August 2004

Environmental Technology Verification Report

CERTEK, INC.
1414RH FORMALDEHYDE GENERATOR/NEUTRALIZER

Prepared by
Battelle

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Environmental Technology Verification Report

ETV Building Decontamination Technology Center

CERTEK, Inc.
1414RH Formaldehyde Generator/Neutralizer

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Notice

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technologies across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of seven environmental technology centers. Information about each of these centers can be found on the Internet at http://www.epa.gov/etv.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. In 2002, EPA established the Building Decontamination Technology Center at Battelle. Battelle plans, coordinates, and conducts verification tests of decontamination technologies and reports the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at http://www.epa.gov/etv/centers/center9.html.

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Contents

Notice ii

Foreword iii

Acknowledgments iv

List of Abbreviations vii

1. Background 1

2. Technology Description 2

3. Test Design and Procedures 4
   - 3.1 Introduction 4
   - 3.2 Test Design 5
   - 3.3 Agents and Surrogates 5
   - 3.4 Test Sequence 6
   - 3.5 Coupon-Scale Testing 6
     - 3.5.1 Preparation of Test Materials 7
     - 3.5.2 Application of Agents to Test Coupons 7
     - 3.5.3 Confirmation of Surface Applications 8
     - 3.5.4 Decontamination 8
       - 3.5.4.1 Verification Testing Apparatus and Parameters 8
       - 3.5.4.2 Formaldehyde Measurement 11
       - 3.5.4.3 Decontamination Efficacy 12
     - 3.5.5 Observation of Surface Damage 13

4. Quality Assurance/Quality Control 14
   - 4.1 Equipment Calibration 14
   - 4.2 Audits 14
     - 4.2.1 Technical Systems Audit 14
     - 4.2.2 Audit of Data Quality 14
   - 4.3 QA/QC Reporting 15
   - 4.4 Data Review 15

5. Statistical Methods 16
   - 5.1 Efficacy Calculations 16
   - 5.2 Statistical Analysis 16

6. Test Results 17
   - 6.1 Efficacy 17
     - 6.1.1 *Bacillus anthracis* Ames Spores 17

6.1.2 *Bacillus subtilis* (ATCC 19659) Spores 21
6.1.3 *Geobacillus stearothermophilus* (ATCC 12980) Spores 24
6.1.4 Statistical Analysis 26
6.2 Damage to Coupons 27
6.3 Other Factors 27
6.3.1 Operation of the 1414RH Unit 27
6.3.2 Operator Bias 28

7. Performance Summary 30

8. References 32

Figures

Figure 2-1. CERTEK, Inc. #1414RH 2
Figure 3-1. Test Materials 4
Figure 3-2. Overview of Plas-Labs Compact Glove Box 9
Figure 3-3. Nebulizers in the Plas-Labs Compact Glove Box 10
Figure 3-4. Formaldehyde Monitor 11
Figure 6-1. Representative Cycle Parameter Data from a Single Experiment 29

Tables

Table 3-1. Test Sequence and Parameters 6
Table 3-2. Material Characteristics 7
Table 4-1. Summary of Data Recording Process 15
Table 6-1. 1414RH Unit Decontamination of *Bacillus anthracis* Ames Spores 18
Table 6-2. Liquid Culture Assessment of *Bacillus anthracis* Ames Spores 19
Table 6-3. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 1 *B. anthracis* Decontamination) 20
Table 6-4. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 2 *B. anthracis* Decontamination) 20
Table 6-5. 1414RH Unit Decontamination of *Bacillus subtilis* Spores 21
Table 6-6. Liquid Culture Assessment of *Bacillus subtilis* Spores 22
Table 6-7. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 1 *B. subtilis* Decontamination) 23
Table 6-8. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 2 *B. subtilis* Decontamination) 23
Table 6-9. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 3 *B. subtilis* Decontamination) 23
Table 6-10. 1414RH Unit Decontamination of *Geobacillus stearothermophilus* Spores 24
Table 6-11. Liquid Culture Assessment of *Geobacillus stearothermophilus* Spores 25

Table 6-12. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 1 *G. stearothermophilus* Decontamination) 26
Table 6-13. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 2 *G. stearothermophilus* Decontamination) 26
Table 6-14. Statistical Analysis of Mean Efficacy (Log Reduction) for Spores 27

List of Abbreviations

ANOVA	analysis of variance
BDT	Building Decontamination Technology
BSC	biological safety cabinet
BWD	bare wood (pine lumber)
CFU	colony-forming unit
cm	centimeter
DL	decorative laminate
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
FMI	Fluid Metering, Inc.
g	gram
GM	galvanized metal ductwork
GS	glass
HEPA	high-efficiency particulate air
IC	industrial-grade carpet
in	inch
MFC	mass flow controller
min	minute
mL	milliliter
PC	painted (latex, semi-gloss) concrete cinder block
ppb	part per billion
ppm	part per million
psig	gauge pressure
PW	painted (latex, flat) wallboard paper
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
SD	standard deviation
TSA	technical systems audit
USAMRIID	United States Army Medical Research Institute of Infectious Diseases

Chapter 1
Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The EPA's National Risk Management Research Laboratory and its verification organization partner, Battelle, operate the Building Decontamination Technology (BDT) Center under ETV. The BDT Center recently evaluated the performance of the CERTEK, Inc., Model# 1414RH formaldehyde gas generator/neutralizer for decontaminating buildings.

Chapter 2
Technology Description

The objective of the ETV BDT Center is to verify the performance characteristics of technologies that can be used to decontaminate indoor surfaces in buildings contaminated with either chemical or biological agents as a result of an intentional attack. This verification report provides results for testing the CERTEK, Inc. 1414RH formaldehyde gas generator/neutralizer. The following is a description of the 1414RH unit, based on information provided by the vendor. The information provided below was not verified in this test.

Figure 2-1. CERTEK, Inc. # 1414RH

The 1414RH unit generates formaldehyde gas for decontaminating a sealed area. Formulas in the operating manual are used to calculate the appropriate amount of water, paraformaldehyde, and neutralizer (ammonium carbonate) based on the volume of the space intended to be decontaminated. The 1414RH unit generates the appropriate relative humidity (50 to 90%), then generates formaldehyde gas for an operator-selected contact time, and finally it generates neutralizer into the decontaminated space. The hexamethylenetetramine formed by the reaction of formaldehyde with the neutralizer is a white powder with a slight “fishy” odor.

The 1414RH unit has a capacity of 240 grams (g) of paraformaldehyde per canister. Based upon the recommendation of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), 0.3 g of paraformaldehyde per cubic foot should be used to decontaminate *Bacillus anthracis*. Therefore, 240 g of paraformaldehyde is sufficient to treat an enclosure of approximately 800 cubic feet (23 cubic meters). The operation of the 1414RH unit can be modified to utilize a second canister filled with 240 g of paraformaldehyde; therefore, 480 g of paraformaldehyde can treat an enclosure of approximately 1,600 cubic feet (45 cubic meters). The 1414RH unit weighs 55 pounds

(25 kilograms), and is 12 inches (in) [30 centimeters (cm)] wide by 20 in (51 cm) in depth by 12 in (30 cm) in height.

The 1414RH unit was attached to a Plas-Labs Compact Glove Box (Model No. 830-ABC) modified for this verification test (see Section 3.5.4.1). The connections between the 1414RH unit and the glove box consisted of flexible supply and delivery gassing hoses connected to high-efficiency particulate air (HEPA) filters. A formaldehyde monitor also was connected to the glove box to measure the concentration of formaldehyde during each run of this verification test. A hygrometer was added inside of the glove box to measure relative humidity.

Chapter 3
Test Design and Procedures

3.1 Introduction

This verification test was conducted according to procedures specified in the *Test/QA Plan for Verification of Formaldehyde Vapor Technologies for Decontaminating Indoor Surfaces Contaminated with Biological or Chemical Agents.*[1] The biological and chemical agents that pose a threat to buildings include toxic industrial chemicals, chemical warfare agents, and biological warfare agents (including biotoxins). The biological agent selected for this verification test was *Bacillus anthracis* (Ames strain). In addition, two biological surrogates were used: *B. subtilis* (ATCC 19659) and *Geobacillus stearothermophilus* (ATCC 12980). Seven materials representing indoor surfaces commonly found in buildings were used for the verification testing. The indoor surfaces tested (Figure 3-1) include

- Industrial-grade carpet (IC)
- Bare wood (pine lumber) (BWD)
- Glass (GS)
- Decorative laminate (DL)
- Galvanized metal ductwork (GM)
- Painted (latex, flat) wallboard paper (PW)
- Painted (latex, semi-gloss) concrete cinder block (PC).

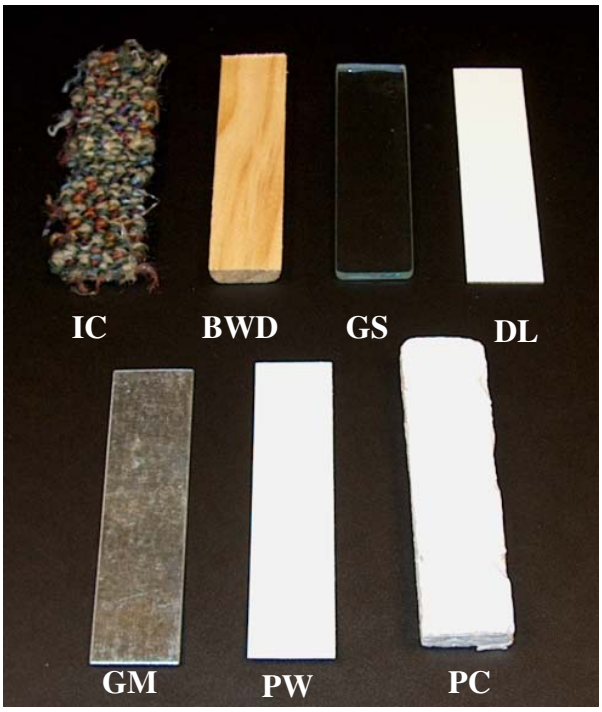

Figure 3-1. Test Materials.

The objective of the verification testing was to evaluate the efficacy of the 1414RH unit to decontaminate a biological agent or surrogate. Efficacy was tested by applying a biological agent and surrogates to the surfaces of test coupons and, after using the 1414RH unit, comparing the number of viable spores on decontaminated and control (non-decontaminated) samples. Visual inspection of the physical integrity of the test materials was performed, and observations were recorded before and after using the 1414RH unit in an effort to detect any degradation or chemical destruction of the material itself.

3.2 Test Design

Coupons were cut from larger pieces of the representative materials for each of the seven indoor surfaces (Section 3.1). These coupons measured 3/4 x 3 in (1.9 x 7.5 cm) and varied in thickness from about 1/32 in (0.079 cm) to 3/8 in (0.95 cm), depending upon the material. In triplicate, the coupons were placed into a biological agent safety hood, and aliquots of an aqueous suspension of the biological agent were added to the surface of each coupon. Based upon the concentration of the spores in the aqueous suspension, the number of spores added to each coupon was calculated. The coupons were allowed to dry overnight. After drying, the inoculated coupons intended for decontamination were transferred into a custom-modified glove box and placed horizontally on a wire rack. Both blank (uncontaminated; N=2) and control (inoculated with spores, but not decontaminated; N=3) coupons were prepared, together with the inoculated coupons that were to be decontaminated (N=3).

Efficacy of the 1414RH unit was determined by comparing the number of viable spores on the control coupons (not decontaminated) to the number present on the decontaminated coupons, expressed as a log reduction. Following extraction of spores from the test, control, and blank coupons, efficacy was further evaluated for each biological agent or surrogate by transferring each coupon into liquid growth medium and assessing bacterial growth after 1 and 7 days.

Physical degradation of the indoor materials used as test surfaces was evaluated informally in conjunction with the efficacy testing procedure. After decontaminating the test coupons, the appearance of the decontaminated coupons was observed; and any obvious changes in the color, reflectivity, and apparent roughness of the coupon surfaces were noted.

3.3 Agents and Surrogates

The following biological agent was used for verification testing:

- *Bacillus anthracis* spores (Ames strain).

To provide correlations with the biological agent results, two biological surrogates also were used:

- *Bacillus subtilis* spores (ATCC 19659)
- *Geobacillus stearothermophilus* spores (ATCC 12980).

Biological indicators and spore strips that were used to evaluate decontamination efficacy included:

- Biological indicators (Apex Laboratories, Apex, North Carolina), approximately 1 x 10^6 spores each: *Bacillus subtilis* (ATCC 19659) and *Geobacillus stearothermophilus* (ATCC 12980) spores on steel disks and sealed Tyvek pouches

- Spore strips (Raven Biological Laboratories, Omaha, Nebraska): with *Bacillus atrophaeus* (ATCC 9372) spores, approximately 1 x 10^6 spores per strip on a filter paper matrix in sealed glassine envelopes.

3.4 Test Sequence

In Table 3-1, a summary of the verification testing of the 1414RH unit is presented. Verification testing was performed during a 7-week period that commenced in November 2003 and concluded in January 2004.

Table 3-1. Test Sequence and Parameters

Test Procedure	Parameters Evaluated	Data Produced
Biological Efficacy Test	Enumerations	Log reduction (Efficacy)
	B. anthracis	
	B. subtilis	
	G. stearothermophilus	
	Liquid culture assessment of coupons	Positive/negative bacterial growth (1 and 7 days)
	B. anthracis	
	B. subtilis	
	G. stearothermophilus	
	Biological indicators/spore strips	Positive/negative bacterial growth (1 and 7 days)
	B. subtilis	
	G. stearothermophilus	
	B. atrophaeus	
Coupon Damage	Damage to test coupons	Visual observation of every test coupon in all biological efficacy tests before and after decontamination

3.5 Coupon-Scale Testing

Coupon-scale testing was used to evaluate the decontamination efficacy of the 1414RH unit by extracting and measuring the viable biological spores on test coupons.

3.5.1 Preparation of Test Materials

Coupons used for biological agent decontamination were cut to about 3/4 x 3 in (1.9 x 7.5 cm) and prepared as shown in Table 3-2 by Battelle staff. Test coupons were visually

inspected, and the condition of each coupon was recorded. The length, width, and thickness of the test coupons were measured and recorded. Chain-of-custody forms were used to ensure that the test coupons were traceable throughout all phases of testing.

Table 3-2. Material Characteristics

Material	Lot, Batch, or ASTM No., or Observation	Manufacturer/ Supplier Name	Approximate Coupon Size, L x W (inch)	Material Preparation
Decorative Laminate	Laminate/ Formica/ White Matte Finish	Solid Surface Design	3 x 3/4	Wiped with 70% isopropanol
Galvanized Metal Ductwork	Industry HVAC standard 24 Gauge Galvanized Steel	Accurate Fabrication	3 x 3/4	Cleaned with acetone; wiped with 70% isopropanol
Glass	C1036	Brooks Brothers	3 x 3/4	Cleaned with acetone; wiped with 70% isopropanol
Industrial-grade Carpet	ShawTek, EcoTek 6	Shaw Industries, Inc.	3 x 3/4	Wiped with 70% isopropanol
Concrete, Cinder Block	ASTM C90	Wellnitz	3 x 3/4	Brush and roller painted all sides. One coat Martin Senour latex primer (#71-1185) and one coat Porter Paints latex semi-gloss finish (#919); wiped with 70% isopropanol
Wallboard Paper	05-16-03; Set-E-493; Roll-3	United States Gypsum Company	3 x 3/4	Roller painted on one side using Martin Senour Paints. One primer (#71-1185) and two finish (flat, #70-1001) coats; wiped with 70% isopropanol
Wood	Screen Molding (Pine Wood)	Kingswood Lumber	3 x 3/4	Wiped with 70% isopropanol

3.5.2 Application of Agents to Test Coupons

Biological agent test coupons were laid flat in a Biological Safety Cabinet (BSC) Class III and contaminated at challenge levels of approximately 1 x 10^8 spores per coupon. Working stock suspensions of the spores at the required concentration were transferred to the coupon using a micropipette by placing the suspension over the surface as small droplets. After contamination with biological agent or surrogate suspension, the test coupons were allowed to dry overnight, undisturbed. The next day, the inoculated test materials intended for decontamination (and one blank) were transferred to the glove box that was attached to the 1414RH unit (see Section 3.5.4.1). The control inoculated test materials (not intended for decontamination) and one blank were left undisturbed in a BSC Class II.

3.5.3 Confirmation of Surface Applications

To confirm the application density of the biological agent and surrogates, the *B. anthracis* and surrogate spore suspensions used to contaminate the coupons were serially diluted and

plated each day of use and enumerated the following day. The plating and enumerating were carried out as described in Section 3.5.4.3.

3.5.4 Decontamination

3.5.4.1 Verification Testing Apparatus and Parameters

A Plas-Labs Compact Glove Box (Model 830-ABC) was utilized as the test chamber (Figure 3-2). The parameters used for this test, as specified by CERTEK, Inc., were according to the *CERTEK Model 1414RH Formaldehyde Generator/Neutralizer Operating Protocol.*[(2)] Specifically, the temperature should be between 60 and 90ºF (16-32ºC) and the relative humidity must be held between 50 and 90%. The concentration of paraformaldehyde and contact time (0.3 g of paraformaldehyde per cubic foot treated volume with a 10-hour contact time) were recommended by the vendor and based upon the recommendations of the USAMRIID (as stated in the *CERTEK Model 1414RH Formaldehyde Generator/Neutralizer Operating Protocol*).

For this verification test, it was difficult to generate the required relative humidity using the capability of the RH1414 unit at the operating level as stated above. To solve this problem, Battelle staff configured a series of six nebulizers (Figure 3-3) inside the glove box to generate water vapor without using the capability of the 1414RH unit. These nebulizers were joined to a HEPA filter that was connected to an air pump. Air was pumped through the nebulizers at 5 to 7 psig (gauge pressure), and a relative humidity of 75% was achieved within 5 minutes. The 1414RH unit has a *Humidify/Bypass* switch, enabling initiation of the *Formaldehyde Insert* mode once 75% relative humidity was achieved.

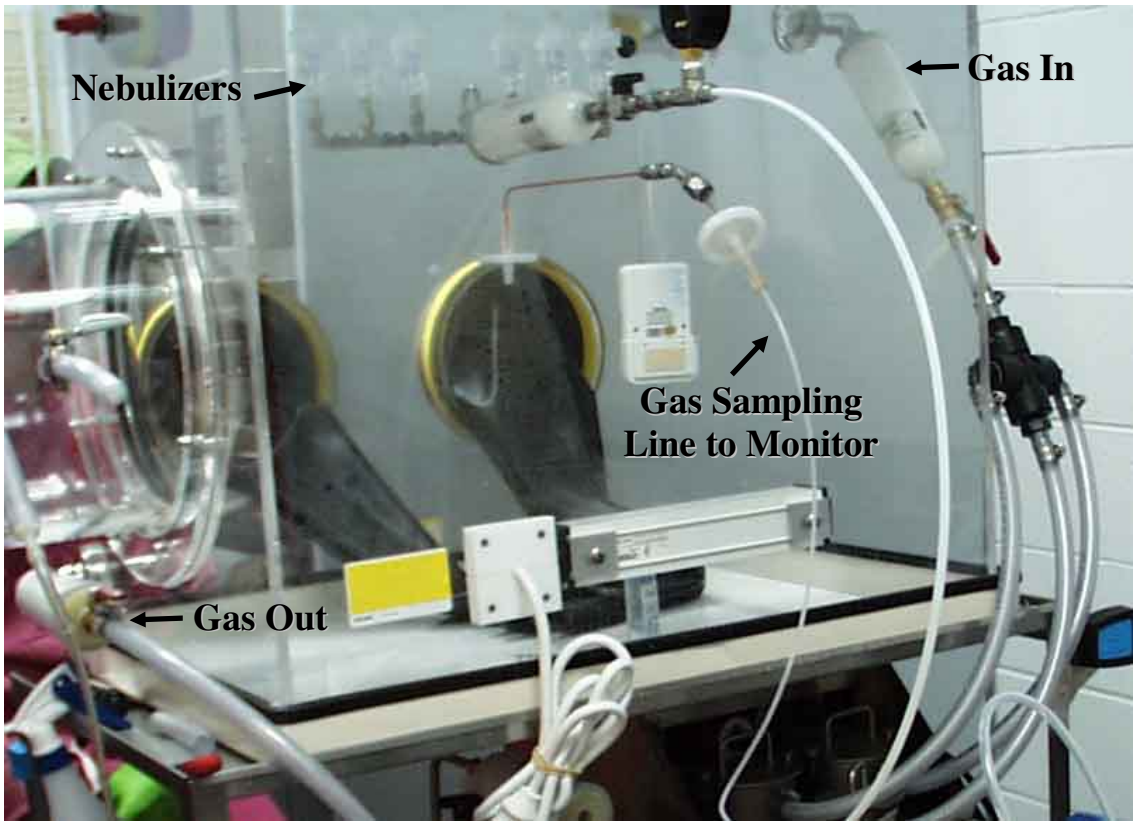

Figure 3-2. Overview of Plas-Labs Compact Glove Box

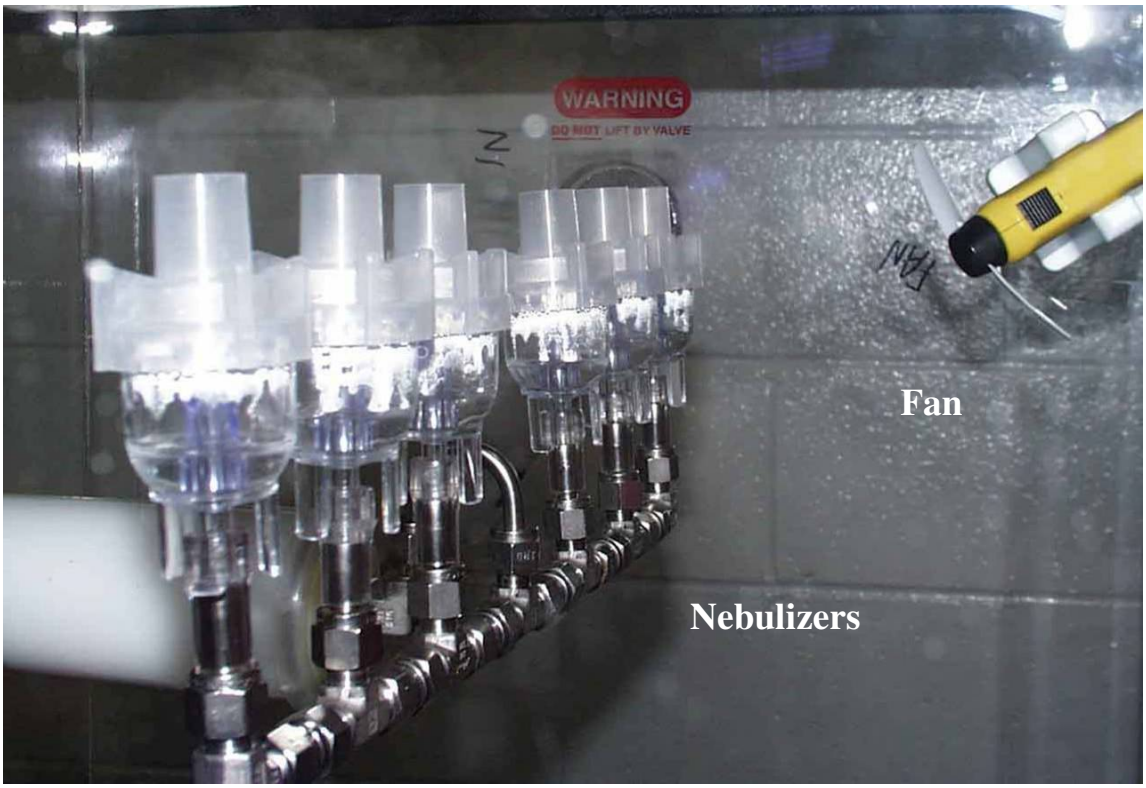

Figure 3-3. Nebulizers in the Plas-Labs Compact Glove Box

Figure 3-4. Formaldehyde Monitor

3.5.4.2 Formaldehyde Measurement

A previously developed monitor[3] was used to measure the formaldehyde concentration within the Plas-Labs Compact Glove Box during each run. This monitor (Figure 3-4) was developed to measure formaldehyde concentrations within a range of approximately 1 part per billion (ppb) to 1 part per million (ppm). For this verification test, the concentration of formaldehyde within the glove box is much higher than 1 ppm (theoretical calculation of approximately 8,600 ppm); therefore, this monitor had to be modified to dilute the gas sample from the glove box approximately 1:10,000. This dilution system was designed as two identical 1:100 systems in series, where each subsystem was made up of a Sierra Instruments mass flow controller (MFC) and a Fluid Metering, Inc. (FMI), valveless rotating and reciprocating piston metering pump. In the 1:100 dilution subsystems, the FMI pumps are set to exactly 10.0 milliliter per minute (mL/min) flow rate. The first FMI subsystem pulled 10 mL/min from the glove box, which was mixed with 990 mL/min air gas stream from a gas cylinder controlled by the MFC. From the exhaust stream of the first FMI, the second FMI subsystem pulled 10 mL/min, which was also mixed with 990 mL/min air gas stream from the same gas cylinder controlled by the second MFC. The sample from

the glove box was then diluted 1:10,000. All gas flows were calibrated against a Buck bubble meter.

For approximately 15 min prior to operating the 1414RH unit, the glove box was monitored for background formaldehyde concentration (ppm). Once a background baseline had been established, the 1414RH unit was operated according to the vendor's instructions. The formaldehyde concentration in the glove box was monitored in real-time throughout the complete operational cycle of the technology, and the data were recorded on a strip chart. Using a formaldehyde standard and the known dilution factor, the data from the strip chart were calculated and expressed as ppm formaldehyde.

3.5.4.3 Decontamination Efficacy

Biological agent or surrogate decontamination efficacy was quantified by measuring the viable spores on both exposed (test) and unexposed (control) coupons. Each coupon was placed in a 50 mL test tube containing 10 mL of sterile phosphate-buffered saline to which 0.1% Triton X-100 had been added. The purpose of the Triton X-100 was to minimize clumping of spores. For spore extraction, the tubes were agitated on an orbital shaker for 15 minutes at room temperature. Each tube was then heat-shocked at 60 to 65ºC for one hour to kill vegetative bacteria. Following the heat-shock, 1.0 mL of each extract was removed, and a series of dilutions through 10^{-7} were prepared in sterile water.

Spore viability was determined by dilution plating, using both the undiluted extracts and the successive dilutions of each extract. One hundred microliters of the undiluted extract and of each serial dilution were plated onto tryptic soy agar plates in triplicate, allowed to dry, and incubated overnight at 35 to 37ºC for *B. anthracis* and *B. subtilis* and at 55 to 60ºC for *G. stearothermophilus*. Plates were enumerated the next day, and the colony-forming units (CFU)/mL were determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Data were expressed as a mean ± standard deviation (SD) of the number of CFUs observed. To calculate the efficacy of the decontamination treatment, the number of spores remaining on the decontaminated test coupons was compared to the number of spores on the control coupons. Efficacy for biological agents was expressed in terms of a log reduction.

An additional qualitative assessment of the 1414RH unit efficacy was conducted following spore extraction. After the extraction process described above, each coupon was transferred to a sterile 50-mL tube containing 20 mL of tryptic soy broth culture medium. The vials were sealed and incubated on an orbital shaker at the appropriate temperatures (see above) for each organism. At 1 and 7 days post-decontamination, the tubes were visually assessed qualitatively for viability as "growth" or "no growth." The biological indicators and spore strips were also evaluated at 1 and 7 days post-decontamination for "growth" or "no growth."

3.5.5 Observation of Surface Damage

Following decontamination, each test surface was examined visually to establish whether decontamination using the 1414RH unit caused any obvious damage to the surface. The coupons were observed immediately after completing the decontamination process, but before post-decontamination sampling. The surface was inspected by comparing the decontaminated test surface with control coupons of the same test material. Differences in color, reflectivity, contrast, and roughness were assessed and recorded.

Chapter 4
Quality Assurance/Quality Control

Quality assurance (QA)/quality control (QC) procedures were performed in accordance with the Quality Management Plan (QMP) for the BDT Center[4] and the test/QA plan for this verification test.[1] QA/QC procedures and results are described below.

4.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, biosafety cabinets) used at the time of testing was verified as being certified, calibrated, or validated.

4.2 Audits

Two types of audit were performed during the verification test: a technical systems audit (TSA) of the verification test performance and an audit of data quality. Audit procedures are described below.

4.2.1 Technical Systems Audit

The Battelle Quality Assurance Unit conducted a TSA on January 21, 2004, to ensure that the verification test was being conducted in accordance with the test/QA plan[1] and the BDT Center QMP.[4] As part of the TSA, test procedures were compared to those specified in the test/QA plan, and data acquisition and handling procedures were reviewed. Observations and findings from the TSA were documented and submitted to the Battelle Verification Test Coordinator for response. None of the findings of the TSA required corrective action. TSA records are permanently stored with the ETV Quality Assurance Manager.

4.2.2 Audit of Data Quality

At least 10% of the data acquired during the verification test were audited. A Battelle Quality Assurance Auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.3 QA/QC Reporting

Each audit was documented in accordance with Section 3.3.4 of the QMP for the ETV BDT Center.[4] Once the audit reports were prepared, the Battelle Verification Test Coordinator ensured that a response was provided for each adverse finding or potential problem and implemented any necessary follow-up corrective action. A Battelle Quality Assurance Auditor ensured that follow-up corrective action was taken.

4.4 Data Review

Records generated in the verification test received a QC/technical review and a QA review before they were used to calculate, evaluate, or report verification results. Table 4-1 summarizes the types of data recorded and reviewed. All data were recorded by Battelle staff. The person performing the QC/technical review added his/her initials and the date to a hard copy of the record being reviewed.

Table 4-1. Summary of Data Recording Process

Data to Be Recorded	Where Recorded	How Often Recorded	Disposition of Data
Dates, times of test events	Data forms	Start/end of test, and at each change of a test parameter	Used to organize/check test results; manually incorporated into spreadsheets as necessary
Test parameters (agent or surrogate identities, concentrations, test surfaces, test conditions, etc.)	Data forms	When set or changed, or as needed to document the sequence of test	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Sampling data	Data forms	At least at start/end of reference sample, and at each change of a test parameter	Used to organize/check test results; manually incorporated into spreadsheets as necessary
Biological enumeration and liquid culture assessment, chain of custody, and results	Data forms	Throughout sample handling and analysis process	Transferred to spreadsheets
Records and observations of 1414RH unit use	Printout from the formaldehyde monitor; data forms	Throughout implementation of the 1414RH unit	Reviewed and summarized to support data interpretation
Surface damage	Data forms	Start/end of test	Used to assess damage of test materials following use of the 1414RH unit

Chapter 5
Statistical Methods

The statistical methods for evaluating the efficacy of the 1414RH unit are presented in this chapter. Qualitative observations also were used to evaluate verification test data.

5.1 Efficacy Calculations

For biological agents and surrogates, decontamination efficacy was calculated as the log reduction in viable organisms achieved by the 1414RH unit. The efficacy (E), or log reduction, for the biological agent, or surrogates was calculated as

$$E = \log (N°/N)$$

where $N°$ is the mean number of viable organisms recovered from the control coupons (i.e., those not subjected to decontamination), and N is the number of viable organisms recovered from each test coupon after decontamination. For decontaminated samples where viable organisms were not detected, the efficacy was calculated as the log of the mean number of viable organisms on the control coupons. Using the calculated log reduction for each test coupon, the mean log reduction (efficacy) ± SD was calculated.

Percent recovery was calculated for each type of test material inoculated with each biological agent or surrogate. Percent recovery (mean ± SD) was calculated by dividing the number of biological organisms in the treated sample by the number of biological organisms in the controls (non-decontaminated).

5.2 Statistical Analysis

For each material and species combination, log reduction was calculated as described above, resulting in a total of 63 log reduction values. In cases where no viable colonies remained after decontamination, one colony was assumed to be present for the purpose of this calculation. A two-way analysis of variance (ANOVA) model with main effects for *Bacillus* species and test material and interactions was fitted to the log reduction data. This model was used to compare each mean to zero, compare each surrogate to *B. anthracis* (within material), and compare each surrogate to *B. anthracis* for porous and non-porous materials. T-tests or statistical contrasts were used for the comparisons, with no adjustment for multiple comparisons. The ANOVA model was fitted using the SAS (Version 8.2) GLM procedure.

Chapter 6
Test Results

The results of the verification test of the 1414RH unit are presented in this section.

6.1 Efficacy

6.1.1 Bacillus anthracis Ames Spores

Exposure of material test coupons contaminated with *B. anthracis* Ames spores to the 1414RH unit, resulted in decontamination that varied according to the type of the test material (Table 6-1). The mean log reduction of detectable viable *B. anthracis* Ames spores ranged from 5.17 to 7.86 across all seven test materials. Three of these test materials (IC, BWD, PC) can be considered porous (on the inoculated surface), while the other four test materials (GS, DL, GM, PW) can be considered non-porous (on the inoculated surface). The log reduction in viable spores detected on the porous materials was ≥ 7.00, ≥ 7.61, and 7.15 for IC, BWD, and PC, respectively. The log reduction in viable spores detected on the non-porous materials was ≥ 7.71, 6.47, ≥ 7.86, and ≥ 5.17 for GS, DL, GM, and PW, respectively. For the PW, the log reduction in viable spores was calculated to be ≥ 5.17, although no viable spores were detected during the enumerations. This suggests that the ≥ 5.17 calculated log reduction may not accurately reflect the decontamination process, but may be a result of the low recovery rate of 0.16%.

A liquid culture growth assessment at 1 and 7 days post-decontamination was performed to determine whether viable *B. anthracis* Ames spores remained on the test materials following the extraction step (Table 6-2). The extraction efficiency for spores on all seven test materials was less than 100%; therefore, it was assumed that viable spores could remain on the test materials. Each test material was wiped with 70% isopropanol prior to inoculation (or non-inoculated blanks) with *B. anthracis* Ames spores; however, this isopropanol wash does not guarantee sterility, especially with the porous materials. The test materials were not autoclaved due to the risk of the materials being damaged during the autoclaving process. Therefore, to maintain equivalent treatment and handling of the test materials, a 70% isopropanol wipe was used. The liquid culture assessment was intended to detect spores that remained on the test material following the extraction step. However, since the materials were not sterilized by autoclaving, this type of assessment may not discriminate between the growth of *B. anthracis* and/or other microorganisms.

Table 6-1. 1414RH Unit Decontamination of *Bacillus anthracis* Ames Spores[a]

Test Material	Inoculum	Total No. Spores	% Recovery	Efficacy
Industrial-Grade Carpet (IC)				
Control	9.33×10^7	$1.01 \pm 0.37 \times 10^7$	10.8 ± 3.98	-[b]
Decontaminated	9.33×10^7	0	0	≥ 7.00 ± 0 (7.00)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Bare Wood (BWD)				
Control	1.02×10^8	$4.03 \pm 0.24 \times 10^7$	39.5 ± 2.37	-
Decontaminated	1.02×10^8	0	0	≥ 7.61 ± 0 (7.61)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Glass (GS)				
Control	9.33×10^7	$5.13 \pm 1.42 \times 10^7$	55.0 ± 15.2	-
Decontaminated	9.33×10^7	0	0	≥ 7.71 ± 0 (7.71)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Decorative Laminate (DL)				
Control	1.02×10^8	$4.58 \pm 0.60 \times 10^7$	44.9 ± 5.89	-
Decontaminated	1.02×10^8	4.87 ± 5.81 x 10	< 0.0001	6.47 ± 1.07 (5.61-7.66)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Galvanized Metal Ductwork (GM)				
Control	1.02×10^8	$7.24 \pm 1.50 \times 10^7$	71.0 ± 14.7	-
Decontaminated	1.02×10^8	0	0	≥ 7.86 ± 0 (7.86)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Wallboard Paper (PW)				
Control	9.33×10^7	$1.49 \pm 0.43 \times 10^5$	0.16 ± 0.05	-
Decontaminated	9.33×10^7	0	0	≥ 5.17 ± 0 (5.17)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Concrete (PC)				
Control	1.02×10^8	$5.76 \pm 0.25 \times 10^7$	56.4 ± 2.45	-
Decontaminated	1.02×10^8	2.23 ± 3.87 x 10	< 0.0001	7.15 ± 1.05 (5.93-7.76)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-

[a]Data are expressed as mean (± SD) total number of spores, percent recovery, and efficacy (log reduction). The efficacy range is shown in parentheses.
[b]Not Applicable

Table 6-2. Liquid Culture Assessment of *Bacillus anthracis* Ames Spores

Test Material		Day 1				Day 7			
		S1	S2	S3	Bl	S1	S2	S3	Bl
Industrial-Grade Carpet (IC)	Control	-	-	-	-	-	-	-	-
	Decontaminated	-	-	-	-	-	-	-	-
Bare Wood (BWD)	Control	+	+	+	+	+	+	+	+
	Decontaminated	-	-	-	-	-	-	-	-
Glass (GS)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Decorative Laminate (DL)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork (GM)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Painted Wallboard Paper (PW)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	+	-	+
Painted Concrete (PC)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
Bl = Blank (not inoculated with *B. anthracis* Ames spores)
"+" = growth; "-" = no growth

Following the extraction step, each test coupon was placed into liquid culture to promote spore germination, thereby enabling the vegetative bacteria to proliferate. Growth was determined if the liquid culture medium turned cloudy, while no growth was determined when the liquid medium remained clear.

None of the liquid culture samples for IC (both control and decontaminated) exhibited bacterial growth. The brand of IC used for this test contains a product known as FlorSept, which is considered a broad spectrum antimicrobial that is effective against Gram-positive and Gram-negative bacteria, as well as mold and fungi. It appears that, under the conditions employed for this verification test, the FlorSept may not be sporicidal since viable *B. anthracis* Ames spores were extracted from the IC and cultured on tryptic soy agar plates. Therefore, it is possible that, in the liquid cultures, FlorSept may inhibit growth of vegetative cells derived from germination of the *B. anthracis* Ames spores. This growth inhibition was also observed for *B. subtilis*, with the exception of one control sample at 7 days (Table 6-6). For *G. stearothermophilus* (Table 6-11), growth was observed in one control sample at Day 1, and all 3 control samples at Day 7.

Qualitative assessments of biological indicators and spore strips are shown in Tables 6-3 and 6-4. For all tests using *B. anthracis*, the control (not exposed to formaldehyde) biological indicators and spore strips exhibited growth in the liquid cultures at both 1 and 7 days. Growth in some of the liquid cultures was also observed at 1 and 7 days for the biological indicators and spore strips subjected to formaldehyde exposure using the 1414RH unit.

Table 6-3. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 1 *B. anthracis* Decontamination)

Indicator (Organism)		Day 1			Day 7		
		S1	S2	S3	S1	S2	S3
Biological Indicator (*B. subtilis* ATCC 19659)	Control	+	+	+	+	+	+
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Control	+	+	+	+	+	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Control	+	+	+	+	+	+
Biological Indicator (*B. subtilis* ATCC 19659)	Decontaminated	-	+	-	-	+	-
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Decontaminated	-	-	-	+	-	-
Spore Strip (*B. atrophaeus* ATCC 9372)	Decontaminated	-	-	-	+	+	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
"+" = growth; "-" = no growth

Table 6-4. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 2 *B. anthracis* Decontamination)

Indicator (Organism)		Day 1			Day 7		
		S1	S2	S3	S1	S2	S3
Biological Indicator (*B. subtilis* ATCC 19659)	Control	+	+	+	+	+	+
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Control	+	+	+	+	+	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Control	+	+	+	+	+	+
Biological Indicator (*B. subtilis* ATCC 19659)	Decontaminated	-	-	-	-	+	+
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Decontaminated	-	-	+	+	-	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Decontaminated	-	-	-	+	-	+

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
"+" = growth; "-" = no growth

6.1.2 Bacillus subtilis (ATCC 19659) Spores

Exposure of test coupons contaminated with *B. subtilis* spores to the 1414RH unit resulted in decontamination that varied according to the type of test material. The log reduction of detectable viable *B. subtilis* spores ranged from approximately 6.02 to ≥ 8.04 for all seven test materials (Table 6-5). The log reduction in viable spores detected on the porous materials was ≥ 8.04, 6.58, and 6.02 for IC, BWD, and PC, respectively. The log reduction in viable spores detected on the non-porous materials was ≥ 7.79, 7.29, 6.24, and ≥ 7.68 for GS, DL, GM, and PW, respectively.

Table 6-5. 1414RH Unit Decontamination of *Bacillus subtilis* Spores[a]

Test Material	Inoculum	Total No. Spores	% Recovery	Efficacy
Industrial-Grade Carpet (IC)				
Control	1.24×10^8	$1.10 \pm 0.08 \times 10^8$	88.4 ± 6.26	-[b]
Decontaminated	1.24×10^8	0	0	≥ 8.04 ± 0 (8.04)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Bare Wood (BWD)				
Control	1.05×10^8	$1.21 \pm 0.41 \times 10^7$	11.6 ± 3.91	-
Decontaminated	1.05×10^8	$1.10 \pm 1.91 \times 10$	< 0.0001	6.58 ± 0.88 (5.57-7.08)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Glass (GS)				
Control	1.24×10^8	$6.21 \pm 2.18 \times 10^7$	50.1 ± 17.6	-
Decontaminated	1.24×10^8	0	0	≥ 7.79 ± 0 (7.79)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Decorative Laminate (DL)				
Control	1.04×10^8	$5.52 \pm 1.27 \times 10^7$	53.1 ± 12.2	-
Decontaminated	1.04×10^8	$2.23 \pm 3.87 \times 10$	< 0.0001	7.29 ± 0.78 (6.38-7.74)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Galvanized Metal Ductwork (GM)				
Control	1.04×10^8	$7.42 \pm 1.89 \times 10^7$	71.4 ± 18.2	-
Decontaminated	1.04×10^8	$1.89 \pm 1.65 \times 10^2$	< 0.001	6.24 ± 1.42 (5.39-7.87)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Wallboard Paper (PW)				
Control	1.24×10^8	$4.82 \pm 1.22 \times 10^7$	38.9 ± 9.81	-
Decontaminated	1.24×10^8	0	0	≥ 7.68 ± 0 (7.68)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Concrete (PC)				
Control	1.05×10^8	$5.47 \pm 0.43 \times 10^7$	52.1 ± 4.10	-
Decontaminated	1.05×10^8	$6.63 \pm 5.77 \times 10$	< 0.0001	6.02 ± 0.35 (5.61-6.22)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-

[a]Data are expressed as mean (± SD) total number of spores, percent recovery, and efficacy (log reduction). The efficacy range is shown in parentheses.
[b]Not Applicable

A liquid culture growth assessment at 1 and 7 days post-decontamination was performed to determine whether viable *B. subtilis* spores remained on the test materials following the extraction step (Table 6-6). As stated above, each test material (or non-inoculated blank) was wiped with 70% isopropanol prior to inoculation with *B. subtilis* spores; however, this isopropanol wash does not guarantee sterility, especially with the porous materials. Therefore, growth observed in some of the test materials not inoculated with *B. subtilis* spores may have resulted from growth of other microorganisms not affected by the 70% isopropanol wash. This type of assessment may not discriminate between the growth of *B. anthracis* and/or other microorganisms.

Table 6-6. Liquid Culture Assessment of *Bacillus subtilis* Spores

Test Material		Day 1				Day 7			
		S1	S2	S3	Bl	S1	S2	S3	Bl
Industrial-Grade Carpet (IC)	Control	-	-	-	-	-	+	-	-
	Decontaminated	-	-	-	-	-	-	-	-
Bare Wood (BWD)	Control	+	+	+	+	+	+	+	+
	Decontaminated	-	-	-	-	-	-	+	-
Glass (GS)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Decorative Laminate (DL)	Control	+	+	+	-	+	+	+	+
	Decontaminated	-	-	-	-	-	-	+	-
Galvanized Metal Ductwork (GM)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Painted Wallboard Paper (PW)	Control	+	+	+	-	+	+	+	+
	Decontaminated	-	-	-	-	+	-	-	-
Painted Concrete (PC)	Control	-	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
Bl = Blank (not inoculated with *B. subtilis* spores)
"+" = growth; "-" = no growth

Qualitative assessment of biological indicators and spore strips are shown in Tables 6-7, 6-8, and 6-9. For all tests using *B. subtilis*, the biological indicators and spore strips not exposed to formaldehyde using the 1414RH unit exhibited growth in the liquid cultures at both 1 and 7 days. No growth in the liquid cultures was observed at 1 and 7 days for the biological indicators and spore strips subject to formaldehyde exposure using the 1414RH unit, with the exception of a single spore strip exhibiting growth at Day 7 for week one of testing.

Table 6-7. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 1 *B. subtilis* Decontamination)

Indicator (Organism)		Day 1		Day 7	
		S1	S2	S1	S2
Biological Indicator (*B. subtilis* ATCC 19659)	Control	+	+	+	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Control	+	+	+	+
Biological Indicator (*B. subtilis* ATCC 19659)	Decontaminated	-	-	-	-
Spore Strip (*B. atrophaeus* ATCC 9372)	Decontaminated	-	-	-	+

S1 = Sample 1
S2 = Sample 2
"+" = growth; "-" = no growth

Table 6-8. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 2 *B. subtilis* Decontamination)

Indicator (Organism)		Day 1		Day 7	
		S1	S2	S1	S2
Biological Indicator (*B. subtilis* ATCC 19659)	Control	+	+	+	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Control	+	+	+	+
Biological Indicator (*B. subtilis* ATCC 19659)	Decontaminated	-	-	-	-
Spore Strip (*B. atrophaeus* ATCC 9372)	Decontaminated	-	-	-	-

S1 = Sample 1
S2 = Sample 2
"+" = growth; "-" = no growth

Table 6-9. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 3 *B. subtilis Decontamination*)

Indicator (Organism)		Day 1			Day 7		
		S1	S2	S3	S1	S2	S3
Biological Indicator (*B. subtilis* ATCC 19659)	Control	+	+	+	+	+	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Control	+	+	+	+	+	+
Biological Indicator (*B. subtilis* ATCC 19659)	Decontaminated	-	-	-	-	-	-
Spore Strip (*B. atrophaeus* ATCC 9372)	Decontaminated	-	-	-	-	-	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
"+" = growth; "-" = no growth

6.1.3 Geobacillus stearothermophilus (ATCC 12980) Spores

Exposure of test coupons contaminated with *G. stearothermophilus* (ATCC 12980) spores to the 1414RH unit resulted in variable decontamination. The log reduction of detectable viable *G. stearothermophilus* spores (ATCC 12980) ranged from approximately 5.68 to ≥ 7.64 for all seven test materials (Table 6-10). The log reduction in viable spores detected on the porous materials was 5.68, ≥ 6.82, and 6.20 for IC, BWD, and PC, respectively. The log reduction in viable spores detected on the non-porous materials was ≥ 7.24, ≥ 7.12, ≥ 7.64, and ≥ 7.19 for GS, DL, GM, and PW, respectively.

Table 6-10. 1414RH Unit Decontamination of *Geobacillus stearothermophilus* Spores[a]

Test Material	Inoculum	Total No. Spores	% Recovery	Efficacy
Industrial-Grade Carpet (IC)				
Control	8.80×10^7	$1.50 \pm 0.19 \times 10^7$	17.1 ± 2.15	-[b]
Decontaminated	8.80×10^7	$1.22 \pm 1.17 \times 10^2$	< 0.001	5.68 ± 1.30 (4.81-7.18)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Bare Wood (BWD)				
Control	7.33×10^7	$6.59 \pm 1.57 \times 10^6$	8.99 ± 2.14	-
Decontaminated	7.33×10^7	0	0	≥ 6.82 ± 0 (6.82)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Glass (GS)				
Control	8.80×10^7	$1.74 \pm 0.05 \times 10^7$	19.8 ± 0.51	-
Decontaminated	8.80×10^7	0	0	≥ 7.24 ± 0 (7.24)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Decorative Laminate (DL)				
Control	7.33×10^7	$1.31 \pm 0.59 \times 10^7$	17.9 ± 8.08	-
Decontaminated	7.33×10^7	0	0	≥ 7.12 ± 0 (7.12)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Galvanized Metal Ductwork (GM)				
Control	7.33×10^7	$4.34 \pm 4.80 \times 10^7$	59.2 ± 65.6	-
Decontaminated	7.33×10^7	0	0	≥ 7.64 ± 0 (7.64)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Wallboard Paper (PW)				
Control	8.80×10^7	$1.53 \pm 0.20 \times 10^7$	17.4 ± 2.30	-
Decontaminated	8.80×10^7	0	0	≥ 7.19 ± 0 (7.19)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Concrete (PC)				
Control	7.33×10^7	$1.94 \pm 0.60 \times 10^7$	26.5 ± 8.15	-
Decontaminated	7.33×10^7	$0.61 \pm 1.06 \times 10^3$	< 0.001	6.20 ± 1.88 (4.03-7.29)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-

[a]Data are expressed as mean (± SD) total number of spores, percent recovery, and efficacy (log reduction). The efficacy range is shown in parentheses.
[b]Not Applicable

A liquid culture growth assessment at 1 and 7 days post-decontamination was performed to determine whether viable *G. stearothermophilus* spores remained on the test materials following the extraction step (Table 6-11). As stated previously, each test material (or non-inoculated blank) was wiped with 70% isopropanol prior to inoculation with *G. stearothermophilus* spores; however, this isopropanol wash does not guarantee sterility, especially with the porous materials. Therefore, growth observed in some of the test materials not inoculated with *G. stearothermophilus* spores may have resulted from growth of other microorganisms not affected by the 70% isopropanol wash. This type of assessment may not discriminate between the growth of *B. anthracis* and/or other microorganisms.

Table 6-11. Liquid Culture Assessment of *Geobacillus stearothermophilus* Spores

Test Material		Day 1				Day 7			
		S1	S2	S3	Bl	S1	S2	S3	Bl
Industrial-Grade Carpet (IC)	Control	-	-	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Bare Wood (BWD)	Control	+	+	+	+	+	+	+	+
	Decontaminated	-	-	-	-	-	-	-	-
Glass (GS)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Decorative Laminate (DL)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork (GM)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Painted Wallboard Paper (PW)	Control	+	+	+	-	+	+	+	+
	Decontaminated	-	-	-	-	-	-	-	-
Painted Concrete (PC)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	+	-	-	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
Bl = Blank (not inoculated with *G. stearothermophilus* spores)
"+" = growth; "-" = no growth

Qualitative assessment of biological indicators and spore strips is shown in Tables 6-12 and 6-13. For all tests using *G. stearothermophilus*, the biological indicators and spore strips not exposed to formaldehyde using the 1414RH unit exhibited growth in the liquid cultures at both 1 and 7 days. Growth in the liquid cultures was observed for one of the biological indicators at day 1 and three biological indicators at Day 7. No growth was observed for the spore strips subjected to formaldehyde exposure using the 1414RH unit.

Table 6-12. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 1 *G. stearothermophilus* Decontamination)

Indicator (Organism)		Day 1			Day 7		
		S1	S2	S3	S1	S2	S3
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Control	+	+	+	+	+	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Control	+	+	+	+	+	+
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Decontaminated	-	-	-	+	-	-
Spore Strip (*B. atrophaeus* ATCC 9372)	Decontaminated	-	-	-	-	-	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
"+" = growth; "-" = no growth

Table 6-13. Liquid Culture Assessment of Biological Indicators/Spore Strips (Week 2 *G. stearothermophilus* Decontamination)

Indicator (Organism)		Day 1			Day 7		
		S1	S2	S3	S1	S2	S3
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Control	+	+	+	+	+	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Control	+	+	+	+	+	+
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Decontaminated	-	-	+	+	-	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Decontaminated	-	-	-	-	-	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
"+" = growth; "-" = no growth

6.1.4 Statistical Analysis

Table 6-14 presents the mean log reduction in spores sorted by material type. Significant differences are denoted in the table as well. All means were significantly different from zero indicating that the technology decontaminated statistically significant numbers of spores on these materials.

Table 6-14. Statistical Analysis of Mean Efficacy (Log Reduction) for Spores

Material		*B. anthracis*	*B. subtilis*	*G. stearothermophilus*
Porous	**Industrial-Grade Carpet (IC)**	≥ 7.00[a]	≥ 8.04[a]	5.68[a, b]
	Painted Concrete (PC)	≥ 7.15[a]	6.02[a]	6.20[a]
	Bare Wood (BWD)	≥ 7.61[a]	6.58[a]	≥ 6.82[a]
Non-Porous	**Glass (GS)**	≥ 7.71[a]	≥ 7.79[a]	≥ 7.24[a]
	Decorative Laminate (DL)	6.47[a]	7.29[a]	≥ 7.12[a]
	Painted Wallboard Paper (PW)	≥ 5.17[a]	≥ 7.68[a, b]	≥ 7.19[a, b]
	Galvanized Metal Ductwork (GM)	7.86[a]	6.24[a, b]	≥ 7.64[a]

[a]Mean significantly different from 0 at the (P ≥ 0.05)
[b]Surrogate significantly different from *B. anthracis* for specified material (P ≥ 0.05).

While there was no significant overall effect of spore species utilized, a significant interaction between the spore species utilized and the test coupon materials was noted in the ANOVA model (P=0.0001). Overall comparisons of the porous and the non-porous materials were not useful due to this interaction, as opposing interactions appeared to cancel each other out. That is, it appears that each of the three spore species interacts with certain test coupons in such a way that the efficacy of the formaldehyde decontamination is influenced. These spore-coupon interactions differ, depending on the spore type; for example, the log reductions for *B. subtilis* and *G. stearothermophilis* are similar for PC (6.58 and 6.82, respectively), but *B. anthracis* was reduced to a greater extent (7.61). Comparisons within each material indicated that the 1414RH unit decontaminated significantly more *B. subtilis* and *G. stearothermophilus* spores than *B. anthracis* spores for PW; significantly fewer *B. subtilis* spores than *B. anthracis* spores for GM; and significantly fewer *G. stearothermophilus* spores than *B. anthracis* spores for IC.

6.2 Damage to Coupons

Subsequent to decontamination, the test coupons were evaluated qualitatively for visible surface damage. No damage (e.g., change in surface texture, color) and no visible changes to any of the test materials were observed during this verification test.

6.3 Other Factors

6.3.1 Operation of the 1414RH Unit

The 1414RH unit was operated for approximately 140 hours during this verification test. By following the user manual, the 1414RH unit was set up for operation within minutes. The only maintenance that was required for the 1414RH unit during this verification test was the

addition of new paraformaldehyde and neutralizer at the beginning of each run. At the end of each run, the hexamethylenetetramine formed during the neutralization step had to be cleaned from all surfaces within the Plas-Labs Glove Box prior to the start of the next run of the 1414RH unit. A towel dampened with ethanol was used to remove the hexamethylenetetramine powder.

The formaldehyde concentration was monitored in real-time, and the data were recorded on a strip chart. Figure 6-1 is a graphical representation of the real-time formaldehyde measurement from 0 to 11.5 hours. Paraformaldehyde was added to the 1414RH unit at the specified concentration, leading to a theoretical concentration of formaldehyde gas in the test chamber of approximately 8,600 ppm. The observed measured concentration of formaldehyde gas in the test chamber averaged approximately 1,100 ppm. Therefore, it appears that the majority of the formaldehyde was deposited on all surfaces within the test chamber. This deposition seems possible since a film formed on all surfaces within the test chamber during operation of the 1414RH unit.

As described in Section 3.5.4.1, a nebulizer system had to be utilized to achieve the appropriate relative humidity (50 to 90%) within the Plas-Labs Compact Glove Box for each run of the 1414RH unit.

6.3.2 Operator Bias

Due to the automated capabilities of the 1414RH unit, there is little room for operator error although operator error was not evaluated in this verification test. Once the appropriate canisters were filled with their respective components (e.g., paraformaldehyde), the timer was set for the appropriate contact time (10 hours for this verification test). Next, the “Start” button was pressed and the 1414RH unit ran through the decontamination cycle. The decontamination and neutralization steps were run overnight and shut off the next morning; therefore, a total run time from start to finish was approximately 16 to 18 hours.

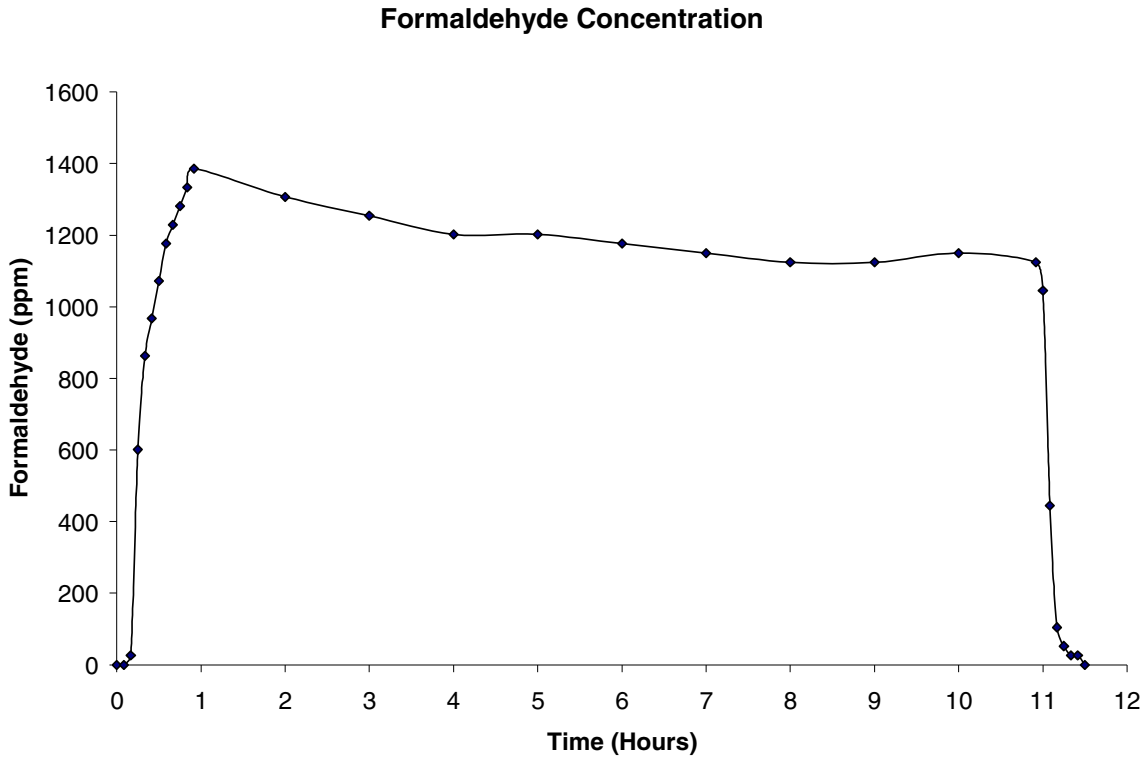

Figure 6-1. Representative Cycle Parameter Data from a Single Experiment

Chapter 7
Performance Summary

For this verification test, the 1414RH unit demonstrated decontamination efficacy for *B. anthracis* Ames, *B. subtilis* (ATCC 19659), and *G. stearothermophilus* (ATCC 12980) on all seven test materials. The test showed that, for all three organisms, material type appeared to influence decontamination. The 1414RH unit promoted a significant decrease in viable spores for all three organisms on all seven test materials.

The ETV testing to measure the effectiveness of the 1414RH unit for inactivating *B. anthracis* Ames strain and surrogate spores on seven indoor surfaces provided a range of results. A quantitative evaluation of the results indicated that the log reduction values for detectable viable *B. anthracis* Ames spores ranged from 5.17 to ≥ 7.86 across all seven test materials. The log reduction values for detectable viable *B. subtilis* spores ranged from 6.02 to 8.04 for all seven test materials. The log reduction values for detectable viable *G. stearothermophilus* spores (ATCC 12980) ranged from 5.68 to ≥ 7.64 for all seven test materials. For the porous materials, a significant difference in efficacy was observed only between *B. anthracis* and *G. stearothermophilus* on industrial carpet. For non-porous materials, significant differences in efficacy between *B. anthracis* and both surrogates were observed for painted wallboard paper, and a significant difference was observed between *B. anthracis* and *B. subtilis* on galvanized metal. No damage was observed for any of the test materials subjected to the 1414RH unit.

A qualitative evaluation of the performance of the 1414RH unit was performed using biological indicators and spore strips. For all procedures for this verification test, the control (not exposed to the 1414RH unit) biological indicators and spore strips used in this test displayed growth in the liquid cultures at both 1 and 7 days. When the biological indicators and spore strips were subjected to exposure by the 1414RH unit, growth was observed in some of the liquid cultures at 1 and 7 days. For these samples, the number of samples exhibiting growth varied among the dates of experimentation, and no clear trend was observed. The 1414RH unit was partially successful in inactivating both the biological indicators (containing *B. subtilis* and *G. stearothermophilus*) and spore strips (containing *B. atrophaeus*), all of which contain spore loads of approximately 1×10^6 spores per indicator or spore strip. It is possible that this partial inactivation resulted from the biological indicators and spore strips remaining in the sealed Tyvek and glassine pouches, respectively. The Tyvek and glassine may have inhibited the penetration of formaldehyde to some extent, thereby preventing complete inactivation of the biological indicators and spore strips.

The 1414RH unit was set up and ready for operation in the laboratory within minutes. The 1414RH unit is not able to measure parameters such as relative humidity and formaldehyde concentration. Within the Plas-Labs Compact Glove Box, the relative humidity was determined by using a traceable hygrometer, and the formaldehyde was measured using a formaldehyde monitor. The importance of operator skill level to using the 1414RH unit, while not verified in this test, should be minimal due to the automated capabilities of the 1414RH unit; which left little room for operator error.

Chapter 8
References

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