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Determination of the Efficacy of Spore Removal from Carpets using Commercially-available Wet/Vacuum Carpet Cleaning Systems

Assessment and Evaluation Report

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National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Disclaimer

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Table of Contents

Disclaimer iii

Acknowledgments iv

Table of Contents v

List of Figures vii

List of Tables vii

List of Appendices viii

List of Acronyms and Abbreviations ix

List of Units x

Executive Summary xi

1 Project Description and Objectives 1

1.1 Purpose 1

1.2 Process 1

1.3 Project Objectives 2

2 Experimental Approach 3

2.1 General Approach 3

2.1.1 Control Chamber (COMMANDER) 5

2.1.2 Material Surfaces 5

2.1.3 Chamber Setups 7

2.1.4 Material Sterilization 9

2.1.5 Spore Preparation 9

2.1.6 Controlled Contamination Procedure 10

2.1.7 Contamination Characterization 11

2.1.8 Decontamination Procedure 11

2.1.9 Final Sterilization 13

2.2 Test Matrix 13

2.3 Sampling Strategy 15

2.4 Sampling/Monitoring Points 16

2.5 Frequency of Sampling/Monitoring Events 18

2.6 Decontamination Event Sequence 18

3 Testing and Measurement Protocols 19

3.1 Methods 19

3.1.1 Surface Sampling 19
3.1.1.1 Wipe Sampling 19
3.1.1.2 HEPA Vacuum Sampling 21
3.1.2 Core Sampling 24
3.1.3 Swab Sampling 25
3.1.4 Rinsate Collection and Sampling Procedures 26
3.2 Prevention of Cross-contamination of Sampling/Monitoring Equipment 26
3.2.1 Preventing Cross-Contamination during Execution of the Decontamination Process 27
3.2.2 Preventing Cross-Contamination during Sampling 28
3.2.3 Preventing Cross-Contamination during Analysis 29
3.3 Representativeness 29
3.4 Sample Quantities 29
3.5 Sample Containers for Collection, Transport, and Storage 29
3.6 Sample Identification 30
3.7 Sample Preservation 30
3.8 Sample Holding Times 31
3.9 Sample Handling and Custody 31
3.10 Sample Archiving 32
4 Testing and Measurement Protocols 33
4.1 Sample Analyses 33
4.1.1 Recovery from HEPA Vacuum Sample 33
4.1.2 Filter Plating 34
4.1.3 Recovery of Core Samples 35
4.2 Analysis Equipment Calibration 35
5 Quality Assurance 36
5.1 Data Quality 36
5.2 Quality Assurance/Quality Control Checks 36
5.3 Data Quality Objectives 37
5.4 Audits 37
6 Results and Discussion 38
6.1 Data Reduction and Validation 38
6.2 Test Results 39

6.2.1 Length of Decontamination Event 46
6.2.2 Physical Impact on Materials and Crew 46
6.2.3 Fate of Spores 46
6.2.4 Health and Safety Effects of Decontamination 46
References 47

List of Figures

Figure 2-1. Conceptual Flowchart for a Test 4
Figure 2-2. Carpet Coupon 6
Figure 2-3. Template for Creating 1' x 1' Carpet Areas 7
Figure 2-4. Carpet Setup in COMMANDER during Inoculation 8
Figure 2-5. Carpet Setup in COMMANDER during Decontamination 9
Figure 2-6. Diffusion Shield 10
Figure 2-7. Century 400 Ninja Carpet Cleaner 11
Figure 2-8. Judson Labs O_2 Pre-Spray and Rinse System 12
Figure 2-9. Decontaminating Carpet B with Spor-Klenz Using the Carpet Extractor 13
Figure 2-10. Carpet Section Template and Sample Grid 17
Figure 3-1. HEPA Sock Sampling a Coupon Section for Viable Spores 22
Figure 6-1. Wipe Sampling Results for Test A 39
Figure 6-2. HEPA Sock Sampling Results for Test B 40
Figure 6-3. HEPA Sampling Results after Decontamination Attempts 41
Figure 6-4. Core Sampling Results after Decontamination Attempts 42
Figure 6-5. Spor-Klenz Applied to a New Carpet with a Backpack Ssprayer 43
Figure 6-6. Spor-Klenz Applied to a New Carpet with an Unheated Vacuum Cleaner 43

List of Tables

Table 2-1. Test Matrix 14
Table 3-1. Cleaning Methods and Frequency for Common Test Materials/Equipment 27
Table 3-2. Sample Quantities for Each Test Setup 30
Table 4-1. Instrument Calibration Frequency 35

Table 5-1. Quality Control Checks 36
Table 5-2. DQIs for Critical Measurements 37
Table 5-3. Acceptance Criteria for Critical Measurements 37
Table 6-1. Spor-Klenz Applied with a Carpet Cleaner* 44
Table 6-2. Spor-Klenz Applied with a Backpack Sprayer* 44
Table 6-3. Log reduction 45

List of Appendices

Appendix A Piping and Instrumentation Diagram of COMMANDER

Appendix B MSDS for Decontamination Solutions

Appendix C Miscellaneous Operating Procedure (MOP) 6535a

List of Acronyms and Abbreviations

APPCD	Air Pollution Prevention and Control Division
ATCC	American Type Culture Collection
B.	*Bacillus*
CFU	Colony Forming Unit(s)
CM	Critical measurement(s)
COC	Chain of Custody
COMMANDER	COnsequence ManageMent ANd Decontamination Evaluation Room
CT	Concentration * time
DCMD	Decontamination and Consequence Management Division
DQI(s)	Data Quality Indicator(s)
DQO(s)	Data Quality Objective(s)
EPA	U.S. Environmental Protection Agency
HEPA	High Efficiency Particulate Air
H_2O_2	Hydrogen peroxide
IDLH	Immediately Dangerous to Life and Health
INL	Idaho National Laboratory
ISO	International Organization for Standardization
LOD	Limit(s) of detection
MOP	Miscellaneous Operating Procedure
NDT	National Decontamination Team
NHSRC	National Homeland Security Research Center
NIST	National Institute for Standards and Technology
ORLS	On-site Research Laboratory Support
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OSWER	Office of Solid Waste and Emergency Response
PBST	Phosphate buffered saline with 0.05% TWEEN®-20
PPE	Personal protective equipment
PVC	Polyvinyl chloride
QA	Quality Assurance
QC	Quality Control

QAPP	Quality Assurance Project Plan
RSD	Relative Standard Deviation
RTP	Research Triangle Park
TRIO	Task Force on Research to Inform and Optimize
TSA	Tryptic Soy Agar
VHP®	Vaporous hydrogen peroxide
VOC	Volatile Organic Compound

List of Units

in	Inch/Inches
ft	Foot/Feet
ft^2	Square feet
mg/L	Milligrams per Liter
mL	Milliliter
ppm	Part(s) per Million
rpm	Revolutions per Minute

Executive Summary

This project supports the U.S. Environmental Protection Agency's (EPA) Homeland Security Research Program (HSRP) to improve the capability to respond to terrorist attacks affecting buildings and the outdoor environments. Given the impact that a few letters containing anthrax spores had on the U.S. Postal Service system in 2001, critical public facilities contaminated following a wide area release could quickly consume the Nation's entire remediation capacity, requiring years to clean up and resulting in enormous economic impacts. Additional quick, effective and economical decontamination methods having the capacity to be employed over wide areas (outdoor and indoor) are required to increase preparedness.

Although some of the facilities in which these letters were processed or received in 2001 were heavily contaminated, they were successfully remediated with approaches such as fumigation with chlorine dioxide or vaporous hydrogen peroxide. In addition, other cleaning methods were used in secondarily contaminated areas or primarily contaminated facilities showing a minimal presence of anthrax spores. These methods included combinations of disposal of contaminated items, vacuuming, and the use of liquid sporicides such as a pH-adjusted bleach solution. Additionally, a combined set of mechanical and chemical procedures (vacuum, scrub/wash and bleach) was used successfully in the decontamination of a small shed contaminated with anthrax spores originating from animal hides during a drum-making process.[1] If proven effective, any approach involving washing and cleaning with readily available equipment would significantly increase EPA's readiness to respond to a wide area release.

This project investigated the decontamination of carpet surfaces contaminated with *Bacillus* spores (i.e., surrogates of *B. anthracis*). Two types of wet/vacuum carpet cleaning systems – unheated (cold) and steam/heated (hot) – were tested for efficacy. In addition, the sporicide Spor-Klenz® Ready to use (Spor-Klenz) (STERIS, Mentor, OH) was used in a carpet cleaner instead of the typical surfactant. This apparatus was compared to application of Spor-Klenz with a backpack sprayer. The goal was to provide information to support the development, use, and/or statement of limitations of these lower-tech decontamination procedures for surfaces.

This work measured the reduction in viable spores on and within the carpet surfaces (effectiveness) as a function of the cleaning technique and duration applied to both new and used carpet. The size of the carpet sections, roughly 4' x 4', was chosen as feasible yet representative of what will likely be encountered in the field (e.g., walkways). Operational parameters such as time, physical impacts on materials or the remediation crew, and the fate of the viable spores (e.g., contamination of equipment carpet cleaner parts, rinsate) were also determined.

The major finding of this research is that spores are very difficult to recover from carpet once the carpet has been wetted. The study suggests that carpet cleaners alone are not effective in completely removing spores (to a non-detectable amount). Carpet cleaners may be effective if used in concert with sporicides to decontaminate a carpet. Neither the High-Efficiency Particulate Air (HEPA) sock sampling nor wipe sampling seemed capable of recovering spores from a wetted carpet, even after the carpet had dried. This problem may have been compounded for used carpet, possibly due to the higher amounts of surface area from dirt, debris, and worn fibers. Extractive techniques were deemed more reliable, but the methods developed for this study need refinement to improve detection limit and recovery.

Test A − Wipe Samples

The first test was conducted with both the hot and cold vacuum cleaners on new carpet, using the manufacturer-recommended pre-spray and extraction solutions, and four sequential decontaminations. Wipe samples taken after the first decontamination seemed to suggest that both vacuum cleaners had removed 99.99% of the spores.

Use of the wet carpet cleaners was not expected to kill the spores but to decontaminate by removing the spores. In this way, the spores could be considered a material, rather than an organism, and a rudimentary type of mass balance could be applied to them. Insufficient spray was used, however, to actually effect such a dramatic log reduction for such a porous material and led to the consideration that the spores had been pushed down within the carpet pile and inaccessible to the wipe sampling.

Test B − HEPA Samples

For Test B, conducted on old carpet, HEPA sock sampling was used instead of wipe sampling. The HEPA sock results suggested that a significant log reduction could be obtained using either the heated or unheated carpet cleaners. However, based on the ineffectiveness of the wipe samples, the data could also suggest that the HEPA socks were no more effective than the wipe sample had been at recovering spores from a wetted carpet.

Test C − HEPA and Core (Extractive) Samples

Results from Tests A and B left questions regarding the effectiveness of the decontamination process based upon uncertainty in the sampling methods. Test C was designed to answer this question by altering the carpet construction method to allow for the collection of the core (extractive) samples in addition to the HEPA sock samples. This test was conducted on new carpet.

Each sample area was first sampled using HEPA socks, and extractive analysis was performed on a small 18 mm diameter core taken from the center of the same sample area. These data show that while HEPA sock sampling suggested that the decontamination methods were removing some amount of spores, subsequent extractive samples showed no or minimal removal. The HEPA sock samples of control areas (not decontaminated) showed minimal downward drift.

The act of decontaminating with the wet/dry vacuums seems to push the spores away from the surface and into the carpet pile, where the HEPA socks are unable to sample effectively. Even though the samples were allowed to dry overnight, residual moisture or detergent may have helped spores adhere to carpet fibers. The apparent log reduction from the HEPA sock samples from Test B showed a greater log reduction than the HEPA sock samples from Test C. The tamped down nature of the older, used carpet, as well as the debris in the fibers, may further reduce recovery by binding to the spores or merely retaining moisture.

Although the results from Tests A and B were inconclusive as to the efficiency of the carpet cleaners, all three tests suggested that there was no statistical difference between the hot (heated) and cold (unheated) cleaners. Test C indicated that the efficacy of the carpet cleaners per the manufacturer's recommendations was poor. Extractive sampling for carpet using core samples appears to be the only reliable sampling method following any dampening of the surface.

Test D – Spor-Klenz in Cold Vacuum versus Backpack Sprayer

A final test (Test D), using new carpet, was conducted to provide information about the benefits of using a carpet cleaner with Spor-Klenz in place of the recommended cleaner versus the application of the sporicide without any vacuuming.

Test D showed a very good log reduction from the use of Spor-Klenz, applied either from the carpet cleaner or the backpack sprayer. The first decontamination showed a 6 log reduction using the HEPA socks (no detection on the core samples, which have a higher detection limit than the HEPA sock samples). Following the second application of Spor-Klenz, no spores were detected using either sampling method, showing at least a 7 log reduction in the HEPA sock results.

Due to the quantity of spores in the small size of the core samples, the core samples could only show a greater than 3 log reduction. The core samples taken for the controlled contamination had lower recovery than expected, possibly due to the rigorous method that was used to extract the core samples causing re-aerosolization of many spores and the high detection limit induced by the small size of the core samples.

The length of the decontamination event was very short using the carpet cleaners (approximately 5 seconds per square foot). Test D with Spor-Klenz suggested that performing the decontamination twice may yield no recoverable spores. If these decontaminations are performed immediately (back-to-back), then there is minimum impact on the remediation crew. If, on the other hand, the carpet is allowed to dry between successive applications, then an extra day is involved. Though it was not a consideration for this test, the ultimate disposal of the carpet cleaners after use in an event may contribute to the cleanup time and expense.

The backpack sprayer method as performed does require an extended duration, allowing for treatment of an extrapolated 192 square feet in 30 minutes, or 6.4 square feet per minute, significantly longer per square foot than when a carpet cleaner is used.

No physical impact on the carpet was noted for any of the decontamination methods. While neither was physically strenuous, any activity inside Level C suits (even with cooling vests) leads to heat stress. Moreover, the use of Spor-Klenz in an area without very high air exchanges could lead to levels of hydrogen peroxide or acetic acid above IDLH (Immediately Dangerous to Life and Health) conditions.

Determining the ultimate fate of spores has proven very complicated due to sampling difficulties. The rinsate recovered from the carpet cleaners used in Tests A, B, and C was very contaminated with organisms of unknown origin, obscuring enumeration of the spores of interest, suggesting that the likelihood our target organism was in the rinsate was very high. The data suggest that only a fraction of the spores were removed from the carpet during Tests A, B, and C, so any spores not removed may be viable, and viable spores may be present in carpet, carpet cleaner parts, and rinsate. The spores in Test D may all be inactivated due to the presence of Spor-Klenz in all locations, but some doubt lingers due to difficulties of sampling the rinsate.

1 Project Description and Objectives

This project supports the mission of U.S. Environmental Protection Agency's (EPA) Homeland Security Research Program (HSRP) by providing relevant information pertinent to the decontamination of contaminated areas resulting from an act of terrorism.

A significant gap in preparedness is in the ability to effectively respond to a wide area release of a biological agent such as *B. anthracis* spores (the causative agent of anthrax and often referred to as such, or anthrax spores). Such a release could potentially result in the contamination of a vast number of personal residences, businesses, public facilities (e.g., hospitals), and outdoor areas. In 2001, the introduction of a few letters containing anthrax spores into the U.S. Postal Service system resulted in the contamination of several facilities. Although some of the facilities in which these letters were processed or received in 2001 were heavily contaminated, they were successfully remediated with approaches such as fumigation with chlorine dioxide or vaporous hydrogen peroxide. However, it is believed that critical public facilities contaminated following a wide area release would quickly consume the Nation's entire remediation capacity, requiring years to clean up and resulting in enormous economic impacts. Additional quick, effective and economical decontamination methods having the capacity to be employed over wide areas (outdoor and indoor) are required to increase preparedness for such a release.

Fumigation has primarily been used in heavily contaminated facilities, while other cleaning methods have been used in secondarily contaminated areas or primarily contaminated facilities showing a minimal presence of anthrax spores. These methods included combinations of disposal of contaminated items, vacuuming, and the use of liquid sporicides such as a pH-adjusted bleach solution. Additionally, a combined set of mechanical and chemical procedures (vacuum, scrub/wash and bleach) was used successfully in the decontamination of a small shed contaminated with anthrax spores originating from animal hides during a drum-making process.[1] If proven effective, any approach involving washing and cleaning with readily available equipment would significantly increase EPA's readiness to respond to a wide area release. Data to quantify the effectiveness of such decontamination techniques are not available.

1.1 Process

The general process being investigated in this project is the decontamination of carpet surfaces contaminated with *Bacillus* spores (i.e., surrogates of *B. anthracis*). Decontamination can be defined as the process of inactivating or reducing a contaminant in or on humans, animals, plants, food, water, soil, air, areas, or items through physical, chemical, or other methods to meet a cleanup goal. In terms of the surface of a material, decontamination can be accomplished by physical removal of the contamination or via inactivation of the contaminant with antimicrobial chemicals, heat, UV light, etc. Physical removal could be accomplished via *in situ* removal of the contamination from the material or physical removal of the material itself (i.e., disposal). Similarly, inactivation of the contaminant can be conducted *in situ* or after removal of the material for ultimate disposal.

During the decontamination activities following the results of the 2001 anthrax incidents, a combination of removal and *in situ* decontamination was used. The balance between the two was facility-dependent and factored in many issues (e.g., physical state of the facility). One factor was that such remediation was unprecedented for the United States Government, and no technologies had been proven for such use at the time. The cost of disposal proved to be very significant and was complicated by the nature of the

waste (e.g., finding an ultimate disposal site). Since 2001, a primary focus for facility remediation has been on improving the effectiveness and practical application of *in situ* decontamination methods and evaluating waste treatment options to be able to provide information necessary to optimize the decontamination/disposal paradigm. This optimization has a very significant impact on reducing the cost of and time for the remediation effort.

In this research, the basis for the specific decontamination procedure is the use of wet/vacuum carpet cleaning systems. Two types of carpet cleaning systems – unheated (cold) and steam/heated (hot) – were tested for efficacy. Completion of the test matrix was expected to provide information to support the development, use, and/or statement of limitations of this lower-tech decontamination procedure for surfaces that can achieve a target cleanup goal while minimizing hazardous waste and the spread of contamination.

1.2 Project Objectives

This work measured the reduction in viable spores on and within the carpet surfaces (effectiveness) as a function of the cleaning technique and duration of application to various carpet types. The size of the carpet sections, roughly 4' x 4', was chosen as feasible yet representative of what will likely be encountered in the field (e.g., walkways). Operational parameters such as time, physical impacts on materials or the remediation crew, and fate of the viable spores (e.g., contamination of equipment, wash water, filters) were also determined.

2 Experimental Approach

This section documents the general approach, test conditions, test equipment, and the methods that were used to evaluate the data related to the project objectives.

2.1 General Approach

The general approaches to meet the objectives of this project were:

1. Use of controlled chambers, standardized sections and spore inoculums;
2. Contamination of large sections of materials via aerosol deposition of bacterial spores;
3. Quantitative assessment of spore contamination by sampling representative sections of carpet sections before decontamination;
4. Application of a prescribed decontamination procedure to the test sections;
5. Quantitative assessment of residual contamination by sampling test sections;
6. Quantitative and qualitative analysis of decontamination procedure residues (e.g., waste water,);
7. Determination of decontamination effectiveness (comparison of results from positive control samples and test sections); and
8. Documentation of operational considerations (e.g., cross-contamination, procedural time, impacts on materials and personnel).

Testing was conducted in the COnsequence ManageMent ANd Decontamination Evaluation Room (COMMANDER) located in H130-A of EPA's Research Triangle Park, NC, facility. For the purposes of this project, effectiveness of a procedure was measured by generating a quantitative estimate of log reduction of viable spores on a surface – a 6 log reduction would be considered very successful. However, factors such as spread of viable spores due to the decontamination procedure itself and recovery were factored into the overall measure of effectiveness. Additionally, procedures showing less than a 6 log reduction may be deemed effective depending upon the circumstantial need (e.g., treatment of scant contamination or repeated treatment of hot spots). Thus, while a log reduction value was reported and may be termed effective, the effectiveness related to use of this procedure is less tangible without the context of the need.

The general test approach is depicted graphically in the flow chart shown in Figure 2-1. The following sections provide details on the approach used to complete the testing.

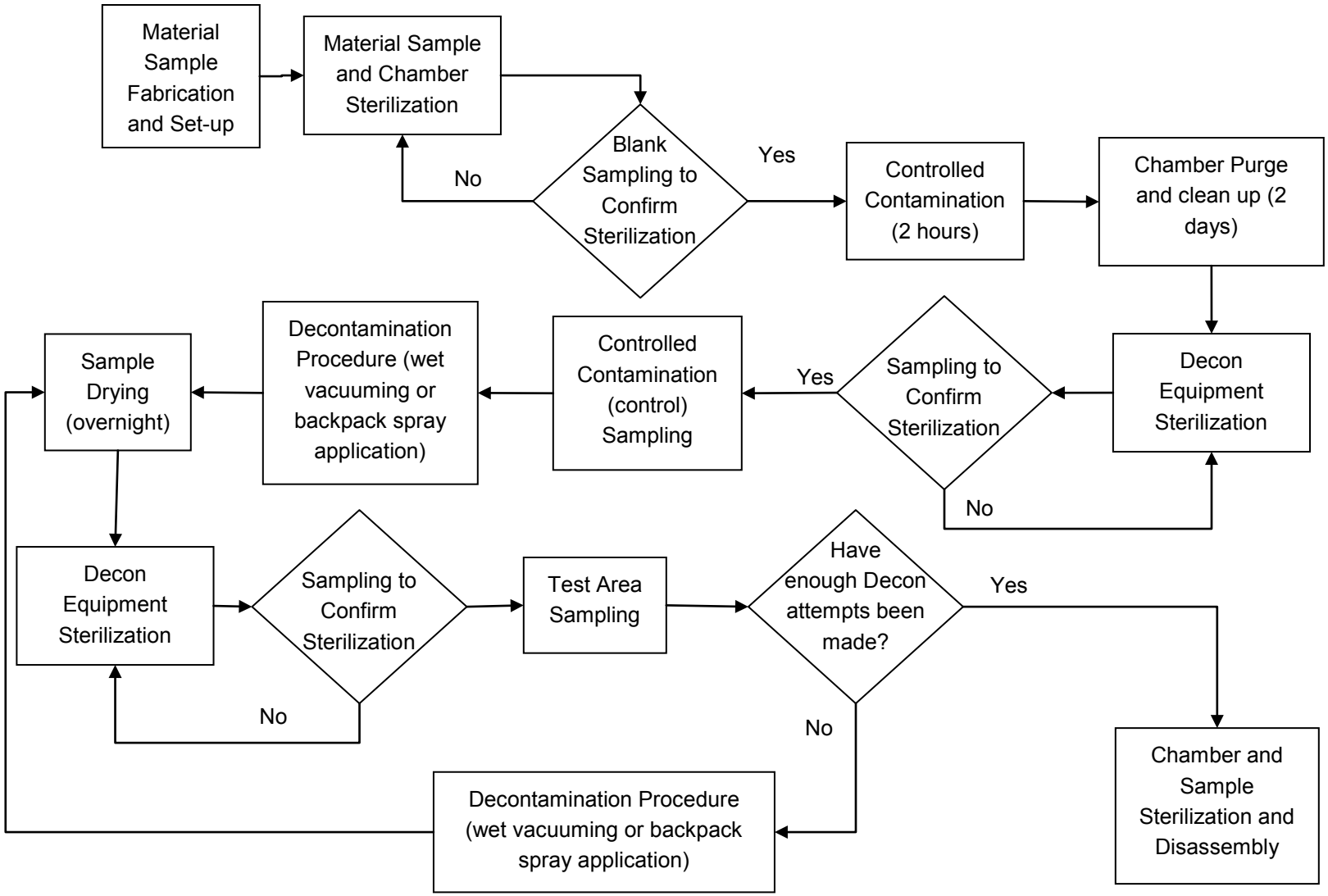

Figure 2-1. Conceptual Flowchart for a Test

2.1.1 Control Chamber (COMMANDER)

All testing was done in the COMMANDER, an enclosed single-access-point chamber (henceforth, may also be referred to as the chamber). COMMANDER meets the following criteria:

1. Supports repeated fabrication of an environment (e.g., furnished office room; outdoor setting) contained within the chamber;

2. Allows for release of biological organisms or chemicals into the chamber;

3. Allows for application of a decontamination technology (including fumigation with toxic corrosive gases);

4. Supports entry into the chamber during all of the above mentioned activities (in appropriate personal protective equipment or PPE);

5. External dimensions of 9 ft. x 12 ft. x 10 ft. high;

6. Contains one air-tight entry/exit port with a window;

7. Contains a 6 ft. x 6 ft. x 8 ft. high airlock with single entry/exit port with a window;

8. Contains entry/exit ports in line with the enclosure double door to allow for large materials to be brought into or out of the chamber; and

9. Complies with all relevant local and national codes.

A piping and instrumentation diagram of COMMANDER is attached in Appendix A.

2.1.2 Material Surfaces

Carpet sections for both the new and “old” carpets were prepared using Sherwood carpet tiles with a Shaw Contract Group Ecoworx® Backing System. These carpet sections were made of 100% nylon woven on a 100% polyvinyl chloride (PVC)–free recyclable backing system with recycled content (made from thermoplastic polyolefin compound with a reinforcing layer). The tiles were manufactured using a multilevel loop construction.

Four individual 2 ft. by 2 ft. carpet tiles were glued directly on a 4 ft. x 4 ft piece of 15/32 in. four-ply plywood, mounted atop a 4 ft. by 4 ft. frame of commercial grade 2 ft. x 3 ft. lumber. A 1 in. x 4 in. border was then attached to the edge of the frame, creating a slight lip to the coupons. A photo of a carpet coupon is shown in Figure 2-2.

Figure 2-2. Carpet Coupon

The used carpet tile came from EPA's Research Triangle Park facility, and was taken from the south corridor adjacent to the elevator lobby of Building C, 6th Floor. The carpet had been in place for approximately eight years.

The 4 ft. x 4 ft. carpet sections were then sectioned into sixteen 1 ft. x 1 ft. sample areas by using the template shown in Figure 2-3. The template was positioned starting at a corner marked on the frame of the carpet section. The resulting 1 ft. x 1 ft. areas were numbered 1 through 16. The only deviation from this sample grid was for the first test, where an 8 x 8 grid of 6 in. squares was used. The change in design for subsequent tests was made both to simplify the sampling process and to provide more information about the homogeneity of the deposition.

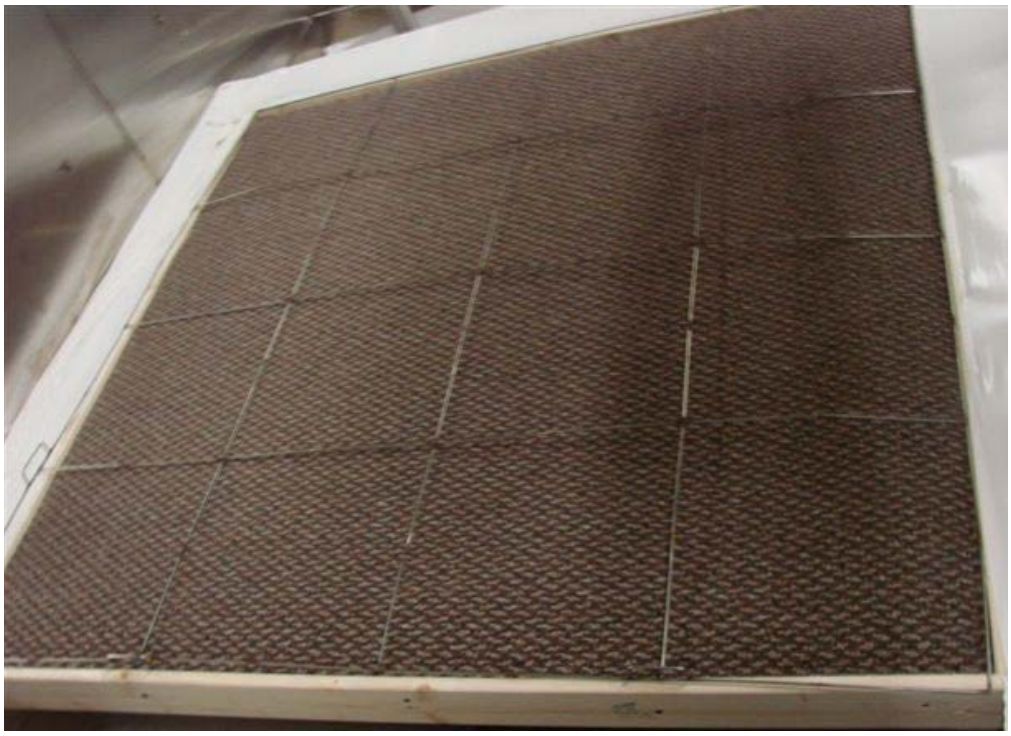

Figure 2-3. Template for Creating 1’ x 1’ Carpet Areas

2.1.3 Chamber Setups

Each test consisted of two carpet sections of the same carpet type. During the inoculation phase, the carpet coupons were centered as shown in Figure 2-4 to allow for a more uniform deposition of aerosolized spores during the spore release.

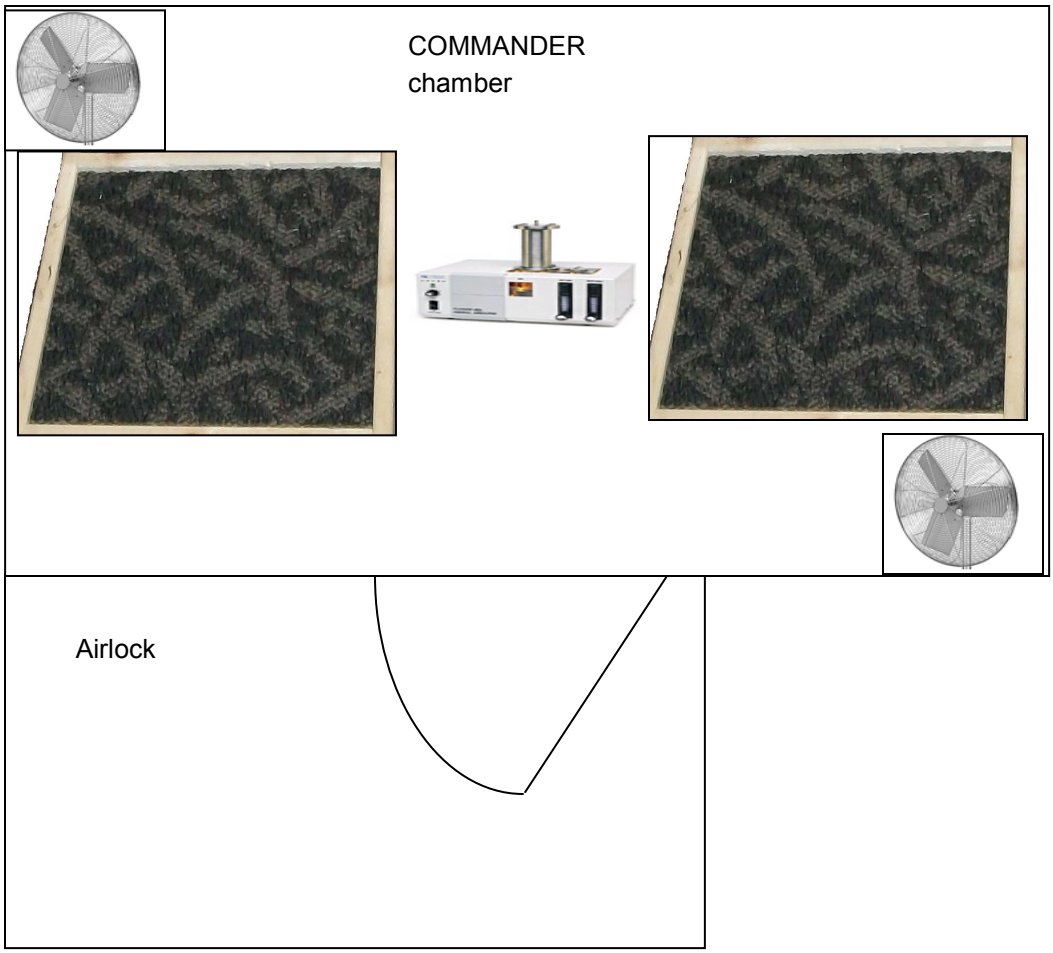

Figure 2-4. Carpet Setup in COMMANDER during Inoculation

The carpet sections were shifted during the decontamination phase to allow a more natural range of motion using the long vacuum cleaner hoses in the relatively small space within COMMANDER. The setup during decontamination is shown in Figure 2-5.

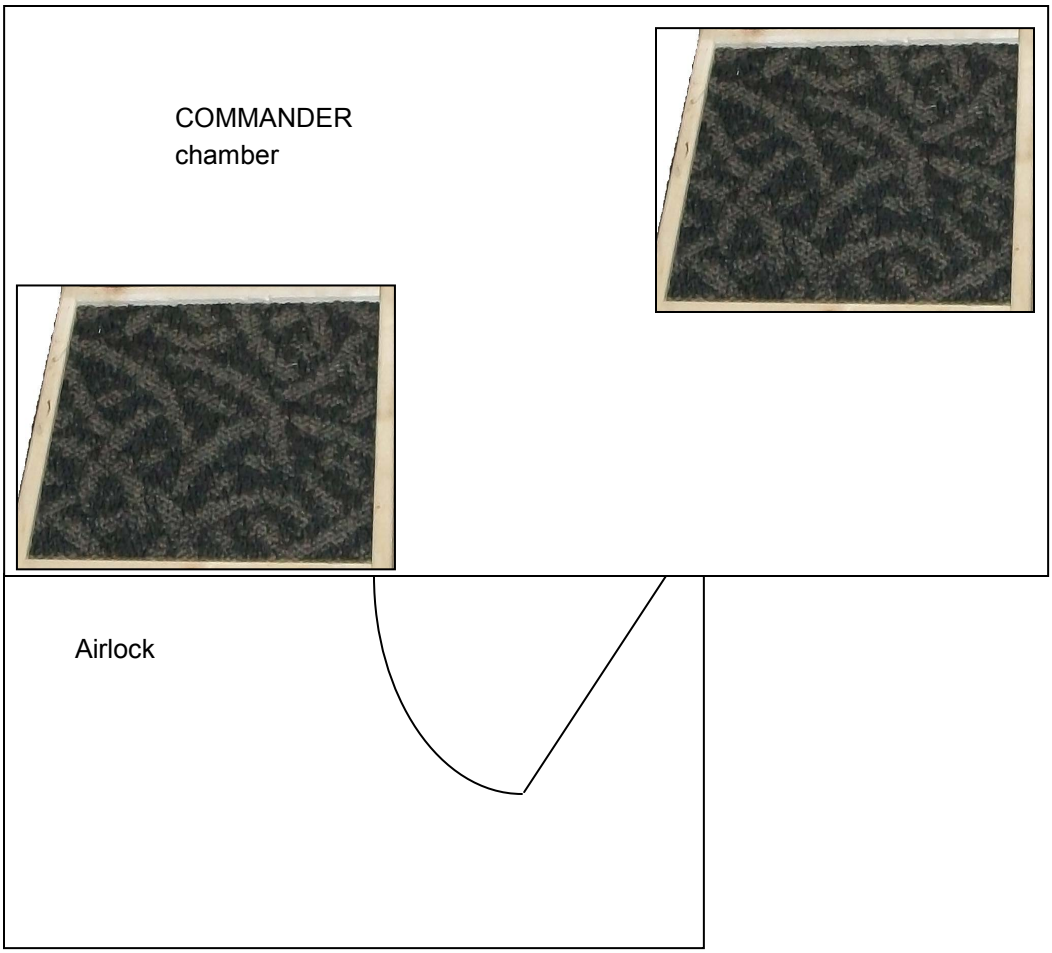

Figure 2-5. Carpet Setup in COMMANDER during Decontamination

2.1.4 Material Sterilization

After the sections were assembled inside COMMANDER, the carpets were sterilized using STERIS vaporous hydrogen peroxide (VHP®). Hydrogen peroxide (H_2O_2) vapor concentration within the chamber was monitored using an Analytical Technology Corp. (Collegeville, PA) H_2O_2 electrochemical sensor (Model B12-34-6-1000-1) to provide real-time concentration readings and control through a feedback loop. A minimum concentration* time (CT) of 1000 parts per million (ppm)*hours was required for the materials to be considered sterile. One test area of each carpet section was sampled (as described in Section 3.1.1.1.2) to test sterility. All sterility checks were negative for growth.

2.1.5 Spore Preparation

The test organism for this work was a powdered spore preparation of *B. atrophaeus* (American Type Culture Collection [ATCC] 9732) and silicon dioxide particles. This bacterial species was formerly known as *B. subtilis* var *niger* and subsequently *B*. *globigii*. The preparation was obtained from the U.S. Army Dugway Proving Ground Life Science Division. The preparation procedure is reported in Brown et al.[2] Briefly, after 80 – 90 percent sporulation, the suspension was centrifuged to generate a preparation of approximately 20 percent solids. A preparation resulting in a powdered matrix containing approximately

$1x10^{11}$ viable spores per gram was prepared by dry blending and jet milling the dried spores with fumed silica particles (Deguss, Frankfurt am Main, Germany). The nominal particulate size was 1 micron. The estimated quantity of spores to be dispersed was approximately $2x10^{10}$ per event.

2.1.6 Controlled Contamination Procedure

The controlled contamination procedure was required to deliver a contamination of $1x10^{6}$ to $1x10^{7}$ recoverable viable spores per square foot to the material samples. This was done by releasing 0.2 g of the spore preparation using a TSI (Shoreview, MN) Model 3400A Fluidized Bed Aerosol Generator. The Model 3400A Fluidized Bed Aerosol Generator was placed in the center of the COMMANDER exposure chamber at a height of 4 feet. A perforated diffusion shield, as shown in Figure 2-6, was placed over the fluidized bed. The shield was made of type 304 stainless steel with 0.25 in. holes and an open area of 58 percent.

Fans inside COMMANDER during the spore release created significant turbulence, forcing spores onto all the surfaces. The real-time concentration of aerosols was monitored using a Dekati ELPI® (Tampere, Finland) Once the aerosol concentration began to subside (indicating that most spores had been released), the fans were turned off (to prevent the turbulence from beginning to remove the spores). The chamber was aerated until no aerosol was detected inside the chamber (typically approximately two hours).Spores were then cleaned from all surfaces except the carpet coupons.

For the first three tests, the walls, floors, and ceiling of COMMANDER were decontaminated using pH-amended bleach to reduce chances of cross-contamination. For the fourth test, this method was not available to use, so the walls, floors, and ceiling were decontaminated with Dispatch® (Caltech Industries, Inc., Midland, MI) wipes.

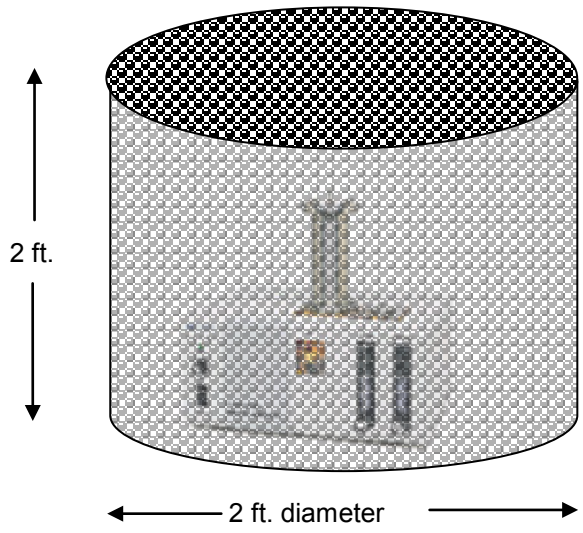

Figure 2-6. Diffusion Shield

2.1.7 Contamination Characterization

The “control” areas (described in Section 2.4 and shown in Figure 2-5 in that section) were sampled (as described in Section 3.1.1.1.2). These “control” areas were the basis for any log reduction as a result of decontamination procedures. This characterization sampling took place on the same day as the decontamination procedure was carried out.

2.1.8 Decontamination Procedure

For Tests A, B and C, two different Century 400 (Chandler, AZ) Ninja Carpet Extractors (Figure 2-7) were used for the decontamination procedure, the notable difference being that one carpet cleaner used a heated surfactant (Century 400 Ninja 150 PSI, 411-22AHMO) while the other did not include a heater (Century 400 Ninja 150 PSI, 411-22AMO). The same pre-treatment and surfactant (Judson Labs [Greenville, S.C.] O_2 Pre-Spray and Rinse System) were used for both carpet cleaners (Figure 2-8). Both cleaners include a 12 in. dual jet head wand. The pretreatment was applied for a target time of 20 seconds at approximately 660 mL/minute before use of the carpet cleaner. The surfactant was placed in the carpet cleaner reservoir and allowed to heat up (if the heater was present). The surfactant is sprayed from the wand when the operator opens a trigger valve on the wand. During the decontamination procedure, the wand was placed in the left corner closest to the operator and the surfactant was applied along the full length of the carpet. The surfactant was then extracted by pulling the wand back in a straight line using a single stroke left to right across the entire coupon. This pattern was repeated at a 90 degree angle to the first pass. Completion of the second pass was considered the end of a procedure.

Figure 2-7. Century 400 Ninja Carpet Cleaner

For Test D, Coupon A was saturated with Spor-Klenz from a backpack sprayer, back and forth across the carpet with 50 percent overlap on each pass. Spor-Klenz was reapplied in this manner at 20 and 40 minutes from initial application.

The carpet extractor was used to decontaminate Coupon B (see Figure 2-7). During the first decontamination, the wand was placed in the center of the coupon on one side. The operator sprayed the Spor-Klenz RTU solution (Steris Corporation, St. Louis, MO) while moving the wand toward the opposite end of the coupon. The wand was then pulled back toward the operator, extracting the Spor-Klenz from the carpet left to right across the top half of the coupon. The procedure was then repeated across the bottom half of the coupon, with an overlap on the top half of approximately 10 percent.

Figure 2-8. Judson Labs O_2 Pre-Spray and Rinse System

For Test D, Coupon A was saturated with Spor-Klenz from the backpack sprayer (ShurFlo 4 ProPack Rechargable Electric Backpack Sprayer, SHURFLO, LLC., Elkhart, Indiana) back and forth across the carpet with 50 percent overlap on each pass. Spor-Klenz was reapplied in this manner at 20 and 40 minutes from initial application.

The carpet extractor was used to decontaminate Coupon B (see Figure 2-9). During the first decontamination, the wand was placed in the center of the coupon on one side. The operator sprayed the Spor-Klenz RTU solution while moving the wand toward the opposite end of the coupon. The wand was then pulled back toward the operator, extracting the Spor-Klenz from the carpet left to right across the top half of the coupon. The procedure was then repeated across the bottom half of the coupon, with an overlap on the top half of approximately 10 percent.

Figure 2-9. Decontaminating Carpet B with Spor-Klenz using the Carpet Extractor

The second decontamination with the carpet extractor was slightly different. Due to the awkward nature of the first decontamination (the COMMANDER was too narrow to pull the wand to the edge of the carpet hence decontamination was performed in halves), the carpet was placed against the wall opposite the operator to allow for more room. The wand was placed in the left corner closest to the operator and Spor-Klenz was applied along the full length of the carpet. The Spor-Klenz was extracted by pulling the wand back in a straight line using a single stroke left to right across the entire coupon.

2.1.9 Final Sterilization

Once all decontamination procedures had been completed (up to four repeat cleanings or until the decontamination procedure was deemed successful), the material sections and COMMANDER were sterilized again with VHP®. After this final sterilization, the material sections were discarded.

2.2 Test Matrix

To fulfill the project purpose (Section 1.1) and meet the objectives (Section 1.3), the test matrix shown in Table 2-1 was developed. In the presentation of results (Section 6), the reasoning behind the change in surface sampling methods is discussed.

Table 2-1. Test Matrix

Test	Carpet Type	Protocol	Carpet Cleaner Liquid	Sampling Techniques
A	New	Hot vs. cold carpet cleaners	Judson Laboratories Oxygen 2 Pre-Spray and Extraction	Wipe Samples
B	Old	Hot vs. cold carpet cleaners	Judson Laboratories Oxygen 2 Pre-Spray and Extraction	HEPA sock samples
C	New	Hot vs. cold carpet cleaners	Judson Laboratories Oxygen 2 Pre-Spray and Extraction	HEPA sock samples and core samples
D	New	Backpack sprayer vs. cold carpet cleaner	Spor-Klenz	HEPA sock samples and core samples

The decontamination procedure was repeated (up to four times) on materials to determine the number of cleanings necessary to remove the spores as completely as possible. These tests were performed according to the following parameters:

1. Each test was run independently.

2. A single test included the completion of all carpet cleaner types within that setup.

3. A material section blank was taken from each carpet section.

4. Following controlled contamination and decontamination, samples were collected for the test sections of each material type.

5. Cleaning of COMMANDER and all equipment used during testing was performed as described in Section 2.1.9 after the completion of each test.

6. Each 4 ft. x 4 ft. carpet section required three positive control samples and three test samples of each carpet section for each of the consecutive decontamination procedures (up to four), as well as vacuum liquid samples from the decontamination step for each carpet section. Hence, if four consecutive decontamination procedures were conducted, a total of 16 vacuum samples was generated for each carpet section (one vacuum sample for each 1 ft. x 1 ft. area). The exception to this procedure was Test 1, using wipe sampling, which had six samples instead of three at each sampling time.

7. The general testing sequence was shown in Figure 2-1. The following steps describe the testing sequence:

    a. All material sections needed for this project were prefabricated before any testing was begun.

    b. All spores for the study were prepared, per the method discussed in Section 2.1.5, prior to the initiation of any testing.

c. The material sections were installed in COMMANDER and sterilized using VHP®.

d. All material sections for a given test were contaminated in accordance with Section 2.1.6.

e. After air purging, personnel wearing appropriate PPE entered the COMMANDER. Initial cleaning of the walls to reduce chances of cross-contamination was conducted.

f. All materials and equipment necessary for the decontamination procedure were gathered and prepared as documented in Section 2.1.8.

g. Sampling of each test area was done according to Section 3.1.1.

h. Decontamination according to Section 2.1.8 was completed on one material section. All decontamination steps were completed before moving on to the next material.

i. After all decontamination was complete, samples were recovered from the wet/dry vacuums in accordance with Sections 3.1.2.

j. After a minimum of 18 hours and when all coupon surfaces were visibly dry, surface sampling was done in accordance with Section 3.1.1.

k. Decontamination procedures were repeated up to four times, followed each time by sampling.

l. Sample analysis was performed as described in Section 4.1. Data reduction and validation were conducted as described in Section 6.1.

2.3 Sampling Strategy

The objective of the study was to assess the effectiveness of a decontamination procedure to decontaminate the surfaces. The effectiveness was measured by the determination of the log reduction calculated per Section 6.1. Hence, surface sampling of the test areas before and after decontamination was required to determine the log reduction after application of the procedure. Because current surface sampling techniques are intrusive, they will also remove viable spores from the surface of the section. Sampling of positive control areas was required to compare to post-decontamination sampling of test sections for this study. Positive controls and test areas are subsections of the carpet section. Positive control areas were sampled in accordance with Section 4, before the decontamination procedure. The entire carpet section, including test areas, is carried through the decontamination procedure, allowed to dry, and subsequently sampled in accordance with Section 4.

The effectiveness of removing contamination from the surface of the sections provides critical information regarding the potential of the procedure; however, field applicability is also dependent upon several other factors including the ultimate disposition (or fate) of the spores. This latter information is required to provide information pertinent to the development of a comprehensive site-specific remediation strategy. For example, if viable spores are washed off materials, remediation field strategies might require rinsate

collection and treatment. Hence, it is important to understand the fate of the spores resulting from the application of the decontamination procedure on the section surface.

To obtain the additional critical information on the fate of the spores, several samples in addition to the surface sampling of the sections was collected. To assess the potential for viable spores to be washed off the surfaces, all liquids used in the decontamination process were collected and quantitatively analyzed as a composite sample for the entire decontamination procedure on a particular carpet section. Quantitative analysis was done on these rinsate samples to provide for an order of magnitude determination of the disposition of viable spores in this media.

There are currently no validated methods for sampling biological agents from porous materials. Hence, results from past field practices[1] and recent studies[2 - 5] were used to define the surface sampling strategy. For rough and/or porous surfaces, HEPA vacuum sampling is the preferred method.[3,6] Limits of detection (LOD) and sensitivities determined from the comparison of wipes on nonporous surfaces and the HEPA vacuum on nonporous and porous surfaces indicate that HEPA vacuuming has a comparable LOD (400-600 colony forming units [CFU] per sample area) to wipe sampling and an order of magnitude greater sensitivity.[2] Of the literature reviewed, however, only one reference provided a direct comparison between HEPA vacuuming and wipe sampling for a porous surface (carpet).[5] While wipe sampling had a higher collection efficiency, the level of detection was lower for the HEPA vacuuming.

2.4 Sampling/Monitoring Points

The front face of each carpet section was the only surface of the sections that was sampled in this study. Two 4 ft. x 4 ft. sampling templates of welded stainless steel wire were made for each carpet section, one for before decontamination and one for post-decontamination sampling. The template, with 16 areas of 1 square foot, is shown in Figure 2-10. This template was placed against the material during the sampling events. The only deviation from this sample grid was for the first test, where an 8 x 8 grid of 6 in. squares was used. Six areas were sampled, rather than the three replicates described here. The change in design simplified the sampling process and provided more information about the homogeneity of the deposition.

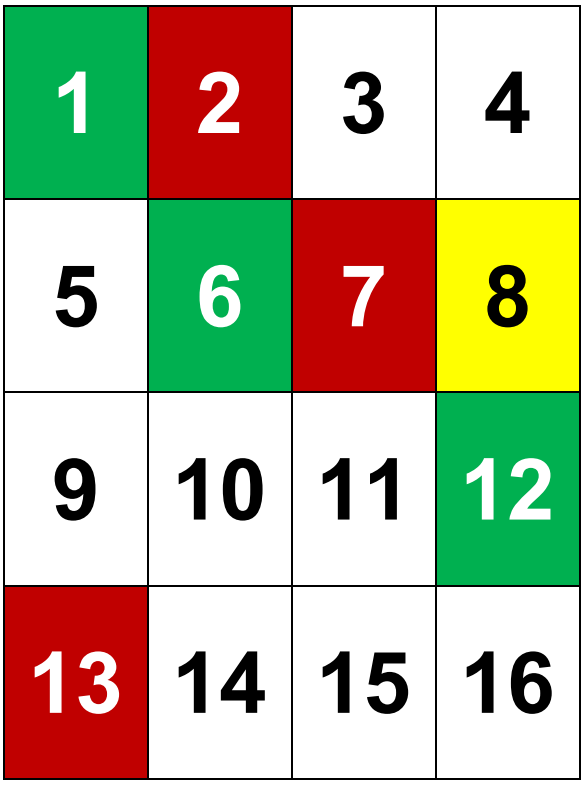

Figure 2-10. Carpet Section Template and Sample Grid

One area was sampled as a blank after sterilization. This area was "randomly" selected based on the last digit of the date the blank sample was taken. A small indelible mark was placed on the sample area to mark it as the starting point for the subsequent samples. As an example, blank sample testing on April 28th would indicate a blank sample taken from area 8. This area has been highlighted yellow in Figure 2-10.

After the controlled contamination, three areas were sampled as controls, i.e., indicative of spore counts *before* decontamination. Starting from the blank sample area, every fifth area was designated as one of these controls. In our example, this would mean that areas 13, 2, and 7 would be control sample areas (highlighted red in Figure 2-10). A small mark in indelible ink was made on each area of the material surface after sampling.

After decontamination, three more areas were sampled. The decontaminated samples were taken from areas with preceding numbers to the control areas. In our example, these would be Areas 12, 1, and 6 (highlighted green in Figure 2-10). A small mark in indelible ink was made on each of these areas of the material surface after sampling.

The decontamination procedure was repeated up to three more times, with successive rounds of sampling being conducted on the sampling areas with preceding numbers to the first post-decontamination sampling areas. In our scenario of blank testing starting on June 28th, areas 11, 16 and 5 would be sampled following the second round of decontamination; areas 10, 15, and 4 after the third round of decontamination; and areas 9, 14, and 3 after the fourth and final round of decontamination. No area was sampled twice.

The liquid in each carpet cleaner recovery tank was analyzed independently. The COMMANDER chamber was cleaned as detailed in Section 2.1.9 after the final sampling round.

2.5 Frequency of Sampling/Monitoring Events

Three surface samples of each carpet section were collected before decontamination (control samples) and three samples after each of the following decontaminations (up to four) and the appropriate drying time post-decontamination. The liquid collected by the cleaners from the carpet surfaces was filtered and labeled appropriately (see Section 3.6) after the conclusion of decontamination of each carpet section.

2.6 Decontamination Event Sequence

For Tests A, B and C, the pre-spray and the solution used in the hot and cold vacuums were those recommended by the manufacturer (Judson Laboratories Oxygen 2/Pre-Spray and Oxygen 2/Extraction, respectively). The STERIS Spor-Klenz used in Test D was ready-to-use as provided by the manufacturer. Material Safety Data Sheets (MSDS) for each decontamination solution are included in Appendix B.

3 Testing and Measurement Protocols

Several types of samples were included in this project. Surface sampling procedures were used to collect samples from the coupon materials. These samples included wipe samples as well as HEPA socks. Core (punch or hole saw) samples were taken for spore enumeration via extractive analysis. In addition, swab sampling was done for each sterilization batch for all equipment used during decontamination (vacuum nozzles, etc), as well as to identify spores captured in the any filter associated with the wet vacuum cleaners used during the decontamination. The rinsate generated during the decontamination procedure was collected for each material type. Details of the sampling procedures are provided below. A laboratory notebook was used to document the details of each sampling event (or test).

3.1 Methods

3.1.1 Surface Sampling

Prior to the sampling event, all materials needed for sampling were prepared. The materials specific to each protocol are included in the relevant sections below. In addition, general sampling supplies were also needed. A sampling material bin was stocked for each sampling event, using the information included in Section 3.4 (Table 3-2). The bin contained enough sampling kits to accommodate all required samples for the specific test. Additional kits of each type were also included for back-up. Sufficient prepared packages of gloves and bleach wipes were also included in the bin. A sample collection bin was used to transport samples back to the Microbiology Laboratory. The exterior of the transport container was decontaminated by wiping all surfaces with a bleach wipe or towelette moistened with a solution of pH-adjusted bleach prior to transport from the sampling location to the Microbiology Laboratory.

3.1.1.1 Wipe Sampling

Wipe sampling is typically used for small sample areas and is effective on nonporous smooth surfaces such as ceramics, vinyl, metals, painted surfaces, and plastics.[7] The general approach is that a moistened sterile non-cotton pad is used to wipe a specified area to recover bacteria, viruses, and biological toxins.[7] The protocol that was used in this project is described below and has been adapted from that provided by Busher et al.[7] and Brown et al.[8], and documented in the INL 2008 Evaluation Protocols.[9] None of these references provides a validated wipe procedure for *Bacillus* spores, as a validated sampling procedure does not currently exist.

The following procedure was used in this study for wipe sampling of each coupon surface:

1. A two-person team was used, employing aseptic technique throughout. The team consisted of a sampler and a support person.

2. All materials needed for collection of each sample were prepared in advance using aseptic technique. A sample kit for a single wipe sample was prepared as follows:

    a. Two sterile sampling bags 10 in. x 15 in. (Fisherband Twirl'Em Sterile Sampling Bags, item 14-955-196, Pittsburg, PA) 5.5 in. x 9 in. (Fisherbrand Sterile Sampling Bags, item 14-955-185, Pittsburgh, PA) and a 50 mL conical tube (Becton Dickson Labware, item 352098, Franklin Lakes, NJ), capped, were labeled. These bags and conical tube had the same label. The 5.5 in. x 9 in. labeled sterile sampling bag was referred to as the sample collection sterile sampling bag.

b. A dry sterile wipe was placed in an unlabeled sterile 50 mL conical tube using sterile forceps and aseptic technique. The wipe was moistened by adding 5 mL of sterile phosphate buffered saline with 0.05% TWEEN®-20 (item P-3563, SIGMA, St. Louis, M.O., USA). The tube was then sealed.

c. The labeled 50 mL conical tube, capped, the unlabeled conical tube containing the pre-moistened wipe, and the 5.5 in. x 9 in. labeled sampling bag were placed into the 10 in. x 15 in. labeled sterile sampling bag. Hence, each labeled sterile sampling bag contained a labeled 50 mL conical tube (capped), an unlabeled capped conical tube containing a pre-moistened wipe, and an empty labeled sterile sampling bag.

d. Each prepared bag was one sampling kit.

3. The sampler and support person placed the template onto the coupon surface.

4. Each member of the sampling team donned a pair of sampling gloves (a new pair per sample); the sampler's gloves were sterile sampling gloves. All members wore N95 dust masks to further minimize potential contamination of the samples.

5. The support person removed a sample kit from the sampling bin and confirmed sample ID to sampler.

6. The support person:

    a. Opened the outer sterile sampling bag touching the outside of the bag.

    b. Touching only the outside of the outer bag, maneuvered the unlabelled conical tube until it was at the opening of the bag. The sampler then grabbed the tube, removed it from the bag, opened the tube, and poured the pre-moistened wipe into the glove of the sampler.

    c. Discarded the unlabelled conical tube.

    d. Maneuvered the labeled 50 mL conical tube to the end of the outer sterile sampling bag and loosen the cap.

    e. Removed the cap from 50 mL conical tube immediately preceding the introduction of the sample into the tube.

7. The sampler:

    a. Squeezed out excess moisture from the wipe before approaching sample area.

    b. Confirmed the area ID of the carpet to sample.

    c. Wiped the surface of the sample horizontally using S-strokes to cover the entire sample area of the coupon using a consistent amount of pressure.

    d. Folded the wipe concealing the exposed side and then wiped the same surface vertically using the same technique.

e. Folded the wipe over again and rolled the folded wipe to fit into the conical tube.

f. Carefully placed the wipe into the 50 mL conical tube that the support person was holding being careful not to touch the surface of the 50 mL conical tube or plastic sterile sampling bag.

8. The support person immediately closed and tightened the cap to the 50 mL conical tube and slid the tube back into the sample collection sterile sampling bag.

9. The support person then put the 50 mL conical tube into the empty labeled 5.5 in. x 9 in. sampling bag and sealed the bag.

10. The support person then sealed the outer sample collection bag containing the capped 50 mL conical tube (containing the sample wipe) inside a sealed 5.5 in. x 9 in. sample collection bag.

11. The support person then decontaminated the outer sample bag by wiping it with a Dispatch® bleach wipe.

12. The support person then placed the triple-contained sample into the sample collection bin.

13. All members of the sampling team removed and discarded their gloves.

14. Steps 3 – 13 were repeated for each sample collected.

3.1.1.2 HEPA Vacuum Sampling

HEPA vacuum sampling is typically used for large porous areas. The general approach is that a collection sock is used to trap dust material. The protocol that was used in this project is depicted below and has been adapted from that provided by Busher et al.[2] and Brown et al.[3] and documented in the INL 2008 Evaluation Protocols[9]. None of these references provides a validated HEPA vacuuming procedure for *Bacillus* spores, as a validated sampling procedure does not currently exist.

The following procedure, shown in Figure 3- 1, was used in this study for HEPA vacuum sampling the surfaces of each area of carpet section or blank coupon:

1. A two person team was used. The team consisted of a sampler and a support person.

2. All materials needed for each sample to be collected were prepared in advance. A sample kit for a single HEPA vacuum sample was prepared as follows:

   a. Two sterile sampling bags were labeled in accordance with Section 3.6. These bags had the same label. An additional unlabeled bag was utilized.

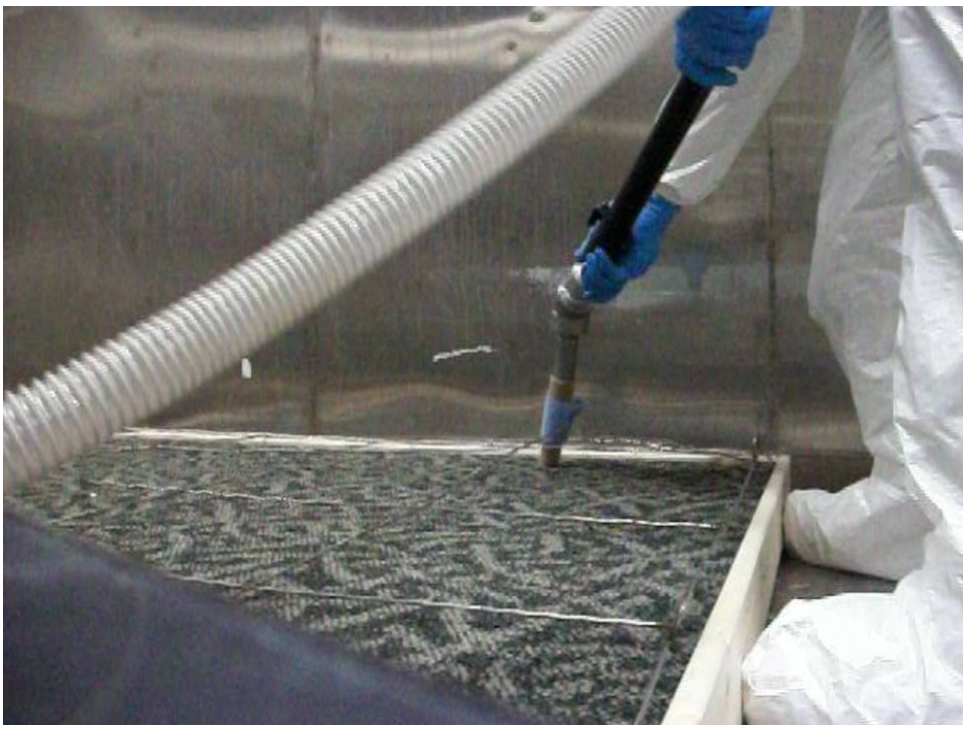

Figure 3-1. HEPA Sock Sampling a Coupon Section for Viable Spores

b. A HEPA sock assembly was placed into one of the unlabeled sterile sampling bags.

c. The bag containing the HEPA sock assembly was placed into a labeled sterile sampling bag; the second unlabeled bag was also placed into the labeled bag. The label was clearly distinguishable through the unlabeled bag.

d. Each prepared bag was one sampling kit.

3. The sampler and support person placed the template onto the coupon surface.

4. Each member of the sampling team donned a pair of sampling gloves (a new pair per sample).

5. The sampler plugged in the HEPA vacuum power cord and then donned his/her gloves.

6. The sampler placed the HEPA vacuum onto a convenient surface and held the vacuum nozzle for the support person to place the HEPA vacuum sock assembly onto the nozzle.

7. The support person opened the sampling supply bin and removed one HEPA vacuum sock sample kit from the bin.

8. The support person recorded the sample collection bag number on the sampling log sheet or laboratory notebook.

9. The support person recorded the coupon code on the sampling log sheet or laboratory notebook next to the corresponding sample collection bag number that was just recorded.

10. The support person:

    a. Opened the outer sampling bag containing the HEPA vacuum sock assembly.

    b. Opened the bag within the outer sterile sampling bag and pushed the HEPA vacuum sock assembly from the bottom to expose the cardboard applicator tube opening.

    c. Placed the HEPA vacuum sock assembly onto the nozzle of the vacuum tube, using the inner sampling bag to handle the HEPA sock assembly, while the sampler held the vacuum nozzle.

11. The sampler:

    a. Turned on the vacuum.

    b. Vacuumed “horizontally” using S-strokes to cover the 1.0 sq. ft. sample area of the template, while keeping the vacuum nozzle perpendicular to the sample surface.

    c. Vacuumed the same area “vertically” using the same technique.

    d. Turned off the vacuum when sampling was completed.

12. The support person opened the labeled sterile sampling bag and removed the HEPA sock assembly from the nozzle by sliding the sampling bag over the HEPA sock assembly and gripping the sock from the outside of the bag.

13. The support person then sealed the inner sterile sampling bag and placed it into the outer sterile sampling bag.

14. The support person then sealed the outer sterile sampling bag and wiped the outer bag with a bleach wipe.

15. The support person then placed the outer sample bag into the remaining labeled sterile sampling bag and disposed of the bleach wipe.

16. The sampler wiped the nozzle (inside and out) and ends of the tubing with bleach wipe, then disposed of the bleach wipe.

17. The support person then placed the triple-contained sample into the sample collection bin.

18. If sampling from the carpet section was completed, the sample handler marked the tested areas as having been sampled and moved the template to the appropriate location for decontamination.

19. All members of the sampling team removed and discarded their gloves.

20. Steps 3 – 19 were repeated for each sample to be collected.

A sterilized stainless steel nozzle was used between the HEPA sock and the vacuum hose. A separate nozzle was used for each carpet cleaning technique.

3.1.2 Core Sampling

The following procedure was used in this study for core sampling of each coupon surface. This procedure involved a two-person team, referred to below as the sampler and the assistant. Punch sampling (utilizing a punch and hammer) was employed for Tests C and D. Because of the difficulty in obtaining core samples with this procedure, it was modified to use a hole saw. The resulting minor modifications to this procedure are shown in *italics.*

1. Kit Assembly

    a. PPE for non-HazMat situations was donned.

    b. Sample IDs were determined.

    c. A sterile 50 mL vortex tube (*or 120 mL sample cup*) was labeled with each sample ID.

    d. An over pack bag was labeled with each sample ID.

    e. The kits were assembled ahead of time. Each kit included a clean, sterile 50 mL tube (*or cup*), a small bag, and a pair of sterile tweezers, all packed into the larger bag.

2. Sample Collection

This procedure followed HEPA Sock sampling.

a. The assistant donned clean gloves.

b. The assistant opened the over pack bag and maneuvered the tweezers from the outside of the over pack until the assistant could grab the package.

c. The sampler recorded the time and the sample ID *(and the size of the hole saw and the pilot bit*).

d. The sampler donned sterile gloves.

e. The assistant opened the tweezers package so that the sampler could grab the sterile tweezers.

f. The assistant opened the bag holding the sterilized punch (*or the bag holding the sterilized hole saw and attaches it to the drill using the outside of the autoclave bag to maintain sterility*).

g. The assistant identified the sample or sample area as indicated by the sample ID on the over pack bag.

h. The assistant used the punch and the hammer (*or drill*) to collect the extractive sample from the center of the carpet sampling area identified in the previous Step g. This often required repeated blows.

i. Once the carpet had been cut, the core sample typically was lodged inside the punch (*or hole saw*). The assistant held the punch such that the sampler could use the tweezers to grab the carpet piece (*or the assistant used the Allen wrench to loosen the pilot bit. The sampler removed*

the bit. The sampler then used the tweezers to grab the carpet piece). If the carpet core did not lodge inside the punch (*or hole saw*), but remained on the carpet surface, the sampler used the tweezers to collect as much of the core as possible. While the sampler was holding the circle of carpet, the assistant maneuvered the 50 mL vial (*or 120 mL sterile cup*) from the outside of the over pack.

j. The assistant opened the vial (*or cup*), touching only the cap.

k. The sampler put the carpet into the vial (*or cup*).

l. The assistant aseptically returned the cap to the vial (*or cup*) and closed the vial (*or cup*).

m. The assistant placed the vial (*or cup*) in the small inner bag and wiped the outside of the bag with a Dispatch® wipe.

n. The inner bag was placed inside the over pack bag, and was wiped with a Dispatch® wipe. (*The assistant then removed the used hole saw from the drill and placed in a bag for eventual re-sterilization*.)

3.1.3 Swab Sampling

Two types of swab samples were collected, though a similar procedure was used for each. Swab sampling was done for three items of each sterilization batch for all equipment used during decontamination (vacuum nozzles, etc). The process for this sampling is described below.

1. The sampler donned a P95 respirator, bouffant cap, gloves, disposable lab coat, and safety glasses

2. The sampler:

   a. Opened the package and removed the BactiSwab™ (Remel, item 12100/12110, Lenexa, KS).

   b. Labeled the plastic tube appropriately using the scheme detailed in Section 3.6.

   c. Removed the cap-swab from the plastic tube.

   d. Swabbed the surface while spinning the cap-swab between the thumb and index fingers.

   e. Returned cap-swab to tube.

   f. Through the sleeve, crushed the BactiSwab™ Transport System ampoule at midpoint.

   g. Held BactiSwab™ tip end to allow the medium to wet the swab.

   h. The sampler dated and initialed each sample tube.

3. All BactiSwab™ Transport Systems were placed in a 5 in. x 9 in. sterile sampling bag.

4. A chain of custody form was completed and the samples relinquished to the Microbiology Laboratory.

This procedure was modified slightly for the final Test D. Disposable booties were added to the PPE to help prevent re-contamination of the COMMANDER during subsequent decontamination and sampling events. Second, the BactiSwab™ ampoule was broken prior to taking the swab sample, as it was determined that a moistened swab increased the collection efficiency. The process for this sampling is described below.

1. The sampler donned a P95 respirator, bouffant cap, gloves, disposable lab coat, disposable booties, and safety glasses

2. The sampler:

    a. Through the sleeve, crushed the BactiSwab™ Transport System ampoule at midpoint.

    b. Held BactiSwab™ tip end up for at least five seconds to allow the medium to wet the swab.

    c. Opened the package and removed the BactiSwab™.

    d. Labeled the plastic tube appropriately using the scheme detailed in Section 3.6.

    e. Removed the cap-swab from the plastic tube.

    f. Swabbed the surface while spinning the cap-swab between the thumb and index fingers.

    g. Returned cap-swab to tube.

3. All BactiSwab™ Transport Systems were placed in a 5 in. x 9 in. sterile sampling bag.

4. A chain of custody form was completed and the samples relinquished to the Microbiology Laboratory.

3.1.4 Rinsate Collection and Sampling Procedures

The liquid collected by the carpet cleaners during the decontamination procedure was collected for a given carpet section. After all steps of the decontamination process had been completed, aliquots of the collected liquid in the cleaner were filtered immediately to remove the spores from the liquid according to the protocol described in Section 4.1.2 below. The filtered spores were then rinsed with DI water to remove any residual surfactant.

3.2 Prevention of Cross-contamination of Sampling/Monitoring Equipment

Several management controls were put in place to prevent cross-contamination. This project was labor intensive and required that many activities be performed on carpet sections or coupons that were intentionally contaminated (test coupons and positive controls). The treatment of these two groups of test areas (positive control and test) varied for each group. Hence, specific procedures were put in place in the effort to prevent cross-contamination among the groups. Adequate cleaning of all common materials

and equipment was critical in preventing cross-contamination. Cleaning methods for this purpose are listed in Table 3-1.

There were four primary activities for each test in the experimental matrix. These activities were preparation of the coupons, execution of the decontamination process (including sample recovery), sampling, and analysis. Coupons were fumigated with VHP® prior to the contamination process. Specific management controls for each of the three following activities are described below.

3.2.1 Preventing Cross-Contamination during Execution of the Decontamination Process

The decontamination process was labor intensive; it required that a multistep procedure be executed repeatedly for each coupon. Additionally, the process occurred using a single test chamber. Hence, controlling the order of processing and actions taken to minimize cross-contamination were essential. The following management controls were followed in an effort to minimize the potential for cross-contamination:

Table 3-1. Cleaning Methods and Frequency for Common Test Materials/Equipment

Material/Equipment	Use	Cleaning Method
COMMANDER	Contain carpet sections during the application of the decontamination procedure being tested	Fumigation with VHP® or washing with pH-adjusted bleach solution in accordance with the wet/dry vacuum cleaning procedure.*
Wet vacuums	Part of the decontamination procedure	pH-adjusted bleach solution
Heads of wet vacuums	Part of the decontamination procedure	pH-adjusted bleach solution
Other Bulk equipment (fans, templates, etc)	Various	Fumigation with VHP® or washing with pH-adjusted bleach solution in accordance with the wet/dry vacuum cleaning procedure.*

* Following use in a test, the wet/dry vacuum (including head assembly) was cleaned by being fumigated with a STERIS VHP® sterilization cycle. This cycle entailed the use of a STERIS VHP® ARD H_2O_2 generator and exposure of all components of the wet/dry vacuum for a minimum concentration * time (CT) of 1000 ppm*hours.

- COMMANDER was cleaned prior to the start of each test in Table 2-2 via fumigation with VHP®.
- COMMANDER was cleaned with a pH-adjusted bleach solution as described above following the controlled contamination procedure and before decontamination of the carpet sections began.
- COMMANDER was cleaned after completion of each test in Table 2-2 via fumigation with VHP®.
- All cleaner wands were decontaminated after use by soaking in a pH-adjusted bleach solution for at least one hour. Wands were put in the solution immediately after use to eliminate any accumulation of contaminated equipment in the test area. The bucket of pH-adjusted bleach solution for test equipment clean-up was clearly identified and maintained separate from the cleaning solutions being used for the test coupon decontamination process.
- Testing was done using a "clean team/dirty team" technique. One dirty team was responsible for chamber wipe-down and moving the carpet sections into the decontamination shower. A clean team (with clean and dirty members) was used for control sampling. A dirty team performed the decontamination procedure. A clean team (with clean and dirty members) was used for test sampling. Only dirty members handled contaminated items and only clean members handled samples.

3.2.2 Preventing Cross-Contamination during Sampling

Sampling poses an additional significant opportunity for cross-contamination of samples. In an effort to minimize the potential for cross-contamination, several management controls were implemented.

- In accordance with aseptic technique, a sampling team made up of a "sampler" and a "support person" was utilized.
- The sampler handled only the sampling media and the support person handled all other supplies. The sampler sampled the surface according to the appropriate procedure described in Section 3.1.1.
- The collection medium (e.g., HEPA filter) was then placed into a sample container that was opened, held and closed by the support person.
- The sealed sample was handled only by the support person.
- All of the following actions were performed only by the support person, using aseptic technique:

.1.1 The sealed bag with the sample was placed into another sterile plastic bag that was then sealed; that bag was then decontaminated using a bleach wipe.

.1.2 The double-bagged sample was then placed into a third sterile bag that was sealed and then placed into a sterile sample container for transport.

.1.3 The exterior of the transport container was decontaminated by wiping all surfaces with a bleach wipe or towelette moistened with a solution of pH-adjusted bleach prior to transport from the sampling location to the Microbiology Laboratory.

- The sampling crew then changed their gloves in preparation for working with the next sample.

3.2.3 Preventing Cross-Contamination during Analysis

Aseptic laboratory technique was followed per the standard operating procedures (SOPs) and miscellaneous operating procedures (MOPs) of the Microbiology Laboratory. The SOPs and MOPs document the aseptic technique employed to prevent cross-contamination. Additionally, the order of analysis (consistent with the above) was as follows: (1) all blank coupons; and (2) all positive control or decontaminated coupons.

3.3 Representativeness

The representativeness of the test material, decontamination procedure, and equipment used were critical attributes to assure reliable test results. Representativeness of the test materials means that the materials used are typical of such materials used in buildings in terms of quality, surface characteristics, structural integrity, etc. The materials chosen for this study (carpet) are representative of surfaces that are likely to contribute significantly to the overall decontamination challenge in the event of a wide area release of *B. anthracis* spores. The particular carpet chosen for this study is representative of modern low Volatile Organic Compound (VOC) carpets used in large government institutions; this carpet is being used by US EPA in the RTP, NC facility. Representativeness was assured by selection of test materials that met government procurement specifications and by obtaining those materials from appropriate suppliers. The material coupons were fabricated to be representative of the bulk surfaces. The size of the carpet sections was chosen to be representative of a large surface area yet manageable within the confines of COMMANDER. The sampling strategy for the 1 ft. x 1 ft. sample areas analyzed 18% of the carpet section both before and after each decontamination. The equipment used in the decontamination procedures was also representative of the equipment actually used in the field. The only minor exception is that the equipment was chosen with a preference to allow for as much quality assurance as possible. During analysis, samples were homogenized to ensure that any aliquots taken were representative of the bulk titer (e.g., viable spores per mL).

3.4 Sample Quantities

For each carpet section, and assuming that four decontaminations were performed, a total of 29 samples were generated. The total numbers of samples of each type for each test are listed in Table 3-2. As discussed in Section 3.3, the samples from each test were analyzed in six batches, represented by “Test Day” in Table 3-2.

3.5 Sample Containers for Collection, Transport, and Storage

For each wipe, the primary containment was an individual sterile 50 mL conical tube. Secondary and tertiary containment were sterile sampling bags. The primary, secondary, and tertiary containment of each HEPA vacuum sock consisted of separate sterile sampling bags. All samples from a single test were then placed in a sterilized plastic bin. Sterilization of all containers prior to their use for the test samples was via autoclaving on a gravity cycle or via use of pH-adjusted bleach solution. After samples were placed in the container for storage and transport to the Microbiology Laboratory, the container was wiped with a towelette saturated with a pH-adjusted bleach solution. A single plastic bin was used for storage in the decontamination laboratory during sampling and for transport to the Microbiology Laboratory.

Table 3-2. Sample Quantities for Each Test Setup

Test Day	Description	Sterility Blank	Control Samples	Test Samples	Liquid Filter samples
1	Carpet Sterility Sampling	2			
2	Post-Controlled Contamination Control Sampling		3 Samples x 2 Decontamination Types (Test A - 6 samples)		
3	Decontamination 1 Sampling			3 Samples x 2 Decontamination Types	2
5	Decontamination 2 Sampling			3 Samples x 2 Decontamination Types	2
7	Decontamination 3 Sampling			3 Samples x 2 Decontamination Types	2
9	Decontamination 4 Sampling			3 Samples x 2 Decontamination Types	2

3.6 Sample Identification

Each carpet section was identified by a description of the material and a unique sample number. The sampling team maintained an explicit laboratory log which included records of each unique sample number and its associated test number, contamination application, any preconditioning and treatment specifics, and the date treated. Each carpet section test area sample was marked with only the material descriptor and unique code number. The wet/dry vacuum samples from each test were identified with an associated test number and carpet section type. The sample codes eased written identification. Once the coupons were transferred to the Microbiology Laboratory for plate counts, each sample was additionally identified by replicate number and dilution. The Microbiology Laboratory also included on each plate the date it was placed in the incubator.

3.7 Sample Preservation

After sample collection, sample integrity was maintained by storage of samples in quadruple containers (1 – sample collection container, 2 – sterile bag, 3 – sterile bag with exterior sterilized during sample packaging process, 4 – sterile container holding all samples from a test). All individual sample containers remained sealed while in the decontamination laboratory or in transport after the introduction of the sample. The locking lid on the container holding all samples remained closed except for the brief period it was opened for sample introduction by the support person of the sampling team. The sampling person did not handle any samples after they were relinquished to the support person during placement into the primary sample container.

In the Microbiology Laboratory, all samples were stored in the refrigerator at approx. 4 °C±2°C until they were analyzed. All samples were allowed to stabilize at room temperature for 1 hour prior to analysis.

3.8 Sample Holding Times

After sample collection for a single test was complete, all samples were transported to the Microbiology Laboratory immediately, with appropriate chain of custody form(s). The samples were stored in accordance with Section 3.7 and no longer than ten days before they were analyzed. A typical holding time for most samples was a maximum of two days.

3.9 Sample Handling and Custody

Careful coordination with the Microbiology Laboratory is required to arrange for successful transfer of uncompromised samples in a timely manner for analysis. Test schedules were confirmed with the Microbiology Laboratory prior to the start of each test. To ensure the integrity of samples and to maintain a timely and traceable transfer of samples, an established and proven chain of custody or possession is mandatory. It is imperative that accurate records be maintained whenever samples are created, transferred, stored, analyzed, or destroyed. The primary objective of these procedures is to create an accurate written record that can be used to trace the possession of the sample from the moment of its creation through the reporting of the results. A sample is in custody if it is in any one of the following states:

- In actual physical possession.
- In view, after being in physical possession.
- In physical possession and locked up so that no one can tamper with it.
- In a secured area, restricted except to authorized personnel.
- In transit.

Laboratory test team members received copies of the test plans prior to each test. Pre-study briefings were held to apprise all participants of the objectives, test protocols, and chain of custody procedures to be followed. These protocols were required to mesh with any protocols established by EPA.

In the transfer of custody, each custodian signed, recorded, and dated the transfer on the Chain of Custody (COC). Sample transfer could be on a sample-by-sample basis or on a bulk basis. The following protocol was followed for all samples as they were collected and prepared for distribution:

- A chain of custody record accompanied the samples. When turning over possession of samples, the transferor and recipient signed, dated, and noted the time on the record sheet. This record sheet allowed transfer of custody of a group of samples from H130-A to the Microbiology Laboratory.
- If the custodian had not been assigned, the laboratory technician had the responsibility of packaging the samples for transport.
- Samples were carefully packed and hand-carried between on-site laboratories.
- The chain of custody record showing the identity of the contents accompanied all packages.

3.10 Sample Archiving

All samples and diluted samples were archived for two weeks following completion of analysis. This time allowed for all data to be processed according to quality control requirements and allowed for the data to be reviewed. Samples were archived by maintaining the primary extract at 4 °C+/-2°C in a sealed extraction tube. Two weeks post-analysis, all samples were discarded.

4 Testing and Measurement Protocols

The primary results from this study were from the analysis of samples in the Microbiology Laboratory, resulting in recovered CFUs per sample expressed on a log-10 scale. This analysis for each sample type is detailed in Section 4.1.

Additional measurements prior to or during the decontamination procedure application were also required to ensure quality control in the testing.

The time for application of each procedural step and time between procedural steps on each coupon was measured using a stopwatch (Table 3-1) and recorded in the laboratory notebook.

4.1 Sample Analyses

The Microbiology Laboratory analyzed all samples for presence (swab samples) or to quantify the number of CFU per sample (wipe, vacuum, or core samples), which were used as per Section 6.1. For all sample types, phosphate buffered saline with 0.05% TWEEN®-20 (PBST) were used as the extraction buffer. After the appropriate extraction procedure (as described in the sections to follow), the buffer was subjected to a four stage serial dilution (10^0 to 10^{-4}) in accordance with MOP 6535a (a revision of MOP 6535 specifically for bacterial spores; attached as Appendix C). The resulting samples were plated in triplicate and incubated overnight. CFU were counted as detailed in MOP 6535a.

The PBST was prepared according to an internal MOP. The extraction procedure used to recover spores will be varied depending upon the different matrices (HEPA filter socks, wipe samples, core samples). The procedures are described in the following subsections.

4.1.1 Recovery from HEPA Vacuum Sample

The recovery of the spores from the HEPA socks was done as follows, as adopted from the Idaho National Laboratory (INL) 2008 Evaluation Protocols:[9]

1. The analyst donned a fresh pair of gloves. Gloves were changed periodically (at least between batches) or after direct contact with a sample to reduce contamination.

2. Sterile 3 oz. specimen cups were pre-labeled as per the sample log corresponding to the batch of samples being processed.

3. The 3 oz. specimen cup sample containers were loaded with 20 mL of PBST.

4. Both sterile sample bags were opened without removing the inner bag from outer bag. The HEPA sock assembly was moved to the opening of the bag using the bag.

5. The HEPA sock was removed from the assembly using sterile forceps while holding the cardboard applicator from the outside of the bag. The HEPA sock was placed into the corresponding pre-labeled specimen cup containing 20 mL PBST. The plastic bag with cardboard applicator was discarded. A new pair of sterile forceps was used for each sample.

6. After use, forceps were placed in a container of pH-adjusted bleach solution. The forceps were soaked for at least one hour before being autoclaved using a gravity cycle in preparation for use with the next sample batch.

7. The HEPA sock was wetted by holding the upper blue portion of the HEPA sock and dipping the lower 1 inch of the HEPA sock into the PBST. The HEPA sock was allowed to soak up the PBST for a few seconds.

8. After the soaking, the HEPA sock was lifted up just above the opening of the specimen bottle. A 1-inch vertical slit was cut up the center from the bottom of the sock using sterile scissors. A new pair of scissors was used for each sample.

9. The HEPA sock was cut horizontally from side to side about 1 inch from the bottom, allowing the two pieces to fall into the specimen bottle. The HEPA sock was only cut where the sock had been wetted (dip, wet, look, cut).

10. Steps 7 – 9 were repeated until the entire white portion of the HEPA sock was cut.

11. The upper top blue portion of the HEPA sock was then discarded.

12. After use, scissors were placed in a container of pH-adjusted bleach solution. The scissors were soaked for at least one hour before being autoclaved using a gravity cycle in preparation for use with the next sample batch.

13. Gloves were changed between samples.

14. Steps 4 – 12 were repeated for each sample in the batch.

15. Twelve samples at a time were loaded into the well plate of the incubator shaker (Lab-Line, Melrose Park, IL).

16. The samples were agitated in the shaker incubator at 300 revolutions per minute (rpm) for 30 minutes with the heat off.

17. The samples were then removed from the shaker incubator and brought to the BioSafety Cabinet (NuAire, Inc., Plymouth, MN) for dilution plating as described in Section 4.1.

4.1.2 Filter Plating

Filter plating was done by the Microbiology Laboratory for the Spor-Klenz tests as well as for the rinsate samples. Three 100 mL aliquots of Spor-Klenz or rinsate were delivered to the Microbiology Laboratory. One of the 100 mL aliquots was filtered by pouring through a 0.2 micron Nalgene filter unit (Thermo-Scientific, Waltham, MA). The filter was then rinsed by pouring 10 mL of sterile deionized water over the filter. The filter was removed from the filtration unit aseptically with disposable thumb forceps and placed onto a Tryptic Soy Agar (TSA) plate, filter side up. The plate was then placed in a 35 °C+/-2°C incubator (Thermo-Scientific, Waltham, MA), for at least 18 hours. Filter plating was also done for any samples for which there were fewer than 30 CFU in the primary dilution sample.

4.1.3 Recovery of Core Samples

The smaller core punches used in Test C were processed in 20 mL PBST tubes. They were sonicated for 10 minutes, vortexed for two continuous minutes, and then vortexed immediately prior to dilution plating as described in Section 4.1. The larger hole saw cores used in Test D were processed in 40 mL PBST specimen cups. The cups were placed in the orbital shaker incubator for 30 minutes at ambient temperatures and were vortexed immediately prior to dilution plating as described in Section 4.1.

4.2 Analysis Equipment Calibration

Standard laboratory equipment such as biological safety cabinets and incubators were routinely monitored for proper performance. All equipment was verified as being certified calibrated or having the calibration validated by the Metrology Laboratory at the time of use. Calibration of instruments was done at the frequency shown in Table 4-1. Any deficiencies were noted. The instrument was adjusted to meet calibration tolerances and recalibrated within 24 hrs. If tolerances were not met after recalibration, additional corrective action was taken, possibly including the replacement of the equipment.

Table 4-1. Instrument Calibration Frequency

Equipment	Calibration/Certification	Expected Tolerance
Thermometer	Compared to independent National Institute for Standards and Technology (NIST) thermometer (this is a thermometer that is recertified annually by either NIST or an International Organization for Standardization (ISO)-17025 facility) value once per quarter.	±1°C
Micropipettes	All micropipettes were verified to be within the calibration date at time of use. Pipettes were recalibrated by gravimetric evaluation of pipette performance to manufacturer's specifications every year by supplier (Rainin Instruments, Oakland, C.A., Ovation, VistaLabs, Brewster, NY).	±5%
Clock	Compared to office U.S. Time @ www.NIST.time.gov every 30 days.	±1 min/30 days
Biological Cabinet	The biological cabinets were verified to be within certification dates at the time of use. Biological Cabinets are adjusted yearly to be within flow tolerances established by the manufacturer.	

5 Quality Assurance

5.1 Data Quality

The objective of this study was to investigate the reduction in viable spores on and within the carpet surfaces (effectiveness) as a function of the cleaning technique and duration of application to various carpet types. This section discusses the Quality Assurance/Quality Control (QA/QC) checks (Section 6.2) and Data Quality Objectives (DQOs; Section 6.3) considered critical to accomplishing the project objectives.

The QAPP[10] in place for this testing was followed with several deviations, many of which were documented in the text above. Deviations included incorporating HEPA sock samples in place of the inefficient wipe samples, and the addition of the core (extractive) samples and the use of Spor-Klenz. These deviations did not substantially affect data quality and were necessitated by the test results themselves.

5.2 Quality Assurance/Quality Control Checks

Quantitative standards do not exist for biological agents. Quantitative determinations of organisms in this investigation did not involve the use of analytical measurement devices. Rather, the CFU were enumerated manually and recorded. Critical QC checks are shown in Table 5-1, with acceptance criteria set at the most stringent level that could routinely be achieved and that were consistent with the DQOs.

Table 5-1. Quality Control Checks

QC Sample	Information Provided	Acceptance Criteria	Corrective Action
Positive Control (sample from carpet section area contaminated with biological agent but not subjected to the test conditions)	Initial contamination level on the coupons; allows for determination of log reduction (see Section 5-1); controls for confounds arising from history impacting bioactivity; controls for special causes.	Target loading of 1E7 CFU per sample with a standard deviation of < 0.5. (5E6 – 5E7 CFU/sample);	Outside target range: discuss potential impact on results with EPA WAM; correct loading procedure for next test and repeat depending on decided impact. .
Blank TSA, Sterility Control (plate incubated, but not inoculated)	Controls for sterility of plates.	No observed growth following incubation.	Incubate additional ten plates. If any additional growth is observed, reject results from the lot.
Stability Control	Verifies that the spores are stable for the length of time required for multiple decontamination cycles	No downward trend over time	Re-evaluate efficacy results based on natural decay.

The positive control samples did not meet acceptance criteria for Tests A and B, but were deemed high enough to continue with the testing. Extractive samples did not meet the criteria because of their smaller size.

All sterility control samples met the acceptance criteria.

The stability control samples did show a downward trend over time, on the order of 0.5 log reduction. This downward trend has not been factored into the log reduction values.

5.3 Data Quality Objectives

The DQOs define the critical measurements (CMs) needed to address the stated objectives and specify tolerable levels of potential error associated with simulating the prescribed decontamination environments. The following measurements were deemed to be critical to accomplish part or all of the project objectives:

- time
- CFU counts.

The Data Quality Indicators (DQIs) listed in Table 5-2 are specific criteria used to quantify how well the collected data meet the DQOs. Detection limits were defined by the QAPP as 50% of the minimum number of detectable spores, or 0.5 CFU.

Table 5-2. DQIs for Critical Measurements

Measurement Parameter	Analysis Method	Accuracy	Detection	Completeness Goals	Actual Completeness
Counts of CFU	Visual counting, See Section 4.1	±10 % of CFU count	0.5 CFU	100%	100%
Streak Plate	Visual detection of growth	N/A*	0.5 CFU	100%	100%

*N/A= not applicable

The quantitative acceptance criteria in terms of precision (%Relative Standard Deviation, %RSD) for each critical measurement are shown in Table 5-3. Tests with conditions falling outside these criteria were rejected and repeated. Decisions to accept or reject tests were based upon engineering judgment used to assess the likely impact of the parameter on the conclusions drawn from the data.

Table 5-3. Acceptance Criteria for Critical Measurements

Measurement Parameter	Target Value	Precision RSD (%)
Test coupon replicates	30 – 300 CFU per quantifiable plate	40%
Negative control CFUs	0-1 CFU per sample area	±0.5 CFU

5.4 Audits

This project was assigned QA Category III and did not require technical systems or performance evaluation audits.

6 Results and Discussion

This work measured the reduction in viable spores on and within the carpet surfaces (effectiveness) as a function of the cleaning technique (hot versus cold vacuum) and duration of application to various carpet types (up to four subsequent decontaminations). The size of the carpet sections, roughly 4' x 4', was chosen as feasible yet representative of what will likely be encountered in the field (e.g., walkways). Operational parameters such as time, physical impact on materials or the remediation crew, and fate of the viable spores (e.g., contamination of equipment, carpet cleaner parts, and rinsate) were also determined. The data reduction and validation procedures used are presented in Section 6.1, followed in Section 6.2 by the results for each of the tests from the test matrix (Table 2-2).

6.1 Data Reduction and Validation

Data reduction was performed to tabulate all results from each test. The data reduction included the total CFU recovered from each replicate sample area, the average recovered CFU and standard deviation for each group of sample areas, log reductions, and total recovered CFU for each wet vacuum liquid sample. The coupons included the following, for each combination of material type and decontamination type:

- Positive control areas (three replicates, average, standard deviation)
- Test areas (three replicates, average, standard deviation)

CFU counts per coupon were calculated according to the equation shown in MOP 6535a (Appendix C). Efficacy is defined as the extent (by log reduction) to which the agent extracted from the coupons after the treatment with the decontamination procedure is reduced below that extracted from positive control areas (not exposed to the decontamination procedure). Efficacy was calculated for each test coupon within each combination of decontamination procedure (i) and test material as:

$$\eta_i = \sum_j Log(CFU_C)/N_C - \sum_k Log(CFU_S)/N_t \qquad (6\text{-}1)$$

where :

η_i = the spore log reduction efficacy of decontamination technique i

$\sum_j Log(CFU_C)/N_C$ = the mean log CFU recovered from the control areas (C= control, j = coupon number, and N_C is the number of coupons (1, j))

$\sum_k Log(CFU_S)/N_t$ = the mean log CFU recovered from the surface of a decontaminated coupon (S= sample from decontaminated carpet, k = coupon number, and N_t is the number of coupons tested (1, k))

When no viable spores were detected, then a value of 0.5 CFU was assigned as the detection limit, and the efficacy was reported as greater than or equal to the value calculated by Eqn. 6-1.

For the recovered liquid samples, the results were reported as CFU per area cleaned.

At least 10% of the data generated during sample analysis from each test was reviewed. This review occurred within one week after the analysis was completed. The review included an independent verification of CFU per plate and the calculation of CFU per sample (per Equation 6-1).

6.2 Test Results

The first test (Test A) was conducted on new carpet using wipe samples. Use of the wet carpet cleaners was not expected to kill the spores but to decontaminate by removing the spores. In this way, the spores could be considered a material, rather than an organism, and a rudimentary type of mass balance could be applied to them.

Figure 6-1 shows the wipe sampling recovery following spore inoculation (loading) and after each of the four subsequent decontaminations. When wipe sampling was used to determine spore concentration for Test A, the results failed to satisfy the mass balance.

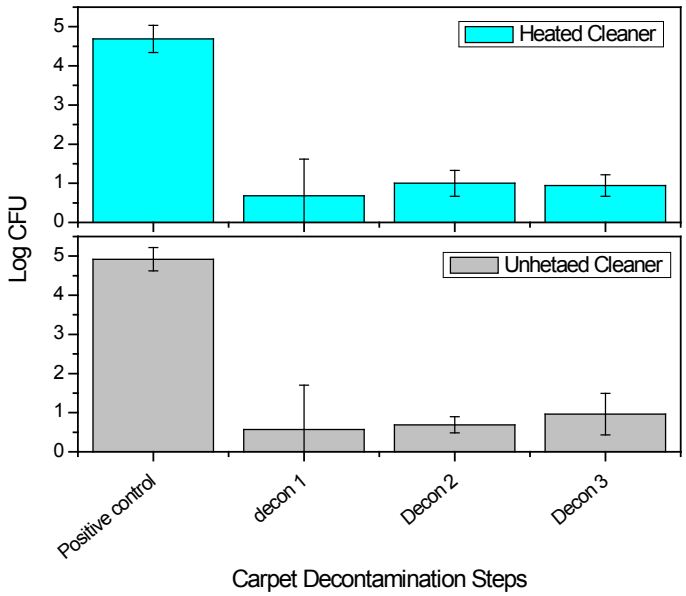

Figure 6-1. Wipe Sampling Results for Test A

The wipe results seemed to suggest that a single use of a carpet cleaner (whether heated or unheated) removed 99.99% of the spores after the first decontamination. This observation would further suggest that the recovery of the surfactant was also 99.99%, if the spores were carried away by the surfactant. This value was beyond the anticipated (or believable) efficacy of the vacuum cleaner itself. For example, the flow rate of the surfactant was 26.6 (±3%) mL/sec for the heated carpet cleaner and 39.8 (±0.5%) mL/sec for the unheated carpet cleaner. The surfactant was applied for approximately 45 seconds. For the unheated carpet cleaner, only 560 mL of the approximately 1200 mL was recovered, much below the 99.99% recovery suggested by the spore results. This observation led to the consideration that the spores were not inactivated or removed at all, but had been pushed down within the carpet pile and made inaccessible to the wipes.

For Test B, conducted on old carpet, HEPA sock sampling was used instead of wipe sampling, and core samples were also intended to be collected for extraction. Because core samples were a late addition to the test plan, the carpet construction method (gluing the carpet down) was not designed to allow for the collection of these samples. The core samples could not be collected for Test B.

Figure 6-2 shows the results after loading and three subsequent decontaminations. The HEPA sock results suggested that a significant log reduction could be obtained with repetition of the decontamination process using either the heated or unheated carpet cleaners. However, based on the ineffectiveness of the wipe samples (and without the core samples for verification), the data would suggest that the HEPA socks were no more effective than the wipe sample had been.

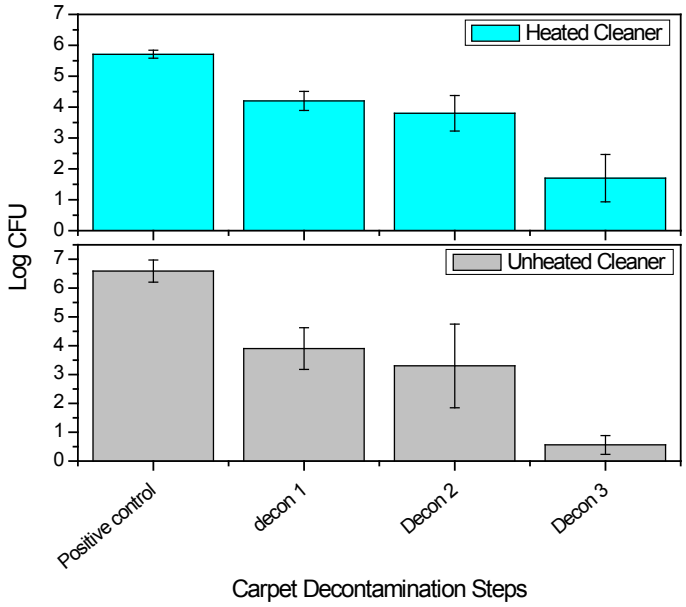

Figure 6-2. HEPA Sock Sampling Results for Test B

Results from Tests A and B suggested that neither of the surface sampling methods – wipe sampling or HEPA sock sampling – was sufficiently efficient at collecting spores once the wet/dry vacuuming operation had been performed. Test C was designed to answer the question of spore recovery by altering the carpet construction method to allow for the collection of the core (extractive) samples in addition to the HEPA sock samples. This test was conducted on new carpet. Figure 6-3 shows the logarithm of the CFU recovered per square foot following multiple decontamination attempts using the HEPA sock samples.

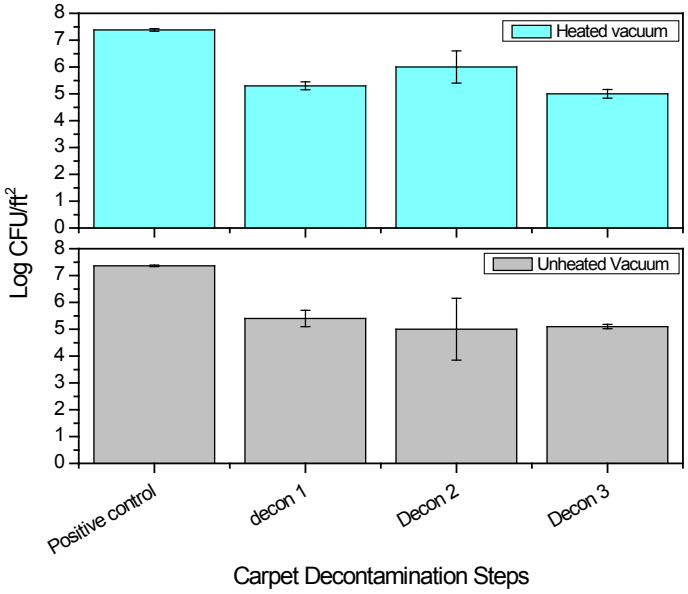

Figure 6-3. HEPA Sampling Results after Decontamination Attempts

Each sample area was first sampled using HEPA socks, then extractive analysis was performed on a small 18 mm diameter core taken from the center of the same sample area. These data show that while HEPA sock sampling suggested that the decontamination methods were removing some spores, subsequent extractive samples showed no or minimal removal (less than 1 log reduction, see Figure 6-4). The HEPA sock samples of control areas (not decontaminated) showed minimal downward drift.

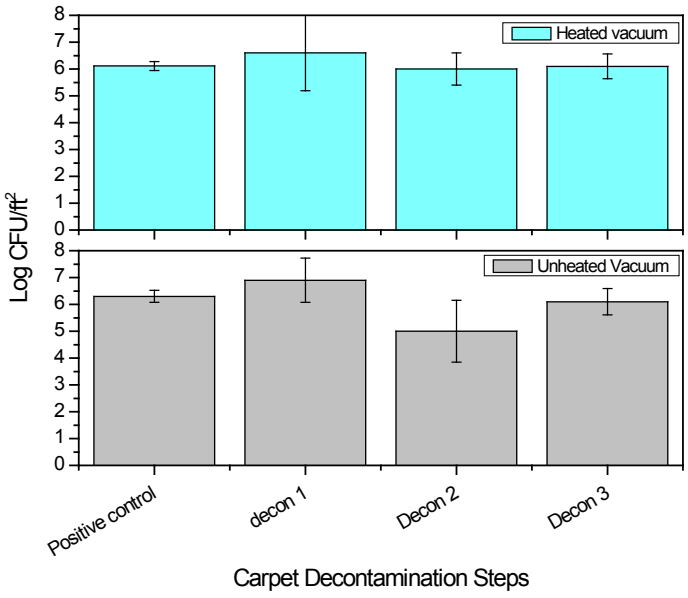

Figure 6-4. Core Sampling Results after Decontamination Attempts

The act of decontaminating with the wet/dry vacuums seems to push the spores away from the surface and into the carpet pile where the HEPA socks were unable to sample effectively. Even though the samples were allowed to dry overnight, residual moisture or detergent may have helped spores adhere to carpet fibers. Because the apparent log reduction from the HEPA sock samples from Test B (Figure 6-2) showed a greater log reduction that the HEPA sock samples from Test C (Figure 6-3), the tamped down nature of the older used carpet as well as the debris in the fibers may further reduce recovery by binding to the spores or merely retaining moisture.

Although the results from Tests A and B were inconclusive regarding the efficiency of the carpet cleaners, all three tests suggested that there was no statistical difference between the hot (heated) and cold (unheated) cleaners. Test C indicated that the efficacy of the carpet cleaners per the manufacturer's recommendations was poor. For carpet, extractive sampling appears to be the most reliable method following any dampening of the surface, especially for used carpet.

One final test (Test D), using new carpet, was conducted to provide information about the benefits of using a carpet cleaner with a sporicide (Spor-Klenz) in place of the recommended cleaner versus the simple application of the sporicide without any vacuuming. These results are shown in Figure 6-5 and Figure 6-6 using a back pack sprayer and an unheated vacuum cleaner, respectively.

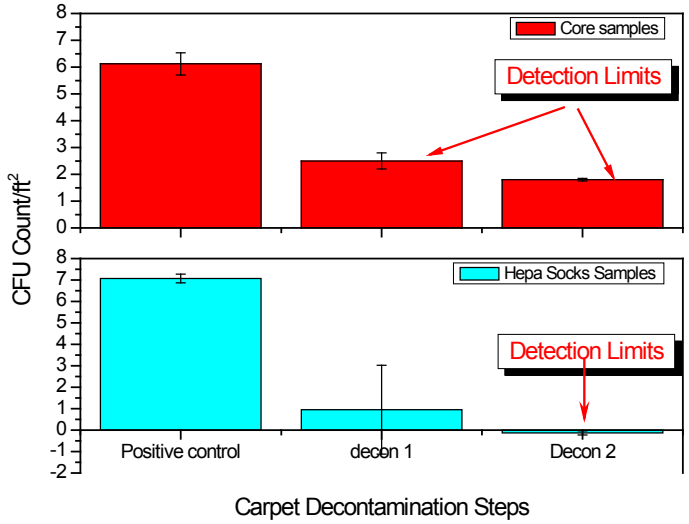

Figure 6-5. Spor-Klenz Applied to a New Carpet with a Backpack Sprayer

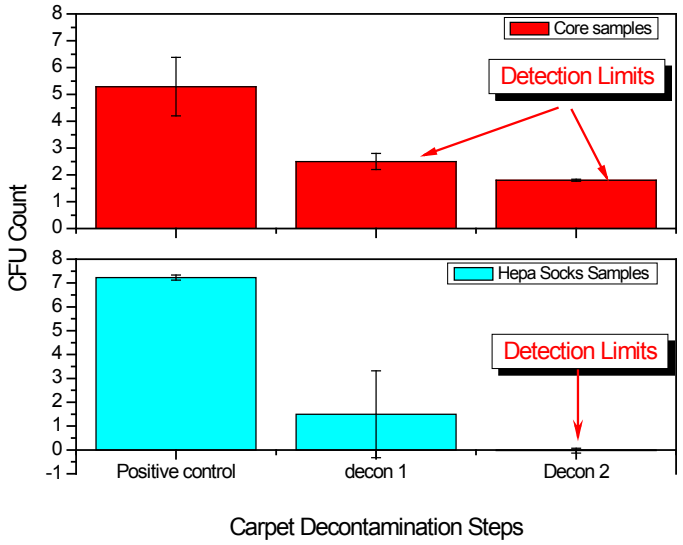

Figure 6-6. Spor-Klenz Applied to a New Carpet with an Unheated Vacuum Cleaner

Test D showed a very good log reduction from the use of Spor-Klenz, applied either from the carpet cleaner (Table 6-1) or the backpack sprayer (Table 6-2). The first decontamination showed a 6 log reduction from the HEPA socks (no detection on the core samples, which have a higher detection limit than the HEPA sock samples). Following the second application of Spor-Klenz, no spores were detected using either sampling method, a 7 log reduction in the HEPA sock results.

The core samples could show only a 3 log reduction, in part because of the higher detection limit. The core samples taken for the controlled contamination had lower recovery than expected, possibly due to the rigorous method that was used to extract the core samples causing re-aerosolization of many spores. The summary of the results for the spore counts log reductions for Test B through Test D are presented in Table 6-3.

Table 6-1. Spor-Klenz Applied with a Carpet Cleaner*

Carpet Status	Date	HEPA socks		Core Samples	
		Mean of log CFU/ft^2	Error (95% Confidence Interval)	Mean of log CFU/ft^2	Error (95% Confidence Interval)
Controlled Contamination	8/25/2010	7.2	0.11	5.29	1.09
After 1st Decontamination	8/30/2010	1.5	1.82	2.5	0.30
After 2nd Decontamination	9/1/2010	-0.03	0.10	1.8	0.04

* Detection Limit values are in **red**.

Table 6-2. Spor-Klenz Applied with a Backpack Sprayer*

Carpet Status	Date	HEPA socks		Core Samples	
		Mean of log CFU/ft^2	Error (95% Confidence Interval)	Mean of log CFU/ft^2	Error (95% Confidence Interval)
Controlled Contamination	8/25/2010	7.07	0.20	6.12	0.41
After 1st Decontamination	8/30/2010	0.95	2.07	2.49	0.3
After 2nd Decontamination	9/1/2010	-0.13	0.10	1.81	0

* Detection Limit values are in **red**.

Table 6-3. CFU Log Reduction with Spor-Klenz Application

Decontamination procedure	HEPA Sock Sampling		Extractive Sampling	
	Decon 1	**Decon 2**	**Decon 1**	**Decon 2**
Spor-Klenz from backpack sprayer	1.7<LR<7.5	6.6<LR<7.5	2.2<LR<5.0	3.4<LR<5.2
Spor-Klenz application with carpet cleaner	1.9<LR<7.4	6.8<LR<7.4	0.07<LR<5.5	1.3<LR<5.7

Confidence intervals (CI) are defined to be:

$$CI = mean \pm z_{\alpha/2} \times standard\ error$$

where the standard error is the standard deviation divided by the square root of the number of observations, and $z_{\alpha/2}$ is the $\alpha/2^{th}$ percentile of the standard normal distribution.

The second decontamination step sampled using core samples yielded zero CFU counts on each of the five samples collected. The size of the samples, 5 out of 360, was too small to determine the confidence interval that the whole carper (1 ft^2) is fully decontaminated (no detectable viable spores). However, the problem can be approached from the point of view of sampling from a distribution with only two possible outcomes. Given the results of the experiment, one can view the observations as: (1) no contamination, that is, a value below the limit of detection or "clean"; (2) contaminated, that is, above the limit of detection or "dirty." Let *p* denote the probability of a dirty or contaminated carpet; this probability can also be interpreted as the fraction of the carpet that remains contaminated. The estimate from this experiment for this carpet sample is *p*=0. To place a 95% confidence interval on this estimate, one can consider the question of "How many dirty spots (i.e., where viable spores are detected) might there be on the carpet, while yielding 5 out of 5 clean (no detectable viable spores) samples with a probability of 5%?" While the use of a binomial probability distribution suggests itself here, the binomial probability distribution is not the best choice for this situation because the binomial would account only for the number of samples actually taken (here 5 squares), not the number taken relative to the size of the entire carpet section (here 360 squares). A better choice is the hypergeometric distribution to answer the question of the upper 95% confidence bound on *p*. (The lower bound on *p* is clearly 0.) Employing the hypergeometric distribution in this case yields an upper bound of 0.45. So, the 95% confidence interval for *p*, the probability of some contamination remaining, is (0, 0.45). That is, at the 95% confidence level, the carpet could be contaminated over (up to) 45% of its surface, and one could still obtain 5 clean samples. Therefore, the extractive method would be more effective if more samples were taken to increase the probability that the full carpet is clean (no detectable viable spores).

6.2.1 Length of Decontamination Event

The decontamination procedure using the carpet cleaners is very short, if only performed once, approximately five seconds per square foot. Test D with Spor-Klenz suggested that performing the decontamination twice would yield no recoverable spores. If the decontaminations are performed immediately (back-to-back), then there is minimum impact on the remediation crew. If, on the other hand, the carpet is allowed to dry between successive applications, then an extra day is involved. Though it was not a consideration for this test, the ultimate disposal of the carpet cleaners after use in an event may contribute to the cleanup time and expense.

The backpack sprayer method as performed does require an extended duration, allowing for treatment of an extrapolated 192 square feet in 30 minutes, or 6.4 square feet per minute, significantly higher than when a carpet cleaner is being used.

6.2.2 Physical Impact on Materials and Crew

No physical impact on the carpet was noted for any of the decontamination methods. While neither decontamination procedure was physically strenuous, any activity inside Level C suits (even with cooling vests) leads to heat stress. Moreover, the use of Spor-Klenz in an area without very high air exchanges could lead to levels of hydrogen peroxide or acetic acid above IDLH conditions.

6.2.3 Fate of Spores

Determining the ultimate fate of spores has proven very complicated due to sampling difficulties. The rinsate recovered from the carpet cleaners used in Tests A, B, and C was very contaminated, obscuring enumeration of the spores of interest. This level of contamination does suggest that conditions are such that the likelihood our target organism was in the rinsate was very high. No data suggest that all spores were removed from the carpet during Tests A, B, and C, so the ultimate fate of those spores is probably viable and present in carpet, carpet cleaner parts, and rinsate. The fate of spores in Test D is probably deactivated due to the presence of Spor-Klenz in all locations, but some doubt lingers due to difficulties sampling the rinsate.

6.2.4 Health and Safety Effects of Decontamination

There were no noxious fumes or gases detected from the use of the carpet cleaner solutions (Judson Laboratories Oxygen 2 Pre-Spray and Extraction) or the Spor-Klenz.

References

[1] After Action Report – Danbury Anthrax Incident, U.S. EPA Region 1, September 19, 2008. (Available upon request from Mike Nalipinski, Nalipinski.Mike@epa.gov, Region 1 On-Scene Coordinator)

[2] Brown, G.S.; Betty, R.G.; Brockmann, J.E.; Lucero, D.A.; Souza, C.A.; Walsh, K.S.; Boucher, R.M.; Tezak, M.S.; Wilson, M.C. Evaluation of Surface Sample Collection Methods for *Bacillus* Spores on Porous and Non-porous Surfaces. In 2006 Workshop on Decontamination, Cleanup and Associated Issues for Sites Contaminated With Chemical, Biological, or Radiological Materials, Proceedings of the 2006 NHSRC Decontamination Workshop, Washington, DC, April 26 – 28, 2006.

[3] Busher, A.;Noble-Wang; J.; Rose, L. Surface Sampling. In *Sampling for Biological Agents in the Environment*; Emanual, P., Roos, J.W., Niyogi, K., Eds.; ASM Press: Washington, DC, 2008; pp 95 – 131

[4] Brown, G.S.; Betty, R.G.; Brockmann, J.E.; Lucero, D.A.; Souza, C.A.; Walsh, K.S.; Boucher, R.M.; Tezak, M.; Wilson, M.C.; Rudolph, T. Evaluation of a Wipe Surface Sample Method for Collection of *Bacillus* Spores from Nonporous Surfaces. *Appl Environ Microbiol* **2007**, 73 (3), 706 – 710

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[6] Mattorano, D. U.S. EPA/OSWER/OEM/National Decontamination Team, Erlanger, KY. Personal communication (regarding the current sampling strategies under development by the Interagency Workgroup for the Validated Sampling Plan for *B. anthracis*), December 12, 2008.

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[8] Brown, G.S.; Betty, R.G.; Brockmann, J.E.; Lucero, D.A.; Souza, C.A.; Walsh, K.S.; Boucher, R.M.; Tezak, M.; Wilson, M.C.; Rudolph, T. Evaluation of a Wipe Surface Sample Method for Collection of *Bacillus* Spores from Nonporous Surfaces. *Appl Environ Microbiol* **2007**, 73 (3), 706 – 710.

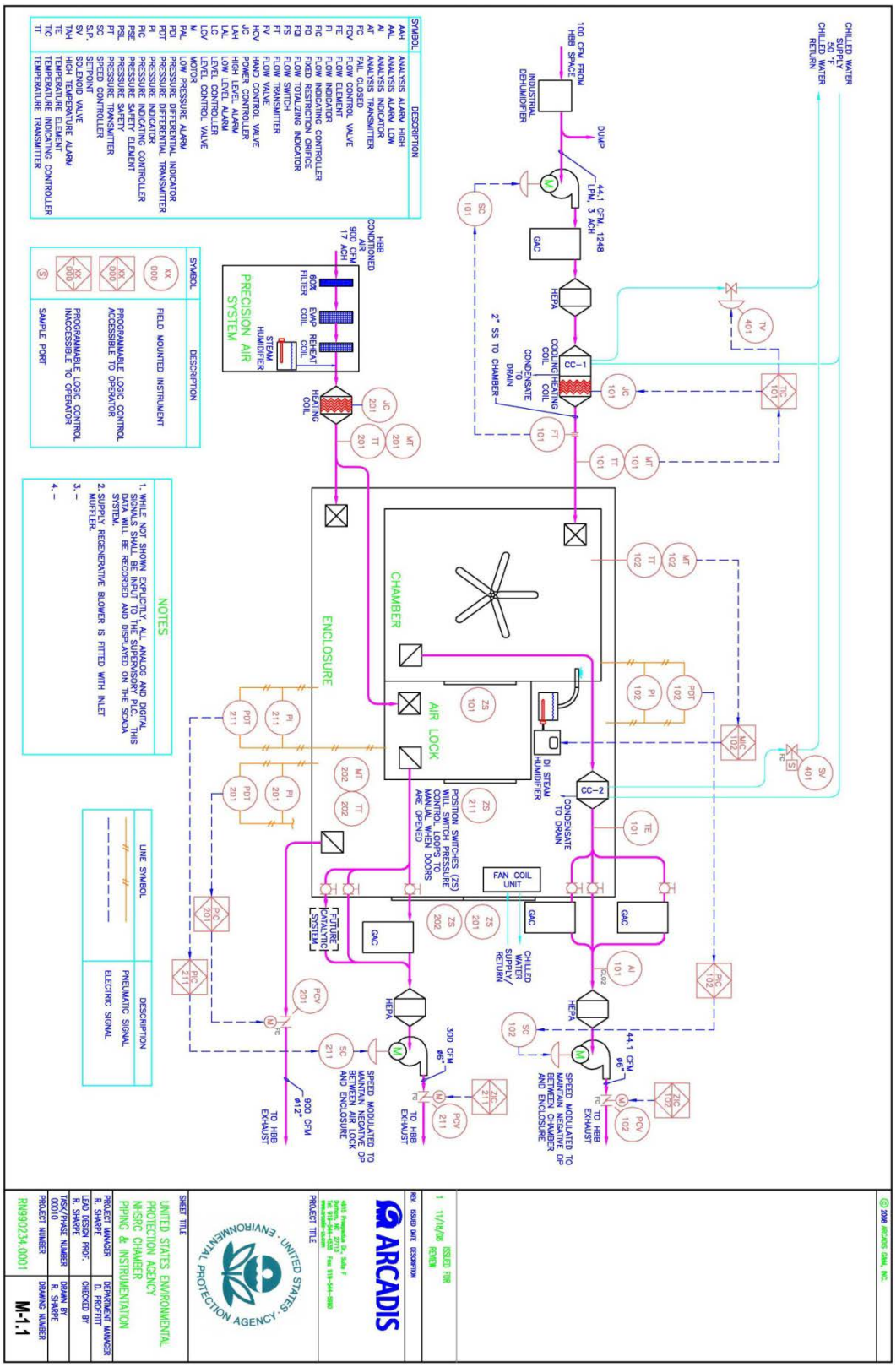
[9] [FOUO] INL 2008 Evaluation Protocols.

[10] ARCADIS G&M, Inc. *Quality Assurance Project Plan for the Determination of the Efficacy of Spore Removal from Carpets using Commercially-available Wet/Vacuum Carpet Cleaning Systems.* Prepared under Contract No. EP-C-04-023, Work Assignment No. 4-35. U.S. Environmental Protection Agency, National Homeland Security Research Center, Research Triangle Park, NC. May 2009. (Available upon request from Shawn Ryan, Ryan.Shawn@epa.gov, DCMD Division Director)

[11] ARCADIS U.S., Inc. *Compatibility of Material and Electronic Equipment with Chlorine Dioxide Fumigation, Assessment and Evaluation Report.* Prepared under Contract No. EP-C-04-023, Work Assignment No. 4-50 for U.S. Environmental Protection Agency, National Homeland Security Research

Center, Office of Research and Development, Research Triangle Park, NC. July 2009. (Available upon request from Shawn Ryan, Ryan.Shawn@epa.gov, DCMD Division Director)

Appendix A: COMMANDER Piping and Instrumentation Diagram



Appendix B: MSDS for Decontamination Solutions

- **Judson Laboratories Oxygen 2 / Pre-Spray**
- **Judson Laboratories Oxygen 2 / Extraction**
- **Steris Spor-Klenz® Ready To Use**

JUDSON LABORATORIES
P.O. BOX 4388
GREENVILLE, SC 29608
(864) 233-6442

MATERIAL SAFETY DATA SHEET

I. IDENTIFICATION

PRODUCT NAME: OXYGEN 2 / PRE-SPRAY
CHEMICAL NAME: ALKALINE DEGREASER
CHEMICAL FAMILY: ALKALI BLENDED/OXYGEN BRIGHTENER/D-LIMONENE SOLVENT/PH-9.3 to 9.9
FORMULA: TRADE SECRET
SYNONYMS: NONE
CAS # AND NAME: TRADE SECRET

II. PHYSICAL DATA

BOILING POINT: 220' F
FREEZING POINT: 32' F
PH: 10.8
SPECIFIC GRAVITY (H2O=1): 1.0002
VAPOR DENSITY (AIR=1): NA
VAPOR PRESSURE AT 20'C: NA
SOLUBILITY IN WATER BY WT: NA
EVAPORATION RATE (D-LIMONENE=1): SLOWER THAN WATER
APPEARANCE & ODOR: WHITE CREAM WITH CLEAN ODOR

EMERGENCY PHONE NUMBERS:
MONDAY - FRIDAY: 9:00AM - 6:00PM (864) 233-6442 / OTHER TIMES (864) 787-3038

****HAZARD RATING SCALE****		1- HEALTH HAZARD - MILD SKIN IRRITANT
0=MINIMAL	3=HIGH	1- FLAMMABILITY - FLASHPOINT ABOVE 110 Deg. F (43 Deg. C)
1=SLIGHT	4=SERIOUS	0- REACTIVITY - STABLE
2=MODERATE	5=SEVERE	G- PROTECTION - SAFETY GLASSES, GLOVES

III. INGREDIENTS

MATERIAL	%	EXPOSURE LIMITS HAZARD
D-Limonene CAS# 5989-27-5	25-33%	HAZARD RATING - 1 50ppm (SKIN) ACGIH TOXIC EYE IRRITATION 100ppm (SKIN) OSHA ORAL LD50-5G/KG,RAT

IV. FIRE AND EXPOSURE HAZARD DATA

FLASH POINT: COMPLETELY NON COMBUSTIBLE, NOT A FIRE HAZARD

FLAMMABLE LIMITS IN AIR, % BY VOLUME: LOWER: NA / UPPER: NA

EXTINGUISHING MEDIA: THIS MATERIAL IS NOT COMBUSTIBLE

SPECIAL FIRE FIGHTING PROCEDURES: N/A

UNUSUAL FIRE AND EXPLOSION HAZARDS: NONE

V. HEALTH HAZARD DATA

EXPOSURE LIMITS: NONE

EFFECTS OF ACCUTE OVER EXPOSURE:
SWALLOWING: INGESTION OF THE PRODUCT CAN CAUSE GASTRIC WALL IRRITATION AND POSSIBLE RED BLOOD CELL HEMOTOSIS

SKIN ABSORPTION: MODERATE SKIN PENETRATION

INHALATION: INHALATION OF AIR CONTAMINATED WITH LARGE AMOUNTS OF MIST SPRAY OR ATOMIZED PRODUCTS WILL IRRITATE MUCUS MEMBRANE

SKIN CONTACT: LONG TERM CONTAMINATION OF SKIN MAY CREATE EXCESSIVE SKIN DRYNESS AND MILD SKIN DERMATITIS BECAUSE OF THE BREAKDOWN OF NATURAL OILS

EYE CONTACT: FLUSH WITH COPIUS AMOUNTS OF WATER

EFFECTS OF REPEATED OVEREXPOSURE: WILL CAUSE THE BREAD DOWN OF NATURAL SKIN OILS CAUSING CONTACT DERMATITIS

MEDICAL CONDITIONS AGGRAVATED BY OVEREXPOSURE: SKIN DISEASES AND CONTACT DERMATITIS AS WELL AS PERSONS HAVING CHRONIC RESPIRATORY DISEASES

EMERGENCY AND FIRST AID PROCEDURES:
SWALLOWING: WASH OUT MOUTH, DRINK LARGE QUANTITIES OF CITRUS JUICE,(EXAMPLE: GRAPEFRUIT JUICE) AND THEN INDUCE VOMITING

SKIN: WASH CONTAMINATED AREA WITH DILUTED VINEGAR AND WATER RINSE

INHALATION: THIS PRODUCT HAS NO HARMFUL VAPOR CHARACTERISTICS BUT DROPLETS FROM SPRAY APPLICATION CAN CAUSE RESPIRATORY IRRITATION. EVACUATE AREA TO FRESH AIR

EYES: FLUSH WITH COPIUS AMOUNTS OF WATER FOR 15 MINUTES, WASH EYES WITH 5% BORIC ACID SOLUTION THEN, CONSULT A PHYSICIAN IMMEDIATELY

NOTES TO PHYSICIAN: TREAT ALL CONTACT PROBLEMS BY NEUTRALIZATION WITH MILD ACIDIC WASH THEN TREAT FOR CHEMICAL BURNS

VI. REACTIVITY DATA

STABILITY: STABLE

CONDITIONS TO AVOID: CONTACT WITH STRONG ACIDS

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS: THIS PRODUCT SHOULD NEVER BE STORED IN ALUMINUM CONTAINERS

HAZARDOUS POLYMERIZATION: NONE

CONDITIONS TO AVOID: FURNITURE, WALLS ECT.

VII. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED: SMALL SPILLS: ABSORB WITH ABSORBENT MATERIAL AND DISCARD IN TRASH RECEPTACLE. LARGE SPILLS: PUMP INTO DRUMS FOR RECOVERY

WASTE DISPOSAL METHOD: SPILL, THEN NEUTRALIZE WASTE WITH HYDROCHLORIC ACID SOLUTION. DILUTE WITH WATER BEFORE DISCHARGING TO SEWER SYSTEM

VIII. SPECIAL PROTECTIVE INFORMATION

RESPIRATORY PROTECTION: THE APPROVED RESPIRATORY MASK SHOULD BE USED IN HIGH PRESSURE SPRAY MIST ENVIRONMENTS, IF SPRAYED INTO THE AIR.

VENTILATION: CLOSE AREAS SHOULD BE PROVIDED WITH FRESH AIR

PROTECTIVE GLOVES: IT IS SUGGESTED RUBBER NEOPRENE GLOVES TO BE WORN

EYE PROTECTION: GOGGLES OR MASK SHOULD BE WORN IF PRODUCT BECOMES IRRITATING

OTHER PROTECTIVE EQUIPMENT: IT IS SUGGESTED ALL SKIN AREAS TO BE COVERED WHEN USING THIS PRODUCT

IX. SPECIAL PRECAUTIONS

STATUS ON SUBSTANCE: THE CONCENTRATIONS SHOWN ARE MAXIUM OR CEILING LEVELS (WEIGHT %) TO BE USED FOR CALCULATIONS FOR REGULATIONS. TRADE SECRETS ARE INDICATED BY "TS"

FEDERAL EPA: COMPREHENSIVE ENVIRONMENTAL RESPONSE, CONPENSATION, AND LIABILITY ACT OF 1980 (CERCLA) REQUIRES NOTIFICATION OF THE NATIONAL RESPONSE CENTER OF RELEASE OF QUANTITIES OF HAZARDOUS SUBSTANCES EQUAL TO OR GREATER THAN THE REPORTABLE QNANTITIES (RQ'S) IN 40 CFR 302.4 THE OPINIONS ARE THOSE OF QUALIFIED SCIENTIST WITHIN JUDSON LABORATORIES. WE BELIEVE THE INFORMATION CONTAINED IS CURRENT AND VALID. SINCE USE OF THIS INFORMATION, OPINIONS AND THE CONDITIONS OF USE OF THE PRODUCT ARE NOT WITHIN THE CONTROL OF JUDSON LABORATORIES, IT IS THE USERS OBLIGATION TO DETERMINE THE CONDITIONS OF SAFE USE OF THE PRODUCT.

JUDSON LABORATORIES
P.O. BOX 4388
GREENVILLE, SC 29608
(864) 233-6442

MATERIAL SAFETY DATA SHEET

I. DENTIFICATION

PRODUCT NAME: OXYGEN 2 / EXTRACTION
CHEMICAL NAME: CLEANER / OXYGEN BRIGHTNER / NEUTRAL AGENT / IPA DRYING AGENT
CHEMICAL FAMILY: NEUTRALIZING AGENT
FORMULA: TRADE SECRET
PH: 7.0
SYNONYMS: NONE
CAS # AND NAME: TRADE SECRET WITH NO CAS NUMBERS ASSIGNED

II. PHYSICAL DATA

BOILING POING: 220' F
FREEZING POINT: 32' F
SPECIFIC GRAVITY (H2O=1): 1.0002
VAPOR DENSITY (AIR=1): NA
SOLUBILITY IN WATER BY WT: COMPLETE
EVAPORATION RATE: 1
APPEARANCE & ODOR: AQUA-BLUE LIQUID WITH CLEAN ODOR

EMERGENCY PHONE NUMBERS:
MONDAY - FRIDAY: 9:00AM - 6:00PM (864) 233-6442 / OTHER TIMES (864) 787-3038

****HAZARD RATING SCALE****

0=MINIMAL	3=HIGH
1=SLIGHT	4=SERIOUS
2=MODERATE	5=SEVERE

III. INGREDIENTS

MATERIAL % EXPOSURE LIMITS HAZARD - 1
SODIUM BORATE 3-8% 25ppm
50ppm (SKIN) OSHA
ORAL 1.48gm/kg (RAT)
INHALATION LD 50-700ppm / 7 HOURS (RAT)

IV. FIRE AND EXPOSURE HAZARD DATA

FLASH POINT: COMPLETELY NON COMBUSTIBLE, NOT A FIRE HAZARD

FLAMMABLE LIMITS IN AIR, % BY VOLUME: LOWER: NA UPPER: NA

EXTINGUISHING MEDIA: NONE REQUIRED

SPECIAL FIRE FIGHTING PROCEDURES: N/A

UNUSUAL FIRE AND EXPLOSION HAZARDS: NONE

V. HEALTH HAZARD DATA

EXPOSURE LIMITS: MODERATE

EFFECTS OF ACCUTE OVER EXPOSURE:
SWALLOWING: INGESTION OF THE PRODUCT CAN CAUSE GASTRIC WALL IRRITATION

SKIN ABSORPTION: MODERATE SKIN PENETRATION

INHALATION: INHALATION OF AIR CONTAMINATED BY THE HIGH PRESSURE SPRAY MIST OF THIS PRODUCT MAY CAUSE RESPIRATORY IRRITATION

SKIN CONTACT: LONG TERM CONTAMINATION OF SKIN MAY CREATE EXCESSIVE SKIN DRYNESS AND MILD SKIN DERMATITIS BECAUSE OF THE BREAKDOWN OF NATURAL OILS

EYE CONTACT: FLUSH WITH COPIUS AMOUNTS OF WATER

EFFECTS OF REPEATED OVEREXPOSURE: MAY CAUSE DRY IRRITATED SKIN DUE TO REMOVAL OF OILS

MEDICAL CONDITIONS AGGRAVATED BY OVEREXPOSURE: CHRONIC SKIN DISEASE PERSONS WITH ALLERGY PROBLEMS AND CHRONIC RESPIRATORY DISEASES MAY EXPERIENCE COMPLICATIONS

EMERGENCY AND FIRST AID PROCEDURES:
SWALLOWING: WASH OUT MOUTH, DRINK LARGE QUANTITIES OF CITRUS JUICE, GRAPEFRUIT AND THEN INDUCE VOMITING

SKIN: WASH CONTAMINATED AREA WITH DILUTED VINEGAR AND WATER

INHALATION: THIS PRODUCT HAS NO HARMFUL VAPOR CHARACTERISTICS BUT MIST FROM HIGH PRESSURE SPRAY MAY BE IRRITAING TO RESPIRATORY SYSTEM SEEK FRESH AIR

EYES: FLUSH WITH COPIUS AMOUNTS OF WATER FOR 15 MINUTES

NOTES TO PHYSICIAN: NONE

VI. REACTIVITY DATA

STABILITY: STABLE

CONDITIONS TO AVOID: STRONG OXIDIZING AGENTS

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS: NONE

HAZARDOUS POLYMERIZATION: NONE

CONDITIONS TO AVOID: NONE

VII. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED: CONTAIN SPILL VACUUM OR BLOT UP WITH MOP OR ABSORB WITH WATER ABSORBING MATERIAL CONTAMINATED SURFACES MAY BE WASHED WITHOUT ADVERSE ENVIRONMENTAL IMPACT

WASTE DISPOSAL METHOD: SPILLED MATERIAL MAY BE RECLAIMED DEPENDENT UPON OTHER CONTAMINATS. WASTE DISPOSAL (INSURE CONFORMITY WITH ALL APPLICABLE DISPOSAL REGULATIONS) INCINERATE OF OTHERWISE MANAGE IN A RCRA PERMITTED WASTE MANAGEMENT FACILITY

VIII. SPECIAL PROTECTIVE INFORMATION

RESPIRATORY PROTECTION: GENERALLY NOT REQUIRED

VENTILATION: USE ADEQUATE VENTILATION

PROTECTIVE GLOVES: SUGGESTED USE OF RUBBER GLOVES IS A GOOD PRACTICE

EYE PROTECTION: FOR SPLASH PROTECTION USE PROTECTIVE GOGGLES OR FACE SHIELD

OTHER PROTECTIVE EQUIPMENT: NO SPECIAL GARMENTS REQUIRED AVOID UNNECCARY SKIN CONTAMINATION WITH MATERIAL

IX. SPECIAL PRECAUTIONS

STATUS ON SUBSTANCE: THE CONCENTRATIONS SHOWN ARE MAXIUM OR CEILING LEVELS (WEIGHT %) TO BE (CERCLA) REQUIRES NOTIFICATION OF THE NATIONAL RESPONSE CENTER OF RELEASE OF QUANTITIES OF HAZARDOUS SUBSTANCES EQUAL TO OR GREATER THAN THE REPORTABLE QUANTITIES (RQS) IN 40 CFR 302.4 THE OPINIONS ARE THOSE OF QUALIFIED SCIENTIST WITHIN JUDSON LABORATORIES. WE BELIEVE THE INFORMATION CONTAINED IS CURRENT AND VALID. SINCE USE OF THIS INFORMATION, OPINIONS AND THE CONDITIONS OF USE OF THE PRODUCT ARE NOT WITHIN THE CONTROL OF JUDSON LABORATORIES, IT IS THE USERS OBLIGATION TO DETERMINE THE CONDITIONS OF SAFE USE OF THE PRODUCT.

Material Safety Data Sheet
May be used to comply with OSHA's Hazard Communication Standard, 29 CFR 1910.1200. Standard must be consulted for specific requirements.

STERIS®

Product Name	Product Description	Product ID
SPOR-KLENZ READY TO USE	**COLD STERILANT** **ALIPHATIC CARBOXYLIC ACID, INORGANIC PEROXIDE, ORGANIC PEROXIDE (STRONG OXIDIZER)**	**6525**

SECTION I

Manufacturer's Name	Emergency Telephone Number
STERIS Corporation	1-314-535-1395 (STERIS); 1-800-424-9300 (CHEMTREC)
Address P.O. Box 147 St. Louis, Missouri 63166	**Telephone Number for Information** 1-800-444-9009 (Customer Service - Scientific Products)
	Date Prepared: July 1, 1999
	Prepared by: M. Ebers

Health	2
Fire	0
Reactivity	0

Least Slight Moderate High Extreme

0 1 2 3 4

SECTION II - HAZARDOUS INGREDIENTS/IDENTITY INFORMATION

Hazardous Components (Specific Chemical Identity; Common Name(s))	% by Wt.	ACGIH TLV	OSHA PEL	Other Limits Recommended
Hydrogen Peroxide (7722-84-1)	0.80	1ppm	1ppm	
Peracetic Acid (79-21-0)	0.06	N/E	N/E	
Acetic Acid (64-19-7)	Proprietary	10ppm	10ppm	

SECTION III - PHYSICAL/CHEMICAL CHARACTERISTICS

Solubility in Water	Complete	**Specific Gravity (H_20=1 @ 25C)**	1.01
Freezing Point F	N/A	**% Volatile by wt @ 105C/1 hr**	N/A
pH @ Solution	N/A	**pH as Distributed**	~ 1.87
Appearance	Clear, colorless liquid	**Odor**	Mild vinegar odor

SECTION IV - FIRE AND EXPLOSION HAZARD DATA

Flash Point (Method Used)	N/A	**Flammable Limits**	N/A	**LEL**	**UEL**
Extinguishing Media	Use water, foam, CO_2, dry chemicals.				

Special Fire Fighting Procedures
As with any chemical fire, the use of a self-contained breathing apparatus and full protective equipment is recommended. Dilute with large volumes of water.

Unusual Fire and Explosion Hazards	N/A

SECTION V - REACTIVITY DATA

Stability	**Unstable**		**Conditions to Avoid**
	Stable	X	Avoid temperatures above 75F. Avoid contact with combustible materials.
Incompatibility (Materials to Avoid)			Heavy metals including iron, copper, copper alloys, brass and aluminum, salts, alkalis, caustics, and formaldehyde.
Hazardous Decomposition or Byproducts			Oxygen and heat. Do not mix with chlorinated products as this could liberate toxic corrosive chlorine gas.
Hazardous Polymerization	**May Occur**		**Conditions to Avoid**
	Will Not Occur	X	None known.

SPOR-KLENZ READY TO USE

SECTION VI - HEALTH HAZARD DATA

A. ACUTE (Primary Route of Exposure)

EYES: Contact with eyes may cause burns.

SKIN: May cause moderate skin irritation, including oxidation (i.e., whitening of the skin). LD_{50} Rat: > 20 g/kg.

INHALATION: May cause irritation to mucous membranes. LD_{50} Rat: >13,439 mg/m^3.

B. SUBCHRONIC, CHRONIC, OTHER: None known.

SECTION VII - EMERGENCY AND FIRST AID PROCEDURES

EYES: In case of contact, immediately flush with water for 15 minutes and get medical attention.

SKIN: For skin contact, wash with soap and water. Get medical attention if irritation persists. Remove and launder contaminated clothes before reuse.

INHALATION: Remove to fresh air.

INGESTION: Do not induce vomiting. Drink large quantities of water. Get immediate medical attention.

SECTION VIII - PRECAUTIONS FOR SAFE HANDLING & USE

Spill Management:	Flush with large quantities of water; mop thoroughly. Do not use absorbent to soak up spill.
Waste Disposal Methods:	Dispose of in accordance with all local, state and federal regulations.
Precautions to be taken in handling and storage:	Store in a cool, dry place below 75F. Do not expose to sunlight. Read and observe all labeled use instructions.
Shipping information:	Not restricted

SECTION IX - PROTECTION INFORMATION/CONTROL MEASURES

Respiratory:	Use only in well-ventilated area		
Eyes:	Safety glasses/goggles/full face shield	**Gloves:**	Rubber or plastic
Other Clothing and Equipment:	Rubber apron		
Ventilation:	Use local exhaust.		

U.S. FEDERAL REGULATIONS:
All components are listed in the Toxic Substance Control Act (TSCA) Chemical Substance Inventory. OSHA Hazardous Communication Standard (29 CFR 1910.1200) Hazardous Class: Corrosive.

Appendix C: Miscellaneous Operating Procedure (MOP) 6535a

BL MOP NO. 6535a **M. Worth Calfee**
4-8-2009, rev. 2.0

Title: SERIAL DILUTION: SPREAD PLATE PROCEDURE TO QUANTIFY VIABLE BACTERIAL SPORES

Scope: Determine the abundance of bacterial spores in a liquid extract

Purpose: Determine quantitatively the number of viable bacterial spores in a liquid suspension using the spread plate procedure to count colony-forming units (CFU)

Materials:

- Liquid suspension of bacterial spores
- Sterile microcentrifuge tubes
- Diluent (sterile deionized water, buffered peptone water or phosphate buffered saline)
- Trypticase Soy Agar plates
- Microliter pipettes with sterile tips
- Sterile beads placed inside a test tube (will be used for spreading samples on the agar surface)
- Vortex mixer

Procedure: (This protocol is designed for 10-fold dilutions.)

1. For each bacterial spore suspension to be tested, label the microcentrifuge tubes as follows: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}... (The number of dilution tubes will vary depending on the concentration of spores in the suspension). Aseptically, add 900 µL of sterile diluent to each of the tubes.

2. Label three Trypticase Soy Agar (TSA) plates for each dilution that will be plated. These dilutions will be plated in triplicate.

3. Mix original spore suspension by vortexing thoroughly for 30 seconds. Immediately after the cessation of vortexing, transfer 100 µL of the stock suspension to the 10^{-1} tube. Mix the 10^{-1} tube by vortexing for 10 seconds, and immediately pipette 100 µL to the 10^{-2} tube. Repeat this process until the final dilution is made. It is imperative that used pipette tips be exchanged for a sterile tip each time a new dilution is started.

4. To plate the dilutions, vortex the dilution to be plated for 10 seconds, immediately pipette 100 µL of the dilution onto the surface of a TSA plate, taking care to dispense all of the liquid from the pipette tip. If less than 10 seconds elapses between inoculation of all replicate plates, then the initial vortex mixing before the first replicate is sufficient for all replicates of the sample. Use a new pipette tip for each set of replicate dilutions.

5. Carefully pour the sterile glass beads onto the surface of the TSA plate with the sample and shake until the entire sample is distributed on the surface of the agar plate. Aseptically remove the glass beads. Repeat for all plates.

6. Incubate the plates overnight at 32 °C – 37 °C (incubation conditions will vary depending on the organism's optimum growth temperature and generation time.)

7. Enumerate the colony forming units (CFU) on the agar plates by manually counting with the aid of a plate counting lamp, and with a marker (place a mark on the surface of the Petri dish over each CFU when counting, so that no CFU is counted twice).

Since each dilution was tested in triplicate, determine the average of the triplicate plate abundances. Plates suitable for counting must contain between 30 - 300 colonies.

Calculations

Total abundance of spores (CFU) within extract:

(Avg CFU / volume (mL) plated) x (1/tube dilution factor) X extract volume

For example:

Tube Dilution	Volume plated	Replicate	CFU
10^{-3}	100 µL (0.1 mL)	1	150
10^{-3}	100 µL (0.1 mL)	2	250
10^{-3}	100 µL (0.1 mL)	3	200

Extract total volume = 20 mL

(200 CFU / 0.1 mL) x ($1/10^{-3}$) X 20 mL = (2000) x (1000) x 20 = 4.0×10^{7} CFU

Note: The volume plated (mL) and tube dilution can be multiplied to yield a 'decimal factor' (DF). DF can be used in the following manner to simplify the abundance calculation.

Spore Abundance per mL = (Avg CFU) X (1 / DF) X extract volume

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