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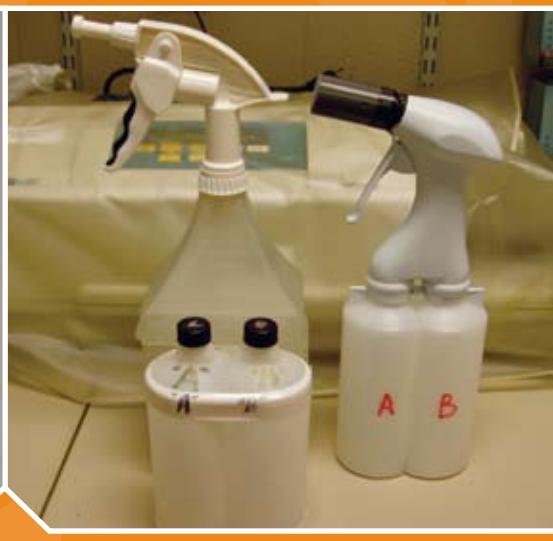
United States
Environmental Protection
Agency

Evaluation of Liquid and Foam Technologies for the Decontamination of *B. anthracis* and *B. subtilis* Spores on Building and Outdoor Materials

DioxiGuard™ (Frontier Pharmaceutical)
pH-Amended Bleach Calcium Polysulfide
CASCAD™ Surface Decontamination Foam (Allen-Vanguard)
Oxonia Active® (Ecolab Inc.)
Minncare® Cold Sterilant® (Minntech Corp.)
SanDes (DTI-Sweden AB)

TECHNOLOGY EVALUATION REPORT



Office of Research and Development
National Homeland Security Research Center

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Errata Sheet

Evaluation of Liquid and Foam Technologies for the Decontamination of B. anthracis and B. subtilis on Building and Outdoor Materials: Technology Evaluation Report (EPA/600/R-09/150), November 2009)

On page 40, Table 9.1, table section labeled “Painted wallboard paper”, in far right column with heading “Decontamination Efficacy ± CI”, the number should be “≥7.42±0.28”

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National Homeland Security Research Center, Decontamination and Consequence Management Division

Notice

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development's National Homeland Security Research Center (NHSRC), funded, directed, and managed this technology evaluation through a Blanket Purchase Agreement (BPA) under General Services Administration contract number GS23F0011L-3 with Battelle. This report has been peer and administratively reviewed and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use of a specific product.

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 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.

In September 2002, EPA announced the formation of the National Homeland Security Research Center (NHSRC). The NHSRC, part of the Office of Research and Development, manages, coordinates, supports, and conducts 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 decontaminate in the event of such 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 created 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. TTEP provides potential users with unbiased, third-party information that can supplement vendor-provided information. Stakeholder involvement 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. Additionally, environmental persistence information is also important for containment and decontamination decisions.

The evaluation reported herein was conducted by Battelle as part of the TTEP program. Information on NHSRC and TTEP can be found at http://www.epa.gov/nhsrc/index.html.

Acknowledgments

The following Battelle staff contributed to this report: Thomas J. Kelly, Young W. Choi, James V. Rogers, Karen B. Riggs, and Zachary J. Willenberg. 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 J. Carlton Kempter, Frank W. Schaefer III, and Anthony Zimmer, all of the EPA, and Martin Hamilton of Montana State University, for their reviews of the test/QA plan for this evaluation, and J. Carlton Kempter, Michael Ottlinger, and Worth Calfee of EPA for their reviews of this Technology Evaluation Report.

Contents

Tables

Abbreviations/Acronyms

ATCC	American Type Culture Collection
BBRC	Battelle Biomedical Research Center
BSC	biosafety cabinet
C	Celsius
CaS_x	calcium polysulfide
CFU(s)	colony-forming unit(s)
CI	confidence interval
ClO_2	chlorine dioxide
cm	centimeter
D/E	Dey/Engley
EPA	U.S. Environmental Protection Agency
g	gram
h	horizontal
hr	hour
L	liter
min	minute
mL	milliliter
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
NS	neutralization solution
ORD	U.S. EPA Office of Research and Development
PBS	phosphate-buffered saline
ppm	parts per million
psi	pounds per square inch
QA	quality assurance
QC	quality control
QMP	quality management plan
RH	relative humidity
rpm	revolutions per minute
SD	standard deviation
SDF	surface decontamination foam
SE	standard error
SFW	sterile filtered water (cell-culture grade)
STS	sodium thiosulfate
TOPO	Task Order Project Officer
TSA	technical systems audit
TTEP	Technology Testing and Evaluation Program
v	vertical
wt	weight

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 of terrorist acts by carrying out performance tests on homeland security technologies. TTEP recently evaluated the performance of liquid and foam decontamination technologies under vendor-specified application conditions to decontaminate test coupons prepared from the materials listed below. These materials include building materials typical of surfaces found in an office building or transportation terminal and outdoor materials such as soil that could become contaminated with biological agents. The first seven materials listed below were used as "indoor" surfaces, and the last four as "outdoor" surfaces, with bare wood and glass being common to the two sets of materials (in one case painted cinder block replaced glass as an outdoor material). For testing, each coupon was placed in an appropriate orientation (vertical or horizontal) for typical use of the material; for some materials either orientation may be appropriate but only one was chosen for testing. The orientation used in testing is indicated in the listing as vertical (v) or horizontal (h):

- Industrial-grade carpet (h)
- Decorative laminate (h)
- Galvanized metal ductwork (v)
- Painted (latex, flat) wallboard paper (v)
- Painted (latex, semi-gloss) cinder block (v)
- Bare wood (pine lumber) (v)
- Glass (v)
- Unpainted concrete (h)
- Topsoil (h).

Test coupons were 1.9 cm by 7.5 cm, except for topsoil which was prepared by filling a Parafilm®- lined 3.5 cm diameter by 1 cm deep petri dish with uncompacted soil.

For testing, coupons were "contaminated" by spiking with the biological warfare agent, *Bacillus anthracis* Ames, or a surrogate, *B. subtilis* (American Type Culture Collection [ATCC] 19659). The technologies evaluated for their ability to inactivate *B. anthracis Ames* or *B. subtilis* on test coupons of either the seven indoor or the four outdoor surface materials were:

- Frontier Pharmaceutical's DioxiGuard™
- pH-Amended bleach (Clorox® bleach diluted with sterile filtered water and 5% acetic acid to obtain pH-amended solution)
- Calcium polysulfide (lime sulfur) solution at 5.8% (wt/wt) (i.e., diluted with sterile filtered water by a factor of 5 from the original 29% solution)
- Allen-Vanguard's CASCAD™ Surface Decontamination Foam (SDF)
- Ecolab Inc.'s Oxonia Active®
- Minntech Corp.'s Minncare® Cold Sterilant
- DTI-Sweden AB's SanDes.

With the exception of pH-amended bleach and calcium polysulfide, each decontaminant was tested using the application apparatus and conditions provided by the respective vendor, and according to the vendor's instructions. For pH-amended bleach and calcium polysulfide, no single vendor exists. Those two products were tested for decontamination of outdoor surfaces using a conventional hand-pumped household garden sprayer to apply the product. Technical descriptions and preparation and application procedures (including the spray device, contact time, and reapplication rate) for all the decontaminants tested are included as appendices to this report. Spray distance, humidity, and temperature were the same for all applications.

The following performance characteristics of the decontamination technologies were evaluated:

- Decontamination efficacy
  - Quantitative assessment of the decontamination efficacy for viable organisms (log reduction)
  - Qualitative assessment for residual spores on the test coupons
- Qualitative assessment of material surface damage following decontamination.

Summary results:

Results for the seven decontaminants tested are summarized in the following paragraphs. Table ES-1 lists the quantitative efficacy results for all decontaminants on all test materials.

DioxiGuard™ - This decontaminant was applied to the test coupons until they were fully wetted, and no reapplication was done. The total contact time before spore extraction was 10 minutes. Quantitative efficacy was 2.6 log reduction or less for *B. anthracis* and 0.87 log reduction or less for *B. subtilis*, on the seven indoor materials. All materials showed the presence of viable spores after decontamination, consistent with the quantitative efficacy results. No damage was observed on any of the materials from DioxiGuard™ immediately after quantitative efficacy testing, or seven days later after completion of the qualitative assessment for residual spores.

pH-Amended bleach - This decontaminant was applied to the test coupons until they were fully wetted, and the product was reapplied if coupons became dry (only one such reapplication was needed, on painted cinder block). The total contact time before spore extraction was 60 minutes. Quantitative efficacy for *B. anthracis* Ames ranged from a log reduction of 7.31 on painted cinder block, to 4.99 on unpainted concrete, to 1.47 on topsoil and 0.81 on bare pine wood. Quantitative efficacy for *B. subtilis* ranged from a log reduction of ≥ 7.22 on painted cinder block, to ≥ 5.63 on unpainted concrete, to

0.18 on topsoil and 0.68 on bare pine wood. Most materials showed the presence of viable spores after decontamination, except that no growth of *B. anthracis* or *B. subtilis* was found on painted cinder block, and none of *B. subtilis* was found on unpainted concrete; these results are consistent with the quantitative efficacy results. No damage was observed on any of the materials from pH-amended bleach immediately after quantitative efficacy testing, or seven days later after completion of the qualitative assessment for residual spores.

Calcium polysulfide - This decontaminant was applied to the test coupons until they were fully wetted, and then reapplied 30 minutes after the initial application. The total contact time before spore extraction was 60 minutes. Quantitative efficacy on the four outdoor materials was very low with both test organisms, with a maximum log reduction of 0.24 for *B. anthracis* Ames (on unpainted concrete) and of 0.33 for *B. subtilis* (on glass). All materials showed the qualitative presence of viable organisms after decontamination, consistent with the low efficacy results. Decontamination with calcium polysulfide left a grayish surface residue on glass and topsoil coupons; the presence of such a residue on bare wood and unpainted concrete could not be confirmed due to the surface characteristics of those materials. The residue remained on the glass surfaces throughout agitation for spore recovery and the subsequent seven-day qualitative assessment for residual spores.

CASCAD™ SDF - This decontaminant was applied to the test coupons until they were fully covered with the foam, and no reapplication was done. The total contact time before spore extraction was 30 minutes. Quantitative efficacy was greater than 7.0 log reduction for both *B. anthracis* and *B. subtilis* on five of the seven indoor materials. Lower efficacy values were found only on painted wallboard paper and bare pine wood. Efficacy results for *B. anthracis* and *B. subtilis* on painted wallboard paper were 4.82 and ≥ 6.14 log reduction, respectively; on bare pine wood the corresponding efficacy results were 2.77 and 1.28 log reduction, respectively. Only those two materials showed the presence of viable organisms after decontamination, consistent with the quantitative efficacy results. The only materials damage observed from decontamination with CASCAD™ SDF was that the top coat of paint peeled away from the primer coat on painted cinder block coupons.

Oxonia Active® - This decontaminant was applied to the test coupons until they were fully wetted, and then reapplied every 10 minutes after the initial application. The total contact time before spore extraction was 60 minutes. Quantitative efficacy of Oxonia Active® was 7.0 log reduction or greater on six of the seven indoor test materials for *B. anthracis* and on five of those seven test materials for *B. subtilis*. Lower efficacy values were found only on bare pine wood and painted wallboard paper. Efficacy results for *B. anthracis* and *B. subtilis* on bare pine wood were 4.64 and 5.15 log reduction, respectively; on painted wallboard paper the efficacy for *B. subtilis* was ≥ 6.69 log reduction. No viable spores were found on any decontaminated coupon after either one or seven days of incubation, consistent with the quantitative efficacy results. No visible damage was observed on any of the test materials after 60 minutes contact time with Oxonia Active®, or seven days later after completion of the qualitative assessment of residual spores.

Minncare® Cold Sterilant - This decontaminant was applied to the test coupons until they were fully wetted, and no reapplication was done. The total contact time before spore extraction was 30 minutes for coupons of industrial-grade carpet, painted cinder block, and bare pine wood, and 10 minutes for coupons of decorative laminate, galvanized metal ductwork, painted wallboard paper, and glass. Quantitative efficacy of Minncare® Cold Sterilant was 7.5 log reduction or greater on six of the seven indoor test materials for both *B. anthracis* and *B. subtilis*. Lower efficacy values were found only on bare pine wood, for which efficacy results for *B. anthracis* and *B. subtilis* were 5.40 and 6.00 log reduction, respectively. No viable spores were found on any decontaminated coupon after either one or seven days of incubation, consistent with the quantitative efficacy results. No visible damage was observed on any of the test materials after either 10 or 30 minutes contact time with Minncare® Cold Sterilant, or seven days later after completion of the qualitative assessment of residual spores.

SanDes - This decontaminant was applied to the test coupons until they were fully wetted, and reapplication was done at 10, 20, 30, and 60 minutes after the initial application. The total contact time before spore extraction was 70 minutes. Quantitative efficacy of SanDes was less than 1.0 log reduction for six of the seven indoor materials for both *B. anthracis* and *B. subtilis*. The exceptions were for *B. anthracis* on glass (4.65 log reduction) and for *B. subtilis* on decorative laminate (1.37 log reduction). Viable spores were found on all decontaminated coupons after one day and seven days of incubation, consistent with the low quantitative efficacy results. No visible damage was observed on any of the test materials after 70 minutes contact time with SanDes, or seven days later after completion of the qualitative assessment of residual spores.

Table ES-1. Summary of Quantitative Efficacy by Decontaminant and Test Material

Table ES-1a

Test Material	Quantitative Efficacy (log reduction) for *Bacillus anthracis* Ames /*Bacillus subtilis*			
	DioxiGuard™	pH-Amended Bleach	Calcium Polysulfide	CASCAD™ SDF
Industrial-Grade Carpet	1.83 / 0.87	--	--	7.40 / ≥ 7.62
Decorative Laminate	2.59 / 0.30	--	--	7.40 / ≥ 7.30
Galvanized Metal Ductwork	0.95 / -0.66	--	--	≥ 7.59 / ≥ 7.60
Painted Wallboard Paper	0.70 / 0.73	--	--	4.82 / ≥ 6.14
Painted Cinder Block	1.77 / -0.49	7.31 / ≥ 7.22	--	≥ 7.84 / ≥ 7.05
Bare Pine Wood	0.75 / 0.31	0.81 / 0.68	0.05 / -0.12	2.77 / 1.28
Glass	2.53 / 0.30	--	-0.04 / 0.33	≥ 7.85 / ≥ 7.51
Unpainted Concrete	--	4.99 / ≥ 5.63	0.24 / 0.12	--
Topsoil	--	1.47 / 0.18	0.21 / 0.21	--

-- Decontaminant not tested with this material.

Table ES-1b

Test Material	Quantitative Efficacy (log reduction) for *Bacillus anthracis* Ames /*Bacillus subtilis*		
	Oxonia Active®	Minncare® Cold Sterilant	SanDes
Industrial-Grade Carpet	7.00 / ≥ 7.42	≥ 7.82 / ≥ 7.91	0.13 / 0.59
Decorative Laminate	≥ 7.61 / ≥ 7.66	≥ 7.58 / ≥ 7.87	0.18 / 1.37
Galvanized Metal Ductwork	≥ 7.87 /≥ 7.64	≥ 7.80 / ≥ 7.89	0.09 / 0.76
Painted Wallboard Paper	≥ 7.42 / ≥ 6.69	≥ 7.53 / ≥ 7.46	0.19 / 0.60
Painted Cinder Block	≥ 7.86 / ≥ 7.29	≥ 8.08 / ≥ 7.93	0.33 / 0.51
Bare Pine Wood	4.64 / 5.15	5.40 / 6.00	0.39 / 0.65
Glass	≥ 7.72 / ≥ 7.03	≥ 7.75 / ≥ 7.95	4.65 / 0.22
Unpainted Concrete	--	--	--
Topsoil	--	--	--

-- Decontaminant not tested with this material.

1.0 Introduction

NHSRC's TTEP works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, scientists, engineers, 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.

TTEP evaluated the performance of liquid and foam sporicidal decontamination technologies using vendor-specified application conditions. The primary objective of testing sporicidal decontamination technologies was to evaluate their ability to inactivate *Bacillus anthracis* (Ames) spores and *Bacillus subtilis* (ATCC 19659) spores on representative indoor or outdoor surface materials. These technologies were selected for testing based on existing information or data indicating potential sporicidal efficacy on building or outdoor materials. Such information or data could include EPA registration as a sterilant on hard non-porous surfaces, or data showing sporicidal efficacy on different materials or under different test conditions. The technologies, which were applied using vendor-specified procedures and evaluated on test coupons of either seven indoor or four outdoor surface materials, included the following:

- Frontier Pharmaceutical's DioxiGuard™
- pH-Amended bleach (Clorox® bleach diluted with certified cell-culture grade sterile filtered water (SFW) and 5% acetic acid to obtain pH-amended solution)
- Calcium polysulfide (lime sulfur) solution at 5.8% (wt/wt) (i.e., diluted with SFW by a factor of 5 from the original 29% solution)
- Allen-Vanguard's CASCAD™ Surface Decontamination Foam (SDF)
- Ecolab Inc.'s Oxonia Active®
- Minntech Corp.'s Minncare® Cold Sterilant
- DTI-Sweden AB's SanDes.

Testing was performed using application procedures specified by each vendor, or (for pH-amended bleach and calcium polysulfide) developed by EPA and Battelle based on likely use of these decontaminants. The application procedures for all decontaminants are included as appendices to this report. The decontaminant test procedures are specified in 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 decontamination technologies were evaluated:

- Decontamination efficacy
  - Quantitative assessment of the decontamination efficacy for viable organisms
  - Qualitative assessment for residual spores
- Qualitative assessment of material surface damage following decontamination.

2.0
Technology Description

Table 2-1 lists the decontamination technologies and the contact times used. The information on product composition in Table 2-1 is based on vendor-provided information (except for pH-amended bleach and calcium polysulfide) and was not confirmed in this evaluation. Detailed technology descriptions and the application procedures used are included as Appendices A to G.

Table 2-1. Technology Information

Product	Vendor	General Description/ Active Ingredients	Components	EPA Registration[a]	Contact Time (min)
DioxiGuard™	Frontier Pharmaceutical	Chlorine dioxide	Sodium chlorite solution; acid solution; contains alcohol.	None	10
Bleach	Clorox®	Sodium hypochlorite, hypochlorous acid	Sodium hypochlorite 5-6% (pH-amended by adding acetic acid 5%)[b]	5813-1 (disinfectant)	60
Calcium polysulfide (lime sulfur)	VGS, Inc.	Calcium polysulfide	5.8% CaS_x[c]	769-558 (fungicide, insecticide, miticide)	60
CASCAD™ SDF	Allen-Vanguard	Hypochlorite	Sodium myristyl sulfate 10-30%, sodium (C14-16) olefin sulfonate 10-30%; ethanol denatured 3-9%; alcohols (C10-16) 5-10%, sodium sulfate 3-7%; sodium xylene sulfonate 1-5%; proprietary mixture of sodium and ammonia salt along with co-solvent >9%; dichloroisocyanuric acid, sodium salt 48-85%; sodium tetraborate 3-7%; sodium carbonate 10-15%.	None	30
Oxonia Active®	Ecolab Inc.	Peroxide/ peroxyacetic acid	Hydrogen peroxide 27.5%, peroxyacetic acid 5.8%.	1677-129 (sterilant, disinfectant, sanitizer)	60
Minncare® Cold Sterilant	Minntech Corp.	Peroxide/ peroxyacetic acid	Stabilized mixture of 4.5% peroxyacetic acid, 22% hydrogen peroxide, and acetic acid.	52252-4 (sterilant, disinfectant, sanitizer)	10 or 30[d]
SanDes	DTI-Sweden AB	Chlorine dioxide	1,500 ppm ClO_2.	None	70

[a] Registered with the EPA Office of Pesticide Programs (OPP). Registration indicates EPA/OPP has evaluated the antimicrobial pesticide to show its effectiveness and that it will not have unreasonable adverse effects on humans, the environment, and non-target species, and EPA/OPP has issued a registration or license for use in the United States. Note: No product is registered for use against *B. anthracis*.

[b] Using procedure recommended by TTEP stakeholders, 5% acetic acid was added to the household bleach to obtain a pH-amended bleach solution. The solution was prepared using 9.4 parts SFW, 1 part commercial household bleach, and 1 part 5% glacial acetic acid to yield a solution having a mean pH of 6.81 ± 0.15 and a mean total chlorine content of 6,215 ± 212 ppm. This "pH-amended bleach" was evaluated for sporicidal activity.

[c] Solution tested was a 1:5 dilution (with SFW) of commercially supplied 29.0% (wt/wt) product.

[d] 10 minutes for decorative laminate, glass, wallboard paper, and metal ductwork; 30 minutes for carpet, cinder block, and bare wood.

Note that Clorox® bleach is registered as a disinfectant, but pH-amended bleach is not.

Below are brief physical descriptions of the decontamination technologies (their form, appearance as received) and preparation instructions. Greater detail on product composition, preparation, and application procedures is provided in Appendices A to G.

- DioxiGuard™ – This two component product was mixed in equal volumes at the time of use. Component A was a sodium chlorite solution and component B was an acid solution. The vendor-provided applicator was a dual spray bottle containing the two component solutions in separate compartments within the bottle, and designed to deliver equal portions of the two reagent solutions through a single spray nozzle to produce the ClO_2 decontaminant.
- pH-Amended bleach – Clorox® bleach purchased in a one gallon container from a local retail store. The diluted, pH-adjusted final solution was applied using a hand-pressurized portable garden sprayer.
- Calcium polysulfide (lime sulfur) − This product was a red clear liquid consisting of 29.0% by weight calcium polysulfide (CaS_x) in water. This solution was diluted by a factor of five with SFW to produce a 5.8% by weight solution for use in testing. The diluted solution was applied using a hand-pressurized portable garden sprayer.
- CASCAD™ SDF – One CASCAD™ solution was prepared by diluting 31.2 g of GP2100 (decontaminant) to 300 mL with SFW, and the other solution was made by diluting 7.2 g of GPB-2100 (buffer) and 18 mL of GCE2000 (surfactant) to 300 mL with SFW. The application process used a dual spray bottle designed to deliver equal portions of the two solutions through a single spray nozzle equipped with a diffuser mesh to produce the foam.
- Oxonia Active® – A decontaminant solution containing 5,000 ppm peroxyacetic acid was prepared fresh daily by diluting 76 mL of Oxonia Active® to 1 L with SFW water. The diluted solution was applied using a hand-pressurized portable garden sprayer.
- Minncare® – A 10% solution of Minncare® Cold Sterilant was prepared fresh shortly before use on each day of testing, by diluting 1 part of the Cold Sterilant with 9 parts of SFW. The 10% Cold Sterilant solution was applied to test coupons using a hand-held plastic spray bottle.
- SanDes – This product was an aqueous solution of 1,500 ppm ClO_2, and was used without dilution. The product was applied to test coupons using a small push-button spray attachment that replaced the cap on a bottle of SanDes.

3.0
Summary of Test Procedures

Test procedures were performed in accordance with the test/QA plan[1] and are briefly summarized here.

3.1 Preparation and Analysis of Test Coupons

B. anthracis Ames and *B. subtilis* spores were spiked onto test coupons in an appropriate biosafety cabinet (BSC-II or -III) according to established Battelle procedures.[3-8] Spiked coupons were prepared fresh for each day of experimental work, by placing coupons flat in the BSC and spiking at approximately 1 x 10^8 colony-forming units (CFUs) per coupon. This spiking was accomplished by dispensing a 100-µL aliquot of a spore stock suspension (approximately 1 x 10^9 CFUs/mL) using a micropipette as 10 droplets (each of 10 µL volume) across the surface of the coupon. This approach provided more uniform distribution of spores across the coupon surface than would be obtained through a single drop of the suspension. After spiking, the coupons remained undisturbed overnight in a BSC to dry. Except in testing of DioxiGuard™ with *B. anthracis*, and in testing of pH-amended bleach with both *B. anthracis* and *B. subtilis*, blank (unspiked) coupons were held in a separate cabinet from the spiked coupons, to avoid contamination of the blanks with spores during the drying period.

On the day following spiking, coupons intended for decontamination (including blanks and controls) were transferred into a glove box (test chamber) where the decontamination technology was applied using the apparatus and application conditions specified in the appendices of this report. The decontamination spray distance of 30 cm (12 inches), humidity (< 70% relative humidity), and temperature (20 to 25 °C) were the same for all applications. For most decontaminants tested, the amount of decontaminant, contact time, spray pressure, application and reapplication procedures, etc., were as specified by the vendor. For pH-amended bleach and calcium polysulfide, these parameters were chosen by EPA and Battelle based on common use of these products and reasonable application procedures for small-scale evaluation.

The materials used for test coupons were:

- Industrial-grade carpet[a]
- Decorative laminate
- Galvanized metal ductwork
- Painted (latex, flat) wallboard paper
- Painted (latex, semi-gloss) concrete cinder block
- Bare wood (pine lumber)
- Glass
- Unpainted concrete
- Topsoil.

With the exception of topsoil, test coupons were sterilized before use by gamma irradiation (carpet, laminate, wallboard paper, cinder block, bare wood) or autoclaving (metal ductwork, glass, unpainted concrete).

The use of topsoil as a test coupon required development of techniques to assure adequate recovery of spiked *B. anthracis* or *B. subtilis* spores, and the absence of interference from native soil microorganisms in counting of recovered spores. A heat shock procedure was found to minimize interference by native microorganisms. Specifically, spiked or blank topsoil was extracted in phosphate-buffered saline (PBS) solution containing Triton X surfactant, and the recovered supernatant was heat-shocked in a water bath at 65 °C for one hour before being serially diluted and plated. Topsoil samples spiked with *B. anthracis* or *B. subtilis* spores each showed the presence of a single homogeneous species, with all colonies of uniform size and morphologically distinctive for the respective *Bacillus* species. Blank topsoil samples showed growth of colonies of other, native, *Bacillus* species, which were not seen with the spiked topsoil samples. Consequently, although topsoil blanks showed some growth, that growth did not occur with extracts of spiked topsoil, so no interference existed in terms of counting recovered spores. The mechanism by which growth of native *Bacillus* is suppressed in the extracts of spiked topsoil was not investigated, but may involve monopolization of nutrients by the large numbers of spiked spores. By this procedure, the recovery of spores spiked onto topsoil was found to be approximately 50% for *B. anthracis* and approximately 34% for *B. subtilis*. The heat shock procedure for use of topsoil differed from the procedure originally stated in the test/QA plan;[1] an appropriate amendment to the plan was prepared and approved before any testing with topsoil coupons was conducted.

In all testing of each decontaminant, test coupons of those materials that are likely to be oriented horizontally in actual use (carpet, decorative laminate, topsoil, unpainted concrete) were placed flat in the BSC for decontamination, whereas coupons of materials likely to be oriented vertically (painted wallboard paper, glass, painted cinder block, bare wood, and metal ductwork) were held vertically in the BSC for decontamination. For some materials (e.g., metal ductwork) either a horizontal or vertical orientation could be realistic, but only one orientation was used in testing. Runoff of the decontaminant from each vertically oriented coupon was captured in a vial placed under the coupon and neutralized after the requisite contact time, as was the decontaminant

[a] Carpet used was treated with zinc omadine (a broad spectrum fungicide-algaecide) during manufacture. This treatment may affect test results on this material.

remaining on the coupon. Decontaminant pooled on top of horizontally positioned coupons was similarly captured and neutralized.

Following decontamination, each coupon (along with any associated run-off or pooled decontaminant) was transferred aseptically to a sterile 50 mL conical vial containing 10 mL of extraction solution. All extraction solutions consisted primarily of sterile phosphate-buffered saline (PBS) solution with Triton X-100 surfactant (i.e., 99.9% PBS solution, 0.1% Triton X-100). In extraction of coupons used with a specific decontaminant, the PBS/Triton X-100 solution also included a neutralizer chosen (or recommended by the vendor) to stop the action of that decontaminant. The required concentration of each neutralizer was determined in trial runs for each decontaminant tested; results of those trial runs are shown in the respective results chapters (Chapters 5 to 11). With the exception of bare concrete, the coupons were then extracted by agitation on an orbital shaker for 15 minutes at approximately 200 revolutions per minute (rpm) at room temperature. For bare concrete, recovery of spores required an alternate procedure in which 45 minutes of sonication was used, instead of the period of agitation. For all coupons, following extraction 1 mL of the coupon extract was removed, and a series of dilutions through 10^{-7} was prepared in SFW. An aliquot (0.1 mL) of the undiluted extract and each serial dilution was then spread plated onto tryptic soy agar plates and incubated overnight at 35 to 37 °C. Plates were enumerated within 18 to 24 hours of plating. The number of CFUs/mL was determined by multiplying the average number of colonies per plate by the reciprocal of the dilution, and accounting for the 0.1 mL volume of extract or dilution that was plated.

Before further decontamination tests, the test chamber was cleaned using the vendor-supplied method for neutralizing the decontamination reagent (see the appendices to this report). If no instructions for neutralization were provided, the test chamber was cleaned following procedures established under the Battelle Biomedical Research Center (BBRC) Facility Safety Plan.[5, 8]

Laboratory blanks controlled for sterility, and procedural blanks controlled for viable spores inadvertently introduced to test coupons. The procedural blanks were spiked with an equivalent amount of 0.1 mL of "stock suspension" that did not contain the biological agent or surrogate. To be considered acceptable for quantitative efficacy determination, extracts of laboratory or procedural blanks had to contain no CFU. The mean percent spore recovery from each coupon type was calculated using results from positive control coupons (spiked, not decontaminated (sprayed with deionized water instead of the decontaminant)), by means of the following equation:

$$\text{Mean \% Recovery} = [\text{Mean CFU}_{pc}/\text{CFU}_{spike}] \times 100 \quad (1)$$

where Mean CFU_{pc} is the mean number of CFUs recovered from five replicate positive control coupons of a single type, and CFU_{spike} is the number of CFUs spiked onto each of those coupons. The value of CFU_{spike} is known from enumeration of the stock spore suspension. Spore recovery was calculated for both *B. anthracis* and *B. subtilis* on each coupon type, and the results are included in Chapters 5 through 11.

3.2 Decontamination Efficacy

The performance or efficacy of the decontamination technology was assessed by determining the number of viable organisms remaining on each test coupon, and in any decontaminant run-off from the coupon, after decontamination. These data were compared with the number of viable organisms extracted from the positive control coupons sprayed with SFW, which was the matrix for the spore suspension used to spike the test coupons.

The number of colony-forming units (CFUs) of *B. anthracis* or *B. subtilis* in extracts of test and positive control coupons was determined to calculate efficacy of the decontaminant. Efficacy is defined as the extent (as $\log_{10}$ reduction) by which viable spores extracted from test coupons after decontamination were less numerous than the viable spores extracted from positive control coupons subjected only to an inert SFW spray, at the same temperature and contact time as the decontaminant application. First, the logarithm of the CFU count value from each coupon extract was determined, and then the mean of those logarithm values was determined for each set of control and associated test coupons, respectively. Efficacy of a decontaminant for a test organism on the i^{th} coupon material was calculated as the difference between those mean log values, i.e.:

$$Efficacy = \overline{(\log CFUc_{ij})} - \overline{(\log CFUt_{ij})} \quad (2)$$

where log $CFUc_{ij}$ refers to the j individual logarithm values obtained from the positive control coupons and log $CFUt_{ij}$ refers to the j individual logarithm values obtained from the corresponding test coupons, and the overbar designates a mean value. In tests conducted under this plan, there were five control and five corresponding test coupons (i.e., $j = 5$). In the case where no CFUs were found in a coupon extract, a CFU count of 1 was assigned, resulting in a log CFU of zero for that coupon. This situation occurred frequently when a decontaminant was highly effective, and no CFUs were found in the plated aliquot of extract from the decontaminated test coupons. In such cases, the final efficacy was reported as greater than or equal to (≥) the value calculated by Equation 2.

The variances (i.e., the square of the standard deviation) of the log $CFUc_{ij}$ and log $CFUt_{ij}$ values were also calculated for both the control and test coupons (i.e., S^2c_{ij} and S^2t_{ij}), and were used to calculate the pooled standard error (SE) for the efficacy value calculated in Equation 2, as follows:

$$SE = \sqrt{\frac{S^2c_{ij}}{5} + \frac{S^2t_{ij}}{5}} \quad (3)$$

where the number 5 again represents the number j of coupons in both the control and test data sets. Thus each efficacy result is reported as a log reduction value with an associated SE value.

The significance of differences in efficacy across different coupon materials and spore types was assessed based on the 95% confidence interval of each efficacy result. The 95% confidence interval (CI) is:

$$95\%\ CI = \textit{Efficacy} \pm (1.96 \times SE) \quad (4)$$

Differences in efficacy were judged to be significant if the 95% CIs of the two efficacy results did not overlap. The efficacy results are presented in a series of tables in Chapters 5 through 11 for each decontaminant technology by coupon material and spore type.

3.3 Qualitative Assessment of Residual Spores

Based on the results of previous decontamination studies,[9-12] spores might not be expected to be completely recovered from coupons by the extraction process. Therefore, viable spores might remain on the test coupons following decontamination and extraction. As in previous decontamination studies, a qualitative assessment was performed to determine whether viable spores remained on the test coupons after extraction, including both the decontaminated test coupons and the positive control coupons not subjected to decontamination. This qualitative assessment involved different conditions and a much longer growth period than the conditions and growth period used in the quantitative assessment of efficacy. The assessment was made to determine whether the decontaminated coupons with zero growth in the quantitative measurement also showed no growth in the qualitative method.

To conduct the qualitative assessment, the test coupons from the quantitative assessment, following extraction, were transferred into tryptic soy broth culture medium and incubated for seven days at appropriate temperatures for growth. The culture media were visually inspected after one day and after seven days of incubation. A cloudy liquid culture after incubation indicated that viable organisms of some type remained on the coupon after decontamination and extraction. For liquid cultures in which cloudiness was observed, a loop of the liquid sample was streaked onto a tryptic soy agar plate and incubated under appropriate conditions for growth. After incubation the plates were examined to determine qualitatively (morphologic comparison performed visually) if the observed growth was a pure culture of the organism that was inoculated onto the coupons, a mixture of the inoculated organism and other endogenous organisms, or a mixture of organisms, such as molds and bacteria. Thus, by itself a cloudy appearance in the growth medium did not necessarily indicate the presence of residual viable organisms that had been spiked onto the test coupon. This morphological comparison is not definitive, but relies on the morphology observed being consistent with the distinctive morphology of the target *Bacillus* species.

3.4 Qualitative Assessment of Surface Damage

Trial runs were conducted before any testing with each decontaminant, using coupons that had not been spiked with spores. In these trial runs the decontaminant was applied exactly as specified in the test/QA plan, and measurements were made with multiple coupons of each material type to determine the amount of the decontaminant that remained on, or ran off from, each material. This information was used in the calculation of efficacy on each respective material, and in trial runs to determine the amount of neutralizing agent needed to stop the action of the decontaminant after the prescribed contact time. In addition, visual inspection of each coupon surface by two test personnel took place after the prescribed decontaminant contact time, through side-by-side comparison of the decontaminated test surface and control coupons of the same test material. Differences in color, reflectivity, and roughness were assessed qualitatively, and observations were recorded by the test personnel. The same inspection was conducted after the conclusion of the seven-day growth period that assessed qualitative efficacy (Section 3.3).

4.0
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, except as noted below. QA/QC procedures are summarized below.

4.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, biological safety cabinets) and monitoring devices (e.g., temperature, relative humidity) used at the time of evaluation were verified as being certified, calibrated, or validated.

4.2 QC Results

Quality control efforts conducted during decontaminant testing included positive control coupons (spiked, not decontaminated), procedural blanks (not spiked, decontaminated), laboratory blanks (not spiked, not decontaminated), and spike control samples (analysis of the stock spore suspension). The results for these QC samples in each decontaminant evaluation are included in the results chapter for each respective decontaminant (i.e., see Chapters 5 through 11).

A common observation was relatively low recovery of spores from coupons of wood and unpainted concrete. However, such recoveries were sufficient to meet QA targets in nearly all cases, and allowed determination of efficacy up to ~6 log reduction.

4.3 Audits

4.3.1 Performance Evaluation Audit

No performance evaluation audit was performed for *B. anthracis* Ames or *B. subtilis* organisms because quantitative standards for these biological materials do not exist.

4.3.2 Technical Systems Audit

Battelle QA staff first conducted a technical systems audit (TSA) at the BBRC during DioxiGuard™ testing on February 7, 2008 to ensure that the evaluation was being conducted in accordance with the test/QA plan[1] and the QMP.[2] A second such TSA was conducted during various activities of the Oxonia Active® testing, on multiple days between November 5 and November 21, 2008. As part of the TSAs, 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 TSAs were documented and submitted to the Battelle Task Order Leader for response. No adverse findings resulted from these TSAs. TSA records were permanently stored with the TTEP QA Manager.

4.3.3 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.

4.4 Test/QA Plan Amendments and Deviations

Two amendments to the test/QA plan were prepared, reviewed, approved, and distributed to all parties involved in this evaluation. One amendment established the heat shocking approach to be used with the soil test material, in place of the soil sterilization approach indicated in the test/QA plan. The second amendment established sonication, rather than agitation, as the spore extraction procedure for unpainted concrete coupons. The TSAs cited in Section 4.3.2 showed that all test procedures followed the test/QA plan, i.e., no deviations were recorded.

4.5 QA/QC Reporting

Each audit was documented in accordance with the QMP.[2] The results of the audits were submitted to the EPA (i.e., to the NHSRC Quality Assurance Manager and the Task Order Project Officer (TOPO)).

4.6 Data Review

Records and data generated in the evaluation received a QC/technical review before they were utilized in calculating or evaluating results and prior to incorporation in reports. All data were recorded by Battelle staff. The person performing the QC/technical 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.

5.0 DioxiGuard™ (Frontier Pharmaceutical) Test Results

5.1 QC Results

In testing of DioxiGuard™, all positive control results were well within the target recovery range of 1 to 150% of the spiked spores. For *B. anthracis* positive control recovery values ranged from 7 to 77%, with the lowest recovery (and the only recovery value below 50%) occurring on bare wood. For *B. subtilis* positive control recovery values ranged from 3 to 42%, with recoveries below 10% on bare wood, painted wallboard, galvanized metal, and painted concrete.

In testing of DioxiGuard™, all procedural and laboratory blanks met the criterion of no observed CFUs in quantitative efficacy testing, with both *B. anthracis* and *B. subtilis*. However, in the qualitative assessment of residual spores, which involves a much longer nutrient growth period, growth was observed from procedural and laboratory blanks used in testing with *B. anthracis*. This finding suggested slight contamination of the blanks during overnight drying of all coupons in the test chamber. Modification of the drying procedure (i.e., placing procedural and laboratory blank coupons in a separate chamber before overnight drying of the coupons) was implemented for *B. subtilis* testing with DioxiGuard™. As a result, no CFUs were observed from any of the procedural or laboratory blanks used in *B. subtilis* testing with DioxiGuard™.

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for two days of *B. anthracis* testing were 1.79×10^8/coupon and 1.25×10^8/coupon, and for two days of *B. subtilis* testing the actual spike values were 1.30×10^8/coupon and 9.57×10^7/coupon.

5.2 Decontamination Efficacy

The decontamination efficacy of Frontier Pharmaceutical's DioxiGuard™ was evaluated for *B. anthracis* Ames and *B. subtilis* on seven indoor material surfaces. The following sections summarize the results found with this decontaminant.

5.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The decontamination efficacy of Frontier Pharmaceutical's DioxiGuard™ was approximately 2.6 log reduction or less on all materials, as shown for *B. anthracis* and *B. subtilis* in Tables 5-1 and 5-2, respectively, and summarized in Table 5-3. For each test material, Tables 5-1 and 5-2 show the number of CFUs inoculated per coupon, the mean of the logs of the five observed spore counts in terms of CFUs found on both control and test coupons, the mean percent recovery (calculated using Equation 1), and the quantitative efficacy value and its 95% confidence interval, calculated using Equations 2 through 4.

The highest efficacy of DioxiGuard™ with *B. anthracis* (over 2.5 log reduction) was seen on non-porous materials (decorative laminate, glass) (Table 5-1). Although galvanized metal is a non-porous material, efficacy for *B. anthracis* on that material (approximately one log reduction, Table 5-1) differed from the efficacy on the other non-porous materials. Intermediate efficacy for *B. anthracis* (i.e., approximately 1.8 log reduction) was seen with industrial-grade carpet and painted concrete. The other porous materials (bare wood, wallboard paper), showed consistently lower efficacy with *B. anthracis* (less than 0.8 log reduction). During the DioxiGuard™ testing, the porous materials appeared wet while the DioxiGuard™ was being sprayed on, but then absorbed the DioxiGuard™ within a few seconds once the application stopped. Wetting or saturation could not be discerned with the industrial grade carpet due to its weave. Therefore, the DioxiGuard™ was continuously sprayed across the surfaces of the five replicates and blank for ten seconds as stated in the application procedure for this decontaminant (Appendix A).

The efficacy of DioxiGuard™ for *B. subtilis* was lower than for *B. anthracis*. As Table 5-2 shows, the highest efficacy for the *B. subtilis* was found with the industrial-grade carpet (0.87 log reduction). On metal ductwork and painted concrete, fewer *B. subtilis* spores were recovered from the control coupons than from the decontaminated coupons, leading to a negative result for efficacy.

Table 5-3 summarizes the efficacy results for DioxiGuard™ on all test materials. Bolded entries in the table indicate materials for which the efficacy results with *B. subtilis* are significantly different from those with *B. anthracis*. Efficacy results for *B. subtilis* on these materials were zero to 0.3 logs.

As Tables 5-1 and 5-2 show, no CFUs were observed from extraction and plating of either the laboratory or procedural blanks for either organism in the quantitative efficacy testing. However, in the subsequent qualitative assessment of residual spores, the blank coupons from *B. anthracis* testing did exhibit some growth. These results are discussed in Section 5.2.2 below.

5.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Tables 5-4 and 5-5 for coupons spiked with *B. anthracis* Ames and *B. subtilis* spores, respectively. In this assessment, cultures showing positive growth (i.e., a cloudy growth medium) were applied to streak plates and the identity of the growing organism was checked by colony morphology. Only *B. anthracis* colonies were found in cultures of coupons used with *B. anthracis*, and only *B. subtilis* colonies were found in cultures of coupons used with *B. subtilis*.

Table 5-4 shows that almost all coupons of all materials showed positive growth for *B. anthracis*, including most of the blank coupons. The growth observed with the blank coupons is most likely due to slight contamination due to the proximity of these blanks to their *B. anthracis*-inoculated replicates during post-spike drying in the test chamber. As noted in Section 5.1, once the positive results on the blanks were observed in the *B. anthracis* testing, a procedural change was made to avoid cross-contamination in the *B. subtilis* testing (i.e., the blanks were placed inside a different Class III BSC from the spore-inoculated materials for drying overnight). As Table 5-5 shows, no growth was observed on any of the blank coupons in the qualitative assessment of residual *B. subtilis* spores, though growth was observed on all the spiked coupons of all materials.

Table 5-1. Inactivation of *Bacillus anthracis* Ames Spores[a]—Frontier Pharmaceutical's DioxiGuard™ (10 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Industrial-Grade Carpet				
Positive Controls[b]	1.79×10^8	8.07 ± 0.08	66.3 ± 10.5	--
Test Coupons[c]	1.79×10^8	6.24 ± 0.50	1.8 ± 2.7	1.83 ± 0.45
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Decorative Laminate				
Positive Controls[b]	1.79×10^8	8.04 ± 0.06	61.4 ± 8.1	--
Test Coupons[c]	1.79×10^8	5.45 ± 0.53	0.26 ± 0.26	2.59 ± 0.47
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Galvanized Metal Ductwork				
Positive Controls[b]	1.25×10^8	7.98 ± 0.05	76.8 ± 8.5	--
Test Coupons[c]	1.25×10^8	7.03 ± 0.13	8.9 ± 2.7	0.95 ± 0.12
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Painted Wallboard Paper				
Positive Controls[b]	1.25×10^8	7.80 ± 0.10	51.7 ± 11.4	--
Test Coupons[c]	1.25×10^8	7.10 ± 0.07	10.2 ± 1.7	0.70 ± 0.11
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Painted Cinder Block				
Positive Controls[b]	1.25×10^8	7.98 ± 0.06	76.9 ± 10.0	--
Test Coupons[c]	1.25×10^8	6.21 ± 0.30	1.6 ± 1.5	1.77 ± 0.27
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Bare Pine Wood				
Positive Controls[b]	1.79×10^8	7.09 ± 0.12	7.1 ± 1.7	--
Test Coupons[c]	1.79×10^8	6.34 ± 0.47	1.8 ± 1.4	0.75 ± 0.42
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Glass				
Positive Controls[b]	1.79×10^8	8.01 ± 0.05	57.7 ± 7.0	--
Test Coupons[c]	1.79×10^8	5.48 ± 1.08	1.7 ± 3.4	2.53 ± 0.95
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--

[a] Data are expressed as mean (± SD) total number of spores (CFUs) observed, percent recovery, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"--" Not Applicable.

Table 5-2. Inactivation of *Bacillus subtilis* Spores[a]—Frontier Pharmaceutical's DioxiGuard™ (10 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Industrial-Grade Carpet				
Positive Controls[b]	1.30×10^8	7.31 ± 0.12	16.0 ± 4.6	--
Test Coupons[c]	1.30×10^8	6.44 ± 1.06	9.7 ± 14.4	0.87 ± 0.94
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Decorative Laminate				
Positive Controls[b]	1.30×10^8	7.67 ± 0.05	36.3 ± 4.0	--
Test Coupons[c]	1.30×10^8	7.38 ± 0.12	18.9 ± 6.0	0.30 ± 0.12
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Galvanized Metal Ductwork				
Positive Controls[b]	9.57×10^7	6.59 ± 0.07	4.1 ± 0.7	--
Test Coupons[c]	9.57×10^7	7.25 ± 0.25	21.0 ± 10.7	-0.66 ± 0.23
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Painted Wallboard Paper				
Positive Controls[b]	9.57×10^7	6.81 ± 0.20	7.3 ± 2.7	--
Test Coupons[c]	9.57×10^7	6.08 ± 0.01	1.3 ± 0.04	0.73 ± 0.18
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Painted Cinder Block				
Positive Controls[b]	9.57×10^7	6.81 ± 0.15	7.1 ± 3.0	--
Test Coupons[c]	9.57×10^7	7.35 ± 0.31	22.8 ± 11.4	-0.49 ± 0.23
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Bare Pine Wood				
Positive Controls[b]	1.30×10^8	6.56 ± 0.19	3.0 ± 1.2	--
Test Coupons[c]	1.30×10^8	6.25 ± 0.22	1.5 ± 0.7	0.31 ± 0.26
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--
Glass				
Positive Controls[b]	1.30×10^8	7.73 ± 0.02	41.8 ± 2.1	--
Test Coupons[c]	1.30×10^8	7.43 ± 0.17	17.5 ± 11.8	0.30 ± 0.15
Laboratory Blank[d]	0	0	0	--
Procedural Blank[e]	0	0	0	--

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"--" Not Applicable.

Table 5-3. Summary of Efficacy Values (Log Reduction) Obtained for Frontier Pharmaceutical's DioxiGuard™ [a]

Material	*B. anthracis* Ames	*B. subtilis*
Industrial-Grade Carpet	1.83	0.87
Decorative Laminate	2.59	**0.30**
Galvanized Metal Ductwork	0.95	**-0.66**
Painted Wallboard Paper	0.70	0.73
Painted Cinder Block	1.77	**-0.49**
Bare Pine Wood	0.75	0.31
Glass	2.53	**0.30**

[a] Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames

The qualitative, liquid culture growth assessment results are consistent with the quantitative, observed efficacy results for all of the materials, except for the industrial-grade carpet, perhaps due to the antibacterial component (zinc omadine) in the carpet. For both *B. anthracis* and *B. subtilis*, this material exhibited only partial growth for the five replicate samples (both decontaminated with DioxiGuard™ and not decontaminated) after Day 1. After Day 7, however, all replicate samples were positive for growth.

5.3 Damage to Coupons

No visible damage was observed on any of the test materials after the 10 minute contact time with DioxiGuard™ in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

5.4 Other Factors

5.4.1 Operator Control

On each day of testing, Frontier Pharmaceutical's DioxiGuard™ was prepared by placing the spray nozzle onto the dual bottle, in which each half of the bottle contained one of the two DioxiGuard™ reagent solutions. Prior to each application, the DioxiGuard™ spray nozzle was primed by repeatedly spraying into an absorbent cloth to clear any air bubbles that may have formed between applications. After each application, the spray nozzle was removed from the bottle and any residual DioxiGuard™ was removed by repeated pulls on the trigger of the spray nozzle. The spray nozzle was then placed onto a dual bottle that contained only SFW to completely clean out the spray nozzle until its next use.

All tests were conducted under ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature of approximately 22 °C. The RH inside the test chamber was monitored with a NIST-traceable hygrometer. Whenever the RH exceeded 40%, the dehumidification system attached to the testing chamber was actuated until the RH dropped below 40%. The dehumidifier was actuated only after the ten minute contact time with the DioxiGuard™. Therefore, the testing chamber was always within 40% RH prior to the decontamination of a new set of materials with DioxiGuard™.

Table 5-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* Ames Spores—Frontier Pharmaceutical's DioxiGuard™

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Industrial-Grade Carpet												
Positive Controls	+	-	+	+	-	-[a]	+	+	+	+	+	+
Test Coupons	+	+	-	+	-	-[b]	+	+	+	+	+	+
Decorative Laminate												
Positive Controls	+	+	+	+	+	+	+	+	+	+	+	+
Test Coupons	+	+	+	+	+	+	+	+	+	+	+	+
Galvanized Metal Ductwork												
Positive Controls	+	+	+	+	+	+	+	+	+	+	+	+
Test Coupons	+	+	+	+	+	+	+	+	+	+	+	+
Painted Wallboard Paper												
Positive Controls	+	+	+	+	+	+	+	+	+	+	+	+
Test Coupons	+	+	+	+	+	+	+	+	+	+	+	+
Painted Cinder Block												
Positive Controls	+	+	+	+	+	+	+	+	+	+	+	+
Test Coupons	+	+	+	+	+	+	+	+	+	+	+	+
Bare Pine Wood												
Positive Controls	+	+	+	+	+	+	+	+	+	+	+	+
Test Coupons	+	+	+	+	+	+	+	+	+	+	+	+
Glass												
Positive Controls	+	+	+	+	+	+	+	+	+	+	+	+
Test Coupons	+	+	+	+	+	+	+	+	+	+	+	+

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. anthracis* Ames spores)); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. anthracis* Ames spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. anthracis* Ames spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

Table 5-5. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus subtilis* Spores—Frontier Pharmaceutical's DioxiGuard™

Test Material	Day 1						Day 7					
	S1	S2	S3	S4	S5	Bl	S1	S2	S3	S4	S5	Bl
Industrial-Grade Carpet												
Positive Controls	-	-	-	+	-	-[a]	+	+	+	+	+	-
Test Coupons	+	-	-	-	+	-[b]	+	+	+	+	+	-
Decorative Laminate												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Galvanized Metal Ductwork												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Painted Wallboard Paper												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Painted Cinder Block												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Bare Pine Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. subtilis* spores)); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. subtilis* spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. subtilis* spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

5.4.2 Technology Spray Deposition

Frontier Pharmaceutical's DioxiGuard™ was applied according to the procedure included as Appendix A of this report. DioxiGuard™ was applied from a distance of 30 cm (12 inches) from the horizontally and vertically oriented materials until the materials appeared fully wetted. For most test materials only a few sprays from the dual bottle, over a few seconds, were required. The one exception was for the industrial-grade carpet. Since it was difficult to discern whether this material was wetted with DioxiGuard™ due to its weave, the carpet was, instead, wetted using several sprays over ten full seconds. No reapplication of the DioxiGuard™ was made on any coupon surface. After the ten minute contact time, each material coupon was placed in the tube that also served to collect excess decontaminant runoff. The horizontally and vertically oriented coupon materials stayed in their respective configurations for the duration of their ten minute contact times.

To assess DioxiGuard™ deposition, triplicate coupons of each test material were weighed prior to application of DioxiGuard™ in trial runs, and these values were recorded. Then the triplicate coupons were sprayed with DioxiGuard™ until the triplicate coupons were fully wetted in their respective vertical or horizontal orientations, allowed a 10 minute contact time, and then each coupon was weighed again. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. Table 5-6 summarizes the results, showing that the amount of DioxiGuard™ deposited on different materials ranged from 0.07 g to 0.34 g; the average deposited amount was approximately 0.2 g (or 200 µL). That average amount was used to determine the amount of sodium thiosulfate (STS) needed to effectively neutralize the DioxiGuard™.

Table 5-6. Deposition/Runoff Weight of Frontier Pharmaceuticals' DioxiGuard™ on Test Materials

Material	Avg. Deposition/Runoff Weight (g)
Industrial-Grade Carpet	0.19
Decorative Laminate	0.10
Galvanized Metal Ductwork	0.34
Painted Wallboard Paper	0.07
Painted Cinder Block	0.26
Bare Pine Wood	0.26
Glass	0.15

5.4.3 Neutralization Methodology

The vendor reported 190 ppm of ClO_2 was present in the delivered DioxiGuard™ formulation. For testing this ClO_2 value was assumed to be correct to calculate the amount of STS needed to neutralize the DioxiGuard™. That calculation was based on the formula weights for ClO_2, the average mass of spray deposition on the test materials, and other factors. The target concentration of STS needed to effectively neutralize the DioxiGuard™ was thus calculated at 0.002% in the PBS/Triton X-100 extraction solution. This calculated STS concentration was coincidentally the same as that used in previous testing,[13] as a result of the higher nominal ClO_2 concentration (i.e., 190 ppm) and lower average deposited amount of DioxiGuard™ (i.e., 200 µL) in this evaluation. However, during the DioxiGuard™ neutralization trial conducted with *B. anthracis* and *B. subtilis*, the extraction media (PBS/Triton/0.002% STS) still showed significant kill after neutralization (*i.e.*, the DioxiGuard™ was not effectively neutralized) (see Tables 5-7 and 5-8). The upper and lower limits for this neutralization trial were set at 0.02% and 0.0002% STS, respectively, to provide a range of neutralization results. The upper limit STS concentration of 0.02% neutralized the DioxiGuard™, whereas the lower limit exhibited total kill (i.e., no neutralization of DioxiGuard™).

As result of these observations, the ClO_2 concentrations from two recent shipments of DioxiGuard™ (saved after the *B. anthracis* and *B. subtilis* tests) were measured to try to explain why the calculated concentration of STS was ineffective. The DioxiGuard™ ClO_2 concentration was measured by the procedure of titration with 0.1 N sodium thiosulfate[14, 15] and found to be over 400 ppm, more than twice the concentration indicated by the vendor. This result explains why the upper limit (0.02%) STS concentration successfully neutralized the DioxiGuard™ but the calculated target (0.002%) STS concentration failed to neutralize the DioxiGuard™. The original target concentration of 0.002% STS was used in tests described above with both *B. anthracis* and *B. subtilis*, so the potential exists for incomplete neutralization of DioxiGuard™ in those tests. Consequently, the efficacy results reported in Section 5.1 must be considered as upper limits to the actual efficacy of DioxiGuard™, in that the action of DioxiGuard™ may not have been promptly neutralized upon addition of the STS.

Table 5-7. Neutralization Testing with *Bacillus anthracis* Ames Spores for Frontier Pharmaceutical's DioxiGuard™

Treatment	Inoculum (CFUs)	Total Observed CFUs	% of Control
DioxiGuard™ + Spores[a]	1.37×10^8	0	0
DioxiGuard™ + PBS + Triton X-100 + Spores[ab]	1.37×10^8	0	0
PBS + Triton X-100 + Spores (Control)[b]	1.37×10^8	1.40×10^8	-
DioxiGuard™ + PBS + Triton X-100 + 0.0002% STS + Spores[ab]	1.37×10^8	0	0
DioxiGuard™ + PBS + Triton X-100 + 0.002% STS + Spores[ab]	1.37×10^8	0	0
DioxiGuard™ + PBS + Triton X-100 + 0.02% STS + Spores[ab]	1.37×10^8	1.25×10^8	89.3

[a] DioxiGuard™ volume of 0.2 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with DioxiGuard™ = 10.2 mL (10 mL PBS+Triton +STS + 0.2 mL DioxiGuard™).
"-" Not Applicable.

Table 5-8. Neutralization Testing with *Bacillus subtilis* Spores for Frontier Pharmaceutical's DioxiGuard™

Treatment	Inoculum (CFUs)	Total Observed CFUs	% of Control
DioxiGuard™ + Spores[a]	1.11×10^8	0	0
DioxiGuard™ + PBS + Triton X-100 + Spores[ab]	1.11×10^8	0	0
PBS + Triton X-100 + Spores (Control)[b]	1.11×10^8	1.14×10^8	-
DioxiGuard™ + PBS + Triton X-100 + 0.0002% STS + Spores[ab]	1.11×10^8	0	0
DioxiGuard™ + PBS + Triton X-100 + 0.002% STS + Spores[ab]	1.11×10^8	0	0
DioxiGuard™ + PBS + Triton X-100 + 0.02% STS + Spores[ab]	1.11×10^8	1.10×10^8	96.5

[a] DioxiGuard™ volume of 0.2 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with DioxiGuard™ = 10.2 mL (10 mL PBS+Triton +STS + 0.2 mL DioxiGuard™).
"-" Not Applicable.

6.0
pH-Amended Bleach Test Results

6.1 QC Results

In testing of pH-amended bleach with *B. anthracis*, percent recovery of inoculated spores from the positive control coupons ranged from about 9 to 77%, with the lowest recovery results on bare wood and unpainted concrete. For *B. subtilis*, positive control recovery values ranged from about 0.5 to 18%, also with the lowest recoveries on bare wood and unpainted concrete. All percent recovery values were well within the acceptable range of 1 to 150% stated in the test/QA plan, except for the value of 0.49% recovery found for *B. subtilis* on unpainted concrete. (In trial runs with this organism on this material, a recovery value of 1.22% had been found.) The EPA Task Order Project Officer (TOPO) was notified of this low recovery value, and he decided to retain the test results, i.e., testing was not repeated with this organism on this material.

All procedural and laboratory blanks met the criterion of no observed CFUs in quantitative efficacy testing, with both *B. anthracis* and *B. subtilis*. In the qualitative assessment of residual spores, which involves a much longer nutrient growth period, growth was observed from the procedural and laboratory blank soil coupons used with both *B. anthracis* and *B. subtilis*, and from the laboratory blank coupons of bare pine wood and painted cinder block used with *B. subtilis*. This finding is discussed in Section 6.2.2. Preliminary tests indicated that extracts of blank soil samples (i.e., not spiked with *B. anthracis* or *B. subtilis*) showed the presence of several colony forming species. However, when spiked with *B. anthracis* or *B. subtilis* spores and extracted, each soil sample showed the presence of a single homogeneous species, with all colonies of uniform size and morphologically distinctive for the respective *Bacillus* species. Therefore, blank soil samples were deemed to be contaminated only if more than the one inoculated species was found in the extracts of inoculated soil samples. This approach was formalized by the approval of the test/QA plan amendment noted in Section 3.1.

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so spore density is known after completion of each day's testing. The target criterion is a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike value for *B. anthracis* testing was 1.22×10^8/coupon, and for *B. subtilis* testing the actual spike value was 9.10×10^7/coupon. Thus all coupons received a spore spike that met the target criterion.

6.2 Decontamination Efficacy

The decontamination efficacy of pH-amended bleach was evaluated for *B. anthracis* and *B. subtilis* on four outdoor material surfaces. The following sections summarize the results found with this decontaminant.

6.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The quantitative efficacy results for pH-amended bleach are presented in Tables 6-1 and 6-2. The decontamination efficacy of pH-amended bleach was highest for the painted cinder block (7.31 log reduction and ≥ 7.22 log reduction for *B. anthracis* Ames and *B. subtilis*, respectively), and relatively high for unpainted concrete (4.99 and ≥ 5.63 log reduction, respectively), but was low for soil (1.47 and 0.18 log reduction) and bare pine wood (0.81 and 0.68 log reduction). The porous bare pine wood tended to absorb some of the control application (i.e, SFW) and the decontaminant. The porous unpainted concrete also appeared to absorb the SFW and decontaminant, but allowed much higher efficacy than did the bare wood.

Table 6-3 summarizes the quantitative efficacy results, and shows that the efficacy of pH-amended bleach for *B. subtilis* was similar to that for *B. anthracis* Ames on most test materials. Only with soil as the test surface was the efficacy for *B. subtilis* significantly different (in this case, lower) than that for *B. anthracis*.

6.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Tables 6-4 and 6-5 for coupons spiked with *B. anthracis* Ames and *B. subtilis* spores, respectively. In this assessment, cultures showing positive growth (i.e., a cloudy growth medium) were applied to streak plates and the identity of the growing organism was checked by colony morphology. Only *B. anthracis* colonies were found in cultures of coupons inoculated with *B. anthracis*, and only *B. subtilis* colonies were found in cultures of coupons inoculated with *B. subtilis*.

Table 6-4 shows that with *B. anthracis*, no growth was observed from decontaminated coupons of painted cinder block after either one or seven days' incubation, but positive growth was observed with the other materials. Similarly, Table 6-5 shows that with *B. subtilis*, little to no growth was observed from decontaminated coupons of painted cinder block and unpainted concrete after either one or seven days' incubation, but positive growth was observed with the other materials. These results are consistent with the quantitative

efficacy observed on the test materials (Table 6-3). As noted in Section 6.1, the laboratory and procedural blanks for topsoil with both organisms, and the laboratory blanks for bare pine wood and painted cinder block with *B. subtilis*, also showed positive growth at both one and seven days' incubation. This growth is likely to have resulted from slight contamination of the blank coupons during storage, after inoculation of the test coupons. In this testing of pH-amended bleach, all coupons for both *B. anthracis* Ames and *B. subtilis*, including blanks, were stored in the same BSC during the overnight drying of the spore-inoculated test coupons. Note that a few of the positive control coupons of unpainted concrete showed no growth of *B. anthracis* or *B. subtilis* after one or seven days' incubation (Tables 6-4 and 6-5), despite not being exposed to the decontaminant. This result may be an artifact of the low spore recovery achieved from this material.

Table 6-1. Inactivation of *Bacillus anthracis* Ames Spores[a] — pH-Amended Bleach (60 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Painted Cinder Block				
Positive Controls[b]	1.22×10^8	7.96 ± 0.13	76.8 ± 21.2	-
Test Coupons[c]	1.22×10^8	0.65 ± 1.45	0.0003 ± 0.0006	7.31 ± 1.27
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Pine Wood				
Positive Controls	1.22×10^8	7.11 ± 0.11	10.8 ± 2.77	-
Test Coupons	1.22×10^8	6.29 ± 0.44	2.20 ± 1.48	0.81 ± 0.40
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Unpainted Concrete				
Positive Controls	1.22×10^8	7.04 ±0.14	9.32 ± 2.94	-
Test Coupons	1.22×10^8	2.05 ± 1.90	0.002 ± 0.003	4.99 ± 1.67
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Topsoil				
Positive Controls	1.22×10^8	7.87 ± 0.12	62.8 ± 16.1	-
Test Coupons	1.22×10^8	6.40 ± 0.95	4.76 ± 3.50	1.47 ± 0.84
Laboratory Blank	0[f]	0	0	-
Procedural Blank	0[f]	0	0	-

[a] Data are expressed as mean of the logs of total number of spores (CFU) observed on individual coupons, percent recovery, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
[f] Endogenous organisms were found in uninoculated soil blanks; no organisms other than *B. anthracis* Ames or *B. subtilis* were found on inoculated coupons.
"-" Not Applicable.

Table 6-2. Inactivation of *Bacillus subtilis* Spores[a] —pH-Amended Bleach (60 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Painted Cinder Block				
Positive Controls[b]	9.10×10^7	7.22 ± 0.06	18.31 ± 2.89	-
Test Coupons[c]	9.10×10^7	0 ± 0.0	0	≥ 7.22 ± 0.06
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Pine Wood				
Positive Controls	9.10×10^7	6.23 ± 0.22	2.06 ± 1.05	-
Test Coupons	9.10×10^7	5.55 ± 0.53	0.64 ± 0.60	0.68 ± 0.50
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Unpainted Concrete				
Positive Controls	9.10×10^7	5.63 ± 0.16	0.49 ± 0.16	-
Test Coupons	9.10×10^7	0 ± 0.0	0	≥ 5.63 ± 0.14
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Topsoil				
Positive Controls	9.10×10^7	7.03 ± 0.15	12.31 ± 4.33	-
Test Coupons	9.10×10^7	6.85 ± 0.23	8.53 ± 3.64	0.18 ± 0.24
Laboratory Blank	0[f]	0	0	-
Procedural Blank	0[f]	0	0	-

[a] Data are expressed as mean of the logs of total number of spores (CFU) observed on individual coupons, percent recovery, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
[f] Endogenous organisms were found in uninoculated soil blanks; no organisms other than *B. anthracis* Ames or *B. subtilis* were found on inoculated coupons.
"-" Not Applicable.

Table 6-3. Summary of Efficacy Values (Log Reduction) Obtained for pH-Amended Bleach[a]

Material	*B. anthracis* Ames	*B. subtilis*
Painted Cinder Block	7.31	≥ 7.22
Bare Pine Wood	0.81	0.68
Unpainted Concrete	4.99	≥ 5.63
Topsoil	1.47	**0.18**

[a]Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames.

Table 6-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* Ames Spores—pH-Amended Bleach

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Painted Cinder Block												
Positive Controls	+	+	+	+	+	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-[b]	-	-	-	-	-	-
Bare Pine Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Unpainted Concrete												
Positive Controls	-	-	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Topsoil												
Positive Controls	+	+	+	+	+	+	+	+	+	+	+	+
Test Coupons	+	+	+	+	+	+	+	+	+	+	+	+

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. anthracis* Ames spores); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. anthracis* Ames spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. anthracis* Ames spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

Table 6-5. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus subtilis* Spores—pH-Amended Bleach

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Painted Cinder Block												
Positive Controls	+	+	+	+	+	+[a]	+	+	+	+	+	+
Test Coupons	-	-	-	-	-	-[b]	+	+	-	-	-	-
Bare Pine Wood												
Positive Controls	+	+	+	+	+	+	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Unpainted Concrete												
Positive Controls	-	+	+	+	-	-	-	+	+	+	-	-
Test Coupons	+	-	-	-	-	-	+	-	-	-	+	-
Topsoil												
Positive Controls	+	+	+	+	+	+	+	+	+	+	+	+
Test Coupons	+	+	+	+	+	+	+	+	+	+	+	+

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. subtilis* spores); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. subtilis* spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. subtilis* spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

6.3 Damage to Coupons

No visible damage was observed on any of the four test materials with pH-amended bleach, either immediately after the 60-minute contact time or seven days after decontamination, at the conclusion of the qualitative efficacy test.

6.4 Other Factors

6.4.1 Operator Control

The pH-amended bleach was prepared according to the procedure described in Appendix B, by mixing 9.4 parts SFW, 1 part commercial household bleach, and 1 part 5% acetic acid. The bleach used was Clorox® brand, obtained through a retail purchase, and the bottle was unopened until the first day of use. The actual resulting solution used for testing with *B. anthracis* had a pH of 6.6 and a total chlorine content of 6,800 ppm; the solution used for *B. subtilis* testing had a pH of 6.45 and a total chlorine content of 6,400 ppm. The pH-amended bleach was freshly prepared prior to each testing day (i.e., the preparation was assigned a one day shelf-life and excess was discarded at the end of the day).

All trials were conducted under ambient conditions inside a climate-controlled laboratory. The temperature inside the testing chamber was equilibrated to the ambient laboratory temperature of approximately 22 °C. The RH was monitored with a NIST-traceable hygrometer. The chamber dehumidifier

was actuated only after the 60 min contact time with the control application (water) or pH-amended bleach. The RH in the test chamber never exceeded 70% during the 60 minute contact time.

6.4.2 Technology Spray Deposition

The pH-amended bleach was applied according to the procedure in Appendix B. The pH-amended bleach was applied 30 cm (12 inches) from the horizontally (soil and unpainted concrete) and vertically (bare pine wood and painted cinder block) oriented materials until the materials appeared saturated with liquid. A handheld garden sprayer was used to apply the control application (SFW) and pH-amended bleach. This sprayer was slightly modified to accommodate a pressure gauge to ensure that the spray was applied using 4 to 6 psi pressure. Close observation of the respective material surfaces was made to ensure that they were thoroughly wetted (approximately 5 sec spray duration was needed to produce wetting across the surfaces of all five replicates and corresponding blank for each material type). Only one material, the painted cinder block, received a reapplication of the decontaminant at the 50 minute mark during the 60 minute contact time. A modest reapplication (approximately 2 sec spray duration) of the pH-amended bleach was done on that material, because the beaded liquid droplets visible on the painted surface appeared to have diminished substantially. After the 60 minute contact time, each material was placed in the 50 mL conical vial that also served to collect excess formulation run-off. The horizontally and vertically oriented materials stayed in their respective configurations throughout the 60 minute contact time.

To assess pH-amended bleach deposition, triplicate coupons of each test material were weighed, and these values were recorded. Then the triplicate coupons were sprayed with pH-amended bleach until fully wetted in their respective vertical or horizontal orientations, allowed a 60 minute contact time, and then each coupon was weighed again. Painted cinder block required a single reapplication at 50 minutes into the 60 minute contact time. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. Table 6-6 shows the results. The amount of pH-amended bleach deposited on bare pine wood, unpainted concrete, and soil coupons ranged from 0.20 g to 0.25 g; the pH-amended bleach deposited on painted cinder block coupons was 0.40 g, including the reapplication. The average of these values (0.27 g, or 0.27 mL based on a density of 1.0) was used to estimate the amount of sodium thiosulfate (STS) needed to effectively neutralize the pH-amended bleach.

Table 6-6. Deposition/Runoff Weight of pH-Amended Bleach on Test Materials

Material	Avg. Deposition/Runoff Weight (g)
Painted Cinder Block	0.40
Bare Pine Wood	0.20
Unpainted Concrete	0.24
Topsoil	0.25

6.4.3 Neutralization Methodology

Neutralization of the pH-amended bleach was achieved with STS. The STS concentration stated in Appendix B for neutralizing the pH-amended bleach was about 0.085% in the PBS/Triton X-100 extraction solution, based on an applied quantity in previous testing similar to those quantities noted above in Section 6.4.2. For performance of a neutralization panel of tests, the upper limit STS concentration was set at twice this target concentration (i.e., at 0.17% STS), and the lower limit was set at half this target concentration (i.e., at 0.042% STS). The results of the neutralization trials for *B. anthracis* and *B. subtilis* are shown in Tables 6-7 and 6-8, respectively. These tables show that all three tested concentrations of STS effectively neutralized the pH-amended bleach. The target concentration of 0.085% STS yielded a percent recovery of 97.6% for *B. anthracis* Ames. Similar results (i.e., a percent recovery of 93.6%) were found with *B. subtilis* at that same STS concentration. Based on the neutralization results from both organisms, 0.085% STS was chosen for neutralization of the pH-amended bleach in all tests.

Table 6-7. Neutralization Testing with *Bacillus anthracis* Ames Spores for pH-Amended Bleach

Treatment	Inoculum (CFUs)	Total Observed CFUs	% of Control
pH-Amended Bleach + Spores[a]	1.27×10^8	0	0
pH-Amended Bleach + PBS + Triton X-100 + Spores[ab]	1.27×10^8	0	0
PBS + Triton X-100 + Spores (Control)[b]	1.27×10^8	1.27×10^8	-
pH-Amended Bleach + PBS + Triton X-100 + 0.17% STS + Spores[ab]	1.27×10^8	1.25×10^8	98.4
pH-Amended Bleach + PBS + Triton X-100 + 0.085% STS + Spores[ab]	1.27×10^8	1.24×10^8	97.6
pH-Amended Bleach + PBS + Triton X-100 + 0.042% STS + Spores[ab]	1.27×10^8	1.22×10^8	96.1

[a] pH-Amended bleach volume of 0.27 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with pH-amended bleach = 10.27 mL (10 mL PBS+Triton +STS + 0.27 mL pH-amended bleach).
"-" Not Applicable.

Table 6-8. Neutralization Testing with *Bacillus subtilis* Spores for pH-Amended Bleach

Treatment	Inoculum (CFUs)	Total Observed CFUs	% of Control
pH-Amended Bleach + Spores[a]	9.80×10^7	0	0
pH-Amended Bleach + PBS + Triton X-100 + Spores[ab]	9.80×10^7	0	0
pH-Amended Bleach + Triton X-100 + Spores (Control)[b]	9.80×10^7	9.76×10^7	-
pH-Amended Bleach + PBS + Triton X-100 + 0.17% STS + Spores[ab]	9.80×10^7	8.26×10^7	84.6
pH-Amended Bleach + PBS + Triton X-100 + 0.085% STS + Spores[ab]	9.80×10^7	9.14×10^7	93.6
pH-Amended Bleach + PBS + Triton X-100 + 0.042% STS + Spores[ab]	9.80×10^7	9.07×10^7	92.9

[a] pH-Amended bleach volume of 0.27 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with pH-amended bleach = 10.27 mL (10 mL PBS+Triton +STS + 0.27 mL pH-amended bleach).
"-" Not Applicable.

7.0
Calcium Polysulfide Test Results

7.1 QC Results

In testing of 5.8% calcium polysulfide (CaS_x) solution with *B. anthracis*, percent recovery of inoculated spores from the positive control coupons ranged from about 18 to 87%, with the lowest recovery results on bare wood and unpainted concrete. For *B. subtilis*, positive control recovery values ranged from about 2 to 70%, also with the lowest recoveries on bare wood and unpainted concrete. All percent recovery values were well within the acceptable range of 1 to 150% stated in the test/QA plan.

All procedural and laboratory blanks met the criterion of no observed CFUs in quantitative efficacy testing, with both *B. anthracis* and *B. subtilis*. In the qualitative assessment of residual spores, which involves a much longer nutrient growth period, growth was observed from the procedural and laboratory blank soil coupons used with both *B. anthracis* and *B. subtilis*. This finding is discussed in Section 7.2.2. Preliminary tests indicated that extracts of blank soil samples (i.e., not spiked with *B. anthracis* or *B. subtilis*) showed the presence of several colony forming species. However, when spiked with *B. anthracis* or *B. subtilis* spores and extracted, each soil sample showed the presence of a single homogeneous species, with all colonies of uniform size and morphologically distinctive for the respective *Bacillus* species. Therefore, blank soil samples were deemed to be contaminated only if more than the one inoculated species was found in the extracts of inoculated soil samples. This approach was formalized by the approval of the test/QA plan amendment noted in Section 3.1.

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so spore density is known after completion of each day's testing. The target criterion is a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike value for *B. anthracis* testing was 1.17×10^8/coupon, and for *B. subtilis* testing the actual spike value was 1.07×10^8/coupon. Thus all coupons received a spore spike that met the target criterion.

7.2 Decontamination Efficacy

The decontamination efficacy of CaS_x was evaluated for *B. anthracis* and *B. subtilis* on four outdoor material surfaces. The following sections summarize the results found with this decontaminant.

7.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The quantitative efficacy results for CaS_x are presented in Tables 7-1 and 7-2. The decontamination efficacy of CaS_x proved to be poor (< 0.4 log reduction for each of the materials for both *Bacillus* species).

Table 7-1. Inactivation of *Bacillus anthracis* Ames Spores[a]–5.8% Calcium Polysulfide (60 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	% Mean Recovery	Decontamination Efficacy ± CI
Bare Pine Wood				
Positive Controls[b]	1.17×10^8	7.35	23.8 ± 16.6	-
Test Coupons[c]	1.17×10^8	7.30	25.0 ± 22.1	0.05 ± 0.47
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Glass				
Positive Controls	1.17×10^8	7.70	43.3 ± 6.80	-
Test Coupons	1.17×10^8	7.74	47.8 ± 8.65	-0.04 ± 0.094
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Unpainted Concrete				
Positive Controls	1.17×10^8	7.21	18.4 ± 15.6	-
Test Coupons	1.17×10^8	6.96	12.3 ± 14.4	0.24 ± 0.50
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Topsoil				
Positive Controls	1.17×10^8	8.00	86.5 ± 8.94	-
Test Coupons	1.17×10^8	7.80	57.1 ± 20.1	0.21 ± 0.17
Laboratory Blank	0[f]	0	0	-
Procedural Blank	0[f]	0	0	-

[a] Data are expressed as mean of the logs of total number of spores (CFUs) observed on individual coupons, percent recovery, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
[f] Endogenous organisms were found in uninoculated soil blanks; no organisms other than *B. anthracis* Ames or *B. subtilis* were found on inoculated coupons.
"-" Not Applicable.

Table 7-2. Inactivation of *Bacillus subtilis* Spores[a]–5.8% Calcium Polysulfide (60 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	% Mean Recovery	Decontamination Efficacy ± CI
Bare Pine Wood				
Positive Controls[b]	1.07×10^8	6.31	2.11 ± 1.05	-
Test Coupons[c]	1.07×10^8	6.43	3.05 ± 2.12	-0.12 ± 0.32
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Glass				
Positive Controls	1.07×10^8	7.62	38.9 ± 3.52	-
Test Coupons	1.07×10^8	7.28	19.6 ± 9.46	0.33 ± 0.18
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Unpainted Concrete				
Positive Controls	1.07×10^8	7.19	16.8 ± 10.1	-
Test Coupons	1.07×10^8	7.08	11.3 ± 1.75	0.12 ± 0.23
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Topsoil				
Positive Controls	1.07×10^8	7.87	70.1 ± 6.34	-
Test Coupons	1.07×10^8	7.66	43.8 ± 9.67	0.21 ± 0.083
Laboratory Blank	0[f]	0	0	-
Procedural Blank	0[f]	0	0	-

[a] Data are expressed as mean of the logs of total number of spores (CFUs) observed on individual coupons, percent recovery, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
[f] Endogenous organisms were found in uninoculated soil blanks; no organisms other than *B. anthracis* Ames or *B. subtilis* were found on inoculated coupons.
"-" Not Applicable.

Table 7-3 summarizes the quantitative efficacy results, and shows that the efficacy of CaS_x for *B. subtilis* was significantly different from the efficacy for *B. anthracis* Ames only on the glass surface. The efficacy value of 0.33 log for *B. subtilis* on that material was the highest found for either organism on any of the test materials.

Table 7-3. Summary of Efficacy Values (Log Reduction) Obtained for 5.8% Calcium Polysulfide[a]

Materials	*B. anthracis* Ames	*B. subtilis*
Bare Pine Wood	0.05	-0.12
Glass	-0.04	**0.33**
Unpainted Concrete	0.24	0.12
Topsoil	0.21	0.21

[a] Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames.

7.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Tables 7-4 and 7-5 for coupons spiked with *B. anthracis* Ames and *B. subtilis* spores, respectively. In this assessment, cultures showing positive growth (i.e., a cloudy growth medium) were applied to streak plates and the identity of the growing organism was checked by colony morphology. Only *B. anthracis* colonies were found in cultures of positive control and test coupons inoculated with *B. anthracis*, and only *B. subtilis* colonies were found in cultures of positive control and test coupons inoculated with *B. subtilis*.

Tables 7-4 and 7-5 show that the positive controls and test coupons for all materials with both organisms were positive for growth. These results are consistent with the low quantitative efficacy values observed on the test materials (summarized in Table 7-3). As noted in Section 7.1 and shown in Tables 7-4 and 7-5, the laboratory and procedural blanks for topsoil with both organisms also showed positive growth at both one and seven days' incubation. This growth is likely to have resulted from native organisms present on the test coupons, and has no impact on the test results. As stated above, topsoil coupons inoculated with *B. anthracis* or *B. subtilis* showed the presence of only the respective inoculated organism in the coupon cultures, at one and seven days post-decontamination.

7.3 Damage to Coupons

A readily visible amount of grayish surface residue was observed on the glass coupons sprayed with 5.8% CaS_x. This residue remained on the glass even after the agitation with spore extraction solution. Such a residue was not readily apparent on the bare wood or unpainted concrete coupons, perhaps due to the surface characteristics of the coupons themselves. The topsoil coupons exhibited a gray hue on the surface of the material (uncompressed topsoil in 3.5 cm petri dish). No such residues were observed on the respective control coupons for glass and topsoil, indicating that the residues were due to the CaS_x decontaminant.

Table 7-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* Ames Spores–5.8% Calcium Polysulfide

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Bare Pine Wood												
Positive Controls	+	+	+	+	+	+[a]	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-[b]	+	+	+	+	+	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Unpainted Concrete												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Topsoil												
Positive Controls	+	+	+	+	+	+	+	+	+	+	+	+
Test Coupons	+	+	+	+	+	+	+	+	+	+	+	+

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. anthracis* Ames spores); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. anthracis* Ames spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. anthracis* Ames spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

Table 7-5. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus subtilis* Spores–5.8% Calcium Polysulfide

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Bare Pine Wood												
Positive Controls	+	+	+	+	+	+[a]	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-[b]	+	+	+	+	+	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Unpainted Concrete												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Topsoil												
Positive Controls	+	+	+	+	+	+	+	+		+	+	+
Test Coupons	+	+	+	+	+	+	+	+	+	+	+	+

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. subtilis* spores); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. subtilis* spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. subtilis* spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

7.4 Other Factors

7.4.1 Operator Control

The 5.8% CaS_x was prepared according to the procedure described in Appendix C, by mixing 800 mL SFW with 200 mL commercially obtained stock solution containing 29% CaS_x by weight. The CaS_x was freshly prepared prior to each testing day (i.e., the preparation was assigned a one day shelf-life and excess was discarded at the end of the day).

All trials were conducted under ambient conditions inside a climate-controlled laboratory. The temperature inside of the testing chamber was equilibrated to the ambient laboratory temperature of approximately 22 °C. All coupons were sprayed (SFW control or CaS_x) at the start of each exposure, the need for re-spraying was assessed every 10 min, the CaS_x was re-sprayed at the 30 min mid-exposure time-point, and then the need for re-spraying was assessed every 10 min as before until 60 total minutes had elapsed since the first application. In practice, reapplication of CaS_x took place only at the 30-min midpoint of the test. The RH was monitored with a NIST-traceable hygrometer. The chamber dehumidifier was actuated only after the 60 minute contact time with the control application (SFW) or CaS_x. The RH in the test chamber exceeded 70% during the 60 minute contact time probably due to the additional spraying step at the 30 min mid-exposure time-point.

7.4.2 Technology Spray Deposition

The CaS_x was applied according to the procedure in Appendix C. The CaS_x was applied 30 cm (12 inches) from the horizontally (soil and unpainted concrete) and vertically (bare pine wood and glass) oriented materials until the materials appeared saturated with liquid. A new handheld garden sprayer was used to apply the control application (SFW) and CaS_x. This sprayer was slightly modified to accommodate a pressure gauge to ensure that the spray was applied using 4 to 6 psi pressure, which resulted in a spray comparable to that from the vendor-provided applicator. Close observation of the respective material surfaces was made to ensure that they were thoroughly wetted (approximately 5 sec spray duration was needed to produce wetting across the surfaces of all five replicates and corresponding blank for each material type). After the 60 minute contact time, each material was placed in the 50 mL conical vial that also served to collect excess formulation run-off. The horizontally and vertically oriented materials stayed in their respective configurations throughout the 60 minute contact time.

To assess CaS_x deposition, triplicate coupons of each test material were weighed and these weights were recorded. Then the triplicate coupons were sprayed with CaS_x until the coupons were fully wetted in their respective vertical or horizontal orientations, the need for re-spraying was assessed every 10 min, the CaS_x was sprayed again at the 30 min mid-exposure time-point, re-spraying was assessed every 10 min as before, and then each coupon was weighed again after 60 min total time. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. As shown in Table 7-6, the amount of CaS_x deposited on bare pine wood, glass, and soil coupons ranged from 0.91 g to 1.49 g; the amount deposited on unpainted concrete coupons was lower at 0.38 g, including the reapplication at the 30 min mid-exposure time-point. The average of these spray-and-weigh results (0.95 g, or 0.95 mL based on an approximate density of 1.0 for the diluted CaS_x) was used to determine the concentration of the neutralizer (Dey/Engley (D/E) Broth) for testing.

Table 7-6. Deposition/Runoff Weight of 5.8% Calcium Polysulfide on Test Materials

Material	Avg. Deposition/Runoff Weight (g)
Bare Pine Wood	1.49
Glass	1.01
Unpainted Concrete	0.38
Topsoil	0.91

7.4.3 Neutralization Methodology

The concentrations of D/E Broth tested as a neutralizer during the neutralization trials were 3%, 6%, 12.5%, 25%, 50%, and 100% in the final extraction solution. That is, the extraction solutions tested ranged from 97% PBS/Triton X-100/3% D-E broth to 100% D-E broth. The results of the neutralization panel for *B. anthracis* are shown in Table 7-7. The results indicate that it did not matter which concentration of D/E Broth was used since each exhibited a high recovery percentage (> 96%) when compared to the control, and the ineffectiveness of CaS_x gave no discrimination as to its neutralization by different D/E concentrations. For the *B. subtilis* spores (Table 7-8), 3% D/E Broth exhibited the highest recovery as a percent of control (108%), whereas the higher concentrations gave recoveries that ranged from 45 to 62%. Therefore, 3% D/E Broth was chosen as the neutralizer for CaS_x for both *B. anthracis* and *B. subtilis*.

Table 7-7. Neutralization Testing with *Bacillus anthracis* Ames Spores for 5.8% Calcium Polysulfide

Treatment	Inoculum (CFUs)	Total Observed CFUs	% of Control
CaS_x + Spores[a]	1.19×10^8	1.04×10^8	114.1
CaS_x + PBS + Triton X-100 + Spores[ab]	1.19×10^8	9.76×10^7	106.7
PBS + Triton X-100 + Spores (Control)[b]	1.19×10^8	9.15×10^7	-
CaS_x + PBS + Triton X-100 + 100% D/E Broth + Spores[ab]	1.19×10^8	9.93×10^7	108.5
CaS_x + PBS + Triton X-100 + 50% D/E Broth + Spores[ab]	1.19×10^8	1.04×10^8	114.0
CaS_x + PBS + Triton X-100 + 25% D/E Broth + Spores[ab]	1.19×10^8	9.17×10^7	100.3
CaS_x + PBS + Triton X-100 + 12.5% D/E Broth + Spores[ab]	1.19×10^8	8.86×10^7	96.9
CaS_x + PBS + Triton X-100 + 6% D/E Broth + Spores[ab]	1.19×10^8	1.02×10^8	111.1
CaS_x + PBS + Triton X-100 + 3% D/E Broth + Spores[ab]	1.19×10^8	1.03×10^8	112.3

[a] CaS_x volume of 0.95 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of D/E broth; total volume for all samples with CaS_x = 10.95 mL (10 mL PBS+Triton +D/E + 0.95 mL CaS_x).
"-" Not Applicable.

Table 7-8. Neutralization Testing with *Bacillus subtilis* Spores for 5.8% Calcium Polysulfide

Treatment	Inoculum (CFUs)	Total Observed CFUs	% of Control
CaS_x + Spores[a]	9.90×10^7	1.02×10^8	54.8
CaS_x + PBS + Triton X-100 + Spores[ab]	9.90×10^7	6.79×10^7	36.6
PBS + Triton X-100 + Spores (Control)[b]	9.90×10^7	1.85×10^8	-
CaS_x + PBS + Triton X-100 + 100% D/E Broth + Spores[ab]	9.90×10^7	9.28×10^7	50.1
CaS_x + PBS + Triton X-100 + 50% D/E Broth + Spores[ab]	9.90×10^7	9.27×10^7	50.0
CaS_x + PBS + Triton X-100 + 25% D/E Broth + Spores[ab]	9.90×10^7	9.34×10^7	50.4
CaS_x + PBS + Triton X-100 + 12.5% D/E Broth + Spores[ab]	9.90×10^7	8.26×10^7	44.6
CaS_x + PBS + Triton X-100 + 6% D/E Broth + Spores[ab]	9.90×10^7	1.15×10^8	62.0
CaS_x + PBS + Triton X-100 + 3% D/E Broth + Spores[ab]	9.90×10^7	2.01×10^8	108.5

[a] CaS_x volume of 0.95 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of D/E broth; total volume for all samples with CaS_x = 10.95 mL (10 mL PBS+Triton +D/E + 0.95 mL CaS_x).
"-" Not Applicable.

8.0
CASCAD™ SDF (Allen-Vanguard) Test Results

8.1 QC Results

In testing of CASCAD™ SDF, all positive control results were within the target recovery range of 1 to 150% of the spiked spores. For *B. anthracis* positive control recovery values ranged from 35 to 145%, with the lowest recovery occurring on unpainted pine wood. For *B. subtilis* positive control recovery values ranged from 2 to 43%, with the lowest recoveries occurring on unpainted pine wood and painted wallboard paper.

In testing of CASCAD™ SDF, all procedural and laboratory blanks met the criterion of no observed CFUs in quantitative efficacy testing, with both *B. anthracis* and *B. subtilis*. Also, no growth was observed for any procedural and laboratory blanks in the qualitative assessment of residual spores, which involves a much longer nutrient growth period. The industrial carpet exhibited the antimicrobial properties seen in previous testing (see Chapter 5) and initially inhibited the growth of the inoculated, non-decontaminated samples. This observation is further explained in Section 8.2.2.

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for two days of *B. anthracis* testing were 9.40×10^7/coupon and 4.73×10^7/coupon, and for two days of *B. subtilis* testing the actual spike values were 1.18×10^8/coupon and 1.04×10^8/coupon. Thus all spore spikes were well within the ±25% tolerance of the 1 x 10^8/coupon target, except for the 4.73×10^7/coupon spike on the second day of *B. anthracis* testing. This relatively low spike amount has no effect on data quality, as spore recoveries were good and efficacy of up to 7.7 logs could be determined.

8.2 Decontamination Efficacy

The decontamination efficacy of Allen-Vanguard's CASCAD™ SDF was evaluated for *B. anthracis* Ames and *B. subtilis* on seven indoor material surfaces. The following sections summarize the results found with this decontaminant.

8.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The decontamination efficacy of Allen-Vanguard's CASCAD™ SDF was greater than approximately 7.0 log reduction for five materials for both *B. anthracis* and *B. subtilis*, as shown in Tables 8-1 and 8-2, respectively, and summarized in Table 8-3. Only painted wallboard paper and unpainted pine wood exhibited lower log reductions for both *Bacillus* species. Table 8-3 shows that efficacy results for *B. subtilis* were significantly different from results for *B. anthracis* for only two materials: painted cinder block (both results exceed 7 log reduction) and bare pine wood (the lowest efficacy results for each organism). In both these cases the efficacy values for *B. subtilis* were less than those for *B. anthracis*.

8.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Tables 8-4 and 8-5 for coupons spiked with *B. anthracis* Ames and *B. subtilis* spores, respectively. In this assessment, cultures showing positive growth (*i.e.,* a cloudy growth medium) were applied to streak plates and the identity of the growing organism was checked by colony morphology. Only *B. anthracis* colonies were found in cultures of coupons used with *B. anthracis*, and only *B. subtilis* colonies were found in cultures of coupons used with *B. subtilis*.

Tables 8-4 and 8-5 show results that are consistent with the efficacy of CASCAD™ SDF. No decontaminated coupons of any material showed growth for *B. anthracis* or *B. subtilis*, with the exception of the two materials that had the lowest log reduction (painted wallboard paper and unpainted pine wood). All laboratory and procedural blanks were negative for growth.

The qualitative, liquid culture growth assessment results are consistent with the quantitative, observed efficacy results for all of the materials, except perhaps for the industrial carpet. At one day of incubation, only two of five of the *B. anthracis*-inoculated and three of five of the *B. subtilis*-inoculated, non-decontaminated industrial carpet positive controls were positive for growth, perhaps due to the antibacterial component (zinc omadine) present in this material. However, all inoculated, non-decontaminated industrial carpet positive control samples were positive for growth at the 7- day assessment, possibly due to the degradation of the antibacterial component over multiple days.

Table 8-1. Inactivation of *Bacillus anthracis* Ames Spores[a]—Allen-Vanguard's CASCAD™ SDF (30 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Industrial-Grade Carpet				
Positive Control[b]	4.73×10^7	7.88 ± 0.08	144.5 ± 20.5	-
Decontaminated[c]	4.73×10^7	0.48 ± 1.08	0	7.40 ± 0.95
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Decorative Laminate				
Positive Control	4.73×10^7	7.72 ± 0.08	111.9 ± 22.0	-
Decontaminated	4.73×10^7	0.31 ± 0.70	0	7.40 ± 0.62
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control	9.40×10^7	7.59 ± 0.04	41.9 ± 3.6	-
Decontaminated	9.40×10^7	0	0	≥ 7.59 ± 0.03
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Wallboard Paper				
Positive Control	4.73×10^7	7.67 ± 0.15	102.5 ± 34.3	-
Decontaminated	4.73×10^7	2.84 ± 1.76	0.02 ± 0.03	4.82 ± 1.55
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Cinder Block				
Positive Control	9.40×10^7	7.84 ± 0.04	73.7 ± 6.8	-
Decontaminated	9.40×10^7	0	0	≥ 7.84 ± 0.04
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Bare Pine Wood				
Positive Control	4.73×10^7	7.13 ± 0.28	34.8 ± 29.4	-
Decontaminated	4.73×10^7	4.36 ± 0.83	0.11 ± 0.10	2.77 ± 0.77
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	0
Glass				
Positive Control	9.40×10^7	7.85 ± 0.14	77.9 ± 27.6	-
Decontaminated	9.40×10^7	0	0	≥ 7.85 ± 0.12
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFUs) observed, percent recovery, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 8-2. Inactivation of *Bacillus subtilis* Spores[a]—Allen-Vanguard's CASCAD™ SDF (30 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Industrial-Grade Carpet				
Positive Control[b]	1.04×10^8	7.62 ± 0.20	43.0 ± 14.8	-
Decontaminated[c]	1.04×10^8	0	0	≥ 7.62 ± 0.18
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Decorative Laminate				
Positive Control	1.04×10^8	7.30 ± 0.21	21.1 ± 9.3	-
Decontaminated	1.04×10^8	0	0	≥ 7.30 ± 0.19
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control	1.18×10^8	7.60 ± 0.08	34.1 ± 6.0	-
Decontaminated	1.18×10^8	0	0	≥ 7.60 ± 0.07
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Wallboard Paper				
Positive Control	1.04×10^8	6.14 ± 0.27	1.6 ± 1.0	-
Decontaminated	1.04×10^8	0	0	≥ 6.14 ± 0.24
Laboratory Blank	0	0	0	
Procedural Blank	0	0	0	
Painted Cinder Block				
Positive Control	1.18×10^8	7.05 ± 0.25	10.7 ± 5.1	-
Decontaminated	1.18×10^8	0	0	≥ 7.05 ± 0.22
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Bare Pine Wood				
Positive Control	1.04×10^8	6.22 ± 0.12	1.6 ± 0.4	-
Decontaminated	1.04×10^8	4.94 ± 0.45	0.1 ± 0.1	1.28 ± 0.41
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Glass				
Positive Control	1.18×10^8	7.51 ± 0.26	31.4 ± 16.2	-
Decontaminated	1.18×10^8	0	0	≥ 7.51 ± 0.23
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFUs) observed, percent recovery, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 8-3. Summary of Efficacy Values (Log Reduction) Obtained for Allen-Vanguard's CASCAD™ SDF[a]

Test Material	*B. anthracis* Ames	*B. subtilis*
Industrial-Grade Carpet	7.40	≥ 7.62
Decorative Laminate	7.40	≥ 7.30
Galvanized Metal Ductwork	≥ 7.59	≥ 7.60
Painted Wallboard Paper	4.82	≥ 6.14
Painted Cinder Block	≥ 7.84	**≥ 7.05**
Bare Pine Wood	2.77	**1.28**
Glass	≥ 7.85	≥ 7.51

[a] Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames.

Table 8-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* Ames Spores—Allen-Vanguard's CASCAD™ SDF

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Industrial-Grade Carpet												
Positive Controls	-	-	-	+	+	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-[b]	-	-	-	-	-	-
Decorative Laminate												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Painted Wallboard Paper												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	-	+	+	+	-	+	-	+	+	+	-
Painted Cinder Block												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Bare Pine Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	+	+	-	-	+	-	+	+	+	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. anthracis* Ames spores); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. anthracis* Ames spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. anthracis* Ames spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

Table 8-5. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus subtilis* Spores—Allen-Vanguard's CASCAD™ SDF

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**B1**	**S1**	**S2**	**S3**	**S4**	**S5**	**B1**
Industrial-Grade Carpet												
Positive Controls	+	-	+	+	-	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-[b]	-	-	-	-	-	-
Decorative Laminate												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Painted Wallboard Paper												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	+	-	-	-	-	-	+	+	-	-	-
Painted Cinder Block												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Bare Pine Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	-	+	-	+	-	+	+	+	-	+	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. subtilis* spores); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. subtilis* spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. subtilis* spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

8.3 Damage to Coupons

The only visible damage observed on the test materials after the 30 minute contact time with CASCAD™ SDF in the quantitative efficacy testing was seen on the painted cinder block. The top-coat (semi-gloss latex paint) peeled away from the primer. This physical change was observed only after the extraction step, when small portions of paint floated in the extraction solution. The non-decontaminated painted cinder block did not undergo this physical change during extraction (*i.e.,* the top-coat remained intact).

8.4 Other Factors

8.4.1 Operator Control

On each day of testing, the two components of Allen-Vanguard's CASCAD™ SDF were prepared according to the vendor's instructions in Appendix D. The spray nozzle was then placed onto the dual bottle, in which each half of the bottle contained one of the two CASCAD™ SDF reagent solutions. Prior to each application, the CASCAD™ SDF spray nozzle was primed by repeatedly spraying into an absorbent cloth to clear any air bubbles that may have formed between applications. After each application, the spray nozzle was removed from the bottle and any residual CASCAD™ SDF was removed by repeated pulls on the trigger of the spray nozzle. The spray nozzle was then placed onto a dual bottle that contained only SFW so as to completely clean out the spray nozzle until its next use.

All tests were conducted at ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature of approximately 22 °C. The RH inside the test chamber was monitored with a NIST-traceable hygrometer. Whenever the RH exceeded 40%, the dehumidification system attached to the testing chamber was actuated until the RH dropped below 40%. The dehumidifier was actuated only after the prescribed contact time with the CASCAD™ SDF. Therefore, the testing chamber RH was always less than 40% prior to the decontamination of a new set of materials with CASCAD™ SDF.

8.4.2 Technology Spray Deposition

Allen-Vanguard's CASCAD™ SDF was applied according to the procedure included as Appendix D of this report. CASCAD™ SDF was applied from a distance of 12 inches from the horizontally and vertically oriented materials, with the aim of covering the materials with approximately a 3/8 inch layer of foam. However, only the industrial carpet came close to the required thickness of foam, likely due to its dense weave which allowed the foam to accumulate. The CASCAD™ SDF foam pooled and spilled over the edges of the other horizontal material (laminate) and only permitted accumulation of approximately 1/8 inch thickness of foam. None of the other materials (all vertically oriented) allowed for the 3/8 inch layer of foam to form since the

applied material almost immediately ran off, but a thin layer of foam remained on the materials. No reapplication of the CASCAD™ SDF was made on any coupon surface. After the 30 minute contact time, each material coupon was placed in the tube that also served to collect excess decontaminant runoff. The horizontally and vertically oriented coupon materials stayed in their respective configurations for the duration of their 30 minute contact times.

To assess CASCAD™ SDF deposition, triplicate coupons of each test material were weighed prior to application of CASCAD™ SDF in trial runs, and those weights were recorded. Then the triplicate coupons were sprayed with CASCAD™ SDF until fully wetted in their respective vertical or horizontal orientations. After a 30 minute contact time, each coupon was then weighed again. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average weights of deposition plus runoff for each of the test materials are shown in Table 8-6.

Table 8-6. Deposition/Runoff Weight of Allen-Vanguard's CASCAD™ SDF on Test Materials

Test Material	Avg. Deposition/Runoff Weight (g) of Foam
Industrial-Grade Carpet	3.35
Decorative Laminate	0.69
Galvanized Metal Ductwork	1.18
Painted Wallboard Paper	0.82
Painted Cinder Block	1.00
Bare Pine Wood	1.15
Glass	1.30

The deposition/runoff weights for six of the seven materials in Table 8-6 ranged from 0.69 to 1.30 g, and averaged approximately 1.02 g. However, for industrial carpet the deposition weight was substantially larger (3.35 g). This difference was due to the ability of the carpet surface to retain a much greater depth of the foam. The density of the CASCAD™ SDF deposited on the test coupons was not measured directly, but was estimated to be slightly greater than 1.0, based on the compositions of the two component solutions that produce the delivered foam (see Appendix D). As a result, the average volume of CASCAD™ SDF deposited on six of the seven materials was estimated to be approximately 1.01 mL, and the volume deposited on carpet was estimated to be approximately 3.3 mL. These volumes were then used in trials to determine the amount of STS needed to neutralize the CASCAD™ SDF (Section 8.4.3).

8.4.3 Neutralization Methodology

Neutralization of the CASCAD™ SDF was achieved with STS. The concentrations of STS tested during the neutralization trial were 0.25, 0.50, and 1.00% in the PBS/Triton X-100 extraction solution. These test STS concentrations were based on historical data. A neutralization trial was done using the 1.01 mL of CASCAD™ SDF that represents deposition on most of the test materials. The results of that neutralization trial with *B. anthracis* and *B. subtilis* are shown in Tables 8-7 and 8-8, respectively, and indicate that the 1% STS concentration was most effective in preventing the sporicidal action of CASCAD™ SDF. Both the *B. anthracis* and *B. subtilis* results were considered in selecting the STS concentration to be used. On the basis of these results, 1% STS was used for neutralization of CASCAD™ SDF in testing with both *B. anthracis* and *B. subtilis*. Subsequently, an additional neutralization panel was conducted to check whether 1% STS was effective at neutralizing the larger amount of CASCAD™ SDF (3.3 mL) deposited on carpet coupons. The results of that neutralization panel are shown for the two organisms in Tables 8-9 and 8-10, and confirmed that 1% STS was effective at neutralizing the CASCAD™ SDF deposited on carpet.

Table 8-7. Neutralization Testing with *Bacillus anthracis* Ames Spores for Allen-Vanguard's CASCAD™ SDF

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
CASCAD™ SDF + Spores[a]	9.40×10^7	0	0
CASCAD™ SDF + PBS + Triton X-100 + Spores[ab]	9.40×10^7	0	
PBS + Triton X-00 + Spores (Control)	9.40×10^7	4.84×10^8	-
CASCAD™ SDF + PBS + Triton X-100 + 0.25% STS + Spores[ab]	9.40×10^7	0	0
CASCAD™ SDF + PBS + Triton X-100 + 0.50% STS + Spores[ab]	9.40×10^7	0	0
CASCAD™ SDF + PBS + Triton X-100 + 1.00% STS + Spores[ab]	9.40×10^7	2.56×10^8	52.8

[a] CASCAD™ SDF volume of 1.01 mL corresponds to mean gravimetric deposition for foam application on most test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with CASCAD™ SDF = 11.01 mL (10 mL PBS+Triton +STS + 1.01 mL CASCAD™ SDF).
"-" Not Applicable.

Table 8-8. Neutralization Testing with *Bacillus subtilis* Spores for Allen-Vanguard's CASCAD™ SDF

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
CASCAD™ SDF + Spores[a]	7.00×10^7	0	0
CASCAD™ SDF + PBS + Triton X-100 + Spores[ab]	7.00×10^7	0	
PBS + Triton X-100 + Spores (Control)[b]	7.00×10^7	9.31×10^7	-
CASCAD™ SDF + PBS + Triton X-100 + 0.25% STS + Spores[ab]	7.00×10^7	6.17×10^7	66.2
CASCAD™ SDF + PBS + Triton X-100 + 0.50% STS + Spores[ab]	7.00×10^7	9.76×10^7	104.9
CASCAD™ SDF + PBS + Triton X-100 + 1.00% STS + Spores[ab]	7.00×10^7	7.96×10^7	85.5

[a] CASCAD™ SDF volume of 1.01 mL corresponds to mean gravimetric deposition for foam application on most test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with CASCAD™ SDF = 11.01 mL (10 mL PBS+Triton +STS + 1.01 mL CASCAD™ SDF).
"-" Not Applicable.

Table 8-9. Additional Neutralization Testing with *Bacillus anthracis* Ames Spores for Allen-Vanguard's CASCAD™ SDF

Treatment	Inoculum (CFUs)	Total Observed (CFUs)	% of Control
CASCAD™ SDF + Spores[a]	6.17×10^7	0	0
CASCAD™ SDF + PBS + Triton X-100 + Spores[ab]	6.17×10^7	0	0
PBS + Triton X-100 + Spores (Control)[b]	6.17×10^7	1.18×10^8	-
CASCAD™ SDF + PBS + Triton X-100 + 1.00% STS + Spores[ab]	6.17×10^7	6.60×10^7	56.1

[a] CASCAD™ SDF volume of 3.3 mL corresponds to mean gravimetric deposition for foam application on carpet.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with CASCAD™ SDF = 13.3 mL (10 mL PBS+Triton +STS + 3.3 mL CASCAD™ SDF).
"-" Not Applicable.

Table 8-10. Additional Neutralization Testing with *Bacillus subtilis* Spores for Allen-Vanguard's CASCAD™ SDF

Treatment	Inoculum (CFUs)	Total Observed (CFUs)	% of Control
CASCAD™ SDF + Spores[a]	8.57×10^7	0	0
CASCAD™ SDF + PBS + Triton X-100 + Spores[ab]	8.57×10^7	0	0
PBS + Triton X-100 + Spores (Control)[b]	8.57×10^7	4.46×10^8	-
CASCAD™ SDF + PBS + Triton X-100 + 1.00% STS + Spores[ab]	8.57×10^7	6.46×10^8	145

[a] CASCAD™ SDF volume of 3.3 mL corresponds to mean gravimetric deposition for foam application on carpet.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with CASCAD™ SDF = 13.3 mL (10 mL PBS+Triton +STS + 3.3 mL CASCAD™ SDF).
"-" Not Applicable.

9.0
Oxonia Active® (Ecolab) Test Results

9.1 QC Results

In testing of Oxonia Active®, all positive control results were within the target recovery range of 1 to 150% of the spiked spores. For *B. anthracis* positive control recovery values ranged from about 13 to 117%, with the lowest recovery occurring on unpainted pine wood. For *B. subtilis* positive control recovery values ranged from 3.4 to about 57%, with the lowest recoveries occurring on painted wallboard paper.

In testing of Oxonia Active®, all procedural and laboratory blanks met the criterion of no observed CFUs in quantitative efficacy testing, with both *B. anthracis* and *B. subtilis*. No growth was also observed in the qualitative assessment of residual spores for all procedural and laboratory blanks, which involves a much longer nutrient growth period. Once again, the industrial carpet exhibited the antimicrobial properties seen in previous testing and initially inhibited the growth of the inoculated, non-decontaminated samples. This growth inhibition by the industrial carpet is further explained in Section 9.2.2.

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for two days of *B. anthracis* testing were 6.93×10^7/coupon and 7.13×10^7/coupon, and for two days of *B. subtilis* testing the actual spike values were 7.80×10^7/coupon and 1.74×10^8/coupon. The spike amounts outside the target range had no effect on data quality, as spore recoveries were good and efficacy exceeding seven logs could be determined.

9.2 Decontamination Efficacy

The decontamination efficacy of Oxonia Active® was evaluated for *B. anthracis* Ames and *B. subtilis* on seven indoor material surfaces. The following sections summarize the results found with this decontaminant.

9.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The efficacy results for Oxonia Active® with *B. anthracis* and *B. subtilis* are shown in Tables 9-1 and 9-2, respectively, and summarized in Table 9-3. The decontamination efficacy was 7.0 log reduction or greater on six of the seven test materials for *B. anthracis* and on five of those materials for *B. subtilis*. For *B. anthracis* efficacy was 4.64 log reduction on bare pine wood, and for *B. subtilis* efficacy was 5.15 log reduction on bare pine wood and ≥ 6.69 log reduction on painted wallboard paper.

Table 9-1. Inactivation of *Bacillus anthracis* Ames Spores[a]—Ecolab's Oxonia Active® (60 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Industrial-Grade Carpet				
Positive Control[b]	7.13×10^7	7.92 ± 0.06	116.8 ± 17.6	-
Decontaminated[c]	7.13×10^7	0.92 ± 2.05	0.01 ± 0.02	7.00 ± 1.79
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Decorative Laminate				
Positive Control	7.13×10^7	7.61 ± 005	57.4 ± 7.1	-
Decontaminated	7.13×10^7	0	0	≥ 7.61 ± 0.05
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control	6.93×10^7	7.87 ± 0.07	107.0 ± 17.9	-
Decontaminated	6.93×10^7	0	0	≥ 7.87 ± 0.06
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Wallboard Paper				
Positive Control	7.13×10^7	7.42 ± 0.32	44.7± 30.7	-
Decontaminated	7.13×10^7	0	0	≥ 7.42 ± 28
Laboratory Blank	0	0	0	
Procedural Blank	0	0	0	
Painted Cinder Block				
Positive Control	6.93×10^7	7.86 ± 0.12	106.8 ± 26.8	-
Decontaminated	6.93×10^7	0	0	≥ 7.86 ± 0.11
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Bare Pine Wood				
Positive Control	7.13×10^7	6.95 ± 0.09	12.7 ± 2.8	-
Decontaminated	7.13×10^7	2.31 ± 2.14	0.01 ± 0.02	4.64 ± 1.87
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Glass				
Positive Control	6.93×10^7	7.72 ± 0.07	77.3 ± 11.5	-
Decontaminated	6.93×10^7	0	0	≥ 7.72 ± 0.06
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, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).

[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 9-2. Inactivation of *Bacillus subtilis* Spores[a]—Ecolab's Oxonia Active® (60 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Industrial-Grade Carpet				
Positive Control[b]	1.74×10^8	7.42 ± 0.13	15.5 ± 4.4	-
Decontaminated[c]	1.74×10^8	0	0	≥ 7.42 ± 0.12
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Decorative Laminate				
Positive Control	1.74×10^8	7.66 ± .0.05	26.3 ± 2.9	-
Decontaminated	1.74×10^8	0	0	≥ 7.66 ± 0.05
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control	7.80×10^7	7.64 ± 0.06	56.9 ± 8.6	-
Decontaminated	7.80×10^7	0	0	≥ 7.64 ± 0.06
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Wallboard Paper				
Positive Control	1.74×10^8	6.69 ± 0.34	3.5 ± 2.4	-
Decontaminated	1.74×10^8	0	0	≥ 6.69 ± 0.29
Laboratory Blank	0	0	0	
Procedural Blank	0	0	0	
Painted Cinder Block				
Positive Control	7.80×10^7	7.29 ± 0.27	30.0 ± 21.6	-
Decontaminated	7.80×10^7	0	0	≥ 7.29 ± 0.24
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Bare Pine Wood				
Positive Control	7.80×10^7	6.55 ± 0.27	5.3 ± 3.5	-
Decontaminated	7.80×10^7	1.40 ± 1.92	0.002 ± 0.003	5.15 ± 1.70
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Glass				
Positive Control	7.80×10^7	7.03 ± 0.15	14.4 ± 4.0	-
Decontaminated	7.80×10^7	0	0	≥ 7.03 ± 0.13
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFUs) observed, percent recovery, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 9-3. Summary of Efficacy Values (Log Reduction) Obtained for Ecolab's Oxonia Active®[a]

Test Material	*B. anthracis* Ames	*B. subtilis*
Industrial-Grade Carpet	7.00	≥ 7.42
Decorative Laminate	≥ 7.61	≥ 7.66
Galvanized Metal Ductwork	≥ 7.87	≥ **7.64**
Painted Wallboard Paper	≥ 7.42	≥ **6.69**
Painted Cinder Block	≥ 7.86	≥ **7.29**
Bare Pine Wood	4.64	5.15
Glass	≥ 7.72	≥ **7.03**

[a] Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames results.

Table 9-3 shows that the efficacy values found for *B. subtilis* were significantly different from those found for *B. anthracis* on four materials; in all four cases the efficacy for *B. subtilis* was lower than that for *B. anthracis*. However, in all four of these cases, no viable spores of either organism were found on the decontaminated test coupons, i.e., Oxonia Active® achieved a complete kill of the inoculated spores, and the efficacy values are shown as "greater than or equal to" (≥) values. Thus the differences in efficacy seen in these four cases are due to limitations in the recovery of the two types of spores from these coupon materials (lower recoveries of *B. subtilis*), and cannot be attributed to actual differences in efficacy toward the two organisms.

9.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Tables 9-4 and 9-5 for coupons spiked with *B. anthracis* Ames and *B. subtilis* spores, respectively. In this assessment, cultures showing positive growth (i.e., a cloudy growth medium) were applied to streak plates and the identity of the growing organism was checked by colony morphology. Only *B. anthracis* colonies were found in cultures of coupons used with *B. anthracis*, and only *B. subtilis* colonies were found in cultures of coupons used with *B. subtilis*.

The qualitative liquid culture growth assessment results in Tables 9-4 and 9-5 are consistent with the quantitative efficacy results, in that no growth was observed for *B. anthracis* or *B. subtilis* on any decontaminated test material. Growth was observed at both one and seven days on all non-decontaminated test materials, as expected, with the exception of the industrial carpet. For both *B. anthracis* and *B. subtilis*, only three of the five inoculated, non-decontaminated industrial carpet coupons were positive for growth after one day, perhaps due to the antibacterial component (zinc omadine) incorporated into this material. All inoculated, non-decontaminated industrial carpet coupons, however, were positive for growth at the seven day assessment, possibly due to the degradation of the antibacterial component over multiple days.

Table 9-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* Ames Spores—Ecolab's Oxonia Active®

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Industrial-Grade Carpet												
Positive Controls	-	-	+	+	+	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-[b]	-	-	-	-	-	-
Decorative Laminate												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Painted Wallboard Paper												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Painted Cinder Block												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Bare Pine Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. anthracis* Ames spores)); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. anthracis* Ames spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. anthracis* Ames spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

Table 9-5. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus subtilis* Spores—Ecolab's Oxonia Active®

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Industrial-Grade Carpet												
Positive Controls	-	-	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Decorative Laminate												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Painted Wallboard Paper												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Painted Cinder Block												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Bare Pine Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. subtilis* spores); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. subtilis* spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. subtilis* spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

9.3 Damage to Coupons

No visible damage was observed on any of the test materials after the 60 min contact time with Oxonia Active®.

9.4 Other Factors

9.4.1 Operator Control

On each day of testing, Oxonia Active® was prepared according to the vendor's explicit instructions as stated in Appendix E. After the Oxonia Active® was diluted in SFW, the product was tested to ensure that the active component (peroxyacetic acid) was within the range specified by the vendor. This check was done using a test kit also provided by the vendor (High Oxonia Active® Test Kit 322). All such checks showed the prepared solution to be in the correct range. The diluted Oxonia Active® was then transferred to a handheld garden sprayer modified with a pressure gauge to ensure that the spray was applied using 4 to 6 psi pressure.

All tests were conducted at ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature of approximately 22 °C. The RH inside the test chamber was monitored with a NIST-traceable hygrometer. Whenever the RH reached 40%, as it did during reapplications of the Oxonia Active®, the dehumidification system attached to the testing chamber was actuated until the RH dropped below 40%. Therefore, the testing chamber was always within 40% RH during the decontamination of a set of material coupons with Oxonia Active®.

9.4.2 Technology Spray Deposition

Oxonia Active® was applied according to the procedure included as Appendix E of this report. Oxonia Active® was applied from a distance of 12 inches from the horizontally and vertically oriented materials until the materials were completely saturated. The respective material surfaces were closely observed to ensure that they were thoroughly wetted (approximately 3-5 sec spray duration was needed to produce wetting across the surfaces of all five replicates and corresponding blank for each material type). Reapplication of the Oxonia Active® was made on all coupon surfaces every 10 minutes. After the 60 minute contact time, each material coupon was placed in its respective 50 mL collection vial that also served to collect excess decontaminant runoff. The horizontally and vertically oriented coupon materials stayed in their respective configurations for the duration of their 60 minute contact times.

To assess Oxonia Active® deposition, triplicate coupons of each test material were weighed prior to application of Oxonia Active® in trial runs, and those weights were recorded. Then the triplicate coupons were sprayed with Oxonia Active® until fully wetted in their respective vertical or horizontal orientations, Oxonia Active® was reapplied at 10-minute intervals, a 60 minute contact time was allowed, and then each coupon was weighed again. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of the Oxonia Active® for each of the test materials is shown in Table 9-6. The average deposited weight of Oxonia Active® over all the test materials was 1.16 g. That average mass of diluted Oxonia Active® (assumed to have a density of 1.0 g/mL) was used in neutralization tests to determine the amount of sodium thiosulfate (STS) needed to effectively neutralize the Oxonia Active®.

Table 9-6. Deposition/Runoff Weight of Ecolab's Oxonia Active® on Test Materials

Test Material	Avg. Deposition/Runoff Weight (g)
Industrial-Grade Carpet	1.60
Decorative Laminate	0.79
Galvanized Metal Ductwork	0.94
Painted Wallboard Paper	1.01
Painted Cinder Block	1.14
Bare Pine Wood	1.70
Glass	0.97

9.4.3 Neutralization Methodology

Neutralization of the Oxonia Active® was achieved with STS. The concentrations of STS used during the neutralization trial were 0.4, 0.8, and 1.6% in the PBS/Triton X-100 extraction solution. These STS concentrations were chosen for the trial based on historical data. The results of the neutralization panel are shown in Tables 9-7 and 9-8. From these results it was determined that a concentration of 0.4% STS in the extraction solution was sufficient for neutralization of Oxonia Active® for both *B. anthracis* and *B. subtilis*.

Table 9-7. Neutralization Testing with *Bacillus anthracis* Ames Spores for Ecolab's Oxonia Active®

Treatment	Inoculum (CFUs)	Total Observed (CFUs)	% of Control
Oxonia Active® + Spores[a]	1.01×10^8	0	0
Oxonia Active® + PBS + Triton X-100 + Spores[ab]	1.01×10^8	9.60×10^5	1.07
PBS + Triton X-100 + Spores (Control)[b]	1.01×10^8	9.00×10^7	-
Oxonia Active® + PBS + Triton X-100 + 1.6% STS + Spores[ab]	1.01×10^8	8.12×10^7	90.3
Oxonia Active® + PBS + Triton X-100 + 0.80% STS + Spores[ab]	1.01×10^8	7.89×10^7	87.7
Oxonia Active® + PBS + Triton X-100 + 0.4% STS + Spores[ab]	1.01×10^8	8.81×10^7	97.8

[a] Oxonia Active® volume of 1.16 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with Oxonia Active® = 11.16 mL (10 mL of PBS/Triton X-100/STS + 1.16 mL Oxonia Active®).
"-" Not Applicable.

Table 9-8. Neutralization Testing with *Bacillus subtilis* Spores for Ecolab's Oxonia Active®

Treatment	Inoculum (CFUs)	Total Observed (CFUs)	% of Control
Oxonia Active® + Spores[a]	9.73×10^7	0	0
Oxonia Active® + PBS + Triton X-100 + Spores[b]	9.73×10^7	0	0
PBS + Triton X-100 + Spores (Control)[c]	9.73×10^7	1.73×10^8	-
Oxonia Active® + PBS + Triton X-100 + 1.6% STS + Spores[b]	9.73×10^7	1.39×10^8	80.3
Oxonia Active® + PBS + Triton X-100 + 0.80% STS + Spores[b]	9.73×10^7	1.20×10^8	69.2
Oxonia Active® + PBS + Triton X-100 + 0.4% STS + Spores[b]	9.73×10^7	1.90×10^8	110

[a] Oxonia Active® volume of 1.16 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with Oxonia Active® = 11.16 mL (10 mL of PBS/Triton X-100/STS + 1.16 mL Oxonia Active®).
"-" Not Applicable.

10.0 Minncare® Cold Sterilant (Minntech) Test Results

10.1 QC Results

During testing of Minncare® Cold Sterilant, all positive control results were well within the target recovery range of 1 to 150% of the spiked spores. For *B. anthracis* positive control recovery values ranged from 26 to 124%, with the lowest recovery occurring on bare pine wood. For *B. subtilis* positive control recovery values ranged from 5 to 93%, with the lowest recoveries occurring on bare pine wood.

All procedural and laboratory blanks met the criterion of no observed CFUs in quantitative efficacy testing, with both *B. anthracis* and *B. subtilis* during Minncare® testing. No growth was also observed for any of the procedural and laboratory blanks in the qualitative assessment of residual spores, which involves a much longer nutrient growth period (up to 7 days). Once again, the industrial carpet exhibited the antimicrobial properties seen in previous testing and initially inhibited the growth of the inoculated, non-decontaminated samples. This inhibition of growth is further explained in Section 10.2.2.

Spiked control samples were taken from the spore suspension on each day of testing, and serially diluted, plated on nutrient media, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for two days of *B. anthracis* testing were 8.93×10^7/coupon and 9.63×10^7/coupon, and for one day of *B. subtilis* testing the actual spike value was 9.87×10^7/coupon.

10.2 Decontamination Efficacy

The decontamination efficacy of Minncare® Cold Sterilant was evaluated for *B. anthracis* Ames and *B. subtilis* on seven indoor material surfaces. The following sections summarize the results found with this decontaminant.

10.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The decontamination efficacy of Minncare® Cold Sterilant was greater than approximately 7.5 log reduction for six materials for both *B. anthracis* (shown in Tables 10-1 and 10-2) and *B. subtilis* (shown in Tables 10-3 and 10-4). Only bare pine wood exhibited log reductions lower than 7.5 for both organisms, with log reductions of 5.40 for *B. anthracis* and 6.00 for *B. subtilis*. Note that Tables 10-1 and 10-3 show results for those materials that were tested with a 10-minute contact time, and Tables 10-2 and 10-4 for those materials that were tested with a 30-minute contact time, as directed by the vendor of Minncare® Cold Sterilant. The efficacy results are summarized in Table 10-5.

Table 10-1. Inactivation of *Bacillus anthracis* Ames Spores[a]—Minntech's Minncare® Cold Sterilant (10 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Decorative Laminate				
Positive Control	8.93×10^7	7.58 ± .0.06	42.6 ± 6.4	-
Decontaminated	8.93×10^7	0	0	≥ 7.58 ± 0.06
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control[b]	8.93×10^7	7.80 ± 0.04	71.4 ± 6.4	-
Decontaminated[c]	8.93×10^7	0	0	≥ 7.80 ± 0.03
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Painted Wallboard Paper				
Positive Control	8.93×10^7	7.53 ± 0.08	38.7 ± 7.7	-
Decontaminated	8.93×10^7	0	0	≥ 7.53 ± 0.07
Laboratory Blank	0	0	0	
Procedural Blank	0	0	0	
Glass				
Positive Control	8.93×10^7	7.75 ± 0.03	63.2 ± 3.7	-
Decontaminated	8.93×10^7	0	0	≥ 7.75 ± 0.02
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, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 10-2. Inactivation of *Bacillus anthracis* Ames Spores[a]—Minntech's Minncare® Cold Sterilant (30 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Industrial-Grade Carpet				
Positive Control[b]	9.63×10^7	7.82 ± .0.13	71.7 ± 22.7	-
Decontaminated[c]	9.63×10^7	0	0	≥ 7.82 ± 0.11
Laboratory Blank[d]	0	0	0	-
Procedural Blank[c]	0	0	0	-
Painted Cinder Block				
Positive Control	9.63×10^7	8.08 ± 0.04	124.0 ± 12.5	-
Decontaminated	9.63×10^7	0	0	≥ 8.08 ± 0.04
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Bare Pine Wood				
Positive Control	9.63×10^7	7.28 ± 0.38	25.7 ± 16.9	-
Decontaminated	9.63×10^7	1.88 ± 1.83	0.0019 ± 0.0020	5.40 ± 1.60
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, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 10-3. Inactivation of *Bacillus subtilis* Spores[a]—Minntech's Minncare® Cold Sterilant (10 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Decorative Laminate				
Positive Control	9.87×10^7	7.87 ± .0.07	76.7 ± 13.4	-
Decontaminated	9.87×10^7	0	0	≥ 7.87 ± 0.06
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control[b]	9.87×10^7	7.89 ± 0.04	79.5 ± 7.4	-
Decontaminated[c]	9.87×10^7	0	0	≥ 7.89 ± 0.03
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Painted Wallboard Paper				
Positive Control	9.87×10^7	7.46 ± 0.17	30.8 ± 10.0	-
Decontaminated	9.87×10^7	0	0	≥ 7.46 ± 0.15
Laboratory Blank	0	0	0	
Procedural Blank	0	0	0	
Glass				
Positive Control	9.87×10^7	7.95 ± 0.10	93.1 ± 24.5	-
Decontaminated	9.87×10^7	0	0	≥ 7.95 ± 0.09
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, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 10-4. Inactivation of *Bacillus subtilis* Spores[a]—Minntech's Minncare® Cold Sterilant (30 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Industrial-Grade Carpet				
Positive Control[b]	9.87×10^7	7.91 ± .0.06	82.8 ± 12.0	-
Decontaminated[c]	9.87×10^7	0	0	≥ 7.91 ± 0.05
Laboratory Blank[d]	0	0	0	-
Procedural Blank[c]	0	0	0	-
Painted Cinder Block				
Positive Control	9.87×10^7	7.93 ± 0.09	88.2 ± 18.8	-
Decontaminated	9.87×10^7	0	0	≥ 7.93 ± 0.08
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Bare Pine Wood				
Positive Control	9.87×10^7	6.69 ± 0.13	5.1 ± 1.6	-
Decontaminated	9.87×10^7	0.69 ± 1.54	0.00056 ± 0.0012	6.00 ± 1.35
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, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW.
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 10-5. Summary of Efficacy Values (Log Reduction) Obtained for Minntech's Minncare® Cold Sterilant[a]

Material	*B. anthracis* Ames	*B. subtilis*
Industrial-Grade Carpet	≥ 7.82[b]	≥ 7.91[b]
Decorative Laminate	≥ 7.58[c]	**≥ 7.87[c]**
Galvanized Metal Ductwork	≥ 7.80[c]	**≥ 7.89[c]**
Painted Wallboard Paper	≥ 7.53[c]	≥ 7.46[c]
Painted Cinder Block	≥ 8.08[b]	**≥ 7.93[b]**
Bare Pine Wood	5.40[b]	6.00[b]
Glass	≥ 7.75[c]	**≥ 7.95[c]**

[a] Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames.
[b] 30 minute contact time.
[c] 10 minute contact time.

Table 10-5 shows that the efficacy values found for *B. subtilis* were significantly different from those found for *B. anthracis* on four materials. However, in all four of these cases, no viable spores of either organism were found on the decontaminated test coupons, i.e., Minncare® Cold Sterilant achieved a complete kill of the inoculated spores, and the efficacy values are shown as "greater than or equal to" (≥) values. Thus the differences in efficacy seen in these four cases are due to limitations in the recovery of the two types of spores from these coupon materials, and cannot be attributed to actual differences in efficacy toward the two organisms.

10.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of coupons at 1 and 7 days post-decontamination are provided in Tables 10-6 and 10-7 for coupons spiked with *B. anthracis* Ames and *B. subtilis* spores, respectively. In this assessment, cultures showing positive growth (i.e., a cloudy growth medium) were applied to streak plates, and the identity of the growing organism was checked by colony morphology. Only *B. anthracis* colonies were found in cultures of coupons used with *B. anthracis*, and only *B. subtilis* colonies were found in cultures of coupons used with *B. subtilis*.

The qualitative liquid culture growth assessment results in Tables 10-6 and 10-7 are consistent with the quantitative efficacy results, in that no growth was observed for *B. anthracis* or *B. subtilis* on any decontaminated test material except bare wood. Growth was observed at both one and seven days on all non-decontaminated test materials, as expected, with the exception of some coupons of the industrial carpet. For *B. anthracis* only three of the five inoculated, non-decontaminated industrial carpet coupons were positive for growth after one day, and for *B. subtilis* only two of the five inoculated, non-decontaminated industrial carpet coupons were positive for growth after one day. This result is likely due to the antibacterial component (zinc omadine) in this material. All inoculated, non-decontaminated industrial carpet coupons, however, were positive for growth at the seven day assessment, possibly due to the degradation of the antibacterial component over multiple days. No growth was observed from any of the blank coupons.

10.3 Damage to Coupons

No visible damage was observed on the test materials after either the 10 min or 30 min contact time with Minncare® Cold Sterilant, or seven days later after completion of the qualitative assessment of residual spores.

10.4 Other Factors

10.4.1 Operator Control

On each day of testing, Minncare® Cold Sterilant was prepared according to the vendor's explicit instructions as described in Appendix F. A 10 percent solution of Minncare® Cold Sterilant was prepared fresh before use on each day of testing by diluting one part of the concentrate with nine parts of SFW. This diluted solution was then transferred to a plastic handheld spray bottle and applied to the test coupons. The respective material surfaces were inspected closely to ensure that each was thoroughly wetted. After the required contact time, each coupon was placed in a 50 mL conical vial that also served to collect any pooled or runoff decontaminant. The horizontally and vertically oriented coupons stayed in their respective configurations throughout the contact time.

Table 10-6. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* Ames Spores—Minntech's Minncare® Cold Sterilant

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Industrial-Grade Carpet												
Positive Controls	+	+	-	+	-	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-[b]	-	-	-	-	-	-
Decorative Laminate												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Painted Wallboard Paper												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Painted Cinder Block												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Bare Pine Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	-	+	+	+	-	+	-	+	+	+	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. anthracis* Ames spores); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. anthracis* Ames spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. anthracis* Ames spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

Table 10-7. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus subtilis* Spores—Minntech's Minncare® Cold Sterilant

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Industrial-Grade Carpet												
Positive Controls	-	-	+	-	+	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-[b]	-	-	-	-	-	-
Decorative Laminate												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Painted Wallboard Paper												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Painted Cinder Block												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Bare Pine Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	+	-	-	-	-	-	+	-	-	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. subtilis* spores); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. subtilis* spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. subtilis* spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

All tests were conducted under ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature of approximately 22 °C. The RH inside the test chamber was monitored with a NIST-traceable hygrometer. Whenever the RH exceeded 40%, the dehumidification system attached to the testing chamber was actuated until the RH dropped below 40%.

10.4.2 Technology Spray Deposition

Minntech's Minncare® Cold Sterilant was applied according to the procedure included as Appendix F of this report, from a distance of 12 inches from the horizontally and vertically oriented materials until the materials were completely wetted. No reapplication of the Minncare® Cold Sterilant was made.

To assess Minncare® Cold Sterilant deposition, triplicate coupons of each test material were weighed prior to application in trial runs, and these weights were recorded. Then the triplicate coupons were sprayed with Minncare® Cold Sterilant until the coupons were fully wetted in their respective vertical or horizontal orientations, allowed the requisite 10 or 30 minute contact time, and then each coupon was weighed again. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of the Minncare® Cold Sterilant for each of the test materials is shown in Table 10-8. The average deposition value over all materials (0.15 g) was then used in trial runs to estimate the amount of neutralization solution needed to effectively neutralize the Minncare® Cold Sterilant.

Table 10-8. Deposition/Runoff Weight of Minntech's Minncare® Cold Sterilant on Test Materials

Test Material	Avg. Deposition/Runoff Weight (g)
Industrial-Grade Carpet	0.18
Decorative Laminate	0.21
Galvanized Metal Ductwork	0.12
Painted Wallboard Paper	0.11
Painted Cinder Block	1.14
Bare Pine Wood	0.12
Glass	0.16

10.4.3 Neutralization Methodology

Neutralization of Minncare® Cold Sterilant was achieved with a vendor-specified neutralization solution (NS). The (NS) was prepared fresh prior to use on each day of testing by diluting 10 g of peptone, 1 g sodium thiosulfate, and 14 g of potassium dihydrogen phosphate to 1 L with SFW. The pH was adjusted to 7 ± 0.5 with HCl and the solution was then autoclaved for 20 minutes at 121 °C. Once this solution cooled to room temperature, catalase was filter sterilized and added just before neutralization to achieve a catalase concentration of 0.005%.

This NS was then mixed in differing proportions with the PBS/Triton X-100 solution to prepare extraction solutions for testing. The compositions of the solutions tested during the neutralization trials ranged from 8.5 mL PBS/Triton X-100 + 1.5 mL NS up to 7 mL PBS/Triton X-100 +3 mL NS for *B. anthracis* and from 9.75 mL PBS/TritonX-100 +0.25 mL NS up to 9 mL PBS/Triton X-100 + 1 mL NS for *B. subtilis*. The results of the final neutralization trials are shown in Tables 10-9 and 10-10. On the basis of these trials, a neutralizer volume of 1.5 mL was used in testing with *B. anthracis*, and a volume of 0.5 mL was used in testing with *B. subtilis*.

Table 10-9. Neutralization Testing with *Bacillus anthracis* Ames Spores for Minntech's Minncare® Cold Sterilant

Treatment	Inoculum (CFUs)	Total Observed (CFUs)	% of Control
Minncare® + Spores[a] (10 min contact)	6.23×10^7	0	0
Minncare® + PBS + Triton X-100 + Spores[ab] (10 min contact)	6.23×10^7	0	0
Minncare® + Spores[a] (30 min contact)	6.23×10^7	0	0
Minncare® + PBS + Triton X-100 + Spores[ab] (30 min contact)	6.23×10^7	0	0
PBS + Triton X-100 + Spores (Control)[b]	6.23×10^7	7.06×10^7	-
Minncare® + PBS + Triton X-100 + 1.5 mL NS[c] + Spores[ab]	6.23×10^7	6.86×10^7	97.3
Minncare® + PBS + Triton X-100 + 2.0 mL NS[c]+ Spores[ab]	6.23×10^7	5.45×10^7	77.2
Minncare® + PBS + Triton X-100 + 3.0 mL NS[c] + Spores[ab]	6.23×10^7	6.01×10^7	85.2

[a] Minncare® Cold Sterilant volume of 0.15 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated volume of NS; total volume for all samples with Minncare® Cold Sterilant = 10.15 mL (10 mL of PBS/Triton X-100/NS + 0.15 mL Minncare Cold Sterilant).
[c] NS = neutralization solution.
"-" Not Applicable.

Table 10-10. Neutralization Testing with *Bacillus subtilis* Spores for Minntech's Minncare® Cold Sterilant

Treatment	Inoculum (CFUs)	Total Observed (CFUs)	% of Control
Minncare®+ Spores[a] (10 min contact)	8.77×10^7	0	0
Minncare® + PBS + Triton X-100 + Spores[ab] (10 min contact)	8.77×10^7	0	0
Minncare® + Spores[a] (30 min contact)	8.77×10^7	0	0
Minncare® + PBS + Triton X-100 + Spores[ab] (30 min contact)	8.77×10^7	0	0
PBS + Triton X-100 + Spores (Control)[b]	8.77×10^7	9.41×10^7	-
Minncare® + PBS + Triton X-100 + 0.25 mL NS[c] + Spores[ab]	8.77×10^7	1.28×10^7	13.6
Minncare® + PBS + Triton X-100 + 0.5 mL NS[c] + Spores[ab]	8.77×10^7	6.92×10^7	73.6
Minncare® + PBS + Triton X-100 + 1.0 mL NS[c] + Spores[ab]	8.77×10^7	5.73×10^7	60.9

[a] Minncare® Cold Sterilant volume of 0.15 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated volume of NS; total volume for all samples with Minncare® Cold Sterilant = 10.15 mL (10 mL of PBS/Triton X-100/NS + 0.15 mL Minncare® Cold Sterilant).
[c] NS = neutralization solution.
"-" Not Applicable.

11.0
SanDes (DTI-Sweden AB) Test Results

11.1 QC Results

In testing of SanDes, all positive control results were well within the target recovery range of 1 to 150% of the spiked spores. For *B. anthracis* positive control recovery values ranged from 12 to 115%, with the lowest recovery occurring on bare pine wood. For *B. subtilis* positive control recovery values ranged from 6 to 98%, with the lowest recoveries occurring on bare pine wood.

In testing of SanDes, all procedural and laboratory blanks met the criterion of no observed CFUs in quantitative efficacy testing, with both *B. anthracis* and *B. subtilis*. No growth was also observed in the qualitative assessment of residual spores for all procedural and laboratory blanks, which involves a much longer nutrient growth period. Once again, the industrial carpet exhibited the antimicrobial properties seen in previous testing and initially inhibited the growth of the inoculated, non-decontaminated samples for the *B. anthracis*. This inhibition of growth is further explained in Section 11.2.2.

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for two days of *B. anthracis* testing were 7.37×10^7/coupon and 8.63×10^7/coupon, and for two days of *B. subtilis* testing the actual spike values were 8.87×10^7/coupon and 9.27×10^7/coupon. The *B. anthracis* spike value of 7.37×10^7/coupon fell slightly outside the ± 25% target criterion, but this spike value was acceptable as spore recoveries were good and efficacy up to 7.87 logs could be determined.

11.2 Decontamination Efficacy

The decontamination efficacy of DTI-Sweden AB's SanDes was evaluated for *B. anthracis* Ames and *B. subtilis* on seven indoor material surfaces. The following sections summarize the results found with this decontaminant.

11.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The decontamination efficacy of DTI-Sweden AB's SanDes was less than 1.0 log reduction for six of the seven materials for both *B. anthracis* and *B. subtilis*. The exceptions were for *B. anthracis* (Ames) spores on glass (4.65 log reduction) and for *B. subtilis* spores on decorative laminate (1.37 log reduction), as shown in Tables 11-1 and 11-2, respectively, and summarized in Table 11-3.

Table 11-1. Inactivation of *Bacillus anthracis* Ames Spores[a]—DTI-Sweden AB's SanDes (70 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Industrial-Grade Carpet				
Positive Control	7.37×10^7	7.81 ± 0.08	89.7 ± 15.7	-
Decontaminated	7.37×10^7	7.68 ±0.06	65.9 ± 9.5	0.13 ± 0.09
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Decorative Laminate				
Positive Control	8.63×10^7	7.86 ± 0.04	84.3 ± 7.8	-
Decontaminated	8.63×10^7	7.68 ± 0.09	56.0 ± 12.5	0.18 ± 0.09
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control[b]	8.63×10^7	7.75 ± 0.13	66.9 ± 19.8	-
Decontaminated[c]	8.63×10^7	7.65 ± 0.10	53.2 ± 11.9	0.09 ± 0.14
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Painted Wallboard Paper				
Positive Control	8.63×10^7	7.86 ± 0.22	93.2 ± 45.8	-
Decontaminated	8.63×10^7	7.67 ± 0.09	55.6 ± 11.3	0.19 ± 0.21
Laboratory Blank	0	0	0	
Procedural Blank	0	0	0	
Painted Cinder Block				
Positive Control	7.37×10^7	7.91 ± 0.07	111.4 ± 18.5	-
Decontaminated	7.37×10^7	7.58 ± 0.07	51.7 ± 8.4	0.33 ± 0.09
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Bare Pine Wood				
Positive Control	7.37×10^7	6.92 ± 0.21	12.2 ± 4.8	-
Decontaminated	7.37×10^7	6.53 ± 0.23	5.3 ± 3.4	0.39 ± 0.27
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Glass				
Positive Control	7.37×10^7	7.74 ± 0.09	75.7 ± 15.5	-
Decontaminated	7.37×10^7	3.09 ± 0.27	0.002 ± 0.001	4.65 ± 0.25
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, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 11-2. Inactivation of *Bacillus subtilis* Spores[a]—DTI-Sweden AB's SanDes (70 minute contact time)

Test Material	Inoculum (CFUs)	Mean of Logs of Observed CFUs	Mean % Recovery	Decontamination Efficacy ± CI
Industrial-Grade Carpet				
Positive Control	9.27×10^7	7.76 ± 0.27	72.5 ± 43.4	-
Decontaminated	9.27×10^7	7.17 ±0.03	15.9 ± 1.1	0.59 ± 0.24
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Decorative Laminate				
Positive Control	8.87×10^7	7.90 ± 0.19	97.7 ± 42.3	-
Decontaminated	8.87×10^7	6.53 ± 0.06	3.9 ± 0.5	1.37 ± 0.17
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control[b]	8.87×10^7	7.73 ± 0.26	70.9 ± 44.3	-
Decontaminated[c]	8.87×10^7	6.98 ± 0.19	11.5 ± 4.4	0.76 ± 0.28
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Painted Wallboard Paper				
Positive Control	8.87×10^7	7.36 ± 0.28	30.0 ± 16.8	-
Decontaminated	8.87×10^7	6.76 ± 0.09	6.6 ± 1.5	0.60 ± 0.25
Laboratory Blank	0	0	0	
Procedural Blank	0	0	0	
Painted Cinder Block				
Positive Control	9.27×10^7	7.76 ± 0.13	63.5 ± 16.2	-
Decontaminated	9.27×10^7	7.24 ± 0.03	18.9 ± 1.2	0.51 ± 0.12
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Bare Pine Wood				
Positive Control	9.27×10^7	6.76 ± 0.07	6.3 ± 1.0	-
Decontaminated	9.27×10^7	6.11 ± 0.09	1.4 ± 0.4	0.65 ± 0.10
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Glass				
Positive Control	9.27×10^7	7.81 ± 0.18	74.3 ± 36.8	-
Decontaminated	9.27×10^7	7.58 ± 0.05	41.6 ± 5.0	0.22 ± 0.16
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFUs) observed, percent recovery, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Inoculated, not decontaminated coupon (sprayed with SFW).
[c] Inoculated, decontaminated coupon.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 11-3. Summary of Efficacy Values (Log Reduction) Obtained for DTI-Sweden AB's SanDes[a]

Test Material	*B. anthracis* Ames	*B. subtilis*
Industrial-Grade Carpet	0.13	**0.59**
Decorative Laminate	0.18	**1.37**
Galvanized Metal Ductwork	0.09	**0.76**
Painted Wallboard Paper	0.19	0.60
Painted Cinder Block	0.33	0.51
Bare Pine Wood	0.39	0.65
Glass	4.65	**0.22**

[a] Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames result.

Table 11-3 shows that for four of the seven materials, the efficacy results with *B. subtilis* were significantly different from the corresponding results with *B. anthracis*. In two such cases, both efficacy results were less than 1.0 log. The largest differences were for glass and decorative laminate, which as noted above resulted in the highest efficacy results for *B. anthracis* and *B. subtilis*, respectively.

11.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Tables 11-4 and 11-5 for coupons spiked with *B. anthracis* Ames and *B. subtilis* spores, respectively. In this assessment, cultures showing positive growth (*i.e.*, a cloudy growth medium) were applied to streak plates and the identity of the growing organism was checked by colony morphology. Only *B. anthracis* colonies were found in cultures of coupons used with *B. anthracis*, and only *B. subtilis* colonies were found in cultures of coupons used with *B. subtilis*.

Tables 11-4 and 11-5 are consistent with the relatively low efficacy of SanDes, in that all inoculated coupons of all materials showed growth for *B. anthracis* and *B. subtilis*. Blank (uninoculated) coupons showed no growth.

These qualitative, liquid culture growth assessment results are consistent with the quantitative, observed efficacy results for all of the materials, except for the industrial carpet inoculated with *B. anthracis*. Only three of the five *B. anthracis*-inoculated, non-decontaminated industrial carpet positive controls were positive for growth at the 1 day assessment, perhaps due to the antibacterial component (zinc omadine) in this material. All inoculated, non-decontaminated industrial carpet positive control samples, however, were positive for growth at the seven day assessment, possibly due to the degradation of the antibacterial component over multiple days.

Table 11-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* Ames Spores—DTI-Sweden AB's SanDes

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Industrial-Grade Carpet												
Positive Controls	+	-	-	+	+	-[a]	+	+	+	+	+	-
Test Coupons	-	-	+	-	+	-[b]	+	+	+	+	+	-
Decorative Laminate												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Galvanized Metal Ductwork												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Painted Wallboard Paper												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Painted Cinder Block												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Bare Pine Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. anthracis* Ames spores)); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. anthracis* Ames spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. anthracis* Ames spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

Table 11-5. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus subtilis* Spores—DTI-Sweden AB's SanDes

	Day 1						Day 7					
Test Material	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**	**S1**	**S2**	**S3**	**S4**	**S5**	**Bl**
Industrial-Grade Carpet												
Positive Controls	+	+	+	+	+	-[a]	+	+	+	+	+	-
Test Coupons	+	+	+	-	+	-[b]	+	+	+	+	+	-
Decorative Laminate												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Galvanized Metal Ductwork												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Painted Wallboard Paper												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Painted Cinder Block												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Bare Pine Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-

S1 to S5 = Sample 1 to Sample 5.
Bl = Blank (not inoculated with *B. subtilis* spores); a = laboratory blank, b = procedural blank.
Positive controls = coupons inoculated with *B. subtilis* spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. subtilis* spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

11.3 Damage to Coupons

No visible damage was observed on any of the test materials after the 70 min contact time with SanDes despite multiple applications of the decontaminant in that time period.

11.4 Other Factors

11.4.1 Operator Control

DTI-Sweden AB's SanDes was provided as a "ready-to-use" formulation straight from the bottle. The product was provided in small bottles (30 mL), and the vendor provided a small push-button attachment for dispensing SanDes as a spray. This attachment produced a very fine spray, and numerous "pumps" were required to fully wet the surface of each test material. Because of the small volume of the SanDes bottles, and the multiple reapplications of SanDes during testing, a bottle was quickly depleted during application and had to be replaced. The SanDes spray attachment did a good job of dispersing the product, but it appeared that the 30 cm (12-inch) application distance exceeded the range at which the attachment would have wetted the testing material surfaces most effectively. Testing staff attempted to apply SanDes in as consistent a fashion as possible, but the spray attachment occasionally stuck in the down position, interrupting the back-and-forth motion needed to wet all six replicate coupons of a single test material (including the blank). Additional spray attachments were provided by the vendor to circumvent this problem. The respective material surfaces were observed closely to ensure that they were thoroughly wetted; approximately 15 seconds of spray duration using the back-and-forth motion was needed to produce wetting across the surfaces of five replicate coupons and a corresponding blank coupon for each material type.

All tests were conducted under ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature of approximately 22 °C. The RH inside the test chamber was monitored with a NIST-traceable hygrometer. Whenever the RH exceeded 40%, the dehumidification system attached to the testing chamber was actuated until the RH dropped below 40%. The dehumidifier was actuated only after the 70 minute contact time with the SanDes. Therefore, the testing chamber was always within 40% RH prior to the decontamination of a new set of materials with SanDes.

11.4.2 Technology Spray Deposition

DTI-Sweden AB's SanDes was applied according to the procedure included as Appendix G of this report. SanDes was applied from a distance of 30 cm (12 inches) from the horizontally and vertically oriented materials until the materials were fully wetted. Reapplication of the SanDes was made on all coupon surfaces at 10, 20, and 30 minutes after the initial application. At 60 minutes after the initial application, one more application of SanDes was made. After 70 minutes total contact time since the initial application, each material coupon was placed in a tube that also served to collect decontaminant that had run off from or pooled on the coupon. The horizontally and vertically oriented coupon materials stayed in their respective configurations for the duration of their 70 minute contact times.

To assess SanDes deposition, triplicate coupons of each test material were weighed prior to application of SanDes in trial runs, and these values were recorded. Then the triplicate coupons were sprayed with SanDes until fully wetted in their respective vertical or horizontal orientations, SanDes was reapplied as described above and allowed a 70 minute contact time, and then each coupon was weighed again. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff from or pooled on each coupon. The average deposition/runoff weight of SanDes from each of the test materials is shown in Table 11-6. The total averaged value of 0.10 g (density assumed = 1.0 g/mL) was then used in trials to determine the amount of sodium thiosulfate (STS) needed to effectively neutralize the SanDes.

Table 11-6. Deposition/Runoff Weight of DTI-Sweden AB's SanDes on Test Materials

Test Material	Avg. Deposition/Runoff Weight (g)
Industrial-Grade Carpet	0.04
Decorative Laminate	0.02
Galvanized Metal Ductwork	0.14
Painted Wallboard Paper	0.13
Painted Cinder Block	0.12
Bare Pine Wood	0.12
Glass	0.14

11.4.3 Neutralization Methodology

Neutralization of the SanDes was achieved with STS. The concentrations of STS used during the neutralization panel were 2.0, 2.5, and 3.0% in the PBS/Triton X-100 extraction solution. These STS concentrations were chosen for the trial based on historical data. The results of the neutralization panel are shown in Tables 11-7 and 11-8. From these results a concentration of 2.0% STS in the extraction solution was determined to be sufficient for neutralization of SanDes for both *B. anthracis* and *B. subtilis*.

Table 11-7. Neutralization Testing with *Bacillus anthracis* Ames Spores for DTI-Sweden AB's SanDes

Treatment	Inoculum (CFUs)	Total Observed (CFUs)	% of Control
SanDes+ Spores[a]	1.08×10^8	0	0
SanDes + PBS + Triton X-100 + Spores[ab]	1.08×10^8	0	
PBS + Triton X-00 + Spores (Control)[b]	1.08×10^8	1.02×10^8	-
SanDes + PBS + Triton X-100 + 2.0% STS + Spores[ab]	1.08×10^8	1.02×10^8	100.0
SanDes + PBS + Triton X-100 + 2.5% STS + Spores[ab]	1.08×10^8	9.69×10^7	95.3
SanDes + PBS + Triton X-100 + 3.0% STS + Spores[ab]	1.08×10^8	9.86×10^7	97.0

[a] SanDes volume of 0.10 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with SanDes = 10.1 mL (10 mL of PBS/Triton X-100/STS + 0.10 mL SanDes).
"-" Not Applicable.

Table 11-8. Neutralization Testing with *Bacillus subtilis* Spores for DTI-Sweden AB's SanDes

Treatment	Inoculum (CFUs)	Total Observed (CFUs)	% of Control
SanDes+ Spores[a]	9.83×10^7	0	0
SanDes + PBS + Triton X-100 + Spores[ab]	9.83×10^7	0	
PBS + Triton X-00 + Spores (Control)[b]	9.83×10^7	9.72×10^7	-
SanDes + PBS + Triton X-100 + 2.0% STS + Spores[ab]	9.83×10^7	9.83×10^7	101.1
SanDes + PBS + Triton X-100 + 2.5% STS + Spores[ab]	9.83×10^7	9.34×10^7	96.1
SanDes + PBS + Triton X-100 + 3.0% STS + Spores[ab]	9.83×10^7	9.72×10^7	100.0

[a] SanDes volume of 0.10 mL corresponds to mean gravimetric deposition on test materials.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with SanDes = 10.1 mL (10 mL of PBS/Triton X-100/STS + 0.10 mL SanDes).
"-" Not Applicable.

12.0 Performance Summary

12.1 DioxiGuard™ Results

- The quantitative decontamination efficacy of DioxiGuard™ was 2.6 log reduction or less for *B. anthracis* Ames, and 0.87 log reduction or less for *B. subtilis*, on the seven test materials. Efficacy values above about 1.8 log reduction for *B. anthracis* were seen only with relatively non-porous materials (glass, laminate, painted concrete) and with carpet.
- Significant differences between efficacy values for *B. subtilis* and *B. anthracis* were found with non-porous materials (glass, laminate, painted concrete, metal ductwork), due primarily to the low efficacy values found with *B. subtilis* on these materials (i.e., zero to 0.3 log reduction).
- In the qualitative tests, coupons of all material types showed the presence of viable organisms after decontamination, consistent with the quantitative efficacy results. Morphological analysis confirmed that the growth observed indicated only *B. anthracis* Ames or *B. subtilis*, respectively, from the spiked coupons.
- In the qualitative tests growth was also observed with blank coupons of all material types used in testing with *B. anthracis*, although no CFUs were found in the quantitative efficacy tests, indicating minimal contamination of the blanks. This result was attributed to contamination of blank materials within the test chamber during overnight drying of coupons spiked with *B. anthracis*. The drying procedure was changed for testing with *B. subtilis* (blanks were removed from the test chamber before overnight drying of spiked coupons) and no growth was observed subsequently with any blank coupons.
- No visible damage was observed on any of the test materials after the 10 minute contact time with DioxiGuard™ in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

12.2 pH-Amended Bleach Results

- The quantitative efficacy of pH-amended bleach was highest for the painted cinder block (7.31 log reduction and ≥ 7.22 log reduction for *B. anthracis* Ames and *B. subtilis*, respectively), and relatively high for unpainted concrete (4.99 and ≥ 5.63 log reduction, respectively), but was low for topsoil (1.47 and 0.18 log reduction) and bare pine wood (0.81 and 0.68 log reduction).
- A significant difference between efficacy values for *B. subtilis* and *B. anthracis* was found only with topsoil as the test surface, with the efficacy for *B. subtilis* (0.18 log reduction) significantly lower than that for *B. anthracis* (1.47 log reduction).
- In the qualitative tests most material types showed the presence of viable organisms after decontamination. However, no viable organisms of either *B. anthracis* or *B. subtilis* were found on painted cinder block, and none of *B. subtilis* were found on unpainted concrete. These results are consistent with the quantitative efficacy results for this decontaminant. Morphological analysis confirmed that the growth observed indicated only *B. anthracis* Ames or *B. subtilis*, respectively, from the spiked coupons.
- In the qualitative tests growth was also observed with the laboratory and procedural blanks for topsoil with both *B. anthracis* and *B. subtilis*, and with the laboratory blanks for bare pine wood and painted cinder block with *B. subtilis*, although no CFU were found on these blanks in the quantitative efficacy tests. This growth is likely to have resulted from slight contamination of the blank coupons in the test chamber during the overnight drying of the spore-inoculated test coupons.
- No visible damage was observed on any of the test materials after the 60 minute contact time with pH-amended bleach in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

12.3 Calcium Polysulfide Results

- The quantitative efficacy of the 5.8% by weight CaS_x solution was very low, achieving maximum log reductions of only 0.24 for *B. anthracis* Ames and 0.33 for *B. subtilis*.
- A significant difference between efficacy values for *B. subtilis* and *B. anthracis* was found only with glass as the test surface, with the efficacy for *B. subtilis* (0.33 log reduction) significantly higher than that for *B. anthracis* (-0.04 log reduction).
- In the qualitative tests coupons of all material types showed the presence of viable organisms after decontamination, consistent with the quantitative efficacy results. Morphological analysis confirmed that the growth observed indicated only *B. anthracis* Ames or *B. subtilis*, respectively, from the spiked coupons.

- A grayish residue was observed on glass and topsoil coupons after decontamination. That residue was not removed from the glass by the agitation used for spore extraction, or by the culturing process used in the seven-day qualitative test for viable spores. The surface characteristics of bare wood and unpainted concrete coupons made it impossible to discern whether a similar residue was also present on those materials.

12.4 CASCAD™ SDF Results

- The quantitative efficacy of CASCAD™ SDF exceeded 7.0 log reduction for both *B. anthracis* and *B. subtilis* on five of the seven test materials. Lower efficacy values were found only on painted wallboard paper and bare pine wood. Efficacy results for *B. anthracis* and *B. subtilis* on painted wallboard paper were 4.82 and ≥ 6.14 log reduction, respectively; on bare pine wood the corresponding efficacy results were 2.77 and 1.28 log reduction, respectively.
- Significant differences between efficacy values for *B. subtilis* and *B. anthracis* were found only with painted cinder block and bare pine wood as the test surfaces. With painted cinder block, no viable spores of either organism were found after decontamination (i.e., the efficacy values were both reported as "≥" values). Thus the difference in efficacy values on that material is due to different efficiencies of recovery of the two spore types, and cannot be attributed to an actual difference in the efficacy of CASCAD™ SDF. On bare pine wood, the efficacy for *B. anthracis* was significantly higher than that for *B. subtilis* (2.77 *vs.* 1.28 log reduction).
- In the qualitative tests, only painted wallboard paper and bare pine wood showed the presence of viable organisms after decontamination, consistent with the quantitative efficacy results. Morphological analysis confirmed that the growth observed indicated only *B. anthracis* Ames or *B. subtilis*, respectively, from the spiked coupons.
- The only materials damage observed from decontamination with CASCAD™ SDF was that the top coat of paint peeled away from the primer coat on painted cinder block coupons.

12.5 Oxonia Active® Results

- The quantitative efficacy of Oxonia Active® was 7.0 log reduction or greater on six of the seven test materials for *B. anthracis* and on five of the seven test materials for *B. subtilis*. Lower efficacy values were found only on bare pine wood and painted wallboard paper. Efficacy results for *B. anthracis* and *B. subtilis* on bare pine wood were 4.64 and 5.15 log reduction, respectively; on painted wallboard paper the efficacy for *B. subtilis* was ≥ 6.69 log reduction.
- Significant differences between efficacy values for *B. subtilis* and *B. anthracis* were found on four materials. However, no viable spores of either organism were found on coupons of any of these materials after decontamination (i.e., the efficacy values were all reported as "≥ " values). Thus the differences in reported efficacy values are due to differing efficiencies of recovery of the two spore types from these materials, and cannot be attributed to actual differences in the efficacy of Oxonia Active®.
- In the qualitative tests, no viable spores were found on any decontaminated coupon after either one or seven days incubation, consistent with the quantitative efficacy results.
- No visible damage was observed on any of the test materials after 60 minutes contact time with Oxonia Active®, or seven days later after completion of the qualitative assessment of residual spores.

12.6 Minncare® Cold Sterilant Results

- The quantitative efficacy of Minncare® Cold Sterilant was 7.5 log reduction or greater on six of the seven test materials for both *B. anthracis* and *B. subtilis*. Lower efficacy values were found only on bare pine wood, for which efficacy results for *B. anthracis* and *B. subtilis* were 5.40 and 6.00 log reduction, respectively.
- Significant differences between efficacy values for *B. subtilis* and *B. anthracis* were found on four materials. However, no viable spores of either organism were found on coupons of any of these materials after decontamination (i.e., the efficacy values were all reported as "≥" values). Thus the differences in reported efficacy values are likely due to differing efficiencies of recovery of the two spore types from these materials, and cannot be attributed to actual differences in the efficacy of Minncare® Cold Sterilant.
- In the qualitative tests, no viable spores were found on any decontaminated coupon after either one or seven day's incubation, consistent with the quantitative efficacy results.
- No visible damage was observed on any of the test materials after either 10 or 30 minutes contact time with Minncare® Cold Sterilant, or seven days later after completion of the qualitative assessment of residual spores.

12.7 SanDes Results

- The quantitative efficacy of SanDes was less than 1.0 log reduction for six of the seven test materials for both *B. anthracis* and *B. subtilis*. Higher efficacy values were found only on glass for *B. anthracis* (4.65 log reduction) and on decorative laminate for *B. subtilis* (1.37 log reduction).

- Significant differences between efficacy values for *B. subtilis* and *B. anthracis* were found on four materials. In two such cases, both efficacy results were less than 1.0 log reduction. The largest differences were for glass (4.65 log reduction with *B. anthracis* and 0.22 log reduction with *B. subtilis*), and for decorative laminate (0.18 log reduction with *B. anthracis* and 1.37 log reduction with *B. subtilis*). As noted above these respective materials exhibited the highest efficacy results for each organism.
- In the qualitative tests, viable spores were found on all of the decontaminated coupons after one day and after seven days of incubation, consistent with the relatively low quantitative efficacy results.
- No visible damage was observed on any of the test materials after 70 minutes contact time with SanDes, or seven days later after completion of the qualitative assessment of residual spores.

13.0
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Appendix A
Dioxiguard™ Description and Application Procedure

General Description

DioxiGuard™ is a chlorine dioxide (ClO_2) disinfectant solution. ClO_2 is released when sodium chlorite ($NaClO_2$) solution is mixed with an acid, in this case producing a concentration of about 190 ppm ClO_2 in the resulting mixture.

DioxiGuard™ is supplied commercially in a dual chambered plastic spray bottle, with each chamber containing one of the two reagent solutions. Sodium chlorite solution is combined with acid solution at the time of use by squeezing the trigger of the spray bottle. The bottle holds 22 oz (650 mL) (total) and is easily hand held. The two solutions are mixed and dispensed in one operation, with ClO_2 immediately produced in the mixed solution. The separate ingredients in the twin bottle are designed to be stable for two years or more.

DioxiGuard™ is designed to suppress both ClO_2 odor in the spray and the corrosion normally expected with ClO_2 preparations. The toxicity of DioxiGuard™ to humans is minimal, and many toxicity reports show the disinfectant liquid can be safely sprayed on the body. As is well known, ClO_2 may bleach clothing and carpets, but not as readily as chlorine bleach.

The DioxiGuard™ system of microorganism deactivation does not depend on ClO_2 alone. Chlorous acid ($HClO_2$) has a much higher oxidation potential than ClO_2, and the product design attempts to maximize concentrations of this transitory molecule. This maximization can be done by adjusting the pH; if the pH is too high, too little $HClO_2$ is formed, if the pH is too low, too much is formed at once. A high concentration of $HClO_2$ immediately disappears and disproportionates to ClO_2:

$$H^+ + NaClO_2 \rightarrow HClO_2 + Na+$$

$$5HClO_2 \rightarrow 4ClO_2 + HCl + 2H_2O$$

In addition, when $HClO_2$ disproportionates as in the second equation above, several free radicals are formed instantaneously in the process. These radicals are short-lived, highly reactive, and strong oxidants. Accordingly, the production of ClO_2 by way of an acid, rather than production directly from the chlorite, offers the advantage of these transient oxidizing species. On the other hand, forming ClO_2 and letting it remain in solution for a time before use will reduce the system's oxidizing capability. In DioxiGuard™, the chlorite is activated by organic acids and the solution also contains alcohol.

Application Procedure for Testing

For evaluation of DioxiGuard™'s efficacy on diverse test surfaces in this evaluation, the following application procedure was used:

- Spray the test coupons with DioxiGuard™ from a distance of about one foot (12 inches) using the vendor-supplied dual spray bottle.
- Squeeze the trigger on the dual spray bottle repeatedly over a period of 10 seconds, or until the coupon surface is fully saturated with solution.
- Let the test coupons remain in place, with the DioxiGuard™ solution on each coupon, for 10 minutes. No reapplication of DioxiGuard™ is needed.

At the conclusion of the 10 minute contact time, each test coupon was placed into a separate aliquot of extraction solution along with any captured runoff of DioxiGuard™ from that coupon, and the DioxiGuard™ was neutralized with sodium thiosulfate (STS). The use of STS to neutralize DioxiGuard™ was established in a previous test program; the STS concentration used in this evaluation was based upon the concentration used previously.

Appendix B
pH-Amended Bleach Description and Application Procedure

General Description

pH-Amended bleach consists of diluted normal household bleach (e.g., Clorox®) with its pH adjusted by addition of a small amount of acetic acid. Specifically, pH-amended bleach contains a total of about 5 to 6% by weight of sodium hypochlorite (NaOCl) in aqueous solution, with pH adjustment achieved by addition of a small amount of 5% acetic acid. The recipe for preparation of pH-amended bleach for use as a decontaminant is as follows:

- Prepare 5% acetic acid solution by diluting 50 mL of glacial acetic acid up to 1 L with SFW in a volumetric flask.
- Mix 9.4 parts SFW, 1 part commercial household bleach, and 1 part 5% acetic acid. The resulting solution will have a mean pH of about 6.8 and a mean total chlorine content of about 6,200 ppm.

The active decontaminating agents in this solution are hypochlorite (OCl^-) and hypochlorous acid. The effectiveness of this reagent as a biological decontaminant is widely known and well demonstrated through the common use of bleach as a sterilant and decontaminant. In testing of pH-amended bleach as a decontaminant under a previous TTEP Task Order,[1] neutralization of the bleach solution was achieved using sodium thiosulfate (STS). Based on the chemical composition of the pH-amended bleach, the amount of that solution (0.325 mL) retained or run off from a test coupon with a specified 10-second application period, and the use of 10 mL of an extraction solution containing phosphate-buffered saline (PBS) + 0.1% Triton X-100, an STS concentration of 0.086% in the extraction solution was determined to be optimal for neutralizing the pH-amended bleach. The application equipment and procedures used in this evaluation differ from those used in previous testing,[1] so the determination of the neutralization procedure was repeated to establish conditions appropriate for this evaluation.

Application Procedure for Testing

Based on information available from previous use of pH-amended bleach,[1] an application procedure for use in testing has been developed. The intent of this procedure is to employ conventional and readily available equipment in a relatively simple application process. Trial runs were conducted to establish the appropriate concentration of STS for neutralization of the pH-amended bleach.

The test coupon materials used with pH-amended bleach were soil, bare wood, bare concrete, and painted cinder block. Good decontaminant efficacy has been demonstrated previously with pH-amended bleach on glass,[1] so that surface was replaced by painted cinder block as an outdoor surface in this test.

The pH-amended bleach was prepared fresh shortly before use on each day of testing, as described above. The pH of the solution was measured and recorded as part of the test data. A non-corroding garden pump sprayer was used to apply the solution of pH-amended bleach to the test coupon surfaces. An identical sprayer was used to apply SFW to positive control test coupons. Each sprayer was fitted with a pressure gauge to indicate the internal delivery pressure of the sprayer. The internal pressure of each sprayer was maintained in a normal range for use (i.e., 4 to 6 psi) throughout all applications. Based on laboratory tests, such a range of pressures produces a stable spray suitable for application on the scale of coupon testing. The step-by-step application procedure was:

- Apply the pH-amended bleach solution to the test coupons (or SFW to the positive control coupons) from a distance of about 30 cm (one foot or 12 inches) using the sprayer at a delivery pressure within the specified range, until the test coupon surfaces are fully wetted by the solution.
- Reapply the solution if test coupon surfaces become dry, but no more frequently than at ten minute intervals.
- If necessary, pump up the pressure in the sprayer before application to maintain pressure within the specified range.

When 60 minutes had elapsed since the start of the first application, the coupons were placed into the extraction solution (containing the neutralization agent) along with any collected runoff of pH-amended bleach.

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Appendix C
Calcium Polysulfide Description and Application Procedure

General Description

Calcium polysulfide (CaS_x), also known as "Lime Sulfur," has been in use as an agricultural fungicide and insecticide since the early 1900s. Calcium polysulfide is also used in veterinary medicine as an effective treatment for various pet and livestock infections. Calcium polysulfide can be purchased from many manufacturers and vendors, with the typical concentration of 29% by weight in water. Calcium polysulfide is a yellow to orange aqueous solution with a density of 1.28 g/mL.

Diluted calcium polysulfide solutions ranging from 0.7 to 2.9% are commonly applied to treat fungus and insect infestation of agricultural crops, by spraying with non-corroding crop and garden sprayers to the point of solution runoff.

Application Procedure for Testing

Based on the information available on calcium polysulfide, an application procedure for use in testing has been developed. The intent of this procedure is to use a concentration of calcium polysulfide that is likely to be effective when applied with conventional and readily available equipment in a relatively simple application process. Trial runs were conducted to establish the appropriate concentration of D-E Neutralizing Agar for neutralization of the calcium polysulfide. Test surfaces used were glass, soil, bare wood, and bare concrete.

For testing, a concentration of 5.8% by weight calcium polysulfide (i.e., a 1:5 dilution with water of the commercial 29% product) was used. The specific product used is Aqua-Clear®, manufactured by VGS, St. Joseph, Missouri (www.calciumpolysulfide.com; site currently under construction).

A non-corroding garden pump sprayer was used to apply the solution of calcium polysulfide to the test coupon surfaces. An identical sprayer was used to apply SFW to positive control test coupons. Each sprayer was fitted with a pressure gauge to indicate the internal delivery pressure of the sprayer. The internal pressure of each sprayer was maintained in a normal range for use (i.e., 4 to 6 psi) in all applications. Based on laboratory tests, such a range of pressures produces a stable spray, suitable for application on the scale of coupon testing. The step-by-step application procedure was:

- Apply the calcium polysulfide solution to the test coupons (or SFW to the positive control coupons) from a distance of about one foot (12 inches) using the sprayer at a delivery pressure within the specified range. Spray the solution onto the coupons until the test coupons are fully wetted, and with no less than a five-second spray duration on any surface.
- Reapply the solution if test coupon surfaces become dry, but no more frequently than at ten minute intervals.
- Regardless of the wetness of the coupons, reapply the calcium polysulfide solution to all coupons 30 minutes after the initial application, again with at least a five-second spray duration on each surface.
- If necessary, pump up the pressure in the sprayer before application to maintain pressure within the specified range.
- When 60 minutes have elapsed since the start of the first application, place the coupons into the extraction solution (containing the neutralization agent) along with any collected runoff of calcium polysulfide solution.

Appendix D
CASCAD™ SDF Description and Application Procedure

General Description

CASCAD™ Surface Decontamination Foam (SDF) uses two liquid solutions (A and B) which react to form a foam as they are mixed upon release from the application device. These two solutions are made from three separate reagents, having chemical composition as follows:

- GPA-2100 (decontaminant) – solid reagent in powder form consisting of dichloroisocyanuric acid sodium salt, 70 to 100% by weight;
- GPB-2100 (buffer) – solid reagent in powder form consisting of sodium tetraborate 10 to 30%, sodium hydroxide 1 to 5 %, and sodium carbonate 40 to 65% by weight;
- GCE-2000 (surfactant) – liquid reagent consisting of sodium myristyl sulfate 10 to 30%, sodium (C14-16) olefin sulfonate 10 to 30%, ethanol denatured 3 to 9%, alcohols (C10-16) 5-10%, sodium sulfate 3 to 7%, sodium xylene sulfonate 1 to 5%, and a proprietary mixture of sodium and ammonium salts along with water and co-solvent >9% by weight.

The A and B solutions are prepared from these reagents by the following procedure:

1. Make solution A by adding 31.2 grams (four 7.8 gram packets) of GPA-2100 to 250 mL of SFW in a graduated cylinder, and then dilute with water to 300 mL.
2. Mix with a micro stir bar until dissolved
3. Make solution B by adding 7.2 grams (four 1.8 gram packets) of GPB-2100 to 250 mL of SFW in a graduated cylinder.
4. Mix with a micro stir bar until dissolved.
5. Add 18 mL (four 4.5 mL packets) of GCE-2000 to the solution from step 4, mix, and then dilute with SFW to a final volume of 300 mL

For use on the small scale needed for testing, a manual spray application bottle developed by Allen-Vanguard (the 600 mL Hand Held Decontamination System) draws solutions A and B from separate compartments and delivers them as a foam through a single spray head. To fill and operate the Hand Held Decontamination System, follow these steps:

1. Pull the Locking Lever on the front of the bottle housing forward and lift to open the housing and expose the solution bottles, which are labeled “A” and “B”.
2. With the housing opened remove the caps (turn counter clockwise) and pull out the solution suction lines from the solution bottles.
3. With the caps and suction lines removed from both the “A” and “B” solution bottles:
    a. Pour solution A into the bottle labeled “A”, and pour solution B into the bottle labeled “B”.
    b. Assure that both bottles are seated in the housing with the “B” bottle at the front.
    c. Place the suction lines back into the “A” and “B” bottles and tighten both the “A” and “B” caps by turning them in a clockwise direction.
4. Hold the suction line up with one hand while closing the top of the housing with the other hand. Make certain that the Locking Lever snaps into its recess when the housing top closes. The suction line may be pinched closed if this procedure is not followed correctly. Check for closure of the line by looking through the housing and checking the suction line.
5. To use the 600 mL Hand Held Decontamination System, grasp the neck of the housing with your dominant hand and place the finger of this hand on the trigger of the foam nozzle. Aim the tip of the foam nozzle in the direction of the area to be decontaminated and pump the trigger. The trigger may have to be squeezed three or four times to evacuate the air in the suction line before foam is discharged.

Application Procedure for Testing

CASCAD™ SDF was applied to test coupons using the vendor-developed dual spray applicator. In previous testing,[1] neutralization of the CASCAD™ SDF was achieved by addition of 0.5% sodium thiosulfate (STS) to the extraction solution. Trial runs were conducted before testing to establish the appropriate STS concentration for neutralization of the applied CASCAD™ SDF.

The step-by-step application procedure for testing was:

- Follow the instructions provided above for preparation of the reagent solutions and loading of the manual spray applicator.
- Squeeze the trigger of the applicator head a few times while pointing the applicator into a laboratory sink or other waste container, until any air is cleared from the applicator and CASCAD™ SDF is delivered from the applicator as a foam.
- Apply the CASCAD™ SDF to the test coupons using the manual applicator from a distance of about 30 cm (12 inches) while moving the nozzle, until the test coupons are entirely covered with no less than one (1) centimeter (3/8") deep foam.

- Allow the foam to remain on the coupons for 30 minutes. Do not reapply.
- When 30 minutes have elapsed since the application, place each coupon into the extraction solution (containing the STS neutralization agent) along with any associated collected runoff of CASCAD™ SDF.
- Empty and clean the manual spray applicator after use according to the instructions below.

Cleaning the Hand Held Decontamination System

Clean the CASCAD™ SDF system after use by the following procedure.

1. Dump any remaining decontamination solution from both the "A" and "B" bottles and dispose of the solutions following appropriate waste procedures.
2. Thoroughly rinse both bottles with SFW, then fill each bottle with SFW.
3. Place the filled bottles back into the housing, insert the suction lines, and close the housing.
4. Pump the trigger until the suction lines and foam nozzle are free from the decontamination solution.
5. Flush the interior and the exterior of the housing, and the caps used while mixing the solution, thoroughly with SFW.

Reference:

1. Rogers, J.V., Richter, W.R, Choi, Y.W., Fleming, E.J., Shesky, A.M., Cui, J., Taylor, M.L., Riggs, K.B., Willenberg, Z.J., Stone, H.J., Wood, J.P.. Evaluation of Spray-Applied Sporicidal Decontamination Technologies. U.S. EPA Technology Testing and Evaluation Program Report, EPA/600/R-06/146, September 2006. (http://www.epa.gov/NHSRC/tte_liquiddecontech.html).

Appendix E
Oxonia Active® Description and Application Procedure

General Description

Oxonia Active® is a liquid sanitizer made by Ecolab Inc., that consists of 27.5 % hydrogen peroxide (H_2O_2) and 5.8% peroxyacetic acid ($CH_3CO(O_2)H$) by weight in water (density = 1.13 g/mL). According to the vendor, Oxonia Active is used for sterilizing a variety of surfaces and containers in food, packaging, and other industries, and can be applied as a liquid or foam, or as droplets by fogging the target area. A temporary approval (crisis exemption) of Oxonia Active® was granted by the U.S. Environmental Protection Agency for decontamination of *Bacillus anthracis* spores on non-porous surfaces, at defined temperatures, contact times, and dilution of the product.

Application Procedure for Testing

An application procedure for use of Oxonia Active® in testing has been developed, based on information provided by the vendor. The aim is to use a relatively simple application process that is likely to be effective when carried out with conventional and readily available equipment. Trial runs were conducted to establish the appropriate concentration of sodium thiosulfate (STS) for neutralization of Oxonia Active®. Test surfaces used include glass, decorative laminate, industrial-grade carpet, galvanized metal ductwork, painted wallboard paper, painted cinder block, and bare pine wood.

For testing, a decontaminant solution containing 5,000 ppm peroxyacetic acid was prepared fresh daily by diluting 76 mL of Oxonia Active® to 1 L with SFW. The Ecolab High Oxonia Active® Test Kit 322 was used for periodic verification of the peroxyacetic acid concentration in the undiluted Oxonia Active® from which the decontaminant solution was prepared.

A non-corroding garden pump sprayer was used to apply the diluted Oxonia Active® solution to the test coupon surfaces. An identical sprayer was used to apply SFW to positive control test coupons. Each sprayer was fitted with a pressure gauge to indicate the internal delivery pressure of the sprayer, which was maintained in a normal range for use (i.e., 4 to 6 psi) in all applications. Based on laboratory tests, such a range of pressures produces a stable spray, suitable for application on the scale of coupon testing. All applications were done at normal room temperature (approximately 20 °C (68 °F)).

The step-by-step application procedure for Oxonia Active® was:

- Apply the decontaminant solution to the test coupons (or SFW to the positive control coupons) from a distance of about one foot (12 inches) using the sprayer at a delivery pressure within the specified range. Spray the solution onto the coupons until the test coupons are visibly wet and excess liquid drips from the coupons.
- Reapply the decontaminant solution if coupon surfaces become visibly dry, and regardless of the wetness of the coupons reapply the decontaminant solution every 10 minutes.
- If necessary, pump up the pressure in the sprayer before application to maintain pressure within the specified range.
- When 60 minutes have elapsed since the start of the first application, place the coupons into the extraction solution (containing the neutralization agent) along with any collected runoff of decontaminant solution.

Appendix F
Minncare® Cold Sterilant Description and Application Procedure

General Description

Minncare® Cold Sterilant is a liquid decontaminant consisting of 22.0% by weight hydrogen peroxide (H_2O_2) and 4.5% by weight peroxyacetic acid ($CH_3C(O)O_2H$) in aqueous solution. Minncare® Cold Sterilant is a clear liquid with a density of 1.1 g/mL and a pH of 0.5 to 1.1. Minncare® Cold Sterilant is thus both an oxidizing agent and a strongly acid solution.

At the direction of Minntech Corp., a solution of peptone, sodium thiosulfate (STS), and potassium dihydrogen phosphate (KH_2PO_4) with a small amount of catalase was used as the neutralizing agent for Minncare® Cold Sterilant. Trial runs were conducted to establish the appropriate chemical quantities and procedures for neutralization in this evaluation.

Preparation of Minncare® Cold Sterilant: A 10% solution of Minncare® Cold Sterilant was prepared fresh shortly before use on each day of testing, by diluting 1 part of the Cold Sterilant with 9 parts of SFW.

Preparation of Neutralization Solution: The neutralization solution was prepared fresh shortly before use on each day of testing, by diluting 10 g of peptone, 1 g of STS, and 14 g KH_2PO_4 to 1 L in SFW, and adjusting the pH to 7 ± 0.5. That solution was then autoclaved for 20 minutes at 121 °C, and then allowed to cool to room temperature. Catalase (Sigma C-9322) was filter sterilized and added to the cooled solution just before neutralization to achieve a catalase concentration of 0.005%.

Application Procedure for Testing

Based on the vendor's instructions, an application procedure for use of Minncare® Cold Sterilant in testing was developed. The intent of this procedure was to employ conventional and readily available equipment in a relatively simple application process. The test coupon materials used in testing of Minncare® Cold Sterilant included decorative laminate, galvanized metal ductwork, painted wallboard paper, glass, industrial-grade carpet, painted cinder block, and bare pine wood.

The 10% Cold Sterilant solution was applied to test coupons using a hand-held plastic spray bottle. A similar bottle was used to apply SFW to positive control test coupons. The step-by-step application procedure was as follows:

- Apply the Minncare® Cold Sterilant 10% solution to the test coupons (or SFW to the positive control coupons) from a distance of about 30 cm (one foot or 12 inches) using the handheld spray bottle, until the test coupon surfaces are fully wetted by the solution.
- No reapplication of the Cold Sterilant solution is required.
- Allow the Cold Sterilant solution to remain in contact with the test coupon surfaces for the following contact times, which differ for different coupon materials:

Decorative laminate	10 minutes
Galvanized metal ductwork	10 minutes
Painted wallboard paper	10 minutes
Glass	10 minutes
Industrial-grade carpet	30 minutes
Painted cinder block	30 minutes
Bare pine wood	30 minutes

- When the allotted contact time has elapsed since the application of the Cold Sterilant solution, place the coupons into the extraction solution (containing the pre-determined amount of neutralization solution) along with any collected runoff of the Cold Sterilant solution.

Appendix G
SanDes Description and Application Procedure

General Description

SanDes, a liquid decontaminant made by DTI-Sweden AB, consists of 1,500 ppm chlorine dioxide (ClO_2) in aqueous solution. SanDes is a light yellow solution with a density of 1.0 g/mL and a pH of less than 1.5. SanDes is thus both an oxidizer and a relatively strong acid solution. At the vendor's direction SanDes was used without dilution for application to test coupons. This application procedure is based on technical information provided by the vendor; that information was not verified as part of the test program.

Based on previous experience with ClO_2 decontaminants,[1] sodium thiosulfate (STS) was used as the neutralizing agent for SanDes. Trial runs were conducted to establish the appropriate chemical quantities and procedures for neutralization in this evaluation.

Application Procedure for Testing

Based on the vendor's instructions, an application procedure for use of SanDes in testing was developed. The intent of this procedure was to employ conventional and readily available equipment in a relatively simple application process. The test coupon materials used in testing of SanDes included decorative laminate, galvanized metal ductwork, painted wallboard paper, glass, industrial-grade carpet, painted cinder block, and bare pine wood.

The undiluted SanDes solution was applied to test coupons using a push-button spray nozzle that replaced the cap on a 30 mL bottle of SanDes. An identical spray nozzle was used to apply SFW to positive control test coupons. The step-by-step application procedure was as follows:

- Apply SanDes to the test coupons (or SFW to the positive control coupons) from a distance of about 30 cm (one foot or 12 inches) using the spray nozzle, until the test coupon surfaces are fully wetted by the solution.
- Reapply SanDes at 10-minute intervals after the original application, or more often if surfaces become dry, until three reapplications have been made.
- Make a final application of the SanDes at 60 minutes after the original application.
- Allow the final application of SanDes to remain in contact with the test coupon surfaces for 10 minutes, resulting in a total contact time of 70 minutes since the original application.
- When the 70 minutes total contact time has elapsed, place the coupons into the extraction solution (containing the pre-determined amount of neutralization solution) along with any collected runoff of the SanDes solution.

Reference:

1. Rogers, J.V., Richter, W.R, Choi, Y.W., Fleming, E.J., Shesky, A.M., Cui, J., Taylor, M.L., Riggs, K.B., Willenberg, Z.J., Stone, H.J., Wood, J.P.. Evaluation of Spray-Applied Sporicidal Decontamination Technologies. U.S. EPA Technology Testing and Evaluation Program Report, EPA/600/R-06/146, September 2006. (http://www.epa.gov/NHSRC/tte_liquiddecontech.html).

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