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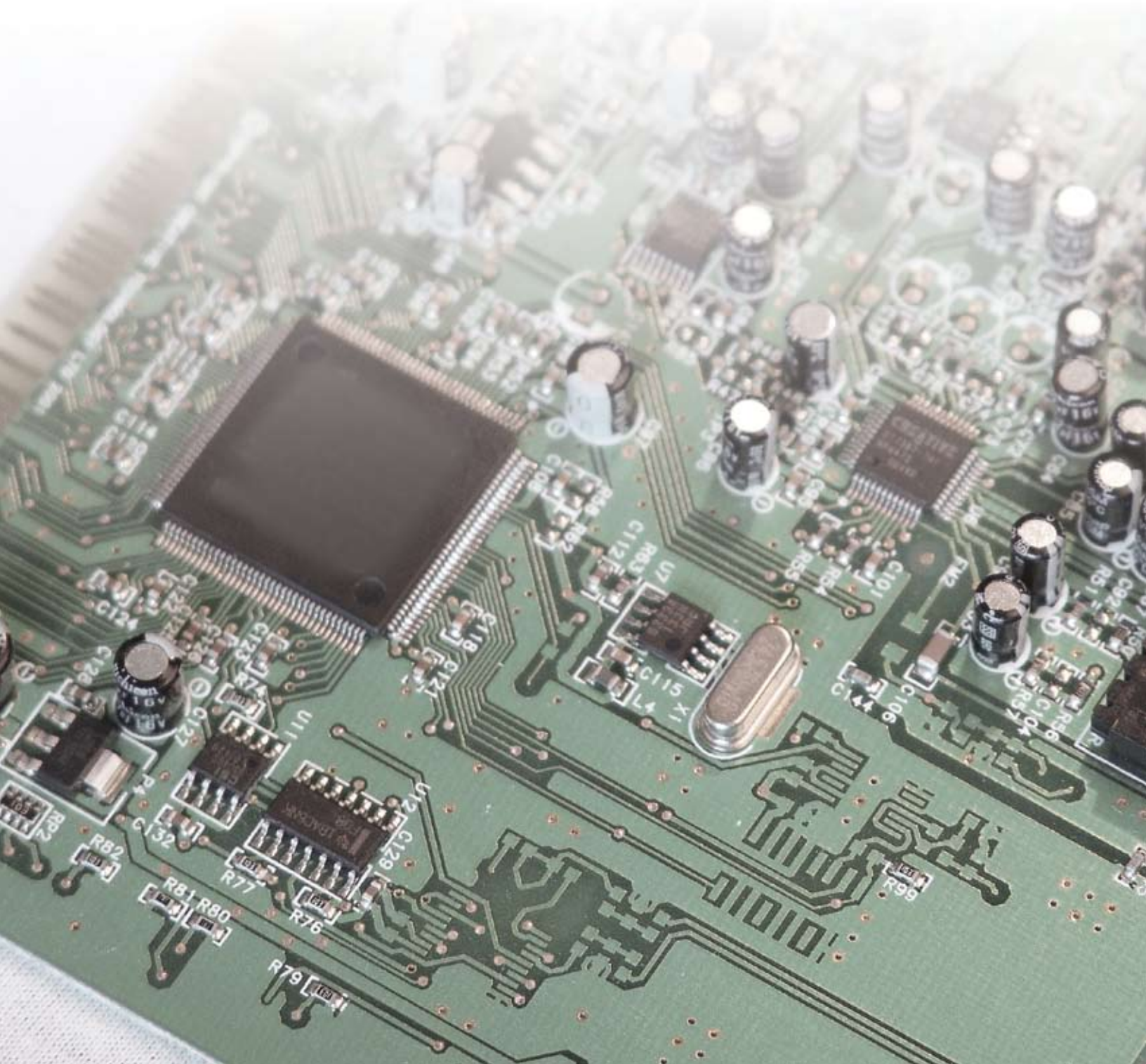
TECHNOLOGY EVALUATION REPORT ON

# Evaluation of Chlorine Dioxide Gas Generator

Office of Research and Development
National Homeland Security
Research Center



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# Technology Evaluation Report

# Evaluation of Sporicidal Decontamination Technology

# Sabre Technical Services Chlorine Dioxide Gas Generator

By

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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 (ORD) provides data and scientific 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.

In September 2002, EPA announced the formation of the National Homeland Security Research Center (NHSRC). The NHSRC is part of the Office of Research and Development; it manages, coordinates, and supports a variety of research and technical assistance efforts. These efforts are designed to provide appropriate, affordable, effective, and validated technologies and methods for addressing risks posed by chemical, biological, and radiological terrorist attacks. Research focuses on enhancing our ability to detect, contain, and clean up in the event of such attacks.

NHSRC's team of scientists and engineers is dedicated to understanding the terrorist threat, communicating the risks, and mitigating the results of attacks. Guided by the roadmap set forth in EPA's Strategic Plan for Homeland Security, NHSRC ensures rapid production and distribution of security related products.

The NHSRC has developed the Technology Testing and Evaluation Program (TTEP) in an effort to provide reliable information regarding the performance of homeland security related technologies. TTEP provides independent, quality assured performance information that is useful to decision makers in purchasing or applying the tested technologies. It provides potential users with unbiased, third-party information that can supplement vendor-provided information.

Stakeholder involvement in TTEP ensures that user needs and perspectives are incorporated into the test design so that useful performance information is produced for each of the tested technologies. The technology categories of interest include detection and monitoring, water treatment, air purification, decontamination, and computer modeling tools for use by those responsible for protecting buildings, drinking water supplies and infrastructure and for decontaminating structures and the outdoor environment.

The evaluation reported herein was prepared by Battelle as part of TTEP. Information on NHSRC and TTEP can be found at http://www.epa.gov/ordnhsrc/index.htm.

## Acknowledgments

The authors wish to acknowledge the support of all those who helped plan and conduct the evaluation, analyze the data, and prepare this report. We also would like to thank Mr. Carlton Kempter, U.S. Environmental Protection Agency's Office of Pesticide Programs, and Dr. Philip Koga, U.S. Army Research, Development, and Engineering Command, for their reviews of this report.

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## Abbreviations/Acronyms

| | |
|---|---|
| ATCC | American Type Culture Collection |
| C | Celsius |
| CFU | colony-forming unit |
| $ClO_2$ | chlorine dioxide |
| cm | centimeter |
| CT | concentration x time |
| EPA | U.S. Environmental Protection Agency |
| hr | hour |
| L | liter |
| min | minute |
| mL | milliliter |
| NHSRC | National Homeland Security Research Center |
| ppm | parts per million |
| QA | quality assurance |
| QC | quality control |
| QMP | quality management plan |
| RH | relative humidity |
| SD | standard deviation |
| TSA | technical systems audit |
| TTEP | Technology Testing and Evaluation Program |

# Executive Summary

The U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center (NHSRC), Technology Testing and Evaluation Program (TTEP) helps to protect human health and the environment from adverse impacts resulting from acts of terror. One way this is accomplished is by carrying out performance tests on homeland security technologies. Under TTEP, Battelle recently evaluated the performance of the Sabre Technical Services chlorine dioxide gas generator. The objective of testing the Sabre chlorine dioxide gas generator was to evaluate its ability to decontaminate *Bacillus anthracis* Ames spores and two surrogates, *Bacillus subtilis* (ATCC 19659) and G*eobacillus stearothermophilus* (ATCC 12980), on indoor surface materials.

The Sabre chlorine dioxide gas generator uses chlorine dioxide fumigation for decontamination of biological agents. The following performance characteristics of the Sabre chlorine dioxide gas generator were evaluated:

- Decontamination efficacy
  - —Quantitative assessment of the log reduction of viable organisms
  - —Qualitative assessment of residual spores
  - —Qualitative assessment of biological indicators and spore strips
- Qualitative assessment of material surface damage following decontamination.

The Sabre chlorine dioxide gas generator demonstrated statistically significant decontamination efficacy for *B. anthracis* Ames, *B. subtilis* (ATCC 19659), and G. *stearothermophilus* (ATCC 12980) on test coupons (1.9 cm by 7.5 cm) of seven materials:

- Industrial-grade carpet
- Bare wood (pine lumber)
- Glass
- Decorative laminate (Formica®, white matte finish)
- Galvanized metal ductwork
- Painted (latex, flat) wallboard paper
- Painted (latex, semi-gloss) concrete cinder block.

Within the limits of the evaluation methodology, the Sabre chlorine dioxide gas generator was effective at eliminating extractable, viable spores from all test coupons under the conditions of this test. After decontamination, no viable spores were extracted from any test coupons contaminated with either *B. anthracis* Ames, *B. subtilis*, or *G. stearothermophilus* spores. In more than 95% of the cases, qualitative analysis showed that no residual microbial organisms were present on the test coupons that had been decontaminated, extracted, and immersed in growth media. In only 5% of the test coupons, extractable, viable microorganisms (either added by inoculation, i.e., *B. anthracis* Ames, *B. subtilis*, or *G. stearothermophilus* spores, or endogenous) were found after decontamination and extraction. The only qualitative (visual) damage observed for any of the test coupons subjected to the Sabre chlorine dioxide gas generator was a bleaching effect on the industrial carpet.

# 1.0 Introduction

The U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center (NHSRC) is helping to protect human health and the environment from adverse impacts resulting from acts of terror. With an emphasis on decontamination and consequence management, water infrastructure protection, and threat and consequence assessment, NHRSC is working to develop tools and information that will help detect the intentional introduction of chemical or biological contaminants in buildings or water systems, contain these agents, decontaminate buildings and/or water systems, and dispose materials resulting from cleanups.

NHSRC's Technology Testing and Evaluation Program (TTEP) works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, scientists, and permitters; and with participation of individual technology developers in carrying out performance tests on homeland security technologies. In response to the needs of stakeholders, TTEP evaluates the performance of innovative homeland security technologies by developing test plans, conducting evaluations, collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure the generation of high quality data and defensible results. TTEP provides unbiased, third-party information supplementary to vendor-provided information that is useful to decision makers in purchasing or applying the evaluated technologies. Stakeholder involvement ensures that user needs and perspectives are incorporated into the evaluation design to produce useful performance information for each evaluated technology.

Under TTEP, Battelle recently evaluated the performance of the Sabre Technical Services chlorine dioxide gas generator. The objective of testing the Sabre chlorine dioxide gas generator was to evaluate its ability to decontaminate *Bacillus anthracis* Ames spores and two surrogates, *Bacillus subtilis* (ATCC 19659) and G*eobacillus stearothermophilus* (ATCC 12980), on representative indoor surface materials. This evaluation was conducted according to a peer-reviewed test/QA plan[(1)] that was developed according to the requirements of the quality management plan (QMP) for the TTEP program.[(2)] The following performance characteristics of the Sabre chlorine dioxide gas generator were evaluated:

- Decontamination efficacy
  - —Quantitative assessment of the log reduction of viable organisms
  - —Qualitative assessment of residual spores
  - —Qualitative assessment of biological indicators and spore strips
- Qualitative assessment of material surface damage following decontamination.

## 2.0 Technology Description

The following is a description of the Sabre chlorine dioxide gas generator based on information provided by the vendor. The information provided below was not confirmed in this evaluation.


**Figure 2-1. Sabre Technical Services Bench-Scale Chlorine Dioxide Gas Generator**

The Sabre chlorine dioxide gas generator includes a 20.3 cm x 20.3 cm base onto which a sparging column (15.2 cm x 15.2 cm, 91.4 cm high) is mounted. A solution is prepared on-site for each testing day in a 19 L container. The $ClO_2$-generating solution (3 L) is prepared by mixing household Clorox® bleach (5-6% sodium hypochlorite), 6N hydrochloric acid, 25% sodium chlorite, and distilled water. Following mixing, this solution can typically generate a $ClO_2$ concentration of 3,000 parts per million (ppm) and a chlorite concentration (ppm) that is at least half of the $ClO_2$ concentration.

The $ClO_2$-generating solution is pumped into the top of the sparging column using a peristaltic pump, and air from the test chamber is pumped as a counter-current against the flow of liquid in the sparging column. This air flow strips $ClO_2$ from the liquid into the air stream that is then pumped into the test chamber to establish the desired gaseous $ClO_2$ concentration. Liquid introduction from the reservoir of $ClO_2$-generating solution to the sparging column is initially at the rate of 60 milliliters per minute (mL/min); when the desired $ClO_2$ concentration in the test chamber is achieved, the liquid introduction into the sparging column is stopped. When needed, the $ClO_2$ concentration is increased in the test chamber by introducing more $ClO_2$-generating liquid into the sparging column and stripping the $ClO_2$ from the liquid with the counter-current air stream.

The spent liquid exiting the sparging column is collected in a reservoir containing 10% sodium hydroxide. The air from the chamber is recirculated into and out of the sparging column. Temperature for the decontamination is maintained in the range of 22 to 35°C, and the relative humidity (RH) is maintained above 70%. A nebulizer (supplied by Battelle for this evaluation) is used to establish the desired humidity level in the test chamber. Total treatment time is 3 hours (hr) at 3,000 ppm $ClO_2$ to achieve a concentration x time (CT) of 9,000 ppm-hr. Following decontamination, the $ClO_2$ in the test chamber is neutralized with activated carbon, while the liquid in the sparging column is treated with a 10% sodium hydroxide solution.

# 3.0 Quality Assurance/Quality Control

Quality assurance/quality control (QC) procedures were performed in accordance with the program QMP[2] and the test/QA plan[1] for this evaluation. QA/QC procedures are summarized below.

## 3.1 Equipment Calibration

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

## 3.2 Audits

### *3.2.1 Performance Evaluation Audit*

No performance evaluation audit was performed for biological agents and surrogates because quantitative standards for these biological materials do not exist. The confirmation procedure, controls, blanks, and method validation efforts support the biological evaluation results.

### *3.2.2 Technical Systems Audit*

Battelle QA staff conducted a technical systems audit (TSA) on June 9, 2005 to ensure that the evaluation was being conducted in accordance with the test/QA plan[1] and the QMP.[2] 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 Task Order Leader for response. None of the findings of the TSA required corrective action. TSA records were permanently stored with the TTEP QA Manager.

### *3.2.3 Data Quality Audit*

At least 10% of the data acquired during the evaluation were audited. A Battelle QA 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.

## 3.3 QA/QC Reporting

Each audit was documented in accordance with the QMP.[2] The results of the TSA were submitted to the EPA.

### 3.4 Data Review

Records generated in the evaluation received a QC/technical review and a QA review before they were used to calculate, evaluate, or report evaluation results. 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. This hard copy was returned to the Battelle staff member who stored the record.

## 4.0 Test Results

The Sabre chlorine dioxide gas generator was evaluated for decontamination efficacy against a biological warfare agent and two surrogates on seven indoor surfaces. The evaluation followed the EPA-approved *Technology Testing and Evaluation Program Test/QA Plan for Evaluation of Sporicidal Decontamination Technologies* (Version 1).[(1)] Various structural, decorative, and functional surfaces typically found inside an office building or a mass transit station were used to evaluate the sporicidal decontamination technology. The test surfaces (coupons measuring 1.9 cm x 7.5 cm) are listed below:

- Industrial-grade carpet
- Bare wood (pine lumber)
- Glass
- Decorative laminate (Formica®, white matte finish)
- Galvanized metal ductwork
- Painted (latex, flat) wallboard paper
- Painted (latex, semi-gloss) concrete cinder block.

The biological agent used to evaluate the sporicidal decontamination technology was *B. anthracis* Ames spores. To provide correlations with the *B. anthracis* results, the surrogates, *B. subtilis* (ATCC 19659) and *G. stearothermophilus* (ATCC 12980), were used.

The following sections summarize the results of these evaluations.

### 4.1 Decontamination Efficacy

No viable spores were found in extracts of the test coupons contaminated with *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus* spores after decontamination by the Sabre chlorine dioxide gas generator.

#### *4.1.1 Quantitative Assessment of the Log Reduction of Viable Organisms*

Decontamination efficacy was calculated as the log reduction in viable organisms achieved by the decontamination technology. Efficacy ($E$) was calculated for each of the triplicate coupons of each biological agent or surrogate and test coupon as:

$$E = log_{10} N/N'$$

where $N$ was the average number of viable organisms recovered from the triplicate positive control coupons (i.e., those inoculated, but not subjected to decontamination) and $N'$ was the number of viable organisms recovered from each test coupon after decontamination. If no viable organisms were

recovered from a test coupon after decontamination, the value 1 was substituted for *N′*. (This is a typical microbiological practice, since any number divided by zero is infinity.) Since the value 1 is greater than the observed value of zero, the estimated efficacy with this substitution becomes a lower bound for the true efficacy.

For all seven test materials, the results were statistically significant for decontamination (95% confidence that efficacy exceeds 0) (Tables 4-1, 4-2, and 4-3). For the quantitative assessment using dilution plating, no colony-forming units (CFU) of any of the three organisms used for the evaluation were observed in the extracts of the test coupons that had been inoculated then decontaminated. Calculating the corresponding efficacy resulted in mean log reduction values of at least 7.14, 6.73, and 6.25 for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus* spores, respectively.

Table 4-4 contains a summary of the mean log reductions obtained for each of the three organisms on each of the seven test materials. It should be noted that average percent recoveries of inoculated organisms as shown in Tables 4-1, 4-2, and 4-3 were, at times, well under 25%. In fact, the lowest average percent recovery was 2.34% [± 1.2 standard deviation (SD)]. These low recoveries of inoculated organisms may be attributed to interactions (adherence or sorption) to the material comprising each test coupon. These recoveries are similar to the recoveries achieved in previous TTEP testing.[3-5] Note also that in the present evaluation as well as previous studies,[3-5] recoveries obtained for *B. anthracis* Ames are generally not the same as the recoveries obtained for the surrogates, *B. subtilis* and *G. stearothermophilus*. To put the impact of a low percent recovery into perspective, in the case of the painted wallboard paper (Table 4-2) a total of 80,000,000 spores were placed or inoculated onto the painted wallboard paper and the number of spores recovered was 5,312,000. Thus, a considerable number of spores was recovered and was available for assessment of the decontamination. The standard deviations for the extractions were consistent, lending confidence in the reliability of the measurements. The mean (± SD) calculated concentration multiplied by time (CT) value for all of the decontamination runs for this evaluation was 9,205 ± 90.03 ppm-hr.

The lack of any observable (recoverable) organisms for decontaminated coupons prevented application of the statistical analysis approach used in previous decontamination technology evaluations.[3-5] Because no viable spores were extracted from any test coupon, the calculated efficacy values of the triplicate test coupons of each agent/surrogate and material were identical. As stated above, the calculated efficacy values were lower bounds based on replacing the number of viable organisms after decontamination in the denominator of the efficacy calculation with the value 1. Since these efficacy values are lower bounds, they do not provide information on the coupon-to-coupon variability in efficacy. Hence, they do not provide a basis for the analysis of variance approach used in previous evaluations[3-5] to determine if the observed efficacy values are (1) statistically significantly greater than zero and (2) statistically significantly different from each other (between agent/surrogates for a particular material or across materials within a specific agent/surrogate). The statistical significance between agent/surrogate and material groups could not be determined.

## Table 4-1. Decontamination of *Bacillus anthracis* Ames Spores[a]

| Test Material | Inoculum | Total Observed CFU | % Recovery | Efficacy |
|---|---|---|---|---|
| **Industrial-Grade Carpet** | | | | |
| Positive Control[b] | $9.53 \times 10^7$ | $5.21 \pm 0.95 \times 10^7$ | $54.7 \pm 9.98$ | - |
| Inoculated, Decontaminated[c] | $9.53 \times 10^7$ | 0 | 0 | $\geq 7.72$ |
| Laboratory Blank[d] | 0 | 0 | 0 | - |
| Procedural Blank[e] | 0 | 0 | 0 | - |
| **Bare Wood** | | | | |
| Positive Control | $1.04 \times 10^8$ | $1.37 \pm 0.02 \times 10^7$ | $13.2 \pm 0.15$ | - |
| Inoculated, Decontaminated | $1.04 \times 10^8$ | 0 | 0 | $\geq 7.14$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Glass** | | | | |
| Positive Control | $1.04 \times 10^8$ | $5.60 \pm 1.06 \times 10^7$ | $53.9 \pm 10.2$ | - |
| Inoculated, Decontaminated | $1.04 \times 10^8$ | 0 | 0 | $\geq 7.75$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Decorative Laminate** | | | | |
| Positive Control | $9.53 \times 10^7$ | $7.80 \pm 0.06 \times 10^7$ | $81.9 \pm 0.61$ | - |
| Inoculated, Decontaminated | $9.53 \times 10^7$ | 0 | 0 | $\geq 7.89$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Galvanized Metal Ductwork** | | | | |
| Positive Control | $9.53 \times 10^7$ | $6.93 \pm 0.12 \times 10^7$ | $72.8 \pm 1.25$ | - |
| Inoculated, Decontaminated | $9.53 \times 10^7$ | 0 | 0 | $\geq 7.84$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Painted Wallboard Paper** | | | | |
| Positive Control | $1.04 \times 10^8$ | $4.15 \pm 0.82 \times 10^7$ | $39.9 \pm 7.86$ | - |
| Inoculated Decontaminated | $1.04 \times 10^8$ | 0 | 0 | $\geq 7.62$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Painted Concrete** | | | | |
| Positive Control | $9.53 \times 10^7$ | $5.86 \pm 0.25 \times 10^7$ | $61.5 \pm 2.62$ | - |
| Inoculated Decontaminated | $9.53 \times 10^7$ | 0 | 0 | $\geq 7.77$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery (± SD), and mean efficacy (log reduction).
[b] Positive Control = inoculated, not decontaminated coupon
[c] Inoculated, Decontaminated = inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated coupon
"-" Not Applicable

## Table 4-2. Decontamination of *Bacillus subtilis* Spores[a]

| Test Material | Inoculum | Total Observed CFU | % Recovery | Efficacy |
|---|---|---|---|---|
| **Industrial-Grade Carpet** | | | | |
| Positive Control[b] | $7.67 \times 10^7$ | $8.21 \pm 1.32 \times 10^6$ | $10.7 \pm 1.72$ | - |
| Inoculated, Decontaminated[c] | $7.67 \times 10^7$ | 0 | 0 | $\geq 6.91$ |
| Laboratory Blank[d] | 0 | 0 | 0 | - |
| Procedural Blank[e] | 0 | 0 | 0 | - |
| **Bare Wood** | | | | |
| Positive Control | $8.00 \times 10^7$ | $5.91 \pm 0.94 \times 10^6$ | $7.39 \pm 1.17$ | - |
| Inoculated, Decontaminated | $8.00 \times 10^7$ | 0 | 0 | $\geq 6.77$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Glass** | | | | |
| Positive Control | $8.00 \times 10^7$ | $1.34 \pm 0.25 \times 10^7$ | $16.7 \pm 3.13$ | - |
| Inoculated, Decontaminated | $8.00 \times 10^7$ | 0 | 0 | $\geq 7.13$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Decorative Laminate** | | | | |
| Positive Control | $8.67 \times 10^7$ | $2.43 \pm 1.00 \times 10^7$ | $28.0 \pm 11.5$ | - |
| Inoculated, Decontaminated | $8.67 \times 10^7$ | 0 | 0 | $\geq 7.39$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Galvanized Metal Ductwork** | | | | |
| Positive Control | $8.67 \times 10^7$ | $1.19 \pm 0.13 \times 10^7$ | $13.7 \pm 1.50$ | - |
| Inoculated, Decontaminated | $8.67 \times 10^7$ | 0 | 0 | $\geq 7.08$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Painted Wallboard Paper** | | | | |
| Positive Control | $8.00 \times 10^7$ | $5.31 \pm 3.12 \times 10^6$ | $6.64 \pm 3.90$ | - |
| Inoculated, Decontaminated | $8.00 \times 10^7$ | 0 | 0 | $\geq 6.73$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Painted Concrete** | | | | |
| Positive Control | $7.67 \times 10^7$ | $1.94 \pm 0.12 \times 10^7$ | $25.3 \pm 1.56$ | - |
| Inoculated, Decontaminated | $7.67 \times 10^7$ | 0 | 0 | $\geq 7.29$ |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery (± SD), and mean efficacy (log reduction).
[b] Positive Control = inoculated, not decontaminated coupon
[c] Inoculated, Decontaminated = inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated coupon
"-" Not Applicable

**Table 4-3. Decontamination of *Geobacillus stearothermophilus* Spores[a]**

| Test Material | Inoculum | Total Observed CFU | % Recovery | Efficacy |
|---|---|---|---|---|
| **Industrial-Grade Carpet** | | | | |
| Positive Control[b] | $7.60 \times 10^7$ | $2.13 \pm 0.62 \times 10^7$ | 28.0 ± 8.21 | - |
| Inoculated, Decontaminated[c] | $7.60 \times 10^7$ | 0 | 0 | ≥ 7.33 |
| Laboratory Blank[d] | 0 | 0 | 0 | - |
| Procedural Blank[e] | 0 | 0 | 0 | - |
| **Bare Wood** | | | | |
| Positive Control | $7.60 \times 10^7$ | $1.78 \pm 0.88 \times 10^6$ | 2.34 ± 1.15 | - |
| Inoculated, Decontaminated | $7.60 \times 10^7$ | 0 | 0 | ≥ 6.25 |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Glass** | | | | |
| Positive Control | $7.60 \times 10^7$ | $1.18 \pm 0.47 \times 10^7$ | 15.5 ± 6.2 | - |
| Inoculated, Decontaminated | $7.60 \times 10^7$ | 0 | 0 | ≥ 7.07 |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Decorative Laminate** | | | | |
| Positive Control | $7.60 \times 10^7$ | $5.60 \pm 1.02 \times 10^6$ | 7.37 ± 1.35 | - |
| Inoculated, Decontaminated | $7.60 \times 10^7$ | 0 | 0 | ≥ 6.75 |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Galvanized Metal Ductwork** | | | | |
| Positive Control | $7.60 \times 10^7$ | $3.03 \pm 0.76 \times 10^7$ | 39.9 ± 10.0 | - |
| Inoculated, Decontaminated | $7.60 \times 10^7$ | 0 | 0 | ≥ 7.48 |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Painted Wallboard Paper** | | | | |
| Positive Control | $7.60 \times 10^7$ | $3.79 \pm 0.97 \times 10^6$ | 4.99 ± 1.28 | - |
| Inoculated, Decontaminated | $7.60 \times 10^7$ | 0 | 0 | ≥ 6.58 |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |
| **Painted Concrete** | | | | |
| Positive Control | $7.60 \times 10^7$ | $4.28 \pm 2.96 \times 10^6$ | 5.64 ± 3.90 | - |
| Inoculated, Decontaminated | $7.60 \times 10^7$ | 0 | 0 | ≥ 6.63 |
| Laboratory Blank | 0 | 0 | 0 | - |
| Procedural Blank | 0 | 0 | 0 | - |

[a] Data are expressed as mean (± SD) total number of spores (CFU) Observed, percent recovery (± SD), and mean efficacy (log reduction).
[b] Positive Control = inoculated, not decontaminated coupon
[c] Inoculated, Decontaminated = inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated coupon
"-" Not Applicable

**Table 4-4. Mean Log Reduction for Spores**

| Material | *B. anthracis* | *B. subtilis* | *G. stearothermophilus* |
|---|---|---|---|
| **Industrial-Grade Carpet** | ≥ 7.72 | ≥ 6.91 | ≥ 7.33 |
| **Bare Wood** | ≥ 7.14 | ≥ 6.77 | ≥ 6.25 |
| **Glass** | ≥ 7.75 | ≥ 7.13 | ≥ 7.07 |
| **Decorative Laminate** | ≥ 7.89 | ≥ 7.39 | ≥ 6.75 |
| **Galvanized Metal Ductwork** | ≥ 7.84 | ≥ 7.08 | ≥ 7.48 |
| **Painted Wallboard Paper** | ≥ 7.62 | ≥ 6.73 | ≥ 6.58 |
| **Painted Concrete** | ≥ 7.77 | ≥ 7.29 | ≥ 6.63 |

### *4.1.2 Qualitative Assessment of Residual Spores*

Based on previous decontamination studies[3-5], it was anticipated that 100% recovery of spores from the inoculated test coupons would not be achieved, and therefore viable spores would remain on the test coupons. As in previous decontamination studies, a qualitative assessment was performed to determine whether viable spores did in fact remain on the decontaminated test coupons. After the organisms were extracted from the test coupons for the efficacy determination, each coupon was transferred into tryptic soy broth culture medium and incubated at appropriate temperatures for growth. For liquid cultures in which a cloudy culture was observed, a loop of the liquid sample was removed and a streak plate on tryptic soy agar was performed. The intent of this assessment was to determine whether the observed growth in the liquid broth was due to the presence of a single organism (e.g., inoculated or endogenous organism) or a mixture of microorganisms that may or may not contain the species used for the inoculation.

Results from the liquid culture growth assessment of coupons at 1 and 7 days post-decontamination are provided in Tables 4-5, 4-6, and 4-7. In this qualitative assessment, a clear liquid medium indicates that no growth of the test organism or other microorganisms (endogenous to the test coupons) occurred, whereas cloudy cultures indicated microorganism growth.[*] For all samples in which cloudy cultures were observed, a loop of the liquid samples was removed and a streak plate on tryptic soy agar was performed. From these streak plates, various types of growth were observed: a pure culture of the organism inoculated onto the coupons, mixed bacterial growth (inoculated and endogenous microorganisms), or mold/bacteria mixtures. The percentage of streak plates displaying only growth from the inoculated organism was 57%, 32%, and 100% for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus*, respectively. The observed 100% growth of *G. stearothermophilus* is most likely a result of these liquid cultures and subsequent streak plates were cultures at 55-60ºC, thereby inhibiting the growth of most, if not all, endogenous microorganisms.

In the case of industrial grade carpet, no growth is observed for the positive controls for *B. anthracis* Ames (Table 4-5) and *G. stearothermophilus* (Table 4-7). This is likely due to the susceptibility of

[*] Results in Tables 4-5, 4-6, and 4-7 indicate that in most cases, chlorine dioxide killed all microorganisms (no growth was observed for either inoculated or endogenous organisms).

vegetative growth to an antibacterial compound in the carpet that leaches into the medium. The brand of industrial-grade carpet 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. Therefore, it is possible that, in the liquid cultures, FlorSept® will inhibit growth of vegetative cells derived from germination of the *B. anthracis* Ames spores and *G. stearothermophilus.* Similar results have been observed in previous testing with industrial carpet.[3-5] It is not clear as to why growth in the liquid cultures was observed for *B. subtilis*. However, similar observations were reported for both *B. subtilis* and *G. stearothermophilus* in a previous TTEP test.[4] It appears that under the conditions employed for the quantitative test, the FlorSept® may not be sporicidal since large numbers of viable *B. anthracis* Ames spores were extracted from the industrial-grade carpet and cultured on tryptic soy agar plates.

For the liquid culture assessments in which positive growth was observed, data were recorded as indicating that the culture consisted of a single organism (e.g., inoculated or endogenous organism) or a mixture of microorganisms. Representative photos of the different observations of microorganism growth of the liquid culture are shown in Figure 4-1. For the blank bare wood and painted wallboard paper, slime molds and other microorganisms completely covered the tryptic soy agar plate and were the predominant organisms that were endogenous to the test coupons.

### *4.1.3 Qualitative Assessment of Biological Indicators and Spore Strips*

The commercial spore strip containing *B. subtilis* var *niger* (*B. atrophaeus* ATCC 9372) spores on paper backing (manufactured by Raven Biological Laboratories), which were the same as those used during decontamination of U.S. Postal Service facilities contaminated with *B. anthracis*, and biological indicators containing *G. stearothermophilus* and *B. subtilis* (Apex Laboratories, Inc.) were included in each decontamination test. Each of these biological indicators contains a spore population of approximately $10^6$ spores on a stainless steel disc packaged in a Tyvek® envelope. For all evaluations, the control (not exposed to $ClO_2$) biological indicators and control spore strips exhibited cloudy cultures (suggesting bacterial growth) at 1 and 7 days. Clear cultures (suggesting no bacterial growth) were observed at 1 and 7 days for the biological indicators and spore strips subjected to $ClO_2$ exposure. A representation of the data from a single test day is shown in Table 4-8.

**Table 4-5. Liquid Culture Assessment of Coupons Inoculated with *Bacillus anthracis* Ames Spores following Extraction**

| Test Material | Day 1 | | | | Day 7 | | | |
|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | Bl | S1 | S2 | S3 | Bl |
| **Industrial-Grade Carpet** | | | | | | | | |
| Inoculated, Not Decontaminated | - | - | - | - | - | - | - | - |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Bare Wood** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | + | + | + | + | + |
| Inoculated, Decontaminated | - | - | + | - | - | - | + | - |
| **Glass** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | - | + | + | + | - |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Decorative Laminate** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | - | + | + | + | - |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Galvanized Metal Ductwork** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | - | + | + | + | - |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Painted Wallboard Paper** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | + | + | + | + | + |
| Inoculated, Decontaminated | + | - | - | - | + | - | - | - |
| **Painted Concrete** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | - | + | + | + | - |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |

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

**Table 4-6. Liquid Culture Assessment of Coupons Inoculated with *Bacillus subtilis* Spores following Extraction**

| Test Material | Day 1 | | | | Day 7 | | | |
|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | Bl | S1 | S2 | S3 | Bl |
| **Industrial-Grade Carpet** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | - | + | + | + | - |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Bare Wood** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | + | + | + | + | + |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Glass** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | + | + | + | + | + |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Decorative Laminate** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | + | + | + | + | + |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Galvanized Metal Ductwork** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | - | + | + | + | - |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Painted Wallboard Paper** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | + | + | + | + | + |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Painted Concrete** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | - | + | + | + | - |
| Inoculated, Decontaminated | - | - | - | - | - | + | - | - |

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

**Table 4-7. Liquid Culture Assessment of Coupons Inoculated with *Geobacillus stearothermophilus* Spores following Extraction**

| Test Material | Day 1 | | | | Day 7 | | | |
|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | Bl | S1 | S2 | S3 | Bl |
| **Industrial-Grade Carpet** | | | | | | | | |
| Inoculated, Not Decontaminated | - | - | - | - | - | - | - | - |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Bare Wood** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | + | + | + | + | + |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Glass** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | - | + | + | + | - |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Decorative Laminate** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | + | + | + | + | + |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Galvanized Metal Ductwork** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | - | + | + | + | - |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Painted Wallboard Paper** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | + | + | + | + | + |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |
| **Painted Concrete** | | | | | | | | |
| Inoculated, Not Decontaminated | + | + | + | + | + | + | + | + |
| Inoculated, Decontaminated | - | - | - | - | - | - | - | - |

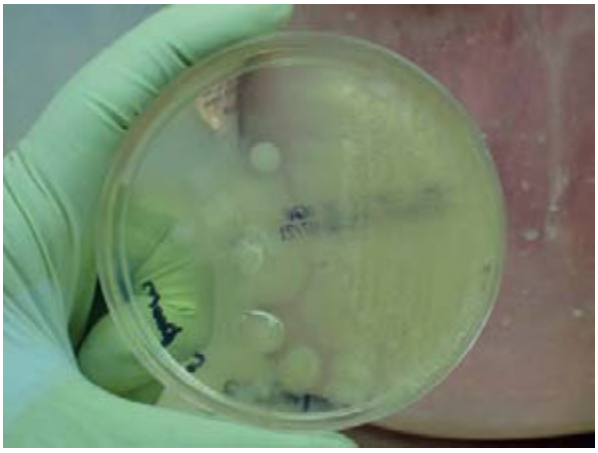
S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
Bl = Blank (not inoculated with *G. stearothermophilus* spores)
"+" = growth; "-" = no growth

## Table 4-8. Representative Liquid Cultures of Biological Indicators/Spore Strips

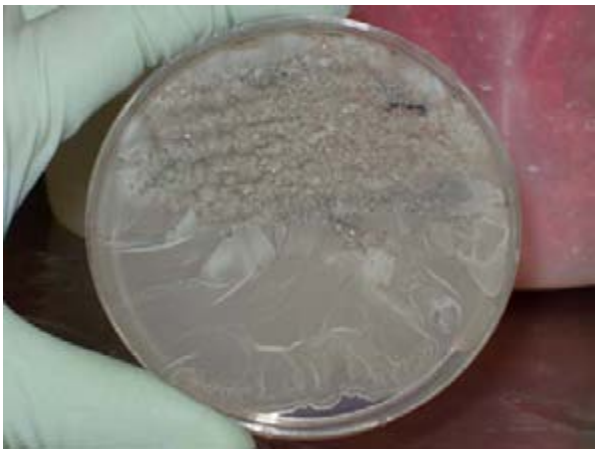
| Indicator (Organism) | Day 1 | | | Day 7 | | |
|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | S1 | S2 | S3 |
| **Biological Indicator (*B. subtilis* ATCC 19659)** | | | | | | |
| Positive Control[a.] | + | + | + | + | + | + |
| **Biological Indicator (*G. stearothermophilus* ATCC 12980)** | | | |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

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
"+" = cloudy culture; "-" = clear culture
a. Positive Control = inoculated, not decontaminated biological indicator or spore strip
b. Decontaminated = inoculated, decontaminated biological indicator or spore strip

**A. Homogeneous *B. anthracis* Culture from Inoculated Glass Control**



**B. Non-homogeneous Bacterial Culture from Inoculated Wood Control**



**C. Mixed Microbial Growth from an Inoculated Painted Wallboard Paper Control**

**Figure 4-1. Representative Plated Liquid Culture Assessment Samples**

## 4.2 Damage to Coupons

Before and after decontamination of the test coupons, the decontaminated coupons were visually inspected; and any obvious changes in the color, reflectivity, and apparent roughness of the coupon surfaces were recorded. No damage (e.g., change in surface texture, color) or visible change was observed during this evaluation to any of the test coupons with the exception of industrial carpet. Exposure to the $ClO_2$ appeared to produce a bleaching effect (all colors in the multicolor weave were affected) of the industrial carpet.

## 4.3 Other Factors

### *4.3.1 Operator Control*

On each day of testing, the $ClO_2$-generating solution was prepared fresh by mixing household Clorox® bleach (5-6% sodium hypochlorite), 6N hydrochloric acid, 25% sodium chlorite, and distilled water according to the vendor's instructions. Titrations determined that in the $ClO_2$-generating solutions prepared, the $ClO_2$ concentration ranged from 3642 to 4182 ppm and the chlorite concentration ranged from 2934 to 3912 ppm.

A HOBO U12 data logger (Onset Computer Corporation, Bourne, Massachusetts) was used for real-time monitoring of temperature and RH. Results from all experiments using the HOBO data logger indicated that the temperature and RH were maintained within the specified range of 22 to 35ºC and >70% RH. The initial RH was increased in the test chamber using a series of six nebulizers containing distilled water; following five minutes of nebulization, the RH was >70%. Figure 4-2 is a screen capture from the HOBO data logger software showing temperature (black line; left axis) and relative humidity (blue line; right axis) data from an actual 3-hr. test. Note that the temperature range for the test was 23.1º to 23.9º C. Figure 4-2 also shows that during testing, the introduction of the sparged $ClO_2$ into the test chamber promoted an increase in RH into the 75 to 90% range for the 3-hr contact time.

The Sabre chlorine dioxide gas generator was operated for a total of approximately 50 hr during this technology evaluation. During the technology evaluation a nebulizer system (supplied by Battelle) was utilized to achieve the appropriate RH (>70%) in the test chamber for each decontamination run. At the end of each run, the $ClO_2$ was neutralized by drawing it out of the test chamber through a cylindrical bed (approximately 90 cm high and 10 cm in diameter) of activated charcoal. The neutralization time took approximately 1 to 2 hr. No maintenance was required for the Sabre chlorine dioxide gas generator.

The $ClO_2$ concentration was monitored every 20 min during each decontamination run using a titration method[6]. Figure 4-3 shows $ClO_2$ concentrations in the test chamber during a typical decontamination run. During the evaluation of the technology, the Sabre chlorine dioxide gas generator was operated by manually regulating the introduction of $ClO_2$ into the exposure chamber. The periodic measurement of $ClO_2$ enabled the operator to maintain the desired concentration of $ClO_2$ within the test chamber. During the 3-hr contact time, a slight decrease in the $ClO_2$ concentration over time was counteracted by the operator, who manually introduced additional $ClO_2$ gas into the test chamber by temporarily turning on the Sabre chlorine dioxide gas generator. The decontamination and neutralization steps were run the day of the evaluation; therefore, a total run time from start to finish was approximately 6 hr.

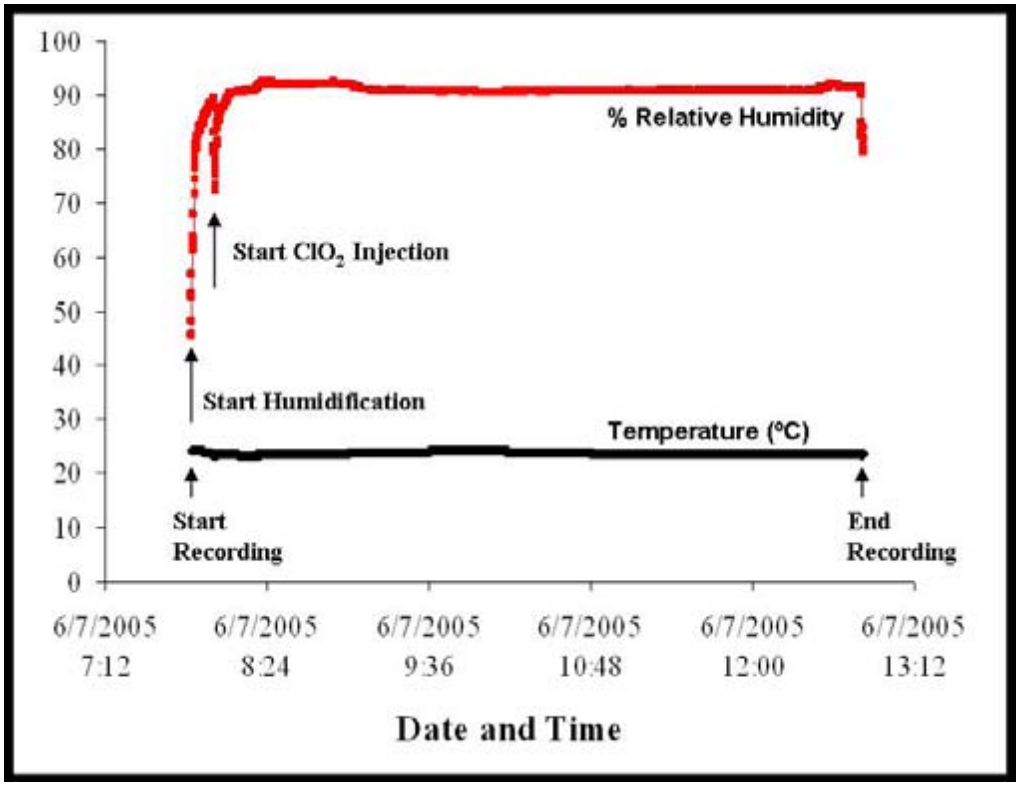



**Figure 4-2. Temperature and Relative Humidity Measurements from a Single Experiment**

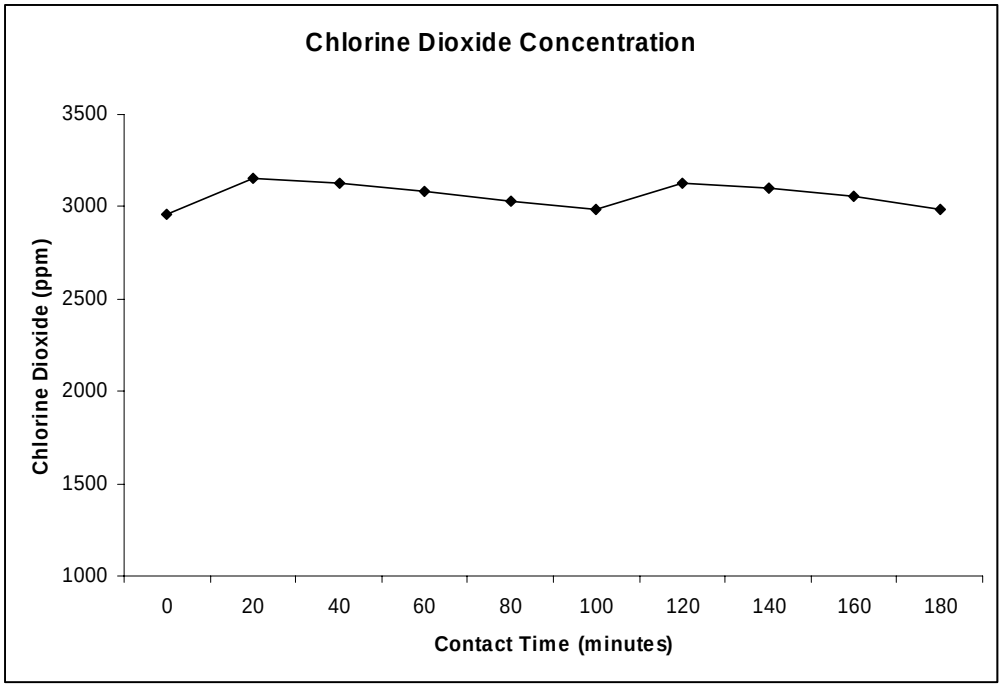


**Figure 4-3. Representative Chlorine Dioxide Concentration from a Single Experiment**

## 5.0 Performance Summary

- The results of all decontamination tests of the Sabre chlorine dioxide gas generator were the same – no viable spores were found in extracts of any test coupons contaminated with *B. anthracis* Ames, *B. subtilis* (ATCC 19659), and *G. stearothermophilus* (ATCC12980) spores after decontamination by the Sabre chlorine dioxide gas generator.

- With no viable spores extracted from any test coupon, mean log reduction values were unbounded but have been conservatively reported (by replacing the "0" value with "1" in the efficacy calculation) to be at least 7.14, 6.73, and 6.25 for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus* respectively (these are the maximum values that can be achieved within the limits of the evaluation).

- The Sabre chlorine dioxide gas generator demonstrated statistically significant decontamination efficacy for each of the three test organisms on all seven types of test coupons. Because of the lack of any detectable viable organisms after decontamination, it was not possible to determine or draw conclusions about the differential efficacy between agent/surrogates and material groups.

- While no viable spores were extracted from any test coupon after decontamination, differences in the initial number of spores on the coupons resulted in decontamination efficacy values varying across the seven test coupons for all three organisms. For *B. anthracis* Ames, there was a mean log reduction in viable spores from every test coupon of at least 7.1.

- To assess whether or not viable spores remained in or on the coupons following decontamination and subsequent extraction (to quantitate extractable, viable spores), both extracted control and extracted decontaminated coupons were placed in tryptic soy broth and incubated for 7 days. The contents of the tubes were examined at 1 and 7 days for cloudiness as an indicator of growth. In most cases, qualitative analysis showed that viable residual organisms were not present on the decontaminated and extracted coupons.

- However, in a few cases, this qualitative analysis indicated that viable inoculated or other endogenous microorganisms in or on the test coupons were still present after decontamination and extraction. For the decontaminated samples, cloudy cultures were observed in only three coupons (bare wood, painted wallboard paper, and painted concrete) at days 1 and 7 (see Tables 4-4 and 4-5). These results suggest that in most of the inoculated coupons subjected to $ClO_2$ exposure, no viable organisms were present on the coupons following decontamination and extraction.

- In a few cases, growth was observed in tubes containing blank and control coupons and in these instances, a sample of the culture was further analyzed by plating on tryptic soy agar and incubating the plates overnight.

- In most cases, the organisms from the liquid cultures that grew on the plates demonstrated morphology consistent with that of the organism used for inoculation. However, in some cases these samples demonstrated a range of microorganism growth, particularly in the case of the bare wood and painted wallboard paper test coupons, were mold and other microorganisms were found.

- The growth on the non-inoculated decontaminated blanks may have been due to ineffective disinfection (the 70% isopropanol wipe did not sterilize the internal portions of the coupons) prior to inoculating the coupons.

- A qualitative evaluation of the performance of the Sabre chlorine dioxide gas generator showed that the control (not exposed to $ClO_2$) biological indicators and spore strips cultured in tryptic soy broth displayed growth (cloudy) at both 1 and 7 days.

- Cultures of biological indicators and spore strips exposed to $ClO_2$ displayed no growth (clear) at 1 and 7 days. Based on these results, the Sabre chlorine dioxide gas generator inactivated in all cases spore loads of approximately $1 \times 10^6$ spores per indicator or spore strip.

- The only visual damage observed for any of the test coupons subjected to the Sabre chlorine dioxide gas generator was a bleaching effect on the industrial carpet.

## 6.0 References


1. *Technology Testing and Evaluation Program Test/QA Plan for Evaluation of Sporicidal Decontamination Technologies*, Version 1, Battelle, Columbus, Ohio, February 2005.

2. *Quality Management Plan (QMP) for the Technology Testing and Evaluation Program (TTEP)*, Version 1, Battelle, Columbus, Ohio, January 2005.

3. Rogers JV, Sabourin CL, Taylor ML, Riggs K, Choi YW, Richter WR, Rudnicki DC. BIOQUELL, Inc. Clarus™ Hydrogen Peroxide Gas Generator. Report submitted to U.S. EPA Environmental Technology Verification, Building Decontamination Technology Center, March 2004. (http://www.epa.gov/etv/verifications/vcenter11-1.html).

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6. American Water Works Association Method 4500-$ClO_2$E, "Chlorine Dioxide by Amperometric Method II", (modified).