September 2004

Environmental Technology Verification Report

CDG RESEARCH CORPORATION BENCH-SCALE CHLORINE DIOXIDE *GAS:SOLID* GENERATOR

Prepared by
Battelle

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The Business of Innovation

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Environmental Technology Verification Report

ETV Building Decontamination Technology Center

CDG Research Corporation Bench-Scale Chlorine Dioxide *Gas:Solid* Generator

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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 six verification 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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List of Abbreviations

ANOVA	analysis of variance
ATCC	American Type Culture Collection
BDT	Building Decontamination Technology
BSC	biological safety cabinet
BWD	bare wood (pine lumber)
CFU	colony-forming unit
ClO_2	chlorine dioxide
cm	centimeter
DL	decorative laminate
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
GM	galvanized metal ductwork
GS	glass
HEPA	high-efficiency particulate air
HVAC	heating, ventilating, and air conditioning
IC	industrial-grade carpet
in	inch
mL	milliliter
PC	painted (latex, semi-gloss) concrete cinder block
ppm	part per million
PW	painted (latex, flat) wallboard paper
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
SAIC	Science Application International Corporation
SD	standard deviation
TSA	technical systems audit
UV	ultraviolet

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. The ETV Program 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.

The ETV Program 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 the ETV Program. The BDT Center recently evaluated the performance of the CDG Research Corporation bench-scale chlorine dioxide (ClO_2) *Gas:Solid* generator (unit) for decontaminating buildings contaminated with a biological agent and surrogates.

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 of the CDG Research Corporation bench-scale unit. The following description of the CDG bench-scale unit is based on information provided by the vendor. The information provided below was not verified in this test.

Figure 2-1. CDG Research Corporation Bench-Scale Unit

The CDG bench-scale unit generates ClO_2 gas for decontaminating a sealed area by producing a blend of ClO_2 gas in nitrogen or air. A mixture of nitrogen (or air) and chlorine gas is passed through a reactor cartridge containing processed pellets of sodium chlorite. The chlorine reacts with the sodium chlorite to produce ClO_2 gas and sodium chloride.

For each molecule of chlorine gas, Cl_2, the reaction produces two molecules of ClO_2. Therefore the volumetric concentration of the ClO_2 produced by the reaction is approximately twice the concentration of the chlorine feed gas. As long as the chlorine concentration in the feed gas never exceeds 5%, the concentration of the ClO_2 can never enter the range (20% or greater) in which it can spontaneously undergo a self-propagating reaction.[1] The production rate of ClO_2 is controlled either by adjusting the flow rate of the nitrogen/chlorine blend or by using a compressed gas with a different chlorine:nitrogen ratio.

The CDG bench-scale unit includes a compressed gas cylinder containing 4% chlorine in nitrogen (vol/vol), a sodium chlorite cartridge containing Saf-T-Chlor thermally stable sodium chlorite pellets, a supply of nitrogen for purging the system prior to shutdown, a

flow meter and valve for controlling the flow rate of nitrogen/chlorine (and thereby controlling the production rate of ClO_2), a pressure regulator and gauge for controlling the gas pressure in the generator, pressure relief valves to protect against over-pressure, and on-off valves for nitrogen/chlorine supply and nitrogen purge.

The CDG bench-scale unit consists of a cabinet about 20 inches (in) [51 centimeters (cm)] high by 16 in (41 cm) wide by 9 in (23 cm) deep, plus the required nitrogen and chlorine (4% chlorine in nitrogen) gas cylinders. The production rate of ClO_2 is controlled and recorded manually.

The CDG bench-scale unit was attached to a Plas-Labs Compact Glove Box (Model 830-ABC) modified for this verification test (see Section 3.5.4.1). The connections between the CDG bench-scale unit and the glove box consisted of flexible supply and delivery gassing hoses connected to the glove box high-efficiency particulate air (HEPA) filters. A ClO_2 monitor also was placed inside the glove box to measure the concentration of ClO_2 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 Chlorine Dioxide Gas Technologies for Decontaminating Indoor Surfaces Contaminated with Biological or Chemical Agents.*[2] 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). The latter two organisms also were used to prepare biological indicators that were used in the tests; spore strips containing *B. atrophaeus* also were used. Seven materials representing indoor surfaces commonly found in buildings were used for the verification testing. The indoor surfaces tested (Figure 3-1) include

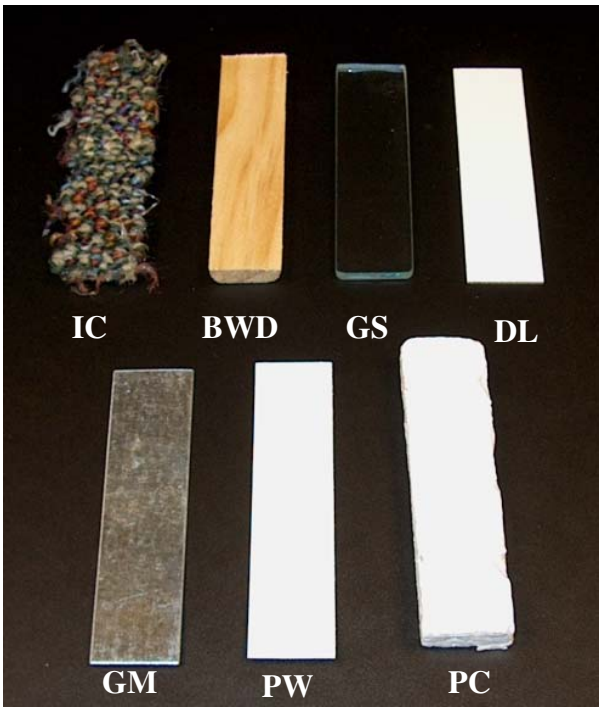

Figure 3-1. Test Materials

- 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).

The objective of the verification testing was to evaluate the efficacy of the CDG bench-scale unit to decontaminate a biological agent/surrogate. Efficacy was tested by applying a biological agent or surrogates to the surfaces of test coupons and, after using the CDG

bench-scale 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 CDG bench-scale 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 0.02 in (0.05 cm) to 0.28 in (0.71 cm), depending upon the material. In triplicate, the coupons were placed into a biological safety cabinet (BSC), and aliquots of an aqueous suspension of the biological agent/surrogate 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 CDG bench-scale unit was determined by comparing the number of viable spores recovered from the control coupons (not decontaminated) to the number recovered from 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/surrogate by transferring each coupon into liquid growth medium and assessing bacterial growth after 1 and 7 days. (Note: The test/QA plan states that bacterial growth will be assessed at 1 day after extraction; however, growth was also assessed at 7 days. Accordingly, a deviation to the test/QA plan was prepared.)

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 visible changes in the color, reflectivity, and apparent roughness of the coupon surfaces were noted. These observations were preliminary in nature and not meant to be definitive.

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 [American Type Culture Collection (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 in 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 CDG bench-scale unit is presented. Verification testing was performed during a 7-week period that commenced in March 2004 and concluded in April 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 CDG bench-scale 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 x H (inch)	Material Preparation
Industrial-grade Carpet	ShawTek, EcoTek 6 http://www.shawcontract.com/html/html/technical/technical.asp	Shaw Industries, Inc.	3 x 3/4 x 0.244	Wiped with 70% isopropanol
Wood	Screen Molding (Pine Wood)	Kingswood Lumber	3 x 3/4 x 0.220	Wiped with 70% isopropanol
Glass	C1036	Brooks Brothers	3 x 3/4 x 0.114	Cleaned with acetone; wiped with 70% isopropanol
Decorative Laminate	Laminate/ Formica/ White Matte Finish	Solid Surface Design	3 x 3/4 x 0.047	Wiped with 70% isopropanol
Galvanized Metal Ductwork	Industry HVAC standard 24 Gauge Galvanized Steel	Accurate Fabrication	3 x 3/4 x 0.028	Cleaned with acetone; wiped with 70% isopropanol
Wallboard Paper	05-16-03; Set-E-493; Roll-3	United States Gypsum Company	3 x 3/4 x 0.020	Roller painted on one side using Martin Senour Paints. One primer (#71-1185) and two finish (flat, #70-1001) coats; wiped with 70% isopropanol
Concrete, Cinder Block	ASTM C90	Wellnitz	3 x 3/4 x 0.280	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

The test materials were not autoclaved due to the risk of the materials being damaged during the autoclaving process. Therefore, to maintain equivalent treatment/handling, each test material was wiped with 70% isopropanol prior to inoculation (non-inoculated blanks were also wiped with 70% isopropanol) with spores; however, this isopropanol wipe does not guarantee sterility, especially with the porous materials.

3.5.2 Application of Agents to Test Coupons

Test coupons were laid flat in a 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 micropipet by placing the suspension (a 100-microliter aliquot of the suspension was applied) over the surface as small droplets. (Note that spore suspension tended to form discrete droplets on the surface of the glass, decorative laminate, painted wallboard paper, and painted concrete, but rapidly penetrated into carpet and wood.) 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 CDG bench-scale 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 agents and surrogates, the *B. anthracis* and surrogate spore suspensions used to contaminate the coupons were re-enumerated on each day of use. This enumeration was 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 inner dimensions of the glove box are 28 inches wide by 23 inches deep by 29 inches high (71 cm by 59 cm by 74 cm). The glove box has a total volume of 11.2 cubic feet (317 liters). This glove box was modified and equipped to enable proper generation of relative humidity and venting during operation of the liquid scrubber. Two computer fans were mounted inside the glove box (Figure 3-3) to promote circulation. ClO_2 is light-sensitive; therefore, the modified test chamber also was wrapped in brown paper. During testing, the lights in the laboratory were turned off, and the only light source used was a flashlight. The parameters used for this test, as specified by CDG Research Corporation, were as follows:

- ClO_2 Concentration: 2,000 parts per million (ppm)
- Exposure Time: 6 hours

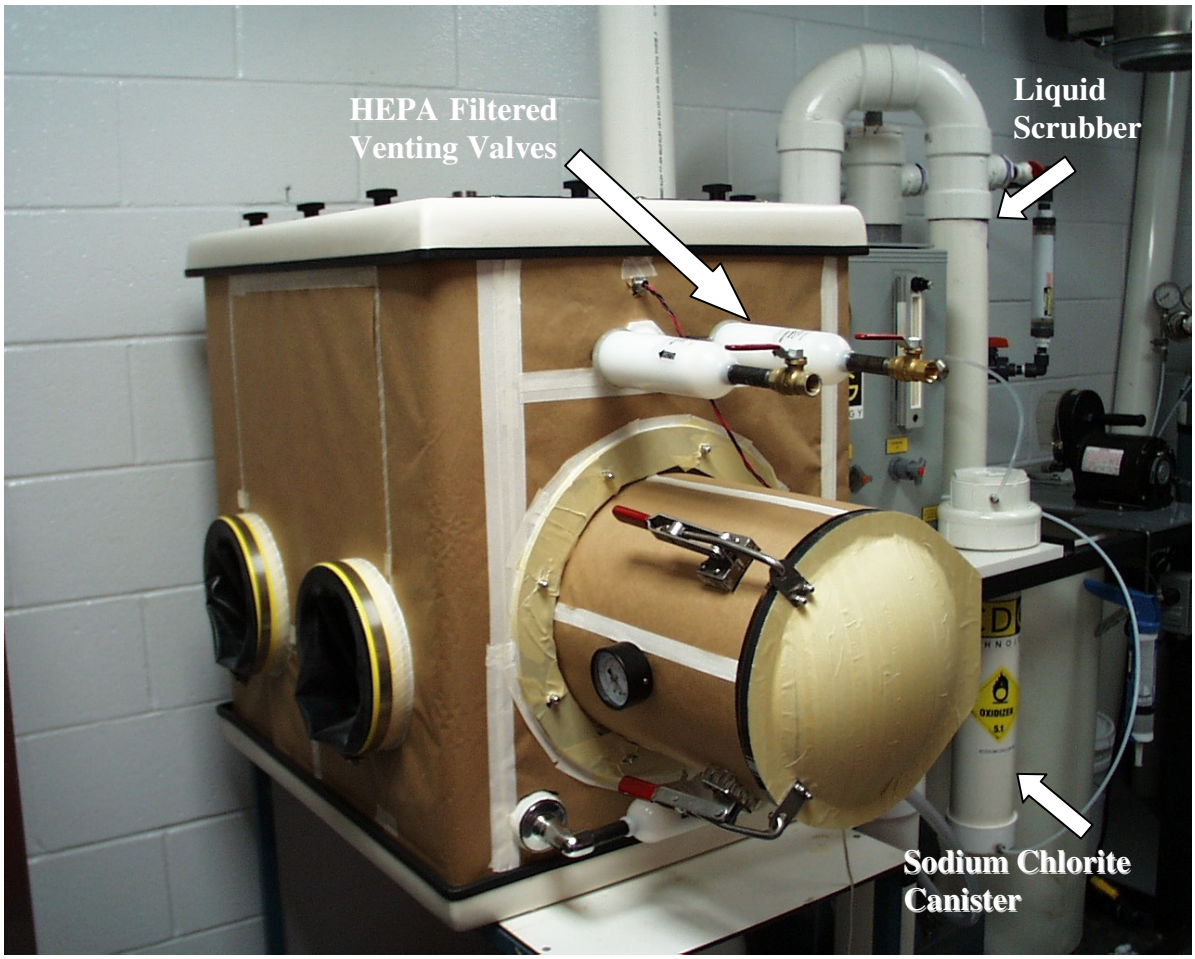

Figure 3-2. Overview of Plas-Labs Compact Glove Box

- Relative Humidity: 70% minimum
- Temperature: room temperature (Actual temperature inside the glove box ranged from 23°C to 27°C during testing.)

For this verification test, the CDG bench-scale unit was not designed to generate the minimum 70% relative humidity required in the test chamber. To solve this problem, Battelle staff configured a series of six nebulizers (Figure 3-4) inside the glove box (nebulizers located inside the box near the top center) to generate water vapor. 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 pounds per square inch gauge (gauge pressure), and a relative humidity of 70 to 80% was achieved within 5 minutes and remained at this level (with no maintenance or changes to the nebulizers) for the duration of the test. A traceable hygrometer was placed inside the glove box to monitor relative humidity.

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

Figure 3-4. Nebulizers in the Plas-Labs Compact Glove Box

To neutralize the ClO_2 removed from the glove box, the test chamber was attached to a liquid scrubber containing 10% sodium hydroxide/10% sodium thiosulfate in water. The liquid scrubber was connected to the facility heating, ventilating, and air conditioning (HVAC) system for subsequent evacuation of the neutralized ClO_2. At the end of each run,

the liquid scrubber was turned on and run for approximately 1 hour. The liquid scrubber was then turned off, but the facility HVAC system continued to draw air through the test chamber. Since running the liquid scrubber as well as the facility HVAC system pulled a vacuum on the testing chamber, the glove box was modified with HEPA-filtered valves (Figure 3-2) that were opened during liquid scrubber operation for appropriate venting. The next morning, ClO_2 was not detectable in the test chamber.

3.5.4.2 Chlorine Dioxide Measurement

Spectroscopic monitoring of the concentration of ClO_2 gas in the test chamber was conducted during the decontamination to enable a real-time measurement of the ClO_2 concentration. This monitoring was accomplished using customized ClO_2 monitoring devices manufactured by Science Application International Corporation (SAIC) under a Defense Advanced Research Projects Agency contract (Figure 3-5). The monitors obtained from SAIC measure the 360-nanometer ultraviolet (UV) absorption of ClO_2 gas. The UV optical beam is produced by a low-power light-emitting diode and detected by a photodiode. The monitor provided an analog (4 to 20 milliamp) signal that was displayed using a Fluke IV multimeter and recorded manually every 15 minutes during the 6-hour exposure time. The analog signal was converted manually to a concentration value (ppm) using a standardized table. Only one monitor was placed inside the box, sitting on the bottom in the back right corner. Note that the monitor is a prototype; although Battelle did ascertain that the monitor yielded data that compared well with data obtained using a published method, the SAIC method has not been fully validated.

3.5.4.3 Decontamination Efficacy

Biological agent/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 milliliter (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 65 ºC for 1 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 37 ºC for *B. anthracis* and *B. subtilis* and at 55 to 60 ºC for *G. stearothermophilus*. [Note: The incubation of *B. anthracis* Ames for 24 hours is based upon in-house standard operating procedures and practical laboratory experience. Within

Figure 3-5. SAIC Chlorine Dioxide Monitor

24 hours, the *B. anthracis* Ames colonies are large (about 0.5 cm in diameter); therefore, incubating the tryptic soy agar plates for an additional 12 to 24 hours would potentially decrease the sensitivity of counting colonies due to the potential for overgrowth on the plates.] 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.

The percent recovery of spores on all seven test materials ranged from 83 to 4%, with an average of 37% recovery; therefore, it was assumed that viable spores could remain on the test materials. 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 to promote spore germination, thereby enabling the vegetative bacteria to proliferate. The vials were sealed and incubated on an orbital shaker at the appropriate temperatures (see above) for *B. anthracis* or the surrogate organism. At 1 and 7 days post-decontamination, the tubes

were visually assessed qualitatively for viability. Viability, "growth," was determined if the liquid culture medium turned cloudy, while "no growth" was determined when the liquid medium remained clear. However, since the test materials were not sterilized by auto-claving, this type of assessment may not discriminate between the growth of *B. anthracis* and other microorganisms. (Therefore, growth in these liquid culture samples should not be interpreted as decreased efficacy.)

The biological indicators and spore strips were also evaluated in a similar manner 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 CDG bench-scale 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 visually 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. These assessments, as stated previously, are qualitative in nature and not intended to be rigorous.

Chapter 4
Quality Assurance/Quality Control

QA/quality control (QC) procedures were performed in accordance with the Quality Management Plan (QMP) for the BDT Center[3] and the test/QA plan for this verification test.[2] QA/QC procedures and results are described below.

4.1 Equipment Calibration

All equipment (e.g., pipets, incubators, BSCs) 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 March 24, 2004, to ensure that the verification test was being conducted in accordance with the test/QA plan[2] and the BDT Center QMP.[3] 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.[3] 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. The results of the TSA were submitted to the EPA.

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 review was involved in the experiments and 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/surrogate identi-ties, 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 assess-ment, chain of custody, and results	Data forms	Throughout sample handling and analysis process	Transferred to spreadsheets
Records and observa-tions of CDG bench-scale unit use	Reading from the SAIC monitor; data forms	Throughout use of the CDG bench-scale 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 CDG bench-scale unit

Chapter 5
Statistical Methods

The statistical methods for evaluating the efficacy of the CDG bench-scale 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 CDG bench-scale 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/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 (3 coupons for each of seven materials analyzed in triplicate). 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* (for each 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 CDG bench-scale 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 CDG bench-scale 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 4.33 to ≥7.79 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 4.62, 4.33, and 7.25 for IC, BWD, and PC, respectively. The log reduction in viable spores detected on the non-porous materials was 5.70, 4.57, ≥ 7.79, and ≥ 7.68 for GS, DL, GM, and PW, respectively.

Results from the liquid culture growth assessment at 1 and 7 days post-decontamination, to evaluate whether viable *B. anthracis* Ames spores may remain on the test materials following the extraction step, are provided in Table 6-2. Clear liquid medium indicates that no growth of *B. anthracis* Ames or other microorganisms in or on the test material occurred during the incubation period. (Note: This type of assessment may not discriminate between the growth of *B. anthracis* or other microorganisms. The presence of growth in media containing blanks indicates that viable microorganisms, other than the spiked *B. anthracis*, may have been present on or in the test material and not killed by either 70% isopropanol wipe or the decontamination treatment.)

None of the liquid culture samples for IC (both control and decontaminated) exhibited bacterial growth. Although it was not known prior to the start of testing, 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* (Table 6-5) and *G. stearothermophilus* (Table 6-8).

After decontamination, GM and PC, in addition to IC, exhibited no growth after seven days incubation in nutrient broth. After decontamination, GS and PW each exhibited growth in only one of the three replicate culture media. After decontamination, BWD and DL each exhibited growth in each of the three replicate culture media.

Table 6-1. CDG Bench-Scale Unit Decontamination of *Bacillus anthracis* Ames Spores[a]

Test Material	Inoculum	Total No. Spores	% Recovery	Efficacy
Industrial-Grade Carpet (IC)				
Control	9.40×10^7	$5.29 \pm 0.36 \times 10^7$	56.2 ± 3.85	-[b]
Decontaminated	9.40×10^7	$2.41 \pm 2.03 \times 10^3$	<0.01	4.62 ± 0.76 (4.11-5.50)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Bare Wood (BWD)				
Control	9.27×10^7	$9.14 \pm 0.72 \times 10^6$	9.86 ± 0.78	-
Decontaminated	9.27×10^7	$4.67 \pm 2.33 \times 10^2$	<0.001	4.33 ± 0.20 (4.10-4.48)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Glass (GS)				
Control	9.40×10^7	$7.77 \pm 0.75 \times 10^7$	82.6 ± 8.03	-
Decontaminated	9.40×10^7	$1.89 \pm 1.34 \times 10^2$	<0.001	5.70 ± 0.35 (5.35-6.06)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Decorative Laminate (DL)				
Control	9.27×10^7	$5.42 \pm 0.75 \times 10^7$	58.5 ± 8.03	-
Decontaminated	9.27×10^7	$1.81 \pm 1.48 \times 10^3$	<0.01	4.57 ± 0.34 (4.19-4.85)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Galvanized Metal Ductwork (GM)				
Control	9.27×10^7	$6.16 \pm 0.02 \times 10^7$	66.4 ± 0.25	-
Decontaminated	9.27×10^7	0	0	≥ 7.79 ± 0 (7.79)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Wallboard Paper (PW)				
Control	9.40×10^7	$4.78 \pm 0.49 \times 10^7$	50.8 ± 5.21	-
Decontaminated	9.40×10^7	0	0	≥ 7.68 ± 0 (7.68)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Concrete (PC)				
Control	9.27×10^7	$5.73 \pm 1.73 \times 10^7$	61.8 ± 18.7	-
Decontaminated	9.27×10^7	$1.10 \pm 1.91 \times 10$	<0.0001	7.25 ± 0.88 (6.24-7.76)
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

For all tests using *B. anthracis*, the control (not exposed to ClO_2) biological indicators and spore strips 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 ClO_2 exposure using the CDG bench-scale unit. A representation of the data from a single test day is shown in Table 6-3.

Table 6-3. Representative Liquid Culture Assessment of Biological Indicators/Spore Strips

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 CDG bench-scale 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 4.44 to 5.57 for all seven test materials (Table 6-4). The log reduction in viable spores detected on the porous materials was 4.44, 4.48, and 4.74 for IC, BWD, and PC, respectively. The log reduction in viable spores detected on the non-porous materials was 5.23, 5.14, 5.57, and 4.62 for GS, DL, GM, and PW, respectively.

Table 6-4. CDG Bench-Scale Unit Decontamination of *Bacillus subtilis* Spores[a]

Test Material	Inoculum	Total No. Spores	% Recovery	Efficacy
Industrial-Grade Carpet (IC)				
Control	8.43×10^7	$3.60 \pm 1.18 \times 10^7$	42.7 ± 14.1	-[b]
Decontaminated	8.43×10^7	$1.37 \pm 0.52 \times 10^3$	<0.01	4.44 ± 0.17 (4.28-4.62)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Bare Wood (BWD)				
Control	8.80×10^7	$4.15 \pm 0.20 \times 10^6$	4.72 ± 0.22	-
Decontaminated	8.80×10^7	$1.67 \pm 1.20 \times 10^2$	<0.001	4.48 ± 0.33 (4.14-4.79)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Glass (GS)				
Control	8.43×10^7	$4.10 \pm 1.37 \times 10^7$	48.6 ± 16.3	-
Decontaminated	8.43×10^7	$2.89 \pm 2.14 \times 10^2$	<0.001	5.23 ± 0.31 (4.89-5.49)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Decorative Laminate (DL)				
Control	8.80×10^7	$6.51 \pm 0.60 \times 10^7$	74.0 ± 6.83	-
Decontaminated	8.80×10^7	$5.45 \pm 3.67 \times 10^2$	<0.001	5.14 ± 0.27 (4.83-5.34)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Galvanized Metal Ductwork (GM)				
Control	8.80×10^7	$7.04 \pm 0.43 \times 10^7$	80.0 ± 4.85	-
Decontaminated	8.80×10^7	$1.89 \pm 0.19 \times 10^2$	<0.001	5.57 ± 0.05 (5.55-5.63)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Wallboard (PW)				
Control	8.43×10^7	$9.63 \pm 0.35 \times 10^6$	11.4 ± 0.42	-
Decontaminated	8.43×10^7	$1.87 \pm 3.15 \times 10^3$	<0.01	4.62 ± 1.20 (3.24-5.47)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Concrete (PC)				
Control	8.80×10^7	$2.85 \pm 0.11 \times 10^7$	32.4 ± 1.23	-
Decontaminated	8.80×10^7	$5.88 \pm 3.85 \times 10^2$	<0.001	4.74 ± 0.26 (4.44-4.93)
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 6-5). Only GS and IC exhibited no growth after decontamination. (Note that IC also showed no growth in control samples. This is attributed to the presence of FlorSept® broad spectrum antibacterial treatment in the carpet samples.) All samples of PC, DL, BWD, and GM (except one decontaminated case), including both control and treatment blanks, exhibited growth in the liquid culture medium. The presence of growth in media containing blanks indicates that viable microorganisms, other than the spiked surrogate, may

have been present on or in the test material (microbes could be indigenous or introduced by handling coupons) and not killed by either the 70% isopropanol wipe or the decontamination treatment.

Table 6-5. 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

For all tests using *B. subtilis* ATCC (19659) and *B. atrophaeus* (ATCC 9372) the control (not exposed to ClO_2) biological indicators and spore strips 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 ClO_2 exposure using the CDG bench-scale unit. A representation of the data from a single test day is shown in Table 6-6.

Table 6-6. Representative Liquid Culture Assessment of Biological Indicators/Spore Strips

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 CDG bench-scale unit resulted in variable decontamination. The log reduction of detectable viable *G. stearothermophilus* spores (ATCC 12980) ranged from approximately 3.22 to 5.79 for all seven test materials (Table 6-7). The log reduction in viable spores detected on the porous materials was 3.22, 3.78, and 5.79 for IC, BWD, and PC, respectively. The log reduction in viable spores detected on the non-porous materials was 3.87, 4.44, 3.43, and 5.62 for GS, DL, GM, and PW, respectively.

Results from the liquid culture growth assessment at 1 and 7 days post-decontamination, performed to determine whether viable *G. stearothermophilus* spores remained on the test materials following the extraction step, are provided in Table 6-8. Similar to findings with other spores, IC exhibited no growth in liquid culture media at 1 or 7 days for controls or decontaminated test coupons. This is likely due to the IC being manufactured with a broad spectrum antimicrobial treatment. All other controls for all materials exhibited growth at 1 and 7 days. Decontaminated test coupons of PC and PW (except one 7-day case) exhibited no growth. After decontamination, BWD, GL (except in one case), DL, and GM exhibited growth after seven day incubation in liquid culture media. In most cases *G. stearothermophilus* or other microbial growth may occur after treatment of test samples.

Table 6-7. CDG Bench-Scale Unit Decontamination of *Geobacillus stearothermophilus* Spores[a]

Test Material	Inoculum	Total No. Spores	% Recovery	Efficacy
Industrial-Grade Carpet (IC)				
Control	1.13×10^8	$8.70 \pm 0.67 \times 10^6$	7.70 ± 0.60	-[b]
Decontaminated	1.13×10^8	$5.30 \pm 0.65 \times 10^3$	<0.01	3.22 ± 0.05 (3.17-3.28)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Bare wood (BWD)				
Control	1.17×10^8	$4.71 \pm 0.64 \times 10^6$	4.03 ± 0.55	-
Decontaminated	1.17×10^8	$7.89 \pm 1.50 \times 10^2$	<0.001	3.78 ± 0.08 (3.70-3.87)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Glass (GS)				
Control	1.13×10^8	$3.19 \pm 0.39 \times 10^7$	28.2 ± 3.41	-
Decontaminated	1.13×10^8	$4.85 \pm 2.63 \times 10^3$	<0.01	3.87 ± 0.29 (3.64-4.20)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Decorative Laminate (DL)				
Control	1.17×10^8	$2.87 \pm 0.14 \times 10^7$	24.5 ± 1.22	-
Decontaminated	1.17×10^8	$1.08 \pm 0.37 \times 10^3$	<0.001	4.44 ± 0.15 (4.29-4.59)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Galvanized Metal Ductwork (GM)				
Control	1.17×10^8	$3.67 \pm 0.82 \times 10^7$	31.3 ± 7.00	-
Decontaminated	1.17×10^8	$1.39 \pm 0.35 \times 10^4$	0.01 ± 0.003	3.43 ± 0.11 (3.33-3.56)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Wallboard Paper (PW)				
Control	1.13×10^8	$7.42 \pm 1.04 \times 10^6$	6.57 ± 0.92	-
Decontaminated	1.13×10^8	6.67 ± 8.84	<0.0001	5.62 ± 1.14 (4.65-6.87)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Concrete (PC)				
Control	1.17×10^8	$8.01 \pm 0.30 \times 10^6$	6.85 ± 0.33	-
Decontaminated	1.17×10^8	$3.33 \pm 3.35 \times 10$	<0.0001	5.79 ± 0.98 (5.08-6.90)
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 6-8. 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

Analysis of indicators containing *G. stearothermophilus,* with and without decontamination, provided consistent results. For all tests using *G. stearothermophilus*, the control (not exposed to ClO_2) biological indicators 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 subjected to ClO_2 exposure using the CDG bench-scale unit. A representation of the data from a single test day is shown in Table 6-9.

Table 6-9. Representative Liquid Culture Assessment of Biological Indicators/Spore Strips

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 6-10 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 CDG bench-scale unit decontaminated statistically significant numbers of spores on these materials.

Table 6-10. Statistical Analysis of Mean Efficacy (Log Reduction) for Spores

Material		*B. anthracis*	*B. subtilis*	*G. stearothermophilus*
Porous	**Industrial-Grade Carpet (IC)**	4.62[a]	4.44[a]	3.22[a, b]
	Painted Concrete (PC)	7.25[a]	4.74[a, b]	5.79[a, b]
	Bare Wood (BWD)	4.33[a]	4.48[a]	3.78[a]
Non-Porous	**Glass (GS)**	5.70[a]	5.23[a]	3.87[a, b]
	Decorative Laminate (DL)	4.57[a]	5.14[a]	4.44[a]
	Painted Wallboard Paper (PW)	≥ 7.68[a]	4.62[a, b]	5.62[a, b]
	Galvanized Metal Ductwork (GM)	≥ 7.79[a]	5.57[a, b]	3.43[a, b]

[a]Mean significantly different from 0 ($P \leq 0.05$).
[b]Surrogate significantly different from *B. anthracis* for specified material ($P \leq 0.05$).

Comparisons within each material indicated that the CDG bench-scale unit decontaminated significantly fewer *B. subtilis* and *G. stearothermophilus* spores than *B. anthracis* spores for PC, PW, and GM. Significantly fewer *G. stearothermophilus* spores were decontaminated by the CDG bench-scale unit compared to *B. anthracis* spores on IC and GS.

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 with the exception of IC. Exposure to the ClO_2 appeared to produce a bleaching effect (all colors in the multicolor design were affected) of the IC.

6.3 Other Factors

6.3.1 Operation of the CDG Bench-scale Unit

The CDG bench-scale unit was operated for approximately 50 hours during this verification test. The CDG bench-scale unit can be set up for operation within minutes. For this verification test, the liquid scrubber took hours to setup due to connections to the glove box and facility exhaust system. As described in Section 3.5.4.1, a nebulizer system had to be utilized to achieve the appropriate relative humidity (75%) within the glove box for each run of the CDG bench-scale unit. At the end of each run, the ClO_2 was drawn out of the glove box using the liquid scrubber as described in Section 3.5.4.1. No maintenance was required for the CDG bench-scale unit or liquid scrubber.

The ClO_2 concentration was monitored in real time, and the data were recorded manually (see Section 3.5.4.2). Figure 6-1 is a graphical representation of ClO_2 concentrations in the glove box (measured in real time using the SAIC spectrometer) during a typical 6-hour run.

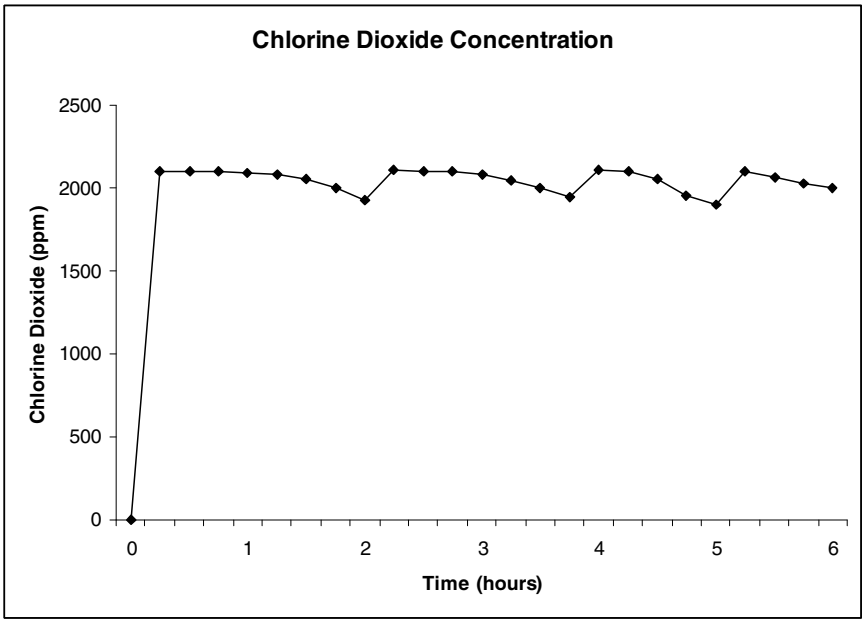

Figure 6-1. Representative Chlorine Dioxide Concentration from a Single Experiment

6.3.2 Operator Bias

The CDG bench-scale unit is operated by manually regulating the introduction of ClO_2 into the exposure chamber. The real-time measurement of ClO_2 enabled the operator to maintain the desired concentration of ClO_2 within the glove box. During the 6-hour contact time, the ClO_2 concentration would decrease slightly over time (concentration of ClO_2 in the glove box during the 6-hour contact time is shown in Figure 6-1). This decrease in the ClO_2 concentration was counteracted by the operator manually introducing additional ClO_2 gas into the glove box by temporarily increasing the flow rate. The decontamination and neutralization steps were run the same day of testing; therefore, a total run time from start to finish was approximately 8 hours. Note that the duration of the humidification phase was 5 minutes, the neutralization phase was 30 to 60 minutes, and further aeration of the box occurred overnight.

Chapter 7
Performance Summary

For this verification test, the CDG bench-scale unit demonstrated a range of decontamination efficacy for *B. anthracis* Ames, *B. subtilis* (ATCC 19659), and *G. stearothermophilus* (ATCC 12980) on all seven test materials. Based on these results, it did not appear that the porosity of the different material types influenced the efficacy of decontamination for the three organisms. However, IC and BWD consistently exhibited the lowest level of spore decontamination for *B. anthracis* Ames, *B. subtilis* (ATCC 19659), and *G. stearothermophilus* (ATCC 12980). The CDG bench-scale unit decontaminated significantly fewer *B. subtilis* and *G. stearothermophilus* spores than *B. anthracis* spores for PC, PW, and GM. Significantly fewer *G. stearothermophilus* spores were decontaminated by the CDG bench-scale unit compared to *B. anthracis* spores on IC and GS.

A quantitative evaluation of the results indicates that the log reduction values for detectable viable *B. anthracis* Ames spores ranged from 4.33 to ≥ 7.79 across all seven test materials. The log reduction values for detectable viable *B. subtilis* spores ranged from 4.44 to 5.57 for all seven test materials. The log reduction values for detectable viable *G. stearothermophilus* spores (ATCC 12980) ranged from 3.22 to 5.79 for all seven test materials. Significant differences in efficacy were observed between *B. anthracis* and *B. subtilis* on PC, PW, and GM. The only damage observed for any of the test materials subjected to the CDG bench-scale unit was a bleaching effect on the IC. The differences in decontamination efficacy across the seven test materials could be a result of the interactions of the different spore types with each substrate. The observed differences in log reductions and recovery rates of *B. anthracis, B. subtilis,* and *G. stearothermophilus* spores suggest that the test material composition and/or porosity affect decontamination efficacy and spore recovery. Although clumping or non-homogenous distribution of spores can occur during the inoculation and subsequent drying on the non-porous materials, it is assumed that the spores remain predominantly at the material surface. However, in addition to clumping or non-homogenous distribution of spores at the surface, the porous characteristics of the industrial carpet, bare pine wood, and painted concrete materials can also lead to spores penetrating and embedding into the test material. Such penetration and embedding of spores into the test materials could preclude the interaction of the decontaminant with the spores, thereby decreasing the potential for inactivation and affecting spore recovery. Therefore, the observed differences in log reductions of all three bacterial spore species across all materials may reflect chlorine dioxide-induced killing of spores at the material surface with little to no effect on spores that may have penetrated into the test material. For differences in recovery,

the penetration of bacterial spores into the porous test materials is plausible and differences in spore coat composition may affect the interactions of the three types of spores with the different materials. Further work in evaluating spore deposition and material matrix interactions would be useful to support these conclusions.

A qualitative evaluation of the performance of the CDG bench-scale unit showed that the control (not exposed to the CDG bench-scale 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 exposed to the CDG bench-scale unit, no growth was observed at 1 and 7 days. Based on these results, the CDG bench-scale unit inactivated both the biological indicators (containing *B. subtilis* and *G. stearothermophilus*) and sporesstrips (containing *B. atrophaeus*), all of which contained spore loads of approximately 1×10^6 spores per indicator or spore strip. On the basis of the biological indicator and spore strip results, the technology effectively inactivated the surrogate and spore strip organisms. However, the results obtained using porous materials indicates that the performance of the technology may be influenced by the matrix in which or on which the microorganisms and dispersed.

In an effort to assess whether viable spores remained in or on the coupons following decontamination and subsequent extraction, both control and decontaminated coupons were placed in tubes containing nutrient broth (as called for in the test/QA plan) and incubated for 7 days. The tubes were examined at 1 and 7 days for cloudiness as an indicator of growth. Besides the controls and decontaminated samples, growth was observed in many of the tubes containing blank coupons (excluding industrial carpet coupons); therefore these results were inconclusive. The unexpected growth may have been due to ineffective sterilization (the 70% isopropanol wipe did not sterilize the internal portions of the coupons) prior to inoculating the coupons. Identification of organisms causing cloudiness in the nutrient broth was beyond the scope of the verification testing and not specified in the test QA plan; therefore, analyses were not performed to identify the organisms that grew in the broth. Thus, the question of whether or not viable spores remained on the coupons after the initial extraction remains unanswered. In the case of industrial carpet coupons, however, no growth was observed when the carpet coupons were incubated following initial extraction of control and decontaminated coupons. The lack of growth was most likely due to the presence of an antimicrobial treatment that was incorporated into the carpet during manufacture.

The CDG bench-scale unit can be set up and ready for operation within minutes. The CDG bench-scale unit cannot measure parameters such as relative humidity and ClO_2 concentration. Within the glove box, the relative humidity was determined using a traceable hygrometer, and the ClO_2 was measured using a ClO_2 monitor. The effect of operator skill level on using the CDG bench-scale unit, while not verified in this test, should be minimal.

Chapter 8
References

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3. *Quality Management Plan (QMP) for the Building Decontamination Technology Center,* Version 2, prepared by Battelle, Columbus, Ohio, March 2004. (This reference is posted on the ETV web site at: http://www.epa.gov/etv/centers/center9.html.)