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# Effectiveness of Physical and Chemical Cleaning and Disinfection Methods for Removing, Reducing or Inactivating Agricultural Biological Threat Agents

**Office of Research and Development**
National Homeland Security Research Center

EPA/600/R-11/092

# Effectiveness of Physical and Chemical Cleaning and Disinfection Methods for Removing, Reducing or Inactivating Agricultural Biological Threat Agents

## Disclaimer

The United States Environmental Protection Agency, through its Office of Research and Development's National Homeland Security Research Center, funded and directed this investigation through EP-C-09-027 WA 1-35 with ARCADIS-US, Inc. This report has been peer and administratively reviewed and has been approved for publication as an Environmental Protection Agency document. It does not necessarily reflect the views of the Environmental Protection Agency. No official endorsement should be inferred. The Environmental Protection Agency does not endorse the purchase or sale of any commercial products or services. This report includes photographs of commercially available products. The photographs are included for purposes of illustration only and are not intended to imply that the Environmental Protection Agency approves or endorses the product or its manufacturer.

Questions concerning this document or its application should be addressed to the principal investigator on this effort.

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## Foreword

Following the events of September 11, 2001, addressing the critical needs related to homeland security became a clear requirement with respect to the U.S. Environmental Protection Agency's (EPA's) mission to protect human health and the environment. Presidential Directives further emphasized EPA as the primary federal agency responsible for the country's water supplies and for decontamination following a chemical, biological, and/or radiological (CBR) attack. To support the EPA mission with respect to response and recovery from incidents of national significance, the National Homeland Security Research Center (NHSRC) was established to conduct research and deliver products that improve the capability of the Agency to carry out its homeland security responsibilities.

One specific goal of NHSRC's research is to provide information on decontamination methods and technologies that can be used in the response and recovery efforts resulting from a biological incident. The complexity and heterogeneity of surface decontamination necessitates the understanding of the effectiveness of a range of decontamination options. In addition to effective volumetric decontamination approaches (e.g., facility fumigation), more rapidly deployable or readily available alternative surface decontamination approaches have also been recognized as a tool to enhance the capabilities to respond to and recover from such incidents.

Through working with EPA's Federal Partners (for example, Department of Homeland Security and Department of Agriculture), NHSRC is attempting to understand and develop useful surface decontamination procedures for agriculturally-relevant situations such as a foreign animal disease incident. This report documents the results of a laboratory study to better understand the effectiveness of surface cleaning and decontamination methods and to develop a readily-deployable treatment procedure for surfaces contaminated with highly pathogenic biological agents. Studies such as this advance our ability to respond and recover from incidents of national significance where biological agent has contaminated commodities and facilities.

These results, coupled with additional information in separate NHSRC publications (available at www.epa.gov/nhsrc) can be used to determine whether a particular decontamination technology can be effective in a given scenario. NHSRC has made this publication available to assist the response community to prepare for and recover from incidents involving biological contamination. This research is intended to move EPA and its Federal Partners one step closer to achieving the nation's homeland security goals and the agency's overall mission of protecting human health and the environment while providing sustainable solutions to our environmental problems.

Jonathan Herrmann, Director
National Homeland Security Research Center

## Acknowledgments

This effort was initiated following the identification of knowledge gaps by the National Science and Technology Council (NSTC) Subcommittee on Foreign Animal Disease Threats (FADT), Decon and Disposal Working Group, which is co-chaired by the US EPA and the US Department of Agriculture (USDA). Emergency response and remediation following a foreign animal disease (FAD) incident will involve numerous federal agencies (particularly those listed), as well as state, local, and private entities. This project addresses closing gaps in our ability to decontaminate and remediate facilities following an agro-terrorism incident. Funding support by the US Department of Homeland Security (DHS) to complete this effort is greatly appreciated.

This effort was directed by the principal investigator from ORD's National Homeland Research Center (NHSRC), utilizing the support of a project team consisting of staff from across the US EPA, DHS, and USDA. The contributions of the following individuals have been a valued asset throughout this effort:

- R. Leroy Mickelsen (US EPA/OSWER/OEM/National Decontamination Team)
- Carlton (Jeff) Kempter (US EPA/Office of Chemical Safety and Pollution Prevention)
- Joseph P. Wood (US EPA/ORD/NHSRC, Decontamination and Consequence Management Division)
- Michelle Colby (DHS)
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## List of Acronyms

| | |
|---|---|
| acm | actual cubic meter |
| ADA | Aerosol Deposition Apparatus |
| APPCD | Air Pollution Prevention and Control Division |
| Ba | *Bacillus atropheus* (formerly identified as *Bacillus subtilis* var. *niger, and B. globigii*) |
| CBR | Chemical, Biological, Radiological |
| CFU | Colony Forming Unit(s) |
| COMMANDER | Consequence Management and Decontamination Evaluation Room |
| DCMD | Decontamination and Consequence Management Division |
| DGM | dry gas meter |
| DHS | US Department of Homeland Security |
| DI | Deionized |
| DPG | U.S. Army Dugway Proving Grounds |
| DQI | Data Quality Indicator |
| DQO | Data Quality Objective |
| ECBC | U.S. Army Edgewood Chemical Biological Center |
| EPA | U.S. Environmental Protection Agency |
| FAC | Free Available Chlorine |
| FAD | Foreign Animal Disease |
| FADT | Subcommittee on Foreign Animal Disease Threats |
| FIFRA | Federal Insecticide, Fungicide, and Rodenticide Act |
| ft | foot, feet |
| g | gram(s) |
| g/L | gram(s) per liter |
| $H_2O_2$ | Hydrogen Peroxide |
| HVAC | heating, ventilation and air conditioning |
| in | inch(es) |
| INL | Idaho National Laboratory |
| ISO | International Organization For Standardization |

| | |
|---|---|
| L | liter |
| Lpm | liters per minute |
| LR | log(s) reduction |
| MDI | Metered Dose Inhaler |
| min | minute(s) |
| mL | milliliter(s) |
| MOP | Miscellaneous Operating Procedure |
| NHSRC | National Homeland Security Research Center |
| NIST | National Institute of Standards and Technology |
| NSTC | National Science and Technology Council |
| ORD | Office of Research And Development |
| OSWER | Office of Solid Waste And Emergency Response |
| pH-AB | pH-Adjusted Bleach |
| PBST | Phosphate Buffered Saline with 0.05% TWEEN®-20 |
| PPE | Personal Protective Equipment |
| ppm | parts per million |
| ppmv | parts per million by volume |
| psi | pounds per square inch |
| QA | Quality Assurance |
| QAPP | Quality Assurance Project Plan |
| QC | Quality Control |
| RSD | Relative Standard Deviation |
| RTU | Ready-to-Use |
| SS | stainless steel |
| sq | square |
| STS | sodium thiosulfate |
| TNTC | too numerous to count |
| TSM | Three-Step Method |
| USDA | US Department of Agriculture |
| USG | US Government |

| | |
|---|---|
| WAM | Work Assignment Manager |

**Executive Summary**

This project supports the U.S. Environmental Protection Agency (EPA), through its National Homeland Security Research Center (NHSRC) Decontamination and Consequence Management Division (DCMD), by providing relevant information pertinent to the decontamination of contaminated animal facilities resulting from an agro-terrorism incident or foreign animal disease (FAD) event. The primary focus of this project is to evaluate and improve the effectiveness and practical application of *in situ,* cost-effective alternative decontamination methods to remediate and restore areas contaminated by biological threat agents. These decontamination techniques rely on equipment (garden hoses, portable chemical sprayers, power washers) and application of liquid decontaminant solutions that are cost-effective and readily available.

The aim of this research was to assess the effectiveness of two decontamination application methods and two decontaminants: the use of either a portable, battery-powered backpack sprayer or a motorized power chemical sprayer to dispense antimicrobial solutions of either pH-adjusted bleach (pH-AB) or Ready-to-Use (RTU) Spor-Klenz® onto contaminated surfaces. The performance of these two decontamination procedures and two decontaminants was evaluated with respect to the physical removal, inactivation, and overall fate of spores on “medium-sized” 35.6 cm by 35.6 cm (14 in by 14 in) and “large-sized” 101.6 cm by 101.6 cm (40 in by 40 in) pressure-treated wood and concrete pieces (coupons). These materials were chosen because of their common occurrence in animal production facilities. Coupon materials were inoculated (loaded) with $1 \times 10^6$ - $5 \times 10^6$ *B. atrophaeus* spores using metered dose inhalers (MDIs) provided by the U.S. Army Edgewood Chemical Biological Center (ECBC) according to a proprietary protocol. *Bacillus* spores were used as surrogates for all FAD biological agents since they are highly resistant to chemical inactivation and represent a conservative estimate of decontamination effectiveness. Each “medium-sized” coupon was inoculated independently by being placed into a separate aerosol deposition apparatus (ADA) designed to fit one 14 in by 14 in coupon of any thickness. For the “large-sized” coupons, inoculations with spores were performed using nine ADAs aligned side-by-side (three rows of three) to cover the entirety of the surface. All coupons were free of dirt or grime.

The effectiveness of each decontamination method was first evaluated using the “medium-sized” coupons in a custom built test chamber, testing three coupons at a time in a vertical orientation, under varying conditions (Task I). Ten different test runs were set up with variations in application methods and antimicrobial solutions, as well as variations in spray time, rinse methods and time, and total contact time. Results from the “medium-sized” coupon tests were then used to develop two decontamination procedures applying antimicrobial solutions to “large-sized” coupons inside an enclosed, single-access-point chamber designated as the “Consequence Management and Decontamination Evaluation Room (COMMANDER)” (Task II). These tests were designed to evaluate the decontamination approach on a pilot scale. The pilot scale offers not only more realistic assessment of the effectiveness of the decontamination procedures than small scale testing (e.g., in a small chamber), but also more insights on the operational parameters such as time, physical impacts on materials and equipment, impact on the remediation crew (e.g., physical exertion), and spore cross-contaminations arising from the by-products of the decontamination processes (rinsate, exhaust, and decontamination equipment).

The major findings from this study are as follows:

- pH-Adjusted bleach was highly effective (approximately 6 log reduction (LR)) on wood and concrete when used with a thirty-minute contact time and two applications.

- Spor-Klenz® was more effective on wood than on concrete.
- For concrete coupons, pH-adjusted bleach was more efficacious than Spor-Klenz®.
- Reduction of the number of pH-adjusted bleach applications and contact time resulted in lower decontamination efficacy for surfaces and greater amounts of spores detected in rinsate and aerosol samples.
- Decontainationefficacy was similar between the two evaluated application devices (backpack sprayer and pressurized sprayer) despite significant differences in volume of decontaminant delivered to the coupon surface.
- Viable biological agent was detected in aerosol and rinsate (runoff) samples during all tests and can therefore be a significant source of cross-contamination during a remediation
- Elimination of a rinse step from the decontamination procedure did not reduce surface decontamination efficacy, and may be a viable option on materials not susceptible to corrosion.
- Worker fatigue may be of concern in an actual remediation as heat and exhaustion were experienced by laboratory workers when conducting scale-up tests that required level C personal protective equipment.

More specifically, most tests performed during Task I achieved the target efficacy from surfaces of greater than 6 Log Reduction (LR), a widely accepted standard for demonstrating sporicidal efficacy (e.g., 1 LR would be a reduction of 10, 2 LR would be a reduction of 100, 6 LR would be a reduction of 1 million, etc.). The decontamination by means of pH-adjusted bleach was accomplished by a combination of removal and inactivation of spores. Viable spores were found in both the rinsate and bioaerosol samples. Of the procedures tested, those incorporating pH-adjusted bleach were more effective for decontamination on concrete and wood than Spor-Klenz®. The lower log reduction (4 LR) seen in one test with wood may have been the result of material demand (i.e., reduction in activity of the decontaminant though reaction with the test material) in conjunction with a single application of the pH-adjusted bleach; one spray application does not appear to provide enough pH-adjusted bleach to overcome the demand of wood. The surface LRs for tests utilizing Spor-Klenz® were comparable to those with pH-adjusted bleach on treated wood, but significantly lower on tests involving concrete (< 3 LR).

Based on the Task I results, the most effective decontamination procedures were developed for further testing in Task II: the use of pH-adjusted bleach by backpack sprayer, sprayed on either concrete or wood, and rinsed or not rinsed. These procedures all used two, 30-second spray times every 15 minutes, for a total of 30 minutes of spray exposure per application. Procedure 1 included a rinse step, and Procedure 2 did not include this step. The results indicate that the two decontamination approaches were equivalent in decontaminating the two types of materials. The results also suggest that rinsing is not needed for these decontamination procedures to be effective on concrete and wood. However, if applications were to be made to surface materials sensitive to bleach (e.g., stainless steel), rinsing might be desirable from that standpoint as bleach and other aggressive oxidants are known to cause corrosion of numerous surfaces. LRs were approximately 6 for concrete and just under 6 for wood.

The overall fate of the biological spores was assessed, not only for the viable spores recovered from the surface of the materials, but also for fugitive viable agent escaping in the rinsate and aerosol fractions. Aerosol samples collected using bioaerosol filter cassettes during testing with the “medium-sized” coupons

show that re-aerosolization of viable spores can be expected during the decontamination process. Although one test with the “large-sized” coupons suggests that spores were dislodged during the first decontamination step and were constantly removed from the chamber (due to air exchange) following that release, further evaluation of the data indicates that there was likely cross-contamination and re-aerosolization of ambient spores in the chamber. However, the data do indicate that spores can be expected to be re-aerosolized in a field decontamination event and could be expected to travel through the Heating, Ventilation and Air Conditioning (HVAC) system (if operating) during decontamination and potentially spread contamination throughout a facility.

For most of the “medium-sized” coupon testing, the number of colony forming units (CFUs) recovered in the rinsate was below the detection limit. However, in the tests where only one short application of pH-amended bleach (pH-AB) was used, a large number of viable spores were physically removed from the surface during the decontamination and rinse steps. Such rinsate would potentially cause contamination to spread if not properly collected and treated.

The collection troughs for the “large-sized” coupon rinsates were immediately contaminated once brought inside the test chamber during test set-up. However, the rinsate contamination was systematically higher for the concrete coupons over the wood coupons and suggests that the contamination is coming from the coupons themselves. The loose material from the concrete coupons might have dropped into the trough while it was being placed under the coupon. Despite the occurrence of viable spores in the troughs prior to testing, the data suggest that active spores were transferred to the rinsate, as viable spore abundance in these samples increased by approximately $1 \times 10^5$ following the decontamination procedure that utilized a rinse step.

## 1. Introduction

Contamination of farm animal facility surfaces and equipment during a Foreign Animal Disease (FAD) outbreak could pose potential risks to human and animal health following an incident. Viable options for returning contaminated items to pre-incident risk levels are of immediate need. In response to data gaps/needs identified by the National Science and Technology Council (NSTC) Subcommittee on Foreign Animal Disease Threats (FADT), Decon and Disposal Working Group, which is co-chaired by the US Department of Agriculture (USDA) and the US Environmental Protection Agency (EPA), the EPA's National Homeland Security Research Center (NHSRC) conducted a study to measure the effectiveness of selected physical and chemical cleaning and disinfection methods for removing, reducing or inactivating FAD threat agents on different surface materials.

This project supports the missions of the USDA and US Department of Homeland Security (DHS) by increasing capabilities to respond and recover from an agro-terrorism or Foreign Animal Disease (FAD) incident. NHSRC's expertise in outdoor decontamination testing and evaluation was sought in order to advance the state of the science and benefit all agencies involved. This project also supports the mission of the NHSRC by providing relevant information pertinent to the decontamination of outdoor surfaces contaminated during a biological incident and supports the NHSRC's mission as delineated in Homeland Security Presidential Directive 5, 7, and 9.

During the decontamination activities following 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., the nature of the contaminant, the physical state of the facility, etc.). One factor was that such remediation was unprecedented for the United States Government (USG) and few technologies had been proven for such a large-scale 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 improving the effectiveness and practical application of *in situ* decontamination methods and evaluating waste treatment options to be able to provide information necessary to make the decontamination/disposal strategy more efficient (i.e., less costly, less time-consuming, and more efficacious).

### 1.1 Objectives

The primary objective of this study was to address decontamination method gaps that currently exist for response and recovery from an FAD outbreak at an animal production facility. *Bacillus* spores were used as surrogates for FAD biological agents since they are highly resistant to inactivation and represent a conservative estimate of decontamination effectiveness.

A number of procedures using two active decontamination solutions were evaluated, using equipment expected to be available at such a facility (i.e., garden hoses, pressure washers, and portable chemical sprayers). The decontamination agents tested were pH-adjusted bleach (pH-AB) and Spor-Klenz® RTU, a broad spectrum disinfectant and sporicide (details of both decontaminants given in Appendix E - Decontamination Process). The effectiveness of combined steps of the procedures was tested on "medium-sized" 35.6 cm by 35.6 cm (14 in by 14 in) pieces (coupons) of the selected materials (Task I) and "large-sized" 101.6 cm by 101.6 cm (40 in by 40 in) pieces (Task II). Both coupon sizes are larger than those used commonly in

other decontamination testing[1-4], but smaller than what will likely be encountered in the field (e.g., roadways, walkways, and walls). The medium-sized coupons allow numerous materials and decontaminants to be tested under varying conditions with replication. In addition, 1 sq. ft. size is the preferred surface area for wipe sampling. The 35.6 cm by 35.6 cm (14 in by 14 in) coupons offer this surface area size for decontamination and sampling. The large-sized coupons were used to provide insight into and a more realistic application of decontamination and sampling methods. Operational parameters such as time, physical impacts on materials, impact on the remediation crew (e.g., physical exertion), and fate of the viable spores (e.g., contamination of equipment, wash water, filters) were also determined.

### 1.2 Experimental Approach

The general approach used to meet the objectives of this project was:

- Use of experimental chambers with controlled environmental conditions, standardized coupons and spore inocula;
- Contamination of medium- and large-sized pieces of materials (coupons) via aerosol deposition of bacterial spores;
- Quantitative assessment of spore contamination by sampling positive control coupons (coupons contaminated with the bacterial spores in the same manner as test coupons, but not subjected to the decontamination treatment being tested prior to sampling);
- Application of a prescribed decontamination procedure to the test coupons and procedural blanks;
- Quantitative assessment of residual contamination by sampling test coupons and procedural blanks;
- Quantitative and qualitative analysis of decontamination procedure residues (e.g., waste water, aerosol samples);
- Determination of decontamination effectiveness (comparison of results from positive controls, negative controls and test coupons); and
- Documentation of operational considerations (e.g., cross-contamination, procedural time, impacts on materials and personnel).

For the purposes of this project, effectiveness of a procedure was evaluated by generating a quantitative estimate of the reduction of viable spores on a surface, measured as “log reduction”. In addition, determining the extent to which viable spores were relocated to rinsate water (runoff) or aerosol droplets is important for implications regarding fugitive emissions and downstream health risks.

Log Reduction (LR) can be defined as the amount of reduction in viable spores required to move the decimal one place, or reduce the exponent in scientific notation by one. If starting with one million spores, a log reduction of 2 would result in a 99% reduction, or a change from $1 \times 10^6$ to $1 \times 10^4$. A 5 LR would be 99.999% reduction, or a change from $1 \times 10^6$ to $1 \times 10^1$.

The general test approach for Task I is depicted graphically in the flow chart shown in Figure 1-1. Details of the types and numbers of materials tested, as well as the procedures used for contamination, decontamination, sampling and testing, are described in Section 2 and in the attached appendices.

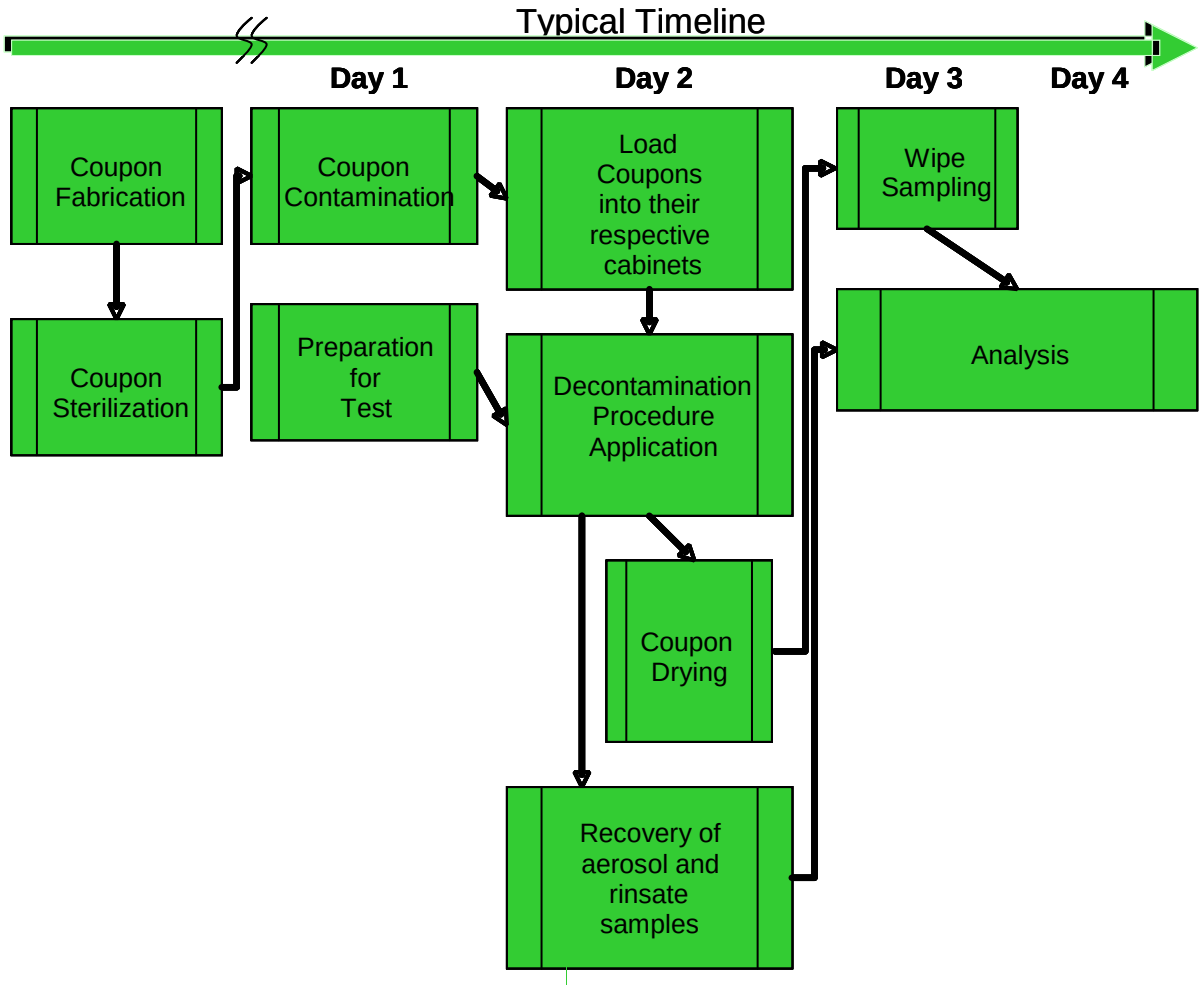


**Figure 1-1. Task I Test Approach Flow Chart**

The two materials investigated in this study were concrete and pressure-treated wood. These materials were chosen due to their common occurrence in animal production facilities. Prior to the start of testing, medium-sized 35.6 cm by 35.6 cm (14 in by 14 in) and large-sized 101.6 cm by 101.6 cm (40 in by 40 in) coupons were fabricated (see Section 2) for Task I and Task II, respectively. The coupons were then sterilized (see Appendix A). The 35.6 cm by 35.6 cm (14 in by 14 in) coupons were sterilized in groups (by autoclave for concrete and by STERIS VHP® 1000ED (STERIS Corporation, Mentor, OH) for pressure-treated wood) identified by sterilization batch number. The 101.6 cm by 101.6 cm (40 in by 40 in) coupons were sterilized in place using 250 ppmv (parts per million volume) vaporized hydrogen peroxide ($H_2O_2$) generated by a VHP® 1000ED for 4 hours.

Prior to use, all test equipment intended to come in contact with coupons or samples was sterilized via autoclave sterilization at 121 °C, 103 kPa (15 psi) or by a STERIS VHP® cycle at 250 ppmv $H_2O_2$ for 4 hours. All laboratory work surfaces were wiped with Dispatch® bleach wipes (Caltech, Midland, MI), rinsed with DI water, and dried with 70 percent ethanol (VWR, West Chester, PA).

In an actual incident, contaminated surfaces must undergo an organic burden reduction step prior to undergoing an effective decontamination with chemicals. This study uses burden-free materials and makes no attempt to determine the effectiveness of decontamination of heavily soiled materials since the removal of organic burden and surface pre-cleaning are assumed. Burden reduction steps would likely require significant additional effort in an actual incident.However, burden reduction may aid in surface contamination removal. Further testing utilizing a standardized burden on material surfaces is currently underway to better understand the effects of grime on decontamination efficacy.

#### 1.2.1 Task I Approach

Day 1 of testing involved coupon inoculation and preparation for testing on Day 2. The required number of pre-sterilized test and positive control coupons were loaded with the target spores. The procedural blank coupons were also located with the test and positive control coupons, but were not intentionally loaded with the target organism. The coupons remained isolated in independent deposition devices throughout this time.

On Day 2, the inoculated (and procedural blank) coupons were removed from the deposition devices and loaded into their respective cabinets (positive controls and test coupons into the Test Coupon Cabinets and the procedural blanks into the Procedural Blank Cabinet) until being retrieved for use in the decontamination test. Task I coupons were tested in the small chamber (see Section 2.3.1) in a vertical orientation. Procedural blank coupons were subjected to the decontamination procedure first, followed by the test coupons. The decontamination procedure was completed on all test coupons of one material type before moving on to the next material. After the decontamination procedure was applied to a coupon or set of coupons, the coupons were moved to the appropriate cabinet for drying (test coupons to the Decontaminated Coupon Cabinet and procedural blanks to the Procedural Blank Cabinet).

The temperature and pH of the pH-adjusted bleach solution and DI water, and the temperature of the Spor-Klenz® were measured at the initiation of a test and prior to the start of each test set (i.e., material type). The flow rate from the backpack sprayer (SRS-600 Propack, SHURflo, Cypress, CA), the pressure washers (John Deere 3300 psi, Model 020382 and Troy Bilt 2550 psi, Model 020337), and the chemical sprayer (Model# PP-UAG1003HU-K, UDOR, USA) were measured at the start and end of testing of each set of three coupons. The spray pattern for the backpack sprayer was confirmed (and adjusted as needed) prior to the start of a test. The 25° nozzle was used with the pressure washers. The chemical sprayer had an adjustable nozzle similar to the garden hose. These measurements were made to ensure that such parameters were in accordance with the data quality objectives (DQOs) defined for the project (see Section 4). Adjustments were made as necessary to achieve the desired set-points, within the acceptable tolerances.

Although surface sampling of the coupons did not occur until Day 3, several other samples were collected to obtain additional information on the fate of the spores. To assess the potential for viable spores to be washed off the surfaces, all liquid runoff (rinsate) generated in the decontamination process was collected and quantitatively analyzed. Rinsate samples were a composite of all replicate coupons of a particular material type per test. Quantitative analysis was conducted on rinsate samples so that the magnitude of spore relocation could be determined. The volume of runoff liquid collected for each coupon set was measured after collection. To quench the decontaminant activity in runoff samples during and after collection, sufficient neutralizer was added to the sample container prior to sample collection to prevent sporicidal activity post sample collection and

provide an accurate estimate of viable spores leaving the contaminated surface in rinse water. Soil or heavily soiled areas receiving biological agent-laden runoff during remediation following an actual FAD incident would be expected to quench most decontaminants in a similar manner.

Bioaerosol samples, using Via-Cell® Bioaerosol Sampling Cassettes (Part# VIA010, Zefon Int., Ocala, FL) , were originally collected during spraying operations (decontamination and rinse steps) in the small chamber to assess the potential for spores to be aerosolized during the decontamination procedure (see Appendix F.4 for details). Bioaerosol samples were collected from the exhaust vent during some tests.

After the completion of each set of coupons, the test chamber was cleaned in accordance with the procedure described in Appendix B. A coupon set for Task I includes all blank coupons or all replicates of one material type. Cleaning between sets reduced the potential for cross-contamination of samples.

On Day 3, after at least 18 hours of drying, sampling of the coupons was performed using pre-wetted gauze wipes (Kendall, 8042) (see section F.2.1). A sampled area of 1,175 $cm^2$ (1.3 $ft^2$) per coupon was used by sampling the interior section of each coupon. A template was used to cover the exterior 0.635 cm (0.25 in) of each 35.6 cm x 35.6 cm (14 in by 14 in) coupon leaving a square, 34.29 cm by 34.29 cm (13.5 in by 13.5 in) exposed for sampling. Surface sampling of each test coupon was conducted only once using the common method of wiping the surface with a wipe in three directions (vertical, horizontal, diagonal), completely covering the surface of the coupon in each direction (Appendix F).

The primary analysis of the samples collected (coupon, rinsate, and bioaerosol) occurred over a three-day period for Task I (note: Day 1 of the microbiological analysis was Day 3 of experimentation). In general, the Microbiology Laboratory extracted and plated the samples on the day of receipt and then counted colonies the next day. In instances when there was insufficient time for wipe samples to be extracted and plated on the day of receipt, they were refrigerated on the day of receipt, with sample extraction and plating on Day 2, and colony counting the following day. Filter plating or additional dilution plating was performed on an as-needed basis.

Appendix C contains Miscellaneous Operating Procedures (MOPs), including the aerosol deposition of spores. Appendices D through G contain additional details of the contamination, decontamination, and sampling and analysis procedures, respectively.

### 1.2.2 Task II Approach

Task II followed a similar pattern, except that an additional wipe sampling step to characterize contamination levels was done before the decontamination procedure, and the first step on Day 2 in Figure 3-1 (loading coupons into their respective cabinets) was not applicable. In addition, the timeline was extended compared to Task I, with the differences detailed below.

Day 1 of testing in the large chamber (referred to as COMMANDER; see Section 2.3.2) involved running a STERIS VHP® cycle in the COMMANDER and airlock to sterilize both the coupons and deposition devices.

On Day 2, the required number of test and positive control coupons were loaded with the target spores in COMMANDER in a horizontal orientation (nine deposition devices per large coupon, see Figure 2-5). Spores were allowed to settle onto the coupon surface for at least 18 hours. The deposition devices were removed on Day 3 and placed in the airlock. The 101.6 cm by 101.6 cm (40 in by 40 in) coupons were placed in vertical positions inside COMMANDER, and the deposition devices and the troughs underwent a STERIS VHP® cycle in the airlock.

Sterility checks (swab samples) were taken of the troughs on Day 4, with the weekend being Days 5 and 6. On the morning of Day 7, provided the troughs were not significantly contaminated (low spore counts were not unexpected since the coupons had been loaded with spores in the airlock), the troughs were placed beneath their assigned coupon inside COMMANDER and another sterility check was taken. For the first test, contamination of the organism of interest was found in the troughs, so the troughs and surfaces were wiped down with Dispatch® bleach wipes and the airlock was subjected to another STERIS VHP® cycle until no growth from sterility samples was observed. Positive control samples were taken immediately prior to the start of the decontamination process.

Unlike Task I, all coupons were inside the test chamber (COMMANDER) together. Completion of the decontamination procedure as well as pre- and post-decontamination sampling were done sequentially, alternating between concrete and pressure-treated wood coupons. Only pH-AB was used for these Task II tests, and pH-AB was applied with the backpack sprayer (SRS-600 Propack, SHURflo, Cypress, CA). During the first test, a garden hose was used to rinse the coupons with deionized (DI) water following the contact time with the decontaminant. Such rinse steps have been included in low-tech remediation of *Bacillus anthracis* contaminations, as rinsing is thought to reduce the amount of corrosion due to residual decontaminants and reduce the amount of chlorine off-gassing in a facility post-decontamination. Elimination of the final rinse step during animal facility remediation is believed to be a potential option; however, previously there have been limited data to support making such changes.

The troughs were used to collect the rinsate from each coupon. Separate bioaerosol samples were collected before, during, and after each individual step of the decontamination process.

On Day 8, post-decontamination sampling was conducted. A stainless steel template was used to create the nine individual sample areas, each 30.5 cm by 30.5 cm (12 in by 12 in). Sampling was conducted only once on any one of the nine sampling locations per coupon.

### 1.3 Definition of Efficacy

The overall effectiveness of a decontamination technique relies on the potential of the technology to inactivate and/or remove the spores from contaminated building material surfaces and the ultimate disposition (or fate) of the spores that would result in secondary contamination of by-products (rinsate) and equipment that would necessitate specific remediation strategies. Surface decontamination efficacies are for the complete procedure and for each specific material. The ultimate fate of the spores is also pertinent in assessing the overall remediation strategy.

The efficacy of each decontamination method (combination of steps) was determined based on the number of viable spores collected from the surface of the decontaminated coupon, as compared to the number of viable spores collected from the surface of control coupons (or coupon areas) not subjected to decontamination procedures. The number of viable spores was measured as colony forming units, or CFU.

#### 1.3.1 Surface Efficacy

CFU counts per coupon or coupon area were calculated according to the equation shown in MOP 6535a (Appendix C). The first step in the calculation of overall efficacy of a treatment to reduce contamination on the surface of the coupons is a separate calculation of efficacy for each individual coupon in a given set of replicates. Efficacy is defined as the extent (by log reduction, or LR) to which the agent extracted from the coupons after the treatment with the decontamination procedure is reduced below that

extracted from positive control coupons (not exposed to the decontamination procedure). Efficacy was calculated for each test coupon within each combination of decontamination procedure (*i*) and test material (*j*) as:

$$LR_{ijk} = \frac{\sum_{c=1}^{c}(\log_{10} C_{ijc})}{N_{ijc}} - \log_{10}\left(N_{ijk}\right) \quad (1\text{-}1)$$

where:

$C_{ijc}$ = the number of viable organisms recovered from c control coupons for the $i^{th}$ decontamination procedure and $j^{th}$ test material.

$N_{ijc}$ = the number of control coupons for the $j^{th}$ test material, $i^{th}$ decontamination procedure

$N_{ijk}$ = number of viable organisms recovered on the $k^{th}$ replicate test coupon for the $i^{th}$ decontamination procedure and $j^{th}$ test material.

The efficacy of the decontamination technique for a specific surface material is evaluated by means of the difference in the logarithm of the CFU before decontamination and after decontamination for that material. This value is reported as a log reduction (LR) efficacy on the specific material surface as defined in Equation 1-2.

$$LR_{ij} = \sum_{c=1} \log(CFU_{ijc}) / N_{ijc} - \sum_{k=1} \log(CFU_{ijk}) / N_{ijk} \quad (1\text{-}2)$$

where:

$LR_{ij}$ = the average log reduction of spores on a specific material surface

$\sum_{c=1} \log(CFU_{cj}) / N_C$ = the average of the logarithm of the number of viable spores (determined by CFU) recovered on the control coupons ($C$= control, $j$ = coupon number, and $N_C$ is the number of coupons (1, $j$))

$\sum_{k} \log(CFU_S) / N_t$ = the average of the logarithm of the number of viable spores (determined by CFU) remaining on the surface of a decontaminated coupon ($S$= decontaminated coupon, $k$ = coupon number, and $N_t$ is the number of coupons tested (1, $k$))

When no viable spores were detected, the detection limit of the sample was used, and the efficacy reported as greater than or equal to the value calculated by Eqn. 1-2. The detection limit of a sample depends on the analysis method and therefore may vary. The detection limit of a plate was assigned a value of 0.5 CFU, but the fraction of the sample plated varied. For instance, the detection limit of a 0.1 mL plating of a 20 mL sample suspension is 100 CFU (0.5 CFU / 0.1 mL * 20 mL), but if all 20 mL of the sample is filter-plated, the detection limit is 0.5 CFU.

The standard deviation of $\mathrm{LR_i}$ is calculated by Eqn 1-3:

$$SD_{\eta_{ij}} = \sqrt{\frac{\sum_{k=1}^{N_s} \left(x_{ijk} - LR_{ij}\right)^2}{N_{ijk} - 1}} \qquad (1\text{-}3)$$

where:

$SD_{\eta_{ij}}$ = standard deviation of $\eta_i$

$LR_{ij}$ = the average log reduction of spores on a specific material surface

$x_{ijk}$ = the average of the log reduction of the *k* replicate test coupon for the $i^{th}$ decontamination procedure and $j^{th}$ test material.

$$x_{ijk} = \frac{\sum_{k} \{\sum_{C=1} \log(CFU_{ijc}) / N_C - \log(CFU_{ijk}))}{N_{ijk}} \qquad (1\text{-}4)$$

where:

$\sum_{c} \log(CFU_{ijc}) / N_{ijc}$ = the “mean of the logs”, the average of the logarithm transformed number of viable spores (determined by CFU) recovered on the control coupons (*C*= control, *j* = coupon number, and $N_C$ is the number of coupons (1, *j))*

$CFU_{ijk}$ = number of CFU on the surface of the $k^{th}$ decontaminated coupon for the $i^{th}$ decontamination procedure and $j^{th}$ test material.

### 1.3.2 Ultimate Fate of Spores

The surface log reduction, as calculated in accordance with Equation 1-4, depicts the effectiveness of the decontamination in mitigating the contamination on materials. The mitigation could be due to inactivation of the spores on the materials (i.e., due to the application of a sporicide) or physical removal from the material (e.g., washed/rinsed off or aerosolized). For physical removal, viable spores may either remain in the rinsate or be re-aerosolized due to the decontamination activity itself. Understanding the ultimate fate of the spores, not just the surface log reduction, is critical to recognizing the utility or appropriate implementation of the decontamination process. Process parameters (as well as the general nature of microbiological sampling) prevented an exact accounting of the fate of spores; however, qualitative measurements were good indications of ultimate fate. For the rinsate sample, the results are reported as Total CFU and CFU per coupon. The Via-Cell® air sample from the vacuum containment cabinet or COMMANDER atmosphere is reported as CFU per actual liter (L) of air sampled.

## 2. Materials and Methods

### 2.1 Coupon Materials and Fabrication

#### 2.1.1 Material Surfaces

This section describes each material and how the medium- and large-sized coupons were fabricated. Both materials are considered porous.

1. **Pressure-Treated Wood** (Figure 2-1). The material used for these coupons is 3/4 in thick, 4 ft by 8 ft Georgia-Pacific ACQ-D (alkaline copper quaternary type D) pressure-treated plywood. Coupons were cut to size (35.6 cm by 35.6 cm (14 in by 14 in) for Task I, 101.6 cm by 101.6 cm (40 in by 40 in) for Task II) with a table saw.

**Figure 2-1. Pressure-treated Wood Coupon Front**

2. **Concrete** (Figure 2-2). Quikrete Sand/Topping mix was used to fabricate 1.5-in thick coupons for Task I (35.6 cm by 35.6 cm (14 in by 14 in)) and 1.0-in thick coupons for Task II (101.6 cm by 101.6 cm (40 in by 40 in)). The mix was prepared and poured into forms. Surfaces were smoothed with a hand trowel, then covered with plastic sheeting and allowed to cure for 24 hours. Once set, the coupons were removed from the form and loose grit was sprayed from the surface with a pressure washer. Task I coupons were then stacked on a pallet where they were further wetted and covered with plastic to cure (more than 20 days). Task II coupons were cured for five days in the shop where they were fabricated.

**Figure 2-2. Curing Concrete (left) and Final Concrete Coupons (right)**

2.1.2 Task I and Task II Coupons

The coupons made from each material for Task I had dimensions of approximately 35.6 cm width by 35.6 cm length (or approximately 14 in width by 14 in length). The dimensions provided an adequate edge for the spore deposition device to seal to the coupon surface and allow for a contaminated surface area of 1 ft by 1 ft. A sample area of 1 sq ft is recommended for wipe samples.[11] Contamination procedures have been developed, tested, and demonstrated by NHSRC in other decontamination studies. The sampled area of 1.3 sq ft per coupon was used for Task I of this study by sampling the interior section of each coupon. The thickness of the coupons varied for each material based upon the fabrication procedures determined to be the most appropriate for each material type. However, each material type had a uniform thickness for all replicate coupons.

Task II coupons prepared from pressure-treated wood and concrete were 101.6 cm by 101.6 cm (40 in by 40 in), and, conceptually, equal to the 3 by 3 square of nine coupons used in Task I. Two replicate coupons of each material were used for each test in Task II. The template used to sample individual coupon areas is shown in Figure-2-3.

All coupons were sterilized as described in Appendix A. There were no visible or documented changes to the structure of the coupons as a result of sterilization.

For the purposes of this project, coupon sets were defined as all blank coupons, groups of replicate test coupons, and all positive control coupons of the same material type.

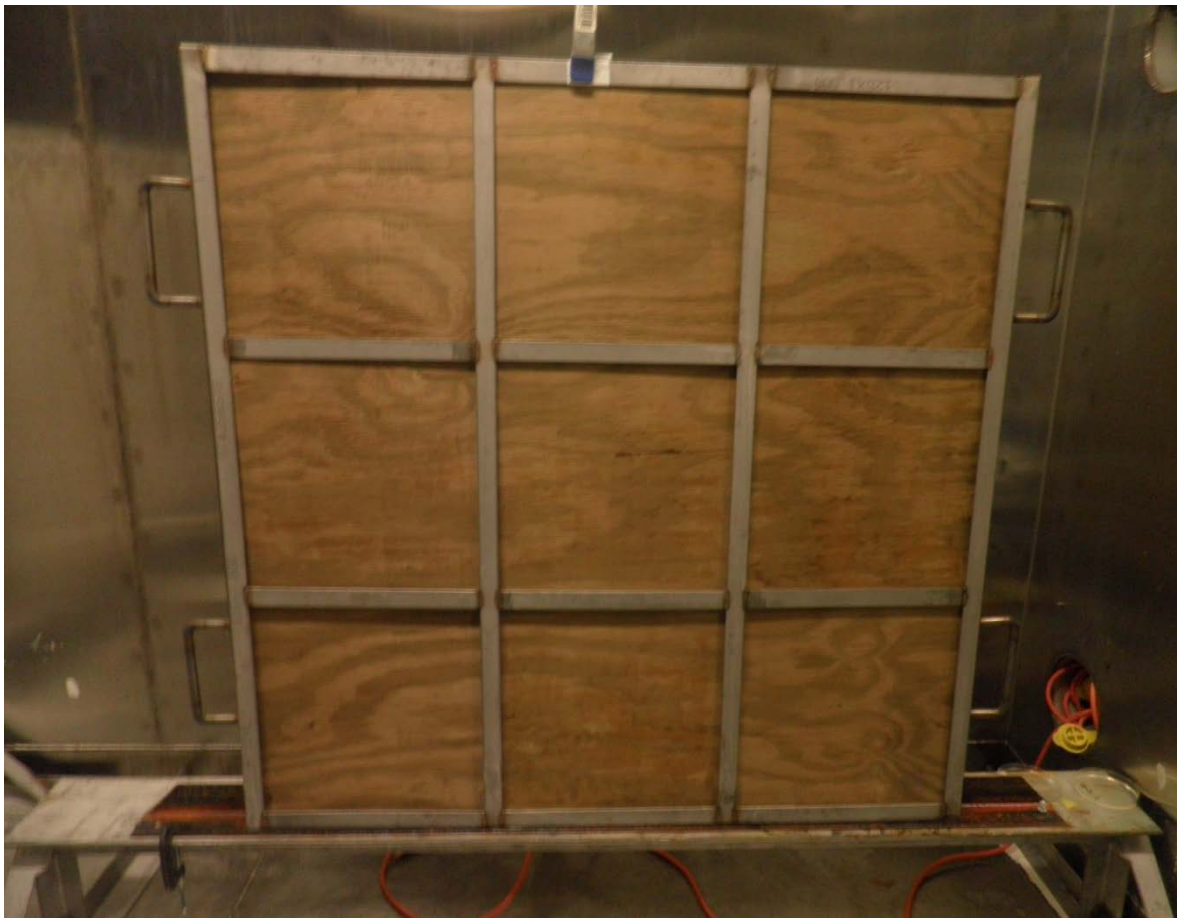

**Figure 2-3. Sampling Template on Task II Pressure-treated Plywood Coupon**

### 2.2 Material Inoculation Procedure

The investigation of the effectiveness of the decontamination procedures required that a target organism be applied to a "sterile" material surface (i.e., material inoculation) at a precise target loading (e.g., spores per piece of material (or coupon)). This section provides detail on the target organism and material inoculation procedures used for this investigation.

#### 2.2.1 *Bacillus* Spore Preparation

The test organism for this work consisted of a *Bacillus atrophaeus* spore preparation infused with silicon dioxide particles. This bacterial species was formerly known as *B. subtilis* var *niger* and previous to that as *B*. *globigii*. The preparation was obtained from the U.S. Army Dugway Proving Grounds (DPG) Life Science Division. The preparation procedure is reported in Brown et al.[12] Briefly, after 80 – 90 percent sporulation, the suspension was centrifuged to generate a preparation of about 20 percent solids. A preparation resulting in a powdered matrix containing approximately 1 x $10^{11}$ 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 powdered preparation was loaded into metered dose inhalers (MDIs) by the U.S. Army Edgewood Chemical Biological Center (ECBC) according to a proprietary protocol. The MDIs are claimed to provide a consistent dose of 1E9

spores per puff. Quality assurance documentation is provided by ECBC with each batch of MDIs. Control checks for each MDI were included in the batches of coupons contaminated with a single MDI as described in Section 2.2.2.

### 2.2.2 Coupon Inoculation Procedure

Coupons were inoculated (loaded) with spores of *B. atrophaeus* from an MDI using the procedure detailed in MOP 6561 (an EPA proprietary method, patent pending). The large 101.6 cm by 101.6 cm (40 in by 40 in) coupons were placed horizontally inside COMMANDER. Nine dosing chambers were arranged on the large coupons, overlapping the inside edges of the dosing chambers. Clamps were placed along the outside edge, and two bars spanning the width of the coupon were clamped down to help stabilize the internal edges for the second Task II test. Each dosing chamber covered a coupon area, as shown in Figure 2-4. Figure 2-5 shows the dosing chambers in place.

Briefly, each coupon (or coupon area for Task II) was contaminated independently by being placed into a separate dosing chamber (aerosol deposition apparatus or ADA) designed to fit one 35.6 cm by 35.6 cm (14 in by 14 in) coupon of any thickness. In accordance with MOP 6561, the MDI was discharged a single time into the dosing chamber. The spores were allowed to settle onto the coupon surfaces for a minimum period of 18 hours. After the minimum 18-hr period, the Task I coupons were then removed from the dosing chamber and moved to an isolated cabinet (Test Coupon Cabinet) which contained all loaded coupons for a single test. The Task II coupons were moved to their test positions in the large chamber following the deposition period. The target recovery range was $1 \times 10^7$ CFU per coupon.

The MDIs are claimed to provide 200 discharges per MDI. The number of discharges per MDI was tracked so that use did not exceed this value. Additionally, in accordance with MOP 6561, the mass of each MDI was determined after completion of the contamination of each coupon. To prevent inadequate inoculation of coupons due to near-empty MDIs, if an MDI had a mass of less than 10.5 g at the start of the contamination procedure described in MOP 6561, it was retired and a new MDI was used. For quality control of the MDIs, an inoculation control coupon was run as the first, middle, and last coupon inoculated with a single MDI in a single test. The contamination control coupon was a stainless steel coupon (35.6 cm by 35.6 cm) inoculated in accordance with MOP 6561, sampled in accordance with Appendix F, and analyzed in accordance with Appendix G.

A log was maintained for each set of coupons or coupon areas that were dosed. Each record in this log recorded a unique coupon identifier (see Appendix D), the MDI unique identifier, the date, the operator, the weight of the MDI before dissemination into the coupon dosing device, the weight of the MDI after dissemination, and the difference between these two weights.

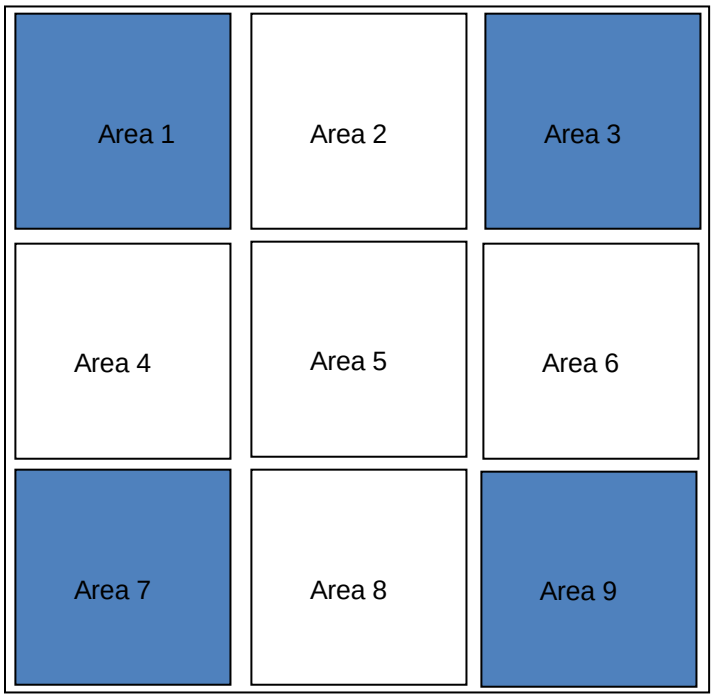


**Figure 2-4. Task II Coupon Sampling Areas (BLUE indicates areas for positive controls)**

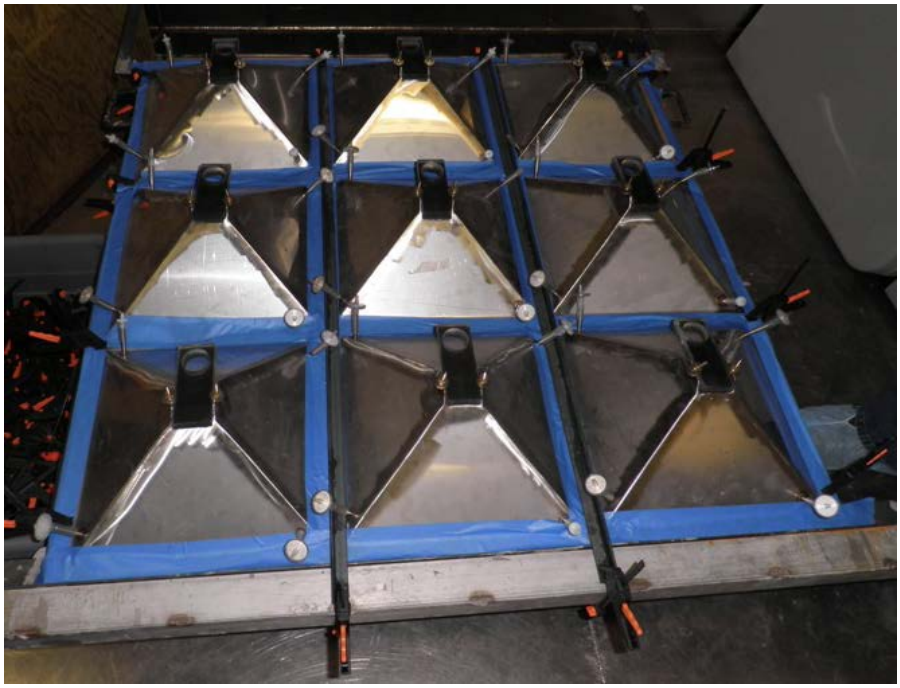

**Figure 2-5. Nine Dosing Chambers on a 101.6 cm by 101.6 cm (40 in by 40 in) Coupon**

The handling of the inoculated coupons, including movement to minimize or control spore dispersal, is described in Appendix D.

### 2.3 Experimental Approach

#### 2.3.1 Task I – Small Chamber

For Task I, application of the decontamination procedures was done in a custom-built test chamber shown in Figure 2-6. The chamber, located in High-Bay Room 130 at EPA's Research Triangle Park facility, has dimensions of 1.2 m high by 1.2 m wide by 1.2 m deep (4 ft high by 4 ft wide by 4 ft deep) and is designed to accommodate three 35.6 cm by 35.6 cm (14 in by 14 in) coupons at a time in either orientation (horizontal or vertical, see below). The chamber is of solid stainless steel construction with the exception of the front face and top which are fabricated from clear acrylic plastic. The front face acrylic section is a door allowing full access to the inside of the chamber while standing outside. The back stainless steel wall contains an assembly to hold the vertically-oriented coupons (maximum three 35.6 cm by 35.6 cm (14 in by 14 in) coupons at one time).

A center-aligned hole in the chamber door is outfitted with a swivel port (see Figure 2-7), allowing spray nozzles to fit and align with the middle of the coupons. The wand is inserted into this center port and moved in and out as necessary to maintain the correct distance from the three coupons while accomplishing the spray pattern described in Appendix E (Decontamination application methods and rinsing with water). Every effort was made to perform this step consistently and maintain the correct distance from all coupons. The port also allows the chamber door to remain closed during application of the decontamination solutions. During the pressure-washing, rinsing steps with the garden hose and the spraying of the decontamination solutions with the backpack sprayer, the front face door was closed and sealed. The seal is designed to contain any splashed liquid. Maintaining the door closed also prevents exposure of the worker to the toxic fumes from decontamination solution during application.

The bottom of the chamber is pyramidal in shape with a 7.6 cm (3 in) diameter drain in the center. The drain can be closed or opened to either collect or release the runoff from the coupons during the decontamination procedure. The bottom of the chamber has a 227 L (50 gal) collection capacity.

The chamber is fitted with connections allowing filtered air to enter and filtered exhaust to exit via a readily accessible connection to the facility's air handling system. Connection to facility point exhaust results in a slight negative pressure inside the spray chamber in relation to the room within which it is contained. The chamber is also designed to be easily decontaminated between runs using either liquids or fumigants, as needed. Decontamination of the chamber is discussed in Appendix B.

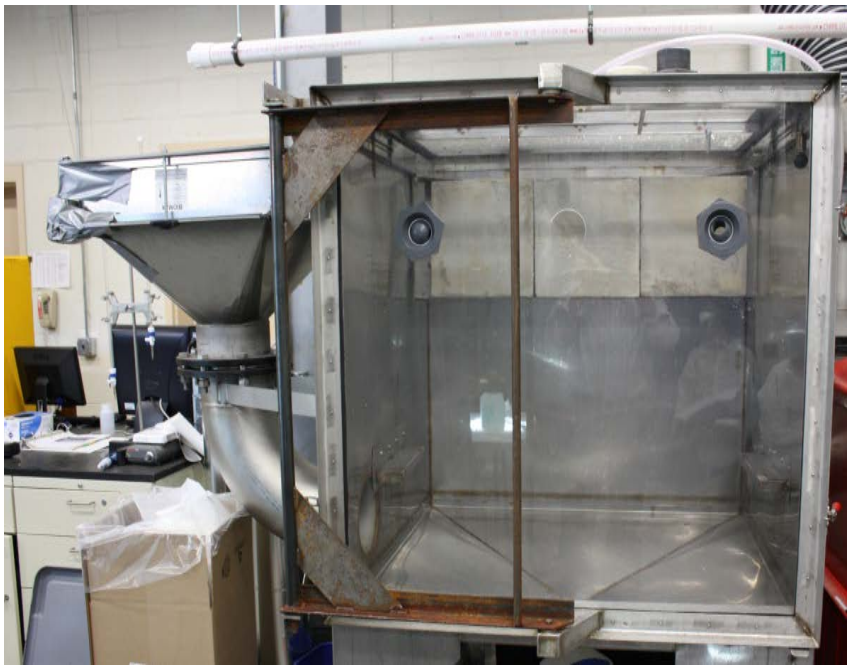

**Figure 2-6. Task I Decontamination Chamber**

**Figure 2-7. Spraying through center-aligned port in the small chamber door**

**Figure 2-8. Airlock in foreground and large chamber (COMMANDER) in background**

### 2.3.2 Task II – Large Chamber (COMMANDER)

For Task II, application of the decontamination procedures was done inside the Consequence Management and Decontamination Evaluation Room (COMMANDER) (Figure 2-8). This room is an enclosed, single-access-point chamber that meets the following criteria:

- Supports repeated fabrication of a representative test environment (e.g., furnished office room, outdoor setting) contained within the chamber
- Allows for release of biological organisms or chemicals into the chamber (Biosafety Level 2, Chemical Safety Level 4)
- Under slight negative pressure in relation to outside environment
- Allows for application of a decontamination technology (including fumigation with toxic, corrosive gases)
- Supports entry into the chamber during all of the above-mentioned activities (in appropriate personal protective equipment (PPE))
- External dimensions of 2.74 m by 3.66 m by 3.05 m high (9 ft by 12 ft by 10 ft high)
- Contains a 1.83 m by 1.83 m by 2.44 m high (6 ft by 6 ft by 8 ft high) airlock with single air-tight entry/exit port with a window
- 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

- Complies with all relevant local and national codes

- For the current study, a trough was placed under the coupons and curtains placed around the coupons, in order to capture and collect the runoff and spray during the decontamination procedures. The curtains were placed to act as a guide during the decontamination steps to facilitate maintaining the correct distances between the nozzles and the surface of the coupon.

### 2.4 Decontamination Procedure

The two procedures tested for application of pH-AB and Spor-Klenz® can be summarized with the following sequential procedural steps. Modifications made to the test matrix are detailed in Section 2.5.

Backpack Sprayer-Applied Decontaminant

1. Apply liquid decontaminant to material surface using a pressurized backpack sprayer.

2. After 15 minutes, reapply the liquid decontaminant to material surface.

3. Once a total of 30 minutes have elapsed since the first application, rinse the material surface with distilled water using a garden hose.

4. Allow material to dry overnight.

5. Sample material surface using sterile non-cotton pre-moistened wipes.

Pressure Washer-Applied Decontaminant

1. Apply liquid decontaminant to material surface using pressure washer and chemical supply tank.

2. After 15 minutes, reapply the liquid decontaminant to material surface.

3. Once a total of 30 minutes have elapsed since the first application, rinse the material surface with distilled water using a pressure washer.

4. Allow material to dry overnight.

5. Sample material surface using sterile non-cotton pre-moistened wipes.

Determining the efficacy of the above-mentioned procedures was the focus of this study, both with respect to the physical removal and the inactivation of spores.

This project employed the use of backpack sprayers, pressure washers, nozzles, garden hoses, pressure regulators, bleach, vinegar, and Spor-Klenz®, as well as carboys, buckets for DI water, and containers for mixing the pH-adjusted bleach solution. The specifications of the materials and equipment used for the decontamination procedural steps are detailed in Table E-1 of Appendix E.

It was critical for this project that each step in the decontamination procedure be implemented as uniformly as possible for all coupons and tests. Changes in technique during the study could lead to highly variable and/or biased data and lead to erroneous conclusions. Therefore, the methods for each step were documented in detail to provide as much standardization as possible. Staff performing the decontamination procedures practiced each step in advance and an attempt was made to add measurable controls. Additional details can be found in Appendix E.

The results of the testing provide information to evaluate the effectiveness of a number of procedures using two active decontamination solutions for removing surface contamination. Additionally, the testing provided information on

viable spore disposition for consideration in the development of remediation strategies (e.g., when/where the procedure might be considered for application, need for water collection and treatment, estimation of waste generation).

### 2.5 Test Matrix

Ten tests in Task I 35.6 cm by 35.6 cm (14 in by 14 in) coupons and two tests in Task II 101.6 cm by 101.6 cm (40 in by 40 in) coupons were performed. Table 2-1 identifies each procedural step for each material type. The original test matrix was amended as the tests progressed, based on the results obtained. These changes were adaptive (altering parameters based upon results of previous tests) and in remediation of unforeseen consequences of testing (replacing of spray devices following failure of the initial device due to incompatibility with the liquid decontaminant).

- Procedural blanks for Task I (coupons of each material not intentionally loaded with the target organism) were run first, followed by the test coupons of each material type. The procedural blank coupons were subjected to the same procedural decontamination steps as the test coupons. On the day of testing, the coupons are moved to their respective storage cabinets (positive controls and test coupons into the Test Coupon Cabinets and the procedural blanks to the Procedural Blank Cabinet) to avoid potential cross-contaminations between coupons. For Task I, a maximum of three coupons were run at a single time in the decontamination chamber. Only one material type was run at a time.

- For Task I, cleaning of the chamber was performed in accordance with Appendix B after the completion of each material type per test.

- For Task II, cleaning of the chamber was performed by running a STERIS VHP® cycle as detailed in Section 1.2 after the completion of each material type per test.

- For Task I, each test required six test coupons, one procedural blank, and six positive control coupons of each material type. Hence, 13 coupons (total) were required for each material type.

- For Task II, each test required two replicate coupons, divided into five test coupon and four positive control sample areas.

- Wipe sampling was used on both the concrete and pressure-treated wood.

**Table 2-1. Test Matrix**

| Task | Test | Date of Decon | Material | Size (in) | Replicates (n) | Application | Decon | Spray Duration (sec) | Reapplication Time (min) | Rinse Duration (sec) | No. of Sprays | Total Exposure (min) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 10/12/2010 | Concrete | 14"x14" | 6 | Sprayer | pH-AB | 30 | 15 | 10 | 2 | 30 |
| 1 | 2 | 10/12/2010 | Wood | 14"x14" | 6 | Sprayer | pH-AB | 30 | 15 | 10 | 2 | 30 |
| 1 | 3 | 12/14/2010 | Concrete | 14"x14" | 6 | Chemical Sprayer | pH-AB | 15 | 15 | 10 | 2 | 30 |
| 1 | 4 | 12/14/2010 | Wood | 14"x14" | 6 | Chemical Sprayer | pH-AB | 15 | 15 | 10 | 2 | 30 |
| 1 | 5 | 10/27/2010 | Concrete | 14"x14" | 6 | Sprayer | Spor-Klenz® | 30 | 15 | 10 | 2 | 15* |
| 1 | 6 | 10/27/2010 | Wood | 14"x14" | 6 | Sprayer | Spor-klenz® | 30 | 15 | 10 | 2 | 15* |
| 1 | 7 | 11/17/2010 | Concrete | 14"x14" | 6 | Pressure Washer | Spor-Klenz® | 15 | 15 | 10 | 2 | 30 |
| 1 | 8 | 11/17/2010 | Wood | 14"x14" | 6 | Pressure Washer | Spor-Klenz® | 15 | n/a | 10** | 1*** | 34 |
| 1 | 9 | 1/18/2011 | Concrete | 14"x14" | 6 | Sprayer | pH-AB | 10 | n/a | 10 | 1 | 15 |
| 1 | 10 | 1/18/2011 | Wood | 14"x14" | 6 | Sprayer | pH-AB | 10 | n/a | 10 | 1 | 15 |
| 2 | C1 | 2/8/2011 | Concrete | 40"x40" | 2 | Sprayer | pH-AB | 30 | 15 | 30 | 2 | 30 |
| 2 | C1 | 2/8/2011 | Wood | 40"x40" | 2 | Sprayer | pH-AB | 30 | 15 | 30 | 2 | 30 |
| 2 | C2 | 2/24/2011 | Concrete | 40"x40" | 2 | Sprayer | pH-AB | 30 | 15 | NA | 2 | 30 |
| 2 | C2 | 2/24/2011 | Wood | 40"x40" | 2 | Sprayer | pH-AB | 30 | 15 | NA | 2 | 30 |

* Coupons were inadvertently rinsed immediately after the second Spor-Klenz® spray, resulting in a total contact time of 15 minutes.

** Rinse applied with garden hose due to power washer failure (34 minute contact time).

*** Power washer failed before second decontaminant application during first set of three replicate coupons. First set had one application; second set was not included in test results.

In Tests 1 and 2, the backpack sprayer was used to spray the coupons twice for 30 seconds with pH-AB, followed by a 15-minute contact time after each spray. This scheme resulted in a total exposure (contact time) of 30 minutes before the DI rinse. Tests 5 and 6 were conducted identically, except that Spor-Klenz® was used as the decontaminant.

Due to concerns over compatibility between pH-AB and the pressure washer, Tests 7 and 8 with Spor-Klenz® were conducted first. Because of the higher flow rate of the pressure washer versus the backpack spray, the duration of the two sprays was reduced to 15 seconds. The total contact time for the concrete coupons remained at 30 minutes. The concrete coupons were subjected to the test procedure first, and the procedure was completed as prescribed. Wood coupons were tested second, and received the first decontamination spray, but the pressure washer could not be restarted to accomplish the second application. Following only one application of Spor- Klenz®, these coupons were rinsed with DI water using a garden hose after 34 minutes of exposure. Ultimately, the pressure washer was rendered inoperable by the Spor-Klenz®.

Tests 3 and 4 were conducted with pH-AB using a chemical sprayer. Known incompatibility with standard pressure washers prevented their use; the UDOR chemical sprayer (Model# PP-UAG1003HU-K, UDOR, USA) was chosen for this test because it was made specifically for use with chlorine (see Appendix E). These tests involved two 15-second sprays of pH-AB with 15-minute contact times after each spray (30 minute total exposure), and a 10-second DI water rinse using the replacement pressure washer.

Based on the pH-AB results from Tests 1 through 4, Tests 9 and 10 reduced the pH-AB backpack spray time to 10 seconds and involved just one 15-minute contact time prior to the DI water rinse. Tests C1 and C2 were conducted in COMMANDER using two replicate coupons of each material for each test. For both tests, the backpack sprayer was used to spray the coupons twice for 30 seconds with pH-AB, followed by a 15-minute contact time after each spray. The difference between these tests was that the coupons in C2 did not receive a DI water rinse.

### 2.6 Sampling and Analytical Procedures

Three types of samples were included in this project. Surface sampling procedures were used to collect samples from the coupon materials. In order to obtain the additional critical information on the fate of the spores, several samples in addition to the surface sampling of the coupons were 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. This sample was a composite for all replicate coupons of a particular material type per test. 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.

To assess the potential for spores to be aerosolized from coupon surfaces during spraying or pressure washing, aerosol samples were collected from the decontamination chamber during any such activities. Quantitative analyses were performed on these samples, so that a concentration (viable spores per volume of air) could be determined. These data are important for understanding the potential for contamination spread and worker risk during the decontamination procedures. Any spores released during this phase may also avoid contact with the decontaminant and therefore remain active. A second decontamination procedure may be needed to decontaminate aerosolized spores that redeposit elsewhere. The materials and equipment used as well as the sampling protocols for sampling are detailed in Appendix F.

### 2.6.1 Factors Affecting Sampling/Monitoring Procedures

Sampling of coupon surfaces was done after coupons that were wetted during the decontamination procedure had become visibly dry. Drying was allowed to occur in the Decontaminated Coupon Cabinet or Procedural Blank Cabinet, or inside COMMANDER (as appropriate), facilitated by a slight air flow due to a constant positive pressure. All coupons were allowed to dry for at least 18 hours. The actual time that each coupon was allowed to dry was recorded.

### 2.6.2 Preparation for Sampling/Monitoring

Sampling kits for wipes were prepared as specified MOP 6568 (see Appendix C). For Task I, all laboratory surfaces intended for use during sampling were wiped with Dispatch® bleach wipes. Precut 50.8 cm by 50.8 cm (20 in by 20 in) sheets of absorbent bench liner were used to cover all work surfaces, replaced after each phase of a test (e.g., coupon contamination is considered one phase, decontamination another, and surface sampling a third). Sampling was conducted on only one coupon at a time. One coupon was moved from the Decontaminated Coupon Cabinet (test coupons), Test Coupon Cabinet (positive controls), or Procedural Blank Coupon Cabinet (procedural blanks) to the sampling space located immediately outside (to the front) of each cabinet. All coupons were placed horizontally for sampling, regardless of their orientation during the decontamination.

Within a single test, surface sampling of the coupons was performed starting with coupons from the lowest level of contamination and ending with the highest level of contamination (i.e., all procedural blank coupons first, followed by all test coupons, and then all positive control coupons). Surface sampling was performed by wipe sampling in accordance with the protocols included in Appendix F. The surface area for all samples was 1175.8 $cm^2$ (1.3 $ft^2$).

A template was used to cover the exterior 0.635 cm (0.25 in) of each coupon leaving a square (34.29 cm by 34.29 cm) exposed for sampling for all coupons. The outer 0.635 cm of each coupon was not sampled in order to avoid edge effects.

A sampling material bin was stocked with all appropriate items (consistent with the protocols in Appendix F) for each sampling event. The bin contained enough wipe sampling kits to accommodate all required samples for the specific test. An additional kit was also included for backup. Enough gloves and bleach wipes needed to complete the test were available. Templates (35.6 cm by 35.6 cm (14 in by 14 in)) with an interior opening of 34.3 cm by 34.3 cm (13.5 in by 13.5 in) were wrapped in aluminum foil and packaged in sterile autoclave-safe bags (autoclave-sterilized by MOP 6570 using a one hour gravity cycle, 10 templates per bag) and transported with the original sterile coupons (concrete and stainless steel procedural blanks). These bags of templates were also included with the sampling kits. 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 Dispatch® bleach wipe prior to transport from the sampling location to the Microbiology Laboratory. To ensure the integrity of samples and to maintain a timely and traceable transfer of samples, an established and proven chain of custody was strictly adhered to for each test.

For Task II, a template (see Figure 2-3) was used to create the nine individual sample areas, each 30.5 cm by 30.5 cm (12 in by 12 in). The sampling templates were sterilized by VHP® or Dispatch® wipes prior to sampling. Coupons were sampled in the vertical position, one material at a time.

### 2.6.3 Wipe Sampling

To assess the effectiveness of the decontamination procedure, wipe sampling was performed for each coupon. Wipe sampling is the method that is anticipated to be used following an FAD incident. 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.[11] 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.[11] The protocol that was used in this project is described in Appendix F and has been adapted from that provided by Busher et al.,[11] Brown et al.,[12] and documented in the INL 2008 Evaluation Protocols.[13] Materials utilized in this study are considered hard and porous. While wipe sampling is not highly efficient on porous materials, few other options exist. In addition, preliminary data suggest that wipe sampling of wood and concrete surfaces routinely allowed recoveries of greater than $1 \times 10^6$ CFU when surfaces were inoculated with $1 \times 10^7$ CFU per $ft^2$. Wipe sampling was therefore utilized for both porous materials used in this study.

### 2.6.4 Rinsate Collection and Sampling

Decontamination procedures utilizing corrosive liquids such as bleach will likely incorporate a final rinse step following a prescribed contact time with the decontaminant to reduce the potential for damage to contacted surfaces. It is important to determine if this "runoff" is a potential risk for spread of contamination, so rinsate samples were sampled and analyzed for viable spores following decontamination.

For Task I, the runoff from the coupons throughout the entire decontamination procedure was collected for a given coupon set (material type or all blanks). After all coupons from a single set were moved to the Decontaminated Coupon Cabinet or Procedural Blank Cabinet, the chamber was rinsed with sterile DI water. The sterile runoff collection carboy was labeled and the volume of liquid collected was recorded. The decontamination liquid was neutralized by sodium thiosulfate (STS) by placing the STS in the collection vessel prior to commencement of the decontamination steps. Neutralization was done in order to standardize the results from all tests, i.e., any sporicidal activity of the runoff was eliminated once the runoff was captured in the carboy preventing run-to-run variability due to differences in the runoff composition. Neutralization of the rinsate was used to simulate a worst case field situation where the residual killing power of the pH-AB or Spor-Klenz® would be removed (i.e., due to material demand from the collection surface (e.g., concrete or pressure-treated wood)).

After collection, rinsate samples were homogenized by shaking and 100 mL aliquots were taken using aseptic technique according to the protocol described in Appendix F. The aliquots were submitted to the Microbiology Laboratory for analysis at the conclusion of each entire test.

For Task II, a trough blank was first collected by adding 1 L of sterile DI water to each trough and taking three 100 mL aliquots for analysis. STS was added to the trough prior to the start of the decontamination procedure. The volume of rinsate collected in each coupon's trough was measured, and 100 mL aliquots were taken as for Task I and submitted to the Microbiology Laboratory for analysis.

### 2.6.5 Bioaerosol Sample Collection

To assess the potential for biological particles to escape the surface of coupons during spraying (decontamination and rinse steps) as aerosols, bioaerosol samples were collected by actively sampling (12 L/min) the air. ViaCell® bioaerosol sampling cassettes were used to collect air from the 1.2 m by 1.2 m (4 ft by 4 ft) spray chamber

and from the COMMANDER chamber during the decontamination procedures. Data obtained from bioaerosol samples were used to indicate whether reaerosolization is possible during decontamination procedures.

### 2.6.6 Sample Analyses

Analyses of all samples were conducted in the on-site Microbiology Laboratory. Phosphate buffered saline with 0.05% TWEEN®-20 (PBST) was used as the extraction buffer. After the appropriate extraction procedure, as described in Appendix F, the samples were plated, incubated, and analyzed (CFU enumerated) in accordance with MOP 6535a (see Appendix C). Appropriate dilutions of the extracted sample (i.e., the initial undiluted sample extraction dilution, and up to a four-stage serial dilution ($10^{-1}$ to $10^{-4}$)) were plated depending on expected CFU concentration. For example, the last two dilutions ($10^{-3}$ and $10^{-4}$) were not plated for a decontaminated sample if a low CFU concentration was expected.

In addition to the analysis in MOP 6535a, additional analysis procedures were used for samples resulting in less than 30 CFU/sample in the undiluted sample extract (e.g., wipe in the extraction buffer). These analyses were conducted in order to lower the current detection limit associated with MOP 6535a. In accordance with MOP 6565, Revision 2 (see Appendix C), samples were filter plated.

The PBST was prepared according to the manufacturer's directions and in accordance with MOP 6562 (see Appendix C), dissolving one packet in one liter of sterile water. The solution was then vacuum-filtered through a sterile 0.22 µm filter unit to sterilize.

The extraction procedure used to recover spores varied depending upon the different matrices (wipes, Via-Cell ® cassette). The procedures are described in Appendix F.

### 2.6.7 Coupon, Material, and Equipment Cleaning and Sterilization

Several management controls were put in place in order to prevent cross-contamination. This project was labor-intensive and required that many activities be performed on coupons that were intentionally contaminated (test coupons and positive controls) and not contaminated (procedural blanks). The treatment of these three groups of coupons (positive control, test, and procedural blank) varied for each group. Hence, specific procedures were put in place in an effort to prevent cross-contamination among the groups.

Due to the amount of waste and reusable items (requiring decontamination after use) generated during this testing (e.g., sterilization bags, sampling templates, etc.), creation of a rigid plan to segregate such items was imperative. Reusable items were clearly distinguished and separated from waste items after use and put in distinct, segregated locations within the testing area.

During the decontamination procedure for Task I, one person (sample handler) was tasked with moving the coupons to the decontamination chamber. A different person was tasked with moving the treated coupons to the appropriate drying cabinet. Disposable laboratory coats were used by the sample handler (tasked with moving the coupons) to further minimize the potential of cross-contamination. The sample handler donned a new disposable laboratory coat after moving a complete set of test samples (i.e., 6) from the test coupon cabinet to the decontamination chamber.

All bins, buckets, and containers remained closed or covered unless in use (e.g., material being placed into or extracted from the bin, bucket, or container). Adequate cleaning of all common materials and equipment was critical in preventing cross-contamination.

Each test in the experimental matrix included four primary activities. These activities were preparation of the coupons, execution of the decontamination process (including sample recovery), sampling, and analysis. Specific management controls for each of these activities are shown in Table 2-2. Appendix A details the coupon sterilization procedures and Appendix B describes the test chamber and equipment cleaning procedures.

**Table 2-2. Cleaning Methods and Frequency for Common Test Materials/Equipment**

| Material/Equipment | Use | Cleaning Method | Frequency |
|---|---|---|---|
| Decontamination Procedure Chamber | Contain coupons during the application of the decontamination procedure being tested | Washing with pH-adjusted bleach solution, or wiping with Dispatch® Bleach Wipes, rinsing with DI water followed by ethyl alcohol | Before/after each test and between test materials |
| Coupon Cabinets | Store coupons prior to testing and/or sampling | pH-Adjusted bleach solution or wiping with Dispatch® Bleach Wipes, rinsing with DI water followed by ethyl alcohol | Before/after each test |
| Distilled water tanks (reservoir) | Utilized during the garden hose rinse and pressure wash rinse procedures | Bleach solution, soak overnight | Treated before each test (within 48 hours of the test start) |
| All work surfaces | Throughout each test | Maintaining the surface wet with a pH-adjusted bleach solution for 10 minutes followed by wiping with 70% ethyl alcohol before wiping dry with a clean towelette. | Before/after each use (cleaning of surfaces between handling of replicate coupons during sampling; cleaning before/after moving all contaminated coupons) |

## 3. Results and Discussion

The primary objective of this study was to evaluate the efficacy of decontaminating building material surfaces as a function of the decontamination method parameters. The parameters were chosen to improve application efficiency while maintaining efficacy. In addition to reduction of contamination from material surfaces, the ultimate fate of the spores was also a critical measurement objective. Combined, this information can inform selection or further development of appropriate, situation-specific decontamination procedures. This section discusses the results of individual decontamination procedures and, when possible, explores the ultimate fate of the spores and decontamination worker exposure due to those procedures.

### 3.1 Surface Sampling Results – Positive Controls

#### 3.1.1 Task I

Most standard or widely used laboratory methods to test the efficacy of decontamination products rely on the contamination of carriers (i.e., uniform pieces of materials, also referred to as coupons) with the target organism using a liquid suspension.[15, 16, 17] Such methods offer the ability to precisely contaminate the material in order to maintain intra- and inter-test consistency. While there are substantial benefits to using liquid inoculation-based test methods in the laboratory measure of efficacy, questions remain as to the representativeness of the results with respect to use in the field on materials contaminated with aerosolized biological agent. Lee et al.[18] describe the development of a novel method to precisely deposit aerosolized spores onto materials at a target loading consistent with that used in liquid inoculation-based methods, i.e., allowing the determination of at least a six-log reduction due to the decontamination process. The method developed in that study was the predecessor of the methods used in the current effort.

The method reported by Lee et al. [18] was modified to be used on the larger coupons required for the current study. The target loading, based upon recovery from the positive controls, was $1 \times 10^6$ spores per coupon with a relative standard deviation (RSD) of 50 percent. The sampling methods used for each material were based on the results of the above-mentioned preliminary comparison test, along with consultation with the Project Team.

Surface sampling results from the positive control coupons of each material demonstrate the ability of the deposition and sampling methods to meet the target criteria. Results shown in Table 3-1 confirm approximately a 6-log recovery (on average) of viable spores from the material surfaces of the positive controls.

**Table 3-1. CFU Abundance and RSD for Positive Controls**

| Decontamination Procedure* | Stainless Steel Control | | Concrete | | Wood | |
|---|---|---|---|---|---|---|
| | CFU/ft$^2$ | RSD | CFU/ft$^2$ | RSD | CFU/ft$^2$ | RSD |
| A= Test 1 & 2 | 1.34E+07 | 27.03% | 1.71E+06 | 40.35% | 3.28E+06 | 47.45% |
| B= Test 3 & 4 | 2.02E+07 | 24.33% | 2.24E+06 | 28.09% | 2.92E+06 | 55.45% |
| C= Test 5 & 6 | 2.87E+07 | 20.95% | 1.93E+06 | 44.63% | 3.87E+06 | 71.33% |
| D= Test 7 & 8 | 1.64E+07 | 17.64% | 2.15E+06 | 53.90% | 4.93E+06 | 44.66% |
| E= Test 9 & 10 | 1.79E+07 | 3.20% | 1.07E+06 | 37.86% | 1.07E+06 | 29.72% |
| | | | | | | |
| Average | 1.93E+07 | 18.63% | 1.82E+06 | 40.96% | 3.28E+06 | 49.72% |

* See Table 2-1 for additional details.

A = pH-AB applied with backpack sprayer (30 minute exposure).

B = pH-AB applied with chemical sprayer (30 minute exposure).

C = Spor-Klenz® applied with backpack sprayer (15 minute exposure).

D = Spor-Klenz® applied with pressure washer (30/34 minute exposure).

E = pH-AB applied with backpack sprayer (15 minute exposure).

Three stainless steel coupons were incorporated into each test as control coupons indicative of the deposition method. The smooth surface of stainless steel allows for optimal recovery of viable spores. Thus, the number of recovered CFU is expected to be higher than from the sample materials. During the inoculation procedure of each Task I test, one stainless steel coupon was loaded with spores before any other coupons; one in the middle of the inoculation series; and one at the end. Thus, these inoculation control coupons could be used to verify the consistency of the spore dispersion apparatus.

The average spore recovery from the positive controls of each material typically fell within 1 log of the stainless steel controls (Figure 3-1). As mentioned above, sampling from the rough, heterogeneous surfaces of concrete and wood was expected to yield lower, more variable CFU than sampling from stainless steel. Recovery from concrete was lower than recovery from wood. During the wipe sampling of concrete, fine particles were present on the sampling surfaces prior to sampling, despite power-washing the coupons prior to sterilization. During sampling, fine particles on the surface of the coupon would cluster together, forming larger masses that would stay behind on the surface of the coupon, presumably with an unknown quantity of the target organism.

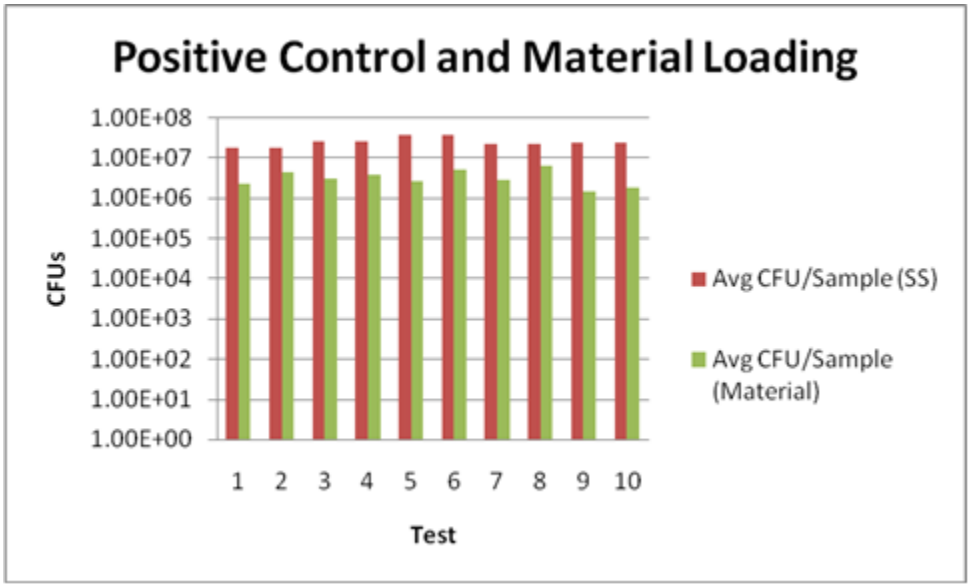


**Figure 3-1. Positive Control and Material Coupon Loading for Task I**

The variation in positive control CFU for each material (Table 3-1) was higher than the anticipated 50 percent for some tests. For the wood coupons in Procedure B and C (Tests 4 and 6), the outliers were higher than the averagepossibly because of natural variations in the coupon surface: a few coupons were smoother than normal and offered superior recovery from wipe samples. For the concrete coupons in Procedure D (Test 7), the outlier was lower than anticipated.

3.1.2 Task II

For Task II, Areas 1, 3, 7, and 9 for positive control determination of the inoculated coupons were sampled immediately prior to the decontamination procedure. The CFU recovered from these sampled areas would be compared to the CFU enumeration recovered from different areas of the same inoculated coupon after the decontamination procedure was complete. This procedure is very similar to the efficacy method that would be used in a field event. There were duplicate coupons for each material. The positive control results are shown in Table 3-2, below.

The positive controls for concrete vertical coupon, replicate A (CVA) in Test C1 were lower than anticipated, but still high enough to provide a potential 6-log reduction. CVA may have had a lower inoculation concentration based on irregularities during inoculation.

**Table 3-2. Task II Positive Controls**

| | Test C1* | | Test C2* | |
|---|---|---|---|---|
| **Material**** | **Avg. CFU/ft$^2$** | **RSD (%)** | **Avg. CFU/ft$^2$** | **RSD (%)** |
| Stainless Steel | 1.49E+07 | 27.3% | 4.51+06 | 19.2% |
| Concrete (CVA) | 5.52E+05 | 48.1% | 3.26+06 | 27.6% |
| Concrete (CVB) | 2.98+06 | 23.1% | 5.29+06 | 31.3% |
| Wood (TWA) | 2.51+06 | 35.7% | 1.96+06 | 21.1% |
| Wood (TWB) | 2.34+06 | 16.8% | 2.39+06 | 20.6% |

* Test C1 = pH-AB applied with backpack sprayer (30 minute exposure) with DI water rinse.
Test C2 = pH-AB applied with backpack sprayer (30 minute exposure); no DI water rinse.

** CVA and CVB, and TWA and TWB, are designations for the replicate coupons.

## 3.2 Task I: Evaluating Decontamination Procedures

### 3.2.1 Surface Sampling Results

To determine the most effective decontamination procedure and to determine which parameters were necessary to achieve desired results, several individual procedures were tested within the test matrix to determine their effect on overall efficacy. Several parameters were modified: application method, spray time, contact time, and overall exposure. Several novel approaches were used in the current study to provide a more directly visible tie of laboratory efficacy testing to field application of decontamination methods (e.g., use of aerosol deposition of biological agent instead of a liquid inoculation, use of field sampling methods instead of coupon extraction methods, and use of large coupons). This section details the results with conclusions that can be drawn from tests completed in this study.

The conditions for each Task I test are shown in Table 3-3. Most tests performed during this task achieved the target log reduction of greater than 6 LR. Figure 3-2 shows the efficacy in terms of log reduction (LR) of the decontamination technique averaged for all material surfaces for each test.

**Table 3-3. Conditions for each Task 1 Test**

| Test | Material | Application | Decon | Spray Time (sec) | Reapplication Time (min) | Number of Sprays | Contact Time (min) | Rinse Method | *LR*‡ |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| 1 | Concrete | Backpack Sprayer | pH-AB | 30 | 15 | 2 | 30 | Garden hose | 6.54 |
| 2 | Wood | Backpack Sprayer | pH-AB | 30 | 15 | 2 | 30 | Garden hose | 6.77 |
| 3 | Concrete | Chemical Sprayer | pH-AB | 15 | 15 | 2 | 30 | Power washer | 6.60 |
| 4 | Wood | Chemical Sprayer | pH-AB | 15 | 15 | 2 | 30 | Power washer | 6.74 |
| 5 | Concrete | Backpack Sprayer | Spor-Klenz® | 30 | 15 | 2 | 15 | Garden hose | 1.63 |
| 6 | Wood | Backpack Sprayer | Spor-klenz® | 30 | 15 | 2 | 15 | Garden hose | 6.80 |
| 7 | Concrete | Power Washer | Spor-Klenz® | 15 | 15 | 2 | 30 | Power Washer | 2.80 |
| 8 | Wood | Power Washer | Spor-Klenz® | 15 | n/a | 1 | 34 | Garden Hose | 6.99 |
| 9 | Concrete | Backpack Sprayer | pH-AB | 10 | n/a | 1 | 15 | Garden Hose | 6.30 |
| 10 | Wood | Backpack Sprayer | pH-AB | 10 | n/a | 1 | 23 | Garden Hose | 4.04 |

‡LR values represent surface log reduction only.

The decontamination by means of pH-adjusted bleach was accomplished by a combination of removal and inactivation of spores. Viable spores were found in both rinsate and Via-Cell® air samples (discussed below). Of the procedures tested, those incorporating pH-adjusted bleach (Tests 1-4, 9-10) were typically most effective (> 6 log reduction) for decontamination. The lower log reduction in Test 10 may be a result of material demand in conjunction with a single application; one spray application may not provide enough pH-adjusted bleach to overcome the demand of wood. The surface log reductions for tests utilizing Spor-Klenz® (Tests 5-8) were comparable to those with pH-adjusted bleach on treated wood (Tests 6,8), but significantly lower on tests involving concrete (Tests 5,7). Reduced efficacy of peroxide-based decontaminants on concrete is consistent with results from previous studies,[19] and suggests that this material may catalyze the destruction of peroxide. Interestingly, efficacy of Spor-Klenz® on wood (Test 6) was not negatively affected by the inadvertent rinse (and therefore reduced contact time) following the second spray application. These results suggest Spor-Klenz® is highly efficacious on wood and is consistent with previous studies.[19]

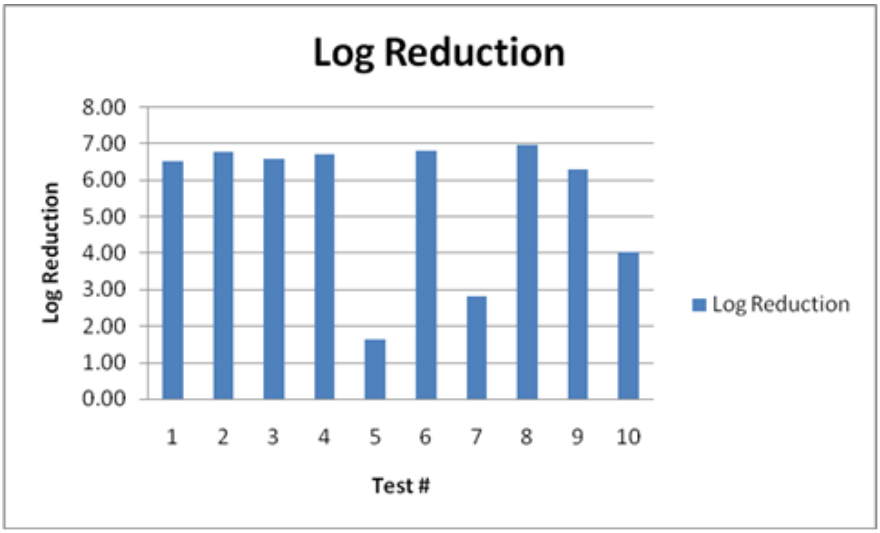


**Figure 3-2. Material Surface Log Reduction for each Test Conducted**

The stability of all concrete coupon samples was called into question when a sample that yielded 'too numerous to count' (TNTC) originally failed to produce any viable CFU six weeks later. The PBST extraction buffer was not strong enough to neutralize the alkalinity of the concrete wipe sample; the extracted sample had a pH of 12 (following the 6-week storage at 4 °C). The pH of the sample matrix may have inactivated the spores over this time period. This result has minimal effect upon study results as most samples were processed within hours of collection. Further, decontamination efficacy was calculated based upon control samples, so antimicrobial activities post-sample collection would have equally impacted recovery from positive controls and thus have little impact on overall efficacy. Another complication arising from samples collected from concrete was that debris from these wipe samples clogged the 200 µL pipette tips used for dilution plating. These tips were graduated to allow for a visual check that the micropipette dispensed the correct volume. While tips with a larger orifice did allow passage of concrete debris, they did not possess graduations and therefore did not provide the same quality assurance during plating.

### 3.2.2 Evaluation of the pH-Adjusted Bleach Application Procedure

For Tests 1-4 and 9-10, pH-adjusted bleach was the sporicidal agent of choice, due in part not only to the previously demonstrated efficacy of this commonly-available solution, but also to the evident incompatibility of Spor-Klenz® with the selected equipment. To optimize the efficacy of low-tech decontamination procedures, several parameters were varied during the course of testing.

- **Tests 1 & 2:** Apply one 30-sec pH-AB spray with backpack sprayer, repeat 30-sec spray after 15 minutes, and then rinse with DI water using a garden hose after 15 minutes (30 minute contact time).

- **Tests 3 & 4:** Apply one 15-sec pH-AB spray with chemical sprayer, repeat 15-sec spray after 15 minutes, and then rinse with DI water using a pressure washer after 15 minutes (30 minute contact time).

- **Tests 9 & 10:** Apply one 10-sec pH-AB spray with backpack sprayer and then rinse

with DI water using a garden hose after 15 minutes (15 minute contact time).

Figure 3-3 shows the LR from the material coupon surface during decontamination in comparison to the number of spores collected in the rinsate (note: bars with values preceded by "<" are detection limit values). The single application (in addition to a shorter spray duration) resulted in a lower total efficacy rate due to the presence of active spores in the rinsate. For tests with 30 minute contact time, the overall surface spore removal was very consistent across both spray durations and material types. The shorter spray duration yielded a higher number of viable spores in the rinsate. This higher number of spores in the rinsate along with the lower total efficacy for a single application of pH-adjusted bleach suggests that a single application would not be as effective or useful for decontamination as two short applications. The effectiveness of the chemical sprayer is consistent with the backpack sprayer used in the remainder of the pH-adjusted bleach tests. Direct comparison of these two methods is complicated by a shorter spray duration used in the chemical sprayer tests. The flow rate for the backpack sprayer is approximately 0.017 L/sec. A 30-second spray dispenses 0.51 L of liquid onto the coupon surface. The flow rate for the chemical sprayer is approximately 0.185 L/sec. A 15-second spray dispenses 2.75 L of liquid onto the coupon surface. These results suggest that smaller amounts of pH-adjusted bleach solution can be just as effective as much larger amounts. Application of decontaminant with both the backpack sprayer and the chemical sprayer resulted in compete wetting of the coupon surface. The increased volume of decontaminant applied with the chemical sprayer likely only increased runoff from the coupon surface and not exposure of spores to decontaminant.

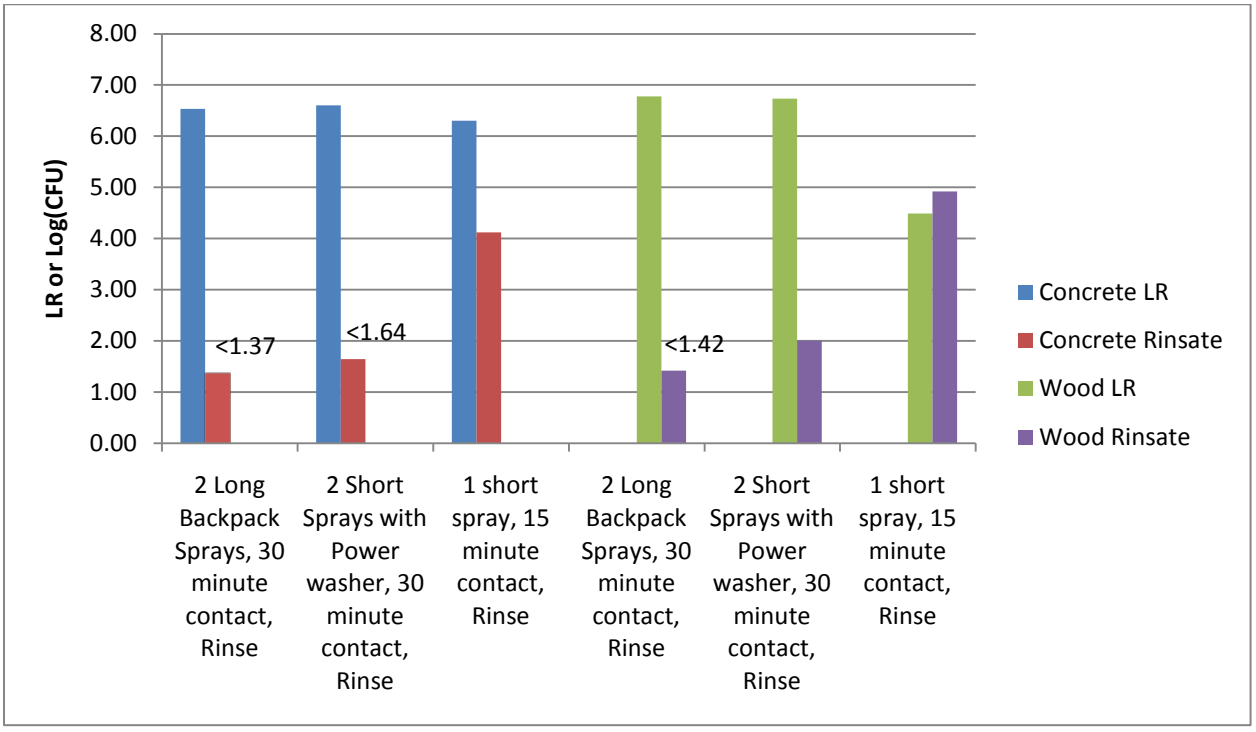


**Figure 3-3. Efficacy of pH-Adjusted Bleach Tests.**

### 3.2.3 Evaluation of the Spor-Klenz® Application Procedure

Tests 5-8 utilized Spor-Klenz® as the sporicidal agent. To optimize the efficacy of this decontamination method, several parameters were modified during the course of testing.

- **Tests 5 & 6 :** Using a backpack sprayer, apply one 30-sec Spor-Klenz® spray, repeat 30-sec spray after 15 minutes. Coupons were inadvertently rinsed with DI water immediately after the second spray, resulting in a contact time of 15 minutes.

- **Tests 7 & 8:** Using a pressure washer, apply one 15-sec Spor-Klenz® spray, repeat 15-sec spray after 15 minutes, and then rinse with DI water after 15 minutes. (Actual parameters varied; see discussion below)

Figure 3-4 shows that Spor-Klenz® was much more effective as a sporicidal agent on wood coupons than on concrete coupons (note: bars with values preceded by "<" are detection limit values). The lack of viable spores in the Spor-Klenz® concrete test rinsate (values in Figure 3-4 are detection limit values) could indicate that the amount of sodium thiosulfate (STS) used to neutralize the Spor-Klenz® was not adequate; the neutralization equivalents used were those of other researchers (USEPA Evaluation Report[19]) and the Spor-Klenz® activity in the rinsate was not independently verified. If the Spor-Klenz® was not sufficiently neutralized, the spores may have continued to be inactivated after the DI rinse until the samples were analyzed by the Microbiology Laboratory. Another plausible explanation is that despite the low decontamination efficacy on concrete coupon surfaces, few viable spores were relocated to the rinsate fraction.

The parameters for Test 8 (pressure washer on wood coupons) were not met as there were unforeseen malfunctions with the pressure washer (apparent vapor lock). Test 8 received only one contact time with Spor-Klenz® and was rinsed with DI water after a 34-minute total exposure time. Overall, Spor-Klenz® seems to be as effective as pH-AB on treated wood but less effective on concrete. Further testing would be necessary to determine its relative effectiveness on other commonly tested materials.

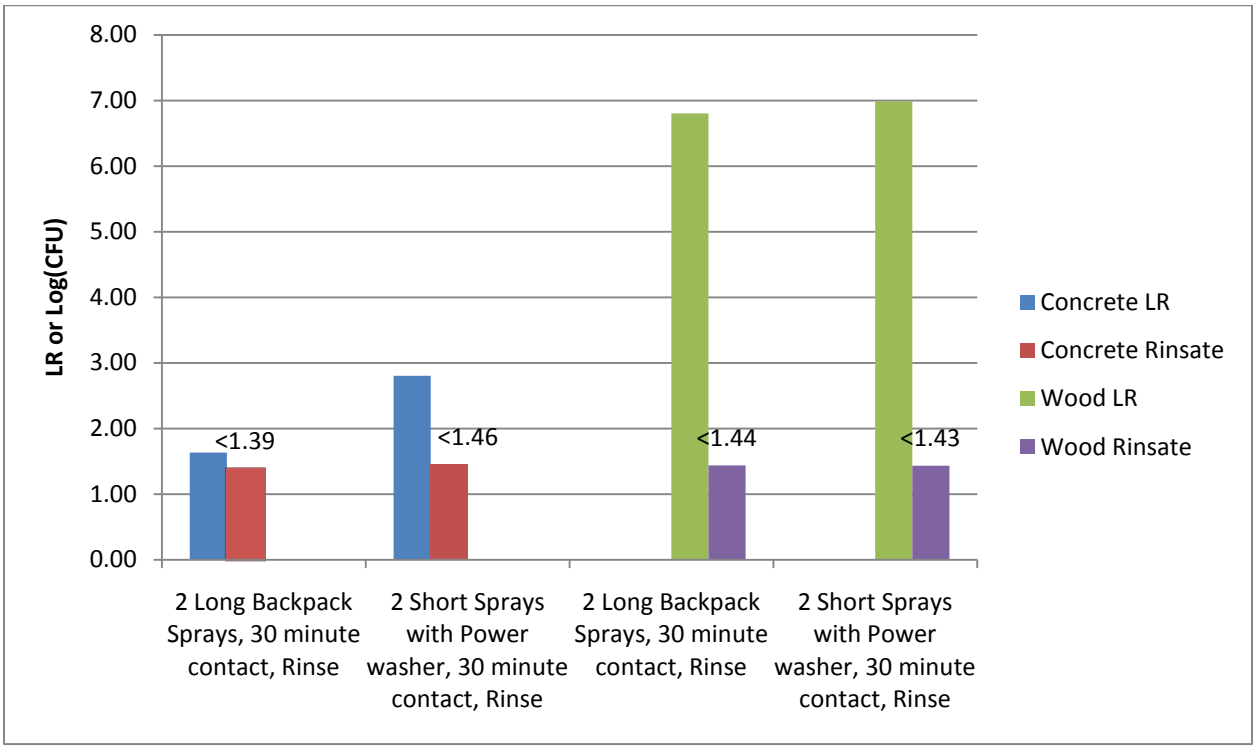


**Figure 3-4. Efficacy of Spor-Klenz® Tests**

### 3.2.4 Ultimate Fate of Viable Spores

An overall assessment of the decontamination procedural steps considers not only the viable spores recovered from the surface of the materials, but also those dislodged from the coupon either through re-aerosolization (as sampled by the Via-Cell ®) or into the rinsate.

#### *3.2.4.1 Aerosol Samples (Via-Cell®) – Task I*

The chamber used for Task 1 decontamination was designed for maximum air flow in order to protect laboratory workers from the hazardous fumes emitted by the decontamination procedure. The aerosol sampling strategy initiallycalled for sampling at a height and distance away from the coupons typical of the breathing zone of a decontamination worker. These bioaerosol sample data are reported as CFU per liter of air sampled, or roughly CFU per two breaths of air. The data are shown in Table 3-4.

**Table 3-4. Bioaerosol Levels**

| Test ID | CFU/L in Aerosol | Sprayer Type |
|---|---|---|
| 1 | 11.8 | Backpack Sprayer |
| 2 | 5.41 | Backpack Sprayer |
| 3 | 70.5 | Chemical Sprayer |
| 4 | 48.3 | Chemical Sprayer |
| 5 | 46.0 | Backpack Sprayer |
| 6 | 6.37 | Backpack Sprayer |
| 7 | 2.01 | Power Washer |
| 8 | 6.71 | Power Washer |
| 9 | 391 | Backpack Sprayer |
| 10 | 386 | Backpack Sprayer |

These samples were collected only during the active spraying and are the maximum expected concentrations in the test chamber. These concentrations should not be viewed as a maximum possible exposure, because it is anticipated that the high rate of air exchange in the chamber removed many of the spores upon resuspension. The time interval over which these concentrations might have been sustained is also unknown. In a real-world area with less air exchange, the concentrations experienced could be much higher.

There was concern during testing that the bioaerosol sample towards the front of the chamber may not be representative of concentrations throughout the chamber due to high flow rates from the exhaust duct at the rear. For Tests 7 and 8, a Via-Cell® cartridge was placed in the duct to monitor the spores exiting the chamber. These, combined with the total flow rate of the duct, could provide a total number of CFU re-aerosolized. These samples were not part of the original sampling strategy, and there were numerous difficulties due to the design of both the Via-Cell® cartridges and the sampling location. The volume of air sampled is not directly known due to the failure of an engineering control, but can be estimated by the sample flow rate and the sample time. These estimations suggest a concentration of nearly $7 \times 10^4$ CFU/L could be re-aerosolized. When extrapolated from the short duration of the sample collection, this concentration represents approximately $1 \times 10^8$ total CFU re-aerosolized from the coupon surfaces. Again, because real-time data were not collected, the duration of time over which these concentrations might be sustained is unknown, or what the total number (or fraction) of spores re-aerosolized might be. The data do show that re-aerosolization of viable spores can be expected during the decontamination process. This re-aerosolization has a broad impact on the efficacy of *in-situ* decontamination. Table 3-4 shows that in Tests 9 and10, at least one order of magnitude higher total number CFU re-aerosolized were present as compared to the rest of the tests. This result parallels the higher number of spores left in the rinsate following a single application of pH-adjusted bleach (see Fig. 3-3). These tests employed only one application with pH-adjusted bleach coupled with a shorter contact time. Re-

aerosolized spores could settle on previously decontaminated surfaces and thus complicate remediation efforts. The purpose of the collection of the bioaerosol samples was to determine if the potential exists for re-aerosolization during decontamination application procedures and not to assess exposure quantitatively.

*3.2.4.2 Rinsate – Task I*

For most Task 1 testing, the number of CFU recovered in the rinsate was below the detection limit and is shown in Table 3-5, below. However, for Tests 9 and 10, a large number of viable spores were physically removed from the surface during the decontamination and rinse steps; these spores could potentially re-contaminate treated surfaces if not properly collected and inactivated. When no viable spores were detected, a value of 0.5 CFU was assigned as the detection limit of the plated amount, and the CFUs were reported as less than the detection limit.

**Table 3-5. Rinsate Sample CFUs**

| Rinsate | |
|---|---|
| **Test #** | Total CFUs |
| | |
| 1 | <24 |
| 2 | <26 |
| 3 | <44 |
| 4 | 100 |
| 5 | <25 |
| 6 | <27 |
| 7 | <29 |
| 8 | <27 |
| 9 | 13000 |
| 10 | 83000 |

Table 3-5 shows that approximately 8 x $10^5$ CFU were present in the rinsate from Tests 9-10. These tests employed only one application with pH-adjusted bleach. These coupons had less contact time with pH-adjusted bleach, which resulted in less chemical inactivation. More viable spores on the coupon at the time of rinsing led to a higher number of viable spores in the rinsate.

## 3.3 Task II Results

3.3.1 Surface Sampling Results – Test Coupons

Based on the Task I results, the decontamination procedure that was most effective was developed for further testing in Task II: the use of pH-adjusted bleach by backpack sprayer, sprayed on either concrete or wood and rinsed with a garden hose. In addition, in an effort to shorten the required time for facility decontamination, a

second test in Task II was conducted, similar to the first test except the rinse step was omitted. Both procedures used two 30-second spray times every 15 minutes, for a total of 30 minutes exposure per application. Again, Procedure 1 included a rinse step, and Procedure 2 did not include this step. The results are shown in Figure 3-5. The results indicate that the two decontamination approaches were equivalent in decontaminating the two types of materials. The results also suggest that a rinse step is not needed for these decontamination procedures to be effective on concrete and wood. However, if applications were to be made to surface materials sensitive to bleach (e.g., stainless steel), rinsing might be desirable. On surfaces and materials where corrosion is not a concern, elimination of the rinse step could streamline the decontamination process and significantly reduce the amount of contaminated wastewater generated. At facilities with minimal ventilation, a rinse step may be necessary to reduce chlorine off-gassing after decontamination.

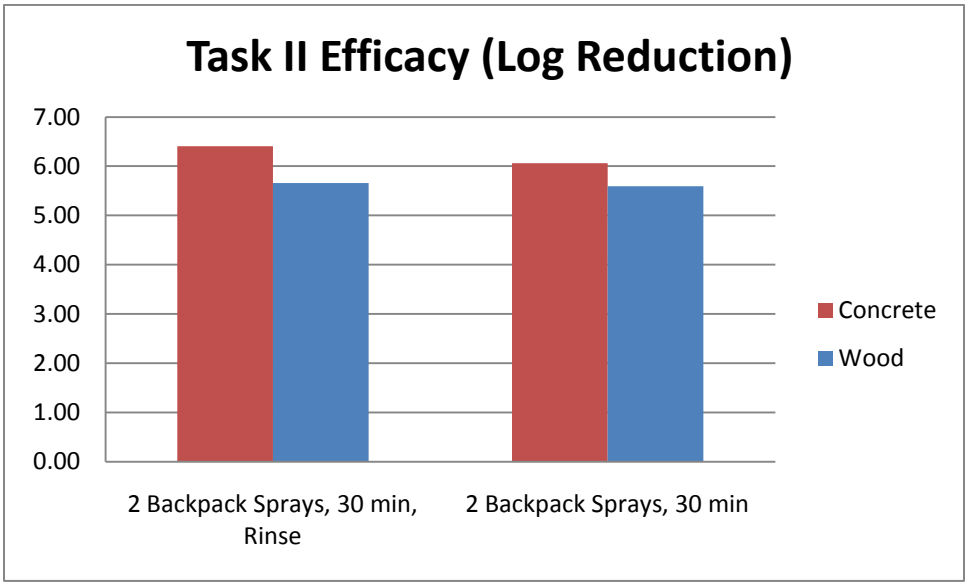


**Figure 3-5. Efficacy of Task II Decontamination Procedures**

### 3.3.2 Ultimate Fate of Viable Spores

#### 3.3.2.1 Aerosol Samples (Via-Cell®) – Task II

The Task II Bioaerosol sample results (Figure 3-6) show some ambiguity. Test C2 suggests that spores were dislodged during the first decontamination step and were constantly removed (due to air exchange) following that release. During Test C1, however, a single aerosol sample was two orders of magnitude above samples taken two minutes before and two minutes after. Given that no intentional activity was done that could have initiated the spike, this high spike in CFU in such a short amount of time may be the result of cross contamination.

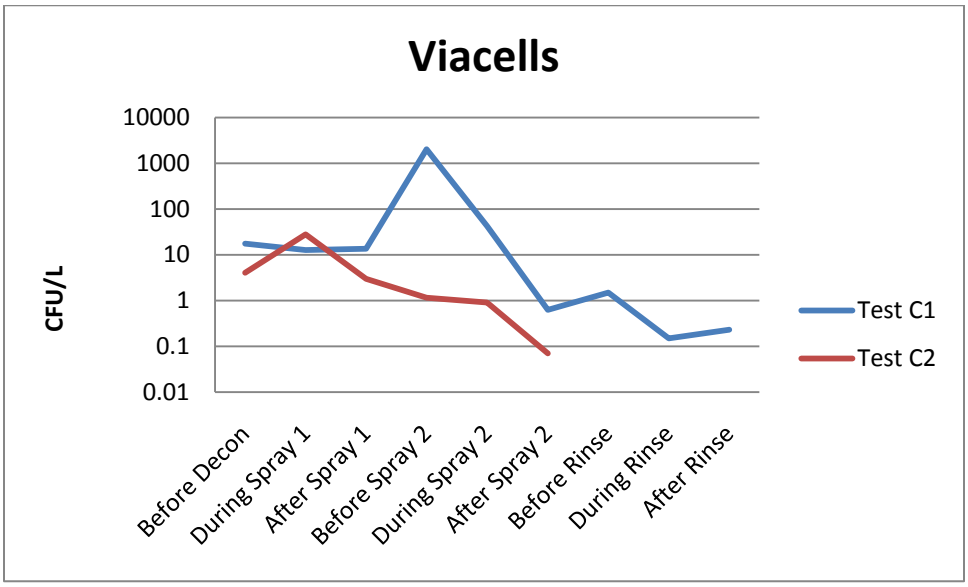


**Figure 3-6. Bioaerosol Levels during Task II**

Discarding this outlier, the aerosol data trend downward as the decontamination progresses, beginning before the decontamination steps were started. Thus, not only is cross-contamination likely, but the original presence of the spores is due to either control sampling or re-aerosolization of ambient spores in the COMMANDER chamber. This result prevented decoupling of airborne spore concentration from environmental conditions such as air exchange rate and the decontamination procedure itself. Hence, unlike in Task I where ambient airborne spore concentration can be shown to increase due to decontamination steps, the data from Task II neither supports nor refutes this proposition due to the complex activity inside COMMANDER before and during the decontamination.

The Task II aerosol sample results shown in Figure 3-6 represent around 9 x $10^4$ re-aerosolized spores during Test C2, demonstrating that airborne spores can be expected in a field decontamination event. The air exchange rate in COMMANDER is higher than could be anticipated in a typical indoor environment, and so could be seen as a best case scenario (i.e., expect higher airborne spore concentrations in a typical indoor environment with a lower air exchange rate). During decontamination, re-aerosolized spores could be expected to move into and through the HVAC system (if operating) during decontamination, thereby spreading contamination to other areas of the facility. In an outdoor environment, or in an indoor facility typical of FAD operations with higher airflow, these airborne contaminants could be removed during decontamination and perhaps contaminate areas adjacent to the initial contamination zone or primary contaminated facility.

#### 3.3.2.2 Rinsate – Task II

For Task II, the rinsate collection troughs were immediately contaminated once brought inside the COMMANDER chamber. The fact that the contamination rate seems systematically higher for concrete coupons than wood coupons suggests that the contamination is coming from the coupons themselves and not from the common environment. The loose material from the concrete coupons might have dropped into the trough while it was being placed under the coupon. While this complicates interpretation of the data, the CFU counts after the decontamination procedure were higher for Test

C1, suggesting that active spores were transferred to the rinsate (Table 3-6). No CFU were detected following the decontamination procedure in Test C2. Failure to detect any CFU seemed unlikely given the presence of spores before the decontamination began. Perhaps excess STS may have caused inhibition of spore outgrowth during cultivation of sample extracts. For Test C1, the STS was quite dilute (due to the presence of the rinse water), so less than 1 percent of the total amount used was present on the filter. For Test C2, STS represented 40 percent of the total rinsate.

**Table 3-6. CFU recovered from Task II Rinsate**

| Coupon | Test C1 | | Test C2 | |
|---|---|---|---|---|
| | Rinsate before Decon | Rinsate after Decon | Rinsate before Decon | Rinsate after Decon |
| CVA | 2.30E+04 | 1.30E+05 | 4.93E+03 | <105 |
| CVB | 2.00E+04 | 2.84E+05 | 1.87E+03 | <398 |
| TWA | 3.30E+03 | 1.51E+05 | 1.00E+03 | <75 |
| TWB | 3.33E+03 | 1.41E+05 | 3.73E+02 | <205 |

## 3.4 Assessment of Operational Parameters

### 3.4.1 Time

The time required to decontaminate a batch of coupons depended on the decontamination procedure being applied. Experience using the backpack sprayer decontamination procedure in Task II suggested that 350 sq ft can be decontaminated by one person before a second application would be needed, which works out to 700 sq ft/hour. The rinse step could be performed quickly at the end of a 4-hour shift, suggesting that 2000-2500 sq ft could be decontaminated per worker per 4-hour shift. Such an application would require approximately 18 gallons/hour of sporicide. This volume would require that the backpack sprayer (5-gallon capacity) be refilled every 15 minutes. Due to safety concerns with fatigue while wearing a NFSA Class C suit, cooling vests may be necessary to sustain a 4-hour shift, especially in hot weather.

### 3.4.2 Physical Impacts on Materials

Treated wood and concrete showed no signs of physical changes after being decontaminated. Spor-Klenz® was incompatible with the commercial off-the shelf pressure washer due probably to its low pH. The apparent vapor lock on the day of decontamination was probably an effect of corrosion, as seen on the nozzle the following morning (see Figure 3-7).

**Figure 3-7. Corrosion on Pressure Washer Nozzle from Contact withSpor-Klenz®**

3.4.3 Impact on Decontamination Workers

For the Task I study, the actual decontamination procedure was moderately intensive with minor discomfort at certain points in the procedure. The procedure included standing in an upright position while decontaminating materials.. Most individual tasks were ergonomic in nature.

Task II tests were performed inside COMMANDER in a completely different environment than Task I tests. Since COMMANDER is an enclosed space in which chlorine levels are a safety hazard to any member of the remediation crew, level B HAZMAT suits were required for any decontamination event in COMMANDER. Supplied air respirators were used and 5 min escape bottles were carried by personnel inside the chamber. The supplied air was fed to the respirators using air lines mounted inside COMMANDER. The backpack sprayers were left on the floor and the remediation crew simply sprayed the coupons from a stationary position. Space is limited inside COMMANDER, causing otherwise simple tasks to require coordination between team members. Although temperatures approximated normal room temperature inside COMMANDER, heat stress was a potential factor while doing work wearing level C suits, so cooling vests were worn inside the suits. At the end of a 2 hr decontamination cycle (including wipe sampling upon entry), the crew was very fatigued and the ice packs in the cooling vests had often melted. For a member of an actual field crew, there would be the added weight of a supplied air cylinder and the need to carry the backpack sprayer from position to position. In addition, if a supplied air cylinder is being worn on the back, it would probably be difficult to wear the backpack sprayer correctly. Wearing a supplied air cylinder would necessitate constant lifting of the sprayer and all of the health and safety risks that are inherent in such actions.

## 3.5 Summary of Results

Most tests performed during Task I achieved the target efficacy from surfaces of greater than 6 LR, a widely accepted standard for demonstrating

sporicidal efficacy (e.g., 1 LR would be a reduction of 10, 2 LR would be a reduction of 100, 6 LR would be a reduction of 1 million, etc.). The decontamination by means of pH-adjusted bleach was accomplished by a combination of removal and inactivation of spores. Viable spores were found in both the rinsate and bioaerosol samples. Of the procedures tested, those incorporating pH-adjusted bleach were more effective for decontamination on concrete and wood than Spor-Klenz®. The lower LR (4 LR) seen in one test with wood may have been the result of material demand (i.e., reduction in activity of the decontaminant though reaction with the test material) in conjunction with a single application of the pH-adjusted bleach. One spray application does not appear to provide enough pH-adjusted bleach to overcome the demand of wood. The surface LRs for tests utilizing Spor-Klenz® were comparable to those with pH-adjusted bleach on treated wood, but significantly lower on tests involving concrete (< 3 LR).

Based on the Task I results, the most effective decontamination procedures were developed for further testing in Task II: the use of pH-adjusted bleach by backpack sprayer, sprayed on either concrete or wood, and rinsed or not rinsed. These procedures all used two 30-second spray times every 15 minutes, for a total of 30 minutes spray exposure per application. Procedure 1 included a rinse step, and Procedure 2 did not include this step. The results indicate that the 2 decontamination approaches were equivalent in decontaminating the two types of materials. The results also suggest that rinsing is not needed for these decontamination procedures to be effective on concrete and wood. However, if applications were to be made to surface materials sensitive to bleach (e.g., stainless steel), rinsing might be desirable from that standpoint as bleach and other aggressive oxidants are known to cause corrosion of numerous surfaces. LRs were approximately 6 for concrete and just under 6 for wood.

The overall fate of the biological spores was assessed, not only for the viable spores recovered from the surface of the materials, but also fugitive viable agent escaping in the rinsate and aerosol fractions. Aerosol samples collected using bioaerosol filter cassettes during testing with the “medium-sized” coupons show that re-aerosolization of viable spores can be expected during the decontamination process. Although one test with the “large-sized” coupons suggests that spores were dislodged during the first decontamination step and were constantly removed from the chamber (due to air exchange) following that release, further evaluation of the data indicates that there was probably cross-contamination and re-aerosolization of ambient spores in the chamber. However, the data do indicate that spores can be expected to be re-aerosolized in a field decontamination event and could be expected to travel through the HVAC system (if operating) during decontamination and potentially spread contamination throughout a facility.

For most of the “medium-sized” coupon testing, the number of CFU recovered in the rinsate was below the detection limit. However, in the tests where only one short application of pH-AB was used, a large number of viable spores were physically removed from the surface during the decontamination and rinse steps. Such rinsate would potentially cause contamination to spread if not properly collected and treated.

The collection troughs for the “large-sized” coupon rinsate were immediately contaminated once brought inside the test chamber during test set-up. However, the rinsate contamination was systematically higher for the concrete coupons over the wood coupons. The contamination may be coming from the coupons themselves. The loose material from the concrete coupons might have dropped into the trough while it was being placed under the coupon. Despite the occurrence of viable spores in the troughs prior to testing, the data suggest that active spores were transferred

to the rinsate as viable spore abundance in these samples increased by approximately 1 x $10^5$ following the decontamination procedure that utilized a rinse step.

The major findings from this study are as follows:

- pH-Adjusted bleach was highly effective (approximately 6 LR) on wood and concrete when used with a thirty-minute contact time and two applications.
- Spor-Klenz® was more effective on wood than on concrete.
- For concrete coupons, pH-adjusted bleach was more efficacious than Spor-Klenz®.
- Reduction of the number of pH-adjusted bleach applications and contact time resulted in lower decontamination efficacy for surfaces and greater amounts of spores detected in rinsate and aerosol samples.
- Decontamination efficacy was similar between the two evaluated application devices (backpack sprayer and pressurized sprayer) despite significant differences in volume of decontaminant delivered to the coupon surface.
- Viable biological agent was detected in aerosol and rinsate (runoff) samples during all tests, and can therefore be a significant source of cross-contamination during a remediation.
- Elimination of a rinse step from the decontamination procedure did not reduce surface decontamination efficacy and may be a viable option on non-corrosive materials.
- Worker fatigue may be of concern in an actual remediation as heat and exhaustion were experienced by laboratory workers when conducting scale-up tests that required level C personal protective equipment.

## 5. Quality Assurance and Quality Control

This project was performed under an approved Category III Quality Assurance Project Plan titled *Effectiveness of Physical and Chemical Cleaning and Disinfection Methods for Removing, Reducing or Inactivating Agricultural Biological Threat Agents (DCMD 3.41B) (August 2010)*.[5]

### 5.1 Calibration of Sampling/Monitoring Equipment

There were standard operating procedures for the maintenance and calibration of all laboratory and Microbiology Laboratory equipment. All equipment was verified as being certified calibrated or having the calibration validated by EPA's Air Pollution Prevention and Control Division (APPCD) on-site (RTP, NC) Metrology Laboratory at the time of use. Standard laboratory equipment such as balances, pH meters, biological safety cabinets and incubators were routinely monitored for proper performance. Calibration of instruments was done at the frequency shown in Tables 4-1 and 4-2. Any deficiencies were noted. The instrument was adjusted to meet calibration tolerances and recalibrated within 24 hours. If tolerances were not met after recalibration, additional corrective action was taken, possibly including, recalibration or/and replacement of the equipment.

**Table 4-1. Laboratory Instrument Calibration Frequency**

| Equipment | Calibration/Certification | Expected Tolerance |
|---|---|---|
| Thermometer | Compare to independent NIST thermometer ( a thermometer that is recertified annually by either NIST or an International Organization for Standardization (ISO)-17025 facility) value once per quarter | ±1°C |
| pH meter | Perform a single point calibration with standard buffers daily. | ± 0.1 pH units |
| Stopwatch | Compare against NIST Official U.S. time at (http://nist.time.gov/timezone.cgi?Eastern/d/-5/java) monthly. | ±1second/min |
| Clock | Compare to office U.S. Time @ www.NIST.time.gov at the start of each test (before coupon loading). | ±1 min/30 days |
| Analytical balance | All analytical balances will be certified as calibrated at time of use. Balances are recalibrated by the Metrology Laboratory using standards. Evaluation of balance performance to manufacturer's specifications conducted yearly. | ±5% |
| Pressure Gauge | Compare to independent NIST Pressure gauge annually. | +2 psi |
| Sampling Pump Flow Rate | Compare to a NIST certified and calibrated soap bubble meter monthly | ± 1 Lpm |

**Table 4-2. Microbiology Laboratory Instrument Calibration Frequency**

| Equipment | Calibration/Certification | Expected Tolerance |
|---|---|---|
| Thermometer | Compare to independent 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 |
| Pipettes | All micropipettes will be certified as calibrated at time of use. Pipettes are recalibrated by gravimetric evaluation of pipette performance to manufacturer's specifications every twelve months by supplier (Rainin Instruments/Ovation) or credible calibration service. | ±5% |
| Analytical balance | All analytical balances will be certified as calibrated at time of use. Balances are recalibrated by the Metrology Laboratory using standards. Evaluation of balance performance to manufacturer's specifications conducted yearly. | ±5% |
| pH Meters | Perform a 2-point calibration with standard buffers that bracket the target pH daily. | ± 0.1 pH units |
| Clock | Compare to office U.S. Time @ www.NIST.time.gov at the start of each test (before coupon loading). | ±1 min/30 days |

### 5.2 Data Quality Indicator (DQI) Goals

Target acceptance criteria for the critical measurements are shown in Table 4-3 along with precision goals.

**Table 4-3. Acceptance criteria and test values for critical measurements**

| Measurement Parameter | Target Value | Test Value |
|---|---|---|
| Free Available Chlorine (FAC) in pH-adjusted bleach solution | 6000 – 6700 ppm | 6200-6800* |
| pH of pH-adjusted bleach solution | >6.5 pH <7.0 | 6.5-6.8 |
| Temperature of liquids | 18 – 28 °C | 10.7*-25.1 |
| Head pressure of rinse water | 55-65 psi | 60 |
| Pressure of backpack sprayer | 30-40 psi | 30-36 |
| Flow rate of backpack sprayer | 850-950 mL/min | 990*-1104* |
| Flow rate of pressure washer | 10 – 11 kg/min | 8.3*-8.7* |
| Positive control CFUs | $5 \times 10^6$ – $5 \times 10^7$ CFU per $ft^2$ | $2.0 \times 10^{6*}$ – $4.9 \times 10^6$ Task 1<br>$6.8 \times 10^{4*}$ – $6.5 \times 10^{5*}$ Task 2 |
| CFU abundance on dilution plate** | 30 – 300 CFU per plate | 19* – 296 CFU per plate |
| CFU abundance on filter plate | < 100 | 0-89 CFU |

* Outside the target range

** This requirement only for plates used for quantification; plates outside this range were not used for quantification.

5.2.1 Free Available Chlorine (FAC) Measurements

The Hach High Range Bleach Test Kit was used to titrate a standard solution of 1000 ppm $NaClO_2$. The Hach test kit returned a value within 10 percent of the standard. The pH-adjusted bleach FAC measurement was higher than the target value for Test 2 during decontamination of wood coupons due to a personnel oversight. The LR for Test 2 may have been slightly elevated in regards to the other tests. As there were spores detected for these samples, the overall effect of the slight elevation of FAC is not expected to be significant.

5.2.2 pH Measurements

The Oakton pH probe was calibrated with certified pH 7.0 buffer solution per manufacturer's instructions at the start of each test day. All the results were within the specified target range.

5.2.3 Temperature Measurements

The contamination prevention protocol required the deionized water reservoir to be filled the day of testing to minimize cross-contamination. Protocol for the daily filling of the deionized reservoir consisted of the following steps: the morning of testing, the reservoir was filled with a diluted bleach solution, let sit for one hour, emptied, triple-rinsed with DI water, then refilled with DI water for testing. Therefore, the water temperature was dependent on the room temperature, and measurements outside the target range were recorded. The temperature of the DI rinse water is expected to have minimal effect on project results, therefore was allowed to remain outside specification without corrective action.

5.2.4 Pressure Measurements

All pressure measurements were consistently within specification.

5.2.5 Flow Measurements

The target flow rates listed in the QAPP for the backpack sprayer were based on water; this study used a non-water solution. The sprayer was set to its lowest setting to provide a spray pattern of 16-in diameter from a distance of 3 ft. The same can be said for the pressure washer. In general, the flow rates were consistent between tests and did not affect the intra-test comparisons.

5.2.6 CFU Counts

Twenty-five percent of all plates containing significant growth (30-300 CFUs) were counted by a second person, and fell within 10 percent of the initial count. The positive control spore counts were in a few instances below the target counts; however, all were within an order of magnitude of the target count and were deemed acceptable by the EPA Work Assignment Manager (WAM). Further, in some instances, the CFU abundances on the dilution plates were taken as they were when their values were near the lower CFU acceptance criterion of 30.

**5.3 Data Quality Audit**

The ARCADIS QA Manager reviewed the final report and randomly selected portions of reported data to trace from the initial acquisition through reduction to final reporting to ensure the integrity of the reported results. Data from two tests from Task I were selected (Test 2 and Test 7) and one test from Task II (Test C2). For each of these tests the following documentation was reviewed: laboratory notebook entries, laboratory test reports, and data tables within the final report. Any discrepancies between reported results and raw data files were brought to the attention of the ARCADIS WAL and revised and/or corrected as appropriate.

**5.4 QA/QC Reporting**

QA/QC procedures were performed in accordance with the QAPP for this investigation.

**5.5 Amendments and Deviations from the Original QAPP**

5.5.1 Formal Amendments

During the course of the projects, some amendments were added to the QAPP by the EPA WAM in response to data results or equipment failures. These amendments, listed below, were submitted by e-mail to the EPA QA officer for formal approval.

Amendment 1 (10/12/2010)

Wipe Sampling Protocol (page 31, step 2b) amended to read, "The wipe will be moistened by adding 2.5 mL of sterile phosphate buffered saline with 0.005% TWEEN-20" (instead of 5 mL).

Amendment 2 (10/22/2010)

Table 4-5, Coupon Sample Coding, was replaced. The original table outlined a sample nomenclature that did not permit easy identification of control samples.

**Table 4-5: Coupon Sample Coding**

| **Coupon Identification: T-S-M-NN** | | |
|---|---|---|
| | **Code** | |
| T/C<br>(Test Number) | 1 – 10 | Test Number preceded with T for Task I(Table 3-2) and preceded with C for tests in Task II (Table 3-4) |
| S<br>(Sample Type) | P | Positive control wipe sample |
| | T | Test wipe sample |
| | PX | Procedural blank |
| | FX | Field Blank |
| | LX | Lab Blank |
| | R | Rinsate |
| | VS | Aerosol sample (Viacell Suspended in chamber) |
| | VD | Aerosol sample (Viacell in Duct) |
| | S | Swab sample |
| M<br>(Material) | CV | Concrete (vertical orientation) |
| | TW | Pressure Treated Wood (vertical orientation) |
| | SS | Stainless Steel |
| | DI | DI Water |
| | XX | Blank |
| NN<br>(Sample number) | ## | Replicate number or sample area number |
| **APPCD Microbiology Laboratory Plate Identification: T-S-M-NN-R-D** | | |
| T-S-M-NN | As above | |
| Replicate | R | A – C |
| Dilution | D | $1 \times 10^0$ - $1 \times 10^4$ |

Amendment 3 (11/08/2010)

Based on data from the first four tests, the Spor-Klenz® power washer testing (Tests 7 and 8) was changed to utilize an application rate of 15 seconds per 3 coupons (rather than 30 seconds) (section 3.1.5.3.2).

Amendment 4 (11/22/2010)

During tests 7 and 8, the John Deere pressure washer failed due to incompatibility with Spor-Klenz®. Section 3.1.5.3.2 of the QAPP was amended to state that sporicides would be applied to the coupons via a Chemical Sprayer

(Model# PP-UAG1003HU-K) made by UDOR USA. The pressure washer would still be used to rinse the decontaminant from the coupons once the contact time had been achieved. In addition, the remainder of tests would be conducted using the following replacement pressure washer:

Troy Bilt
M# 020337
S# 1017273115
Max psi=2550
Max GPM=2.3

Amendment 5 (02/01/2011)

Based on test results, Task II decontaminant spray to the large 101.6 cm by 101.6 cm (40 in by 40 in) coupons would be for 30 seconds per application (rather than 90 seconds).

Amendment 6 (02/23/2011)

The last two Task II tests would be conducted exactly as the first two tests, except that a rinse step would not be conducted following the contact time.

5.5.2 Deviations from the QAPP

Most of the data quality indicators for the critical measurements were within their specified target ranges as indicated in Table 4-3. However, in some instances, some small deviations were noted such as deionized water temperatures, sprayer flow rates, or CFU counts. These small deviations in measurements, although critical, were consistent throughout the tests and did not affect the intra-test comparisons

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**Appendix A: Coupon Sterilization**

Task I: Pressure-Treated Wood Coupons 35.6 cm by 35.6 cm (14 in by 14 in)

The pressure-treated wood coupons underwent sterilization using a STERIS VHP® sterilization cycle. This cycle entails the use of a STERIS VHP® ARD hydrogen peroxide ($H_2O_2$) generator. The coupons were individually enclosed in $H_2O_2$ vapor-permeable sterilization bags (General Econopak, Inc.; Steam Component Autoclave Bag, White, 20" by 20"; Item # 62020TW) and exposed to $H_2O_2$ at 250 ppmv for 240 minutes by maintaining this minimum concentration in the airlock of COMMANDER. The coupons were sterilized in batches. The number of coupons per batch was determined so that all coupons in the chamber would be exposed to the vapor without shielding (e.g., no coupons were physically on top of others) and appropriate mixing of the $H_2O_2$ occurs in the chamber. After sterilization, coupons of the same type were placed in a sterile container for storage prior to use and transport to the testing location. The container was marked with the contents, including the date of sterilization. One coupon from each material type and sterilization cycle was sampled according to the sterilization sampling procedure described in Appendix F. The samples from each material were analyzed qualitatively for the presence of any potentially confounding contamination. Batches found to have the presence of contamination were re-sterilized. If after a second sterilization cycle the batch was determined to still be contaminated, all coupons from the batch were discarded.

Test parameters such as temperature, relative humidity and concentration were monitored and recorded to ensure STERIS's defined quality standards were met. The quality of the cycle was considered in compliance with STERIS's label as long as all parameters were within the manufacturer's specifications.

Task I: Concrete Coupons 35.6 cm by 35.6 cm (14 in by 14 in)

The STERIS VHP® sterilization cycle described above was determined to be inadequate for the sterilization of the concrete coupons. These coupons were therefore sterilized by steam autoclave utilizing a one-hour gravity cycle program consistent with an APPCD Microbiology Laboratory internal MOP 6570 (included in Appendix C). Confirmation of sterilization was conducted as described above with respect to the coupons sterilized using the STERIS VHP® sterilization cycle. Prior to sterilization, concrete coupons were cleaned by pressure-washing each with water to remove excess grit and loose agglomerations of concrete.

Task II: Pressure-Treated Wood and Concrete Coupons 101.6 cm by 101.6 cm (40 in by 40 in)

The large coupons used for Task II underwent sterilization using the STERIS VHP® sterilization cycle described above for the Task I pressure-treated wood coupons, but the large coupons were not enclosed in sterilization bags. These coupons were tested in COMMANDER following sterilization.

MDI Control Check Stainless Steel Coupons

In addition to the test materials, metered dose inhaler (MDI) control coupons made of stainless steel 35.6 cm by 35.6 cm (14 in by 14 in) were also used as coupon inoculation controls. These coupons were sterilized prior to use by steam autoclave utilizing a one-hour gravity cycle program consistent with an APPCD Microbiology Laboratory internal MOP 6570. Confirmation of sterilization was done by sampling.

## Appendix B: Test Chamber and Equipment Cleaning Procedures

The pH-adjusted bleach solution to be used for cleaning surfaces of equipment in both the decontamination and microbiology laboratories will be prepared as a 1:10 dilution of bleach in DI water, pH-adjusted to ~6.8 using glacial acetic acid.

The following steps will be followed for cleaning the decontamination chamber between each material type and before/after each test.

a. Using the backpack sprayer, the interior surfaces will be kept wet with pH-adjusted bleach solution for 10 minutes.
b. With the drain open, the surfaces will then be rinsed with DI water. The rinsate will be collected in a carboy and ultimately discarded.
c. After ensuring all rinsate is removed from the chamber, the valve will be closed in preparation for the next test.
d. A mop assembly with a disposable pad will be used to wipe down the interior of the chamber with isopropyl alcohol or ethanol.
e. The pad will be then removed and placed in a bucket of amended bleach solution for decontamination prior to disposal.

The following steps will be followed for cleaning the work surfaces before and after use.

a. Wet all surfaces with pH-adjusted bleach solution or using Dispatch® bleach wipes.
b. Rinse with DI water.
c. Wet and wipe surfaces with isopropyl alcohol or ethanol.
d. Air dry prior to re-use.
e. Alternatively, cover paper can be used and replaced before/after each use.

The sampling templates will be autoclaved before/after each use.

The following steps will be followed for cleaning the coupon cabinets before and after use.

a. Wet and wipe all surfaces with pH-adjusted bleach solution or using Dispatch® bleach wipes.
b. Rinse with DI water.
c. Wet and wipe surfaces with isopropyl alcohol or ethanol.
d. Air-dry prior to re-use.

The gaskets used during the contamination procedure were cleaned via fumigation with the STERIS VHP® sterilization cycle. This cycle entails the use of a STERIS VHP® ARD hydrogen peroxide ($H_2O_2$) generator and exposure of all components of the wet/dry vacuum to $H_2O_2$ at 1000 ppmv for 60 minutes by maintaining this constant concentration in a decontamination chamber.

Bins used in the study will either be filled with pH-adjusted bleach and left covered for at least 60 minutes, rinsed with DI water, and air-dried or cleaned by the following procedure:

a. Wet and wipe all surfaces with pH-adjusted bleach solution or using Dispatch® bleach wipes.

b. Rinse with DI water.

c. Air-dry prior to re-use.

Alternatively to the use of pH-adjusted bleach for the sterilization of the materials and equipment used in each decontamination test, the STERIS VHP® sterilization cycle may be used. The equipment/materials will be placed in either the ~900 cu. ft. stainless steel chamber, or the COMMANDER main chamber or airlock. The sterilization cycle shall be a minimum of 250 ppmv $H_2O_2$ for at least 4 hours. Dehumidification to less than 40% RH shall be done prior to the injection of $H_2O_2$ vapor. A minimum of 1000 ppmv-hours (dose or CT = concentration by time) shall be achieved with the concentration above the minimum target of 250 ppmv (i.e., the CT clock shall be stopped if the concentration falls below this value.). VHP® will be used for all sterilization events in COMMANDER.

**Appendix C: Miscellaneous Operating Procedures (MOPs)**

MOP 3135 Procedure for Sample Collection using BactiSwab™ Collection and Transport Systems

MOP 6535a: Serial Dilution: Spread Plate Procedure to Quantify Viable Bacterial Spores

MOP 6561: Aerosol Deposition of Spores onto Material Coupon Surfaces Using the Aerosol Deposition Apparatus (An EPA proprietary method, unable to be disclosed at the time of writing this report, patent pending)

MOP 6562: Preparing Pre-Measured Tubes with Aliquoted Amounts of Phosphate Buffered Saline with Tween 20 (PBST)

MOP 6565: Filtration and Plating of Bacteria from Liquid Extracts

MOP 6567: Recovery of *Bacillus* Spores from Wipe Samples

MOP 6568: Aseptic Assembly of Wipe Kits

MOP 6570: Use of STERIS Amsco Century SV 120 Scientific Prevacuum Sterilizer

MOP 6571: Recovery of *Bacillus* Spores from Via-cell Aerosol Sampling Cassettes

**MOP 3135**

TITLE: Procedure for WA 1-25: Procedure for Sample Collection using BactiSwab™ Collection and Transport Systems

SCOPE: This MOP describes the procedure for collecting swab samples for Low Tech Decontamination Technique Testing

PURPOSE: The purpose of this MOP is to ensure that all swab sampling is performed in a consistent manner.

**Equipment/Reagents**

- Disposable laboratory coat
- Nitrile examination gloves
- P95 Respirator
- Shoe covers
- Bouffant cap
- Safety glasses
- BactiSwab™ Collection and Transport System

## 1.0 PROCEDURE

1. Enter the COMMANDER airlock wearing appropriate, project-specific PPE (at a minimum gloves, laboratory coat, and safety glasses), making sure the airlock door is closed.

2. Through the sleeve, crush the BactiSwab™ ampule at midpoint.

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

4. Open the package and remove the BactiSwab™.

5. Label the plastic tube appropriately using the following scheme:

   **X-Y-N** where,

   **X** is the test number,
   **Y** is the material abbreviation, and **N** is the material number.

6. Remove the cap-swab from the plastic tube.

7. Swab the surface following the recommend guidelines for each material while spinning the cap-swab between the thumb and index fingers.

   **a. Brushes (B).**

   Pull the cap-swab through the brush bristles using one continuous stroke moving top to bottom and left to right.

   **b. Nozzles (N).**

   Swab around the squeegee, inside the divisions, and inside the opening for the hose attachment.

   **c. Buckets (P).**

   Swab the sides and the bottom surfaces in an "S" pattern.

   **d. Brush Handles (BH).**

   Swab the top quarter of the handle top to bottom then bottom to top, turning the handle as you go.

   **e. Hoses (VH).**

   Swab inside and outside the hose opening that attaches to the nozzle.

   **f. Vacuums (V).**

Randomly swab the folds of the HEPA filter, swab the bottom of the vacuum lid, then swab the walls and bottom of the canister. Swab the inside of the exhaust port.

8. Return cap-swab to tube.

9. Date and initial each sample tube. Enter this information into the laboratory notebook.

10. Complete the chain of custody form and relinquish the samples to the BioLab.

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 centrifuge 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 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 uL of sterile diluent to each of the tubes.

2- Label three Trypticase Soy agar 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 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 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 = $4.0 \times 10^{7}$

**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**

**MOP-6562**

**TITLE: PREPARING PRE-MEASURED TUBES WITH ALIQUOTED AMOUNTS OF PHOSPHATE BUFFERED SALINE WITH TWEEN® 20 (PBST)**

SCOPE: This MOP provides the procedure for preparing PBST.

PURPOSE: This procedure will ensure that that the PBST is prepared correctly and that all measured tubes are filled aseptically.

## 1.0 PREPARING STERILE PHOSPHATE BUFFERED SALINE WITH TWEEN® 20 (PBST)

Phosphate Buffered Saline with Tween® 20 (PBST) is prepared 1 L at a time in a 1 L flask.

1. Add 1 packet of SIGMA Phosphate Buffered Saline with Tween® 20 (P-3563) to 1 L of deionized (DI) water.

2. Shake vigorously to mix until dissolved.

3. Label bottle as “non-sterile PBST” and include date and initials of person who made PBST.

4. Filter sterilize into two 500 mL reagent bottles using 150 mL bottle top filter (w/ 33mm neck and .22 µm cellulose acetate filter) for sterilization. Complete this procedure by pouring the liquid into the non-sterile PBST into the top portion of the filtration unit 150 mL at a time, while using the vacuum to suck the liquid through the filter. Continue to do this until 500 mL have been sterilized into a 500 mL bottle. Change bottle top filter units between each and every 500 mL bottle.

5. Change label to reflect that the PBST is now sterile. Include initials and date of sterilization. The label should now include information on when the PBST was initially made and when it was sterilized and by whom.

6. Each batch of PBST should be used within 90 days.

## 2.0 PREPARING 20 ML/5 ML PBST TUBES FOR USE DURING EXPERIMENTATION

Twenty (20) mL or five (5) mL of the prepared PBST will be added to each sterile 50-mL conical tube as detailed below. Each flat of conical tubes contains 25 tubes, so one 500 mL sterile bottle of PBST should fill approximately one flat when 20 mL tubes are needed and four flats when 5 mL tubes are needed.

1. Prepare the hood by wiping down with ethanol, followed by bleach, followed by DI water and a clean Kimwipe or Texwipe. Then stock the hood with the following items if they are not already there:
    - The flats of sterile conical tubes you need to fill with PBST.
    - Sufficient bottles of sterile PBST to fill these tubes.
    - Ample 25 mL serological pipettes (at least 3 per flat) for 20 mL transfers and 10 mL serological pipettes for the 5 mL transfers.
    - Serological pipetter (automatic, hand-held pipette).
    - Burner and striker.

2. Light the burner and adjust the flame for a width adequate to flame the lips of the PBST bottles.

3. Take one flat of sterile conical tubes and loosen each cap on the outside edges (about ½ turn).

4. Open a serological pipette and insert into the serological pipetter, taking care to not touch the tip to any surface.

5. Hold the pipetter with the first three fingers of your right (or dominant) hand. With your left hand (or non-dominant hand), pick up a bottle of the PBST and use the bottom of your right hand to unscrew the lid. Place the lid upside down on the benchtop and quickly flame the lip of the bottle. Turn the bottle and repeat, taking care to thoroughly flame the lip without getting the glass so hot that it shatters.

6. Inset the tip of the pipette into the bottle and fill to the 20 mL line. Flame the bottle lip and place the bottle on the benchtop.

    **Note:** If the tip of the pipette touches the outside of the bottle or any other surface in the hood, consider it contaminated. Discard the pipette and reload a new one.

7. Quickly pick up one of the tubes that you have loosened the cap on, and use the bottom of your right hand to remove the cap. Completely discharge the entire pipette into the tube, taking care to not touch anything with the tip of the pipette. Recap the tube and place back into the flat (the lid does not have to be tight – you will tighten the lids after you have completed filling the 10 outside tubes).

    Note**:** If the tip touches the outside or rim of the tube (or any other surface in the hood), consider the tube and pipette contaminated. Discard both the tube and the pipette.

8. Pick up the PBST bottle and flame the lip. Repeat Steps 6 and 7 until all 10 of the tubes on the outside of the flat have been filled. Flame the lip of the PBST bottle and replace the cap. Slide the used pipette back into the plastic sleeve and put to the side of the hood for disposal. Then tighten the lid of each tube you just filled. But rather than placing it back into its original spot in the flat, switch it for the empty tube from the next row. When this has been completed, go around the

outside of the flat again and loosen the lids of these 10 tubes. Repeat steps 4 through 7 to fill and cap these tubes.

9. This same procedure is used to fill the middle row of tubes from the flat, and if more than one flat of tubes is being filled, can be done at the same time as the outside rows of a second flat.

10. When all tubes have been filled, label each flat as follows, and place on the shelf in room E390B:

    "PBST Tubes (20 ml or 5 ml)"
    Date prepared
    Your initials

11. These tubes should be made at least 14 days before they need to be used so that they can be verified as sterile. Any tubes that are cloudy or that have any floating matter/turbidity should be discarded. The tubes are stable for and should be used within 90 days.

## 3.0 CLEANUP FOR 20 ML/5 ML PBST TUBES

1. Dispose of the used pipettes in the nonregulated waste.

2. Plug in the serological pipetter so that it can recharge.

3. Replace any unused PBST in the liquid containment on the shelf. Make sure that the bottle is labeled as having been opened (date opened and initials of whomever used it).

4. Turn off the burner.

5. Wipe down the hood benchtop with ethanol, followed by bleach, followed by DI water and a clean Kimwipe or TexWipe.

## 4.0 PREPARING 900 µL PBST TUBES FOR USE DURING EXPERIMENTATION

1. Prepare the hood by wiping down with ethanol, followed by bleach, followed by DI water and a clean Kimwipe or Texwipe. Then stock the hood with the following items if they are not already there:
   - A sterile beaker of microcentrifuge tubes.
   - Sufficient tubes of sterile PBST to fill these tubes (PBST may be aseptically transferred to 50 mL conical tubes for an easier aseptic transfer to the microcentrifuge tubes- it is easier than working from a 500 mL reagent bottle. Make certain that these 50 mL conical tubes are labeled to when the PBST was made, sterilized, etc.).

   - 1000 µL micropipette. - 1000 µL sterile pipette tips
   - Microcentrifuge tube racks.
   - Labeled beaker or waste container used to hold non-regulated waste, such as tips, under the hood.

2. Carefully remove the microcentrifuge tubes one at a time from the beaker and close the top on each one before placing it in the tube rack. Place the tubes in the rack skipping every other row. Fill up two racks doing this.

3. Add 900 µL of PBST to the microcentrifuge tubes by aseptically transferring the PBST from the sterile 50 mL conical tube containing the PBST. Do this by using the 1000 µL micropitte and tips. Change tips whenever after two rows of tubes are completed or whenever a contamination event (such as touching the outside of the 50 mL tube or the microcentrifuge tube) occurs. Put the dirty tips in the beaker or container used to contain waste (tips, tubes) in the hood. If any 900 µL tubes are contaminated during the transfer, dispose of them in the waste container used to hold tips under the hood. If a new box of tips has to be opened, make certain the date it was opened and initials of the person who opened it are clearly labeled on the box.

4. After both racks are full, carefully move all the tubes from one rack to fill in the empty rows on the other rack. In this manner, one rack should be completely filled with tubes at this point.

5. Label the rack of tubes as “Sterile 900 µL PBST Tubes”, along with the name of the person who completed the transfer, along with the date. Also, include the date that the original stock of PBST was made and the date it was sterilized, along with the initials of the person who completed those steps.

## 5.0 CLEANUP FOR 900 µL PBST TUBES

1. Dispose of the waste that was put in the labeled beaker or waste container (micropipette tips and tubes) in the nonregulated waste. Then, place this beaker in the “To be decontaminated via sterilization- contaminated glassware” bin or if it is a disposable container, then it can be put in the non-regulated waste container.

2. Put the unused sterile tips and the micropipetter back in its original location.

3. Replace any unused 50 mL conicals of PBST in the liquid containment on the shelf. Make sure that the tube is labeled as having been opened (date opened and initials of whomever used it). If the tube could possibly be contaminated in any way, dispose of it in non-regulated waste.

4. Wipe down the hood benchtop with ethanol, followed by bleach, followed by DI water and a clean Kimwipe or TexWipe.

**Title:** **FILTRATION AND PLATING OF BACTERIA FROM LIQUID EXTRACTS**

**Scope**: This MOP outlines the procedure for filtration and subsequent cultivation of bacterial spores from a liquid extract.

**Purpose**: This method is deployed when results from spread-plate methods yield less than 30 colony-forming units (CFU) per plate. This method allows a lower limit of detection for bacterial recovery/survivorship assays. This method can also be used to analyze liquid samples such as decontamination rinsates.

**Materials**: Petri dishes with appropriate agar

0.2 µm pore-size disposable analytical filter units (2-3 per sample)

P1000 pipette and sterile tips

Sterile forceps

Pipetman and sterile serological pipettes

**Procedure**:

1- For each liquid sample to be analyzed, gather the required number of disposable analytical filter units and Petri dishes containing the desired sterilized/QC'd media.

(Note: for analysis of 5 to 30 mL extracts, 1 mL and remainder should be filtered; for 31 to 200 mL samples, 1 mL, 10 mL, and remainder should be filtered; for samples over 200 mL, more filter samples may be needed)

(Note #2: For previously plated samples where 10 – 19 CFU were observed, replating using a 400 µL inoculum, and plates where 20 – 29 CFU were observed, replating using a 200 µL inoculum can be executed rather than filter plating. For inocula greater than 200 µL, a sterile spreader should be used rather than the bead method).

2- Label plates.

3- Vortex liquid extract vigorously for 2 minutes, using 10 second bursts. (For larger volume samples, a vigorous mixing by shaking of the sample container can be substituted for vortex mixing)

4- Using a P1000, sterile tip, and aseptic techniques, immediately following vortexing, pipette 1 mL of the extract into one of the filter units.

5- Apply vacuum to the filter unit to pull the liquid through the filter and collect the spores on the surface of the filter.

6- Using a sterile serological pipette, rinse the filter unit by pipetting 10 mL of sterile deionized water along the inner sides of the unit while it is under vacuum.

7- Aseptically remove the filter from the filter apparatus using sterile forceps and lay the filter onto the agar surface within the Petri dish (spore side up).

8- Vortex the liquid extract vigorously for 10 seconds.

9- Use the appropriate volume serological pipette to transfer the remaining aliquots into their respective filtration units (one at a time).

10- Repeat steps 5 through 7 taking time to vortex or mix the sample 10 seconds immediately before removing an aliquot.

Important: Be sure to note and record the volume of the “remainder” sample.

11- Incubate all plates at the optimal growth temperature for the organism used for 16 – 28 hours.

12- Enumerate and record the number of CFU on each plate.

**Data Calculations**

Utilize the following equation to determine the total abundance of recovered spores:

$$N = CFU \times \frac{V_{Extract}}{V_{filtered}}$$

where N is the total number of spores recovered in the extract, CFU is the abundance of colonies on the agar plate, $V_{Extract}$ is the total volume of the extract (before any aliquots were removed), $V_{Filtered}$ is the volume of the extract filtered.

**Scope**: This MOP outlines the procedure for recovering *Bacillus* spores from wipe samples

**Purpose**: To aseptically extract and quantify *Bacillus* spores from wipe samples in order to determine viability and obtain quantifiable data.

## 1.0 MATERIALS

- PPE (gloves, laboratory coat, safety goggles)
- Biological Safety Cabinet (Class II)
- pH-Amended bleach
- Deionized water
- 70% Solution of denatured ethanol
- Kimwipes
- Dispatch® bleach wipes
- Non-regulated waste container
- 50 mL sterile conical tubes containing 20 mL of sterile phosphate buffered saline with Tween® 20 solution (PBST) (MOP 6562)
- Vortex mixer
- Cart
- Wire or foam rack for 50 mL conical tubes
- Tryptic soy agar plates
- 900 uL tubes of sterile PBST
- Pipettor and pipette tips for dilutions
- Incubator set to appropriate growth temperature for target organism (35 °C or 55 °C)
- Light box for counting colonies
- Laboratory notebook
- QAPP for project that is utilizing the wipe samples

## 2.0 PROCEDURE

1. Begin by donning PPE (gloves, laboratory coat, and protective eyewear).

2. Obtain wipe samples that may contain *Bacillus* spores. Wipe samples should be received as one wipe/sponge in a sterile 50 mL conical tube delivered in secondary containment. Make certain that all of the samples are labeled. Review any chain of custody forms that may accompany the samples to ensure that all of the labels are consistent and that there is no notable variation in the samples. If variation has occurred, make a note of it in the notebook.

3. Clean the workspace (biological safety cabinet) by wiping surfaces with pH-amended bleach, next with deionized water, and lastly with a 70-90 % solution of denatured ethanol. Wipe with a Kimwipe to remove any excess liquid. Make sure the workspace is clean and free of debris. Gather all

necessary items to perform the task, place these items on a clean cart beside the biological safety cabinet, within arm's reach so that once the procedure has begun, the task may be performed without interruptions.

4. Discard gloves and replace with fresh pair.

5. One at a time, under the biological safety cabinet, remove the sample tube containing the wipe sample from the secondary containment bag in which it arrived. Using the Dispatch® bleach wipes, wipe each sample tube with one wipe, and then wipe it with a clean Kimwipe. Discard the used bleach wipe and the used Kimwipe in the secondary containment bag and place them in the non-regulated waste container. Remove gloves and don a fresh pair of gloves. Repeat this procedure for every sample. After each sample has been cleaned, place the tubes containing the wipe samples in an appropriate-sized wire or foam rack to hold the tubes in an upright vertical position.

6. Leaving the tubes in the rack underneath the biological safety cabinet, aseptically add 20 mL of PBST solution (this should be in a pre-measured, sterile conical tube, per MOP 6562) to each sample tube containing a wipe, one a time. Remove the rack containing wipe samples from the hood when all samples have had the PBST added. Place the rack with the samples on the cart.

7. Using the procedure to clean the biological safety cabinet, as found in Step 3, clean the biological safety cabinet again. Afterwards don a fresh pair of gloves.

8. Using a vortex mixer, agitate the wipe samples, four at a time, in a biological safety cabinet, for ten second bursts for two minutes total. Make certain to clean the biological safety cabinet after each set of four samples and change gloves between each set of samples.

   **Note**: The reason that four samples are done at one time is to limit the time between agitation and plating. The samples need to be processed immediately after agitation, and agitation of more than four samples at a time leaves too much time between agitation and spread plating.

9. Using tryptic soy agar media plates that are appropriately labeled with the sample number, dilution set and date, complete dilution plating for the wipe samples immediately after the two-minute agitation step is completed. The samples should also be agitated again for ten seconds directly prior to removing an aliquot from the sample tube. Each dilution tube should also be agitated for ten seconds prior to removal of aliquots. Dilutions should be completed using the techniques and methodology as described in MOP 6535a, and the 900 uL tubes should be made with sterile PBST to stay consistent with materials/solutions. Plating in this manner should be repeated for all samples, with any changes in protocol noted in the laboratory notebook.

10. Once the dilution plating has been completed, the plates are to be placed in an incubator. For non-thermophilic *Bacillus* species, the plates should be placed at 35 °C ± 2 °C for 12-24 hours. For thermophilc *Bacillus* species, such as *Geobacillus stearothermophilis*, the plates should be incubated at 55 °C ± 2°C for 12-24 hours. The target *Bacillus* organism that will be used for the wipe samples will be specific to the project and noted in the QAPP.

11. After the plates have incubated for a sufficient amount of time (12-24 hours) and the growth from any *Bacillus* colonies is quantifiable, the colonies should be manually counted using the light box and the data should be properly recorded as dictated per project by the QAPP. All results will be checked for quality assurance and all data will be reported to the proper personnel as listed in the QAPP.

**MOP 6568**

**Title:** **ASEPTIC ASSEMBLY OF WIPE KITS**

**Scope**: This MOP outlines the procedure for the aseptic assembly of wipe kits.

**Purpose**: To aseptically assemble kits that will be used to collect wipe samples from which quantifiable data will be derived.

## 1.0 MATERIALS

- PPE (gloves, laboratory coat, safety goggles)
- Biological Safety Cabinet (Class II)
- pH-Adjusted bleach
- Deionized water
- 70% Solution of denatured ethanol
- Kimwipes
- Sterile, sealed Twirl-em® bags in two sizes, 10”x15” and 5.5”x9”
- Sterile Kendall 4-ply all-purpose sponges
- Sterile, disposable thumb forceps
- 50 mL conical tubes containing 5 mL PBST tubes (MOP 6562)
- Sharpie
- Wire or foam rack for 50 mL conical tubes
- Secondary containment such as a large Tupperware bin
- Laboratory notebook
- QAPP for project that is utilizing the wipe samples

## 2.0 PROCEDURE

### 2.1 Preparation for Wipe Kit Assembly

Prior to wipe kit assembly, 50 mL sterile conical tubes containing 5 mL of sterile PBST and a sterile 2-ply sponge must be put together. They are assembled in the following manner:

1. Begin by donning PPE (gloves, laboratory coat, and protective eyewear)**.**

2. Clean the workspace and biological safety cabinet by wiping surfaces with pH-adjusted bleach, followed by deionized water, and then with a 70% solution of denatured ethanol. Wipe the surfaces with a Kimwipe to remove any excess liquid. Make sure the workspace is clean and free of debris. Gather all necessary items to perform the task, place these items on a clean cart beside the biological safety cabinet, within arm's reach so that once the procedure has begun, the task may be performed without interruptions.

3. Discard gloves and replace with fresh pair.

4. Place the sterile 50 mL conical tubes containing 5 mL PBST tubes under the biological safety cabinet in a foam or wire rack designed to hold 50 mL conical tubes. Using two sterile, disposable thumb forceps, aseptically transfer one half of a 4-ply sterile all-purpose sponge to each of the tubes. Complete the transfer by using the two forceps together to first separate the 4-ply sponge in half to create two 2-ply sponges. Then remove a cap from one of the tubes, carefully fold one of the 2-ply sponges using the forceps together and aseptically place it in the opening of the tube so that it sits at the top portion of the tube, while the 5 mL of PBST remains at the bottom of the tube. Replace the cap on the tube. Repeat this process until all of the tubes have sponges in them. Once all of the tubes contain sterile sponges, then label the tube rack appropriately with the action completed, the date and your initials and place the tubes on the shelf. These tubes are shelf-stable for up to three months.

### 2.2 Assembly of Wipe Kits

Wipe kits are assembled in the following manner:

1. No more than 48 hours prior to testing or collecting samples, assemble the wipe kits. Wipe kits can be assembled outside the biological safety cabinet, in a dry, clean area. Make certain to use proper PPE, including gloves, while handling all wipe kit materials. Gather all materials to assemble the kits before assembly. These materials include:

    - 50 mL conical tubes containing both a sterile wipe sponge and 5 mL PBST
    - Twirl-em® bags in two sizes, 10"x15" and 5.5"x 9"
    - Sharpie

- Vortex mixer

2. Obtain a copy of the labeling scheme for the samples. This scheme may be detailed in the QAPP. For each wipe kit, use a Sharpie and label a large 10" x 15" Twirl-em® bag and a 50 mL conical tube containing the sponge and PBST.

3. Once all of the tubes are labeled, use the vortex mixer on the highest setting to agitate the tube. This will mix the sponge, which was placed at the top of the tube, with the 5 mL of PBST.

4. Open the labeled, 10" x 15" Twirl-em® bags one at a time. Place the labeled, agitated tubes in the 10" x 15" Twirl-em® bags that have the corresponding label (that matches the tube). Add a non-labeled, sealed 5.5" x 9" Twirl-em® bag into the 10"x 15" Twirl-em® bag, along with the tube containing the wipe sponge to complete the wipe kit assembly. Record the time and date on which the wipe kits were assembled in the laboratory notebook; include the labeling schematic for the wipe kits.

5. Place the assembled wipe kits into a secondary containment, such as a large Tupperware bin. Use within 48 hours. When moving the kits to a sampling location, always have them in secondary containment.

**BL MOP NO. 6570** July 2010

Title: Use of STERIS Amsco Century SV 120 Scientific Prevacuum Sterilizer

Scope: Basic instructions for use of the autoclave

Purpose: To outline proper use of the autoclave, using preprogrammed cycles, to effectively sterilize media, supplies, or waste.

**Materials**

Amsco Century SV 120 Scientific Prevacuum Sterilizer

Items to be sterilized (liquids, solids, waste, etc)

Pouches to contain materials to be sterilized and maintain that state until later use

Aluminum foil Autoclave Indicator Tape

Sterilization Verification Ampoules

Thermally resistant gloves or tongs

Deionized (DI) water

**Procedure:**

Basic start up:

1. Turn on the autoclave. The power switch is located behind the door in the top right corner. The digital touch screen on the front of the unit will power up and indicate that a memory test is in progress.
2. After the memory test is complete, the device will request that it be flushed. This flushing should be conducted daily to minimize scaling inside the boiler. The flush valve is located on the bottom left of the device (yellow handle). Actuate the valve to the open position and then press the “Start Timer” button on the touch screen. The flush will run for 5 minutes and will alert at completion with a single chime.
3. Once the flush is complete, close the flush valve and press the “Continue” button on the touch screen. The screen should then return to its default menu which has two choices “Cycle Menu” and “Options”

Basic Operation:

1. Prepare any items that need to be sterilized. The items must be carefully wrapped or sealed in sterilization pouches in order to maintain sterility when removed from the autoclave. Examples of this wrap or seal include: wrapping any orifices with aluminum foil, placing whole items in autoclave pouches, loosely applying a cap on a bottle (to allow for the pressure changes inside).
2. Once prepared, each item should be outfitted with a sterility indicator. This indicator can be completed by applying a small piece of autoclave indicator tape to an item or by utilizing an autoclave pouch with a built-in sterility indicator strip. These indicators provide a visual verification that the sterilizing temperature (121 °C) was reached.
3. To add items to the autoclave, open the autoclave door by pressing down on the foot pedal on the bottom right corner on the front of the device.
4. Place items that need to be sterilized into the autoclave, adding or moving racks to accommodate the load. If liquids are being autoclaved, then they must have secondary containment (usually a large plastic autoclave-safe tray) to contain any fluids in the event of a leak, spill or boil-over.
5. Once the autoclave is loaded, press the foot pedal to close the autoclave door.
6. Once the door is sealed, a menu of the cycles can be seen by pressing the button on the touch screen labeled “Cycle Menu”. Then choose the appropriate cycle by touching the corresponding button. If the cycle chosen is the one desired for the sterilization process, press the “Start Cycle” button. Otherwise, press “Back” to return to the prior menu screen.
7. After the cycle has started, the type of cycle, the number of the cycle, the items placed in the autoclave during the cycle, the time, whether or not a QC ampoule was included in the load, and the initials of the person starting the cycle must be recorded in the autoclave log book, located in the drawer across form the unit labeled “Autoclave Supplies.”
8. QC ampoules, usually Raven Prospore Ampoules with *Geobacillus stearothermophilus,* are added to one cycle each day to ensure that the autoclave is functioning properly. These ampoules are used according to manufacturer’s instructions.
9. Upon completion of a cycle, the autoclave will alarm with a repeating beep for approximately one minute. Any time after this alarm starts, it is safe to open the main door (take caution because the steam escaping the chamber will be very hot when the door is opened). The contents of the autoclave will be very hot; thermal protection for the hands is therefore required to remove the items (thermally resistant cloth gloves or tongs).
10. Place the contents of the autoclave in an appropriate place to cool and close the autoclave door using the foot pedal.

**Cycles:**

Gravity Cycles:

Gravity cycles are used to sterilize glassware and other utensils which are not submerged in nor contain any volume of liquid. These cycles are typically used for “dry” materials.

Currently there are two different gravity cycles programmed for daily operations: a 1-hour cycle and a 30-minute cycle. The time that the chamber is held at the sterilization temperature (121 °C) is the only difference between these two cycles. The different sterilization times allow for the compensation of the various sizes of materials and more resilient organisms. The 30-minute cycle is primarily used for a small quantity of material. The 1-hour cycle is used for large loads or items containing a large amount of contamination. The 1-hour cycle is recommended for inactivation of gram positive spore-forming bacteria.

Liquid Cycles:

Liquid cycles are used to sterilize a variety of liquids and solutions. The solutions are typically mixed prior to sterilization. It is important to have secondary containment to contain any fluids in the event of a leak, spill or boil-over. The 30-minute liquid cycle is used to sterilize small volumes of liquid (usually less than 2 L total). When attempting to sterilize any volume larger than 2 L, the 1-hour liquid cycle should be used to ensure complete sterilization. The 1-hour liquid cycle is the preferential cycle used as the destruction cycle for waste. In the event of materials (liquid or otherwise) being contaminated/exposed to microorganisms, the 1-hour liquid cycle will be used as the initial means of decontamination. When completing a decontamination cycle, if there is no liquid inside a container, then deionized water must be added to the container or the item must be submerged prior to the start of the cycle. Only items that are being decontaminated can go in destruction cycles. Decontamination cycles cannot be mixed with sterilization cycles.

**MOP 6571**

**Title:** **RECOVERY OF *BACILLUS* SPORES FROM VIA-CELL® AEROSOL SAMPLING CASSETTES**

**Scope**: This MOP outlines the procedure for recovering *Bacillus* spores from Via-Cell® aerosol sampling cassettes

**Purpose**: To aseptically extract and quantify *Bacillus* spores from Via-Cell® samples in order to determine viability and obtain quantifiable data.

## MATERIALS

- Via-Cell® Bioaerosol Sampling Cassettes (Zefon International, Ocala, FL, Part# VIA010)
- PPE (gloves, laboratory coat, safety goggles)
- Biological Safety Cabinet (Class II)
- pH-Amended bleach
- Deionized water
- 70% Solution of denatured ethanol
- Kimwipes
- Dispatch® bleach wipes
- Non-regulated waste container
- 50 mL sterile conical tubes containing appropriate volume of buffer
- Vortex mixer
- Cart
- Wire or foam rack for 50 mL conical tubes
- Sterile blade
- Sterile, disposable forceps
- Tryptic soy agar plates
- 900 µL tubes of sterile PBST
- Pipettor and pipette tips for dilutions
- Incubator set to appropriate growth temperature for target organism (35 °C or 55 °C)
- Light box for counting colonies
- Laboratory notebook
- QAPP for project that is utilizing the wipe samples

## PROCEDURE

1. Begin by donning fresh PPE (gloves, laboratory coat, and protective eyewear).

2. Obtain Via-Cell® samples that may contain *Bacillus* spores. Via-Cell® samples should be received as one Via-Cell® cassette delivered in secondary containment. Make certain that all of the samples are labeled. Review any chain of custody forms that may accompany the samples to ensure that all of the labels are consistent and that there is no notable variation in the samples. If variation has occurred, make a note of it in the notebook.

3. Clean the workspace (biological safety cabinet) by wiping surfaces with pH-amended bleach, next with deionized water, and lastly with a 70-90 % solution of denatured ethanol. Wipe with a Kimwipe to remove any excess liquid. Make sure the workspace is clean and free of debris. Gather all necessary items to perform the task, place these items on a clean cart beside the biological safety cabinet, within arm's reach so that once the procedure has begun, the task may be performed without interruptions.

4. Discard gloves and replace with fresh pair.

5. One at a time, under the biological safety cabinet, remove the sample cassette. Using a sterile blade, cut through the tape around the outside of the cassette. Twist apart the cassette and discard the top portion not containing the sample slide (portion of the cassette where the sample is collected). Using sterile, disposable forceps, remove the slide and place into the appropriate amount of buffer solution. Repeat this procedure for every sample.

6. Using the procedure to clean the biological safety cabinet, as found in Step 3, clean the biological safety cabinet again. Afterwards don a fresh pair of gloves.

7. Using a vortex mixer, agitate the Via-Cell® samples, four at a time, in a biological safety cabinet, for ten second bursts for two minutes total. Make certain to clean the biological safety cabinet after each set of four samples and change gloves between each set of samples.

   **Note**: The reason that four samples are done at one time is to limit the time between agitation and plating. The samples need to be processed immediately after agitation, and agitation of more than four samples at a time results in excessive lag-time between agitation and plating.

8. Using tryptic soy agar (or other appropriate growth media) media plates that are appropriately labeled with the sample number, dilution set and date; conduct dilution plating for the Via-Cell® samples immediately after the two-minute agitation step is completed.-The samples should also be agitated again for ten seconds directly prior to removing an aliquot from the sample tube. Each dilution tube should also be agitated for ten seconds prior to removal of aliquots. Dilutions should be completed using the techniques and methodology as described in MOP 6535a, and the 900 µL tubes should be made with the appropriate buffer to stay consistent with materials/solutions. Plating in this manner should be repeated for all samples, with any changes in protocol noted in the lab notebook.

9. Once the dilution plating has been completed, the plates are to be placed in an incubator. For non-thermophilic *Bacillus* species, the plates should be placed at 35 °C ± 2 °C for 18-24 hours. For thermophilc *Bacillus* species, such as *Geobacillus stearothermophilis*, the plates should be incubated at 55 °C ± 2 °C for 18-24 hours. The target *Bacillus* organism that will be used for the wipe samples will be specific to the project and noted in the QAPP.

10. After the plates have incubated for a sufficient amount of time (18-24 hours) and the growth from any *Bacillus* colonies is quantifiable, the colonies should be manually counted using the light box and the data should be properly recorded as dictated per project by the QAPP. All results will be checked for quality assurance and all data will be reported to the proper personnel as outlined in the QAPP.

## Appendix D: Spore Deposition and Handling Procedures

The handling of the contaminated coupons for Task I, including movement to minimize or control spore dispersal, was done in accordance with the MOP 6561 (a proprietary method unable to be disclosed at the time of writing this report). One person was tasked with removing the clamps holding the dosing chamber to the coupon and the removal of the dosing chamber and gasket from the coupon. A second person was then tasked with moving the coupon to the proper location (e.g., test and positive control coupons to the Test Coupon Cabinet and blank coupons to the Blank Coupon Cabinet).

For Task II, two personnel were used to move the 101.6 cm by 101.6 cm (40 in by 40 in) coupons into their vertical positions in COMMANDER following removal of the deposition devices. This was the only time the coupons were handled, and this handling occurred a minimum of two days prior to sampling.

For Task I, the Test Coupon Cabinet was a steel cabinet (48 in wide by 24 in deep by 78 in high) with twelve shelves each 6 in apart. Each cabinet held a total of 36 coupons, so two Test Coupon Cabinets were needed for a test. These cabinets were labeled as Test Coupon Cabinet 1 and Test Coupon Cabinet 2. Test and positive control coupons were arranged in each cabinet according to material types. A single material type was not split among cabinets. Procedural blank coupons of each material/orientation to be used in a single test were contained in a separate isolated cabinet (Blank Coupon Cabinet) of similar construction, however, with dimensions of 48 in wide by 24 in deep by 36 in high with 3 shelves.

Each MDI was claimed to provide 200 discharges. The number of discharges per MDI was tracked so that use did not exceed this value. Additionally, in accordance with MOP 6561 (a proprietary method), the weight of each MDI was recorded after completion of the contamination of each coupon. If an MDI weighed less than 10.5 g at the start of the contamination procedure, the MDI was retired and a new MDI used. For quality control of the MDIs, a contamination control coupon was run as the first, middle, and last coupon contaminated with a single MDI in a single test. The contamination control coupon was a stainless steel coupon (1.2 ft by 1.2 ft) that was contaminated, sampled, and analyzed.

A log was maintained for each set of coupons that were dosed via the method of MOP 6561 (a proprietary method). Each record in this log recorded a unique coupon identifier (see Table D-1), the MDI unique identifier, the date, the operator, the weight of the MDI before dissemination into the coupon-dosing device, the weight of the MDI after dissemination, and the difference between these two weights. The coupon codes were pre-printed on the log sheet prior to the start of coupon contamination (dosing).

Additionally, after a coupon was dosed via the above procedure, the coupon was labeled with the unique identifier using the coding outlined in Table D-1. The label was printed on the side of the coupon using a permanent marker (e.g., black or silver Sharpie®). 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 coupon was marked with only the material descriptor and unique code number. Once the coupons were transferred to the APPCD Microbiology Laboratory for plate counts, each sample was additionally identified by replicate number and dilution.

**Table D-1. Coupon Sample Coding**

| **Coupon Identification: T-S-M-NN** | | |
|---|---|---|
| | **Code** | |
| T/C<br>(Test Number) | 1 – 10 | Test Number preceded by T for Task 1 (Table 2-1) and preceded by C for tests in Task 2 (Table 2-1) |
| S<br>(Sample Type) | P | Positive control wipe sample |
| | T | Test wipe sample |
| | PX | Procedural blank |
| | FX | Field Blank |
| | LX | Laboratory Blank |
| | R | Rinsate |
| | V | Aerosol sample |
| | S | Swab sample |
| M<br>(Material) | CV | Concrete (vertical orientation) |
| | TW | Pressure Treated Wood (vertical orientation) |
| | SS | Stainless Steel |
| | DI | DI Water |
| | XX | Blank |
| NN<br>(Sample number) | ## | Replicate number or sample area number |
| **APPCD Microbiology Laboratory Plate Identification: T-S-M-NN-R-D** | | |
| T-S-M-NN | As above | |
| Replicate | R | A – C |
| Dilution | D | $1 \times 10^{0}$ - $1 \times 10^{4}$ |

## Appendix E: Contamination Prevention and Quality Control Measures

### Coupon Storage Cabinets

On the decontamination procedure test day, the procedural blank, test, and positive control coupons have been placed into the appropriate cabinets. A total of three cabinets were used to contain the coupons prior to decontamination (one for the procedural blanks and two containing the contaminated (positive controls and test) coupons). One additional cabinet was used to store test coupons for drying after the decontamination procedure had been applied. The cabinets with their intended purpose are listed in Table E-1.

**Table E-1. Coupon Storage Cabinets**

| Cabinet Name | Description |
|---|---|
| Test Coupon Cabinet #1<br>Test Coupon Cabinet #2 | For storage of contaminated coupons (both positive control and test coupons); each cabinet can hold 36 coupons, so two cabinets will be needed for all tests |
| Procedural Blank Cabinet | For storage of procedural blank coupons; the cabinet will be under slight positive pressure in order to prevent contamination from the test environment (i.e., laboratory) and allow passive air flow to promote drying. |
| Decontaminated Coupon Cabinet | For storage of all test coupons after application of the decontamination procedure; the cabinet will be under slight positive pressure in order to prevent contamination from the test environment (i.e., laboratory) and allow passive air flow to promote drying. |

### Material and Equipment

The material and equipment used for the decontamination procedure were standardized as much as possible and are listed in Table E-2. Decontamination steps are described in the subsequent sections of this Appendix.

**Table E-2. Material and Equipment Used in the Decontamination Procedural Steps**

| Material/Equipment | Description |
|---|---|
| Pressure Washer | John Deere 3300 psi, 3.2 gallon per minute, Model 020382 |
| Pressure Washer | Troy Bilt 2550 psi, 2.3 gallon per minute Model 020337 |
| Chemical Sprayer | UDOR USA, Model# PP-UAG1003HU-K<br>300 PSI @ Max of 10.5 GPM<br>AG Spray Gun |

| Material/Equipment | Description |
|---|---|
| Backpack Sprayer (Total of 2 units) | SRS-600 Propack Rechargeable Electric Backpack Sprayer (SHURflo, Cypress, CA), 4 Gallon, 12 Volt http://legacy.shurflo.com/pdf/industry/general/911/911-624.pdf |
| Bleach | Ultra Clorox® Regular Bleach (EPA Reg. No. 67619-8) (http://www.clorox.com/products/overview.php?prod_id=clb) 6.15% sodium hypochlorite; <1% sodium hydroxide (http://www.thecloroxcompany.com/products/msds/bleach/cloroxregularbleach0505_.pdf) |
| Vinegar | 5% v/v technical grade acetic acid |
| Container for mixing pH-adjusted bleach solution | 5 gallon plastic carboy |
| Spor-Klenz® | STERIS Spor-Klenz® Ready-To-Use (EPA Reg. No. 1043-119) Peracetic acid /Hydrogen Peroxide liquid decontaminant http://www.steris.com/products/view.cfm?id=253 |
| Nozzle | Standard Adjustable-Flow Garden Hose Nozzle, Standard Brass, 4" Length, McMaster-Carr, P/N 7484T1 |
| Garden hose | 75 ft.; 5/8 in diameter |
| Pressure regulator | Bronze Pressure Regulator-Plumbing-Code Rated Standard, 3/4" NPT Female, 25-75 PS |
| Bucket of DI water | 3 gallons in a 5-gallon plastic pail |
| Carboy container (Total of 9) | Carboys; Nalgene; Heavy Duty; polypropylene; Autoclavable; Leak proof. For full vacuum applications up to 8 Hours; USP class VI, vacuum rated for intermittent vacuum use only; 83B Closure size; capacity: 5.25 gal. (20 L) |
| Pump | NSF-Certified Rotary Vane Pump for Water with Motor, Brass, 4.3 Max GPM, 3/4 Horsepower |

**pH-Adjusted Bleach Solution**

The pH-adjusted bleach (pH-AB) solution was prepared by mixing 8 parts sterile distilled water with 1 part 5% acetic acid, and 1 part Clorox® bleach. The pH was then adjusted to 6.5 – 7.0 by adding more vinegar, and FAC was adjusted to 6000 – 6700 ppm by diluting with water. The pH-AB solution was prepared just prior to the initiation of testing on a particular day and was used within a window of 3 hours from the time of preparation. After 3 hours, the bleach solution was discarded and a fresh pH-AB solution was prepared. However, a single preparation was used within a single coupon set. Additionally, technical grade acetic acid (5% v/v) was used instead of off-the-shelf white vinegar. This change was expected to reduce the variability in the pH-AB solution for the purpose of this study.

The pH-AB solution was applied to each coupon using a backpack sprayer (Method 1) and with a chemical sprayer (Method 2) (see Table 2-1).

**Spor-Klenz® Ready to Use (RTU) Solution**

Spor-Klenz® RTU is a broad spectrum disinfectant and sporicide that is registered with EPA under FIFRA (registration #1043-119). Spor-Klenz® is a mixture of 1.0% hydrogen peroxide, 0.08% peroxyacetic acid, and 98.92% inert proprietary ingredients. The Ready-to-Use (RTU) variety of Spor-Klenz® was used for this study, as opposed to the concentrate (registration #1043-120), to reduce the variation between experiments. Preparation of diluted Spor-Klenz® from the concentrate for each day of testing would introduce unwanted variation. Spor-Klenz® RTU requires no dilution prior to use. A new container of Spor-Klenz® RTU was used for each day of testing. Unused Spor-Klenz® RTU was discarded appropriately. Since Spor-Klenz® RTU is produced under manufacturer quality assurance criteria, only temperature was imposed as a critical measurement for this liquid (see Section 4).

Spor-Klenz® has sterilant/sporicidal claims for nonporous surfaces when a 5.5 hour (20 °C) contact time is used. While this contact time far exceeds the planned contact times for this study, our test aims to evaluate technologies at conditions that are realistic of their use in homeland security-related remediation events. Maintaining a 5.5 hour contact time in an animal facility would likely be unrealistic for the amount of surface area needing to be decontaminated. Prior EPA research[10] on post-anthrax incident carpet cleaning has suggested that Spor-Klenz® RTU can be effective at much shorter contact times, so a contact time of 30 minutes was utilized.

**Backpack Sprayer Application of Decontaminant**

Prior to the start of testing, the spray pattern from the backpack sprayer was tested by spraying at the appropriate distance (1 ft) onto a piece of 1.2 ft by 1.2 ft blue construction paper mounted in the position of the test coupon. The spray was discharged into the center of the paper and the pattern was visually assessed for consistency with that shown in Figure E-1. The diameter of the spray was checked to ensure that the spray was within the acceptable limits (set at 16 in).

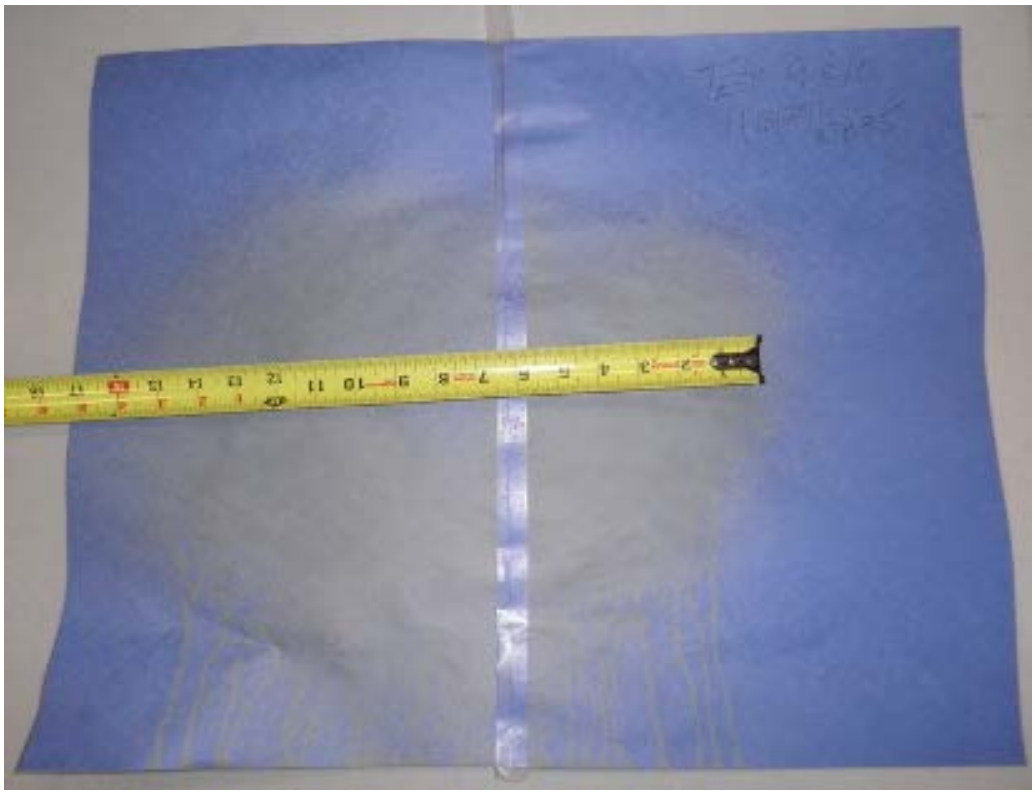

**Figure E-1. Backpack Sprayer - Spray Pattern (diameter of spray = 16 in) from Tests 9 and 10**

For Task I, the spray wand from the backpack sprayer (SRS-600 Propack, SHURflo, Cypress, CA; see Figure E-2) was inserted into the center port on the chamber. For Task II, the spray wand was inserted between the curtains. From a distance of 1 ft, the coupons were sprayed to completely wet (or flood) the surface of the materials. Each set of three Task I coupons was sprayed twice for 30 seconds with pH-AB and Spor-Klenz®, with a third set sprayed once for 10 seconds with pH-AB. Task II coupons were sprayed twice for 30 seconds with pH-AB.

The spray wand was moved back and forth to cover the surface of all three coupons evenly and completely (Task I) or moved back and forth while moving downward to cover the surface of Task II coupons completely. The Task I coupons were sprayed with three side-to-side strokes moving downward, starting first from the top of the left-most coupon, across all three coupons, and finishing at the bottom of the right-most coupon in the decontamination chamber. This step was repeated as often as necessary to satisfy the required spray duration. The decontamination staff practiced the movement before the tests until the sprayer could be operated in a repeatable manner. Data recorded included both the duration of the step and the time of day when the step was started for each coupon.


**Figure E-2. Backpack sprayer**

The constant spray pressure of 35 psi was maintained by the backpack sprayer. At this constant pressure, the flow rate was maintained at 1046 mL/min (average over all tests) with a cone spray pattern of 16 in diameter when held at a distance of 1 ft from the surface. For Task I, the spray wand was inserted at the same distance in the port. The spray pattern is shown in Appendix E.

The flow rate was checked at the start and end of each test and before and after use on each coupon set to ensure proper operation of the sprayer. The flow rate was measured by spraying into a graduated cylinder for 10 seconds and measuring the final volume. A 30 minute contact time, with two applications, one at 0 minutes and one at 15 minutes, would be optimal and most realistic of effort expended during an actual FAD remediation. For example, the decontamination solution would be reapplied once (once 15 minutes has elapsed) during the 30 minute contact time. Section 2.5 presents the Test Matrix and describes how it was modified based on these initial test results.

**Pressure Washer Application of Decontaminant**

Commercial pressure washers are not recommended for use with bleach. Being concerned about the effects of the pH-AB on this equipment, the first pressure washing application test (John Deere 3300 psi, Model 020382; see Figure E-3) was conducted with Spor-Klenz®. A fixed volume of Spor-Klenz® was to be dispensed onto the coupon surface by pressure washing for a fixed amount of time (planned as two sprays of 15 seconds per set of three coupons). The supply line of the pressure washer was connected by the garden hose to a reservoir containing the decontamination solution (at the final concentration). This reservoir was the sole supply of liquid to the pressure washer during the application procedure. The contents of the reservoir were therefore not diluted with water during use of the sprayer. The Task I coupons were sprayed with side-to-side strokes starting first from the top of the left-most coupon, working downward and the spray was moved across all three coupons in the decontamination chamber (Figure E-4). The start time and duration for this action were recorded and spray of the coupons was performed as consistently as possible across all coupons. A 25° angle nozzle was used with the pressure washer at full throttle. At a distance of 3 ft from the coupon surface, this nozzle produces a fan of approximately 12 in. The nozzle was oriented so that the fan was vertical.


**Figure E-3. John Deere pressure washer**

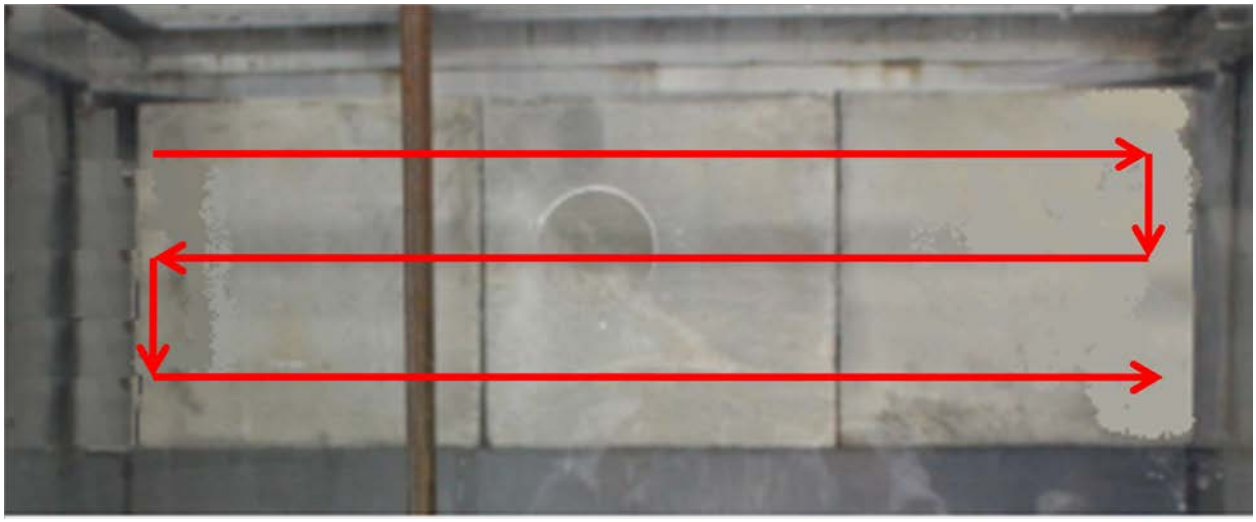

**Figure E-4: Center of spray during Task 1 decontamination procedures**

Large volumes of rinsate were expected. For example, spraying the surface of a set of coupons with a 3300 psi/3.2 gpm pressure washer for a total of 30 seconds would generate 1.6 gallons of rinsate.

After the decontamination solution had been applied to the first set of coupons, the pressure washer was rinsed with DI water by connecting a second supply hose (a hose dedicated for distilled water only) to the pressure washer and actuating the pressure washer for 30 seconds into a waste container. The coupons were then rinsed with DI water using the pressure washer.

During the second set of three coupons, the pressure washer was noticeably being negatively affected by the Spor-Klenz® (i.e., running irregularly). Further, upon attempting to accomplish the second application of sporicide during Test 8, the pressure washer failed to start initially and then ran roughly. The initial application of the Spor-Klenz® was made to the third set of three coupons (second material), but the pressure washer was unable to be restarted to make the second planned application for wood. This procedural anomaly is noted in the Table 2-1 footnote.

**Chemical Sprayer Application of Decontaminant**

Due to the detrimental effects of Spor-Klenz® on the pressure washer, a chemical sprayer (Model# PP-UAG1003HU-K, UDOR, USA; see Figure E-5) was obtained to conduct the Task I pH-AB decontamination procedure. This procedure was conducted in the same manner as the procedure for the pressure washer in Section 2.4.1.2 above (two sprays of 15 seconds per set of three coupons). The only other variation was that a new pressure washer (Troy Bilt 2550 psi, Model 020337; see Figure E-6) was used for the DI rinse step.

**Figure E-5. Chemical sprayer**


**Figure E-6. Troy Bilt pressure washer**

**Rinsing with Water**

Rinsing of the coupons was accomplished using a standard garden hose (with nozzle) for Method 1 (backpack sprayer decon application) and for all Task II tests. For the pressure washer application of Spor-Klenz® (Method 2), the pressure washer was also used to rinse the first two sets of coupons (first material). However, when the pressure washer could not be restarted after the initial application on the first set of coupons for the second material, the garden hose (with nozzle) was used to rinse the coupons. For the chemical sprayer application of pH-adjusted bleach, an alternative (Troy Bilt) pressure washer was used for the rinse step.

The water used in this study was DI water produced by a Dracor water purification system (Model 34RC3). An Oakton pH probe was maintained in the water reservoir to continually monitor the pH and temperature. For Task I, the three coupons were sprayed with side-to-side strokes starting first from the top of the left-most coupon, working downward and the spray was moved across all three coupons in the decontamination chamber. Subsequent passes overlapped the previous by 50 percent. This was done from the central port on the chamber. For Task II, the coupon was sprayed starting at the top left in an alternating left to right, right to left motion, moving downward such that strokes overlapped by 50 percent, and finishing at the bottom right corner. For both Tasks, the start time and duration for this action was recorded and was performed as consistently as possible across all coupons.

**Rinsing with a Garden Hose**

For the garden hose, the water was supplied to the nozzle through a 75 ft garden hose of 5/8 in diameter. The head pressure was maintained constant at approximately 60 psi using a pressure regulator listed in Table E-1 of Appendix E. The water was supplied via a closed loop system having a 60-gallon tank as the reservoir and a pump to provide pressurized stream and continual recirculation (Figure E-7). Via adjustment of the nozzle, the spray pattern was controlled to be 1 ft in diameter measured at 3 ft from the nozzle. Application was for 10 seconds during Task I and for 30 seconds during Task II Test C1.

**Figure E-7: DI water supply system**

### Rinsing with a Pressure Washer

For the pressure washer, the water was supplied via a 75 ft garden hose of 5/8 in diameter. A circulation pump was used to supply water from the tank to the pressure washer. The original pressure washer used for the Spor-Klenz® test reports a pressure of 3300 psi and a flow rate of 3.2 gallons per minute. The replacement pressure washer used for the pH-AB test rinse reports a pressure of 2550 psi and a flow rate of 2.3 gallons per minute. The 25º angle nozzle attachment was used during this study. Application was for 10 seconds during Task I.

### Quality Control Measures

Additional measurements prior to or during the decontamination procedure application are also required in order to ensure quality control in the testing. These measurements include quality control checks on the reagents and equipment being used in the decontamination procedure. The pH and chlorine concentration of the pH-adjusted bleach solution have been shown to have a significant impact on the inactivation of *Bacillus* species spores. After preparation of the pH-adjusted bleach solution, the pH was measured using an Oakton pH probe. Additionally, the pH was measured during the decontamination testing after each set

of coupons was run within a test. The $Cl_2$ concentration was measured after preparation of the pH-adjusted bleach solution by Hach High Range Bleach Test Kit, Method 10100 (Model CN-HRDT). The temperature was also measured after the mixture was prepared and prior to the start of a new set of coupons within a test using a NIST-traceable thermometer.

The water pressure at the head of the garden hose (i.e., faucet) was controlled with a pressure regulator. The pressure was confirmed prior to each use of the hose. The flow rate and spray pattern from the hose were checked prior to the start of the decontamination test. The flow rate was measured using an inline flow meter. The spray pattern was visually verified to be nominally a 1 ft diameter (10 – 14 in) at the coupon surface from a distance of 3 ft between the nozzle and coupon face.

The pressure wash rinse used the 25º attachment, to minimize the amount of overspray and maximize the surface area covered by the spray pattern. This nozzle was also deemed the most appropriate for field use. The chemical sprayer had an adjustable nozzle similar to the garden hose, and the pattern was set at 1 ft diameter from a distance of 3 ft.

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

## Appendix F: Sampling Procedures

### F.1 Sampling Material and Equipment

The materials and equipment used for sampling are listed in Table F-1.

**Table F-1. Material and Equipment Used in Sampling**

| Material/Equipment | Description |
|---|---|
| Nonpowdered sterile surgical gloves | KIMTECH PURE* G3 Sterile Nitrile Gloves, Kimberly-Clark (VWR P/N HC61110 for extra-large; VWR P/N HC61190 for large; VWR P/N HC61180 for medium) |
| Nonpowdered nonsterile surgical gloves | Exam gloves (Fisherbrand Powder-Free Nitrile Exam Gloves, Fisher P/N 19-130-1597D (for large);19-130-1597C (for medium)) |
| Dust Masks | 3M Particulate Respirator 8271, P95 |
| Disposable laboratory coats | Kimberly-Clark Kleenguard A10 Light Duty Apparel, P/N 40105 |
| Disposable Bench Liner | |
| Phosphate Buffered Saline | Phosphate Buffered Saline with TWEEN® 20 (Sigma Aldrich, P/N: P3563-10PAK) |
| 50 mL conical tubes | BD Falcon® BlueMax Graduated Tubes, 15 mL (Fisher Scientific P/N 14-959-70C) |
| Sterile sampling bags | Fisherbrand Sterile Sampling Bags (TWIRL'EM®)<br>Overpack Size : 10” by 14”, P/N 01-002-53<br>Inner bag size: 5.5" by 9" (wipe);<br>Sample Bag Size: 5.5” by 9 “ |
| Bleach wipes | Dispatch® Bleach Wipes, P/N 69260 |
| Wipes | Kendall Curity Versalon absorbent gauze sponge 2" by 2" sterile packed (rayon/polyester blend)<br>(http://www.mfasco.com/) |
| Swabs | Bacti Swab®<br>(http://www.remelinc.com/Industrial/<br>CollectionTransport/BactiSwab.aspx) |
| Carboys (2) | Nalgene autoclavable carboys with tabulation (20 L) (Fisher Cat# 02-690-23) |
| Analytical Filter Units | 150 mL Nalgene Analytical Filter Units (0.2 µm Cellulose Acetate) (Fisher Cat# 130-4020) |
| Vacuum pump | Gas oil-free vacuum Pump with adjustable suction (Fisher Cat# 01-092-25) |
| Tubing | Fisher PVC clear tubing (1/2“ i.d., 1/16” thickness) (Fisher Cat# 14-169-7J) |

| Material/Equipment | Description |
|---|---|
| | Fisher PVC clear tubing (3/8" i.d., 1/16" thickness) (Fisher Cat# 14-169-7G)<br><br>Fisher PVC clear tubing (vacuum tubing)<br>(3/8" i.d., 1/8" thickness)<br>(Fisher Cat# 14-169-7H) |
| Filter cassettes | Via-Cell® Bioaerosol Sampling Cassette P/N VIA010<br>http://www.zefon.com/store/via-cell-bioaerosol-sampling-cassette.html |
| Sampling pump | Isokinetic Method 5 Source Sampling Console<br>Model 511E<br>http://www.apexinst.com/products/consoles.htm |

### F.2 Surface Sampling Procedures

Within a single Task I test, surface sampling of the coupons was completed for all procedural blank coupons first, followed by all test coupons, and then followed by all positive control coupons. Task II coupon areas were tested on different days, in the following order: blanks (day X), positive control (day X), test (day X). Surface sampling was done by wipe sampling in accordance with the protocol documented below. The surface area for all samples was 1.3 sq ft. A template was used to cover the exterior 0.25 in of each Task I coupon leaving a 13.5 in by 13.5 in square exposed for sampling. The outer 0.25 in around each coupon was not sampled in order to avoid unrepresentative edge effects. A large stainless steel template covering the entire coupon was used for Task II sampling. This template also prevented the outer edges from being sampled, and provided a 0.5 in border between samples

Prior to the sampling event, all materials needed for sampling were prepared using aseptic technique. The materials specific to each protocol are included in the relevant sections below. In addition, general sampling supplies were needed. A sampling material bin was stocked for each sampling event, using the information included in these sampling protocols. The bin contained enough wipe sampling kits to accommodate all required samples for the specific test. Additional kits were also included for backup. Enough prepared packages of gloves and bleach wipes were also included in the bin. Extra gloves and wipes were also included. Task I templates (1.2 ft by 1.2 ft with an interior opening of 13.5 in by 13.5 in) were prepared, sterilized, and packaged in sterile bags (7 templates per bag). These bags of templates were included with the sampling kits. A sample collection bin was used to transport samples back to the APPCD 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 APPCD Microbiology Laboratory.

### F.2.1 Coupon 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. 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. The protocol that was used in this project is described below and has been adopted from that provided by Busher et al.[11] Brown et al.[12], and documented in the INL 2008 Evaluation Protocols.[13] 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 Task I wipe sampling of each coupon surface:

1. A three-person team was used, employing aseptic technique throughout. The team consisted of a sampler, coupon handler, and 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” by 14”, 5.5” by 9 “) and a 50 mL conical tube, capped, were labeled in accordance with Appendix D. These bags and conical tube had the same label. The 5.5” by 9” 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 0.005% TWEEN®-20. 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” by 9” labeled sampling bag were placed into the 10” by 14” labeled sterile sampling bag. 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. All members 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 dust masks to further minimize potential contamination of the samples.
4. The coupon handler removed the coupon from the appropriate cabinet and placed the coupon on the sampling area. The sampling area was covered with a new piece of laboratory bench cover for each coupon.
5. The support person recorded the coupon code on the sampling log sheet.
6. The support person removed a template from the bag and handed it to the sampler.
7. The sampler placed the template onto the coupon surface.
8. The support person removed a sample kit from the sampling bin and recorded the sample tube number on the sampling log sheet next to the corresponding coupon code just recorded.

9. The support person:
    a. Opened the outer sterile sampling bag touching the outside of the bag.
    b. Touching only the outside of the overpack bag, removed and opened the unlabelled conical tube and poured the pre-moistened wipe onto the sample.
    c. Discarded the unlabeled conical tube.
    d. Maneuvered the labeled 50 mL conical tube to the end of the outer sterile sampling bag and loosened the cap.
    e. Removed the cap from 50 mL conical tube immediately preceding the introduction of the sample into the tube.
10. The sampler:
    a. 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.
    b. Folded the wipe concealing the exposed side and then wiped the same surface vertically using the same technique.
    c. Folded the wipe over again and rolled up the folded wipe to fit into the conical tube.
    d. 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.
11. The support person then immediately closed and tightened the cap to the 50 mL conical tube and slid the tube back into the sample collection sterile sampling bag.
12. The support person then put the 50 mL conical tube into the empty labeled 5.5” by 9” sampling bag and sealed the bag.
13. The support person then sealed the outer sample collection bag now containing the capped 50 mL conical tube (containing the sample wipe) inside a sealed 5.5” by 9” sample collection bag.
14. The support person then decontaminated the outer sample bag by wiping it with a Dispatch® bleach wipe.
15. The support person then placed the triple-contained sample into the sample collection bin.
16. If sampling from the coupon was completed, the coupon handler moved the coupon and template to the appropriate location for archival or discarding.
17. All members of the sampling team removed and discarded their gloves.
18. Steps 3 – 17 were repeated for each sample to be collected.

A very similar method was used for collecting the samples for Task II coupons. Changes were necessitated by the orientation of the coupon and the use of areas as samples.

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

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” by 14”, 5.5” by 9 “) and a 50 mL conical tube, capped, were labeled in accordance with Appendix D. These bags and conical tube had the same label. The 5.5” by 9” 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 0.005% TWEEN®-20. 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” by 9” labeled sampling bag were placed into the 10” by 14” 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.

2. While wearing gloves, the sampling team affixed a sterile sampling template to the sample. No personnel touched the coupon surface itself. Gloves were removed and discarded following template placement.
3. All members of the sampling team each donned a pair of sampling gloves (a new pair per sample); the sampler’s gloves were sterile sampling gloves. All members wore dust masks to further minimize potential contamination of the samples.
4. The support person recorded the coupon code and area on the sampling log sheet.
5. The support person removed a sample kit from the sampling bin and recorded the sample tube number on the sampling log sheet next to the corresponding coupon code just recorded.
6. The support person:

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

f. Touching only the outside of the overpack bag, removed and opened the unlabeled conical tube and poured the pre-moistened wipe into the hands of the sampler.

g. Discarded the unlabeled conical tube.

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

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

7. The sampler:
    a. Squeezed out the excess liquid from the wipe.
    b. 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.
    c. Folded the wipe concealing the exposed side and then wiped the same surface vertically using the same technique.
    d. Folded the wipe over again and roll up the folded wipe to fit into the conical tube.
    e. 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 then 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 put the 50 mL conical tube into the empty labeled 5.5” by 9” sampling bag and sealed the bag.
10. The support person then sealed the outer sample collection bag now containing the capped 50 mL conical tube (containing the sample wipe) inside a sealed 5.5” by 9” 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 4 – 17 were repeated for each sample to be collected.

### F.2.2 Swab Sampling

Swab sampling was used for sterility checks on coupons and equipment prior to use in the testing. A single swab sample was collected from each item and coupon. MOP 3135 was followed (see Appendix C), which employs a pre-moistened swab.

## F.3 Rinsate Collection and Sampling Procedures

During application of the decontamination procedure for each set of Task I coupons, the drain in the decontamination test chamber remained open. The runoff from the coupons throughout the entire decontamination procedure being tested was collected for a given coupon set (material type or all blanks) into a vessel which was pre-dosed with sodium thiosulfate (STS). The volume of STS needed to neutralize the total volume of decontamination liquid to be applied was determined by titration, and was set to 150% excess. After all coupons from a single set had been moved to the Decontaminated Coupon Cabinet or Procedural Blank Cabinet, the chamber was rinsed with DI water. For Task II, a rinsate collection vessel (trough) was placed under the coupon, and curtains arranged so that splashing rinsate drained into the trough. The trough was also dosed with enough STS to neutralize the decontamination liquid.

Analysis of the liquid was accomplished by filter-plating triplicate 100 mL aliquots of each rinsate sample. The collection procedure for the 100 mL aliquots was performed as follows:

1. Sampler donned a face mask, pair of examination gloves, disposable laboratory coat, and bouffant cap.
2. The contents of the carboy were agitated to ensure homogeneous mixing.
3. The carboy cap was removed.
4. Using a new 50 mL sterile pipette tip, 100 mL of sample was aseptically pipetted into a sterile 100 mL container.

Step 4 was repeated until triplicate samples were obtained.

The rinsate aliquots are triple-contained and transported to the Microbiology Laboratory for submission and analysis at the conclusion of the entire test according to MOP 6565 (see Appendix C). Briefly, spores in the rinsate sample were collected onto 0.2 µm pore-size analytical filters by vacuum filtration (Figure F-1). The filter was then placed (particulate side up) onto bacterial growth media and incubated 18 ± 2 hours at the optimal growth temperature. After incubation, colonies were enumerated on the filter surface by visual inspection as shown in Figure F-2 for Ba agent.

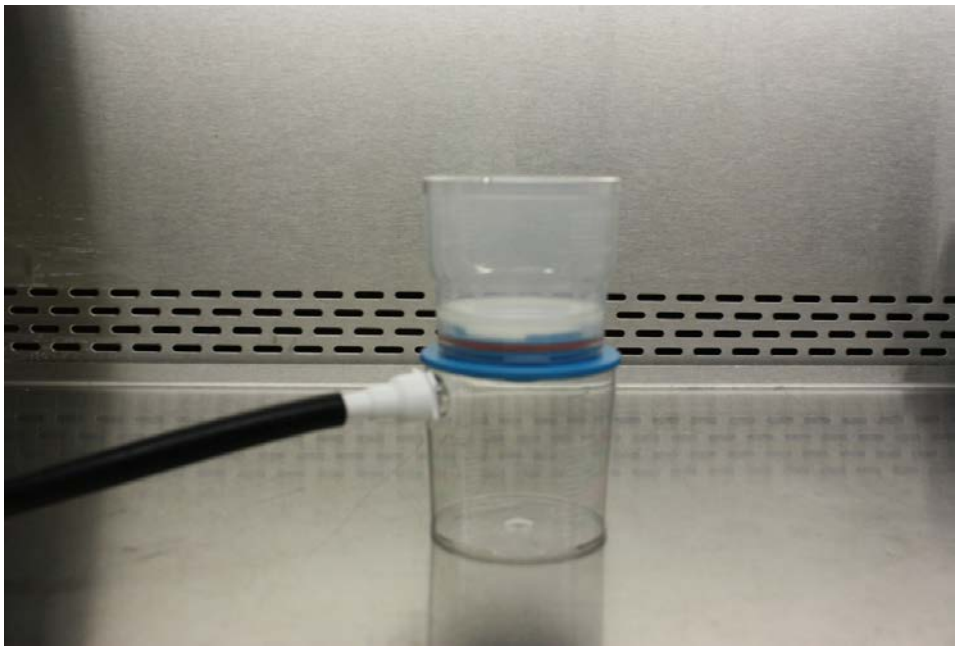

**Figure F-1. Nalgene Analytical Filter Unit connected to a Filter Unit.**

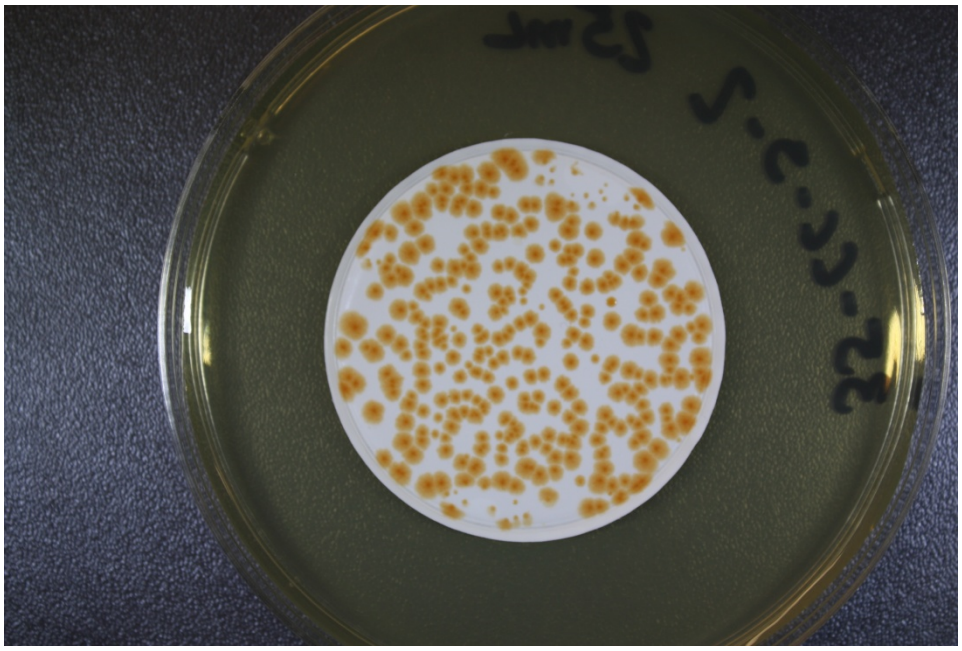

Figure F-2. Ba CFU on a Filter.

### F.4 Aerosol Sampling Procedures

The use of high-pressure hoses and pressure washers is expected to generate aerosols. There is potential for generated aerosols to contain viable spores removed from the coupon surfaces. Bioaerosol samples were collected from the decontamination chamber during all spraying activities. Zefon Via-Cell® Bioaerosol Sampling Cassettes (Figure F-3) were used to collect aerosol samples. During aerosol sample collection, the air concentration of chlorine gas (during pH-amended bleach application) or hydrogen peroxide vapor (during Spor-Klenz® application) was also monitored.

The Via-Cell® sampler was operated and analyzed according to the manufacturer's recommendations. (http://www.zefon.com/analytical/download/Via-Cell_Lab_Manual_Booklet.pdf). During Task I, separate aerosol samples were collected during the liquid decontamination application and the DI water rinse application. During Task II, separate aerosol samples were collected before each decontamination step, two samples during the decontamination step, and after the decontamination procedure to provide some baseline data similar to the procedural blank during Task I. The aerosol samples were analyzed according to MOP 6571 (see Appendix C).


**Figure F-3 Via-Cell® BioAerosol Cassette**

Filters are analyzed to determine viable CFU collected per volume of air sampled.

The following sampling procedure was used to collect the Via-Cell® samples:

1. With a clean pair of gloves, the Via-Cell® was removed from the foil pouch. The cassette and the pouch were labeled with the sample ID.
2. The small blue plug was removed and the cassette connected to the dry gas meter pump.
3. A leak-check was performed by turning on the pump with the inlet to the Via-Cell® closed capped off. The flow of air should have stopped. If not, all connections were checked.
4. The cap of the Via-Cell® was removed and affixed in the ambient air around the coupon to be decontaminated.
5. The starting volume on the dry gas meter (DGM) was recorded and the timer reset.
6. When time to collect a sample, the two switches on the meter box for the pump and the timer were simultaneously turned on. The sample ID, the time of day and the meter temperature were recorded.
7. The valve settings on the meter box were adjusted so that the delta H pressure reading was 1.1” water.
8. At the end of sample collection, the two switches on the meter box for the pump and the timer were simultaneously turned off. The final reading on the DGM, the meter temperature, and the elapsed time were recorded.
9. The cap of the Via-Cell® was replaced and the pump disconnected. The outlet plug was reinserted.
10. The Via-Cell® was placed in the foil pouch. The exterior of the pouch was wiped with a Dispatch® wipe, and placed in secondary containment.

## F.5 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.

After sample collection for a single test was complete, all samples were transported to the APPCD Microbiology Laboratory immediately, with appropriate chain of custody form(s).

In the APPCD Microbiology Laboratory, all samples were stored in the refrigerator at approximately 4 $^{o}$C until they were analyzed. All samples were allowed to stabilize at room temperature prior to analysis.

### F.6 Sample Holding Times

All samples were stored in accordance with Section F.5 and no longer than 10 days before being analyzed. A typical holding time for most samples was less than or equal to 2 days.

During the analysis procedure, samples could be stored in the refrigerator overnight after extraction and prior to the dilution plating. All samples were allowed to equilibrate to room temperature and were vortexed for 10 seconds prior to plating.

## Appendix G: Sampling Analyses

### G.1 Sample Analyses

The APPCD Microbiology Laboratory located in E-288 of the RTP, NC, campus facility analyzed all samples to quantify the number of viable spores per sample. For all sample types, phosphate buffered saline with 0.05% TWEEN®-20 (PBST) was 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 ($10^{-1}$ to $10^{-4}$), plated, incubated, and analyzed (CFU abundance) in accordance with MOP 6535a (see Appendix C).

In addition to the analysis in MOP 6535a, two additional analysis procedures were used for samples resulting in less than 30 CFU/sample in the zero tube (undiluted sample). These analyses were conducted in order to lower the current detection limit associated with MOP 6535a. First, 1 mL of the extract was filter plated in accordance with MOP 6565 (see Appendix C). The remainder of the sample was then filter plated in accordance with the MOP 6565.

The PBST was prepared according to the manufacturer's directions and in accordance with MOP 6562 (see Appendix C), dissolving one packet in one liter of sterile water. The solution was then vacuum-filtered through a sterile 0.22 µm filter unit to sterilize.

The extraction procedure used to recover spores was varied depending upon the different matrices (wipes, liquids, filter cassettes). These procedures are described in the following subsections.

### G.1.1 Recovery from Wipe Samples

The recovery of the spores from the wipe samples was done as follows (MOP 6567, Appendix C):

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. The 50 mL conical tube containing the sample wipe was removed from the double sterile bag and wiped with a bleach wipe. The analysts changed gloves after the wipe step.
3. A volume of 20 mL of PBST was added to each 50 mL conical tube by aseptically pouring a pre-measured volume.
4. The sample was then vortexed for 2 minutes in 10 second bursts, leaving the wipe in the same tube.
5. If the sample sat for more than one minute after Step 4, the sample was re-vortexed individually to homogenize prior to dilution plating. To complete dilution plating, the conical tube was uncapped and the cap placed underside up on the Bio Safety Cabinet surface while the aliquot was removed from the tube. Immediately after the aliquot was removed, the cap was aseptically replaced.
6. Each sample was processed individually. Steps 1-5 were repeated for each sample in the batch. Dilution plating occurred as described in Section G.1.

### G.1.2 Recovery from Liquid

Abundance of viable spores in the rinsate samples was determined by filtration of rinsate aliquots (MOP 6565). Filter samples were cultured on bacterial growth media, and recovery was determined by enumerating colony forming units (CFU). The abundance of spores in the original runoff water was determined by multiplying the calculated abundance of spores per milliliter of aliquot by the total runoff volume.

### G.1.3 Recovery from Air Sample

The extraction of the spores from the filters was done in accordance with MOP 6571 (see Appendix C). In short, the filter housing allows for in-situ extraction using 2 mL DI water. This suspension was then dilution plated in triplicate in accordance with Section G.1. The concentration of spores in the air was determined by dividing the total abundance of spores by the total sampled air volume.

**Appendix H: Test Reports**

## DCMD 3.41B: Effectiveness of Physical and Chemical Cleaning and Disinfection Methods for Removing, Reducing or Inactivating Agricultural Biological Threat Agents

### >>> Test Report <<<

Test Date: 10/12/2010
Test Number: 1 & 2
Test Team: R. Delafield, S. Terll K. Egler, S. Payne

Sampling Date: 10/13/2010
Sampling Team: R.Delafield, S.Terll, S. Payne

Analysis Date: 10/13/2010
Analyst: C. Slone, N. Griffin

**Surface Samples**

| | | Positive Controls | | | Blank Coupons | Test Coupons | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Material | Sample Type | Avg. CFU/sample | Mean of Logs | RSD (%) | CFU/ sample | Avg. CFU/sample | Mean of Logs | RSD (%) | LR | SD | Detection limit value? |
| Stainless Steel | wipe | 1.83E+07 | 7.25 | 27.03% | | | | | | | |
| Concrete | wipe | 2.33E+06 | 6.34 | 40.35% | 1.4E+01 | 6.32E-01 | -0.20 | 2.7% | 6.54 | 0.01 | TRUE |
| Wood | wipe | 4.46E+06 | 6.62 | 47.45% | 6.3E-01 | 7.24E-01 | -0.16 | 35.8% | 6.77 | 0.13 | FALSE |
| Blank | wipe | NA | NA | NA | NA | 6.25E-01 | -0.20 | 0.0% | NA | NA | TRUE |

| Decon Sets | Rinsate (Total CFUs) | Ambient Air CFU/L |
|---|---|---|
| Blanks | 2.68E+01 | <0.0292 |
| Concrete | <23.73 | 1.18E+01 |
| Wood | <26.05 | 5.41E+00 |

Detection limit values are in Yellow

Observations/Comments:

## DCMD 3.41B: Effectiveness of Physical and Chemical Cleaning and Disinfection Methods for Removing, Reducing or Inactivating Agricultural Biological Threat Agents

### >>> Test Report <<<

Test Date: 12/14/2010
Test Number: 3 and 4
Test Team: Rob Delafield, Stella Payne, Steve Terll

Sampling Date: 12/15/2010
Sampling Team: Rob Delafield, Stella Payne, Steve Terll

Analysis Date: 12/17/2010
Analyst: Griffin Gatachalian, Slone

**Surface Samples**

| Material | Sample Type | Positive Controls Avg. CFU/sample | Positive Controls Mean of Logs | Positive Controls RSD (%) | Blank Coupons CFU/ sample | Test Coupons Avg. CFU/sample | Test Coupons Mean of Logs | Test Coupons RSD (%) | LR | SD | Detection limit value? |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Stainless Steel | wipe | 2.75E+07 | 7.43 | 24.33% | | | | | | | |
| Concrete | wipe | 3.05E+06 | 6.46 | 28.09% | 6.7E-01 | 7.64E-01 | -0.14 | 36.6% | 6.60 | 0.13 | FALSE |
| Wood | wipe | 3.97E+06 | 6.55 | 55.45% | 5.9E-01 | 6.53E-01 | -0.19 | 3.7% | 6.74 | 0.02 | TRUE |
| Blank | wipe | NA | NA | NA | NA | 5.65E+00 | 0.41 | 125.8% | NA | NA | FALSE |

| Decon Sets | Rinsate (Total CFUs) | Ambient Air during decon CFU/L | Ambient Air during CFU/L |
|---|---|---|---|
| Blanks | <28.18 | <0.0584 | <0.19 |
| Concrete | <43.75 | 70.50 | 0.12 |
| Wood | 100.92 | 48.33 | <0.12 |

Detection limit values are in Yellow

Observations/Comments: The only deviation from the test parameters was that one set (3) of each coupon type was rinsed for 15 seconds instead of 10 seconds. The blanks (2 coupons) were rinsed for 10 seconds instead of 7 seconds. The viacells during decon (VD) and for rinse (VR) are for all 6 test coupons of each type.

## DCMD 3.41B: Effectiveness of Physical and Chemical Cleaning and Disinfection Methods for Removing, Reducing or Inactivating Agricultural Biological Threat Agents

### >>> Test Report <<<

Test Date: 10/27/2010
Test Number: 5 and 6
Test Team: Rob Delafield, Matt Clayton, Steve Terll

Sampling Date: 10/28/2010
Sampling Team: Rob Delafield, Stella Payne, Steve Terll

Analysis Date:
Analyst: Griffin Gatachalian, Slone

**Surface Samples**

| Material | Sample Type | Positive Controls Avg. CFU/sample | Positive Controls Mean of Logs | Positive Controls RSD (%) | Blank Coupons CFU/ sample | Test Coupons Avg. CFU/sample | Test Coupons Mean of Logs | Test Coupons RSD (%) | LR | SD | Detection limit value? |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Stainless Steel | wipe | 3.90E+07 | 7.58 | 20.95% | | | | | | | |
| Concrete | wipe | 2.62E+06 | 6.38 | 44.63% | 7.7E-01 | 6.06E+04 | 4.75 | 40.2% | 1.63 | 0.19 | FALSE |
| Wood | wipe | 5.27E+06 | 6.65 | 71.33% | 8.0E-01 | 7.03E-01 | -0.15 | 4.8% | 6.80 | 0.02 | TRUE |
| Blank | wipe | NA | NA | NA | NA | 6.60E-01 | -0.18 | 11.6% | NA | NA | TRUE |

| Decon Sets | Rinsate (Total CFUs) | Ambient Air CFU/L |
|---|---|---|
| Blanks | <11.74 | 0.06 |
| Concrete | <24.93 | 45.95 |
| Wood | <27.35 | 6.37 |

Detection limit values are in Yellow

Observations/Comments:

## DCMD 3.41B: Effectiveness of Physical and Chemical Cleaning and Disinfection Methods for Removing, Reducing or Inactivating Agricultural Biological Threat Agents

### >>> Test Report <<<

Test Date: 11/17/2010
Test Number: 7 and 8
Test Team: Rob Delafield, Kim Egler, Steve Terll

Sampling Date: 11/18/2010
Sampling Team: Rob Delafield, Stella Payne, Steve Terll

Analysis Date: 11/19/2010
Analyst: Griffin Gatachalian, Slone

**Surface Samples**

| Material | Sample Type | Positive Controls Avg. CFU/sample | Positive Controls Mean of Logs | Positive Controls RSD (%) | Blank Coupons CFU/ sample | Test Coupons Avg. CFU/sample | Test Coupons Mean of Logs | Test Coupons RSD (%) | LR | SD | Detection limit value? |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Stainless Steel | wipe | 2.23E+07 | 7.34 | 17.64% | | | | | | | |
| Concrete | wipe | 2.93E+06 | 6.40 | 53.90% | 6.5E+00 | 7.30E+03 | 3.60 | 117.4% | 2.80 | 0.55 | FALSE |
| Wood | wipe | 6.71E+06 | 6.79 | 44.66% | 6.3E-01 | 6.32E-01 | -0.20 | 1.8% | 6.99 | 0.01 | TRUE |
| Blank | wipe | NA | NA | NA | NA | 1.00E+00 | -0.03 | 47.1% | NA | NA | FALSE |

| Decon Sets | Rinsate (Total CFUs) | Ambient Air in Duct CFU/L | Ambient Air in Chamber CFU/L |
|---|---|---|---|
| Blanks | <11.86 | <0.0457 | 6.19 |
| Concrete | <28.88 | 0.23 | 2.01 |
| Wood | <27.12 | 0.28 | 6.71 |

Detection limit values are in Yellow

69204.15225

Observations/Comments: Equipment failure prevented the second decon application to the T8-T-TW (3-5) test coupons.
The T8-T-TW (3-5) test coupons were rinsed with the garden hose after a total contact time of 34 minutes.
The T8-T-TW (6-8) test coupons were not done as a result of the equipment failure.
For these tests the application time of the decontaminate was reduced to 15 seconds from 30 seconds. The rinse time remained 10 seconds.
A second application was applied after 15 minutes for a total contact time of 30 minutes.
The duct viacell fell apart and was pulled inside the duct during the concrete decon and rinse.
The ambient air in duct data is not available because the meter box ran backwards due to the vacuum created by the exhaust.

## DCMD 3.41B: Effectiveness of Physical and Chemical Cleaning and Disinfection Methods for Removing, Reducing or Inactivating Agricultural Biological Threat Agents

### >>> Test Report <<<

Test Date: 1/18/2011
Test Number: 9 and 10
Test Team: Rob Delafield, Stella Payne, Steve Terll

Sampling Date: 1/19/2011
Sampling Team: Rob Delafield, John Nash, Steve Terll

Analysis Date: 1/19/2011
Analyst: Griffin Gatachalian, Slone

**Surface Samples**

| | | Positive Controls | | | Blank Coupons | Test Coupons | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Material | Sample Type | Avg. CFU/sample | Mean of Logs | RSD (%) | CFU/ sample | Avg. CFU/sample | Mean of Logs | RSD (%) | LR | SD | Detection limit value? |
| Stainless Steel | wipe | 2.43E+07 | 7.39 | 3.20% | | | | | | | |
| Concrete | wipe | 1.46E+06 | 6.14 | 37.86% | 1.3E+01 | 7.02E-01 | -0.17 | 33.3% | 6.30 | 0.12 | FALSE |
| Wood | wipe | 1.88E+06 | 6.25 | 29.72% | 9.4E+00 | 1.50E+03 | 2.21 | 175.1% | 4.04 | 1.19 | FALSE |
| Blank | wipe | NA | NA | NA | NA | 4.14E+00 | 0.33 | 121.3% | NA | NA | FALSE |

| Decon Sets | Rinsate (Total CFUs) | Ambient Air during decon CFU/L | Ambient Air during rinse CFU/L |
|---|---|---|---|
| Blanks | <5.57 | #DIV/0! | #DIV/0! |
| Concrete | 1.31E+04 | 390.66 | 0.36 |
| Wood | 8.32E+04 | 385.54 | 4.78 |

Detection limit values are in Yellow

Observations/Comments: Due to the power washer running out of gas, the contact time on the second set of treated wood (T10-T-TW-(6-8)) was 22 min 55sec instead of 15 minutes. There did not seem to be any significant change in efficacy based on this variation.

The concrete results are based on a single spore on one coupon, so, while not a detection limit value, it is just above the detection limit.

**DCMD 3.41B: Effectiveness of Physical and Chemical Cleaning and Disinfection Methods for Removing, Reducing or Inactivating Agricultural Biological Threat Agents**

**>>> Test Report <<<**

Test Date: 2/8/2011
Test Number: C1
Test Team: Rob Delafield, Stella Payne, Tim McArthur

Sampling Date: 2/9/2011
Sampling Team: Rob Delafield, Matt Clayton, Tim McArthur

Analysis Date: 2/10/2011
Analyst: Griffin Gatachalian, Slone

**Surface Samples**

| Material | Sample Type | Positive Controls Avg. CFU/sample | Positive Controls Mean of Logs | Positive Controls RSD (%) | Blank Coupons CFU/ sample | Test Coupons Avg. CFU/ sample | Test Coupons Mean of Logs | Test Coupons RSD (%) | LR | SD | Detection limit value? |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Stainless Steel | wipe | 2.02E+07 | 7.29 | 27.27% | | | | | | | |
| Concrete (CVA) | wipe | 7.51E+05 | 5.84 | 48.1% | | 6.06E-01 | -0.22 | 2.1% | 6.1 | 0.01 | TRUE |
| Concrete (CVB) | wipe | 4.06E+06 | 6.60 | 23.1% | | 7.46E-01 | -0.15 | 37.8% | 6.7 | 0.14 | FALSE |
| Wood (TWA) | wipe | 3.42E+06 | 6.51 | 35.7% | | 1.28E+01 | 0.94 | 86.6% | 5.6 | 0.45 | FALSE |
| Wood (TWB) | wipe | 3.18E+06 | 6.50 | 16.8% | | 8.00E+00 | 0.75 | 89.1% | 5.7 | 0.44 | FALSE |

| Decon Sets | Ambient Air Before CFU/L | Ambient Air During CFU/L | Ambient Air After CFU/L |
|---|---|---|---|
| Decon 1 | 17.47 | 12.75 | 13.55 |
| Decon 2 | 2025.27 | 42.15 | 0.62 |
| Rinse | 1.50 | 0.15 | 0.23 |

| Coupon | Rinsate before Decon Total CFU | Rinsate after Decon Total CFU |
|---|---|---|
| CVA | 2.30E+04 | 1.30E+05 |
| CVB | 2.00E+04 | 2.84E+05 |
| TWA | 3.30E+03 | 1.51E+05 |
| TWB | 3.33E+03 | 1.41E+05 |

Observations/Comments:

Detection limit values are in Yellow

There was high contamination of air in COMMANDER prior to decon steps - this reduces usefulness of ViaCell data during and after Decon steps

Rinsates were also contaminated before decon, but levels after decon were higher.

Only 3 positive control samples for CWA

**DCMD 3.41B: Effectiveness of Physical and Chemical Cleaning and Disinfection Methods for Removing, Reducing or Inactivating Agricultural Biological Threat Agents**

**>>> Test Report <<<**

Test Date: 2/24/2011
Test Number: C2
Test Team: Rob Delafield, Matt Clayton, Tim McArthur

Sampling Date: 2/25/2011
Sampling Team: Rob Delafield, Stella Payne, Tim McArthur

Analysis Date: 2/28/2011
Analyst: Griffin Gatachalian, Slone, Levine

**Surface Samples**

| Material | Sample Type | Positive Controls Avg. CFU/sample | Positive Controls Mean of Logs | Positive Controls RSD (%) | Blank Coupons CFU/ sample | Test Coupons Avg. CFU/ sample | Test Coupons Mean of Logs | Test Coupons RSD (%) | LR | SD | Detection limit value? |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Stainless Steel | wipe | 8.33E+06 | 6.99 | 1.92E-01 | | | | | | | |
| Concrete (CVA) | wipe | 4.43E+06 | 6.63 | 27.6% | | 7.13E+01 | 1.27 | 96.5% | 5.4 | 1.10 | FALSE |
| Concrete (CVB) | wipe | 7.20E+06 | 6.84 | 31.3% | | 4.49E+00 | 0.09 | 193.0% | 6.8 | 0.68 | FALSE |
| Wood (TWA) | wipe | 2.67E+06 | 6.42 | 21.1% | | 1.25E+01 | 0.81 | 82.7% | 5.6 | 0.67 | FALSE |
| Wood (TWB) | wipe | 3.25E+06 | 6.50 | 20.6% | | 1.67E+01 | 0.92 | 95.4% | 5.6 | 0.67 | FALSE |

| Decon Sets | Ambient Air Before CFU/L | Ambient Air During CFU/L | Ambient Air After CFU/L |
|---|---|---|---|
| Decon 1 | 4.07 | 27.93 | 2.97 |
| Decon 2 | 1.15 | 0.91 | 0.07 |
| | | | |

| Coupon | Rinsate before Decon Total CFU | Rinsate after Decon Total CFU |
|---|---|---|
| CVA | 4.93E+03 | <105.11 |
| CVB | 1.87E+03 | <398.33 |
| TWA | 1.00E+03 | <74.65 |
| TWB | 3.73E+02 | <204.67 |

Observations/Comments: Detection limit values are in Yellow

Some rinsate samples have returned lower CFUs in subsequent plating (see C2-R-CVA-2) - Possibly due to a decontaminating agent in the rinsate itself. (Bleach, STS)?

No rinse during this test.


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