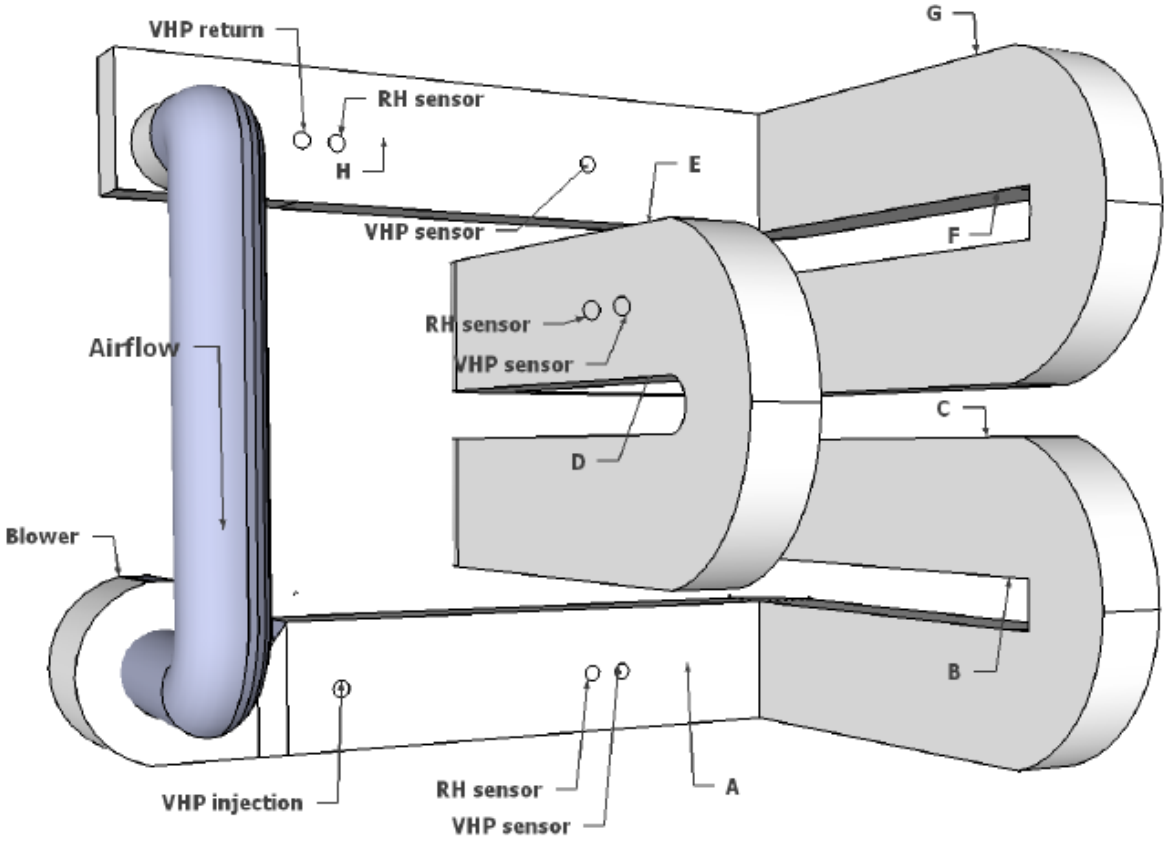

Figure 2-6. Duct Testing Facility with Sampling and Monitoring Locations Indicated by Letters A-H

Table 2-2. Frequency of Sampling Monitoring Events

Sample Type	Sample Number	Sample/Monitoring Frequency	Sample Location	Purpose
Test coupon	4 per sampling location, each at a spatially distinct height within the duct	1 set per location per fumigation	Shown in Figure 2-6 as letters A-H	To determine the number of viable spores after fumigation
Negative control coupon	1 per sampling location	1 set per location per fumigation	Shown in Figure 2-6 as letters A-H	To determine extent of cross-contamination
Positive control coupon	8 to 12 – a set of 4 inoculated at the beginning, middle, and end of test coupon inoculations	1 set per inoculation	NA	To determine the number of viable spores deposited onto the coupons
Field blank coupons	3 coupons which are co-located with test coupons	1 set per inoculation	NA	To determine extent of cross-contamination
Laboratory blank coupons	3 sterile coupons	1 set per fumigation	NA	To demonstrate sterility of coupons and extraction materials.
Biocontaminant Laboratory material blanks	3 per material	One set per use of material	NA	To demonstrate sterility of extraction and plating materials
H_2O_2 monitors	3 real-time instruments	Real time during H_2O_2 fumigations	Shown in Figure 2-6 at three locations	To determine exposure experienced by the coupons and to determine and degradation within the duct
H_2O_2 wet chemistry samples	3 every 2 hours	Once per port every 2 hours	Shown in Figure 2-6 at three locations	To verify proper operation of H_2O_2 monitors
H_2O_2 wet chemistry sample blank	1	1 per H_2O_2 fumigations	NA	To demonstrate correct operation of MOP 3143
Flow rate	1	Logged every 10 seconds	Collocated with RH sensors shown in Figure 2-6 at 4 locations	To determine the flow rate within the duct.
Pressure of Duct	4	Logged every 10 seconds	Co-located with RH sensors shown in Figure2-5 at 4 locations	To help determine the leak rate of the duct
RH/Temp	4	Logged every 10 seconds	Shown in Figure 2-6	To determine environmental conditions inside the duct

Table 2-3. Critical and Non-Critical Measurements

Sample Type	Critical Measurements	Non-critical Measurement
Test coupon	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Negative control coupon	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Positive control coupon	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Field blank coupons	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Laboratory blank coupons	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Biocontaminant Laboratory material blanks	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
VHP monitors	H_2O_2 concentration	NA
Flow rate	Velocity pressure across duct	Temperature and RH of duct
Pressure in duct	NA	Pressure in duct, relative to atmospheric pressure
RH/Temperature	RH and temperature of duct	NA

NA = Not applicable

2.5.1.2 Electrochemical Sensor for H_2O_2 Concentration Measurement

H_2O_2 concentration within the duct was monitored using Analytical Technology Corp. (Collegeville, PA) electrochemical sensors (model B12-34-6-1000-1). The sensors are factory preset to measure from 0 to 1000 ppm H_2O_2 within an accuracy of 5% of the measured value.

The sensors were also calibrated before each test by exposing the transmitter to the head space of a known concentration and temperature of hydrogen peroxide solution. MOP 3136 describes the details of the general procedure for calibration of ATI H_2O_2 transmitters using wells.

2.5.1.3 Duct Flow Rate

Pressure differential traverses were performed on the straight line duct using the AIRDATA™ MULTIMETER ADM-860 electronic micro manometer from Shortridge Instruments, Inc. (Scottsdale, AZ). This meter measures air velocities and differential pressures when used with a pitot tube and automatically corrects for density variation due to local temperatures and barometric pressures. A sampling grid of 24 points was created (3 horizontal lines at 6, 8, and 10 inches from the vertical direction of the duct and eight equally spaced sampling points along the three horizontal lines.

2.5.2 Microbiological Analysis

The NHSRC Biocontaminant Laboratory analyzed all samples qualitatively for spore presence (swab samples) or quantitatively for the number of viable spores per coupon sample.

Details of the sampling and analysis procedures are provided below. A laboratory notebook was used to document the details of each sampling event (or test).

2.5.2.1 Coupon Spore Enumeration

The day after duct fumigation, 18 mm test, procedural blanks, and positive control coupons were transferred aseptically into empty 50 mL sterile vials. This operation was performed in H122 at the site of the duct, so that no spores would be lost in the transfer. The sample vials were then transported to the NHSRC Biocontaminant Laboratory, where 10 mL of sterile Phosphate Buffered Saline plus Tween®20 (PBST) was aseptically added. The sample vials were then sonicated for 10 minutes using an 8510 Branson (Danbury, CT) ultrasonic cleaner at 44 kHz and 250 Watts. The sonication step was immediately followed by two continuous minutes of vortexing to further dislodge any viable spores. Each vial was briefly re-vortexed immediately before any solution was withdrawn for analysis. The solution was subjected to a five-stage serial dilution following MOP 6535a. Each dilution (0.1 mL) was inoculated onto trypic soy agar (TSA) plates, spread with sterile beads according to MOP 6555, and incubated at 35 ± 2 °C for 18-24 hours. Plates with 30-300 CFU were counted manually. Any samples below countable criteria (30 CFU) on the primary dilution plates were filtered following MOP 6565. The filters were incubated at 35 ± 2 °C for 18-24 hours prior to manual enumeration.

2.5.2.2 Swab Samples

Swab sampling was used for sterility checks of the ductwork prior to each use in the testing. A swab equipped with a long handle was used to sample each of the eight test points (A through H) in Figure 2-6. MOP 6563 was followed for collection of swab samples. Swabs were streaked onto TSA and incubated at 35 ± 2 °C for 18-24 hours prior to qualitative growth analysis (presence / absence determination).

2.5.2.3 Method Verification

While there are no approved methods for spore enumeration, the use of positive control samples as the baseline for log reduction calculations includes a built-in verification of the deposition and enumeration methods.

2.6 Sampling Handling and Custody

2.6.1 Prevention of Cross-contamination of Sampling/Monitoring Equipment

Several management controls were instituted to prevent cross-contamination. This project was labor intensive and required that many activities be performed on coupons that were intentionally contaminated (test coupons and positive controls). Specific procedures were put in place in the effort to prevent cross-contamination among the groups. Adequate cleaning of all common materials and equipment was critical in preventing cross-contamination.

There were four primary activities for each test in the experimental matrix. These activities were preparation of the coupons, execution of the decontamination process (including sample recovery), sampling, and analysis. The unlined duct coupons were sterilized prior to use by steam autoclave utilizing a gravity cycle program consistent with NHSRC Biocontaminant Laboratory MOP 6570. Lined coupons were fumigated with ethylene oxide using an Andersen (Haw River, NC) EOGas 333 sterilization system to prevent the heat of the autoclave cycle from melting the liner. Specific management controls for each of the three following activities are described below.

2.6.2 Preventing Cross-Contamination during Execution of the Decontamination Process

The following management controls were followed in an effort to minimize the potential for cross-contamination:

- Negative control coupons were present for each test location. Growth on these coupons would indicate contamination during fumigation or handling.

- Swab samples were taken from inside the duct following the sterilization (reset) fumigation. Growth of these swab samples would indicate the failure of the sterilization fumigation, and new conditions would be assigned to the sterilization fumigation. Nearly all initial swabs indicated that the duct was sterile following reset. In a few instances swab samples indicated the presence of residual background contamination and sterilization conditions were revised and conducted to reset the duct.

2.6.3 Preventing Cross-Contamination during Sampling

Sampling poses an additional significant opportunity for cross-contamination of samples. In an effort to minimize the potential for cross-contamination, several management controls were followed.

- Only one coupon holder was handled at a time. Only the outside surfaces of the holders were touched.
- The coupons were placed in the sterile 50 ml conical tube immediately following post-decontamination, at the site of the duct.
- The coupons were constructed as separate removable discs, so that the stub did not transfer any cross-contaminants.
- Cross-contamination was tracked by the negative *in situ* coupons.

2.6.4 Preventing Cross-Contamination during Analysis

General aseptic laboratory technique was followed and is embedded in the standard operating procedures (SOPs) and MOPs used by the NHSRC Biocontaminant Laboratory to recover and plate samples. The SOPs and MOPs document the aseptic technique employed to prevent cross-contamination. Additionally, the order of analysis was always as follows: (1) all blank coupons; (2) all test coupons; and (3) all positive control coupons.

2.6.5 Sample Quantities

The sample quantities were outlined previously in Table 2-2. In brief, for each test in Table 2-1, there were eight coupon sample locations, which yielded 32 test coupons, 8 negative controls for the test coupons, 8 to 12 positive control coupons, 3 field blank coupons, and 3 laboratory blank coupons.

2.6.6 Sample Containers for Collection, Transport, and Storage

Samples were initially held in the sample holders designed to attach to the duct. These holders were removed from the duct, and sterile forceps were used to transfer samples to individual, sterile 50 mL conical tubes. Swabs of the duct interior were placed in the sterile swab containers and then bagged in

two individual sterile sampling bags as secondary and tertiary containment, prior to transfer to the NHSRC Biocontaminant Laboratory.

After sample collection for a single test was complete, all biological samples were transported to the NHSRC Biocontaminant Laboratory immediately, with appropriate COC form(s). Samples were stored (4 ± 2 °C) no longer than five days before the primary analysis. Typical hold times, prior to analyses, for most biological samples was ≤ 2 days. All samples were allowed to equilibrate at room temperature for one hour prior to analysis.

2.6.7 Sample Identification

Each coupon was identified by a unique sample number. The sampling team maintained an explicit laboratory log which included records of each unique sample number and its associated test number, contamination application, any preconditioning and treatment specifics, and the date treated. The sample codes eased written identification. Once the coupons were transferred to the APPCD Biocontaminant Laboratory for microbiological analysis, each sample was additionally identified by replicate plate (Petri dish) number and dilution. Table 2-4 specifies the sample identification. The NHSRC Biocontaminant Laboratory also included the date each plate was placed in the incubator.

Table 2-4. Coupon Sample Coding

Coupon Identification: 65-TN-LC-RS		
Category	**Example Code**	
65	65	Work Assignment designation
TN	01	Test Number (from Table 2-2)
LC(p) Location Code	A(p)	A through H as shown in Figure 2-1.(p) denotes post test off-gassing sample
	PA, Pp (1-2)	First set of positive controls (at beginning of puffing)
	PM	Middle set (if applicable) of positive controls
	PZ, Pp (3-4)	Last set of positive controls(at end of puffing)
	FB	Field Blank
	BN	Negative stub sample
RS Replicate Sample	1	The replicate sample ID is dictated by the placement in the holder or stage. The positive control RS is shown in Figure 3-2, while the sample RS will be similarly stamped with the numbers 1 through 5. Field and laboratory blank samples are interchangeable, and are simply assigned a value of 1 through 3 in the order of processing.
Biocontaminant Lab Plate Identification: 65-TN-LC-RS -R-D		
65-TN-LC-RS	As above	
R (Replicate)	R	A – C
D (Dilution)	1	0 to 4, corresponding to 1×10^{0} to 1×10^{-4}

Swabs collected as sterility checks were identified by the code 65-TN-SW-LC. The swabs were collected from each sample location shown in Figure 2-6 according to MOP-3135.

2.6.8 Sample Custody

Careful coordination with the NHSRC Biocontaminant Laboratory was required to achieve successful transfer of uncompromised samples in a timely manner for analysis. Test schedules were confirmed with the Biocontaminant Laboratory prior to the start of each test. To ensure the integrity of samples and to maintain a timely and traceable transfer of samples, an established and proven chain of custody or possession is mandatory. Accurate records were maintained whenever samples were created, transferred, stored, analyzed, or destroyed. The primary objective of these procedures was to create an accurate written record that can be used to trace the possession of the sample from the moment of its creation through the reporting of the results. A sample was in custody in any one of the following states:

- In actual physical possession
- In view, after being in physical possession
- In physical possession and locked up so that no one can tamper with it
- In a secured area, restricted except to authorized personnel
- In transit

Laboratory test team members received copies of the test plans prior to each test. Pre-study briefings were held to apprise all participants of the objectives, test protocols, and COC procedures to be followed.

In the transfer of custody, each custodian signed, recorded, and dated the transfer on the COC. Sample transfer could be on a sample-by-sample basis or on a bulk basis. The following protocol was followed for all samples as they were collected and prepared for distribution:

- A COC record accompanied the samples. When turning over possession of samples, the transferor and recipient signed, dated, and noted the time on the record sheet. This record sheet allowed transfer of custody of a group of samples from Highbay room H130-A to the NHSRC Biocontaminant Laboratory.
- If the custodian had not been assigned, the laboratory operator had the responsibility of packaging the samples for transport. Samples were carefully packed and hand-carried between on-site laboratories. The COC record showing the identity of the contents accompanied all packages.

2.6.9 Sample Archiving

All samples and diluted samples were archived for two weeks following completion of analysis. This time allowed for review of the data to determine if any re-plating of selected samples was required. Samples were archived by maintaining the primary extract at 4 ± 2 °C in a sealed 50 mL conical tube.

3 Results and Discussion

This section presents the results of each test, with details on how and why the concentration, exposure time, and flow rate parameters were modified for subsequent tests. The investigation of the effectiveness of H_2O_2 fumigation required some initial characterization of the duct flow rate, flow pattern, and low pressure at turns at angular and curved elbows, before commencement of the biological testing. The results of the duct flow characterization are discussed in Section 3.1. The results of the decontamination testing for unlined HVAC duct and lined HVAC duct are reported and discussed in Sections 3.2 and 3.3, respectively. Note that some additional tests were incorporated in the lined duct test matrix to investigate the effect of out-gassing on the decontamination effectiveness.

3.1 HVAC Duct Flow Characterization

The air velocity inside the unlined duct was characterized as a function of the blower speed and sampling location. A variable frequency inverter was used to operate the blower at three speeds (15 Hz, 30 Hz, and 60 Hz) that resulted in calculated Reynolds (Re) numbers for the unlined duct all above 10^5. This value suggests that the overall bulk flow inside the duct is highly turbulent at all tested flow rates. The flow rate in the lined duct was not measured, but is still expected to be in the turbulent flow region.

3.1.1 Flow Velocity versus Blower Speed

The flow velocities inside the duct were characterized at the three blower speeds by performing pitot tube traverses on the straight line of the duct (Location H before the inlet of the blower and location A downstream of the outlet of the blower).

The velocity profiles at locations A and H inside the duct are shown in Figure 3-1 for each blower speed. The results show that the flow velocities vary linearly with the speed of the blower, and minimal losses are registered between the two locations, A and H.

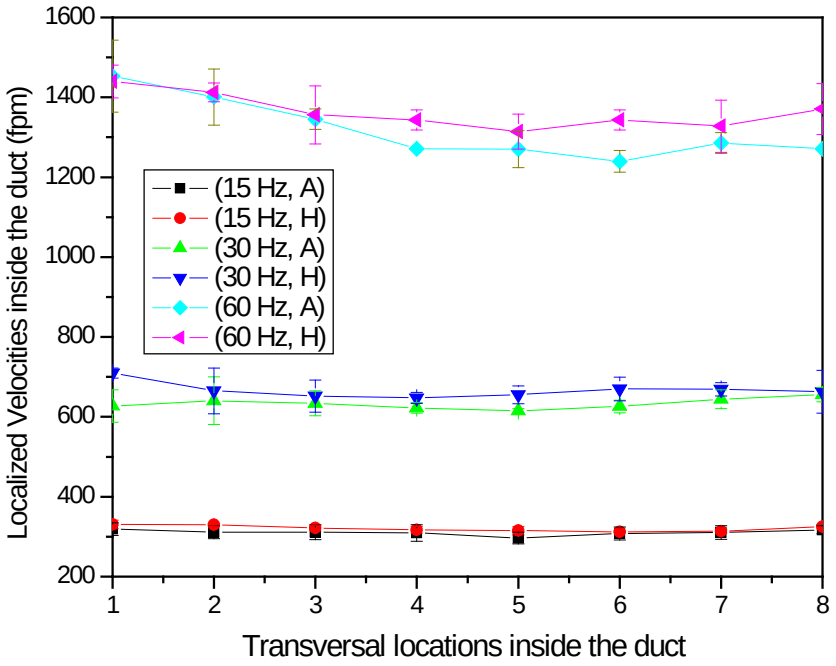

Figure 3-1. Pre- and Post-Blower Velocity Traverses Inside the Duct

Note that the geometry of the duct did not provide a position with straightened flow, thus the standard U.S. EPA Method 2[9] procedure for measuring flow could not be followed.

3.1.2 Flow velocity Profile near an Elbow

The flow pattern near a round elbow (Location D and E) was characterized at three blower speed ratings (15 Hz, 30 Hz, and 60 Hz), using a sampling number of 48 points (a 3 x 16 grid).

These measurements were made in the plane of the duct that includes Sample Location E (zero inches inside the duct) and Sample Location D (16 inches inside the duct). The results shown in Figure 3-2 demonstrate that the flow is affected by the elbow upstream of Location E, with higher flow outside the bend of the elbow (1 to 8 inches) and decreasing on the inside of the bend (9 to 16 inches) causing flow reversal and flow separation. While the total flux of fumigant across this plane of the duct is equal to the total flow rate of the system, the flux at any one point is unknown due to the flow separation. It is unknown whether there was any flux at sample location D, or whether the flow was simply recirculation. The calculated bulk Re was greater than 4000, a benchmark for the transition from intermediary to turbulent flow.

The design of the duct system did not allow similar measurements to be performed at other locations with preceding elbows, due to the limited space at these locations.

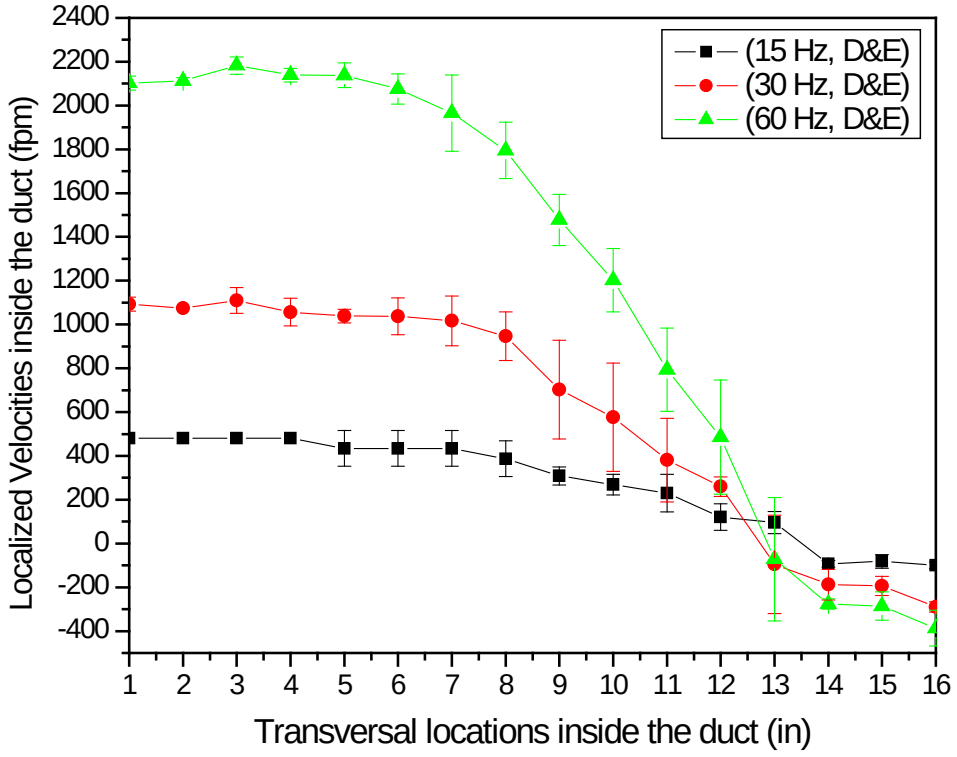

Figure 3-2. Velocity Traverse Inside the Duct at Locations D and E

3.2 H_2O_2 Fumigations – Unlined Duct Results

The first series of tests was completed on the unlined HVAC duct at a H_2O_2 concentration time of 250 ppmv for 4 hrs per the crisis exemption under Section 18 of FIFRA that authorizes EPA to allow an unregistered use of a pesticide for a limited time if EPA determines that an emergency condition exists (http://www.epa.gov/opp00001/factsheets/chemicals/vhp_factsheet.htm). The STERIS registration claim is that Vaprox® hydrogen peroxide is effective as a Sterilant, Sporicide, Bactericide, Virucide, and Fungicide at 250 ppm for 90 minutes in sealed enclosures up to 4,000 ft^3. http://www.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-000595_3-Apr-06_a.pdf.[10] The second parameter investigated was the blower speed (15 Hz and 60 Hz) to determine the effects of the flow velocity, if any, on the fumigant sporicidal effectiveness.

As discussed in Section 2.4.1, there were three locations for H_2O_2 and RH sensors. Table 3-1 shows the average H_2O_2 concentration during fumigations. Location A was nearest the point of injection, and Location H was farthest from injection. Spikes in H_2O_2 concentration were typically short-lived.

Table 3-1. Average H_2O_2 Concentrations and RH during Fumigation

	H_2O_2 Location A (ppm) Average/ (± Standard Deviation)	H_2O_2 Location D-E (ppm) Average/ (± Standard Deviation)	H_2O_2 Location H (ppm) Average/ (± Standard Deviation)	RH Location A (%)	RH Location D-E(%)	RH Location H (%)
Test 13	247(±37)	232 (±34)	225 (±34)	58.2	59.0	59.8
Test 13b	249 (±11)	246 (±13)	215 (±11)	48.0	47.7	51.0
Test 14	250(±9)	232(±9)	219(±10)	47.3	49.8	49.1
Test 14b	255 (±10)	231 (±10)	221 (±12)	75.1	72.7	79.0
Test 15	243 (±27)	244(±27)	228(±26)	51.8	52.4	56.7
Test 16	242(±21)	230 (±20)	208 (±19)	45.1	44.4	47.3

The measured concentration at Location H was consistently lower than the other two locations. The sensor may or may not have been in a position of high flux, but the response time was quick for all sensors, as shown in Figure 3.3. This observation suggests that there was some degradation of H_2O_2 in the duct. The products of H_2O_2 decay include water, so, if there was decay of the H_2O_2, a rise in RH throughout the duct may be expected. The generally rising RH values in Table 3-1 during some tests may further indicate H_2O_2 decay as the vapor traverses the duct (from A to H).

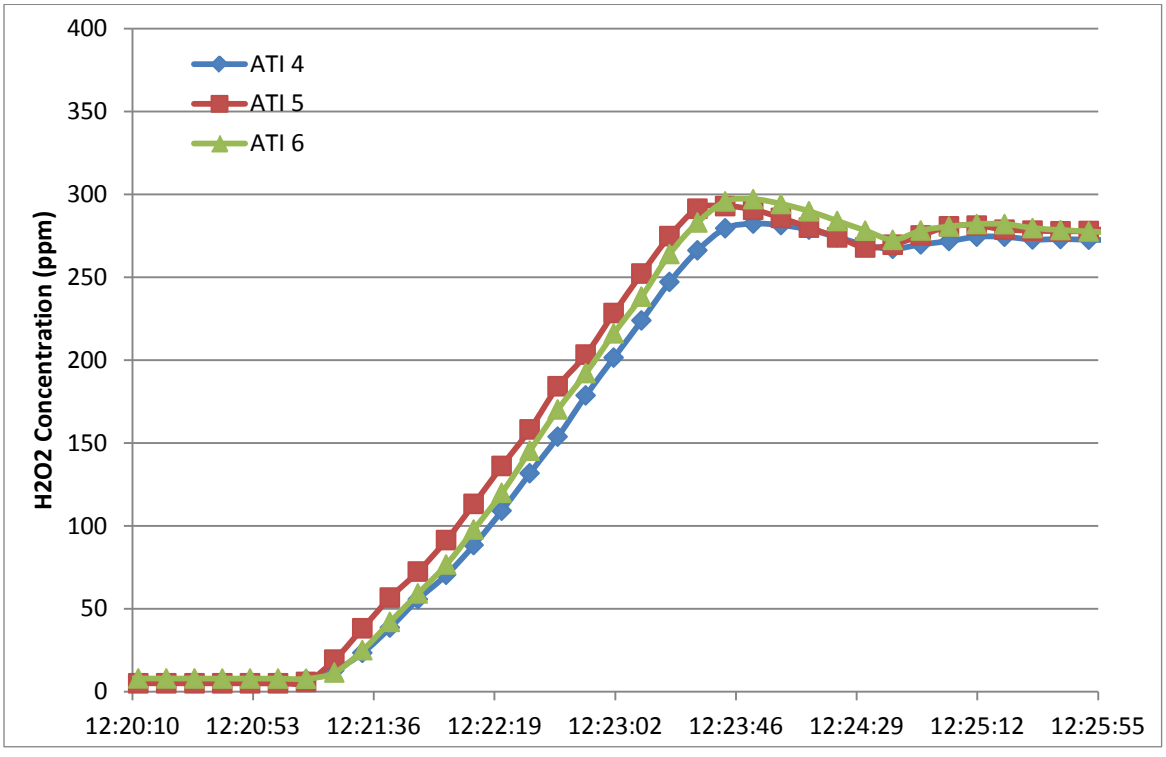

Figure 3-3. Sample Response Time for the ATI Sensors

Two or three sets of positive control coupons were inoculated alongside test coupon sets. These positive control coupons were done at the beginning and the end, and for later tests, also in the middle of inoculations. The CFU recovered from these sets of coupons are shown in Table 3-2.

Table 3-2. Positive Controls Inoculation Results (n = 4)

	First set (CFU/sample)	Middle set (CFU/sample)	End set (CFU/sample)
Test 13	2.95E+07 ± 2.09+07		2.39E+07 ± 1.47+07
Test 13b	1.36E+07 ± 6.57+06	2.16E+07 ± 2.04+07	1.39E+07 ± 8.36+06
Test 14	1.46E+07 ± 3.81+06		6.03E+06 ± 1.75+06
Test 14b	1.53E+07 ± 1.02+07	8.95E+06 ± 4.04+06	1.47E+07 ± 6.90+06
Test 15	2.52E+07 ± 1.26+07		1.71E+07 ± 2.69+06
Test 16	5.98E+06 ± 2.10+06		7.15E+06 ± 6.00+06

While all of these values met the target dose QA requirements and allow for a 6 log reduction, care must be taken when interpreting the data not to compare LR values without considering the initial loading.

There was high variability in the post-decontamination recovery (efficacy) data between tests. Tests 13, 13b, and 15 were all replicate tests. The average CFU recovered from these replicate tests are shown in Table 3-3 and Figure 3-4.

Table 3-3. Average CFU Recovered from Test Coupons from Unlined Duct Sample Points (n = 4)

Test ID	Location A	Location B	Location C	Location D	Location E	Location F	Location G	Location H
13	1	1	1	3500	1	5	1	1
13b	29	11300	1	2	3	7	4.51×10^5	1
15	1	Samples lost*	1	1.88×10^6	463	1200	5.08×10^6	1910

*These samples were mistakenly absent from the duct during exposure.

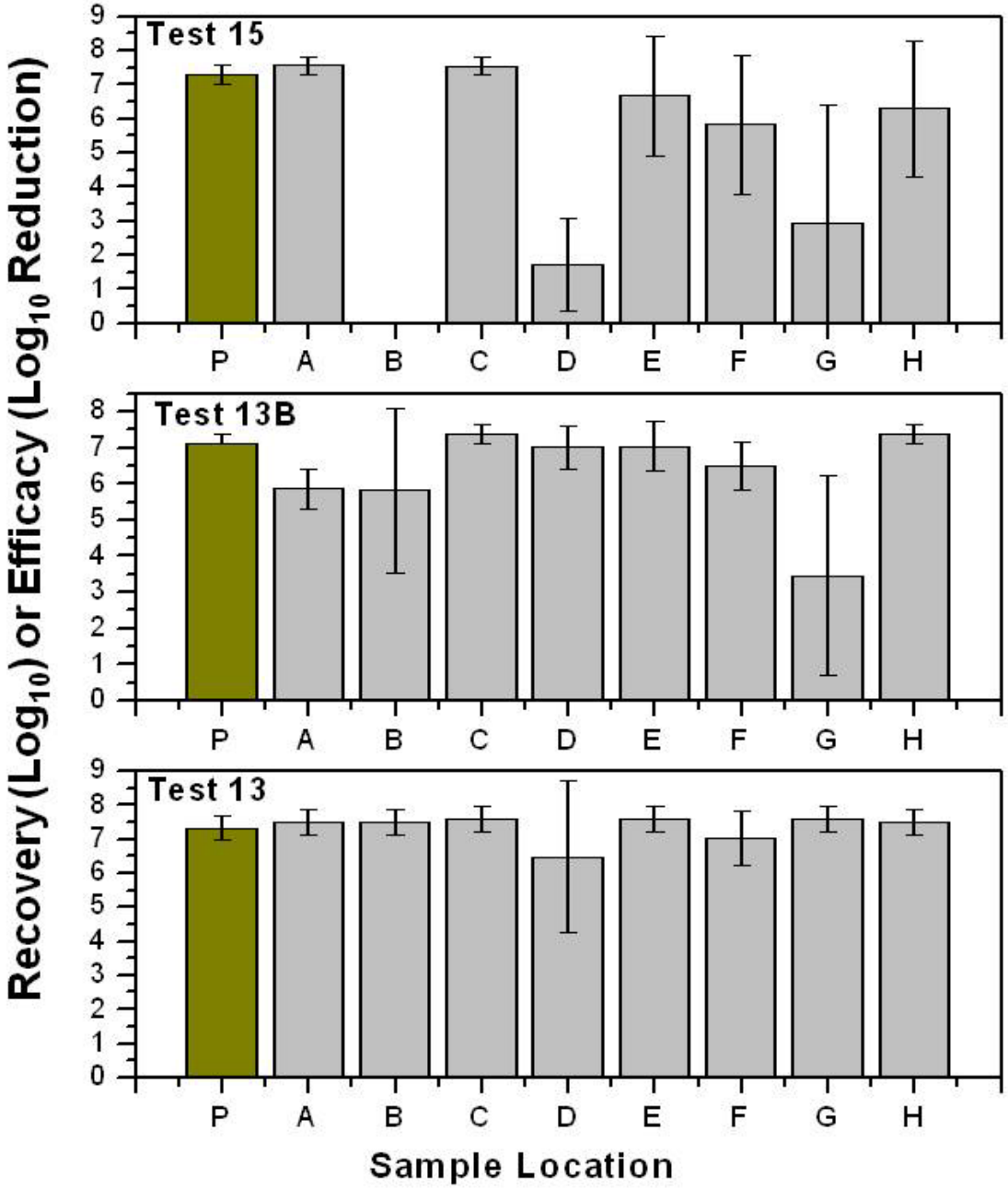

Figure 3-4. Recovery of Positive Controls (green bars) and Spatial Efficacy Results (gray bars) for Unlined Duct (250 ppm x 4 hours). Efficacy data are reported as mean log reduction (gray bars) from four replicate coupons per location. Green bars represent mean Log_{10} positive control recoveries from one replicate coupon collected from each of the eight sampling locations. Sample locations (A through H) from Figure 2-6.

These data show the difficulty in replicating fumigations for localized efficiency measurements, as there was much variation both within the duct for a particular fumigation and within a single location between fumigations. Reasons for the high variability include the following:

- Unknown, non-linear kill kinetics: Small variations in RH or spikes in H_2O_2 concentration could be much more effective against spores than the average condition.
- Leaks from the coupon holders may have offered protection to some coupons. (i.e., in areas of low local pressure, a curtain of fresh air entering the duct near the coupon could have protected it from the fumigant)
- Flow patterns in the duct may depend on (variable) initial conditions.

Several conclusions can be drawn from these data. For sets of coupons that were collocated a distance from the blower near a flow disturbance, one set, or side, experiences higher fumigant flux than the other side. The high flow at Location E and low re-circulating flow at Location D (discussed in Section 3.1), seems to have influenced the efficacy of the fumigant. Location G was also much more difficult to decontaminate than Location F, located across the duct. Location G would seem to be on the high pressure side of the curve, but the duct was intentionally designed to create complex flow patterns that were not easily predicted. Small perturbations in the inside of the duct may have directed flow downward at that location.

Table 3-4 shows log reduction values for all tests as a function of coupon location. Cells with values based on detection limit values have been colored blue. Cells with values based on a very small number (<10) of spores are indicated in red. The lack of contamination observed on any of the negative control coupons suggests these values were not caused by cross-contamination. A comparison between 90-minute exposures and 240-minute exposures suggests that, while longer exposure times may provide higher efficacy, there is no guarantee that higher efficacy will occur, suggesting a non-linear kill curve.

Rather than higher flow rates inside the duct improving contact of the fumigant to the coupons, increasing blower speed seems to have offered some protection to the spores (T-test comparing 15 Hz LR after 240 minute exposure to 60 Hz LR gives a p-value of 0.0003). Further investigation is needed to explain this outcome.

Table 3-4. Average Log Reduction During Testing of the Unlined Duct by Sample Location (n = 4).

Blower Setting (Hz)	Exposure (min)	A	B	C	D	E	F	G	H	Avg	SD
15	240	≥7.50	≥7.50	≥7.57	6.47	≥7.57	7.02	≥7.57	≥7.50	7.34	0.39
		5.86	5.83	≥7.38	7.01	7.05	6.49	3.47	≥7.38	6.31	1.30
		≥7.55		≥7.54	1.71	6.66	5.83	2.95	6.30	5.50	2.29
	90	≥7.22	6.57	6.50	2.15	5.16	3.88	2.51	3.61	4.70	1.94
		≥7.32	≥7.33	≥7.32	6.73	≥7.32	5.46	2.44	≥7.33	6.41	1.73
60	240	3.92	6.28	5.40	4.62	4.29	4.98	3.25	≥6.99	4.97	1.23

NOTE: Data in blue cells are based upon detection limit values (no CFUs detected), LR data in red cells are based upon low post-decon recoveries (<10 CFU).

3.3 H_2O_2 Fumigations – Lined Duct

Internally lined HVAC duct presented a much different fumigation scenario. There are two main differences in the behavior of airflow in the lined duct versus the unlined duct:

- There are fewer leaks in the lined duct because the presence of the liner covers gaps in the duct joints. Note that the presence of the liner did not change the possibility of leaks near the coupon holders.

Note: While leaks were anticipated in both the lined and unlined ducts, these leaks were very minimal compared to the total amount of bulk airflow inside the ductwork. In addition, the ducts were constructed using materials and methods typical of residential and commercial ductwork, and thus any leaks experienced are expected to mimic real-world conditions.

- The liner adsorbs and desorbs fumigant leading to longer aeration times and longer exposures. This phenomenon is not specific to our facility, but will vary as a function of the material and liner manufacturer.

The second difference means that the aeration phase of the lined duct is fundamentally different from unlined duct, even with the exact same fumigation conditions. Due to the desorption of the fumigant over a long period of time, a series of tests was completed to determine the sporicidal effectiveness of the fumigant at low fumigant concentration exposure (i.e., low concentrations resulting from fumigant desorption following a fumigation). A test blank run (no fumigant added) was added to the test matrix to evaluate any non-fumigant related sporicidal effect on the test coupons. The blank test was conducted and sampled the same way as the other test runs.

3.3.1 Exposure Phase

The test and procedural blank coupons were present in the duct during the conditioning phase, the decontamination phase, and for the aeration phase, for a total of approximately 24 hours. It is important to understand the difference in the fumigation minutes and the exposure minutes for the lined duct. For the unlined duct, there was no measurable material absorbance and, at the end of the fumigation, the fumigant concentration declined rapidly. The lined duct, however, exhibited significant desorption during the aeration period. Figure 3-5 shows a trace of the control sensor during all phases of exposure for a lined and unlined test at 250 ppm for 4 hours, as well as the hysteresis response of the sensor when being removed from exposure directly to ambient air.

The trailing concentration during aeration phase of the lined duct (about 30 ppm) contributed a significant portion of the total exposure in terms of Concentration x Time (CT) or ppm*hours. For instance, the CT target fumigation was 1000 ppm*hours (250 ppm x 4 hours) for Test 1, but the overall exposure was 75 percent higher due to the contribution of the aeration phase. The contribution of the aeration phase was an even greater portion of the overall exposure for Tests 02 and 04.

The mean log reduction values for each test are shown in Table 3-5.

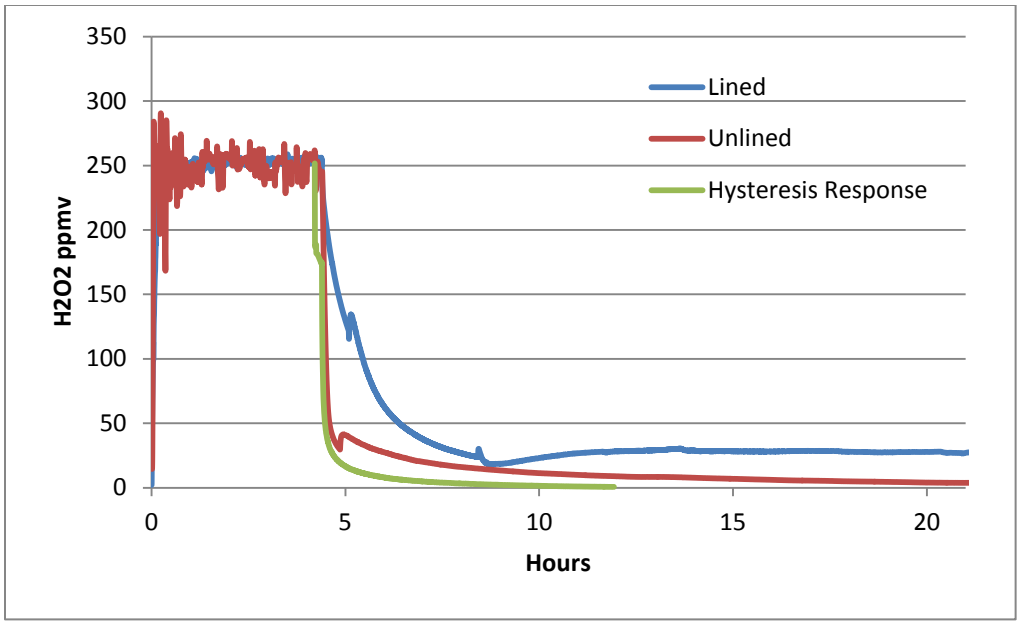

Figure 3-5. H_2O_2 Concentration during Exposure and Aeration Phases for the Lined and Unlined Duct

Table 3-5. Average Log Reduction in Duct (n = 32)

Test Exposure	Exposure ppm	Fumigation Minutes	Total ppm*hours	Avg LR	RSD
Test 01	250	240	1760	>7.4	0.1%
Test 02	250	90	1400	>7.3	1.3%
Test 04	50	90	280	4.6	18.9%

Both fumigation conditions used in Test 01 and Test 02 were very effective, with recovery of fewer than 10 CFU for all samples. Though the exposure time seemed different, the exposure in terms of ppm*hours was quite similar because of desorption during the aeration phase.

Test 4 was performed with the aim of determining the minimum exposure needed for decontamination. Ideally, the CT for Test 4 would have been similar to the target CT for Test 02 (375 ppm*hours), but the kinetics of adsorption/desorption were not well enough understood to predict accurately. The Test 04 conditions were deemed moderately effective, providing only a 4.6 log reduction.

3.3.2 Desorption from Lined Duct

Figure 3-6 shows the sensor responses during Test 01, showing the H_2O_2 concentration in the lined duct over a period of 48 hours. The test and procedural blank coupons experienced the CT represented as the integration of the concentration curve to the left of the first vertical line. The concentration inside the duct seems to have increased while those coupons were removed (the bump between the two vertical lines in Figure 3-6), perhaps because of the physical disturbance of the duct or changes in the movement of air

around the sensors during this operation. Coupons for the desorption test were placed inside the duct after the exposure coupons were removed (the CT to the right of the second vertical line).

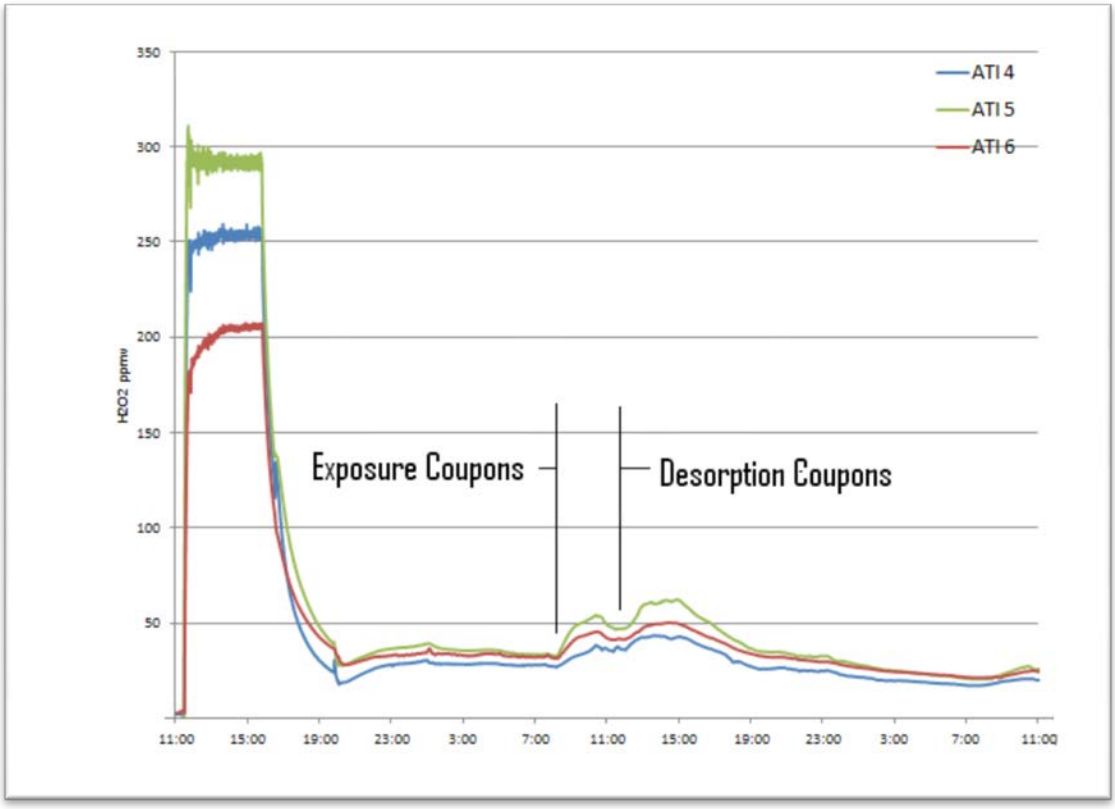

Figure 3-6. H_2O_2 Concentration during the Two Exposure Periods (initial exposure and subsequent desorption) for the Lined Duct (Test 01).

Table 3-6 shows the average concentration, the CT, and the LR of the coupons during the desorption test. Test 3 (discussed below) was conducted identically to Tests 01, 02, and 04, but with no exposure to H_2O_2.

Table 3-6. Conditions and Efficacy during Desorption Tests

Test ID	Average H_2O_2 ppm (Position A)	Average H_2O_2 ppm (Position E)	Average H_2O_2 ppm (Position H)	CT (ppm*hours)	LR
Test 01p	26.3	34.7	31.6	631	>7.3
Test 02p	23.6	22.5	56.0	570	>7.4
Test 03	5.8*	-3.9*	5.5*	9	0
Test 04p	9.6*	6.7*	7.2*	233	0

*These values are below the reported detection limit of the sensor

"p" at the end of each Test ID indicates the test was conducted following (i.e., post-test) the test with similar Test ID (without the "p")

Given the very high efficacy observed for coupons placed in the lined duct following H_2O_2exposure, Test 3 (no H_2O_2) was added to the test matrix to verify that residual H_2O_2, and not some other component of the duct lining, was causing the inactivation. This control test verified that simple exposure to the duct did not reduce recovery compared to positive control samples, indicating that exposure to even very low concentrations of H_2O_2 over long times can be effective at inactivating spores in lined ducts.

Figure 3-7 shows the LR as a function of CT for all lined tests. These results suggest a critical CT value around 550 ppm*hours provided very effective decontamination of the lined duct, and that this CT may be provided with low concentrations of H_2O_2 vapor. Much higher CTs were required to decontaminate the unlined duct.

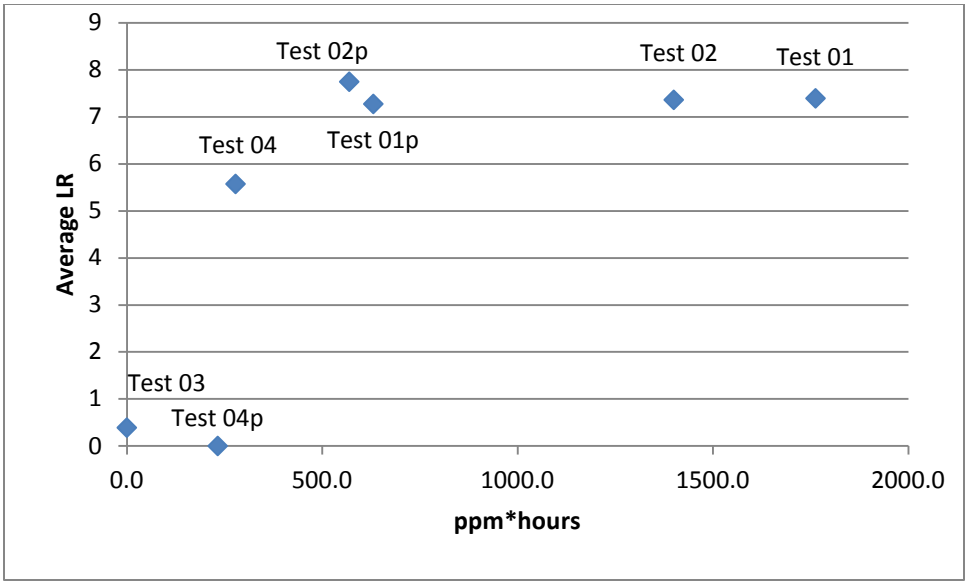

Figure 3-7. Average LR as a Function of CT (lined duct tests)

3.3.3 Comparison of Lined and Unlined Duct

Table 3-7 shows a comparison of Lined and Unlined duct for test conditions common to both duct types.

Table 3-7. Comparison of Lined and Unlined Duct. For each set of conditions (250 ppmv H_2O_2 for 4 hours, or 250 ppmv H_2O_2 for 90 minutes) decontamination efficacy (LR) from the lined test was compared by T-test to efficacy of unlined tests, p-values are reported in the last row of each unlined test column. Exposure (ppmv*hours) is reported as the cumulative CT over 24 hours, as this is the amount of time coupons were inside the duct.

	250 ppmv H_2O_2 for 4 hours				**250 ppmv H_2O_2 for 90 minutes**		
	Lined test 01	**Unlined Test 13**	**Unlined Test 13b**	**Unlined Test 15**	**Lined test 02**	**Unlined Test 14**	**Unlined Test 14b**
H_2O_2 ppmv*hours	1748	NA	1250	1100	1084	482	486
LR	7.4	7.3	6.3	5.5	7.2	4.7	6.4
Student's T-test p-value		0.88	$8.3x10^{-4}$	$2.7x10^{-4}$		$4.9x10^{-8}$	0.013

A Student's T-test comparing the log reductions for lined and unlined ducts was performed for each replicate test on the unlined duct (i.e., lined duct versus each of the unlined test replicates for each test condition). With the exception of Test 13, which had unknown fumigation conditions because of a data acquisition failure, the p-values of the T-test indicate that lined and unlined duct are systems with statistically significant differences. Lined duct were more easily decontaminated than unlined duct at the same target fumigation conditions.

4 Quality Assurance

This project was performed under an approved Category III Quality Assurance Project Plan titled *Evaluation of Medium and High Tech Methods for HVAC Decontamination (July 2011)*.[1]

4.1 Sampling, Monitoring, and Analysis Equipment Calibration

There were standard operating procedures for the maintenance and calibration of all laboratory and NHSRC Biocontaminant Laboratory equipment. All equipment was verified as being certified calibrated or having the calibration validated by EPA's on-site (RTP, NC) Metrology Laboratory at the time of use. Standard laboratory equipment such as balances, pH meters, biological safety cabinets and incubators were routinely monitored for proper performance. Calibration of instruments was done at the frequency shown in Tables 4-1 and 4-2. Any deficiencies were noted. The instrument was adjusted to meet calibration tolerances and recalibrated within 24 hours. If tolerances were not met after recalibration, additional corrective action was taken, possibly including, recalibration or/and replacement of the equipment.

Table 4-1. Sampling and Monitoring Equipment Calibration Frequency

Equipment	Calibration/Certification	Expected Tolerance
Meter box	Volume of gas is compared to NIST-traceable dry gas meter annually	± 2 %
Flow meter	Calibration using a flow hood and a Shortridge manometer	± 5 %
RH sensor	Compare to 3 calibration salts once a week.	± 5 %
Stopwatch	Compare against NIST Official U.S. time at http://nist.time.gov/timezone.cgi?Eastern/d/-5/java once every 30 days.	±1 min/30 days
Clock	Compare to office U.S. Time @ time.gov every 30 days.	±1 min/30 days
Pressure gauges	Compare to independent NIST Pressure gauge annually.	± 2 %full scale

Table 4-2. Analysis Equipment Calibration Frequency

Equipment	Calibration Frequency	Calibration Method	Responsible Party	Acceptance Criteria
Pipettes	Annually	Gravimetric	External Contractor	±1% target value
Pressure Manometer	Annually	Compared to NIST-traceable Heiss gauge	ARCADIS	±3% reading
Incubator thermometers	Annually	Compared to NIST-traceable thermometer	ARCADIS Metrology Laboratory	± 0.2 °C
Scale	Before each use	Compared to Class S weights	ARCADIS	± 0.01% target

4.2 Data Quality

The primary objective of this project was to determine the efficacy of various fumigation methods on deactivating spores inside an HVAC duct. Secondary objectives were to determine the effect that flow rate, distance from injection point, flow and pressure points at turns such as elbows, and inlet concentration of fumigant may have on the efficacy. This section discusses the Quality Assurance/Quality Control (QA/QC) checks (Section 4.3) and Acceptance Criteria for Critical Measurements (Section 4.4) considered critical to accomplishing the project objectives.

The Quality Assurance Project Plan (QAPP)[1] in place for this testing was followed with several deviations, many of which were documented in the text above. Deviations included the flow rate in the duct and the H_2O_2 wet chemistry. The original test matrix listed the air flows at 450, 900, and 1350 CFM. Due to excessive air turbulence and lack of an accurate method to measure the velocity, the variable frequency inverter setting was used to vary flow rate to ensure repeatability. The H_2O_2 wet chemistry method proved to be very unreliable and provided no correlation with the actual set point. The ATI sensors used to monitor the H_2O_2 concentration were calibrated before each test and were relied on instead. These deviations did not substantially affect data quality and were necessitated by the test results themselves. Lined coupons and coupon holders were sterilized using ethylene oxide rather than autoclave due to the potential incompatibility of the lining material with high temperatures.

4.3 QA/QC Checks

Uniformity of the test materials was a critical attribute to assuring reliable test results. Uniformity was maintained by obtaining a large enough quantity of material that multiple material sections and coupons could be constructed with presumably uniform characteristics. Samples and test chemicals were maintained to ensure their integrity. Samples were stored away from standards or other samples which could cross-contaminate them.

Supplies and consumables were acquired from reputable sources and were NIST-traceable when possible. Supplies and consumables were examined for evidence of tampering or damage upon receipt and prior to use, as appropriate. Supplies and consumables showing evidence of tampering or damage were not used. All examinations were documented and supplies were appropriately labeled. Project

personnel checked supplies and consumables prior to use to verify that they met specified task quality objectives and did not exceed expiration dates.

Quantitative standards do not exist for biological agents. Quantitative determinations of organisms in this investigation did not involve the use of analytical measurement devices. Rather, the CFU were enumerated manually and recorded. Critical QC checks are shown in Table 4-3. The acceptance criteria were set at the most stringent level that could be routinely achieved and are consistent with the data quality objectives described in Section 4.4. Positive controls and procedural blanks were included along with the test samples in the experiments so that well-controlled quantitative values were obtained. Background checks were also included as part of the standard protocol. Replicate coupons were included for each set of test conditions. Qualified, trained and experienced personnel using SOPs/MOPs ensure data collection consistency. When necessary, training sessions were conducted by knowledgeable parties, and in-house practice runs were used to gain expertise and proficiency prior to initiating the research.

4.4 Acceptance Criteria for Critical Measurements

The Data Quality Objectives (DQOs) define the critical measurements (CM) needed to address the stated objectives and specify tolerable levels of potential errors associated with simulating the prescribed decontamination environments. The following measurements were deemed to be critical to accomplish part or all of the project objectives:

- enumeration of spores on the surface of the duct coupons
- concentration measurements to characterize the fumigation conditions

The Data Quality Indicators (DQIs) listed in Table 4-4 are specific criteria used to quantify how well the collected data met the DQOs. Failure to provide a measurement method or device that meets these goals results in the rejection of results derived from the CM. For instance, if the plated volume of a sample is not known (i.e., is not 100% complete), then that sample is invalid. In contrast, for the real-time H_2O_2 measurements, some missing data would not invalidate a test.

Table 4-3. QA/QC Sample Acceptance Criteria

Sample Type	Purpose	Acceptance Criteria	Corrective Actions	Frequency
Negative control coupons	Determine extent of cross-contamination within duct	None	Values on test coupons of the same order of magnitude will be considered to have resulted from cross-contamination	1 per sample location
Field blank coupons	Verify the process of moving coupons does not introduce contamination	No detectable spores	Determine source of contamination and remove	3 per test
Laboratory blank coupons	Verify the sterility of coupons following autoclaving	No detectable spores	Determine source of contamination and remove	3 per test
Laboratory material coupons	Verify the sterility of materials used to analyze viable spore count	No detectable spores	Determine source of contamination and remove	3 per material per test
Blank Tryptic Soy Agar sterility control (plate incubated, but not inoculated)	Controls for sterility of plates.	No observed growth following incubation.	All plates are incubated prior to use, so any contaminated plates will be discarded.	Each plate
Positive control coupons	Used to determine the extent of inoculation on the coupons	1e6 CFU, ±0.5 log	Outside target range: discuss potential impact; correct loading procedure for next test and repeat depending on decided impact.	8 per test
Puffing control coupons	Used to determine drift and variance in the MDI	The CFU recovered from the first set of positive controls must be within 0.5 log of the second set of positive controls	Reject results and repeat test.	2 sets of 4 coupons
Fumigation extraction blank samples	Validated baseline of extractive techniques	Non-detect	Obtain new reagents	1 per test
Post-test calibration of ATI H_2O_2 and Vaissala RH sensors	Used to validate sensor operation	The post-test calibration check readings must be within 5% of target reading	Reject results. Repeat test.	1 per test

Table 4-4. Critical Measurement Acceptance Criteria

Critical Measurement	Measurement device	Accuracy	Precision	Detection Limit	Completeness
Plated volume	Pipette	±2 %	±1 %	NA	100%
CFU/plate	Enumeration by sight	±10% (between 2 counters)	±10%	1 CFU	100%
Fumigation Time	Timer	±1 second	± 1 second	1 second	100%
H_2O_2 concentration	ATI sensor	±10% range	±5%	10 ppm	90%
RH of fumigation	Vaissala HMT40Y	±5%	±3%	NA	90%

Plated volume critical measurement goals were met. All pipettes are calibrated yearly by an outside contractor (Calibrate, Inc.).

Plates were quantitatively analyzed (CFU/plate) using a manual counting method. For each set of results (per test), a second count was performed on 25 percent of the plates with significant data (data found to be between 30-300 CFU). All second counts were found to be within 10 percent of the original count.

There are many QA/QC checks used to validate microbiological measurements. These checks include samples which demonstrate the ability of the NHSRC Biocontaminant Laboratory to culture the test organism, as well as to demonstrate that materials used in this effort do not themselves contain spores. The checks include:

- Negative control coupons: sterile coupons placed in duct and fumigated
- Field blank coupons: sterile coupons carried to fumigation location but not fumigated
  Laboratory blank coupons: sterile coupons not removed from NHSRC Biocontaminant Laboratory
- Laboratory material coupons: includes all materials, individually, used by the NHSRC Biocontaminant Laboratory in sample analysis
- Positive control coupons: coupons inoculated but not fumigated
- Inoculation control coupons: aluminum coupons puffed at beginning, middle, and end of each inoculation campaign, not fumigated, to assess the stability of the puffer during the inoculation operation.

The ATI H_2O_2 sensors and Vaissala RH meters were zeroed and spanned prior to each test and were within the factory specifications during each fumigation.

4.5 Data Quality Audits

This project was assigned QA Category III and did not require technical systems or performance evaluation audits.

4.6 QA/QC Reporting

QA/QC procedures were performed in accordance with the QAPP for this investigation.

4.7 Amendment to Original QAPP

The following amendment was added to the QAPP in response to changes necessitated by project results.

Amendment 1 (11/09/2011)

Table 4-5 (below) was submitted as Amendment #1, and was to replace this table in the original QAPP. The results from Test 13 showed that H_2O_2 fumigation was very effective at decontaminating the coupons at the lowest flowrate. Higher flowrates were expected to further improve efficacy by improving mixing. Even at the lowest flow rate, concentrations in the duct were similar at all test points. Due to the lack of H_2O_2 demand presented by the galvanized duct and to the efficacy of the first tested H_2O_2 condition (Test 13), this amendment was needed to modify the test matrix. For Test 14, the exposure time rather than the duct flow rate was changed from conditions in Test 13. New conditions for Test 14 were 250 ppm for 90 minutes (compared to 250 ppm for 240 minutes in Test 13). The results of Test 14 were to be used to determine the conditions for Test 15, and perhaps Tests 2 and 3.

Table 4-5. Proposed Test Matrix

Test	Fumigant	Concentration (ppm)	Exposure Time (min)	Flow Rate in Duct (acfm)	Duct Work Lined?
1	VHP	250	240	450	Lined
2	VHP	250	240	900	Lined
3	VHP	250	240	1350	Lined
4	ClO_2	3000	180	450	Lined
5	ClO_2	3000	180	900	Lined
6	ClO_2	3000	180	1350	Lined
7	ClO_2	200	480	450	Lined
8	ClO_2	200	480	900	Lined
9	ClO_2	200	480	1350	Lined
10	Fog	TBD	TBD	450	Lined
11	Fog	TBD	TBD	900	Lined
12	Fog	TBD	TBD	1350	Lined
13	VHP	250	240	450	Un-lined
14	VHP	250	90	450	Un-lined
15	VHP	TBD	TBD	450	Un-lined
16	ClO_2	3000	180	450	Un-lined
17	ClO_2	3000	180	900	Un-lined
18	ClO_2	3000	180	1350	Un-lined
19	ClO_2	200	480	450	Un-lined
20	ClO_2	200	480	900	Un-lined
21	ClO_2	200	480	1350	Un-lined
22	Fog	TBD	TBD	450	Un-lined
23	Fog	TBD	TBD	900	Un-lined
24	Fog	TBD	TBD	1350	Un-lined

5 Summary and Recommendations

The efficacy of fumigation with H_2O_2, using the STERIS VHP® 1000ED, in the unlined duct varied based on the location in the duct. For a single fumigation condition, the average LR per location ranged from 0.6 LR to full decontamination (7.4 LR). The results suggest that flow patterns can be very complex in ductwork, and those complexities can make gaseous decontamination more difficult in certain locations within the ductwork. Flow separation, eddying, and flow reversal occurred at certain locations in the duct immediately following elbows. These locations were very difficult to decontaminate in the unlined, metal duct. Increasing the flow rate through the duct seemed to exacerbate these effects, though more studies are needed to confirm this result.

Lined duct proved easier to decontaminate than metal duct. The lining absorbed H_2O_2, and desorbed it over a period of more than 48 hours. This desorption contributed a significant portion of the overall exposure. The results demonstrate that fumigation with H_2O_2, per the VHP® process, can be an effective decontaminant on lined duct even at low concentrations for a prolonged period of time (24 hours). Exposures with a CT of 550 ppm-hours provided more than a 6 log reduction.

Based on these results, the following recommendations can be made:

- Fumigation with H_2O_2 shows promise for decontaminating internally-insulated ductwork. Its efficacy on other types of insulation should be investigated.

- Lower concentrations of H_2O_2 for longer exposure times can be used as an effective decontaminant in lined duct.

- Desorbing materials could be investigated as a method for H_2O_2 delivery.

- Given the surprising effect of flow rate in unlined metal duct, efficacy of H_2O_2 in metal ducts should be studied under very low flow conditions.

Note: This study utilized the STERIS VHP® 1000ED to generate H_2O_2 vapor. Results obtained using other methods of H_2O_2 vapor generation may differ from those of the current study.

References

[1] Rupert, R. "Federal On-Scene Coordinator's Report for the Capitol Hill Site, Washington, DC" US EPA, Region 3, Philadelphia, PA, 125 pages, GS-10F-0076K.

[2] Schaudies, R.P. “Analysis of chlorine dioxide remediation of Washington, DC: *Bacillus anthracis* contamination,” Appendix 15, Hart Senate Office Building HVAC Fumigation Final Report, U.S. Environmental Protection Agency, Region III, EPA 903-R-03-013.

[3] Canter, D.A., D. Gunning, P. Rodgers, L. O’Connor, C. Traunero, C.J. Kempter. “Remediation of *Bacillus anthracis* contamination in the U.S. Department of Justice mail facility,” *Biosecurity and Bioterrorism*. 2005;3(2):119–127.

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