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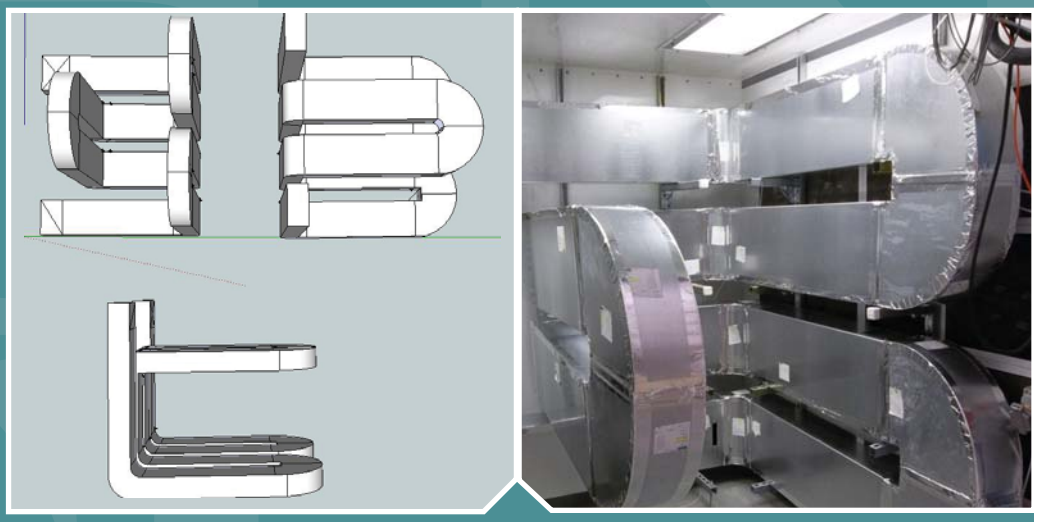
Evaluation of Chlorine Dioxide Gas and Peracetic Acid Fog for the Decontamination of a Mock Heating, Ventilation, and Air Conditioning Duct System

Assessment and Evaluation Report



Office of Research and Development
National Homeland Security Research Center

EPA 600-R-14-014

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U.S. Environmental Protection Agency

Research Triangle Park, NC 27711

Disclaimer

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List of Acronyms and Abbreviations

APPCD	Air Pollution Prevention and Control Division
ATCC	American Type Culture Collection
B.	*Bacillus*
ClO_2	Chlorine dioxide
CBR	Chemical, Biological, or Radiological
CBRN	Chemical, Biological, Radiological, and Nuclear
CFM	Cubic feet per minute
CFU	Colony Forming Units(s)
CM	Critical Measurement
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
DQI	Data Quality Indicator
DQO	Data Quality Objective
ECBC	Edgewood Chemical Biological Center
EMS	Environmental Monitoring System
EPA	U. S. Environmental Protection Agency
GMP	A product name, rather than an acronym
H_2O_2	Hydrogen peroxide
HP	horse power
HSPD	Homeland Security Presidential Directive
HSRP	Homeland Security Research Program
HVAC	Heating, Ventilation, and Air Conditioning
KIPB	Potassium iodide phosphate buffer
LR	Log reduction
MDI	Metered Dose Inhaler
MOP	Miscellaneous Operating Procedure
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OEM	Office of Emergency Management
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OSWER	Office of Solid Waste and Emergency Response
PAA	Peracetic acid
PBST	Phosphate Buffered Saline with Tween 20

ppm	part(s) per million
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
Re	Reynolds
RH	Relative Humidity
SD	Standard deviation
SEM	Scanning Electron Microscope/Microscopy
SOP	Standard Operating Procedure
TSA	Tryptic Soy Agar
VHP	Vaporized H_2O_2
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 investigation was to determine the efficacy of chlorine dioxide (ClO_2) fumigation and fogging with peracetic acid (PAA) for inactivating bacterial spores (using a surrogate for *Bacillus anthracis*) inside a pilot-scale heating, ventilation and air conditioning (HVAC) ductwork system. Tests were conducted at both high (3,000 parts per million [ppm]) and low (200 ppm) levels of ClO_2 with varying contact times or fogging with PAA at for two different PAA quantities in the fogger. The overall goal of the study was to provide an understanding of the performance of these decontamination technologies to guide their use and implementation in homeland security applications for hard-to-decontaminate environments such as HVAC duct systems. In the assessment of options for decontamination following an intentional release of *B. anthracis* spores, it is important to know what products or methods may be used successfully and how operational factors can impact the decontamination efficacy.

This investigation focused on decontamination of two types of HVAC duct: galvanized metal and galvanized metal lined internally with fiberglass duct insulation. Decontamination efficacy tests were conducted with spores of *B. subtilis*, a surrogate for *B. anthracis*. Decontamination efficacy was quantified in terms of log reduction (LR), based on the difference in the number of bacterial spores (quantified as colony forming units) recovered from the positive controls (duct samples not exposed to the decontaminant) and test samples (placed at eight locations along the length of the duct). Tests were conducted with varying operational parameters (e.g., contact time, decontaminant concentration, relative humidity) to assess the effect of these parameters on decontamination efficacy. For example, some tests were conducted at relatively low concentrations of ClO_2 gas (200 ppm) but longer contact times, because this approach could potentially allow for lower capacity ClO_2 generation technologies to be used.

Summary of Results

Chlorine Dioxide – Unlined Duct

With the unlined duct, three tests were conducted at 3,000 ppm ClO_2 and three tests were conducted at 200 ppm ClO_2. For the three tests conducted at 3,000 ppm ClO_2, all achieved a >6 LR, and all but two sampling locations (out of a total of 24) had no viable spores recovered. (Sporicides achieving a LR ≥ 6 are considered effective under efficacy testing requirements developed under the Federal Insecticide, Fungicide, and Rodenticide Act.) For the three tests conducted at the 200 ppm level, ClO_2 was somewhat less effective than at 3,000 ppm. At 200 ppm, the average LR ranged from 5.7 ± 0.26 (at a four-hour contact time) to 7.2 ± 0.19 (with an eight-hour contact time), with 15 out of 24 sampling locations having no viable spores recovered (from the three tests conducted at 200 ppm).

Chlorine Dioxide – Lined Duct

With the fibreglass-lined duct, two tests were conducted at 3,000 ppm ClO_2, and two tests were conducted at 200 ppm ClO_2. The average LR for the two tests conducted at 3,000 ppm was 6.4 ± 0.13, while the average LR for the two tests conducted at 200 ppm was 5.9 ± 0.55. Of the two tests conducted at 200 ppm ClO_2, the one test conducted with a contact time of four hours resulted in all eight sampling

locations having viable spores recovered. While in the other test with 200 ppm, an eight-hour contact time was used and resulted in only three sampling locations (out of eight) at which viable spores were recovered.

Peracetic Acid Fog – Unlined Duct

Two tests were conducted with PAA fog, using varying initial amounts of PAA solution in the fogger. Each test resulted in an average LR of 6.7 ± 0.67. Each test also resulted in having only one sampling location (out of eight) at which no viable spores were recovered.

Efficacy as a Function of Location in Duct

While there was some variability in the efficacy results by location within the duct system - *for each particular test*, there was no specific location within the duct system that tended to be easier or more difficult to decontaminate in the overall study.

1 Introduction

This project supports the mission of the U.S. Environmental Protection Agency's (EPA) Homeland Security Research Program (HSRP) by providing relevant information pertinent to the decontamination of contaminated areas resulting from an act of terrorism. Under Homeland Security Presidential Directive (HSPD)-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, 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.

EPA's HSRP provides expertise and products that can be used widely to prepare for, respond to, and recover from public health and environmental emergencies arising from terrorist threats and incidents. The HSRP's research on biological agent decontamination supports EPA's Office of Solid Waste and Emergency Response (OSWER) and the Office of Pesticide Programs (OPP). OSWER and its Special Teams, which include the Chemical, Biological, Radiological, and Nuclear (CBRN) Consequence Management Advisory Team (CMAT), support the emergency response functions carried out by the Regional Offices. The OPP 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 applicable laws. Close collaboration between 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 *Bacillus anthracis* spores into the U.S. Postal Service system resulted in the contamination of several facilities. Some of the facilities where these letters were processed or received in 2001 were heavily contaminated. While the overall facilities were successfully remediated with approaches such as fumigation with vaporized hydrogen peroxide (VHP®) or chlorine dioxide (ClO_2), including treatment of the heating, ventilation and air conditioning (HVAC) ducts [1,2], data specific to the decontamination efficacy of the HVAC system were lacking. Furthermore, while these decontamination methods have been studied extensively for decontamination of surfaces found in the open spaces of a building (walls, floors, windows, etc.), the present research helps to determine the efficacy of the decontamination method within the confined spaces of an HVAC system and on the materials found within these systems. This study builds on earlier work conducted with VHP® and published by EPA.[3]

1.1 Process

The general process being investigated in this project is decontamination of HVAC system 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.[4] The balance between the two approaches 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., difficulty in finding an ultimate disposal site).[4,5] Since 2001, a primary focus for facility remediation has been improvement of the effectiveness and practical application of *in situ* decontamination methods and evaluation of 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, coupons of HVAC duct material were loaded with spores using an aerosol deposition device. The 18 mm-diameter coupons were prepared from the same materials as the duct. Test and procedural blank coupons were placed in the test duct and decontaminated using ClO_2 fumigation or using a fog of peracetic acid solution, or PAA. After decontamination (fumigation or fogging), the coupons were removed for spore extraction and quantification. Positive control coupons (i.e., contaminated with spores but not subjected to the decontamination process) were used to determine the pre-treatment (i.e., inoculum) loading on each coupon type. Quality control (QC) samples included procedural blank coupons (coupons that underwent the decontamination process, but which were not inoculated) and negative controls (which did not undergo the decontamination process).

1.2 Project Objectives

The primary objective of this project was to determine the efficacy of the ClO_2 fumigation method and fogging with PAA on inactivating spores inside an HVAC duct. Tests were conducted with varying operational parameters (e.g., contact time, decontaminant concentration, relative humidity) to assess the effect of these parameters on decontamination efficacy. For ClO2, some tests were conducted at 3,000 ppm ClO_2, the typical or standard level used for anthrax spore decontamination. Other tests were conducted at relatively low concentrations of ClO_2 gas (200 ppm), but for longer contact times, because this approach could potentially allow for lower capacity chlorine dioxide generation technology to be used. Using lower capacity chlorine dioxide generation technology, in turn, would allow for greater numbers of contractors that could employ ClO_2 gas in the event of a wide area release of *B. anthracis* spores, in which numerous structures would need to be decontaminated. For PAA fogging, the objective was to determine the decontamination efficacy of a PAA fog (via a few screening tests, for proof of concept) for the same mock duct system.

In addition to efficacy testing, other aspects relative to the HVAC system operation were examined, including flow characterization tests (velocity measurements), which were conducted at several points inside the unlined duct system, to determine if flow irregularities could affect decontamination efficacy. Aeration time for the duct following fumigation was also assessed, because residual fumigant off-gassing could affect efficacy. Lastly, visual qualitative effects of the decontaminants on the HVAC materials (galvanized metal, fiberglass insulation) were observed and are noted in this report.

1.3 Experimental Approach

A closed loop duct was constructed and subjected to ClO_2 decontamination under different operating conditions. For the fogging tests with PAA, a modification was made to the supply duct so that the fogger could be inserted. In both cases, 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 either the ClO_2 decontamination technique or fogged with PAA.

Testing was conducted in test ductwork fabricated 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 and built upon as the results of completed tests were analyzed. In general, each test 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 control coupon) at eight defined testing locations along the length of the ductwork. These locations were chosen specifically to determine a) the potential effects of spatial 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 turns.

4. Application of a prescribed fumigation sequence. The ClO_2 gas was provided by a ClorDiSys Cloridox -GMP Sterilization System. The target test condition (fumigant concentration, duct flow rate, and exposure time) was set and controlled at the fan outlet of the ductwork. Relative humidity (RH) was controlled at the inlet of the ductwork while temperature during testing was monitored but not controlled. The ClO_2 fumigant concentration was monitored continuously at three locations (inlet, mid-duct, and at the end of the closed loop duct) to determine the concentration profile as a function of distance from the injection point and the time in the duct. After the exposure time was reached, the ductwork was aerated immediately until fumigant concentrations were low enough to allow safe removal of the test coupons for analysis.

   For the fogging tests, the hydrogen peroxide (H_2O_2) vapor concentration was monitored (as an indicator for PAA, because PAA is produced in equilibrium with acetic acid and H_2O_2)[6] continuously at the same three locations (fan outlet, mid-duct, and at the fan inlet of the closed loop duct).

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

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

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

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

1.3.1 Definitions of Effectiveness

The sporicidal effectiveness (efficacy) of a decontamination technique is a measure of the ability of the method to inactivate and/or remove the spores from 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 colony forming units (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 sample location as defined in Equation 1-1.

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

where:

η_i	=	Surface decontamination effectiveness; the average log reduction of spores on a specific sample location (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)
$\frac{\sum_{k=1}^{N_s} \log(CFU_{S,k})}{N_s}$	=	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 allowed differentiation between detect (1 CFU) and non-detect, a vital distinction in a field event.

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

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

where:

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

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

x_k = The average of the log reduction from the surface of a test coupon (Eqn. 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.

In this report, decontamination efficacy is generally reported in terms of LR for a particular duct location. We also occasionally report results by noting whether the average LR for a particular test is ≥ 6.0, since a decontaminant that achieves ≥ 6 LR is considered effective[7]. Lastly, we also sometimes characterize efficacy in terms of the number of sample locations in which no spores were detected, implying the highest decontamination efficacy quantifiable and achievable.

2 Materials and Methods

2.1 Facility Design

Testing was conducted in a test ductwork assembly that was fabricated at EPA's Research Triangle Park facility. Figures 2-1 and 2-2 show a diagram of the test duct and a photograph of 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 building exhaust system). The design was chosen to maximize overall duct length, provide complex flow regions including elbows, and fit inside the spray booth chamber. The test duct included both the square 90° turns typical of many HVAC systems and radial turns included to reduce the total pressure drop. A blower (Dayton Model 7C651, modified with a ½ horsepower (HP) inverter duty motor; Dayton Electric Manufacturing, Niles, IL) provided recirculation of fumigant within the ductwork and a full dynamic range of flow rates (with corresponding duct velocities typical of full-scale systems), when desirable. The ductwork was made to be disassembled easily and to 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 duct test condition, the duct was internally coated with Knauf Sonic XP 1.5# 1" fiberglass duct liner (Knauf Insulation, Shelbyville, IN).

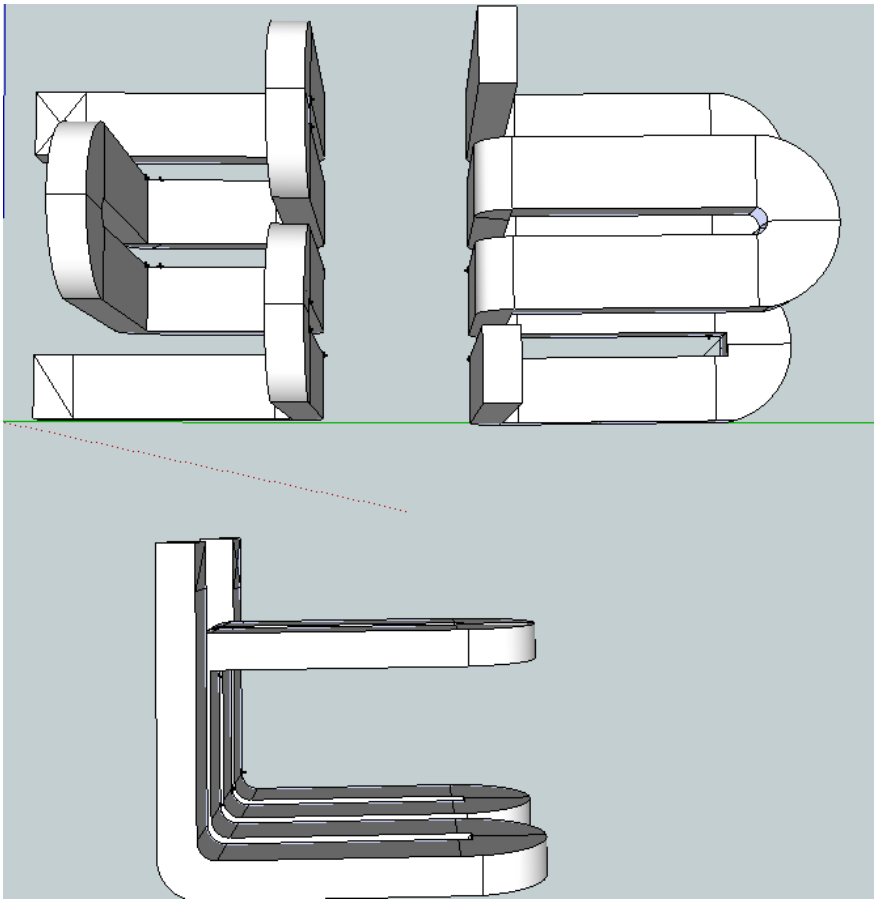

Figure 2-1. Front (top left), Side (top right), and Top views (bottom) of Duct Design (motor and round duct connections not shown in this diagram, see next figure)

Figure 2-2. Photo of Actual Testing Facility

A modification was made to the duct system for the fogging tests (Figure 2-3) to allow for the fogger to be positioned upright so that the fog could be directed initially upward through the riser duct. A **T** was inserted at the elbow so that the fogger (Mini Dry Fog® System; Mar Cor Purification, Minneapolis, MN) could be inserted into the duct. A damper was installed to section off the duct to prevent recirculation. The fog was pulled through the ductwork and removed from the duct system via a line connected to the air pump built into a STERIS VHP® generator (1000 ED, Steris Corporation, Mentor OH, USA). This modified configuration allowed for the fogger to be positioned upright, but resulted in air flow in the opposite direction than the air flow used in the ClO_2 tests. (The air flow direction is arbitrary and change in air flow direction was not expected to affect overall efficacy.)

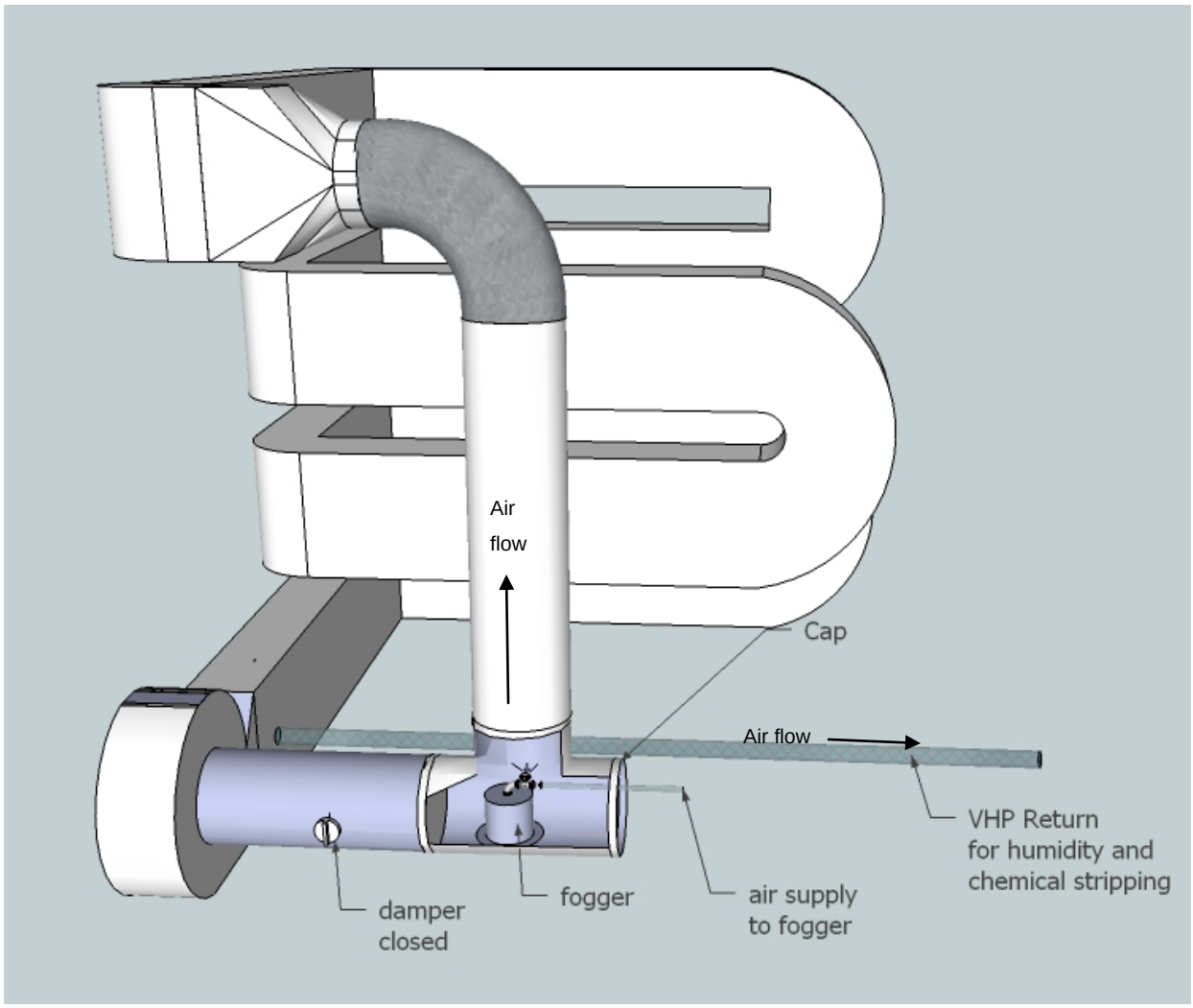

Figure 2-3. HVAC Duct Modification for Fogging Tests

2.2 Decontamination Cycle

2.2.1 Chlorine Dioxide

Chlorine dioxide (ClO_2) gas was generated using a Cloridox -GMP Sterilization System (ClorDiSys, Inc., Lebanon, NJ). The generator operates as a closed loop (Figure 2-4) providing an injection point and a return sampling point. This closed loop construction enables the generator to monitor and control the concentration of ClO_2 in the duct testing facility. ClO_2 was injected at the duct blower outlet, and the return was located downstream but ahead of any duct fittings. In addition, two ClorDiSys Environmental Monitoring Systems (EMS) ClO_2 gas monitors were located at the duct mid- and endpoints. These gas monitors were used for monitoring purposes only.

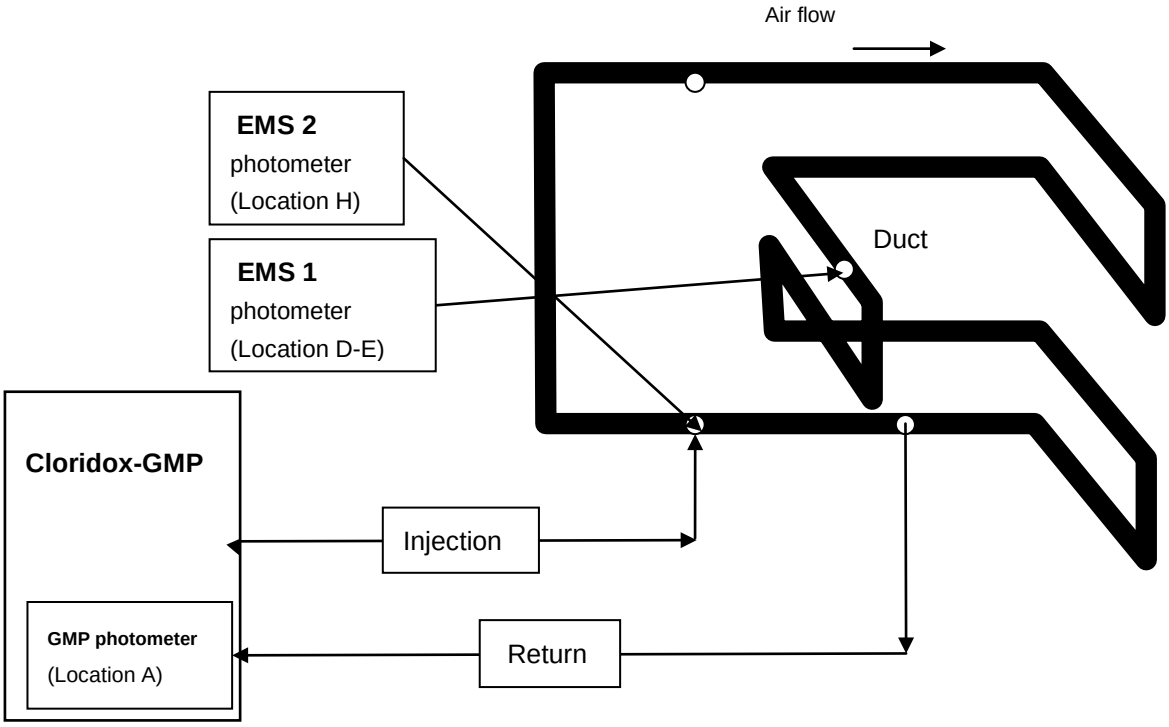

Figure 2-4. Control Loop Schematic

The Cloridox-GMP was placed in the spray booth containing the duct system and was connected to a control screen outside the booth. The booth exhaust system was then turned on as a safety measure.

- *Pre-test Conditioning Phase*: Utilizing a steam injection humidity bottle (Model HF-HBA, Fuel Cell Technologies, Albuquerque, NM), the humidity was brought to the set point specified for the test. The control sensor for humidity was located near the control for the ClO_2 injection. Once the humidity set point was reached and stablized, ClO_2 injection was started.

- *Charge Phase*: The GMP injected ClO_2 until the target concentration was reached and stabilized.

- *Exposure Phase***.** The GMP injected sufficient ClO_2 to maintain the target concentration.

2.2.2 Peracetic Acid Fog

Fogging with PAA was conducted as follows:

- The fogger was prepared with a specified initial amount of PAA solution and deionized (DI) water. (The capacity of the fogger reservoir is 500 mL.) The source of PAA was Minncare® Cold Sterilant (Minntech Corp., Minneapolis, MN), which contains 4.5 % PAA and 22 % H_2O_2.

- The fogger was placed in the duct that was then sealed with a cap. Refer to Figure 2-3.

- An air supply was connected to the fogger.

- A line was connected at the end of the duct to a STERIS VHP® generator to facilitate air flow and removal of decontaminant and humidity.

- A damper separated the fog injection point and the STERIS return line.

- During testing, the duct blower remained off.

- The sequence of operation was as follows:

    1. The STERIS unit and the fogger were started and allowed to run until a maximum of 85 % RH was reached on one of the sensors to keep the maximum RH in the duct between 75-85 %, in accordance with the fogger manufacturer's recommendation. The amount of water added to the fogger reservoir will impact RH levels. Wood et al.6 provide further information regarding operation of the fogger.

    2. Once 85 % RH was reached, the STERIS unit (pump) and the fogger air supply were stopped.

    3. The system was allowed to dwell (i.e., pause in PAA fogging) for 15 minutes.

    4. After the 15 minute dwell, the STERIS unit was restarted to reduce the humidity to 65 %, after which time the fogger was also restarted.

    5. Fogging continued until 85 % RH was reached again, at which time the STERIS unit and fogger were both stopped and another dwell cycle began.

    6. This sequence was repeated until the PAA/water solution was anticipated to be depleted in the fogger. The fogger and STERIS unit were then turned off and the PAA was allowed to dwell overnight.

2.3 Coupon Preparation

2.3.1 Test Coupons

Test materials were 18 mm diameter coupons prepared from the same materials as the duct: galvanized steel (18 gauge ,P/N 01170, Eastcoast Metal Distributors, Durham, NC) and liner (Knauf 1.5# 1" fiberglass, Knauf Insulation, Shelbyville, IN). The liner coupon consisted of a 1 mm-thick slice of the liner (including the inner 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. Liner coupons were sterilized using ethylene oxide. All test procedures were performed in accordance with a pre-approved Quality Assurance Project Plan (QAPP).[8]

A set of five coupons (four test coupons and one negative coupon) were collocated on a test coupon holder (Figure 2-5) and inserted at each testing location in the duct immediately before the start of each 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 flush with the inner surface of the duct, thereby minimizing flow disruptions.

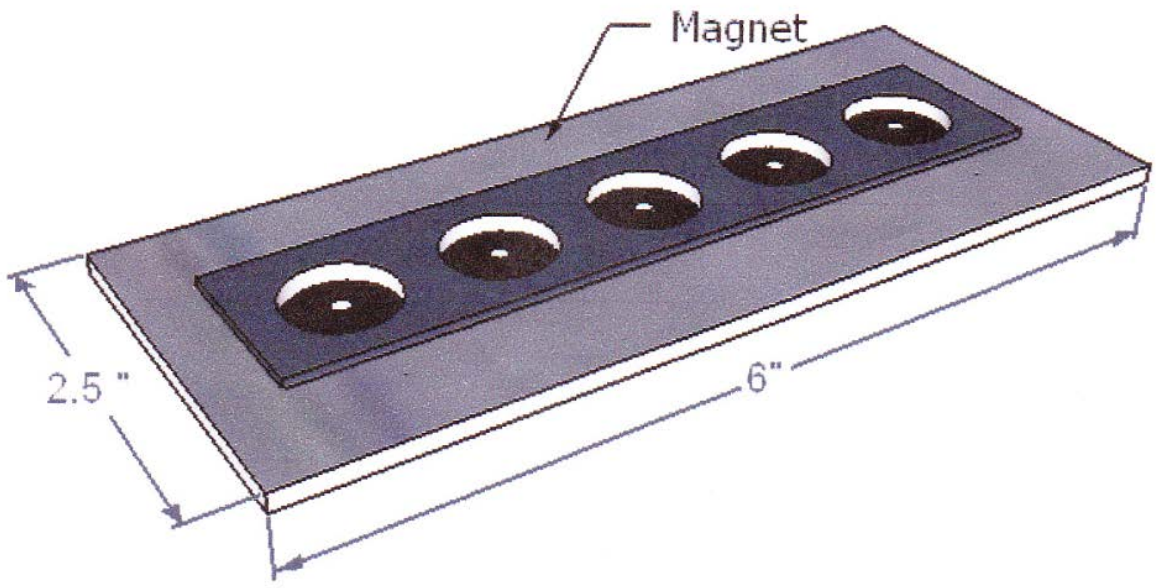

Figure 2-5. Test Coupon Holder Setup

2.3.2 Positive Control Coupons

The positive control coupon holders are slightly different from the test coupon holders, as shown in Figure 2-6. 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 spore inoculation levels were similar for both positive controls and test coupons.

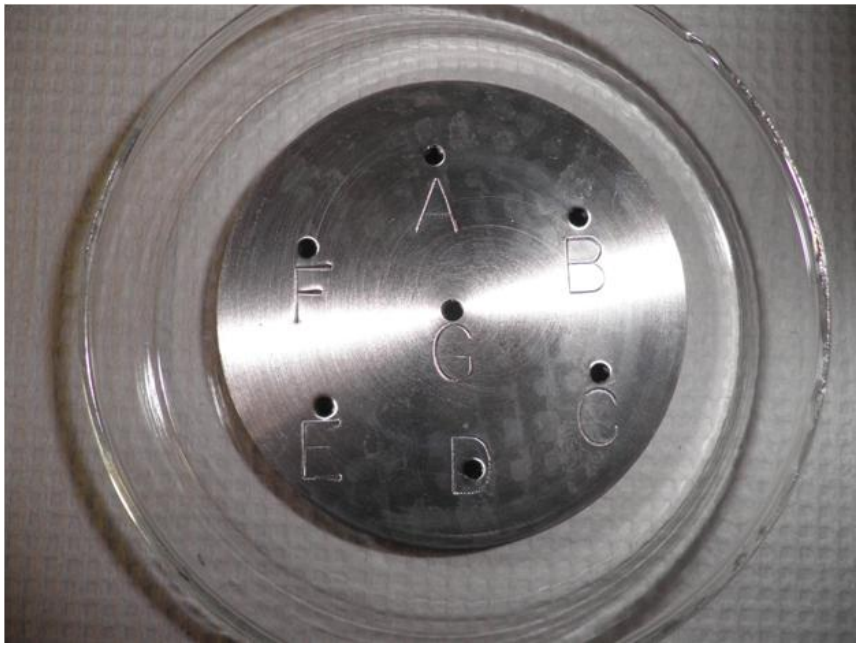

Figure 2-6. Positive Control Coupon Holder

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 MDIs[9] by the U.S. Army Edgewood Chemical Biological Center (ECBC) according to a proprietary protocol.[10,11] Quality assurance (QA) 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 the HVAC materials were inoculated (loaded) with spores of *B. subtilis* using an MDI. The inoculation procedure involved 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 was transferred to a new sterile stub, and the original inoculated stub was discarded. This process was 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 (four total)
2. Inoculate the first four sets of four test coupons (16 total)
3. Inoculate the second set of four positive control coupons. Inoculate the second four sets of four test coupons (16 total)

4. Inoculate the last set of four positive control coupons (four total)

The MDIs are set to provide up to 200 discharges before the spore quantity per puff is expected to diminish. The number of discharges per MDI was tracked so that use did not exceed this value. Additionally, 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, the MDI was retired and a new MDI was used.

A log was maintained for each set of coupons that was dosed. 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 the 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 ClO_2 fumigation or fogging with PAA. 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.

Table 2-1. Test Matrix

Test #	Fumigant	Concentration ClO_2 (ppm) or PAA quantity (mL)	Exposure time (min)	Inverter frequency (blower speed indicator)	RH (%)	Lined
1	ClO_2	200	240	15 Hz	75	No
2	ClO_2	200	480	15 Hz	75	No
3	ClO_2	3000	180	15 Hz	75	No
4	ClO_2	3000	360	15 Hz	65	No
5	ClO_2	200	600	15 Hz	75	No
6	ClO_2 (Blank)	0	600	15 Hz	75	No
7	ClO_2	3000	360	15 Hz	45	No
10	ClO_2	200	240	15 Hz	75	Yes
11	ClO_2	200	480	15 Hz	75	Yes
12	ClO_2	3000	180	15 Hz	75	Yes
13	ClO_2 (Blank)	0	480	15 Hz	75	Yes
14	ClO_2	3000	180	15 Hz	65	Yes
8	PAA	200	Overnight dwell	0 Hz	NA	No
9	PAA	300	Overnight dwell	0 Hz	NA	No

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.

- 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 included fumigant concentration, velocity of air flow in duct, RH, and temperature. Figure 2-7 shows all sampling and monitoring locations in the duct (letters indicate coupon locations). 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.

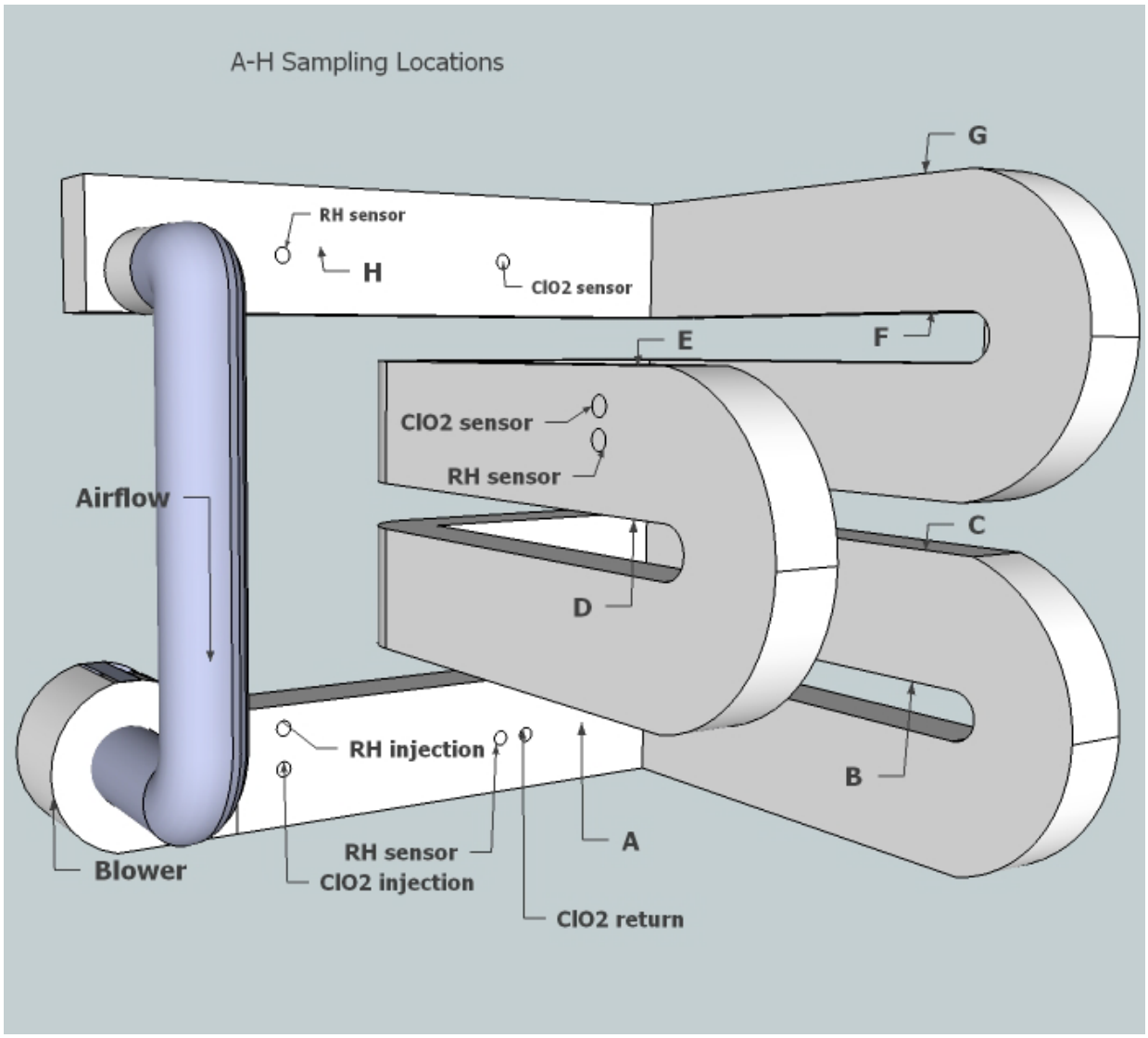

Figure 2-7. Duct Testing Facility (ClO_2) with Sampling and Monitoring Locations Indicated by Letters A-H

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 2-7 as letters A-H	To determine the number of viable spores after fumigation
Negative control coupon	1 per sampling location in duct	1 set per location per fumigation	Shown in Figure 2-7 as letters A-H. See also Figure 2-5.	To determine extent of cross-contamination
Positive control coupon	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 control positive coupons	1 set per inoculation	Carried to test location but not inserted into duct or fumigated	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
Biolab material blanks	3 per material	One set per use of material	NA	To demonstrate sterility of extraction and plating materials
ClO_2 monitors	3 real-time instruments	Real time during ClO_2 fumigations	Shown in Figure 2-4 at three locations	To determine exposure experienced by the coupons and to determine any degradation within the duct
ClO_2 wet chemistry samples	3 every hour	Once per port every hour	Shown in Figure 2-7 at three locations	To verify proper operation of ClO_2 monitors
H_2O_2 monitors	3 real-time instruments	Real time during PAA fogging	At the inlet, mid-duct, and outlet locations (same as ClO_2 sensors shown in Figure 2-4)	To determine exposure experienced by the coupons and to determine any degradation within the duct
Pressure of duct	3	Logged every 10 seconds	Collocated with RH sensors shown in Figure 2-7 at 3 locations	For indication of airflow
RH/temperature	3	Logged every 10 seconds	Shown in Figure 2-7 (temperature measured by RH sensor)	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
Biolab material blanks	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
ClO_2 monitors	ClO_2 concentration	NA
H_2O_2 monitors	H_2O_2 component of PAA solution	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 ClO_2 Concentration Measurement

Chlorine dioxide gas concentration within the duct system was monitored using three photometric instruments (two ClorDiSys EMS instruments and a photometric instrument incorporated into the GMP generator). The three photometric sensors were calibrated before each test using an optical reference filter (Optek-Danulat, Inc., Germantown, WI) at 7.04 mg/L.

To verify the EMS and GMP data, ClO_2 levels in the duct were also measured each hour at each gas sample location using a non-continuous gas sampling method. The gas is sampled through a series of impingers containing a potassium iodide phosphate buffer (KIPB) solution. Gas samples were taken from each of the three sampling ports every hour. Further details on this sampling and analytical method can be found elsewhere[12]. The ClO_2 data shown in the results section of this report are based on the use of this "4500" method.

2.5.1.3 Electrochemical Sensor for H_2O_2 Concentration Measurement

For the two tests using the PAA fog, H_2O_2 vapor concentration within the ductwork (in this case, the H_2O_2 is used as an indicator of the PAA because PAA is in solution in equilibrium with H_2O_2) was monitored using an Analytical Technology Corp. (Collegeville, PA) electrochemical sensor (model B12-34-6-1000-1). The sensors are factory-preset to measure from 0 to 1000 parts per million (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 H_2O_2 solution.

2.5.1.4 Duct Flow Rate

Velocity measurement traverses were performed on the straight line duct in two locations using the AIRDATA^{TM} Multimeter ADM-860 electronic micromanometer from Shortridge Instruments, Inc. (Scottsdale, AZ). This meter measures air duct velocities when used with a pitot tube and automatically corrects for density variation due to local temperatures and barometric pressures.

At point A (in Figure 2.7), a sampling grid of 24 points was used: that is, 3 horizontal traverse lines at 6, 8, and 10 inches from the bottom of duct (16 inches in the vertical direction) and 8 equally spaced sampling points along the 3 horizontal lines. When sampling from point E to D (16 inches in the vertical direction), a sampling grid of 48 points (3 vertical traverses, with 16 samples taken in each traverse) was used.

2.5.2 Microbiological Analysis

The NHSRC Biolab located at the EPA facility in Research Triangle Park, NC analyzed samples either qualitatively for spore presence (swab samples) or quantitatively for the number of viable spores per coupon sample as CFU..

Details of the sampling 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, each18 mm test, procedural blank, and positive control coupon was transferred aseptically into a clean 50 mL sterile vial. The sample vials were then transported to the NHSRC Biolab, where 10 mL of sterile Phosphate Buffered Saline with Tween 20 (PBST) was aseptically added. The sample vials were then sonicated for 10 minutes using an 8510 Branson Ultrasonic Cleaner (Danbury, CT) at 44 kHz and 250 Watts. The sonication step was followed immediately with 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. A 0.1 mL aliquot of each dilution was inoculated onto trypic soy agar (TSA) plates, spread with sterile beads and incubated at 35 $\pm$ 2 °C for 18-24 hours. CFU were counted manually.

Any samples below countable criteria (30-300 CFU) on the primary dilution plates were subsequently filter plated to reduce the detection limit. The filters were incubated at 35 $\pm$ 2 °C for 18-24 hours prior to manual enumeration.

2.5.2.2 Swab Samples

For the first four tests, swab sampling was used for sterility checks of the ductwork prior to 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 2-7. Swabs were streaked onto TSA and incubated at 35 $\pm$ 2 °C for 18-24 hours prior to qualitative growth analysis (presence / absence determination). After the fourth test, a negative (non-inoculated) coupon was used at each sampling set location to address the potential issue of cross contamination.

2.5.2.3 Method Verification

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 Sample 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. 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 control was followed in an effort to minimize the potential for cross contamination.

- For the first four tests, swab sampling was used for sterility checks of the ductwork. Thereafter, negative control coupons were present for each test location. Growth on these coupons would indicate contamination during fumigation or handling.

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.
- New sterile forceps were used for each sample.
- 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 control coupons.

2.6.4 Preventing Cross Contamination during Analysis

General aseptic laboratory techniques were followed and are embedded in all procedures used by the NHSRC Biolab to recover and plate samples. 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, 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 (taken after sterilization of the duct for the first four tests) were placed in the sterile swab containers and then bagged in two individual sterile sampling bags as secondary and tertiary containment.

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 NHSRC Biolab 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 Biolab also included, on each plate, the date it 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	1	Test Number (from Table 2-1)
LC Location Code	B	A through H as shown in Figure 2-7.
	BN	Negative at each location B
	PA	First set of positive controls (at beginning of puffing)
	PM	Second set of positive controls (at the middle of the puffing)
	PZ	Third set of positive controls(at end of puffing)
	FB	Field Blank
	LB	Laboratory blank
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 2-6, 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.
Biolab 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, for 10E0 to 10E4

2.6.8 Sample Preservation

Following transfer to the NHSRC Biolab, all samples were stored at 4 ± 2 °C until they were analyzed. All samples were allowed to equilibrate at room temperature for one hour prior to analysis.

2.6.9 Sample Holding Times

After sample collection for a single test was complete, all biological samples were transported to the NHSRC Biolab immediately, with appropriate COC form(s). Samples were stored no longer than five days before the primary analysis. Typical hold time prior to analyses for most biological samples was ≤ 2 days.

2.6.10 Sample Custody

Careful coordination with the NHSRC Biolab was required to achieve successful transfer of uncompromised samples in a timely manner for analysis. Test schedules were confirmed with the Biolab

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; or
- 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. These protocols were required to be consistent with any protocols established by EPA.

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 Biolab.
- If a sample 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.

2.6.11 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 be performed 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 decontamination of lined and unlined HVAC ductwork using either ClO2 fumigation or fogging with PAA. Due to the boundary layer on duct walls created by airflow and the turbulent nature of the airflow, decontamination of ductwork could be inherently difficult. The investigation of the effectiveness of the ClO_2 decontamination technique required some initial characterization of the duct flow rate/flow pattern; 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 using ClO_2 are reported and discussed in Sections 3.2 and 3.3, respectively. The results of fogging with PAA are reported in Section 3.5.

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 (FRNF50C1S-6U; Fuji Electric Co., Tokyo, Japan) was used to control the blower speed to three different levels using three different frequencies (15 Hz, 30 Hz, and 60 Hz), resulting in calculated Reynolds (Re) numbers (a parameter used to characterize flow turbulence) for the unlined duct all above 1 $x10^5$. These calculations showed that the overall bulk flow inside the duct was highly turbulent at all tested flow rates.

3.1.1 Flow Velocity versus Blower Speed

The flow velocities inside the duct were characterized at the three different blower speeds (indicated by the three variable frequency inverter settings) by performing pitot tube traverses on the straight line of the duct.

The velocity profiles at Location A inside the duct are shown in Figure 3-1 for each blower speed (indicated as inverter frequency) in the unlined duct. The results show that the air velocities vary linearly with the speed of the blower. Note that the geometry of the duct did not provide a position with sufficient length of straight flow, thus the standard US EPA Method 2[13] procedure for measuring flow could not be followed. While this may have some impact on the accuracy of the velocity measurements, the general trends of how velocity may vary by location or fan speed (the aim of these measurements) would not be expected to be affected.

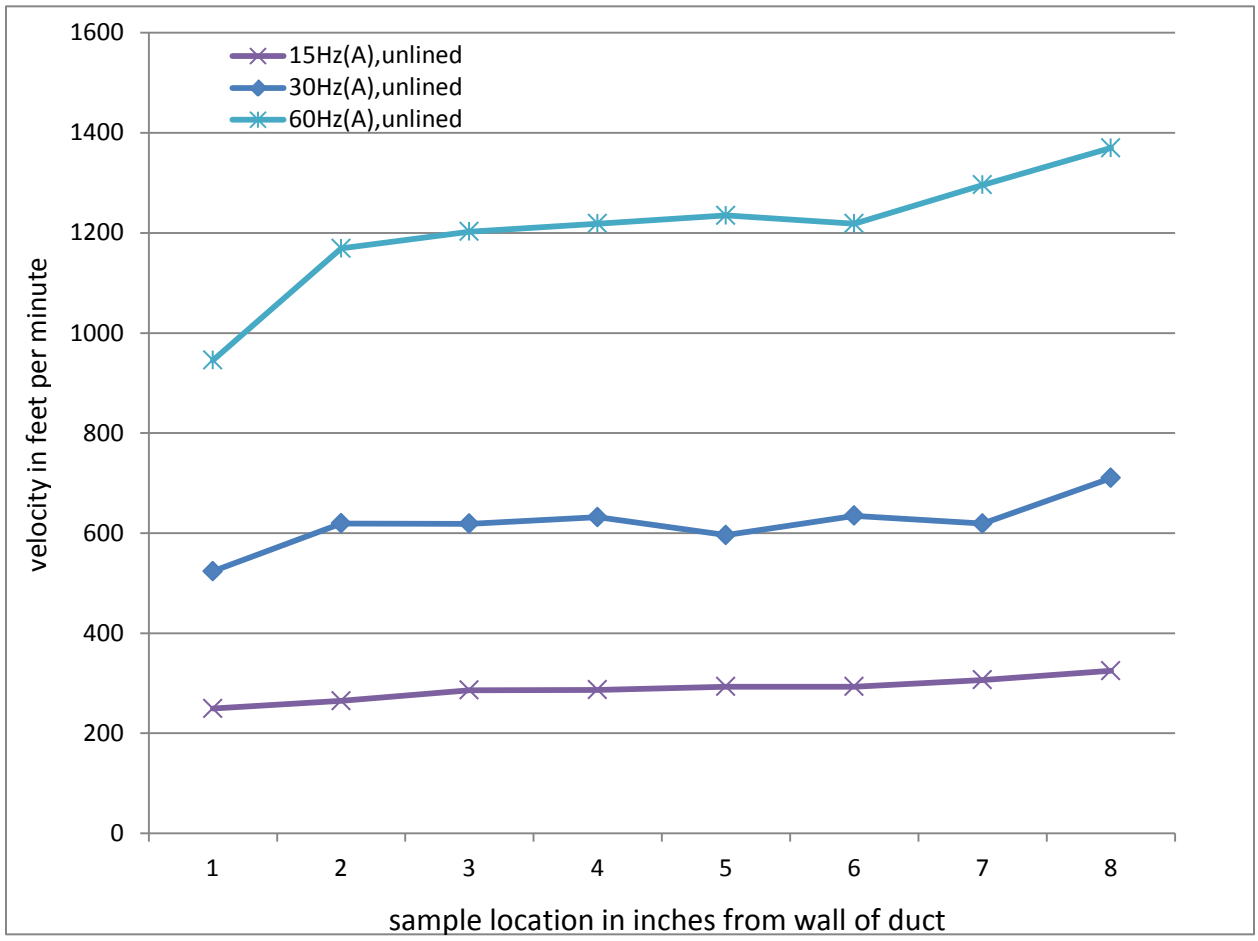

Figure 3-1. Post-Blower Velocity Traverses Inside Unlined Duct at Point A.

3.1.2 Flow Velocity Profile Near an Elbow

The flow pattern near a round elbow (traversing from Location E to Location D) was characterized at three blower motor inverter frequency settings (15 Hz, 30 Hz, and 60 Hz),

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 was affected by the elbow upstream of Location E, with higher flow outside the bend of the elbow (1 to 8 inches), decreasing on the inside of the bend (9 to 16 inches), causing flow reversal and potential flow separation. While the total flux of fumigant across this plane of the duct is proportional to the total flow rate of the system, the flux at any one point is unknown due to the flow separation. The calculated bulk Re was greater than 4000, a benchmark for the transition from intermediate 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.

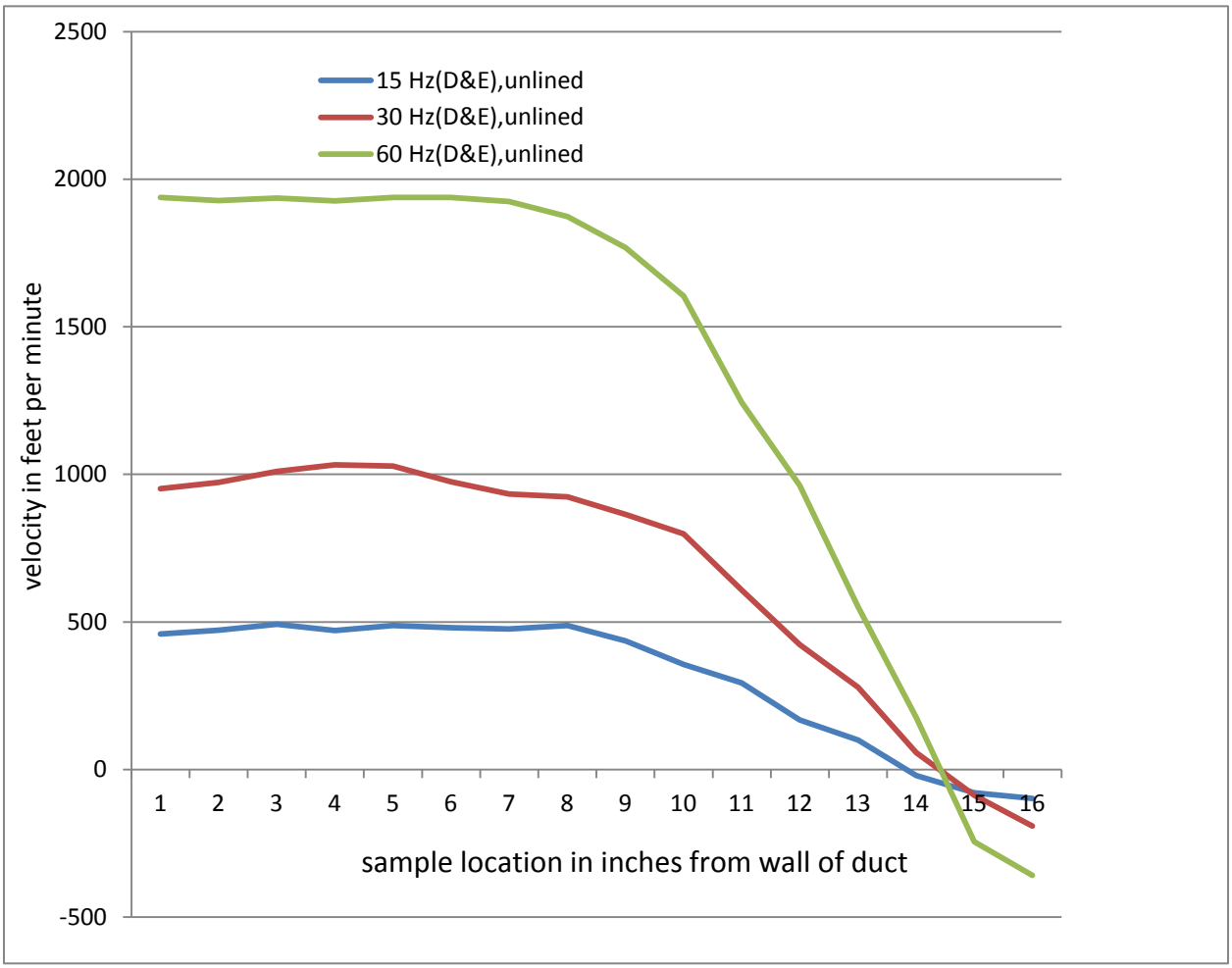

Figure 3-2. Velocity Traverse Inside the Duct from Location E to D for Unlined Duct.

3.2 ClO_2 Fumigations – Unlined Duct Results

Table 3-1 shows the average ClO_2 concentrations and RH during each unlined test.

Table 3-1. Average ClO_2 Concentrations and RH during Unlined Duct Fumigation

Test Target fumigation concentration (ppm) and RH (%)	ClO_2 Concentration (ppm)			RH (%)		
	Location A	Location D-E	Location H	Location A	Location D-E	Location H
	Average (±Standard Deviation)			Average		
Test 1 (200)/75 %	215 (±4.5)	204 (± 6)	219 (± 2)	74	76	76
Test 2 (200)/75 %	192 (±23)	198 (±30)	110 (±43)	75	74	75
Test 3 (3000)/75 %	2784 (±415)	2388 (±819)	2518 (±78)	75	73	75
Test 4 (3000)/65 %	3238 (±116)	3139 (±87)	3353 (±41)	65	65	66
Test 5 (200)/75 %	179 (±14)	174 (±72)	186 (±77)	74	75	80
Test 6 (0)75 %	0	0	0	75	75	76
Test 7 (3000)/45 %	3347 (±61)	3165 (±22)	3454 (±27)	45	47	48

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

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

	First Set CFU/sample(± Standard Deviation)	Middle Set CFU/sample (± Standard Deviation)	End Set CFU/sample (± Standard Deviation)
Test 1	$7.66 \times 10^6 \pm 7.56 \times 10^6$	$8.65 \times 10^5 \pm 5.76 \times 10^5$	$2.52 \times 10^4 \pm 1.98 \times 10^4$
Test 2	$1.15 \times 10^7 \pm 6.35 \times 10^6$	$1.97 \times 10^7 \pm 1.30 \times 10^7$	$3.09 \times 10^7 \pm 2.34 \times 10^7$
Test 3	$2.68 \times 10^7 \pm 1.81 \times 10^7$	$2.73 \times 10^7 \pm 2.21 \times 10^7$	$6.00 \times 10^6 \pm 5.74 \times 10^6$
Test 4	$7.85 \times 10^6 \pm 1.83 \times 10^6$	$2.24 \times 10^7 \pm 2.24 \times 10^7$	$5.92 \times 10^6 \pm 2.21 \times 10^6$
Test 5	$5.91 \times 10^6 \pm 6.96 \times 10^6$	$1.13 \times 10^7 \pm 7.71 \times 10^6$	$9.78 \times 10^6 \pm 4.43 \times 10^6$
Test 6	$1.62 \times 10^7 \pm 8.43 \times 10^6$	$1.56 \times 10^7 \pm 6.32 \times 10^6$	$2.25 \times 10^7 \pm 1.21 \times 10^7$
Test 7	$1.02 \times 10^7 \pm 5.48 \times 10^6$	$2.04 \times 10^7 \pm 6.86 \times 10^6$	$1.44 \times 10^7 \pm 3.12 \times 10^6$

With the exception of Test 1, all of these values met the target dose QA requirements and allow for a six LR. (Refer to Section 1.3.1 for further details regarding the relevance of a 6 LR.) The results suggest a relatively steady inoculation level for all coupons in a test.

Table 3-3 summarizes the average CFU recovered from test coupons (four at each location) during unlined duct tests. Many of the values are at or below the detection limit (indicated with a "≤" symbol). The detection limit varied due to the presence or absence of oxidation particles on the galvanized steel coupon surfaces. Oxidation products (dark colored particles) were observed from the ClO_2 fumigations, but were especially noticeable at the 3,000 ppm tests. These particles made filter plate colony counting difficult when relatively larger volumes (e.g., > 6 mL) of extraction solution were filter plated. Test 3 was the only test with high concentration ClO_2 for which large volume filter plates were performed. Filter plate analysis was limited to 1 mL for subsequent tests when using a 3,000 ppm target level.

The dark colored particles were observed at the cut edge of the galvanized steel coupons. A white, flaky substance was also observed on the surface of the coupons, presumably a reaction product between the ClO_2 gas and the galvanized surface, possibly zinc chloride.

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

Test Target fumigation concentration and RH	**Fumigation Time**	**Average CFU Recovered by Location**							
ppmv ClO_2/RH	**Minutes**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Test 1 (200)/75 %	**240**	3 ± 4.7	1±0.3	1±0.4	59±116	≤1±0	≤1±0	≤1±0	≤1±0
Test 2 (200)/75 %	**480**	≤1± 0	≤1± 0	5± 10	≤1± 0	≤1± 0	≤1± 0	5± 8	5± 10
Test 3 (3000)/75 %	**180**	≤1± 0	≤1± 0	2± 2	1± 0	≤1± 0	≤1± 0	≤1± 0	≤1± 0
Test 4 (3000)/65 %	**360**	≤5± 0	≤5± 0	≤5± 0	≤5± 0	≤5± 0	≤5± 0	≤5± 0	≤5± 0
Test 5 (200)/75 %	**600**	≤5± 0	6± 3	≤5±0	≤5±0	24±38	≤5±0	≤5±0	≤5±0
Test 7 (3000)/45 %	**360**	≤5± 0	≤5± 0	≤5± 0	≤5± 0	≤5± 0	≤5± 0	≤5± 0	≤5± 0

Table 3-4 shows the log reduction as calculated by Equation 1. Note that LR values are a function of positive control recovery and variations in detection limits.

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

Test Target fumigation concentration in	Fumigation Time	Locations								Positive Controls
ppm/RH	min	A	B	C	D	E	F	G	H	Average Log ± SD
Test 1 (200)/75 %	**240**	5.57± 0.63	5.79± 0.17	5.70± 0.20	5.14± 1.26	≥5.88	≥5.88	≥5.88	≥5.88	5.62 ± 1.24
Test 2 (200)/75 %	**480**	≥7.39	≥7.40	7.02 ±0.75	≥7.40	≥7.40	≥7.39	7.04± 0.71	7.03± 0.76	7.19 ± 0.21
Test 3 (3000)/75 %	**180**	≥7.35	≥7.35	7.11± 0.49	≥7.35	≥7.35	≥7.35	≥7.35	≥7.35	7.09 ± 0.42
Test 4 (3000)/65 %	**360**	≥6.25	≥6.25	≥6.25	≥6.25	≥6.25	≥6.25	≥6.25	≥6.25	6.94 ± 0.24
Test 5 (200)/75 %	**600**	≥6.12	6.04± 0.15	≥6.12	≥6.12	5.82± 0.60	≥6.12	≥6.12	≥6.12	6.82 ± 0.26
Test 7 (3000)/45 %	**360**	≥6.43	≥6.43	≥6.43	≥6.43	≥6.43	≥6.43	≥6.43	≥6.43	7.13 ± 0.17

Note: Data in bright yellow cells and shown with "≥" are based on detection limit values (no CFU detected) and had SD values = 0.0.

Note from Table 3-3 that average spore levels greater than 10 were found on only two fumigation tests (indicating less than ideal decontamination conditions), both at 200 ppmv ClO_2 and 75 % RH. For most test sites and fumigation conditions, ClO_2 was an effective fumigant.

3.3 ClO_2 Fumigations – Lined Duct Results

The HVAC duct internally lined with insulation presented a different fumigation scenario. Table 3-5 shows the average ClO_2 concentrations and RH during each lined duct test.

Table 3-5. Average ClO_2 Concentrations and RH during Lined Fumigations

Test # Target fumigation concentration (ppm) and RH (%)	Fumigation Time (min)	ClO_2 (ppm) by location (average ± SD)			Average RH (%)		
		A	D-E	H	A	D-E	H
Test 10 (200)/75 %	240	206 (±27)	215 (±29)	217 (±23)	62	67	67
Test 11 (200)/75 %	480	203 (±35)	185 (±10)	188 (±9)	73	72	75
Test 12 (3000)/75 %	180	1677 (±66)	1425 (±13)	1653 (±4)	75	80	76
Test 14 (3000)/65 %	180	2339 (±250)	1838 (±44)	2379 (±157)	65	69	67

The fiberglass liner was expected to create a demand for the ClO_2 gas, thus preventing the Cloridox-GMP from achieving a target concentration of 3,000 ppm for Tests 12 and 14. Comparing Test 12 and Test 14, lowering the target RH did enable the GMP to reach a higher concentration (due to less air being introduced into the duct via the humidification system). No desorption of ClO_2 was noted following fumigation, in contrast to tests conducted with VHP®, in which significant desorption of VHP® occurred when using lined duct[3]. The presence of fiberglass could require larger fumigant generators than required for the duct alone. Lastly, no visual effect of the ClO_2 fumigant on the insulation was observed.

Three sets of positive control coupons were inoculated alongside test coupon sets. These positive control coupons were generated at the beginning, middle and the end of inoculations. The CFU recovered from these sets of coupons are shown in Table 3-6.

Table 3-6. Positive Controls Inoculation Results for Lined Fumigations (n = 4)

	First Set CFU/sample	Middle Set CFU/sample	End Set CFU/sample
Test 10 (200)/75 %	$9.75 \times 10^6 \pm 1.61 \times 10^6$	$7.85 \times 10^6 \pm 1.36 \times 10^6$	$8.22 \times 10^6 \pm 1.31 \times 10^6$
Test 11 (200)/75 %	$9.54 \times 10^6 \pm 5.75 \times 10^6$	$1.23 \times 10^7 \pm 8.16 \times 10^6$	$8.31 \times 10^6 \pm 2.38 \times 10^6$
Test 12 (3000)/75 %	$1.44 \times 10^7 \pm 1.96 \times 10^6$	$1.65 \times 10^7 \pm 1.28 \times 10^6$	$2.36 \times 10^7 \pm 7.02 \times 10^6$
Test 14 (3000)/65 %	$1.44 \times 10^7 \pm 1.59 \times 10^6$	$1.44 \times 10^7 \pm 2.35 \times 10^6$	$1.53 \times 10^7 \pm 2.39 \times 10^6$

All of these values met the target dose QA requirements and allow for a potential 6 LR determination.

Table 3-7 shows the average CFU recovered from the test coupons in the lined duct tests.

Table 3-7. Average CFU Recovered from Test Coupons from Lined Duct Sample Points (n = 4)

Test ID ClO_2 (ppm)/RH (%)	Fumigation Time (minutes)	Location							
		A	B	C	D	E	F	G	H
Test 10 (200)/75 %	240	29± 28	6± 3	10± 7	9± 3	185± 324	9± 3	* 5.02 x 10^4± 1.0 x 10^5	13± 9
Test 11 (200)/75 %	480	≤5	≤5	29± 20	≤5	230± 433	≤5	6± 3	≤5
Test 12 (3000)/75 %	180	≤5	≤5	≤5	19± 28	131± 253	6± 3	≤5	≤5
Test 14 (3000)/65 %	180	≤5	≤5	6± 3	≤5	≤5	≤5	≤5	6± 3

Note: Data reported as "≤" are based on detection limit values (no CFU detected) and had SD values = 0.0.

*Contamination or other inadvertent error is suspected for one of the coupons, since other coupons at this location for Test 10 had CFU values ranging from 2-33.

Table 3-8. Log Reduction during Testing of the Lined Duct by Sample Location (n=4)

Test ID ClO_2 (ppm)/RH (%)	Fumigation Time (min)	Location								Positive controls
		A	B	C	D	E	F	G	H	Average Logs ± SD
Test 10 (200)/75 %	240	5.64± 0.47	6.15± 0.15	6.00± 0.29	6.00± 0.15	5.43± 1.02	6.00± 0.15	4.10± 1.73	5.93± 0.35	6.93 ± 0.05
Test 11 (200)/75 %	480	≥6.26	≥6.26	5.63± 0.45	≥6.26	5.50± 1.06	≥6.26	6.18± 0.15	≥6.26	6.96 ± 0.07
Test 12 (3000)/75 %	180	≥6.54	≥6.54	≥6.54	6.28± 0.54	6.04± 1.00	6.54± 0.15	≥6.54	≥6.54	7.24 ± 0.10
Test 14 (3000)/65 %	180	≥6.46	≥6.46	6.39± 0.15	≥6.46	≥6.46	≥6.46	≥6.46	6.39± 0.15	7.16 ± 0.01

Note: Data in yellow cells are based on detection limit values (no CFU detected) and had SD values = 0.0.

With regard to comparing results for lined versus unlined duct results, there were three fumigation conditions that were common to both the unlined and lined duct tests. In Tests 1 and 10, the fumigation was conducted at 200 ppm, 75% RH, for 240 minutes. Tests 2 and 11 were both conducted at 200 ppm, 75% RH, but for 480 minutes. And Tests 3 and 12 were both conducted at 3000 ppm, 75% RH, for 180 minutes. However, comparing the efficacy results for the lined vs. unlined duct results is problematic, due to other differences in test conditions. For example, the average spore loading for Test 1 was 5.62 log CFU, while the spore loading for Test 10 was 6.93 log CFU. Further, even with comparable spore loadings, the detection limit for the other unlined duct tests (Tests 2 and 3) was approximately a log lower compared to the lined duct tests (Tests 11 and 12) . In any event, Tests 2 and 11 both resulted in having

five sample locations with no spores detected. Tests 3 and 12 had six and five sampling locations with no spores detected, respectively. In summary, although there are a limited number of tests to compare, and there are some experimental differences between these tests, the tests indicate preliminarily similar or comparable results for the two duct conditions.

3.4 Test Blank Runs – Lined and Unlined Duct

A test blank run for lined and unlined duct (no fumigant added) was added to the test matrix to evaluate any non-fumigant related loss of CFU on the test coupons and demonstrate that the LR from fumigated tests was indeed from the fumigation. The blank test was conducted and sampled the same way as the other test runs. The data suggest that there is some small LR from simple operation of the duct or manipulation of the sample holders (Table 3-9); however, the effect is very small.

Table 3-9 Log Reduction of the Blank Tests for Unlined and Lined Duct

Test ID	Location							
	A	B	C	D	E	F	G	H
Unlined (Test 6)	0.31	0.29	0.43	0.13	0.24	0.32	0.35	1.93
Lined (Test 13)	0.38	0.06	0.36	0.29	0.20	0.15	0.13	0.21

A statistical test was performed to compare the results for the blank test coupons to the results for the positive controls. The p-value of the Student's t test was calculated and is shown in Table 3-10. The results show that the CFU values for the blank test coupons are significantly less at the 95 % confidence level than the positive controls.

Table 3-10. Results of t-Tests on Blank Tests

Test #	Average CFU from Test Coupons	SD	Average CFU from Positive Controls	SD	P Value
6	8.71×10^6	5.19×10^6	1.81×10^7	8.98×10^6	0.0042
13	5.01×10^6	1.56×10^6	8.43×10^6	2.17×10^6	0.0001

3.5 PAA fog

After completing the ClO_2 testing on the unlined duct, two fogging tests were performed using PAA on the unlined duct. These tests were conducted as "proof of concept", to determine if the technology demonstrates any efficacy, and thus if further tests may be warranted. The ductwork was modified (Figure 2-3) to allow for the insertion of the fogger.

Table 3-11 presents some of the fogging operational parameters for the two tests. Different initial amounts of PAA were added to the fogger for each test (200 mL for Test 8 and 300 mL for Test 9), and then DI water was added to fill the 500 mL reservoir to capacity. Based on previous experience with the fogger, the 500 mL PAA/water solution was expected to be fogged in the amount of time the fogger was operated, but this was not the case. For various unknown reasons (possibly due to inadequate air pressure), only a portion of the fogging solution was fogged. The actual amount of PAA fogged was calculated based on the volume of solution remaining. For Test 8, 158 mL of PAA was fogged, and for Test 9, 66 mL PAA was fogged. After fogging, the system was allowed to dwell overnight. The following morning, the duct was aerated.

Table 3-11. Conditions for PAA Screening Tests

Test #	Minncare start volume (mL)	DI Water volume (mL)	Total solution volume (mL)	Solution volume after fogging (mL)	Actual Minncare volume fogged (mL)	Fogger operation time (minutes)	System Dwell (hours)
8	200	300	500	104	158	51.5	23
9	300	200	500	390	66	75	20

Figure 3-3 shows the H_2O_2 concentrations over time from the three H_2O_2 vapor sensors used during Test 9. The maximum H_2O_2 level of 197 ppm was reached at the sensor midway through the HVAC duct circuit. The initial negative response of the sensors is not understood at this time, but may be an interference response to the PAA. H_2O_2 vapor levels are not presented for Test 8 due to malfunction of the data acquisition system.

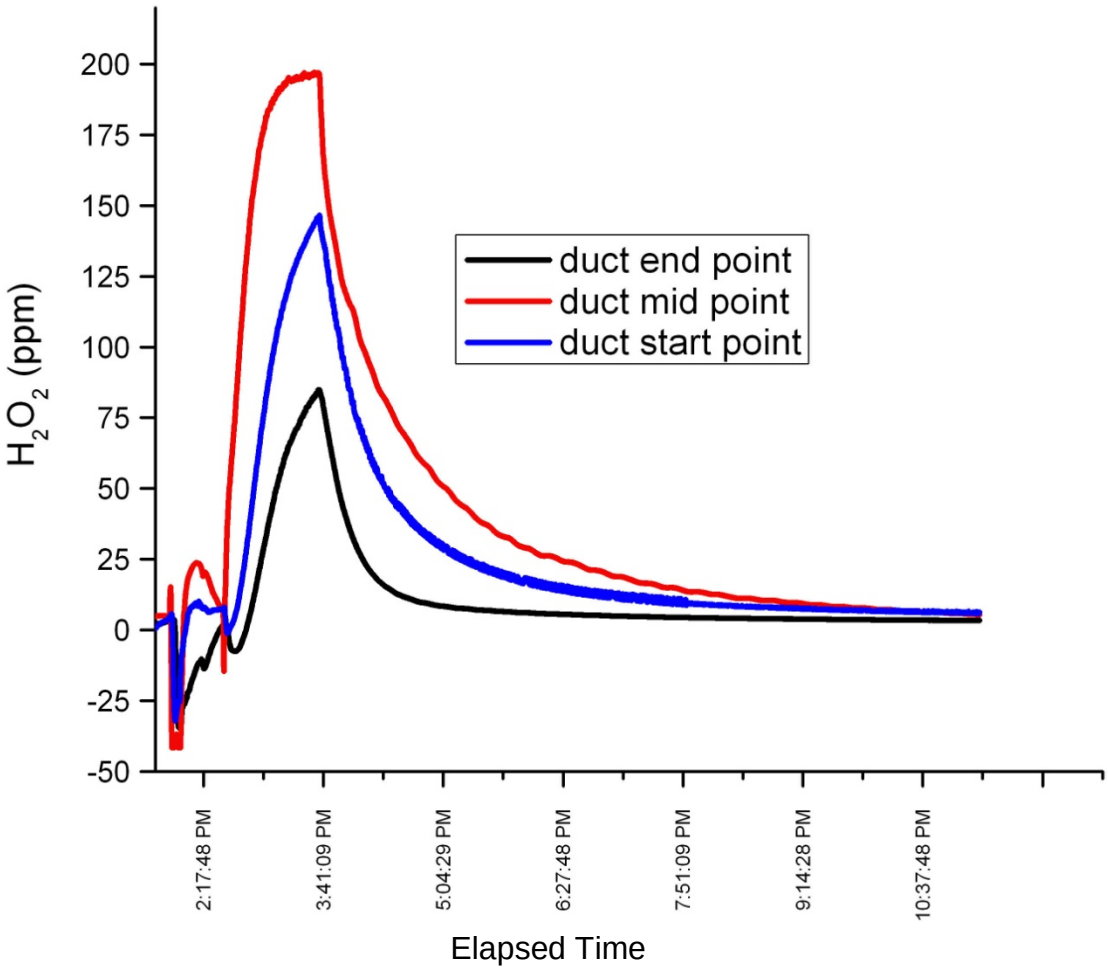

Figure 3-3. H_2O_2 Levels During Fogging Test 9

Maximum RH levels for Tests 8 and 9 ranged from 81-90 % and from 74-86 %, respectively. The average temperature during Test 8 was 17 °C, while for Test 9, the average temperature was 21 °C.

Table 3-12 shows the positive control data and average LR achieved for the two fogging tests. Although only one sampling location per test showed complete kill (no spores detected), fogging of PAA provided a high LR (over 6) for nearly all sample locations. This decontamination technology, thus, shows promise for use in HVAC systems, although a more thorough investigation is warranted.

Table 3-12. Average Log Reduction and Positive Control Levels for Testing of PAA on Unlined Duct

Test #	A	B	C	D	E	F	G	H	Average of Positive Controls (Log value ± SD)
8	7.16 ± 0.46	6.83± 1.09	5.95± 0.26	≥7.40	6.21± 0.87	6.84± 0.50	6.49± 0.74	6.57± 1.48	7.20 ± 0.15
9	7.18± 0.15	6.85± 0.35	5.11± 1.45	7.11± 0.30	6.70± 1.13	6.92± 0.54	6.58± 0.87	≥7.28	7.07 ± 0.12

Note: Data in yellow cells are based on detection limit values (no CFU detected) and had SD values = 0.0.

Lastly, we note that the impacts on coupons observed after ClO_2 fumigation on the unlined duct (corrosion seen on the edges of the coupons, as well as a white flaky material formed on the surface of the coupons) were not observed after the two PAA fog tests.

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)*.[8]

4.1 Sampling, Monitoring, and Analysis Equipment Calibration

There were standard operating procedures for the maintenance and calibration of all laboratory and NHSRC Biolab equipment. All equipment was verified as being certified calibrated or having the calibration validated by EPA RTP's on-site 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 National Institute of Standards and Technology (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	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 inactivating spores inside an HVAC duct. This section discusses the 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)[8] in place for this project was followed with deviations noted as follows:

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. Coupon holders and magnetic stubs were sterilized using ethylene oxide rather than the autoclave to prevent the heat from the autoclave damaging the magnetic material.

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 that could cause cross contamination.

Supplies and consumables were acquired from reputable sources and were NIST-traceable when available. 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, 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 achieved routinely 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 ensured 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 (CMs) 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;
- RH measurements for fumigation conditions; and
- Exposure time.

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, then that sample is invalid. In contrast, for the real-time ClO_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	No detectable spores	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 TSA 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	1×10^6 CFU ±0.5 log	Outside target range: discuss potential impact on results with EPA WAM; correct loading procedure for next test and repeat depending on decided impact.	12 per test
4500-B wet chemistry	Validate concentration	15 % of photometric reading	Repeat	1 per location (3) per hour
Fumigation extraction blank samples	Validated baseline of extractive techniques	Non-detect	Obtain new reagents	1 per test
Post-test calibration of RH sensors (Vaisala, Helsinki, Finland)	Used to validate sensor operation	The post-test calibration check readings must be within 5 % of target reading	Reject results. Repeat test as deemed appropriate.	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	Hand counting	±10 % (between 2 counters)	±10 %	1 CFU	100 %
ClO_2 concentration	4500-B	±15 % of photometric value	±5 %	10 ppm	90 %
H_2O_2 concentration	ATI sensor	±10 % range	±5 %	1000 ppm	90 %
Flow rate (velocity pressure across duct)	differential pressure transducer	±1 %	±0.25 % FS	±0.5" WC	90 %
Fumigation Time	Timer	±1 second	± 1 second	1 second	100 %
RH/temp of fumigation	Vaisala HMD40Y	±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 that demonstrate the ability of the NHSRC Biolab 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 Biolab;
- Laboratory material coupons: includes all materials, individually, used by the NHSRC Biolab in sample analysis;
- Positive control coupons: coupons inoculated but not fumigated; and
- 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 ClO_2 photometer calibrations were checked 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.

5 Summary and Recommendations

The primary objective of this investigation was to determine the efficacy of ClO_2 fumigation and fogging with PAA for inactivating bacterial spores (using *B. subtilis* as a surrogate for *B. anthracis*) inside a pilot-scale HVAC system. The investigation focused on decontamination of either unlined galvanized metal duct or galvanized metal lined internally with fiberglass duct insulation. Test samples were placed at eight locations along the duct circuit to assess whether location within the system impacted results. (Location within the duct system was generally not a factor in decontamination efficacy.) Tests were conducted with varying operational parameters (e.g., contact time, decontaminant concentration, relative humidity) to assess the effect of these parameters on decontamination efficacy.

Overall, ClO_2 was found to be an effective decontaminant for use in both lined and unlined HVAC duct systems for many of the test conditions tested. Although the overall decontamination efficacy was higher at the higher concentration (3,000 ppm ClO_2) compared to the lower concentration (200 ppm ClO_2), as expected, extending the contact time for the 200 ppm condition improved efficacy in most cases. For example, average decontamination efficacy values of nearly 7.0 LR (when ≥ 6.0 LR is considered effective) were obtained for the 200 ppm ClO_2 tests when contact times of eight hours or more were used. Since some duct material degradation (evidenced by the presence of oxidation particles in coupon extraction fluid) was observed when fumigating at 3,000 ppm ClO_2, fumigating at lower concentrations but longer contact times may be an effective decontamination approach and may mitigate potential impacts on duct material.

Two tests were conducted to assess the feasibility and efficacy of fogging PAA in an HVAC (unlined) system. Both of these screening tests resulted in average LR values greater 7.0 (LR ≥ 6.0 considered effective), although only a few of the sample locations were completely decontaminated. One benefit, however, of using PAA fog was that no oxidation of duct materials was observed (an issue with high levels of ClO_2).

A modification was made to the HVAC duct system for the fogging tests to allow for the fogger to be positioned upright so that the fog could initially be directed upward through the riser duct. This modification to the HVAC duct system was required for the type of fogger we used in the study. Other types of foggers may not require this type of modification or be required to be positioned in a certain manner. Additional tests of PAA fog, including using different types of fogging devices, are recommended to further assess operational or environmental factors that may enhance or diminish efficacy in HVAC systems.

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] U.S. Environmental Protection Agency. “Evaluation of H_2O_2 fumigation for HVAC decontamination. Assessment and evaluation report.” U.S. Environmental Protection Agency, National Homeland Security Research Center, Research Triangle Park, NC. EPA/600/R-12/586. August 2012.

[4] 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.

[5] Canter, D.A., T.J. Sgroi, L. O'Connor, C.J. Kempter, “Source reduction in an anthrax-contaminated mail facility,” *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science*. 2009, 7(4): 405-412.

[6] Wood, J.P., M.W. Calfee, M. Clayton, N. Griffin-Gatchalian, A. Touati, K. Egler. Evaluation of peracetic acid fogging for the inactivation of *Bacillus* spores. *Journal of Hazardous Materials*. 2013, 250-251, 61-67.

[7] Determining the Efficacy of Liquids and Fumigants in Systematic Decontamination Studies for *Bacillus anthracis* Using Multiple Test Methods. US EPA Report 600/R-10/088, December 2010.

[8]ARCADIS U.S., Inc., “Quality assurance project plan for the evaluation of medium and high tech methods for HVAC decontamination,” Prepared under Contract No. EP-C-09-027, Work Assignment No. 2-65. U.S. Environmental Protection Agency, National Homeland Security Research Center, Research Triangle Park, NC. July 2011. (available upon request)

[9] Lee, S.D., S.P. Ryan, E.G. Snyder. “Development of an aerosol surface inoculation method for Bacillus spores,” *Applied and Environmental Microbiology*. 2011, 77(5):1638-1645.

[10]Rastogi, V.K., L. Wallace, L.S. Smith, S.P. Ryan, B. Martin. “Quantitative method to determine sporicidal decontamination of building surfaces by gaseous fumigants, and issues related to laboratory-scale studies,” *Applied and Environmental Microbiology.2009,* 75(11):3688–3694.

[11]Rogers, J.V., C.L. Sabourin, Y.W. Choi, W.R. Richter, D.C. Rudnicki, K.B. Riggs, M.L. Taylor, J. Chang, “Decontamination assessment of *Bacillus anthracis*, *Bacillus subtilis*, and *Geobacillus stearothermophilus* spores on indoor surfaces using a H_2O_2 gas generator,” *Journal of Applied Microbiology*. 2005, 99(4): 739– 748.

[12]Wood, J.P., S.P. Ryan, E.G. Snyder, S.D. Serre, A. Touati, M.J. Clayton. Adsorption of chlorine dioxide gas on activated carbons. *Journal Of the Air & Waste Management Association. 2010.* 60(8): 898-906.

[13] US Environmental Protection Agency. Method 2 – Velocity – S-Type Pitot. http://www.epa.gov/ttnemc01/methods/method2.html (accessed January 28, 2014).

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