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Low-Concentration Hydrogen Peroxide (LCHP) Vapor for Bioremediation

Assessment and Evaluation Report

JACOBS®

Contract EP-C-15-008

Office of Land and Emergency Management (OLEM)
Consequence Management Advisory Division (CMAD)

Low-Concentration Hydrogen Peroxide (LCHP) Vapor for Bioremediation

Assessment and Evaluation Report

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Disclaimer

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The goal of this research effort is to demonstrate the efficacy of the fumigant distribution method in a full-scale structure and the variables that affect the fumigant distribution effort. This research effort evaluates the sporicidal efficacy of low-concentration hydrogen peroxide (LCHP) vapor in a typical residential home. The LCHP vapor was generated from a 3 to 4% hydrogen peroxide (HP) aqueous solution in water placed in commercial off–the-shelf (COTS) humidifiers.

R. Leroy Mickelsen from EPA's CBRN CMAD, the principal investigator, directed this research effort with support from a project team consisting of staff from across the EPA. In addition, the individuals listed below contributed to this research effort.

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Acronyms and Abbreviations

Ba	*Bacillus anthracis*
Bg	*Bacillus atrophaeus* var. *globigii*
BI	biological indicator
BioLab	NHSRC Research Triangle Park (RTP) Microbiology Laboratory
CBRN	Chemical, Biological, Radiological, and Nuclear
CFU	colony-forming unit
CMAD	Consequence Management Advisory Division
COTS	commercial off-the-shelf
CT	concentration-time
CTH	Cary Test House
DQI	data quality indicator
DTRL	Decontamination Technologies Research Laboratory
EPA	U.S. Environmental Protection Agency
EtO	ethylene oxide
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
ft^2	square foot
GM	galvanized metal
G/NG	growth or no growth
Gs	*Geobacillus stearothermophilus*
HP	hydrogen peroxide
HPV	hydrogen peroxide vapor
HVAC	heating, ventilation, and air conditioning
IDLH	immediately dangerous to life and health
LCHP	low-concentration hydrogen peroxide
LR	log reduction
MDI	metered dose inhaler
mL	milliliter
mm	millimeter
ND	Non detect
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OEM	Office of Emergency Management
OLEM	Office of Land and Emergency Management
PARTNER	Program to Align Research and Technology with the Needs of Environmental Response
PBST	phosphate-buffered saline with 0.05% Tween® 20
ppmv	part per million by volume
QA	quality assurance
QAPP	Quality Assurance Project Plan

QC	quality control
RH	relative humidity
SD	standard deviation
TSA	tryptic soy agar
TSB	tryptic soy broth
WA	work assignment

Executive Summary

The U.S. Environmental Protection Agency's (EPA) Chemical, Biological, Radiological, and Nuclear (CBRN) Consequence Management Advisory Division (CMAD) of the Office of Emergency Management (OEM) and the National Homeland Security Research Center (NHSRC) are providing information to the response community on decontamination technologies for restoring sites contaminated with biological, chemical, or radiological agents. For biological remediation technologies, the EPA has focused on evaluating "low-tech" solutions for decontaminating building materials contaminated with *Bacillus anthracis* Ames strain (*Ba*: anthrax) spores. Additional focus has been placed on correlating the effectiveness of decontamination efficiency with *Bacillus anthracis* (Ba) surrogates and technology operating parameters for the most promising technologies through systematic decontamination studies.

The goal of this research effort was to demonstrate the efficacy of the fumigant distribution method in a full-scale structure and identify the variables that affect the fumigant distribution. This research effort evaluated the sporicidal efficacy of low-concentration hydrogen peroxide (LCHP) vapor in a typical residential home. The LCHP vapor was generated from a 3 to 4% hydrogen peroxide (HP) aqueous solution in water placed in commercial off–the-shelf (COTS) humidifiers. The potential application of this method would be for homes and small businesses near the Exclusion Zone but not in it. These structures may be contaminated with low amounts of *Ba* spores. Decontamination resources will be occupied inside the EZ leaving homes and small businesses near the contaminated area without access to typical remediation options.

The technical approach for this study involved systematic field research on the efficacy of decontaminating building materials inoculated with *Bacillus* spores as a function of operating conditions, materials present, and fumigation method. Tests were conducted using spores of *Bacillus atrophaeus* var*. globigii (Bg)* Gibbons, et.al. (2011) and *Geobacillus stearothermophilus (Gs),* both non-pathogenic surrogates for *Bacillus anthracis. Bg* and *Gs,* like *Bacillus anthracis,* are gram-positive, spore-forming bacteria. Coupons and biological indicator (BI) discs containing *Bg* and *Gs* spores, respectively, were placed in a typical single-family home (the Cary Test House [CTH]), which was subsequently fumigated.

The laboratory technique developed for systematic evaluation of the efficacy of biological warfare agents was adapted for this effort. The technique consisted of (1) inoculating coupons of typical home materials (carpet and galvanized metal [GM]), (2) exposing the coupons (inoculated with *Bg*) and BI discs (pre-inoculated with *Gs*) to a range of decontamination conditions, and (3) evaluating the viable spores recovered from the coupons and BI discs after decontamination and comparing the results to positive controls (inoculated but non-exposed coupons and BI discs).

The results of this study show that using LCHP vapor generated from HP aqueous solution in water disseminated by COTS humidifiers can be an effective sporicidal surface decontamination technique to help reduce indoor *Bacillus anthracis* contamination. In some configurations, test coupons exhibited a log reduction (LR) of 6 in recovered spores.

The results of this study are summarized below.

- LCHP vapor was efficacious under certain specific operating conditions for full decontamination (complete spore kill) of indoor living areas.

- LCHP vapor was efficacious (6 LR) for carpet and GM coupons inoculated with *Bg* and BI discs inoculated with *Gs* (*Bg* and *Gs* are surrogates for *Bacillus anthracis*).
- The efficacious dose was 12 gallons of 3 to 4% HP for the inside of a 1,200-square-foot (ft^2), 9,600 cubic feet home (1 gallon per 800 ft^3).
- There were several ways to distribute the humidifiers to obtain efficacious results. Placement of the humidifiers near the heating, ventilation, and air conditioning (HVAC) air return ducts and setting the HVAC fan to operate continuously was an effective LCHP vapor distribution method.
- LCHP vapor penetrated through sheets, thin clothing, a bedding comforter, five sheets of paper, and some closed drawers, resulting in full decontamination of *Gs* spores on BI discs.
- LCHP vapor did not penetrate into light switch boxes, thick clothing, books, rugs, furniture cushions, 10 sheets of paper, and some closed drawers, resulting in less efficacious decontamination of *Gs* spores on BI discs.
- The attic and to a lesser extent, the crawl space, were the most challenging spaces for the decontamination method because vents in these locations allowed outside air exchange.
- The results of this study may not be directly applicable to any given site, and spore inactivation results on the tested BI discs and coupons may not apply to other site-specific materials and conditions.

1 Introduction

The U.S. Environmental Protection Agency's (EPA) Chemical, Biological, Radiological, and Nuclear (CBRN) Consequence Management Advisory Division (CMAD) of the Office of Emergency Management (OEM) and the National Homeland Security Research Center (NHSRC) are providing information to the response community on decontamination technologies for restoring sites contaminated with biological, chemical, or radiological agents. For biological remediation technologies, the EPA has focused on evaluating "low-tech" solutions for decontaminating building materials contaminated with *B. anthracis* Ames strain spores. Additional focus has been placed on correlating the effectiveness of decontamination efficiency with *Ba* surrogates and technology operating parameters for the most promising technologies through systematic decontamination studies.

Specifically, the Office of Land and Emergency Management (OLEM) CBRN CMAD scaled up the Office of Research and Development's NHSRC laboratory research findings to a full-scale venue that may be directly applicable in a real-life emergency situation. Results and documents generated from this study may be directly applicable for real-world recovery operations.

This report discusses the efficacy of the fumigant distribution method in a full-scale structure and the variables that affect fumigant distribution. This research effort evaluated the sporicidal efficacy of low-concentration hydrogen peroxide (LCHP) vapor in a typical residential home. The LCHP vapor was generated from a 3 to 4% hydrogen peroxide (HP) aqueous solution in water placed in commercial off–the-shelf (COTS) humidifiers.

The technical approach for this study involved systematic field research on the efficacy of decontaminating building materials inoculated with *Bacillus* spores as a function of operating conditions, materials present, and fumigation method. Tests were conducted using spores of *Bacillus atrophaeus* var*. globigii (Bg)* and *Geobacillus stearothermophilus (Gs),* both surrogates for *Ba. Bg* and *Gs*, are gram-positive, spore-forming bacteria. Coupons and biological indicator (BI) discs inoculated with *Bg* and *Gs* spores respectively were placed in a typical single-family home (the Cary test house [CTH]), which was subsequently fumigated.

The following sections discuss the project background and the project description and objectives.

1.1 Project Background

The EPA's Homeland Security Research Program strives to provide expertise and products that can be widely used to prevent, prepare for, and recover from public health and environmental emergencies arising from terrorist threats and other contamination incidents. OLEM, through its Special Teams, which includes the CBRN CMAD, supports the emergency response functions carried out by EPA Regional Offices. The Office of Chemical Safety and Pollution Prevention supports the decontamination effort by providing expertise on biological agent inactivation and ensuring that the use of pesticides and other inactivation agents in such efforts is conducted in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Close collaboration between the different program offices having homeland security responsibilities is required to increase EPA's capabilities in helping the United States recover from a terrorist event involving the intentional release of CBRN materials. Such collaboration is fostered

through efforts such as the Program to Align Research and Technology with the Needs of Environmental Response (PARTNER)[1] (EPA 2014).

Contamination incidents may result from intentional or accidental releases of biological materials or human or animal disease outbreaks. All scenarios pose significant challenges with regard to determining the extent of contamination, containing the contaminant spread, and remediating the event so that re-occupancy or reuse can occur. The project that is the subject of this report supports multiagency objectives of better understanding and preparing for the remediation of *Bacillus anthracis* in homes after a biological contamination incident.

Resources for responding to a large *Bacillus anthracis* (anthrax) release are limited. After a large *Bacillus anthracis* release, there will be a high demand for response and recovery and few resources to meet that demand. Limited resources result in longer recovery times and higher negative impacts. Many efficacious remediation products produce highly toxic environments and require very specialized equipment and expertise. For example, chlorine dioxide, an unstable chemical, must be produced on location. In addition, chlorine dioxide, methyl bromide, and typically HP are used at concentrations 20 to 100 times higher than the concentration that is immediately dangerous to life and health (IDLH) (TLVS and BEIS. 2017), requiring specialized equipment and expertise for safe handling.

HP typically is used at a concentration of 400 parts per million by volume (ppmv) for 4 hours (concentration × time = 1,600 ppm-hours). Wood, et al. (2016) conducted a laboratory experiment using test coupons of small sections of common materials such as carpet, concrete, ceiling tiles, metal, and drywall. In this experiment, a lower concentration of HP vapor (HPV) (5 parts per million [ppm] over 4 to 7 days = 480 to 840 ppm-hour) resulted in a six-log reduction of spores on all materials tested with the exception of concrete. Wood, et al. (2016) also demonstrated that the LCHP vapor could be disseminated using COTS humidifiers.

Based on these laboratory findings, this project was developed to evaluate the efficacy of LCHP vapor to clean indoor areas of residential homes contaminated with surrogates of *Bacillus anthracis* (the causative agent of anthrax) spores. Low HPV concentrations (less than 25 ppm) and easy-to-deploy techniques were targeted for this effort. The higher the fumigant concentration deployed, the more expertise required for deployment. Conversely, the lower the fumigant concentration, the safer the technique is to deploy. For HP the IDLH is 75 ppm, and the threshold limit value and the permissible exposure limits are both 1 ppm (CDC, NIOSH, OSHA). HP levels used in this study were all below the IDLH and are less hazardous than typical HP fumigations (400 ppm). Even at these low HP concentrations, HP exposure should be avoided. The project that is the subject of this report supports multi-agency objectives of better understanding and preparing for the remediation of a typical residential structure using LCHP vapor.

1.2 Project Description and Objectives

The purpose of this project was to evaluate the efficacy of the LCHP vapor fogging approach in a full-scale structure. Specifically, this project evaluated the sporicidal efficacy of LCHP vapor in a typical residential home represented by an EPA test house, the CTH. The LCHP vapor was generated from 3 to 4% liquid HP placed in COTS humidifiers.

Tests were conducted using spores of *Bg* and *Gs,* both surrogates for *Ba*. The laboratory technique developed by Wood, et al. (2016) for systematic evaluation of the efficacy of remediation of biological warfare agents was adapted for this effort. This technique consisted of (1) inoculating coupons constructed of typical household materials (carpet and GM), (2) exposing the coupons and BI discs pre-inoculated with *Bg* and *Gs,* respectively, to a range of decontamination conditions, and (3) evaluating the viable spores recovered from the coupons and BI discs before and after decontamination. The coupons and BI discs containing the test spores were placed in a typical single-family home (the CTH). Carpet and GM were selected for this study because these materials were the most difficult (excluding concrete) to decontaminate in the laboratory using LCHP vapor, based on findings reported by Wood, et al. (2016).

2 Experimental Approach

The overall experimental approach consisted of preparing coupons of carpet and galvanized metal (GM) and inoculating each coupon with *Bg* spores. In addition, BI steel discs pre-inoculated with *Gs* spores were used. The coupons and BI discs were placed throughout a 1,200-ft^2, three-bedroom residential home. The home was decontaminated using LCHP vapor generated by seven COTS humidifiers charged with a 3 to 4% HP aqueous solution. The humidifiers were set to run continuously until all liquids were dispensed.

HP vapor sensors were placed in various locations throughout the CTH in living areas, the attic, and the crawl space. The CTH's heating, ventilation, and air conditioning (HVAC) system was used during testing (thermostat placed on "heat" setting) to maintain the household temperature at 70 ^oF, and the HVAC circulation fan operated continuously. Oscillating fans also were used to aid in air mixing. A real-time relative humidity (RH) and temperature meter was set up to monitor the CTH throughout each test. A meteorology station was set up outside the CTH to monitor wind velocity, temperature, RH, and rainfall so that these data combined with the data inside the house could be used to estimate air exchange rates.

After fumigation, the test coupons and BI discs were collected and analyzed for spore survival using a plating and colony-counting methodology. Inoculated but unexposed carpet and GM coupons and BI discs were used as positive controls.

The general experimental approach used to meet project objectives is described below.

- **Preparation of representative coupons of test materials:** Coupons of carpet and GM with a diameter of 18 mm each were prepared as discussed in Section 3.4.
- **Sterilization of the coupon materials:** Prior to use, the GM and carpet coupons were sterilized for 18 hours using ethylene oxide (EtO) as discussed in Section 3.4.1.
- **Inoculation of coupons:** Positive control and test coupons were inoculated using the aerosol deposition method described in Section 3.4.2. Briefly, a known quantity of the surrogate organism (2×10^7 colony-forming units [CFU] of *Bg* spores) was deposited onto the center of each coupon using a metered-dose inhaler (MDI). Inoculation was conducted for both types of coupons on the same day, and then the coupons were placed into the CTH for fumigation within 48 hours after inoculation.
- **Decontamination:** The decontamination approach consisted of fumigating the CTH indoor area using 3 to 4% HP liquid fumigant placed in COTS humidifiers as discussed in Section 3.6. LCHP

fumigation typically lasted 24 to 48 hours, with subsequent off-gassing and aeration continuing for the remainder of the week.

- **Decontamination Evaluation:** As discussed in Section 4.2, after fumigation, coupon samples were analyzed for viable spores (CFUs), and BI discs pre-inoculated with ≥ 1×10^6 *Gs* spores were cultured and analyzed for growth or no growth (G/NG). The fumigation atmosphere for HPV concentration, RH, and temperature were tabulated and summarized as discussed in Section 5. Decontamination effectiveness was measured as an LR value for viable spores on the inoculated coupons exposed to fumigation compared to inoculated but un-fumigated control coupons. Decontamination effectiveness for the BI discs was evaluated based on G/NG results, with NG results indicating effectiveness.

3 Experimental Methods and Materials

This section describes the experimental testing methods and materials, including the CTH, experiment set-up, determination of baseline conditions, coupon preparation, BI discs, decontamination, and the test matrix.

3.1 Cary Test House

The CTH is a three-bedroom, ranch-style house with a crawl space, a central forced-air heating system that uses natural gas, and an electric air-conditioning system. The total floor area is 1,200 ft^2, and the house has a total volume of approximately 9,600 cubic feet. Figure 3-1 shows the CTH floor plan.

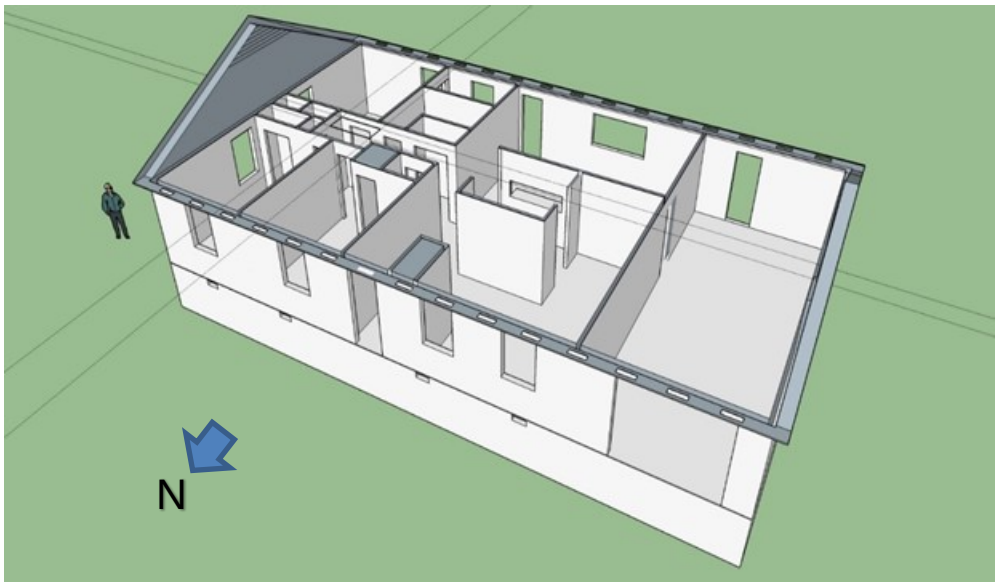

Figure 3-1. CTH Floor Plan

3.2 Experiment Set-up

Fifteen sampling locations were selected to place test coupons to evaluate the efficacy of the decontamination process over the entire CTH. Variables included locations of humidifiers, amount of HP solution used to charge the humidifiers, and whether or not spaces were sealed (such as the crawl space and attic). Initial conditions called for placing one humidifier in each of the following areas: kitchen, den, and the three bedrooms; two humidifiers in the living room; and three humidifiers each in both the attic and crawl space, for a total of 13 humidifiers. Humidifiers, as shown in Figure 3-2, generally were placed in or near the center of the room. Each humidifier was charged with 2 gallons of 3 or 4% liquid HP solution. All windows and exterior doors were closed, and the attic and crawl space were loosely sealed. The door for each room was left open during the decontamination event. An oscillating fan (model 2521, Lasko Products, Inc., West Chester, PA) was placed in each room that did not have a ceiling fan to aid in mixing.

Figure 3-2. Humidifier Placement in Kitchen

The garage was used for personnel and instrument staging and was not fumigated. An HPV sensor capable of detecting 0 to 10 ppm HPV was placed in the garage and set to alarm at 0.5 ppm in case of accidental release of fumigant to this area.

The typical sampling set (as shown in Figure 3-3) for each location consisted of the following items:

(1) Three carpet and three GM inoculated coupons.

(2) One carpet and one GM un-inoculated (blank) coupon – designated locations only.

(3) One BI disc was co-located with each coupon set. Two additional BI discs were located nearby in the same room in hard-to-reach places.

(4) a HOBO® RH/T Onset Data Logger (Model U12, Onset Computers, Bourne, MA) to monitor temperature and RH.

(5) an SPS Medical GPS-250R VH202 Chemical Indicator Strip (SPS Medical Supply Corp., Rush, NY) to monitor exposure to HP.

(6) a Dräger HP tube (Dräger™ Short-Term Detector Tubes – Hydrogen Peroxide, Cat. No 8101041, Dräger, Inc., Houston, TX) with one end open to monitor (similar to chemical indicator test strips) HPV diffusion into the tube.

Figure 3-3. Typical Sampling Set with Coupons in Plastic Holders, Temperature and RH Monitor, Three BI discs in Tyvek Envelopes, HP Test Strip, and Dräger Tube

Dräger tubes are designed to estimate the HP concentration in an atmosphere based on pulling a known volume of atmosphere through the tube. For this study, the Dräger tubes were re-purposed by opening only one end of the tube and allowing the fumigant to diffuse into the tube, with the objective of developing a correlation between the Dräger tube cumulative diffusion reading and fumigant efficacy. A total of 16 Dräger tubes were used for each test run, one at each coupon location.

3.3 Air Infiltration Estimates

A meteorology station (Vantage Pro2, Product # 6152, Davis Instruments, Hayward, CA) and a data acquisition system (WeatherLink®, Windows, USB, Product # 6510USB. Davis Instruments, Hayward, CA) were placed outside the CTH to monitor wind velocity, temperature, RH, and rainfall. The weather data outside and temperature inside the CTH were used to estimate the air infiltration rate (N) using Equation 3-1 below. Equation 3-1 and constants A, B, and C were developed in a previous CTH study conducted in 1995 by Guo, Sparks, and Bero. The CTH configuration may differ slightly from the test house used in the previous study, so the calculated N values are gross estimations.

$$N = A + B\Delta T + C\,V \qquad (3\text{-}1)$$

Where

N = Air infiltration rate (hour^{-1})
ΔT = Air temperature difference (°C)
V = Outdoor wind speed (meter per second)
A = 0.184 ± 0.05
B = 0.0129 ± 0.0004
C = 0.0882 ± 0.003

3.4 Coupon Preparation

The representativeness and uniformity of test materials are essential in achieving defensible evaluation results. Materials are considered representative if they are typical of materials currently used in facilities and buildings in terms of quality, surface characteristics, and structural integrity. Material uniformity means that all test materials are equivalent. Uniformity was maintained by obtaining and preparing a quantity of material sufficient to allow the preparation of multiple test coupons with presumably uniform characteristic. Test coupons were cut from the interior rather than the edge of a large piece of material. These material coupons were randomly selected for use as test, blank, and positive control coupons.

For this study, 18-mm-diameter coupons made of carpet and GM were used. GM was punched from 18-gauge galvanized steel (P/N 01170, Eastcoast Metal Distributors, Durham, NC). Discs with an 18-mm-diameter were cut from carpet (Multiplicity 54594, Shaw Industries Group, Dalton, GA). The coupons then were mounted to 18-mm aluminum stubs (P/N 16119, Ted Pella, Inc., Redding, CA) using double-sided adhesive tape (P/N 16073-2, Ted Pella, Inc., Redding, CA). The coupons were sterilized and inoculated as summarized below.

3.4.1 Coupon Sterilization

The coupons were sterilized using an Andersen EtO sterilizer system (PN:333 EOGas®, Haw River, NC). The sterilization procedure is summarized below.

1. The coupons were randomly selected and loaded into stainless-steel stages (see Figure 3-4).
2. The stage loaded with the coupons was placed in a glass petri dish and loosely covered with a crystallization petri-dish (see Figure 3-4).
3. Each petri dish was placed into an appropriate sterilization bag (PN:333 EOGas®, Haw River, NC).
4. The sterilization bags were loaded into a cabinet for sterilization using EtO.
5. The sterilization bags were removed from the EtO cabinet with the crystallization dishes covering the petri dishes to maintain coupon sterility.

Figure 3-4. Stainless-Steel Stage for Coupon Sterilization

3.4.2 Coupon Inoculation

The test organism for coupon inoculation was a powdered spore preparation of *Bacillus atrophaeus* var. *globigii*, mixed with silicon dioxide particles obtained from the U.S. Army Dugway Proving Ground Life Sciences Division. The preparation procedure is described in Brown, et al. (2007). After 80 to 90% sporulation, the suspension was centrifuged to generate a preparation of approximately 20% solids. A preparation resulting in a powdered matrix containing approximately 1×10^{11} viable spores per gram was prepared by dry blending and jet milling the dried spores with fumed silica particles (Degussa, Frankfurt am Main, Germany).

Positive control and test coupons were inoculated on the same day with approximately 2×10^{7} aerosolized spores using an aerosol deposition method. Briefly, each coupon was placed inside a chamber and positioned in front of an MDI canister containing *Bg* spores suspended in ethanol solution and HFA-134A propellant gas. The MDI is situated inside an actuator such that each time the actuator is depressed a repeatable amount of spores is deposited on the coupon. (Lee, Ryan, and Snyder 2011). The MDI actuator is a small plastic tube in which the MDI is inserted (see Figure 3-5). Each MDI was charged with a volume of spore preparation plus propellant sufficient to deliver 200 discharges of 50 microliters (µL) per discharge. MDI use was tracked so that the number of discharges did not exceed 200. Additionally, MDIs selected for testing were required to weigh more than 10.5 grams. MDIs weighing less than 10.5 grams were retired and no longer used. Test and positive control coupons were inoculated a maximum of 48 hours before decontamination.

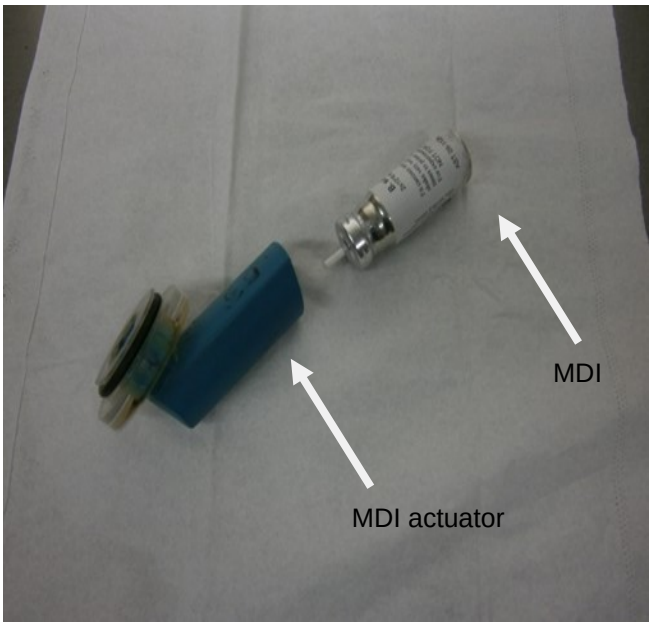

Figure 3-5. MDI and MDI Actuator

3.5 Biological Indicator discs

The commercial BI discs used for this study were purchased from Mesa Laboratories Inc. (P/N HMV-091, Lakewood, CO). Each 0.35-inch diameter x 0.008-inch-thick stainless steel (Grade 304) disc was inoculated with a minimum of 1 x 10^6 *Gs* spores and came from the manufacturer enclosed in a Tyvek pouch.

3.6 Decontamination Approach

Whole-house COTS humidifiers (Model HCN-6009, Honeywell, Morristown, NJ) were charged with 3 to 4% HP solution instead of water. This type of humidifier uses a small fan to push air through a sponge that wicks up the reservoir liquid. Each humidifier holds 3.4 gallons. For this study, 13 humidifiers were deployed throughout the CTH living areas, crawl space, and attic. The HVAC system was set to hold the CTH indoor temperature at 70 ºF, and the fan was set to run continuously. A real-time RH and temperature meter (Model HMD53, Vaisala, Helsinki, Finland) was set up to monitor the CTH inside RH and temperature.

Seven days after beginning the decontamination process, a sampling team verified that the HPV concentration was below 1 ppm using a Dräger HP tube (Dräger™ Short-Term Detector Tubes – Hydrogen Peroxide, Cat. No 8101041, Dräger, Inc., Houston, TX). The sampling team then entered the CTH to collect samples. Positive control and blank coupons that remained outside of the CTH were collected at the same time.

3.7 Test Matrix

- **Test 0 (Humidity Adjustment Only)**

As part of a scoping test, the humidity profile for the CTH was determined during baseline testing (Test 0, Humidity Adjustment only). The temporal effect of high humidity (no HPV presence) on spore survivability also was assessed.

To evaluate the efficacy of the LCHP vapor decontamination technique, five tests were run under this project. For Test 1, test conditions were scaled up from efficacious laboratory results for Wood, et al. (2016) and then adjusted for subsequent tests based on whether or not a 6 LR was achieved for all materials at all locations. Based on test results, site-specific changes were made during Test 2 by changing the placement of fans, the number or placement of humidifiers, or the amount of liquid HP solution deployed. When a 6 LR was achieved, then new and more challenging test conditions, such as adding furniture or reducing the amount of liquid HP deployed, were utilized during Tests 3 through 5.

Unless otherwise noted, the test procedure was as summarized below.

1. Start the HPV monitoring equipment.
2. Ensure the HVAC system controls were set properly.
3. Place the coupons, temperature and RH monitor, BI discs, HP test strip, and Dräger tube at each sampling location throughout the CTH.
4. Fill the humidifiers, measure the volume of filled solution, and place the humidifiers.
5. Start the fans.
6. Turn on the humidifiers.
7. Secure the CTH until fumigation is completed (after 3 to 7 days).

The conditions for each of the five tests are described below. Table 3-2 summarizes the operating conditions of the overall test matrix.

- **Test 1**
  - One humidifier in the kitchen, one in the den, two humidifiers in the living room, one humidifier in each of the three bedrooms, and three humidifiers each in both the crawl space and attic.
  - Each humidifier charged with 2 gallons of 3% liquid HP solution.
  - HVAC fan on continuously to circulate air.
  - HVAC system on to maintain the household temperature at 70 °F.
  - Humidifiers, fans, test coupons, and BI discs located throughout the CTH as shown in Figure 5-1 in Section 5.

- **Test 2**
  - Same conditions as Test 1 with the following modifications:
    - Humidifiers in crawl space loaded with 3.4 gallons of 3.8% HP aqueous solution; all other humidifiers charged with 2 gallons of 3.8% HP aqueous solution.
    - One living room humidifier moved to hallway under the air return; other humidifier remained in living room.

        - One fan added to master bedroom, and one fan removed from den.
        - Additional test coupons added to both den and living room.
    - Humidifiers, fans, test coupons, and BI discs located throughout the CTH as shown in Figure 5-14 in Section 5.

- **Test 3**
    - Same conditions as Test 2 with the following modifications:
        - Humidifiers in crawl space loaded with 3.4 gallons of 3% HP aqueous solution; all other humidifiers charged with 2 gallons of 3% HP aqueous solution.
        - Furniture and clothing added as shown in Figure 5-21.
        - Additional BI discs placed in furniture and hard-to-reach places as shown in Figure 5-22 through Figure 5-25.
        - Added extra test coupons to den and living room to be collected after 3 days.

- **Test 4**
    - For this test, several adjustments made to fan placement; in the corner and middle bedrooms, fans moved closer to closets, and fan added near closet in living room (see Figure 5-29).
    - Number of humidifiers in living areas reduced from seven to two and placed in hallway under air return; both humidifiers filled to maximum capacity of 3.4 gallons, and run sequentially 24 hours apart; all humidifiers (including ones in crawl space and attic) filled to maximum capacity of 3.4 gallons with 3.0% HP aqueous solution.
    - Additional BI discs placed in furniture and hard-to-reach places as discussed in Section 5.5 and shown in Figure 5-30 through Figure 5-32.
- **Test 5**
    - Same conditions as Test 4 except the two humidifiers were refilled after 72-hours of operation: one with 3.2 gallons and the other with 2 gallons of 3% HP aqueous solution.
    - Additional BI discs placed in furniture and hard-to-reach places as discussed in Section 5.6.
    - Humidifiers, fans, test coupons, and BI discs located throughout the CTH as shown in Figure 5-36 in Section 5.

Table 3-2. Test Matrix (Humidifiers operations and locations)

Test 0 (Humidity Adjustment Only)			
Location	**Di-Water**		
	Number of Humidifiers	**Volume Spent (gallons)**	**Concentration (%)**
Master Bedroom	1	2.0	No HP
Den	1	2.0	
Corner Bedroom	1	2.0	
Middle Bedroom	1	2.0	
Kitchen	1	2.0	
Living Room	1	3.8	
Crawl Space Periphery	3	1.6	
Crawl Space Central Location			
Attic Periphery	3	6.0	
Attic Central Location			

Test 1			
Location	HP Aqueous Solution		
	Number of Humidifiers	Volume Spent (gallons)	Concentration (%)
Master Bedroom	1	2.0	3.0
Den	1	1.9	3.0
Corner Bedroom	1	2.0	
Middle Bedroom	1	2.0	
Kitchen	1	1.9	
Living Room	2	3.8	
Crawl Space Periphery	3	5.4	3.0
Crawl Space Central Location			
Attic Periphery	3	5.5	
Attic Central Location			

Test 2			
Location	Number of Humidifiers	Volume Spent (gallons)	Concentration (%)
Master Bedroom	1	2.0	3.8
Den	1	1.9	3.8
Corner Bedroom	1	1.9	
Middle Bedroom	1	1.9	
Kitchen	1	1.9	
Living Room	1	1.8	
Hallway Air Return	1	2.0	
Crawl Space Periphery	3	8.8	
Crawl Space Central Location			
Attic Periphery	3	5.5	
Attic Central Location			

Test 3			
Location	Number of Humidifiers	Volume Spent (gallons)	Concentration (%)
Master Bedroom	1	2.0	3.0
Den	1	1.9	3.0
Corner Bedroom	1	1.9	
Middle Bedroom	1	1.9	
Kitchen	1	1.9	
Living Room	1	2.0	
Hallway Air Return	1	2.0	
Crawl Space Periphery	3	8.3	
Crawl Space Central Location			
Attic Periphery	3	9.6	
Attic Central Location			

Test 4			
Location	Number of Humidifiers	Volume Spent (gallons)	Concentration (%)
Hallway Air return	2	6.6	3.0
Crawl Space Periphery	3	8.2	
Crawl Space Central Location			
Attic Periphery	3	9.8	
Attic Central Location			

Test 5			
Location	Number of Humidifiers	Volume Spent (gallons)	Concentration (%)
Hallway Air Return	2	11.6	3.0
Crawl space Periphery	3	9.8	
Crawl Space Central Location			
Attic Periphery	3	9.9	
Attic central Location			

4 Testing and Measurements

The fumigated areas of the CTH were monitored continuously for HP concentration, RH, and temperature using the HP sensors and monitoring points discussed in Section 4.1. Coupon samples were analyzed for the number of CFUs, and BI discs were analyzed for G/NG as discussed in Section 4.2.

4.1 Hydrogen Peroxide Sensors and Monitoring Points

Three types of electrochemical HP sensors were used during fumigation: one HP sensor capable of detecting 0 to 10 ppm HP (Analytical Technology Inc. (ATI) Model B12-34-1-0010-1, Collegeville, PA) for low concentrations and safety monitoring, three HP sensors capable of detecting 0 to 25 ppm HP (ATI, Model B12-34-5-0025-1), and seven HP sensors capable of detecting 0 to 100 ppm HP (ATI, Model B120100-1). Each sensor was wired into the data acquisition system and had a variable output monitored in real time. The three HP sensors capable of detecting 0 to 25 ppm HP were placed in the center of the CTH, in the crawl space, and in the attic during fumigation, along with one real-time RH and temperature probe (Vaisala, Model HMD53, Vaisala, Helsinki, Finland) in the center of the house. The seven 0- to 100-ppm sensors were distributed throughout the CTH. In addition, as discussed in Section 3.2, RH and temperature sensors were placed at each coupon location.

4.2 Analytical Procedures

The following sections discuss coupon analysis, BI disc analysis, and data reduction.

4.2.1 Coupon Analysis

The sampling team collected coupons from each sampling location in the CTH using gloves and handling only the outside of the coupon holder. Sterile forceps were used to aseptically transfer individual coupons to sterile 50-milliliter (mL) conical tubes. Positive control coupons were transferred last to prevent cross contamination. All sample tubes were transported to the NHSRC RTP Microbiology Lab (BioLab) for quantitative analysis of the number of viable spores recovered per sample (CFUs). Test coupons (positive controls), BI discs, and field blanks (negative control coupons) were deployed and collected after fumigation in separate containers to reduce cross contamination among the different sample types. The extraction and analysis of the coupon samples are discussed below.

To each 50-mL conical tube containing a coupon, 20 mL sterile phosphate-buffered saline with 0.05% Tween® 20 (PBST) was added. The tubes then were sonicated for 10 minutes and vortexed continuously for 2 minutes. Aliquots were plated in triplicate using a spiral plater (Autoplate 5000, Advanced Instruments Inc., Norwood, MA), which deposits the sample in exponentially decreasing amounts across a rotating agar plate in concentric lines to achieve three 10-fold serial dilutions on each plate. Plates were incubated at 35 ± 2 °C for 16 to 19 hours. During incubation, the colonies develop along the lines where the sample was deposited (see Figure 4-1). The colonies on each plate were enumerated using a QCount® colony counter (Advanced Instruments Inc., Norwood, MA).

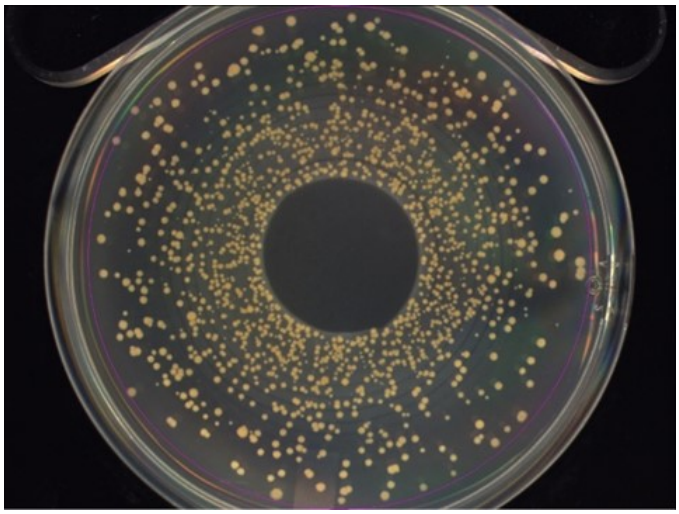

Figure 4-1. Bacterial Colonies on Spiral-plated Agar Plate

Positive control samples were diluted 100-fold (10^{-2}) in PBST before spiral plating, while samples of unknown concentration were plated with no dilution and with a 100-fold dilution. Samples with known low concentrations were plated with no dilution. The QCount® colony counter automatically calculates the CFU/mL in a sample based on the dilution plated and the number of colonies that develop on the plate. The QCount® records the data in an MS Excel (2007, Version 6.1.7600) spreadsheet.

Only plates meeting the threshold of at least 30 CFUs were used for spore recovery estimates. After quantitation with the QCount® colony counter, samples with plate results below the 30-CFU threshold were either re-spiral plated with a more concentrated sample aliquot or filter-plated to achieve a lower detection limit. The filter plate volume was based on the CFU data from the QCount® result. The filters were placed onto tryptic soy agar (TSA) plates and incubated at 35 ± 2 °C for 20 to 24 hours before manual enumeration. Figure 4-2 shows a filter plate with colonies of *Bg*.

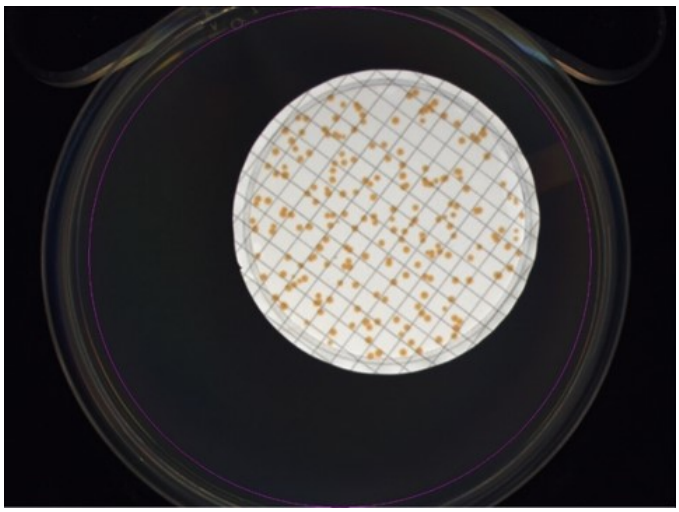

Figure 4-2. Bacterial Colonies on Filter Plate

4.2.2 Biological Indicator Disc Analysis

BI discs containing *Gs* were aseptically transferred to 15-mL polypropylene culture tubes (USA Scientific Inc., P/N 169897, Ocala, FL) containing up to 10 mL of tryptic soy broth (TSB). The tubes were statically incubated at 55 ± 2 °C. After 7 days, the medium in each tube was visually inspected for turbidity (Figure 4-3). To confirm the qualitative culture tube results and to verify that the turbidity was caused by the target organism, a 0.1-mL aliquot was plated to confirm that the growth was from the target organism (based on colony morphology). All sample tubes with no turbidity also were plated (0.1 mL) to confirm that there was no target organism growth.

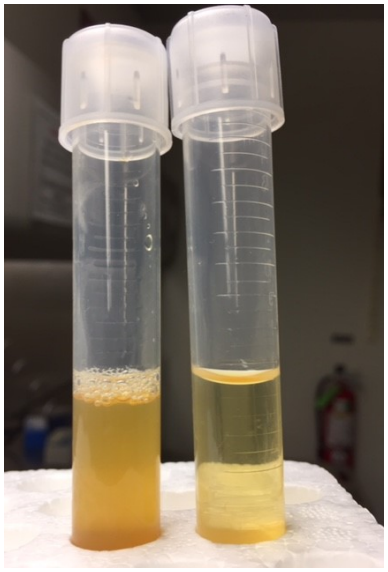

Figure 4-3. BI Turbidity Analysis: Left – TSB Turbid (Growth); Right – TSB Clear (No Growth)

4.2.3 Data Reduction

For the coupon sample results, data reduction was performed using measurements of the total viable spores (CFUs) recovered from each replicate coupon to calculate average recovered CFUs and standard deviation (SD) for each group of coupons. The groups of coupons and BI discs included the following for each combination of material type and extracted sample type at each sampling location:

- Positive control coupons (replicates, average, SD)
- Test coupons and BI discs (replicates, average, SD)
- Procedural blank coupons

Efficacy is defined as the extent (based on LR) to which the agent recovered from the surfaces of the coupons after decontamination has been reduced compared to the agent recovered from the positive control coupons (not exposed to the decontamination procedure). Efficacy was calculated using Equation 4-1 below for each material within each combination of decontamination procedure (*i*) and test material (*j*).

$$\mathrm{LR}_{jk} = \frac{\sum_{c=1}^{c} (\log_{10} C_{jc})}{N_{jc}} - \log_{10}\left(N_{jk}\right) \qquad (4\text{-}1)$$

Where

C_{jc} = Number of viable organisms recovered from *C* positive control coupons for *j*th test material

N_{jc} = Number of positive control coupons for the *j*th test material

N_{jk} = Number of viable organisms recovered on the *k*th replicate coupon for the *j*th test material

If no viable spores were detected, then the detection limit of the sample was used and the efficacy reported as greater than or equal to the value calculated using Equation 4-2 below. The detection limit of a sample depends on the analysis method and so may vary. The detection limit of a plate was assigned a value of 0.5 CFU, but the fraction of the sample plated varies. For example, the detection limit of a 0.1-mL plating of a 20-mL sample suspension would be100 CFUs, but if all 20 mL of the sample is filter plated, the detection limit would be 0.5 CFU.

The standard deviation (SD) of LR_{jk} is calculated by Equation 4-2:

$$SD_{\eta_j} = \sqrt{\frac{\sum_{k=1}^{N_s} \left(x_{jk} - LR_j\right)^2}{N_{jk} - 1}} \qquad (4\text{-}2)$$

Where:

LR_j is the average LR of spores on a specific material surface, and

x_{jk} is the average of the LR of a decontaminated coupon calculated using Equation 4-3.

$$x_{ik} = \frac{\sum_{k} \{\sum_{C=1} \log(CFU_{jc}) / N_C - \log(CFU_{jk})\}}{N_{jk}} \qquad (4\text{-}3)$$

5 Results and Discussion

This section discusses the results for the baseline conditions and Tests 1 through 5. For each test, the following operational parameters are discussed: living area fumigation conditions (including temporal HPV concentration, temporal RH, and temporal temperature), crawl space and attic fumigation conditions (including temporal HPV concentration, temporal RH, and temporal temperature), and decontamination efficacy.

5.1 Test 0 (Humidity Adjustment Only)

As part of a scoping test, the humidity profile for the CTH was determined during baseline testing. The temporal effect of high humidity (no HPV presence) on spore survivability also was assessed. Seven humidifiers were used in the living area: one in the kitchen, one in the den, two in the living room, and one in each of the three bedrooms. In addition, the crawl space and attic contained three humidifiers each. Each humidifier was charged with 2 gallons of de-ionized water.

5.1.1 Living Area Fumigation Conditions

This section discusses the environmental conditions in the living areas for the initial CTH set-up shown in Figure 5-1, including air infiltration estimates, and temporal RH in each section of the living areas. Further, the CTH environmental conditions were evaluated to determine if high RH affects spore viability on the coupons. The humidifiers operated continuously until the fumigant was depleted. After a 1-week baseline testing period, the coupons were collected and analyzed. During the baseline testing, the HVAC fan continuously circulated air throughout the testing sequence, which lasted 7 days, and the temperature in the living areas was constantly maintained at 70° F.

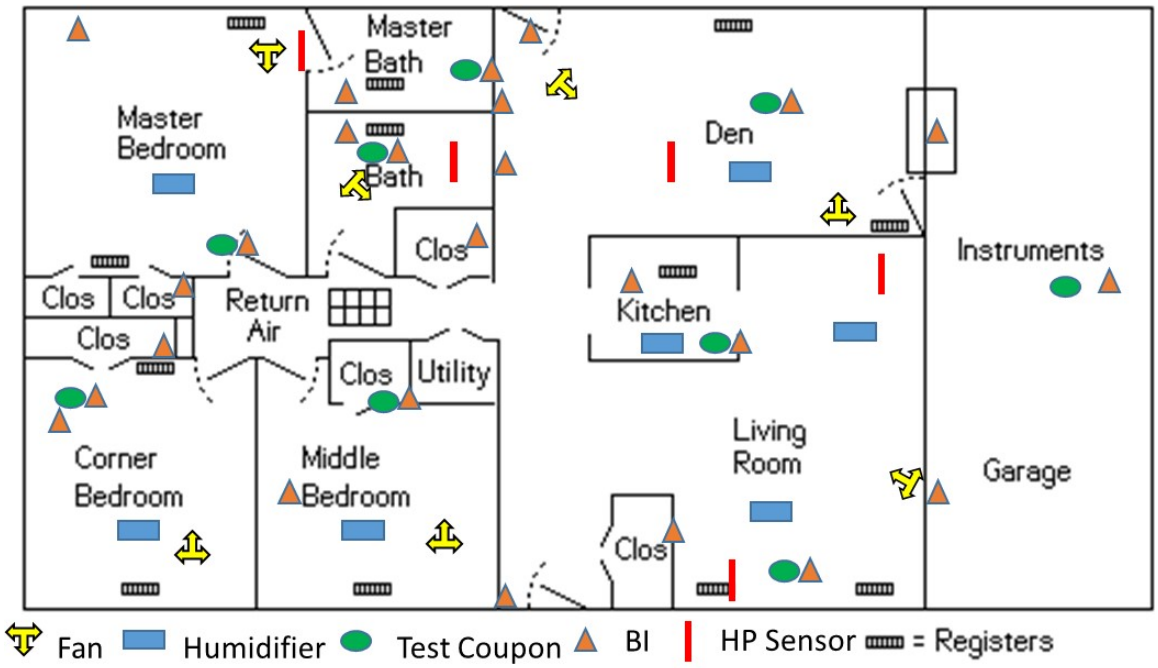

Figure 5-1. CTH Test Set-up

5.1.1.1 Cary test House Air Infiltration Rates

A cyclical pattern was observed for the CTH air infiltration rates throughout the testing sequence. The air exchange was highest during the cooler nights, and fell off during the days, as shown in Figure 5-2 for Test 1. These fluctuations were controlled solely by the outside temperature, since the living areas were temperature controlled. Table 5-1 summarizes the exchange rates over the five 7-days testing events. The average air exchange for this house was found to be 0.32 hr^{-1}, with maximum air exchanges less than 0.55 hr^{-1}, which shows that this home is relatively airtight.

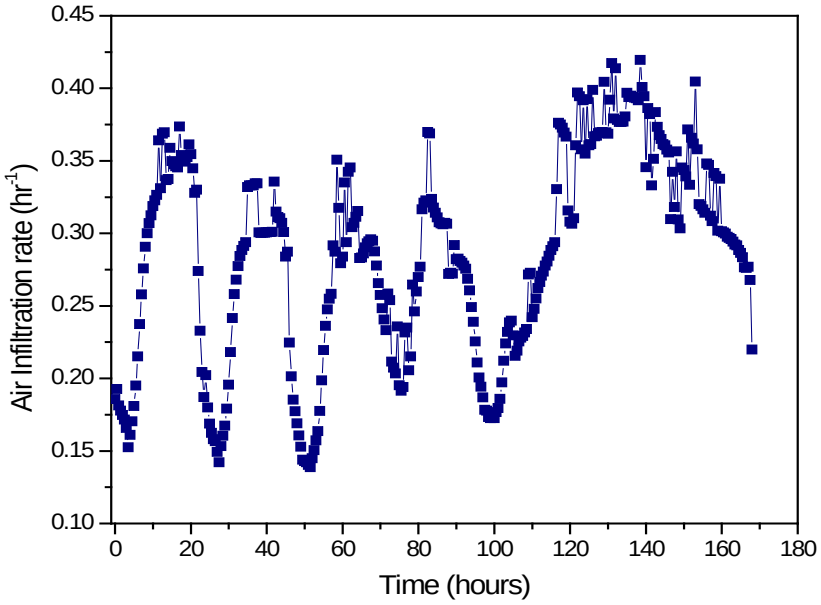

Figure 5-2. Test 1 Temporal Air Exchange Rates in Living Areas

Table 5-1. CTH Air Exchange Rates

Air Infiltration Rate (hr^{-1})			
Test Number	Min	Mean	Max
Test 1	0.14	0.29	0.42
Test 2	0.17	0.32	0.47
Test 3	0.19	0.35	0.52
Test 4	0.19	0.36	0.55
Test 5	0.16	0.30	0.43

5.1.1.2 Temporal RH in Living Areas

Figure 5-3 shows the effect of the humidifiers on RH throughout the CTH living areas for eight locations (master bedroom, master bathroom, corner bedroom, middle bedroom, hallway bathroom, kitchen, den, and living room).

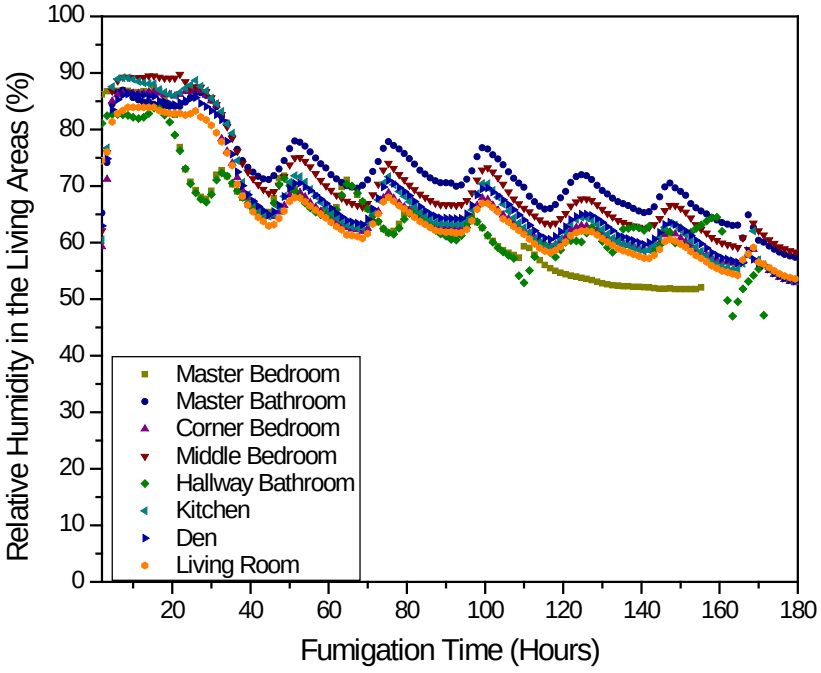

Figure 5-3. Baseline Test Temporal RH in Living Areas

The results show that the RH increased rapidly from about 54% to greater than 75% in all living areas, even in the master bathroom, where no humidifiers were placed. The humidification process resulted in greater than 75% RH within 2 hours and maintained an elevated RH level (especially for 48 hours when the humidifiers were still generating vapor) for the 7 days of the baseline testing period. The HVAC fan continuously circulated air, transporting humidity from locations with humidifiers to other locations with no humidifiers such as the bathrooms. The observed oscillations observed for the RH inside the living areas may be due to diurnal cycling of the outdoor temperature/RH.

5.1.2 Temporal Environmental Conditions in Crawl Space and Attic

5.1.2.1 Crawl Space

The effect of the three humidifiers on the RH and temperature inside the crawl space was monitored for more than 7 days at three locations (east side, central location, and west side). Figure 5-4 shows the RH results for the crawl space. These results indicate that the RH increases rapidly from about 50 to 55% to greater than 80% uniformly throughout the crawl space in less than 1.5 hours and that this RH level was maintained for more than 1 week. The temperature is relatively stable through the testing sequence.

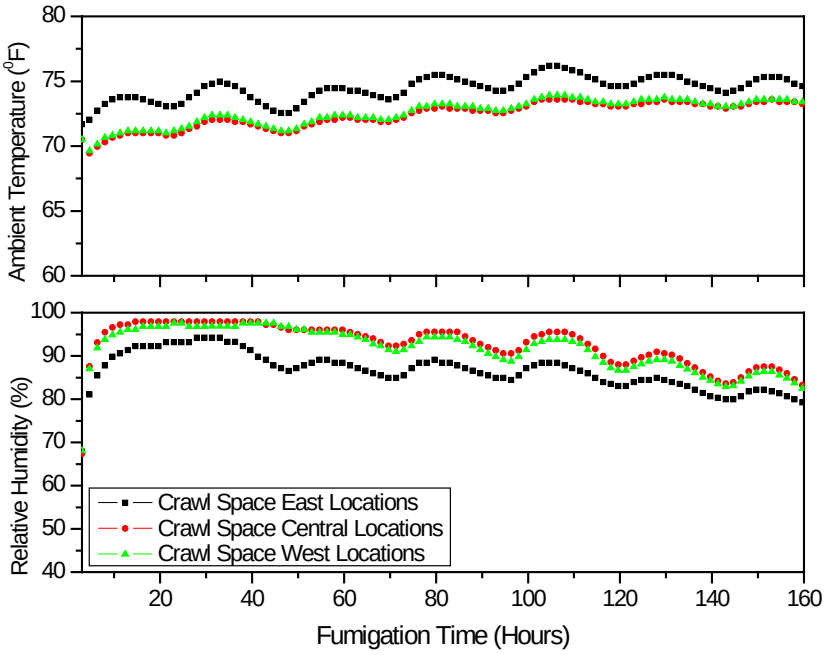

Figure 5-4. Baseline Test Temporal Environmental Conditions in Crawl Space

5.1.2.2 Attic

The effect of the three humidifiers on the RH/temperature in the attic was monitored for more than 7 days at three locations (east side, central location, and west side). Figure 5-5 shows the RH results for the attic. These RH results show a cyclical process between days and nights, regardless of sampling location. The oscillations observed for the temperature/RH inside the attic may be due to the cycling of the outdoor temperature/RH between day and night. The cyclical process includes RH ranges well below 50% that could affect decontamination efficacy and fumigation effectiveness for HP.

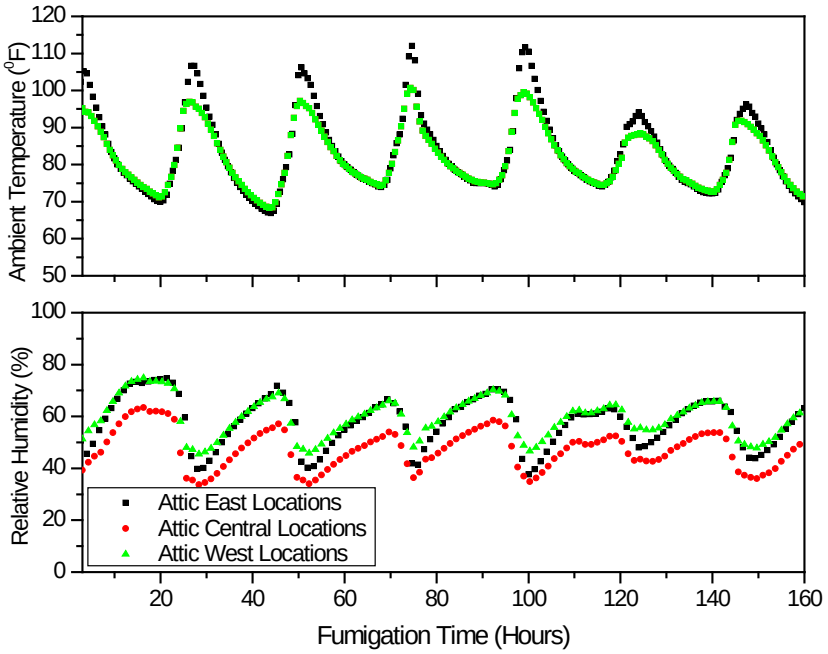

Figure 5-5. Baseline Test Temporal Environmental Conditions in Attic

5.1.3 Temporal Survivability of Spores

In Test 0, exposure of the positive control coupons inoculated with *Bg* and the BI discs containing *Gs* exhibited full spore recovery, regardless of material type, location, and environmental conditions. Table 5-2 summarizes the results, including three replicates (Rep) for each location, which demonstrate that spore viability was not affected by high humidity alone.

Table 5-2. Test 0 (Humidity Adjustment Only) Recoveries on Test Coupons and BI discs

Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)				
Location	**Sample Type**	**Rep 1**	**Rep 2**	**Rep 3**
Master Bedroom Floor	Carpet coupon	2.98×10^7	2.77×10^7	2.14×10^7
	GM coupon	5.26×10^6	2.95×10^6	5.28×10^6
	BI disc	G	G	G
Master Bathroom Floor	Carpet coupon	2.90×10^7	2.89×10^7	2.48×10^7
	GM coupon	5.68×10^6	3.71×10^6	7.56×10^6
	BI disc	G	G	G
Bathroom Sink	Carpet coupon	3.06×10^7	2.66×10^7	2.44×10^7
	GM coupon	5.90×10^6	4.40×10^6	4.12×10^6
	BI disc	G	G	G
Center of Den	Carpet coupon	3.10×10^7	2.78×10^7	1.87×10^7
	GM coupon	6.64×10^6	5.28×10^6	3.08×10^6
	BI disc	G	G	G

Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)				
Location	**Sample Type**	**Rep 1**	**Rep 2**	**Rep 3**
Corner Bedroom Floor	Carpet coupon	2.46×10^7	3.88×10^7	1.91×10^7
	GM coupon	5.88×10^6	3.92×10^6	3.03×10^6
	BI disc	G	G	G
Middle Bedroom Floor	Carpet coupon	2.52×10^7	2.99×10^7	2.64×10^7
	GM coupon	6.59×10^6	4.80×10^6	1.16×10^7
	BI disc	G	G	G
Kitchen Floor	Carpet coupon	2.50×10^7	2.13×10^7	2.78×10^7
	GM coupon	3.92×10^6	2.43×10^6	4.70×10^6
	BI disc	G	G	G
Living Room Floor	Carpet coupon	2.30×10^7	2.47×10^7	2.04×10^7
	GM coupon	4.03×10^6	3.41×10^6	2.42×10^6
	BI disc	G	G	G
Crawl Space Under Corner Bedroom	Carpet coupon	2.07×10^7	2.49×10^7	2.72×10^7
	GM coupon	1.59×10^6	7.40×10^5	1.04×10^6
	BI disc	G	G	G
Crawl Space Under Kitchen	Carpet coupon	2.66×10^7	2.88×10^7	2.08×10^7
	GM coupon	8.46×10^5	6.07×10^5	5.53×10^5
	BI disc	G	G	G
Crawl Space Under Den	Carpet coupon	3.06×10^7	2.57×10^7	2.56×10^7
	GM coupon	1.05×10^6	5.20×10^5	8.47×10^5
	BI disc	G	G	G
Attic Over Master Bath	Carpet coupon	2.60×10^7	1.79×10^7	2.28×10^7
	GM coupon	7.05×10^6	4.72×10^6	9.06×10^6
	BI disc	G	G	G
Center of Attic	Carpet coupon	2.42×10^7	2.18×10^7	2.70×10^7
	GM coupon	8.00×10^6	3.91×10^6	5.02×10^6
	BI disc	G	G	G
Attic over Den	Carpet coupon	2.72×10^7	2.66×10^7	2.88×10^7
	GM coupon	8.44×10^6	4.19×10^6	5.67×10^6
	BI disc	G	G	G

5.2 Test 1

Test 1 was the reference test for this project. The test layout was similar Test 0 (Humidity Adjustment only). Each humidifier was charged with 2 gallons of 3% off-the-shelf HP aqueous solution. The HVAC fan continuously circulated air throughout the testing sequence, which lasted 7 days, and the temperature in the living areas was constantly maintained at 70 °F.

The average results for the living areas, attic, and crawl space fumigation conditions: HPV concentration. (ppm), overall calculated HPV exposure (concentration-time [CT] in ppm-hours), and RH (%) are tabulated in Table 5-3. The temporal HPV concentration, RH, and temperature are discussed in Sections

5.2.1.1 through 5.2.1.3 for living areas, and in Sections 5.2.2.1 through 5.2.2.3 for the crawl space and attic. Finally, the decontamination efficacy of the test coupons inoculated with *Bg* spores, and growth/no growth assessments of BI discs co-located with the coupons are discussed in Section 5.2.3.

Table 5-3. Test 1 Fumigation Conditions

Test 1 - Fumigation and Relative Humidity Conditions by Location				
Location	**HPV Max (ppm)**	**CT (ppm-hour)**	**RH Average (%)**	**RH Max (%)**
Master Bedroom	43	1310	72	86
Master Bathroom	Not Measured		74	85
Hallway Bathroom	7.9	270	72	86
Center of Den	38	1260	72	86
Corner Bedroom	Not Measured		69	85
Middle Bedroom	Not Measured		72	88
Kitchen	Not Measured		69	86
Living Room Floor	73	2030	71	88
Air return	32	1060	65	82
Crawl Space Center	18	787	82	92
Crawl Space North East	0.7	18	83	94
Crawl Space South West			81	92
Center of Attic	37	1077	78	90
Attic Over Master Bath	5.5	177	77	90
Attic over Den			78	90

5.2.1 Living Area Fumigation Conditions

5.2.1.1 Temporal HPV Concentration

The real-time HPV concentration was monitored at five locations using HP sensors at the locations shown in Figure 5-1: master bedroom, bathroom, hallway air return, living room, and den (see Figure 5-6). The highest HPV concentration was found in the living room (73 ppm), which contained two humidifiers, and the lowest HPV concentration was in the hallway bathroom, with a maximum of 7.9 ppm, which contained no humidifiers. The hallway air return location showed a comparable HPV concentration to the bedrooms, living room, and den, which had one humidifier each.

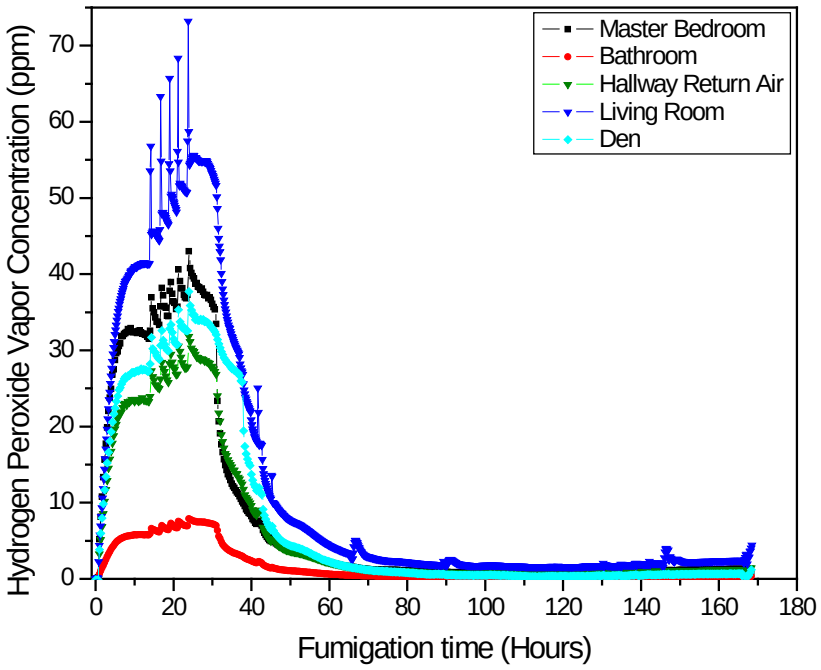

Figure 5-6. Test 1 Temporal HPV Concentration in Living Areas

5.2.1.2 Temporal RH

The effect of the humidifiers on the RH in the CTH living areas was monitored during the fumigation sequence at eight locations (master bedroom, master bathroom, corner bedroom, middle bedroom, hallway bathroom, kitchen, den, and living room), as shown in Figure 5-7. The outside RH measurements were monitored via a weather station and are illustrated in Figure 5-7. RH increased rapidly to values greater than 70% in all living areas, even in the hallway bathroom, where no humidifiers were placed. The HVAC fan continuously circulated air, transporting humidity from the locations with humidifiers to other locations with no humidifiers. Furthermore, the RH was maintained above 60%, even when the HPV concentration decreased to less than 10 ppm. The high swings in outdoor RH seem to have little or no effect on the indoor living areas.

5.2.1.3 Temporal Temperature

The temperature inside the living areas was monitored during the fumigation sequence at eight locations (master bedroom, master bathroom, corner bedroom, middle bedroom, hallway bathroom, kitchen, den, and living room), as shown in Figure 5-8. The outside temperature measurements were monitored via a weather station, and illustrated also in Figure 5-8. The central AC unit was set to “heat” with a setpoint of 70 °F, and the fan was set to run continuously. As expected, the temperatures in the living areas were uniform and were affected neither by the humidification process, nor by fluctuations in the outdoor temperature.

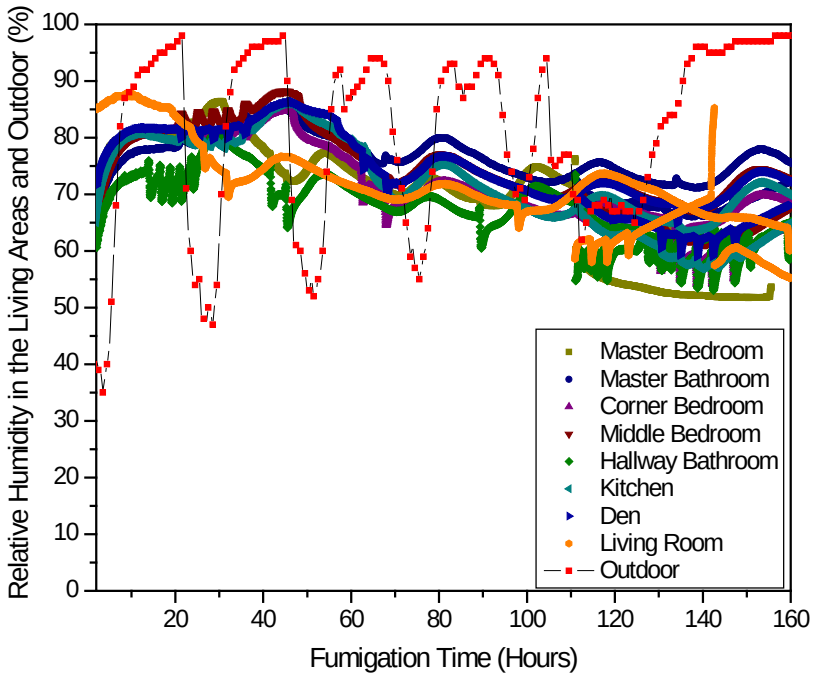

Figure 5-7. Test 1 Temporal RH in Living Areas vs. Outdoors

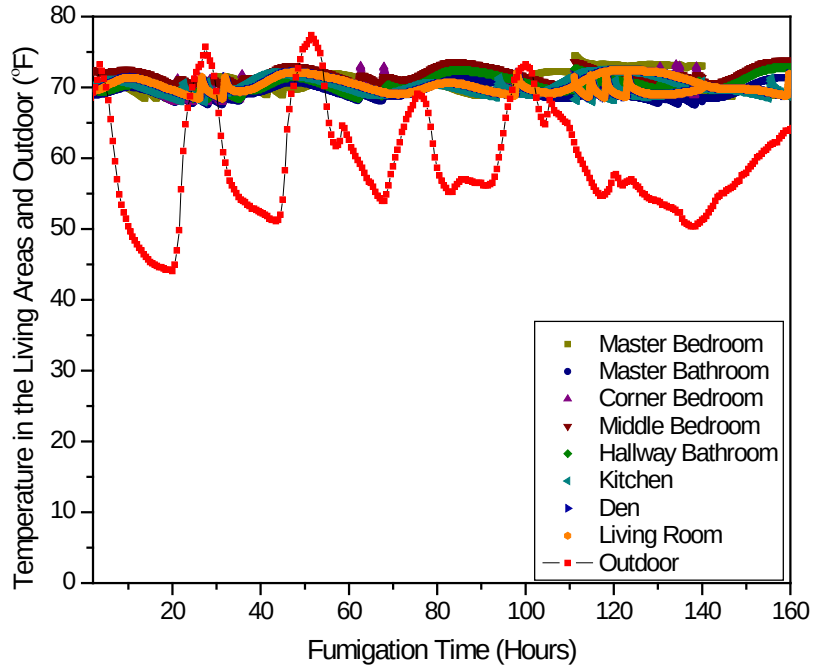

Figure 5-8. Test 1 Temporal Temperature in Living Areas vs. Outdoors

5.2.2 Crawl Space and Attic Fumigation Conditions

5.2.2.1 Temporal HPV Concentration

The temporal HPV concentrations of the crawl space and attic area were monitored at their central and periphery locations (Figure 5-9). The highest HPV concentrations were detected in the central locations of the crawl space and attic, and these concentrations were comparable to those observed in indoor rooms. The peripheral locations had much lower HPV concentrations which may have been caused by leaks of fresh air through the vents.

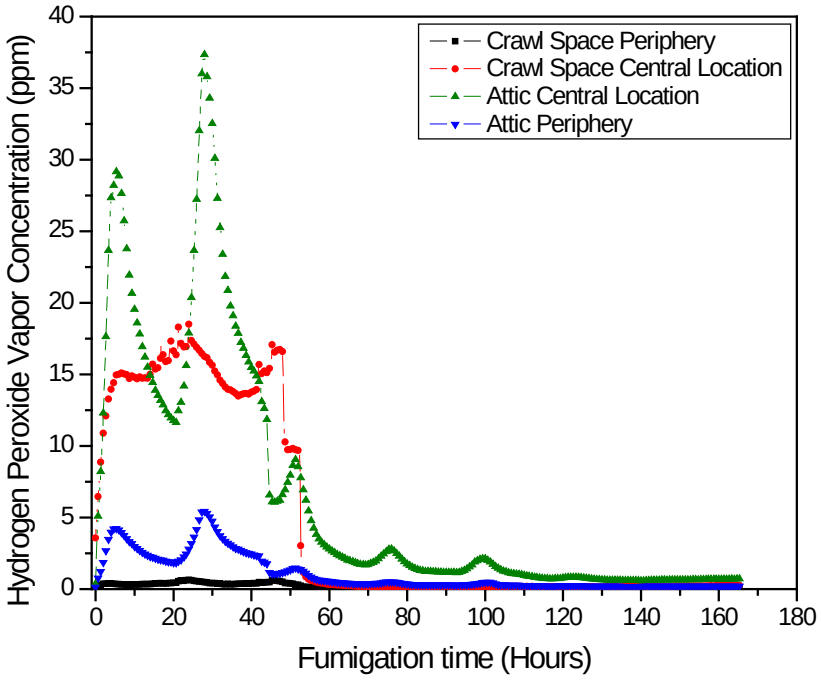

Figure 5-9. Test 1 Temporal HPV Concentration in Crawl Space and Attic

5.2.2.2 Temporal RH and Temperature

Crawl Space

The effect of the humidifiers on the RH and temperature was monitored during the fumigation sequence at three locations in the crawl space: east, central, and west locations (see Figure 5-10). Despite large fluctuations in the outdoor conditions, between days and nights, the temperatures in the crawl space, were unaffected and remained constant throughout the testing sequence. The outside RH appeared to have little to no impact on the crawl space RH, as it stayed relatively high (above 70%) even after the HPV concentration decreased to less than 10 ppm.

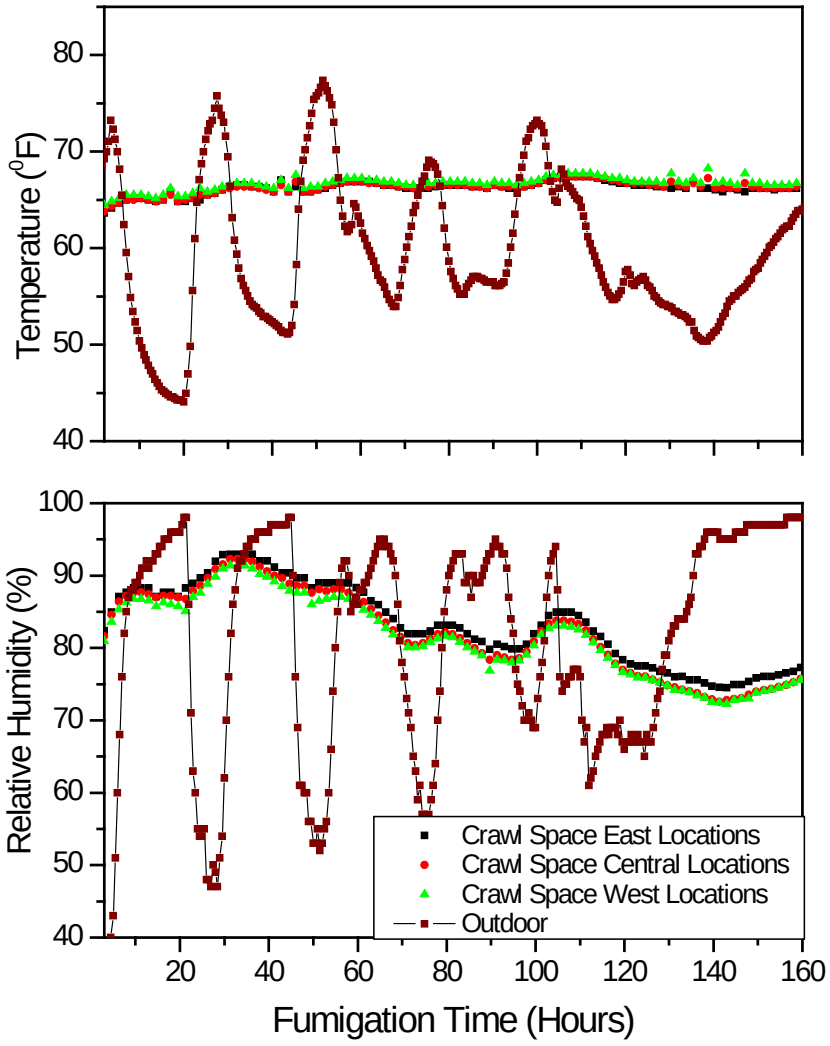

Figure 5-10. Test 1 Temporal RH in Crawl Space vs. Outdoors

Attic

The effect of the humidifiers on the RH was monitored during the fumigation sequence at two locations in the attic: east and west locations (see Figure 5-11). The temperature and RH inside the attic mimic the cyclical pattern of the outdoor environmental conditions. This would suggest that the driving forces for the attic conditions are the outdoor environmental conditions.

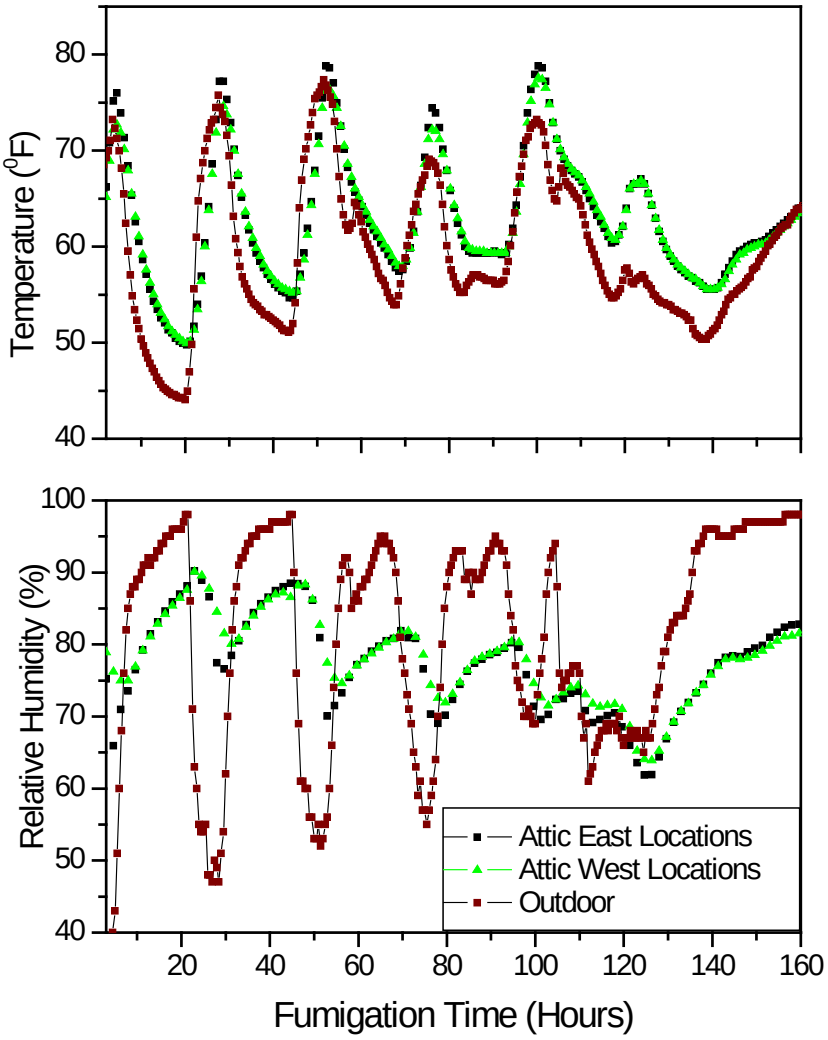

Figure 5-11. Test 1 Temporal RH in Attic vs. Outdoors

5.2.3 Decontamination Efficacy

Test coupons inoculated with *Bg* spores exhibited at least a 6 LR in viable spore count, despite growth of spores for some of the test coupons placed in living areas, crawl spaces, and the attic. These results were mostly independent of test coupon material type, location, or decontamination conditions (HPV concentration, RH, and temperature). Most of the detected results were observed for the GM coupons (8 detections out of 45), with co-located carpet coupons experiencing almost full decontamination (44 non-detects out of 45). Table 5-4 summarizes spore recovery results for the test coupons (three replicates each) and G/NG results for the BI discs (three replicates each).

Table 5-4. Test 1: Post-Decontamination Recoveries on Test Coupons and BI discs

Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)					LR (CFUs)
Location	Sample Type	Rep 1	Rep 2	Rep 3	
Master Bedroom Floor	Carpet coupon	ND	ND	ND	7.45
	GM coupon	35	ND	53	7.04
	BI disc	NG	NG	NG	Not Applicable
Master Bathroom Floor	Carpet coupon	ND	ND	ND	7.45
	GM coupon	1	ND	6	7.04
	BI disc	G	G	NG	Not Applicable
Bathroom Sink	Carpet coupon	ND	ND	ND	7.45
	GM coupon	ND	ND	ND	7.04
	BI disc	NG	G	NG	Not Applicable
Center of Den	Carpet coupon	ND	ND	ND	7.45
	GM coupon	ND	ND	ND	7.04
	BI disc	NG	NG	NG	Not Applicable
Corner Bedroom Floor	Carpet coupon	ND	ND	1	7.45
	GM coupon	ND	ND	54	7.04
	BI disc	NG	NG	NG	Not Applicable
Middle Bedroom Floor	Carpet coupon	ND	ND	ND	7.45
	GM coupon	ND	ND	ND	7.04
	BI disc	NG	NG	NG	Not Applicable
Kitchen Floor	Carpet coupon	ND	ND	ND	7.45
	GM coupon	ND	6	ND	7.04
	BI disc	NG	NG	G	Not Applicable
Living Room Floor	Carpet coupon	ND	ND	ND	7.45
	GM coupon	ND	ND	ND	7.04
	BI disc coupon	NG	NG	NG	Not Applicable
Crawl Space Under Corner Bedroom	Carpet coupon	ND	ND	ND	7.45
	GM coupon	ND	ND	ND	7.04
	BI disc	G	G	G	Not Applicable
Crawl Space Under Kitchen	Carpet coupon	ND	ND	ND	7.45
	GM coupon	ND	ND	ND	7.04
	BI disc	G	G	G	Not Applicable
Crawl Space Under Den	Carpet coupon	ND	ND	ND	7.45
	GM coupon	ND	ND	ND	7.04
	BI disc	G	G	G	Not Applicable
Attic Over Master Bathroom	Carpet coupon	ND	ND	ND	7.45
	GM coupon	ND	ND	ND	7.04
	BI disc	NG	NG	NG	Not Applicable
Center of Attic	Carpet coupon	ND	ND	ND	7.45
	GM coupon	ND	ND	ND	7.04
	BI disc	NG	NG	NG	Not Applicable
Attic Over Den	Carpet coupon	ND	ND	ND	7.45
	GM coupon	16	ND	ND	7.04
	BI disc	NG	NG	NG	Not Applicable
A/C Duct	Carpet coupon	ND	ND	ND	7.45
	GM coupon	ND	9	ND	7.04
	BI disc	NG	NG	NG	Not Applicable

Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)					LR (CFUs)
Location	Sample Type	Rep 1	Rep 2	Rep 3	
Positive Controls	Carpet coupon	3×10^7	3×10^7	3×10^7	
	GM coupon	8×10^6	8×10^6	3×10^7	
	BI disc	G	G	G	

ND = Non-detect	NG = No growth (BI)	G = Growth (BI)

Most of the *Gs* BI discs recovered after the fumigation events showed no growth in the living areas except in locations such as the master bathroom (two out of three BI discs showed growth). HP concentration was not monitored in the master bathroom. Also, one out of three BI discs placed in the hall bathroom sink showed growth, and this one positive may be due to the low HPV concentration (maximum concentration of 8 ppm) in the hall bathroom. No humidifiers were used in or near this location. One BI disc out of three in the kitchen also showed growth.

All the *Gs* BI discs in the crawl space showed growth despite the observed full decontamination of the co-located test coupons. Three of those BI discs were co-located with the coupons, while other BI discs were placed in the periphery of the crawl space as shown in Figure 5-12. The periphery of the crawl space had very low HPV concentrations (less than 1 ppm), and therefore less HPV exposure.

Figure 5-12. BI Disc Package Placement at Periphery of Crawl Space

The Test 1 results demonstrated significant differences in decontamination efficacy between *Bg* inoculated on the test coupons and purchased *Gs* BI discs. These results are consistent with the findings of previous studies (Klapes and Vesley 1990; Kokubo, Inoue, and Akers 1998), where *Gs* spores were found to be more resistant to HP fumigation than *Bg* spores. The *Gs* BI discs were not enumerated and "growth" can be observed even when a disc's residual spore quantity is very low.

This low concentration HPV decontamination approach can be considered an effective sporicidal surface decontamination treatment because LR values exceeded 6 in the entire CTH, regardless of location and material type. For example, even though the HPV concentration was less than 8 ppm in the hallway bathroom, where no humidifier was deployed, two out of three BI discs showed no growth.

A Dräger HP tube with one end open was used to determine that HPV diffused into the tube in the living areas. Figure 5-13 shows a typical Dräger tube. Dräger tube results generally were less than 3 ppm, regardless of location. Since the Dräger tubes were used as passive samplers, and no flow was pulled through them, per manufacturer's instructions, these results should be considered qualitative at best. However, the Dräger tube results can be used as indicators of HPV presence in various test areas.

Figure 5-13. Post-Decontamination Dräger Tube in Living Area

5.3 Test 2

For Test 2, the HP concentration in the aqueous solution was increased from 3 to 3.8%, but all other fumigation conditions remained the same as Test 1 The humidifiers in the crawl spaces were loaded with 3.4 gallons of HP aqueous solution, and all other humidifiers were charged with 2 gallons. One humidifier from the living room was moved to the hallway under the air return vent. Further, one fan was added to the master bedroom, and one fan was removed from the den. Additional test coupons were added to both the den and the living room shown in light green in Figure 5-14.

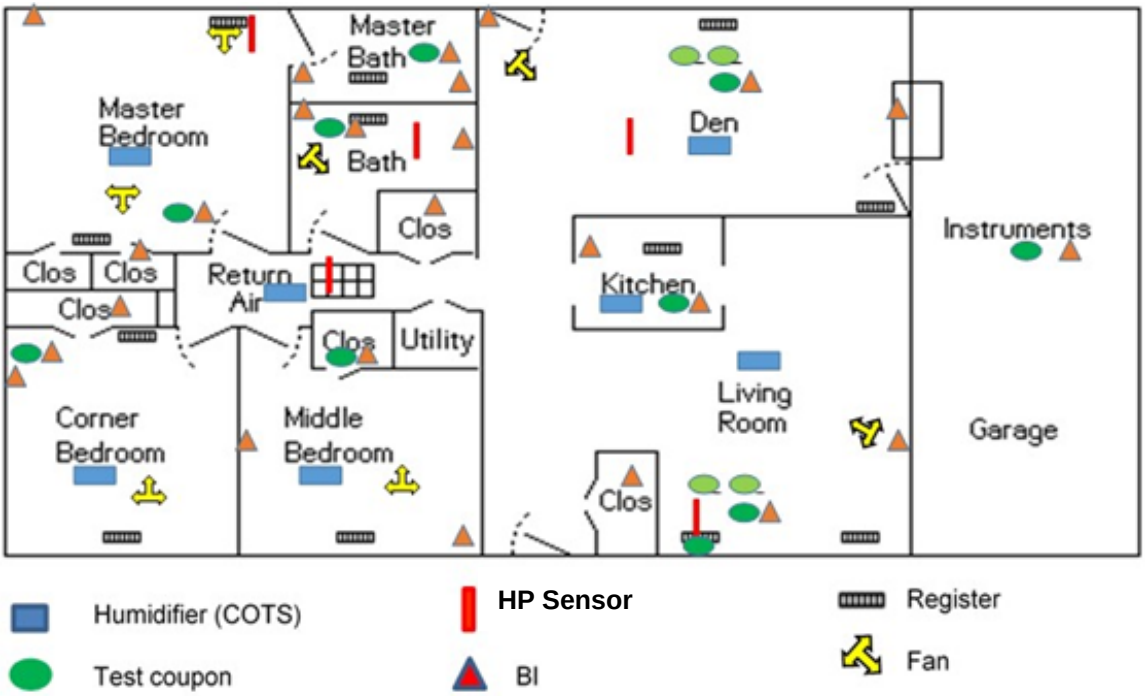

Figure 5-14. CTH Equipment and Sampling Locations for Test 2

The average results for the living areas, attic, and crawl space fumigation conditions including HPV concentration (ppm), overall HPV calculated exposure (concentration-time [CT] in ppm-hours), and RH (%) are tabulated in Table 5-5. The temporal HPV concentration, RH, and temperature are discussed in Sections 5.3.1.1 through 5.3.1.3 for living areas, and in Sections 5.3.2.1 through 5.3.2.2 for the crawl space and attic. Finally, the decontamination efficacy of the test coupons inoculated with *Bg* spores, and growth/no growth assessments of BI discs co-located with the coupons are discussed in Section 5.3.3.

Table 5-5. Test 2 Fumigation Conditions

Test 2 - Fumigation and Relative Humidity Conditions by Location				
Location	**HPV Max (ppm)**	**CT (ppm-hour)**	**RH Average (%)**	**RH Max (%)**
Master Bedroom	76	1930	70	73
Master Bathroom	Not Measured		68	85
Hallway Bathroom	26	976	73	85
Center of Den	51	1540	67	86
Corner Bedroom	Not Measured		70	89
Middle Bedroom	Not Measured		61	77
Kitchen	Not Measured		63	83
Living Room	167	3990	70	85
Air Return	57	1790	Not Measured	
Crawl Space Center	18	1540	77	93
Crawl Space North East	1.2	130	83	100
Crawl Space South West			83	100
Center of Attic	35	1370	79	92
Attic North East	5.9	266	82	100
Attic South West			79	95

5.3.1 Living Area Fumigation Conditions

5.3.1.1 Temporal HPV Concentration

The HPV concentration was monitored using HP sensors at the five following locations: master bedroom, bathroom, hallway air return, living room, and den (see Figure 5-15). The increase in concentration from 3 to 3.8% of the HP aqueous solution resulted in an overall increase in HPV concentration throughout the CTH. The results indicate that moving one humidifier to the hallway resulted in an overall increase in HPV concentration and CT ppm-hours in the hallway, bathroom, and air return areas.

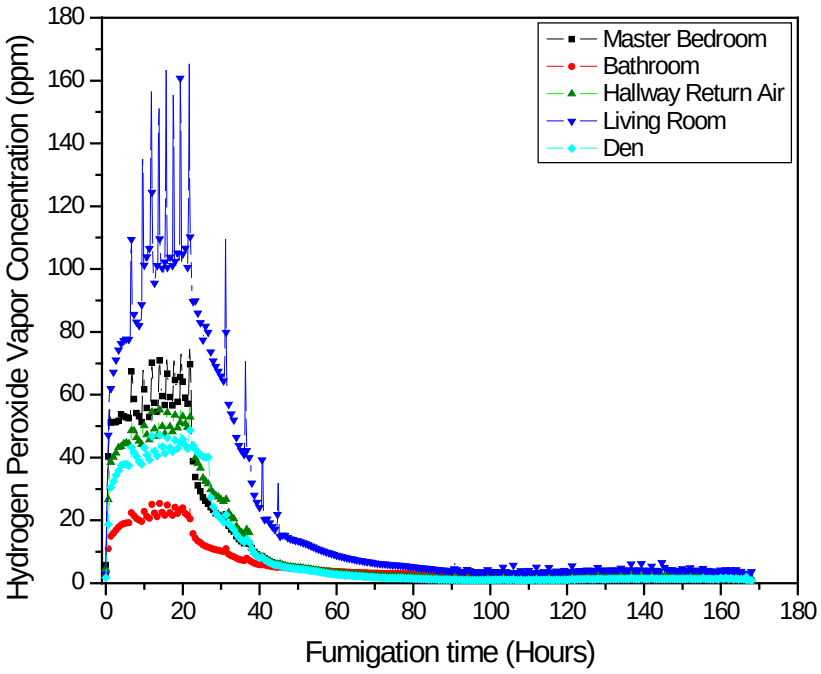

Figure 5-15. Test 2 Temporal HPV Concentration in Living Areas

5.3.1.2 Temporal RH

The effect of the humidifiers on the RH in the CTH living areas was monitored during the fumigation sequence at eight locations (master bedroom, master bathroom, corner bedroom, middle bedroom, hallway bathroom, kitchen, den, and living room), as shown in Figure 5-16. The outside RH measurements were monitored via a weather station, and illustrated also in Figure 5-16. The results show that the RH was maintained above 70% for the first 3 days of the fumigation event, regardless of location in the CTH living area, and despite the large swings in the outdoor RH between days and nights. These results suggest that the driving force for the high RH in the living areas is the continuous fumigation in the living areas. An overall decrease of the RH was observed after the humidifiers were depleted of fumigant.

5.3.1.3 Temporal Temperature

The temperature inside the living areas was monitored throughout the fumigation sequence at eight locations as shown in Figure 5-17. As observed during Test 1, the temperature inside the living areas was uniform and was affected neither by the humidification process nor by the outside environmental conditions.

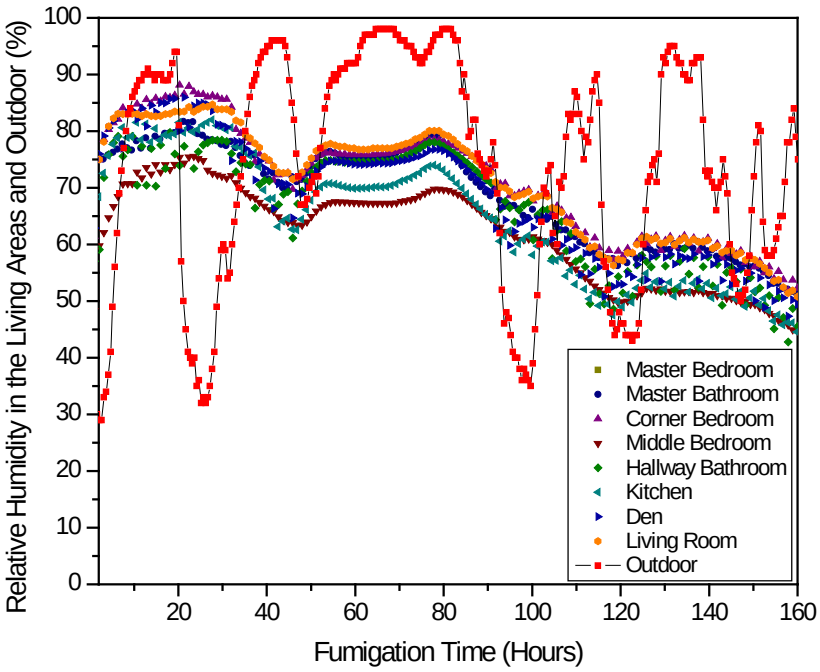

Figure 5-16. Test 2 Temporal RH in Living Areas

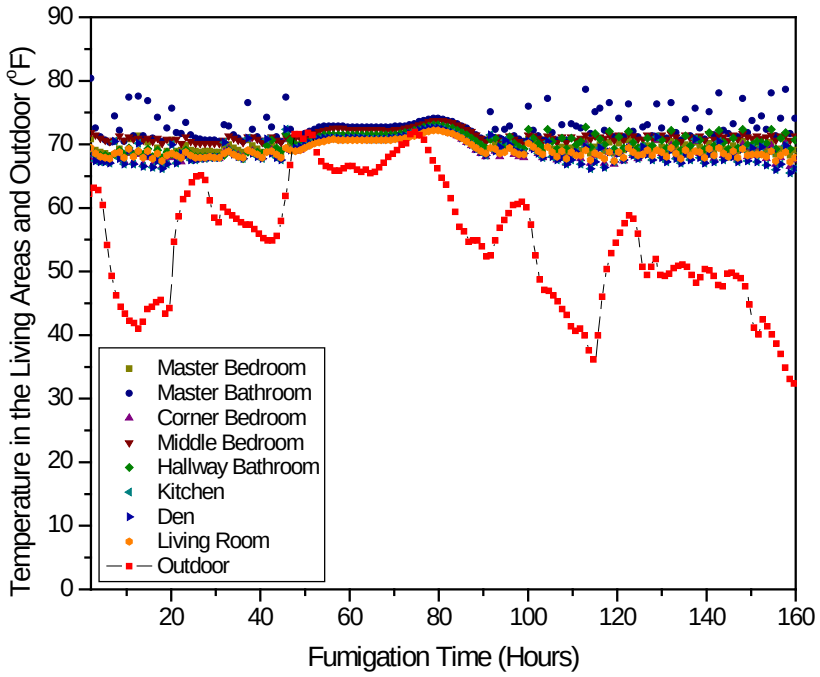

Figure 5-17. Test 2 Temporal Temperature in Living Areas

5.3.2 Crawl Space and Attic Fumigation Conditions

5.3.2.1 Temporal HPV Concentration

The temporal HPV concentrations in the crawl space and attic areas were monitored at their central and periphery locations (see Figure 5-18). The test conditions in the crawl space and attic were the same as Test 1, except that the liquid HP concentration was increased from 3 to 3.8% and more liquid was added to the crawl space humidifiers. The HPV results were of the same order of magnitude as the Test 1 results.

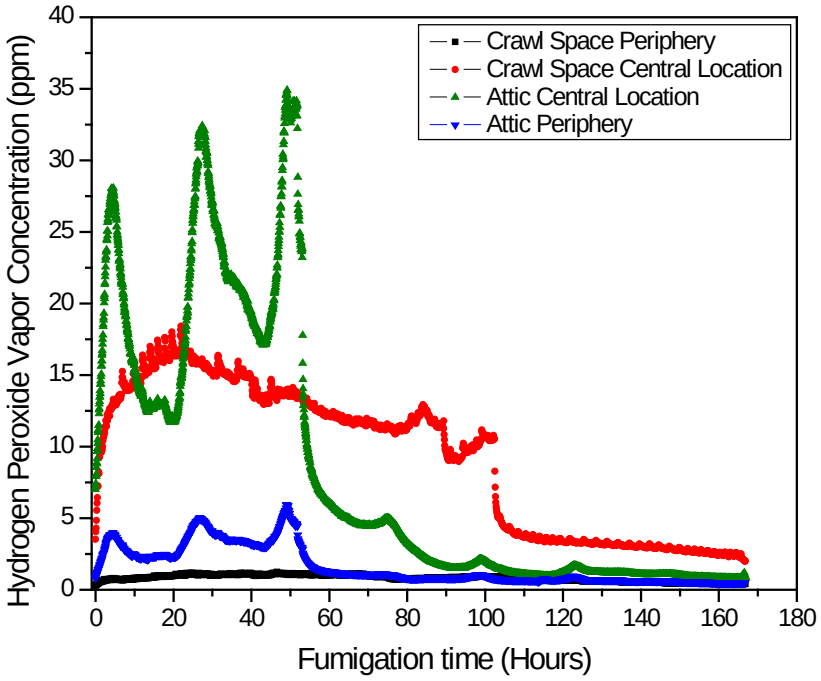

Figure 5-18. Test 2 Temporal HPV Concentration in Crawl Space and Attic

5.3.2.2 Temporal RH

Crawl Space

The effect of the humidifiers on the RH and temperature was monitored during the fumigation sequence at three locations in the crawl space: east, central, and west locations (see Figure 5-19). Despite large fluctuations in the outdoor conditions, between days and nights, the temperatures in the crawl space, were mostly unaffected and remained relatively constant throughout the testing sequence. Likewise, the wide fluctuations observed for the outdoor conditions appear to have little effect on the crawl space RH. After the humidifiers were depleted of fumigant, an overall decrease in RH was observed but the RH stayed high (near 70%) even when HPV concentration levels decreased to single-digit ppm levels. These results are similar to the Test 1 results.

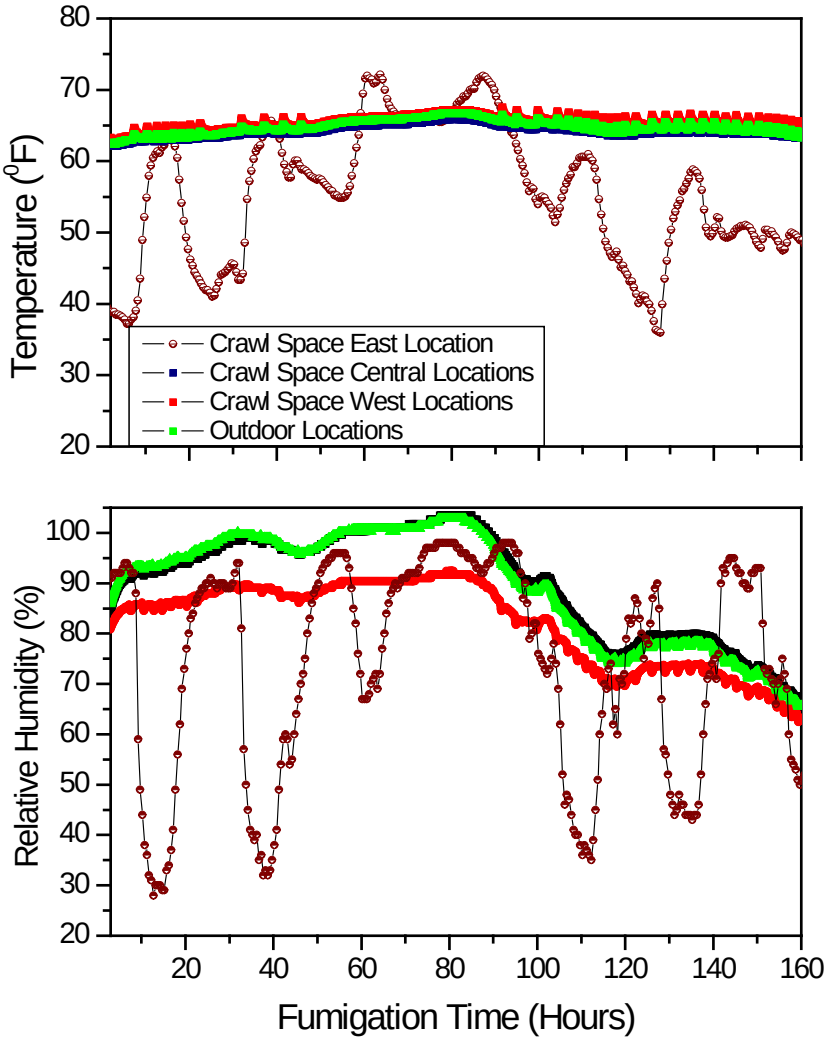

Figure 5-19. Test 2 Temporal RH in Crawl Space

Attic

The effect of the humidifiers on RH was monitored during the fumigation sequence at three locations in the attic: east, central, and west locations (see Figure 5-20). The driving force for the cyclical pattern observed in the attic seems to be the outdoor environmental conditions; however, the large swings in the outdoor RH (between less than 30% to almost saturation) was dampened in the attic, where the RH was above 70% throughout the testing sequence.

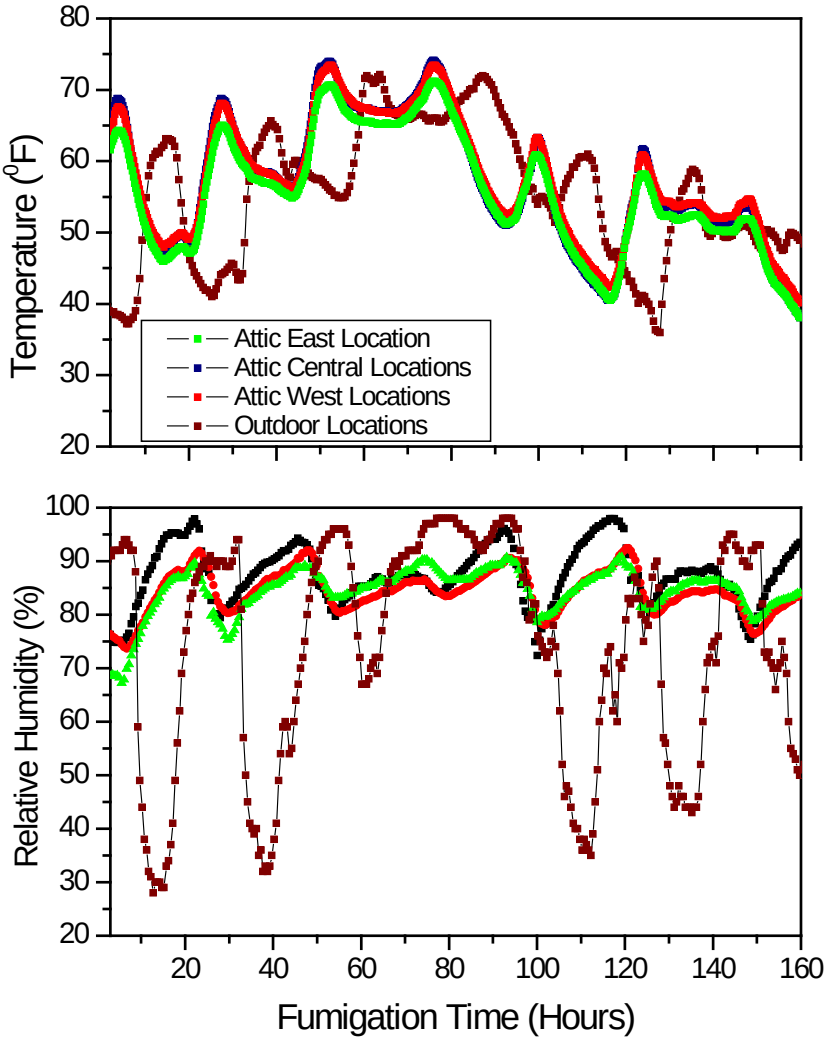

Figure 5-20. Test 2 Temporal RH in Attic

5.3.3 Decontamination Efficacy

The increase from 3 to 3.8% HP aqueous solution in the living areas resulted in full decontamination, with the test coupons inoculated with *Bg* spores exhibiting at least a 6 LR in viable spore count for all the test coupons, regardless of decontamination conditions (HPV concentration, RH, and temperature). During Test 2, two extra sets of coupons (sample sets “a” and “b”) were deployed in the CTH den and living room along with the primary sample set. The “b” sample set was extracted at the end of the third day, while the “a” sample set was extracted on the seventh day along with the primary sample set that underwent the full 7-day decontamination period. The “b” coupon samples exhibited full decontamination, indicating that 3 days of decontamination is sufficient to achieve full decontamination in the CTH living areas. Only 1 of 24 BI discs placed in the CTH living areas showed growth. Table 5-6 summarizes spore recovery results for all the test coupons and G/NG results for the BI discs.

Table 5-6. Test 2: Post-Decontamination Recoveries on Test Coupons and BI discs

Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)					LR (CFUs)
Location	Sample	Rep 1	Rep 2	Rep 3	
Master Bedroom Floor	Carpet coupon	ND	ND	ND	7.62
	GM coupon	ND	ND	ND	7.08
	BI disc	NG	NG	NG	Not Applicable
Master Bathroom Floor	Carpet coupon	ND	ND	ND	7.58
	GM coupon	ND	ND	ND	7.08
	BI disc	NG	NG	NG	Not Applicable
Bathroom Sink	Carpet coupon	ND	ND	ND	7.59
	GM coupon	ND	ND	ND	7.10
	BI disc	NG	NG	NG	Not Applicable
Center of Den	Carpet coupon	ND	ND	ND	7.61
	GM coupon	ND	ND	ND	7.09
	BI disc	NG	NG	NG	Not Applicable
Corner Bedroom Floor	Carpet coupon	ND	ND	ND	7.61
	GM coupon	ND	ND	ND	7.10
	BI disc	NG	NG	NG	Not Applicable
Middle Bedroom Floor	Carpet coupon	ND	ND	ND	7.61
	GM coupon	ND	ND	ND	7.10
	BI disc	NG	NG	NG	Not Applicable
Kitchen Floor	Carpet coupon	ND	ND	ND	7.61
	GM coupon	ND	ND	ND	7.11
	BI disc	NG	NG	NG	Not Applicable
Living Room Floor	Carpet coupon	ND	ND	ND	7.61
	GM coupon	ND	ND	ND	7.10
	BI disc	NG	NG	G	Not Applicable
Living Room Floor (a)	Carpet coupon	ND	ND	ND	7.62
	GM coupon	ND	ND	ND	7.10
Living Room Floor (b)	Carpet coupon	ND	ND	ND	7.60
	GM coupon	ND	ND	ND	7.08
Crawl Space Under Corner Bedroom	Carpet coupon	ND	ND	ND	7.59
	GM coupon	ND	ND	ND	7.10
	BI disc	G	G	G	Not Applicable
Crawl Space Under Kitchen	Carpet coupon	ND	ND	ND	7.61
	GM coupon	ND	ND	ND	7.11
	BI disc	NG	NG	NG	Not Applicable
Crawl Space Under Den	Carpet coupon	ND	ND	ND	7.53
	GM coupon	ND	ND	ND	7.11
	BI disc	NG	G	G	Not Applicable
	Carpet coupon	ND	ND	ND	7.60

Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)					LR (CFUs)
Location	Sample	Rep 1	Rep 2	Rep 3	
Attic Over Master Bathroom	GM coupon	9.80×10^3	8.86×10^4	3.73×10^3	2.79
	BI disc	NG	NG	G	Not Applicable
Center of Attic	Carpet coupon	ND	ND	ND	7.62
	GM coupon	ND	ND	ND	6.27
	BI disc	NG	NG	NG	Not Applicable
Attic over Den	Carpet coupon	6.70×10^5	2.36×10^5	1.18×10^4	2.39
	GM coupon	1.07×10^7	3.83×10^6	5.48×10^6	0.14
	BI disc	G	G	NG	Not Applicable
A/C Duct	Carpet coupon	ND	ND	ND	7.60
	GM coupon	ND	ND	ND	7.11
Center of Den (a)	Carpet coupon	ND	ND	ND	7.62
	GM coupon	ND	ND	ND	7.00
Center of Den (b)	Carpet coupon	ND	ND	ND	7.59
	GM coupon	ND	ND	ND	7.07
Positive 1	Carpet coupon	7.50×10^7	1.14×10^7	3.32×10^7	
	GM coupon	6.38×10^6	6.81×10^6	1.13×10^7	
	BI disc 1,2,3	G	G	G	
Positive 2	Carpet coupon	1.64×10^7	1.97×10^7	2.64×10^7	
	GM coupon	4.74×10^6	1.11×10^7	9.99×10^6	

ND = Non-detect	NG = No growth (BI)	G = Growth (BI)

The GM test coupons placed in the attic spaces over the den and over the master bathroom showed LR values of less than 2.8 to almost no decontamination, despite the increase in HPV concentration. Three out of nine BI discs in the attic and five out of nine BI discs placed in the crawl space exhibited growth after the decontamination process.

In summary, the Test 2 results confirm the Test 1 results, suggesting that this low concentration HPV decontamination approach may be considered an effective sporicidal surface decontamination treatment because LR values exceeded 6 in the living areas of the CTH, regardless of location and material type. LR values exceeded 6 even when the exposure time for Test 2 was 3 days in the living areas.

In the crawl space, LR values exceeded 6 when there was a 7-day exposure time. Despite some growth observed for BI discs in the crawl space, full decontamination was observed on the co-located coupons suggesting that a LR of 6 to 7 can be achieved in much of the crawl space. However, for BI discs (providing non-quantitative assessments) placed near ventilation inlets, some spores survived the HPV treatment. *Gs* spores have been shown to be more hardy than *Bg* spores during HPV exposures (Klapes and Vesley 1990; Kokubo, Inoue, and Akers 1998),

For the attic, very limited decontamination occurred over 7 days. The attic is not well sealed (the ridge vent remained open during fumigation) and the roof is not insulated. The RH data showed a cyclical fluctuation between days and nights.

5.4 Test 3

For Test 3, the humidifiers, fan, test coupons, and BI disc placements were kept the same as Test 2. All humidifiers were loaded with 3.0% HP aqueous solution. The humidifiers in the crawl space and attic were each loaded with 3.4 gallons of HP aqueous solution, and the humidifiers in the living areas were charged with only 2 gallons of this solution. Further, for this test, furniture was added to the CTH, including three beds with bedding and pillows, clothing, five rugs, two couches, two chairs, and three boxes of paper and books. Figure 5-21 shows the placement of some of these items in the CTH.

Master Den Living

Figure 5-21. Furniture Added to CTH for Test 3

Furthermore, BI discs were placed in the following hard-to-reach areas to test the efficacy of the fumigation process:

- Between couch cushions (Figure 5-22)
- Under carpet (jute-backed) (Figure 5-23)
- Inside a closed bathroom drawer (Figure 5-24)
- Under one and also under five pieces of paper (Figure 5-25)

Figure 5-22. Test 3 Placement of BI discs Between Couch Cushions

Figure 5-23. Test 3 Placement of BI discs Under Carpet

Figure 5-24. Test 3 Placement of BI discs in Bathroom Drawer (closed during testing)

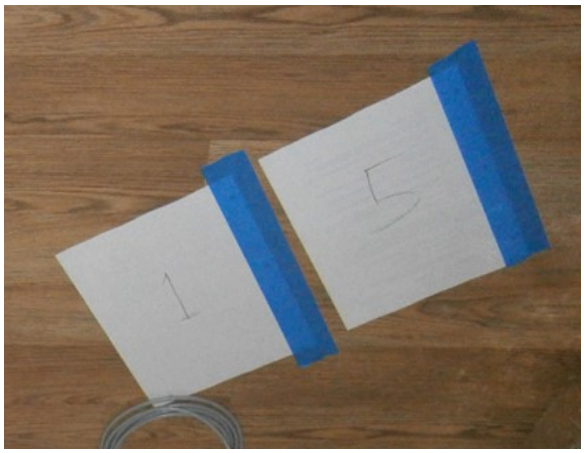

Figure 5-25. Test 3 Placement of BI discs Under One and Five Pieces of Paper

The average results for the living areas, attic, and crawl space fumigation conditions which include HPV concentration (ppm), overall calculated HPV exposure (concentration-time [CT] in ppm-hours), and average RH (%) are tabulated in Table 5-7. The temporal HPV concentration, RH, and temperature are discussed in Sections 5.4.1.1 through 5.4.1.2 for living areas, and the decontamination efficacy of the test coupons inoculated with *Bg* spores, and growth/no growth assessments of BI discs co-located with the coupons are discussed in Section 5.4.2.

Table 5-7. Test 3 Fumigation Conditions

Test 3 - Fumigation and Relative Humidity Conditions by Location				
Location	**HPV Max (ppm)**	**CT (ppm-hour)**	**RH Average (%)**	**RH Max (%)**
Master Bedroom	46	1480	60	86
Master Bathroom	Not Measured		61	83
Hallway Bathroom	17	655	55	81
Center of Den	30	1090	62	85
Corner Bedroom	Not Measured		59	85
Middle Bedroom	Not Measured		59	83
Kitchen	Not Measured		61	89
Living Room	116	2780	58	84
Air return	35	1210	50	78
Crawl Space Center	27	2130	73	92
Crawl Space North East	9.9	503	75	95
Crawl Space South West			78	97
Center of Attic	18	1620	82	94
Attic North East	11	643	83	92
Attic South West			82	93

5.4.1 Living Area Fumigation Conditions

5.4.1.1 Temporal HPV Concentration

The HPV concentration was monitored using HP sensors at the same five locations used during Test 2: master bedroom, bathroom, hallway air return, living room, and den (see Figure 5-26). Adding furniture did not significantly increase the demand for HP aqueous solution or change the CT (ppm-hour) results, and the maximum HPV concentrations in the living areas were not much different than the Test 1 results. There was better distribution of HP to the hall bathroom.

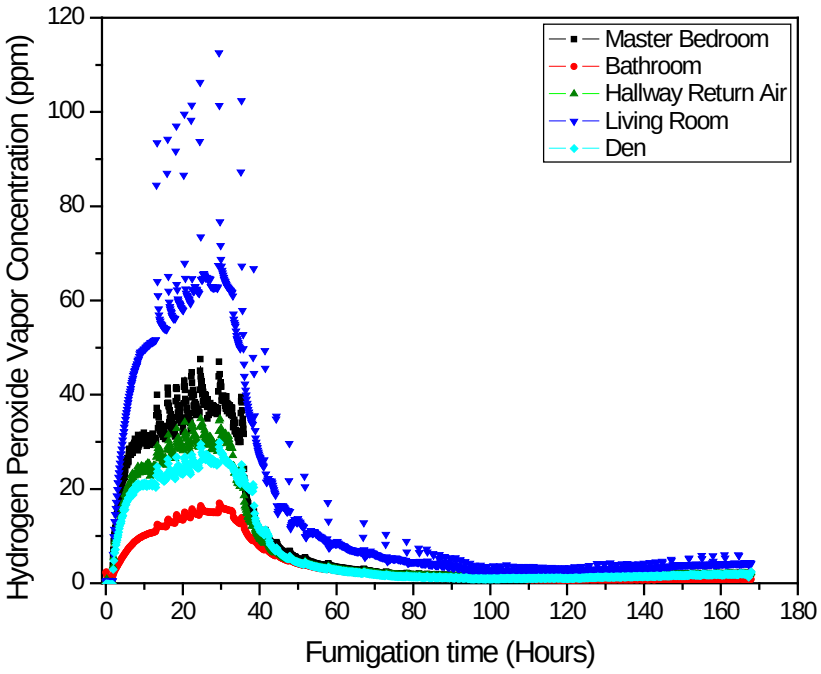

Figure 5-26. Test 3 Temporal HPV Concentration in Living Area

5.4.1.2 Temporal RH and Temperature

The effect of the humidifiers on the RH in the CTH living areas was monitored during the fumigation sequence at eight locations (master bedroom, master bathroom, corner bedroom, middle bedroom, hallway bathroom, kitchen, den, and living room), as shown in Figure 5-27. The outside RH measurements was monitored via a weather station, and illustrated also in Figure 5-27. The results show that the RH was maintained above 70% for the first 3 days of the fumigation event, and slowly decreased after the humidifiers were depleted of fumigant. Temperature (data not shown), as expected, was steadily maintained near 70 °F by the CTH HVAC system.

The large fluctuations in the outdoor conditions seem to have a negligible effect on the indoor conditions. These results suggest that the driving force for the high RH in the living areas is the continuous

fumigation in the living areas. As shown in Figure 5-27, the RH dropped below 70% after about 70 hours, when the fumigant was depleted below 10 ppm in the living areas.

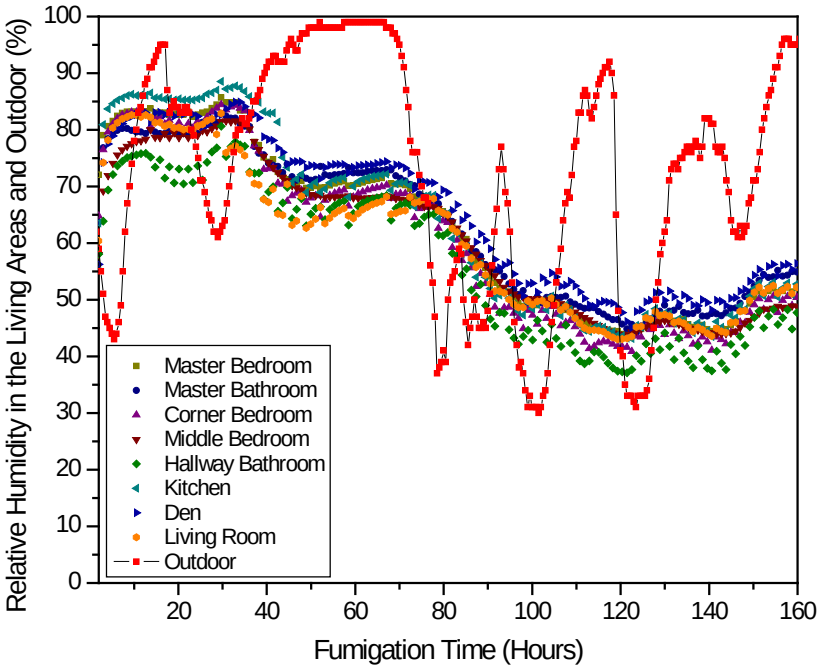

Figure 5-27. Test 3 Temporal RH in Living Areas

5.4.2 Decontamination Efficacy

The addition of furniture to the CTH had little to no effect on the decontamination efficacy in the CTH living areas. Full decontamination with a LR value of at least 6 was observed for all the test coupons in the living areas, except for the coupons placed in the living room hallway and on the master bathroom floor (where no humidifier was located), but even these samples resulted in low CFU counts after fumigation. During Test 3, three extra sets of coupons (sample set “b”) were deployed in the den, bathroom sink, and living room along with the primary set of coupons. On the third day of the 7-day test period, the “b” coupons were recovered and extracted. The “b” sample set results were similar to results for the primary samples that underwent the full 7-day decontamination period. These samples also exhibited full decontamination, indicating that 3 days of decontamination is sufficient, for those two locations, to achieve full decontamination in the living areas of the CTH. Table 5-8 summarizes spore recovery results for the test coupons and G/NG results for the BI discs.

Table 5-8. Test 3: Post-Decontamination Recoveries on Test Coupons and BI discs

Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)					LR
Location	Sample Type	Rep 1	Rep 2	Rep 3	
Master Bedroom Floor	Carpet coupon	ND	ND	ND	7.56
	GM coupon	ND	ND	ND	7.13
	BI disc	NG	NG	NG	Not Applicable
Master Bedroom Floor (b)	Carpet	ND	ND	ND	7.60
	GM	ND	ND	ND	7.15
	BI disc	NG	Not measured		Not Applicable
Master Bathroom Floor	Carpet coupon	ND	ND	ND	7.56
	GM coupon	3	220	ND	6.10
	BI disc	NG	NG	NG	Not Applicable
Bathroom Sink	Carpet coupon	ND	ND	1	7.47
	GM coupon	ND	ND	ND	7.14
	BI disc	NG	NG	NG	Not Applicable
Bathroom Sink (b)	Carpet coupon	ND	ND	1	7.48
	GM coupon	ND	ND	ND	7.15
	BI disc	NG	Not measured		Not Applicable
Center of Den	Carpet coupon	2	ND	ND	7.46
	GM coupon	ND	ND	ND	7.13
	BI disc	NG	NG	NG	Not Applicable
Center of Den (b)	Carpet coupon	ND	ND	ND	7.48
	GM coupon	ND	ND	ND	7.15
	BI disc	NG	Not measured		Not Applicable
Corner Bedroom Floor	Carpet coupon	ND	ND	ND	7.56
	GM coupon	ND	ND	ND	7.12
	BI disc	NG	G	NG	Not Applicable
Middle Bedroom Floor	Carpet coupon	ND	ND	ND	7.56
	GM coupon	ND	ND	ND	7.13
	BI disc	NG	NG	NG	Not Applicable
Kitchen Floor	Carpet coupon	ND	ND	ND	7.57
	GM coupon	ND	ND	ND	7.12
	BI disc	NG	NG	NG	Not Applicable
Living Room / Hallway	Carpet coupon	ND	2	1	7.37
	GM coupon	ND	ND	ND	7.13
	BI disc	NG	NG	G	Not Applicable
Living Room Floor (b)/ Dinning Area	Carpet coupon	ND	ND	ND	7.58
	GM coupon	ND	ND	ND	7.04
	BI disc	NG	NA	NA	Not Applicable
Crawl Space Under Corner Bedroom	Carpet coupon	ND	ND	ND	7.56
	GM coupon	1	8.80×10^2	1.56×10^3	4.98
	BI disc	G	G	G	Not Applicable

Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)					LR
Location	Sample Type	Rep 1	Rep 2	Rep 3	
Crawl Space Under Kitchen	Carpet coupon	ND	ND	ND	7.59
	GM coupon	6.30×10^2	ND	ND	6.15
	BI disc	NG	G	G	Not Applicable
Crawl Space Under Den	Carpet coupon	ND	ND	ND	7.56
	GM coupon	ND	ND	ND	7.14
	BI disc	NG	G	G	Not Applicable
Attic Over Master Bathroom	Carpet coupon	ND	3	7.82×10^5	5.36
	GM coupon	1.97×10^5	1.78×10^5	1.07×10^5	1.77
	BI disc	NG	NG	NG	Not Applicable
Center of attic	Carpet coupon	ND	ND	ND	7.56
	GM coupon	1.29×10^3	1.79×10^4	8.60×10^3	3.20
	BI disc	NG	NG	NG	Not Applicable
Attic over Den	Carpet coupon	6	1.36×10^2	1.38×10^1	6.09
	GM coupon	3.40×10^5	5.21×10^5	3.88×10^5	1.35
	BI disc	G	NG	G	Not Applicable
A/C Duct	Carpet coupon	ND	ND	ND	7.55
	GM coupon	ND	ND	ND	7.13
	BI disc	NG	NG	NG	Not Applicable
Positive 1	Carpet coupon	2.94×10^7	2.79×10^7	3.10×10^7	
	GM coupon	6.19×10^6	1.20×10^7	1.07×10^7	
	BI disc 1,2,3	G	G	G	
Positive 2	Carpet coupon	1.94×10^7	2.75×10^7	3.15×10^7	
	GM coupon	1.11×10^7	6.53×10^6	8.73×10^6	

ND = Non-detects	NG = No growth (BI)	G = Growth (BI)	NA: BI Not deployed in replicates

Consistent with the results for Tests 2 and 3, the GM coupons placed in the attic showed LR values of less than 3.2 to almost no decontamination. On the other hand, the carpet coupons showed more than a 5 LR to full decontamination.

For the crawl space, LR values of 6 to full decontamination were observed for the carpet coupons, but not for the GM coupons. Additionally, seven out of the nine BI discs in the crawl space showed growth, indicating that full decontamination was not achieved, particularly for locations near vents in the crawl space. For the coupons in the crawl spaces, the GM coupons were less prone to LCHP vapor decontamination than carpet coupons, as shown in Figure 5-28. This finding highlights potential differences between real world conditions and those obtained when Rogers, et. al (2005) treated seven different surface materials for 20 minutes with ≥ 1,000 ppm HPV in a closed chamber and observed better inactivation of *Bacillus* spores on non-porous surfaces than on porous surfaces. See also Ryan, et al. (2008).

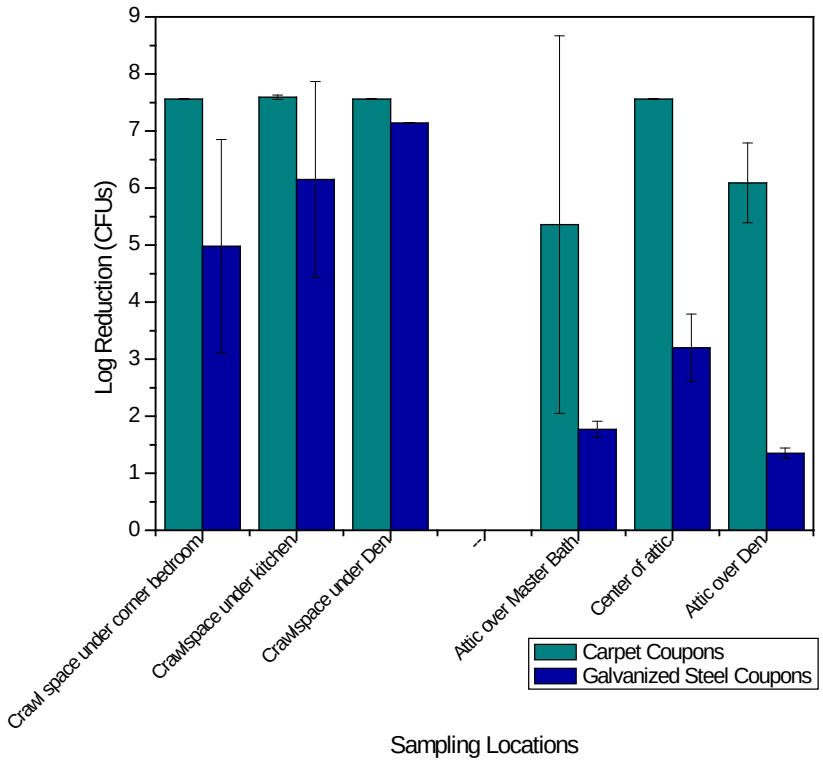

Figure 5-28. Test 3 Decontamination Efficacy on Carpet and GM Coupons

For the hard-to-reach places, the decontamination efficacy depended on the placement of the BI discs and the type of materials shielding them. The BI discs placed between the couch cushions and under the rug showed little, if any, decontamination, while the BI discs placed under one and five sheets of paper and inside the closed bathroom drawer showed full decontamination. The jute-backed carpet and couch cushions evidently prevented the HPV from reaching and affecting the spores on the BI discs. Table 5-9 summarizes the spore count and G/NG results for the BI discs in hard-to-reach places.

Table 5-9. Test 3 Post-Decontamination Recoveries on Hard-to-Reach BI discs

Location	Quantitative Analysis of BI discs (CFUs)				
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Between couch cushions	2.0×10^6	2.1×10^6	2.1×10^6	2.2×10^6	1.9×10^6
Under rug	1.3×10^6	2.7×10^5	1.3×10^5	2.1×10^5	8.3×10^5
Positives	2.1×10^6	2.2×10^6	2.3×10^6	NA	NA
Location	**Qualitative Analysis of BI discs**				
Between couch cushions	NG	G	G	NG	NG
Under rug	G	G	G	G	G
Under one piece of paper	NG	NG	NG	NA	NA
Under five pieces of paper	NG	NG	NG	NA	NA
In closed bathroom drawer	NG	NG	NG	NG	NG

NA: BI Not deployed in replicates	NG = No growth (BI)	G = Growth (BI)

5.5 Test 4

For Test 4, several adjustments were made to the placement of fans. In the corner and middle bedrooms, the fans were moved closer to the closets, and a fan was added near the closet in the living room. The number of humidifiers in the living area was reduced from seven to two. The two humidifiers were placed in the hallway under the air return vent. The first humidifier was started while the second was on a delay timer set to turn on 24 hours after the start of first one. This approach allowed time for the first humidifier to fully dispense its contents and shut off. All the humidifiers, including the ones in the crawl space and the attic, were filled to maximum capacity with 3.4 gallons of 3.0% HP aqueous solution. Figure 5-29 shows the test layout.

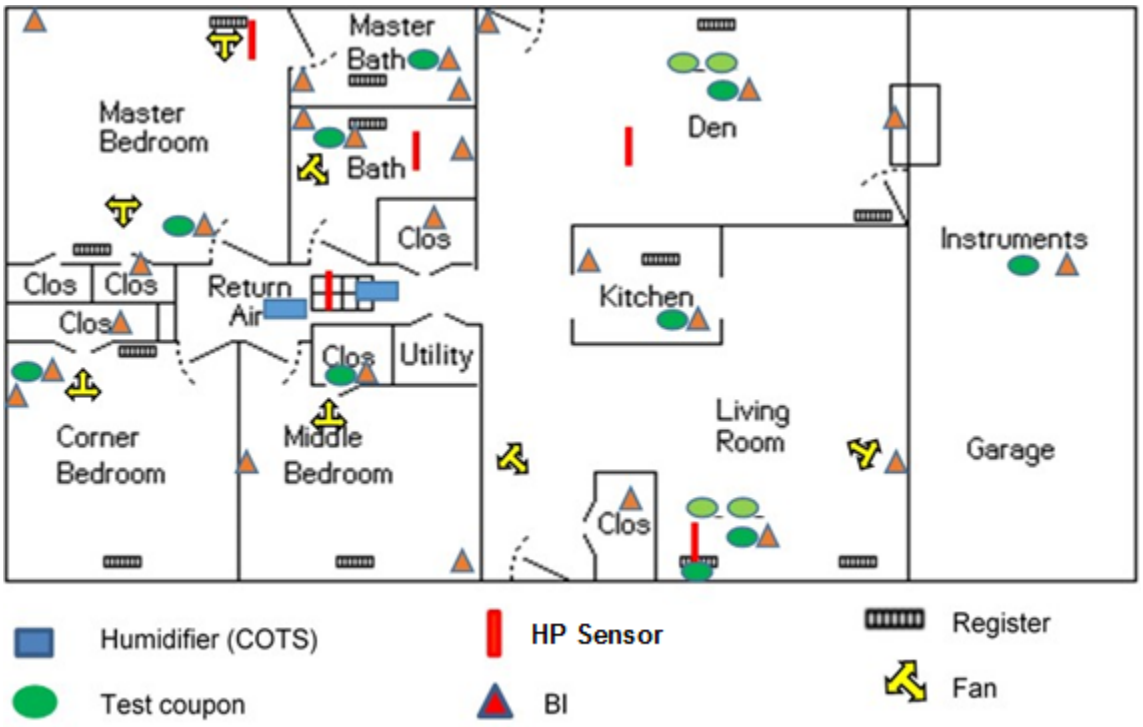

Figure 5-29. CTH Equipment and Sampling Locations for Test 4

Furthermore, BI discs were placed in the following hard-to-reach areas to test the efficacy of the fumigation process:

- Closed book in the living room (Figure 5-30)
- Master bedroom inside a pants pocket
- Entry closet inside a coat pocket (Figure 5-31)
- Dining room behind light switch plate (Figure 5-32)
- Master bedroom window east wall
- Front door jamb
- Den deck door jamb
- Den behind switch plate on outside wall

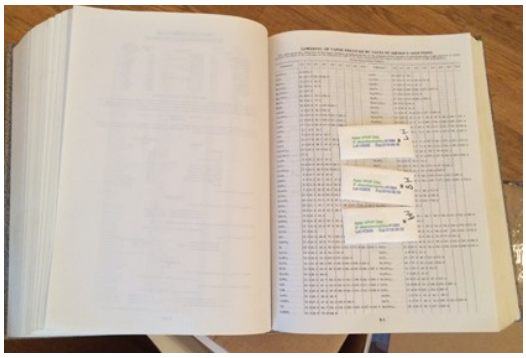

Figure 5-30. Test 4 BI discs Inside Book (closed during the test)

Figure 5-31. Test 4 BI disc Inside Coat Pocket in Entry Closet

Figure 5-32. Test 4 BI discs Behind Light Switch Plate

The average results for the living areas, attic, and crawl space fumigation conditions including: HPV concentration (ppm), overall calculated HPV exposure (concentration-time [CT] in ppm-hours), and RH (%) are tabulated in Table 5-10. The temporal HPV concentration, RH, and temperature are discussed in Sections 5.5.1.1 through 5.5.1.2 for the living areas. The decontamination efficacy of the test coupons inoculated with *Bg* spores, and growth/no growth assessments of BI discs co-located with the coupons are discussed in Section 5.5.2.

Table 5-10. Test 4 Fumigation Conditions

Test 4 - Fumigation and Relative Humidity Conditions by Location				
Location	**HPV Max (ppm)**	**CT (ppm-hour)**	**RH Average (%)**	**RH Max (%)**
Master Bedroom	20	788	41	58
Master Bathroom	Not Measured		40	55
Hallway Bathroom	13	525	31	46
Center of Den	13	432	47	55
Corner Bedroom	Not Measured		38	50
Middle Bedroom	Not Measured		47	61
Kitchen	Not Measured		43	56
Living Room	18	873	45	55
Air return	36	1320	44	55
Crawl Space Center	51	2465	62	81
Crawl Space North East	2.9	113	73	90
Crawl Space South West			68	86
Center of Attic	134	6130	63	81
Attic North East	17	800	63	82
Attic South West			66	83

5.5.1 Living Area Fumigation Conditions

5.5.1.1 Temporal HPV Concentration

The HPV concentration was monitored using HP sensors at the same five locations used during Test 2: master bedroom, bathroom, hallway air return, living room, and den (see Figure 5-33). Reducing the number of humidifiers in the living area from seven to two and placing them in the hallway under the air return vent decreased the maximum concentration of HPV in the living room by more than 84% when compared to Test 3, but decreased the HPV concentration in the other rooms much less. The HPV concentration in the whole house decreased rapidly to about 5 ppm after 24 hours of fumigation before increasing again with the start of the second humidifier.

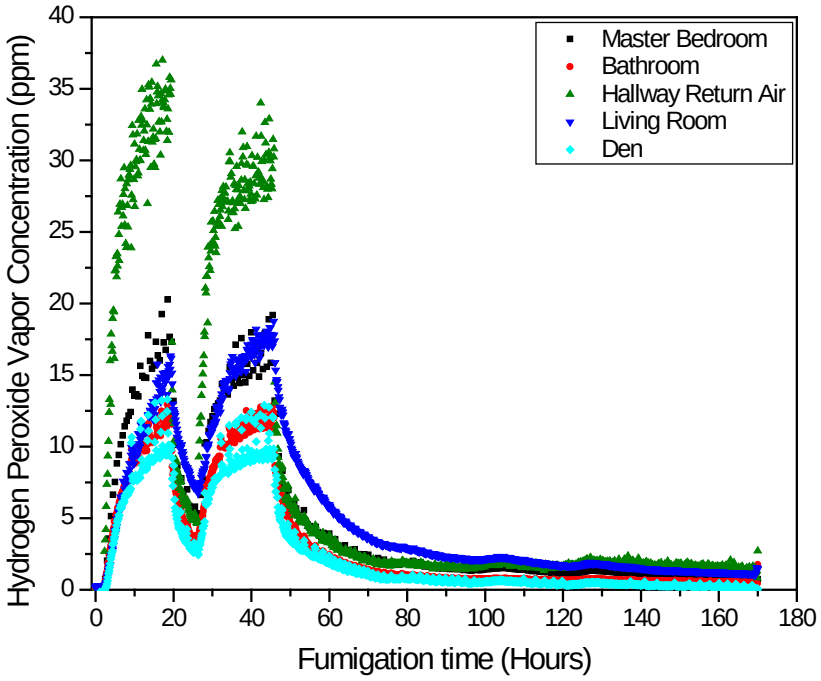

Figure 5-33. Test 4 Temporal HPV Concentration in Living Areas

5.5.1.2 Temporal RH and Temperature

The results shown in Figure 5-34 illustrate fumigation RH conditions, using only two humidifiers run sequentially 24 hours apart, throughout the decontamination testing. The RH for most of the living areas, except for the den, remained below 50% for most of the fumigation period. The temperature, as expected, remained steady near 70 ˚F as maintained by the CTH HVAC system (data not shown). As observed for the prior tests, outdoor conditions appear to have little bearing on the RH conditions observed for the living areas inside the CTH. As shown in Figure 5-34, outdoor RH widely fluctuated, but the indoor RH had a more even trend and remained mostly under 50% throughout the test period.

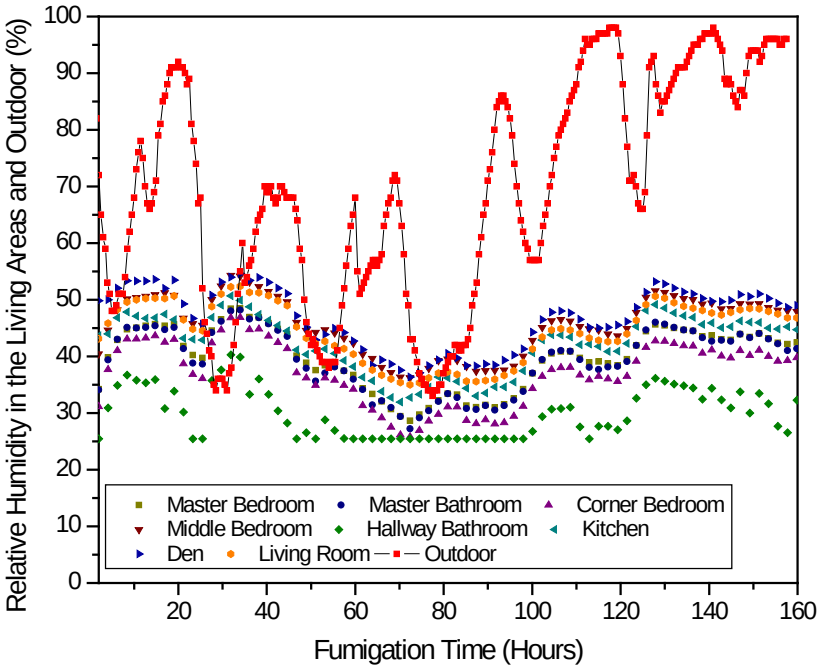

Figure 5-34. Test 4 Temporal RH in Living Areas

5.5.2 Decontamination Efficacy

Reducing the number of humidifiers in the living areas from seven to two greatly affected decontamination efficacy (see Table 5-11 and Figure 5-35). The humidifiers were placed in the hallway under the air return vent and run sequentially 24 hours apart. Most of the test coupons in the living areas did not achieve LR values of 6. The HPV concentrations were lower than the HPV concentrations observed in the prior three tests.

During Test 4, three extra sets of coupons (sample set "b") were deployed in the master bedroom closet, the outside wall of the den, and the middle bedroom closet along with the primary set of coupons. On the third day of the 7-day test period, the "b" coupons were recovered and extracted. The "b" sample set results were similar to results for the primary samples that underwent the full 7-day decontamination period, demonstrating that extending the exposure time beyond 3 days did not improve the decontamination efficacy. Table 5-11 summarizes spore recovery results for the test coupons and G/NG results for the BI discs. Interestingly, there were many NG for BIs with *Gs* spores which are reported to be more difficult to inactivate than *Bg*, but a different conclusion is indicated here.

Table 5-11. Test 4 Post-Decontamination Recoveries on Test Coupons and BI discs

Location	Sample Type	Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)			LR (CFUs)	
		Rep 1	Rep 2	Rep 3	Average	SD
Master Bedroom Closet	Carpet coupon	2.20×10^5	6.11×10^5	6.09×10^5	1.9	0.3
	GM coupon	1.71×10^3	4.30×10^3	5.06×10^3	3.7	0.3
	BI disc	NG	NG	NG	Not Applicable	
Master Bedroom Closet (b)	Carpet coupon	3.29×10^6	4.36×10^5	7.66×10^4	1.9	0.8
	GM coupon	1.14×10^4	4.35×10^3	5.00×10^4	3.0	0.5
Master Bathroom Floor	Carpet coupon	2.27×10^5	1.37×10^5	2.39×10^5	2.3	0.1
	GM coupon	1.81×10^4	1.78×10^3	9.79×10^3	3.3	0.5
	BI disc	NG	NG	NG	Not Applicable	
Bathroom Sink	Carpet coupon	4.35×10^5	3.95×10^4	1.25×10^4	2.8	0.8
	GM coupon	3.57×10^3	ND	1	6.0	2.1
	BI disc	G	NG	NG	Not Applicable	
Center of Den	Carpet coupon	4.88×10^5	2.09×10^4	6.99×10^4	2.6	0.7
	GM coupon	1.39×10^3	2.67×10^2	3	5.2	1.4
	BI disc	NG	NG	NG	Not Applicable	
Outside Wall of Den (b)	Carpet coupon	2.08×10^5	8.64×10^4	1.59×10^4	2.7	0.6
	GM coupon	4.44×10^3	3.81×10^3	1.92×10^3	3.7	0.2
Corner Bedroom Floor	Carpet coupon	2.74×10^5	1.24×10^5	1.39×10^5	2.3	0.2
	GM coupon	9.07×10^1	1.77×10^3	4.20×10^2	4.6	0.6
	BI disc	NG	NG	NG	Not Applicable	
Middle Bedroom Closet	Carpet coupon	2.51×10^5	2.73×10^5	5.44×10^4	2.4	0.4
	GM coupon	3.18×10^4	1.55×10^4	1.87×10^4	2.9	0.2
	BI disc	G	NG	NG	Not Applicable	
Middle Bedroom Closet (b)	Carpet coupon	4.91×10^5	3.31×10^6	2.45×10^6	1.4	0.4
	GM coupon	3.35×10^4	4.24×10^3	4.08×10^3	3.3	0.5
Kitchen Floor	Carpet coupon	1.09×10^6	1.11×10^6	4.73×10^5	1.6	0.2
	GM coupon	6.14×10^4	3.35×10^5	2.39×10^3	2.6	1.1
	BI disc	G	NG	NG	Not Applicable	
Living Room Floor	Carpet coupon	4.45×10^5	6.99×10^4	1.12×10^5	2.4	0.4
	GM coupon	9.93×10^4	3.40×10^2	ND	4.7	2.6
	BI disc	NG	NG	G	Not Applicable	
Entry Closet	Carpet coupon	3	ND	ND	2.0	0.5
	GM coupon	7.00×10^1	ND	2.84×10^4	3.3	0.4
	BI disc	NG	NG	NG	Not Applicable	
Duct	Carpet coupon	3.58×10^5	1.08×10^5	5.13×10^4	2.5	0.4
	GM coupon	ND	2.50×10^1	ND	6.8	0.9
Crawl Space Under Corner Bedroom	Carpet coupon	3	ND	ND	7.5	0.3
	GM coupon	7.00×10^1	ND	2.84×10^4	5.1	2.3
	BI disc	NG	G	NG	Not Applicable	
Crawl Space Under Kitchen	Carpet coupon	ND	ND	1.11×10^4	6.3	2.4
	GM coupon	ND	ND	ND	7.3	0.0
	BI disc	NG	G	G	Not Applicable	

Location	Sample Type	Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)			LR (CFUs)	
		Rep 1	Rep 2	Rep 3	Average	SD
Crawl Space Under Den	Carpet coupon	2	5.56×10^5	8.50×10^2	4.6	2.8
	GM coupon	ND	ND	ND	7.3	0.0
	BI disc	G	G	G	Not Applicable	
Attic Over Master Bathroom	Carpet coupon	8.30×10^2	ND	ND	6.7	1.8
	GM coupon	1	ND	ND	7.2	0.2
	BI disc	NG	NG	NG	Not Applicable	
Center of Attic	Carpet coupon	ND	4.18×10^4	9.26×10^4	4.4	2.8
	GM coupon	ND	ND	ND	7.3	0.0
	BI disc	NG	NG	NG	Not Applicable	
Attic Over Den	Carpet coupon	1.29×10^7	1.42×10^4	1.28×10^7	1.4	1.7
	GM coupon	4.83×10^4	4.21×10^5	1.18×10^5	2.1	0.5
	BI disc	NG	G	NG	Not Applicable	
Positive 1	Carpet coupon	4.81×10^7	3.72×10^7	4.25×10^7		
	GM coupon	1.63×10^7	1.46×10^7	1.18×10^7		
	BI disc	G	G	G		
Positive 2	Carpet coupon	2.43×10^7	Not available	3.55×10^7		
	GM coupon	1.55×10^7	1.62×10^7	3.35×10^7		

ND = Non-detect	NG = No growth (BI)	G = Growth (BI)

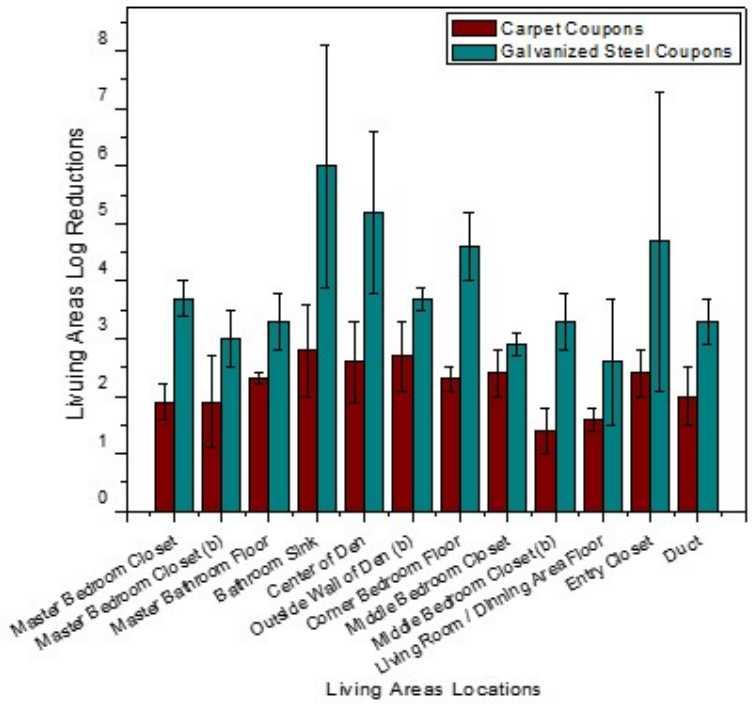

Figure 5-35. Test 4 LR Values in Spore Counts for Coupons in Living Areas

In the living areas there were higher LR values for the GM coupons than the co-located carpet coupons. For the BI discs, 23 out of the 27 samples showed no growth. One possible suggestion for this result may be that the reduction of RH did not hinder the inactivation of spores on GM but did for spores on carpet.

In the crawl space, where the fumigation conditions were maintained the same as during the previous tests, 11 of 18 coupons exhibited full decontamination. In the attic, 8 of 18 coupons exhibited full decontamination (greater than 6 LR). For the hard-to-reach places, 21 of 24 BI discs exhibited growth, demonstrating that Test 4 was not very efficacious. Table 5-12 summarizes G/NG results for the BI discs in hard-to-reach places.

Table 5-12. Test 4 Post-Decontamination Recoveries on Hard-to-Reach BI discs

Location	Qualitative Analysis of BI disc (CFUs)		
	Rep 1	Rep 2	Rep 3
Inside Closed Book	G	G	G
Master Bedroom Pants Pocket	NG	G	G
Inside Coat Pocket in Entry Closet	G	G	G
Dining Room Behind Light Switch Plate	G	G	G
Master Bedroom Window East Wall	G	NG	NG
Front Door Jamb	G	G	G
Den Deck Door Jam	G	G	G
Den Behind Switch Plate on outside wall	G	G	G

NG = No growth (BI)	G = Growth (BI)

5.6 Test 5

Figure 5-36 shows the layout for Test 5 with the equipment placement the same as for Test 4. Test 5 used two humidifiers, each filled and operated in a staggered sequence as follows:

- Day 0:
  - Two humidifiers (Humidifiers 1 and 2) both filled with 3.4 gallons of 3.0% HP aqueous solution placed in hallway under the air return
  - Humidifier 1 started, and Humidifier 2 set to start 24 hours later
- After Day 1:
  - Humidifier 2 automatically started after 1-day delay
- After Day 3:
  - Humidifier 1 refilled with 3.2 gallons of 3.0% HP aqueous solution and started
  - Humidifier 2 refilled with 2 gallons of 3.0% HP aqueous solution and set to start 24 hours later Collection of “b” sample sets

Furthermore, BI discs were placed in the following hard-to-reach areas to test the efficacy of the fumigation process:

- Between couch cushions
- Under one piece of carpet (jute-backed)
- Under one piece of paper
- Under five pieces of paper
- Under 10 pieces of paper
- Under door mat, entryway
- Inside a pants pocket, Master bedroom closet
- Inside a coat pocket, entry closet
- Inside a book
- Behind light switch plate, dining room
- Behind light switch plate, exterior porch
- Inside a pillowcase, middle bedroom
- Between bed sheets, middle bedroom
- Under comforter, middle bedroom
- Inside light fixture. middle bedroom
- Hall linen closet with closet door closed
- Closed drawer, kitchen
- Open drawer, kitchen
- Window, master bedroom
- Door jambs (front and back)

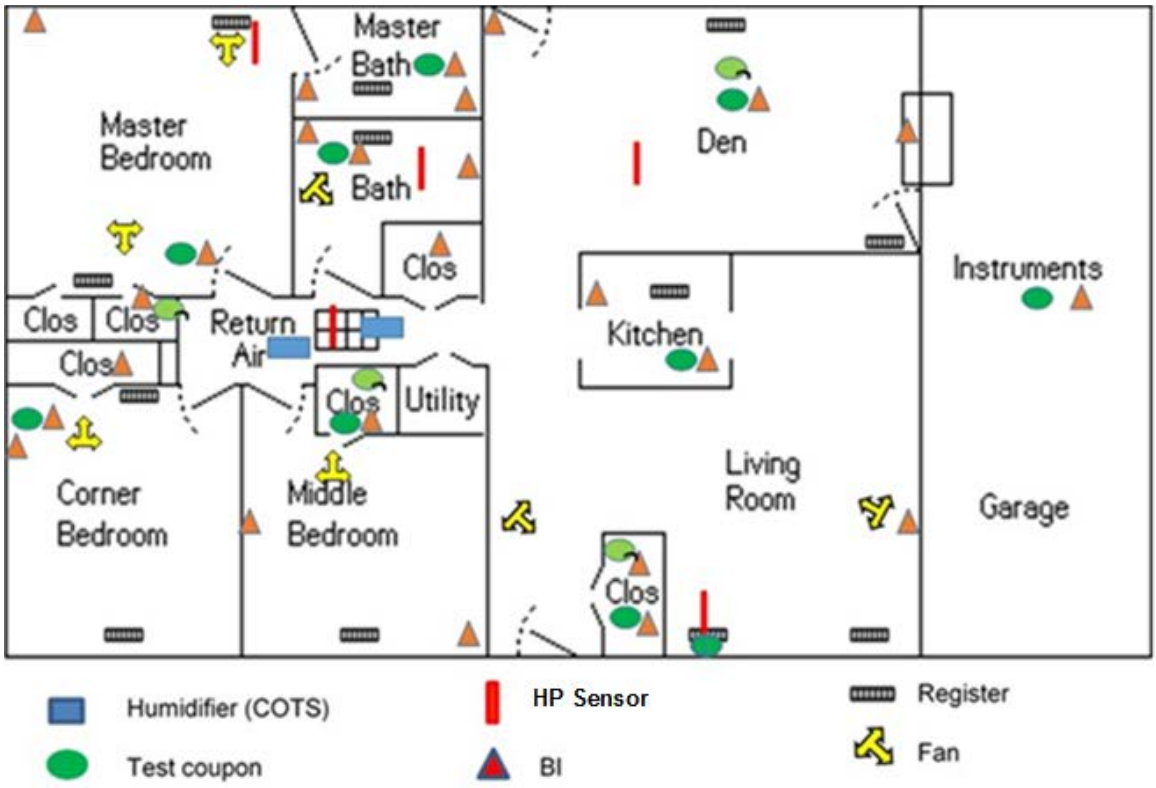

Figure 5-36. CTH Equipment and Sampling Locations for Test 5

The average results for the living areas, attic, and crawl space fumigation conditions including HPV concentration (ppm), overall calculated HPV exposure (concentration-time [CT] in ppm-hours), and RH (%) are tabulated in Table 5-13. The temporal HPV concentration, RH, and temperature are discussed in Sections 5.6.1.1 through 5.6.1.2 for living areas, and the decontamination efficacy of the test coupons inoculated with *Bg* spores, and growth/no growth assessments of BI discs co-located with the coupons are discussed in Section 5.6.2.

Table 5-13. Test 5 Fumigation Conditions

Test 5 - Fumigation and Relative Humidity Conditions by Location				
Location	**HPV Max (ppm)**	**CT (ppm-hour)**	**RH Average (%)**	**RH Max (%)**
Master Bedroom	16	1217	49	57
Master Bathroom	Not Measured		51	60
Hallway Bathroom	9	767	52	61
Center of Den	29	1239	52	63
Corner Bedroom	Not Measured		49	57
Middle Bedroom	Not Measured		45	60
Kitchen	Not Measured		52	63
Living Room	36	2274	50	61
Crawl Space Center	44	3304	70	83
Crawl Space North East	4	219	76	89
Crawl Space South West			74	86
Center of Attic	167	7537	47	68
Attic North East	29	881	48	71
Attic South West			51	77

5.6.1 Living Area Fumigation Conditions

5.6.1.1 Temporal HPV Concentration

The HPV concentration was monitored using HP sensors at the same five locations used during Test 4: master bedroom, bathroom, hallway air return, living room, and den (see Figure 5-37). The results show that staggering the operation of the humidifiers over 4 days increased the exposure time while reducing the maximum HP concentration reached during testing as compared to Tests 1 and 3.

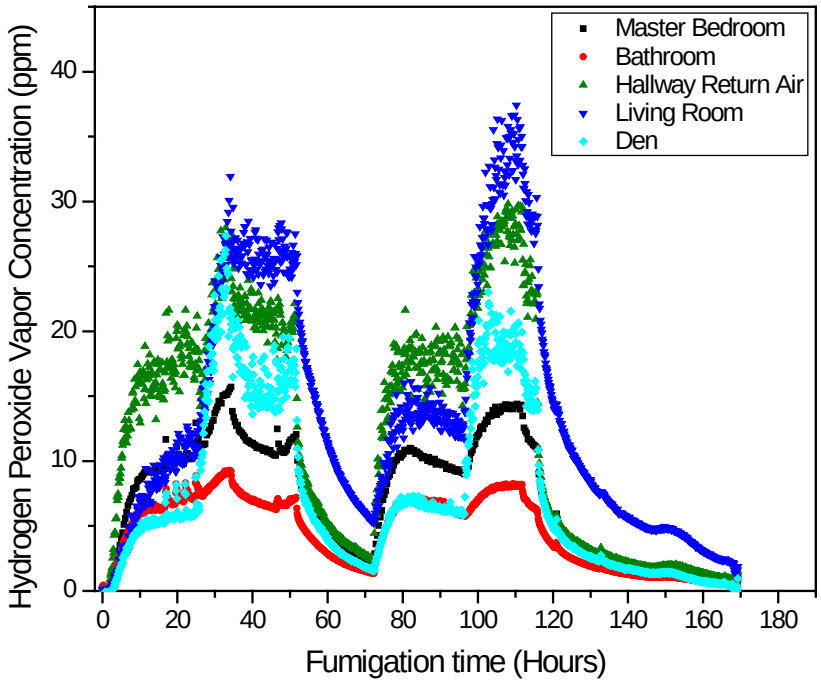

Figure 5-37. Test 5 Temporal HPV Concentration in Living Areas

5.6.1.2 Temporal RH and Temperature

The results shown in Figure 5-38 illustrate fumigation RH conditions throughout the decontamination testing, using the staggered start and refilling humidifier operation, at eight locations: master bedroom, master bathroom, corner bedroom, middle bedroom, hallway bathroom, kitchen, den, and living room.

The staggered start and refilling humidifier operation was able to maintain indoor RH above 50% throughout the entire fumigation period, exceeding the Test 4 overall humidification process. The large swings in the outdoor environmental conditions, as for all other tests, had minimal effects on the indoor environmental conditions. Temperature, as expected, remained steady near 70 ˚F as maintained by the CTH HVAC system (data not shown).

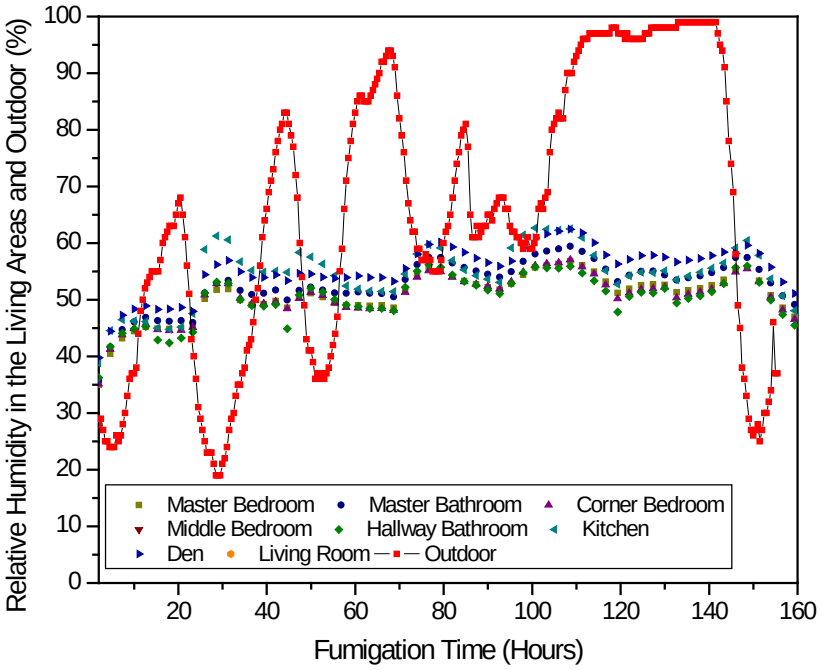

Figure 5-38. Test 5 Temporal RH in Living Areas

5.6.2 Decontamination Efficacy

The increase in exposure time resulted in LR values greater than 6 for 46 of 54 test coupons placed in the living areas. Results for carpet coupons in the master bedroom closet did not show full decontamination for either sample set “b” recovered and extracted after the third day of the test or for two of the three samples recovered and extracted on the seventh day of the sampling event. For 26 of 27 BI discs placed in the living areas, no growth was observed. LR values greater than 6 were observed for test coupons located in the crawl space, but six of nine BI discs exhibited growth. In the attic, only 11 of 18 coupons exhibited greater than a 6 LR. Table 5-14 summarizes spore recovery results for the test coupons and G/NG results for the BI discs.

Table 5-14. Test 5: Post-Decontamination Recoveries on Test Coupons and BI discs

Location	Sample Type	Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)			LR (CFUs)	
		Rep 1	Rep 2	Rep 3	Average	SD
Master Bedroom Closet	Carpet coupon	1.06×10^3	ND	2.58×10^3	5.42	1.96
	GM coupon	ND	ND	ND	7.25	0.00
	BI disc	NG	NG	NG	Not Applicable	
Master Bedroom Closet (b)	Carpet coupon	3.79×10^4	1.01×10^4	1.18×10^4	3.30	0.31
	GM coupon	9.80×10^2	ND	ND	6.16	1.80
Master Bathroom Floor	Carpet coupon	5.20×10^3	ND	ND	6.35	2.21
	GM coupon	ND	ND	ND	7.23	0.04
	BI disc	NG	NG	NG	Not Applicable	

Location	Sample Type	Test Coupon Recovery (CFUs) and BI disc Recovery (G/NG)			LR (CFUs)	
		Rep 1	Rep 2	Rep 3	Average	SD
Bathroom Sink	Carpet coupon	ND	4.60×10^2	ND	6.70	1.60
	GM coupon	ND	ND	ND	7.22	0.06
	BI disc	NG	NG	NG	Not Applicable	
Center of Den	Carpet coupon	6	ND	ND	7.34	0.49
	GM coupon	ND	ND	ND	7.23	0.02
	BI disc	NG	NG	NG	Not Applicable	
Outside Wall of Den (b)	Carpet coupon	ND	2	ND	7.54	0.23
	GM coupon	ND	ND	ND	7.26	0.03
Corner Bedroom Floor	Carpet coupon	ND	ND	6.50×10^2	6.66	1.70
	GM coupon	ND	ND	ND	7.24	0.04
	BI disc	NG	NG	NG	Not Applicable	
Middle Bedroom Closet	Carpet coupon	ND	1	1.12×10^3	6.48	1.75
	GM coupon	2.00×10^1	ND	ND	6.75	0.85
	BI disc	NG	NG	NG	Not Applicable	
Middle Bedroom Closet (b)	Carpet coupon	1.16×10^4	4.31×10^4	ND	4.64	2.58
	GM coupon	ND	ND	ND	7.25	0.03
Kitchen Floor	Carpet coupon	ND	7.13×10^3	ND	6.30	2.29
	GM coupon	ND	ND	ND	7.23	0.03
	BI disc	NG	NG	NG	Not Applicable	
Living Room Floor	Carpet coupon	ND	ND	6	7.34	0.51
	GM coupon	ND	ND	ND	7.27	0.02
	BI disc	G	NG	NG	Not Applicable	
Entry Closet	Carpet coupon	9.30×10^2	ND	ND	5.96	1.56
	GM coupon	ND	ND	ND	7.26	0.02
	BI disc	NG	NG	NG	Not Applicable	
Entry Closet (b)	Carpet coupon	1.29×10^2	1	9.34×10^3	5.43	1.90
	GM coupon	ND	6.90×10^2	ND	6.23	1.73
Crawl Space Under Corner Bedroom	Carpet coupon	ND	ND	ND	7.65	0.01
	GM coupon	2.00×10^1	ND	ND	6.75	0.85
	BI disc	NG	G	G	Not Applicable	
Crawl Space Under Kitchen	Carpet coupon	ND	ND	ND	7.67	0.01
	GM coupon	ND	ND	ND	7.24	0.03
	BI disc	NG	G	G	Not Applicable	
Crawl Space Under Den	Carpet coupon	3.33×10^1	ND	ND	7.10	0.96
	GM coupon	ND	2.00×10^1	ND	6.76	0.86
	BI disc	NG	G	G	Not Applicable	
Attic Over Master Bathroom	Carpet coupon	1.35×10^3	ND	ND	6.55	1.88
	GM coupon	ND	ND	ND	7.24	0.04
	BI disc	G	NG	NG	Not Applicable	
Center of Attic	Carpet coupon	ND	ND	3	7.45	0.34
	GM coupon	1	ND	1	7.06	0.17
	BI disc	NG	NG	NG	Not Applicable	
Attic Over Den	Carpet coupon	8.51×10^5	4.82×10^5	7.98×10^4	2.01	0.54
	GM coupon	4.46×10^4	1.81×10^4	5.11×10^4	2.53	0.24
	BI disc	G	NG	NG	Not Applicable	
Positive 1	Carpet coupon	3.33×10^7	3.83×10^7	3.97×10^7		
	GM coupon	6.95×10^6	8.58×10^6	1.44×10^7		
	BI disc	G	G	G		
Positive 2	Carpet coupon	3.17×10^7	2.68×10^7	2.59×10^7		
	GM coupon	1.68×10^7	1.45×10^7	9.58×10^6		

ND = Non-detects	NG = No growth (BI)	G = Growth (BI)

For the hard-to-reach places, once again efficacy was reduced when BI discs were placed under cushions, under carpet, inside clothing, or behind walls. Table 5-15 summarizes qualitative results for the BI discs in hard-to-reach places.

Table 5-15. Test 5 Post-Decontamination Results for Hard-to-Reach BI discs

Test Location	BI disc Recovery (G/NG)		
	Rep 1	Rep 2	Rep 3
Between couch cushions	G	G	G
Under carpet (jute-backed)	G	G	G
Under one piece of paper	NG	NG	NG
Under five pieces of paper	NG	NG	NG
Under 10 pieces of paper	G	G	G
Under entry door mat	G	G	G
Master bedroom pants pocket	NG	NG	NG
Inside coat pocket in entry closet	G	G	G
Inside book	G	G	G
Switch plates (interior)	G	G	G
Switch plates (exterior)	G	G	G
Inside pillowcase in middle bedroom	NG	NG	NG
Between sheets in middle bedroom	NG	NG	NG
Under comforter in middle bedroom	NG	NG	NG
Inside light fixture in middle bedroom	NG	NG	NG
Closed hall linen closet	NG	NG	NG
Closed drawer in kitchen	G	G	G
Open drawer in kitchen	NG	NG	NG
Window in master bedroom	NG	NG	NG
Door jambs (front)	G	G	G
Door jambs (back)	G	G	G

6 Quality Assurance and Quality Control

This section discusses the quality assurance (QA) and quality control (QC) procedures for this study, including project documentation, integrity of samples and supplies, instrument calibration, critical measurements, QC and NHSRC BioLab checks, and QA assessments and response actions.

6.1 Project Documentation

This project was performed under the approved Category III Quality Assurance Project Plan (QAPP) entitled, “Cary Test House Low-Concentration Hydrogen Peroxide Vapor Decontamination Tests,” prepared by Jacobs and approved by EPA on September 23, 2015. All test activities are documented in laboratory notebooks, digital video footage, and digital photographs. The documentation includes a record for each sampling procedure and any deviations from the QAPP. All tests were conducted in-house in accordance with developed Decontamination Technologies Research Laboratory (DTRL) and NHSRC BioLab procedures to ensure repeatability and adherence to the data quality validation criteria for this project.

6.2 Integrity of Samples and Supplies

Samples were carefully maintained and preserved to ensure their integrity. Samples were stored away from standards and other samples to prevent cross contamination. Supplies and consumables were acquired from reputable sources and were National Institute of Standards and Technology (NIST)-traceable whenever possible. Supplies and consumables were examined for evidence of tampering or damage upon receipt and before use as appropriate. Supplies and consumables showing evidence of tampering or damage were discarded. All examinations were documented, and supplies were appropriately labeled.

6.3 Instrument Calibration

For this project, established and approved operating procedures were used to maintain and calibrate all laboratory equipment. All laboratory measuring devices were certified as having been recently calibrated or were calibrated by the on-site EPA Metrology Laboratory at the time of use. Table 6-1 summarizes the instrument calibration frequency. Any deficiencies were noted and the instrument replaced or repaired as needed to meet calibration tolerances.

Table 6-1. Instrument Calibration Frequencies and Expected Tolerances

Equipment	Calibration/Certification	Expected Tolerance
Thermometer	Compare to independent NIST thermometer (a thermometer recertified annually by either NIST or an International Organization for Standardization [ISO]-17025 facility) value once per quarter	± 1 °F
RH meter	Three-point calibration using NIST-traceable salt cells performed before and after each test	± 5%
HP sensor	Annual calibration provided by the manufacturer	± 1% full scale
Clock	Compare to office U.S. time at time.gov every 30 days	± 1 minute / 30 days
Pipettes and Burets	Check calibration gravimetrically once a quarter	± 3%

6.4 Critical Measurements

The following measurements were deemed critical in achieving the project objectives:

- Volume or mass of HP
- Concentration of liquid HP decontaminant
- Run time
- HPV concentration
- RH
- Temperature, including incubation temperature
- Plated volume
- CFU counts

Table 6-2 lists the data quality indicators (DQI) for the critical measurements. These DQIs were used to determine if the collected data met the project QA objectives.

Table 6-2. DQIs and Acceptance Criteria for Critical Measurements

Measurement Parameter	Analysis Method	Acceptance Criteria	Actual	Pass or Fail Test
Mass of decontaminant (HP)	Scale	Accuracy: 0.1 g	0.05 g	Pass
Volume	Serological pipette tips	0.1 mL	± 10% of target value	Pass
Concentration of liquid HP decontaminant	Preparation of 3% HP solution in batches using titration (Envirotech 2013)	Precision: ± 10% of target value	Test 1: 3%; Test 2: 3.8 ± 0.13%; Test 3: 3 ± 0.2%; Test 4: 3 ± 0.13%; Test 5: 3 ± 0.12%;	Pass
Run time	NIST-calibrated stopwatch	± 1 minute per hour	± 2 minutes (2 × ± 1 min)	Pass
HPV Concentration	Pre- and post-calibration by manufacturer	Bias: ± 10%	Bias: ± 7.7%	Pass
RH	Vaisala	Bias: ± 5%		Pass
Temperature	NIST-traceable thermometer (daily)	± 2°C	Not applicable	Pass
Plated volume (liquid)	Pipette	2%	± 1%	Pass
CFU counts per plate	QCount® colony counter	1.82×10^4 < QC plate < 2.3×10^4	Within range of QC plate, Part # 510014, Spiral Biotech, Inc.	Pass

Decisions to accept or reject test results were based on engineering judgment used to assess the likely impact of the failed criterion on the conclusions drawn from the data. The acceptance criteria were set at the most stringent levels that can routinely be achieved. All the DQIs were within the target acceptance criteria set for this project as shown in Table 6-2.

Several QC checks were used for the measurement instruments to ensure that the data collected met the criteria listed in Table 6-2. Sample integrity was evaluated during collection and analysis. Qualified, trained, and experienced personnel utilized validated operating procedures to ensure data collection consistency. When necessary, knowledgeable parties conducted training, and in-house practice runs were used to gain expertise and proficiency before research began. The QC checks performed for this project are detailed in Section 6.5.

In addition to the measurement instrument checks, positive control samples and procedural blanks were included along with the test samples, so that optimal spore recovery and unintentional contamination of test coupons could be assessed. Replicate coupons were included for each set of test conditions to assess the variability of each test procedure.

6.5 QC and NHRSC BioLab Checks

Quantitative standards do not exist for biological agents. Viable spores were counted using an Advanced Instruments QCount® colony counter. Counts greater than 300 or less than 30 CFUs were considered outside of the targeted range. If the CFU count for bacterial growth did not fall within the target range, the sample was re-plated., and then re-counted.

Before each batch of plates was enumerated, a QC plate was analyzed, and the result was verified to be within the range indicated on the back of the QC plate. As the plates were being counted, a visual

inspection of colony counts made by the QCount® colony counter was performed. Obvious count errors made by the software were corrected by adjusting the settings (such as colony size, light, and field of view) and recounting using an edit feature of the QCount® software that allows manual removal of erroneously identified spots or shadows on the plate or by adding colonies that the QCount® software may have missed.

The acceptance criteria for the critical CFU counts were set at the most stringent level that could routinely be achieved. Positive controls were included along with the test samples so that spore recovery from the different surface types could be assessed. Background checks also were included as part of the standard protocol to check for unanticipated contamination. Replicate coupons were included for each set of test conditions to characterize the variability of the test procedures.

Further QC samples were collected and analyzed to check the ability of the NHSRC BioLab to culture the test organism as well as to demonstrate that the test materials used did not contain pre-existing spores. The checks included the following:

- Procedural blank coupons: Material coupons sampled in the same fashion as test coupons but not contaminated with the surrogate organism before sampling
- GM and carpet positive control coupons: coupons inoculated in tandem with the test coupons to demonstrate the highest level of contamination recoverable from a particular inoculation event

Table 6-3 lists the additional QC checks built into the BioLab procedures designed to provide assurances against cross-contamination and other biases in the microbiological samples.

Table 6-3. Additional Quality Checks for Biological Measurements

Sample Type	Frequency	Acceptance Criteria	Information Provided	Corrective Action
Positive control coupon: material coupon sample contaminated with biological agent and sampled using extraction method	Minimum of three per test	1×10^7 for *Bg*, 50% relative standard deviation between coupons in each test set	Used to determine extent of recovery of inoculum on target coupon type	If outside range, discuss in the results section of this report
Procedural blank coupon (without biological agent) that underwent sampling procedure	One per test	Non-detect	Controls for sterility of materials and methods used in the procedure	Analyze extracts from procedural blank without dilution; identify and remove source of contamination if possible
Blank TSA sterility control (plate incubated but not inoculated)	Each plate	No observed growth after incubation	Controls for sterility of plates	All plates incubated before use; contaminated plates discarded before use
Replicate plating of diluted microbiological samples	Each sample	Reportable CFU count of triplicate plates within 100%; reportable CFU counts between 30 and 300 CFUs per plate	Used to determine precision of replicate plating	Re-plate sample
Unexposed field blank sample	One per test	Non-detect	Level of contamination present during sampling	Clean up environment; sterilize sampling materials before use

6.6 QA Assessments and Response Actions

The QA assessment and corrective action procedures of this project were intended to provide rapid detection of data quality problems. No contamination in QC procedural blank samples was observed after the completion of testing. Table 6-4 summarizes the QA/QC assessment of spore recoveries for the various sample types. Project personnel were intimately involved with the data on a daily basis so that any data quality issue became apparent soon after it occurred.

Table 6-4. QA/QC Assessment of Spore Recoveries for Various Sample Types (CFUs/Sample)

Test No.	Sample Material	Positive Controls		Negative Controls
		Average	SD	Average
1	Carpet	2.82×10^7	3.96×10^6	ND
	GM	1.09×10^7	4.62×10^6	ND
2	Carpet	3.04×10^7	2.32×10^7	ND
	GM	8.39×10^6	2.76×10^6	ND
3	Carpet	2.78×10^7	4.41×10^6	ND
	GM	9.21×10^6	2.45×10^6	ND
4	Carpet	3.69×10^7	8.11×10^6	ND
	GM	1.52×10^7	1.87×10^6	ND
5	Carpet	3.26×10^7	5.70×10^6	ND
	GM	1.18×10^7	3.95×10^6	ND

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