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United States
Environmental Protection
Agency

Assessment and Evaluation Report

Evaluation of Surface Sampling for *Bacillus* Spores Using Commercially-available Cleaning Robots

Office of Research and Development
National Homeland Security Research Center

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Disclaimer

The United States Environmental Protection Agency, through its Office of Research and Development's National Homeland Security Research Center, funded and managed this investigation through EP-C-09-027 WA 2-29 and 3-29 with ARCADIS U.S., 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. This report includes photographs of commercially available products. The photographs are included for purposes of illustration only and are not intended to imply that EPA approves or endorses the product or its manufacturer. Environmental Protection Agency does not endorse the purchase or sale of any commercial products or services.

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Acknowledgments

This effort was managed by the principal investigator from ORD's National Homeland Research Center (NHSRC), and funded through the Wide Area Recovery and Resiliency Program by the Department of Homeland Security Science and Technology Directorate (DHS S&T) under interagency agreement (# RW-70-95812401). The authors acknowledge Chris Russell (formerly, DHS S&T) and Lori Miller (DHS S&T) for their support of this work.

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This effort was completed under U.S. EPA contract #EP-C-09-027 with ARCADIS-US, Inc. The support and efforts provided by ARCADIS-US, Inc. are gratefully acknowledged. The support provided by Tanya Medley (NHSRC) in acquiring the vast quantities of supplies required for the completion of this project is also acknowledged.

Additionally, the authors would like to thank the peer reviewers for their significant contributions. Specifically, the efforts of Frank Schaefer (NHSRC), Rebecca Connell (US EPA), and Don Bansleben (DHS S&T) are recognized.

Table of Contents

List of Figures

List of Tables

List of Appendices

List of Acronyms and Abbreviations

ADA	Aerosol Deposition Apparatus
AFSD	Automatic Floor Sampling Device
ATCC	American Type Culture Collection
B.	*Bacillus*
BSC	Biological Safety Cabinet
CBRN	Chemical, Biological, Radiological, and Nuclear
CFU	Colony Forming Units(s)
CM	Critical Measurements
CMAT	Consequence Management Advisory Team
COC	Chain of custody
COMMANDER	Consequence Management and Decontamination Evaluation Room
COTS	Consumer Off-the-Shelf
CR	Comparable Recovery
CT	Concentration x Time
DCMD	Decontamination and Consequence Management Division
DHS	Department of Homeland Security
DI	Deionized
DF	Decimal Factor
DPG	Dugway Proving Ground
DQI	Data Quality Indicator
DQO	Data Quality Objective
ECBC	Edgewood Chemical Biological Center
EPA	U. S. Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GMP	A product name, rather than an acronym
HEPA	High-Efficiency Particulate Air
lpm	Liter per minute
LR	Log reduction
m	Meter
MDI	Metered Dose Inhaler
MOP	Miscellaneous Operating Procedure
NDT	National Decontamination Team
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
ORLS	On-Site Research Laboratory Support
OPP	Office of Pesticides Programs
ORD	Office of Research and Development
OSWER	Office of Solid Waste and Emergency Response

PARTNER	Program to Align Research and Technology with the Needs of Environmental Response
PBST	Phosphate Buffered Saline with Tween20
PPE	Person Protective Equipment
ppm	parts per million
ppmv	parts per million by volume
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RH	Relative Humidity
AFSD	Automated Floor Sampling Device
SOP	Standard Operating Procedure
TBD	To Be Determined
TSA	Tryptic Soy Agar
VHP	Vaporized Hydrogen Peroxide
WAM	Work Assignment Manager

Executive Summary

The existing surface sampling strategy for a post-terror incident involving the release of *Bacillus anthracis* spores requires the use of various methods depending on the surface type. The established surface sampling methods for *B. anthracis* spores include wet wipes (for smooth nonporous surfaces) or wet sponge wipes, vacuuming (for rough and porous surfaces), and wet swabs (for small and/or hard to sample areas such as keyboards). These methods can be labor intensive and expensive to deploy since they require sampling personnel to wear appropriate personal protective equipment (PPE) to reduce the risk of exposure to pathogenic agents. The general process being investigated in this project is to assess an alternative cost-effective, reliable sampling technique for various surfaces contaminated with *Bacillus* spores (i.e., surrogates of *B. anthracis*) using commercially available, off-the shelf Automated Floor Sampling Devices (AFSD).

Three commercially available autonomous (robotic) vacuum-based cleaning robots (R1, R2 and R3), one wipe-based robot (R4) and one wet vacuum-based robot (R5) were evaluated as AFSD for their sampling efficiency on non-porous surfaces (laminate and tile). The first three vacuum-based AFSD were also evaluated on a porous surface (carpet). The two wipe and wet vacuum-based AFSD were not tested on carpet because of their recommended usage only on hard surfaces, according to the instruction manuals. The evaluation criteria for the robotic cleaners included vacuum efficiency, availability, and cost. The top two performers were then further evaluated to investigate their sampling capabilities at multiple levels of contamination. In addition, these two AFSD were each challenged with two contamination scenarios, a low level, large spatial extent contamination (wide contamination) scenario in which ~40% of the total area sampled was experimentally inoculated with spores (~0.1 and 10 colony forming units (CFUs) per cm^2), and a high level, small spatial extent contamination (hot spot) scenario in which ~2% of the test area was experimentally loaded with spores (~10^4 CFUs per cm^2).

The sampling efficiencies of these AFSD were assessed by comparing their recoveries (CFUs) to recoveries obtained using currently-used surface sampling methods. The overall results show that sampling via AFSD is a viable option, when compared to traditional sampling methods. Some AFSD for porous and non-porous materials were as efficacious as the respective surface sampling methods that are currently recommended.

The AFSD sampling comparative recovery (CR) results for a laminate surface were higher for the wet wipe and wet vacuum-based AFSD (up to 62% and 32%, respectively) than for the vacuum-based AFSD (CR less than 10%) that were tested. The sampling process of the wet wipe-based AFSD is similar to the well established wet wipe surface sampling method since both methods use a wetted cloth in conjunction with a rubbing action on the surface. Low CRs from vacuum units were expected since previous sampling studies have shown that the surface sampling using the wet wipe or the sponge wipe method on nonporous surfaces has higher recovery efficiency than vacuum-based sampling methods.

The CRs for porous material (carpet) sampling were determined by comparison of the number of spores (CFUs) recovered using three vacuum-based AFSD to that of the vacuum sock sampling method. The test results showed CR values on the same order or greater (in some cases up to 161%) than the vacuum sock sampling method. The differences in CRs among the three vacuum-based AFSD may be related to the unique design and operating conditions of each device.

Two AFSD types from the scoping test were selected for further evaluation in a more complicated environment, such as a larger spatial scale. The test results demonstrated the capability of AFSD sampling of spores from carpet and laminate surfaces under two test scenarios (hot spot and wide contamination). Further, only minimal contamination of the non-inoculated adjacent surfaces was observed. The same AFSD were tested on larger floor areas inoculated at lower concentrations and showed comparable results to the comparative surface sampling methods. This information may help design targeted decontamination strategies, and possibly assist in the determination of the spatial distribution of the spore attack.

Aerosol recoveries of spores observed during sampling for all five types of AFSD and all type of materials tested showed small, but detectable, spore re-aerosolization. The observed relative differences in the level of spore re-aerosolization for each AFSD/material combination are presumably due to the presence of surface agitation devices (brush or a beater bar) on these units, and the type of AFSD sampling scheme (vacuum-based versus wet-wipe sampling).

The current test method focused only on the sampling mechanism of the individual AFSD by limiting sampling surface area. Varying the area cleaning logics or algorithms of individual AFSD was not part of this study. However, varying the area cleaning logics or algorithms of individual AFSD could be a way to increase collection efficiency when sampling a wide area.

Currently available AFSD have various convenient functions such as self-recharging, mapping, navigation, etc. These functions will allow large contaminated areas to be sampled systematically. Two obvious benefits of using AFSD for wide area sampling rather than the currently used sampling methods include (1) fewer samples, because one composite sample is generated per deployment, and (2) less risk of personnel exposure to *B. anthracis* spores. In addition to wide area sampling, these AFSD could be deployed to areas where human sampling is difficult, such as inside HVAC ductwork and in highly contaminated areas (hot zones). However, for real world application, these AFSD need further evaluation with various surfaces, deposition types, surface loadings, and environmental conditions (relative humidity variation, exposure duration, etc.).

1 Introduction

After the 2001 intentional *Bacillus anthracis* spore contamination incidents in the U.S., many studies have been conducted to develop and improve the remediation process of contaminated buildings [1-6]. Since 2001, surface sampling studies have been especially emphasized because of their direct impact on decision making during on-site remediation activities.[7, 8] Accordingly, sound and defensible protocols and implementation plans for surface sampling are needed but not yet adequately developed.[9] Numerous studies have tested surface sampling methods to evaluate and/or validate their efficacy on various surface types under numerous environmental conditions.[10-18] As a result, surface sampling methods have been improved and optimized for real world application. However, there are still large gaps surrounding sampling and analysis following a large urban area biological terror attack. [19] A *B. anthracis* release over a wide and highly populated area would tremendously increase the time, cost and complexity to return the contaminated area to normalcy. Currently-used sampling methods are limited to small areas {10 cm^2 to 1 m^2 (0.01 ft^2 to 10.8 ft^2)} and would require the collection of a large number of samples in order to be representative if deployed over a large spatial scale. Such a sample burden would strain sample processing laboratories during characterization and remediation and delay the overall recovery. Although efforts have been made to increase the number of laboratories capable of processing biological agent samples, the current capacity may be a limiting resource during recovery operations following a wide-area attack.[9, 20]

The currently-used surface sampling methods include wet wipes, vacuuming, and wet swabs. The existing spore sampling strategy requires the use of varied methods depending on the surface types, e.g., wet wipes for smooth nonporous surfaces, vacuuming for rough and porous surfaces, and wet swabs for small and/or hard to sample areas such as keyboards. These methods can be labor intensive and expensive to deploy since they require sampling personnel to wear appropriate personal protective equipment (PPE) to reduce the risk of exposure to pathogenic agents. Commercially-available domestic cleaning robots could be an alternative for *B. anthracis* spore surface sampling. These cleaning robots were introduced and commercialized since the early 1980s for home and industrial use.[21] The cleaning mechanisms of these robots are similar to the current surface sampling methods such as vacuuming, sweeping, and scrubbing. Robots have been developed with various convenient functions and sensors to improve cleaning performance[22], and can clean approximately 2 to 4 rooms {100 to 400 m^2 (1076 ft^2 to 4305 ft^2)} per charge according to the manufacturers' claims. Using such cleaning robots as "automated floor sampling devices" (AFSD) for *B. anthracis* spore surface sampling would reduce the number of required samples (consequently reducing the burden on laboratories) and personnel compared to the current sampling methods. AFSD can collect composite samples, thereby sampling numerous buildings and large surface areas efficiently and economically. This study investigates the collection efficiency of AFSD for *B. anthracis* spore sampling compared to the current, conventional, surface sampling methods.

1.1 Process

The general process being investigated in this project is sampling of surfaces contaminated with *Bacillus* spores (i.e., surrogates of *B. anthracis*). Commercially available cleaning robots (AFSD) were evaluated for their suitability and robustness for such surface sampling. The cleaning robots for this study were selected based on their availability in the United States and price ($50 - $500). Three vacuum-based AFSD and two mopping-based AFSD were chosen for laboratory evaluation of their efficacy at sampling *Bacillus* spores. The top two performers based upon comparative sampling efficiencies were then further

evaluated at multiple levels of contamination and on several spatial scales. In all cases, the AFSD were compared to traditional surface sampling techniques.

1.2 Project Objectives

This work was designed to evaluate AFSD by generating data on the effectiveness of the device for collection of *Bacillus* spores on different materials and under varied environmental conditions compared to currently-used surface sampling methods.

1.3 Experimental Approach

In this study, AFSD were evaluated for their ability to collect *Bacillus* spores from environmental surfaces. The current study determined the sampling efficiency of each AFSD, without modifying the sensors, algorithms, or logics set by the manufacturers. Aerosol deposited *B. atrophaeus* spores were used as a surrogate of *B. anthracis* spores. Test results were compared to currently-used surface sampling methods (vacuum sock and sponge wipe). Air was sampled using a bio filter sampler to evaluate the potential for re-aerosolization of spores during the sampling process using AFSD.

1.3.1 Testing Approach

Coupons representing three types of flooring materials were fabricated and sterilized before use. Floor types included laminate flooring, carpet and tile. Coupons were inoculated with *Bacillus atrophaeus* spores by aerosol inoculation using custom designed dose chambers. After inoculation, coupons were transported into the Consequence Management and Decontamination Evaluation Room (COMMANDER), a specially constructed enclosed, single-access-point chamber (henceforth, chamber) within the current Homeland Security Enclosure located within High-Bay Room 130 (H130) at EPA's Research Triangle Park, NC campus. The dosing chambers were removed from the coupons and then each coupon was placed in a small secondary isolation AFSD testing chamber to prevent cross-contamination and to help quantify re-aerosolized spores. AFSD were then used to sample the inoculated material, during a predetermined or robot-determined amount of time. Collected spores were then recovered from the collection bins and filters of each AFSD using liquid extraction-based techniques. Culture-based methods were subsequently used to quantify the number of spores recovered by enumeration of CFUs on microbiological growth media after plating serially-diluted aliquots of sample extracts. Therefore, in this report "recovery" is defined by the number of CFUs observed following sample collection, extraction, and analysis (dilution-plating or filter-plating).

2 Materials and Methods

2.1 AFSD Testing Chamber

Each AFSD testing chamber (91cm x 91cm x 46 cm) was constructed of clear acrylic material (5 mm thickness) and the inside surface was coated with antistatic film (chemical-resistant PVC (Type I) antistatic Film, McMaster-Carr, Princeton, NJ). The diagram of the chamber is shown in Figure 2.1. The chamber had one port located on the lid that was used for air sampling. Another port was located on the front of the chamber and was outfitted with a HEPA filter so that sterile make-up air could be supplied during sampling. Chamber air was sampled (15 LPM for 20 min) using a bio filter sampler (Via-Cell® Bioaerosol Sampling Cassette, p/n VIA010, Zefon International, Inc., Ocala, FL) to determine the potential for re-aerosolization of spores during AFSD sampling.

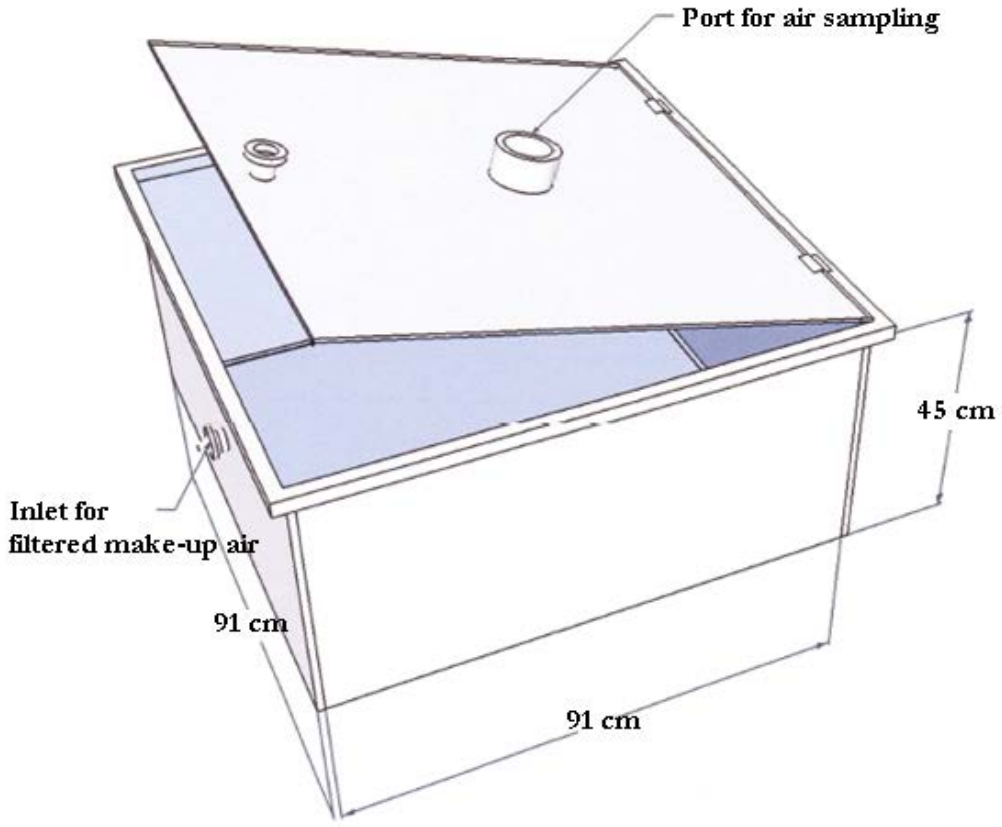

Figure 2-1: Isolation chamber for coupon and AFSD

2.2 AFSD

The five commercially-available cleaning robots purchased from an internet retail store and evaluated as AFSD are summarized in Table 2-1.

Table 2-1. List of AFSD evaluated in the current study

AFSD	Model	Manufacturer	Cleaning type	Applicable Surfaces
R1	Roomba 760	iRobot	Vacuum with bristle brush	All surfaces
R2	XV-11	Neato	Vacuum with silicone flat beater	All surfaces
R3	P3 P4920	P3 International	Vacuum (no surface agitation tool)	All surfaces
R4	Mint 4200	Evolution Robotics	Sweep and mop	Hard floor
R5	Scooba 390	iRobot	Wet vacuum	Hard floor

R1, R2, and R3 are vacuum-based cleaning AFSD and were tested on both carpet and laminate surfaces. R4 and R5 are wet wipe- and wet vacuum- based AFSD, respectively, and were tested only on laminate surfaces (not on carpet) as instructed by the factory manuals. All AFSD were removed from the shipping box and sterilized inside COMMANDER by exposure to 250 ppmv of vaporized hydrogen peroxide (VHP®, 1000ED, Steris, Mentor, OH) for 4 hours before testing. The sterilized AFSD were degassed one to three days to remove residual fumigants. All AFSD retained their factory settings during testing, and each AFSD was used only once before being discarded. All AFSD, except R3, possess internal logic that allows the device to sample a discrete or predefined space and subsequently deactivate itself. For these AFSD, the sampling duration was, therefore, determined by the AFSD itself. R3 units were manually operated for an amount of time equivalent to those AFSD with the longest sampling duration (R1 for carpet and R5 for laminate). When operating manuals required liquid inputs, sterile Phosphate Buffered Saline with Tween-20 (PBST) was used rather than water or soapy water. For example, the R4 wipe material was soaked with the PBST and the R5 “clean tank” was filled with PBST before testing. Spore recovery efficiencies from the collection components (i.e., filters or collection bins) of individual AFSD types were separately evaluated prior to conducting coupon-based testing. For these preliminary recovery tests, a predetermined amount of *B. atrophaeus* spores (in PBST) were spiked onto the filters and collection bins of each AFSD and allowed to dry. Extraction procedures were conducted according to the procedures outlined in Section 2.3.5. Extraction efficiencies were determined by comparing recoveries from AFSD to recoveries where extraction buffer was directly spiked with the same liquid inoculum.

2.3 Test Materials and Deposition

2.3.1 Test Coupons Preparation

AFSD sampling tests were conducted with three floor surface types: laminate (Pergo Estate Oak , PE-191113), carpet (Beaulieu Laredo Sagebrush loop carpet, Model 6666-01-1200-AB), and tile (Marazzi Island Sand Glazed Ceramic Tile, Model UG4W). These materials were purchased from a local retail store (Home Depot, Durham, NC). Coupons were fabricated from all three surface types into 107 cm x 107 cm and 71 cm x 71 cm size pieces for AFSD sampling tests and 36 cm x 36 cm for vacuum or sponge wipe sampling tests. Both coupon types were backed with an equal-sized piece of 1.1 cm thick Oriented Strand Board (OSB) plywood. Prior to use in tests, carpet coupons were vacuumed to remove

the detachable foreign debris and particles, while laminate and tile coupons were cleaned with a dry wipe (SIMWyPE tack cloth). After surface cleaning, coupons were sterilized by exposure to VHP® (250 ppmv hydrogen peroxide vapor for 4 hours). The sterilized coupons were stored in sterilization bags (General Econopak Inc., Philadelphia, PA, P/N 63636TW) until tested. After sterilization, coupons were degassed for a minimum of three days before testing. The sterility of the coupons and other equipment needed for the inoculation were confirmed by sampling at least one coupon per sterilization batch and one representative piece of each inoculation equipment by using a Bactiswab (Remel Products, Lenexa KS, P/N R12100) for sampling their respective surfaces. The swabs were subsequently streaked onto tryptic soy agar (TSA) (BD, Franklin Lakes, NJ) plates, and the plates were incubated at 35 ± 2°C for at least 18 hours before being visually inspected to determine if bacterial growth (i.e., contamination) was present.

2.3.2 Bacillus Spore Preparation

The *B. anthracis* surrogate used for this study was a powdered spore preparation of *B. atrophaeus* (ATCC 9372, Manassas, VA) and silicon dioxide particles. This bacterial species was formerly known as *B. subtilis* var *niger* and subsequently *B*. *globigii*. The preparation was obtained from the U.S. Army Dugway Proving Grounds (DPG) Life Science Division. The preparation procedure is reported in Brown et al. [23] 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 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) or by ARCADIS-US, Inc., according to a proprietary protocol. The MDI preparation and characteristics can be found in Lee et al. and the references therein.[24]

2.3.3 Coupon Inoculation

The sterilized coupons were inoculated using the method described in the study by Calfee et al. [25, 26] Coupons were inoculated with between ~5 x 10^2 and ~1 x 10^9 spores, depending on the desired target inoculum levels. The consistency and loading levels of inoculums were verified using four stainless steel control coupons during each inoculation event. These inoculation control stainless steel coupons were sterilized via steam autoclave (Steris, Mentor, OH), inoculated, and then sampled with the sponge wipe method as described in Miscellaneous Operating Procedure (MOP) 3165. Stainless steel surfaces were inoculated concurrently with test samples and used to verify the magnitude and repeatability of the inoculation procedures, since stainless steel surfaces have been shown to provide highly repeatable recoveries.[10, 12, 27] Test coupons used for AFSD sampling tests were inoculated in the centermost 30 cm x 30 cm area of the coupon. The same size area was inoculated on the comparative surface sampling method coupons. All test coupons underwent the same inoculation procedures, and were stored together until used in testing. Each coupon was inoculated independently using separate dosing chambers, originally designed for inoculation of the centermost 30 cm x 30 cm area of 36 cm x 36 cm coupons. Following a metered dissemination, spores were allowed to settle onto the coupons for a minimum period of 18 hours.

2.3.4 AFSD Testing Procedure

AFSD sampling tests were conducted inside COMMANDER, a controlled chamber. COMMANDER was controlled for the temperature (22 ± 0.7°C) and relative humidity (57 ± 5%). More detailed information about COMMANDER can be found in prior publications.[28, 29] AFSD sampling tests were conducted with carpet, laminate, and tile surfaces inside AFSD testing chambers. Each test (one AFSD and one surface type) was conducted with four AFSD testing chambers (one blank and three test replicates) inside COMMANDER. First, the test started with a blank AFSD sampling. A sterilized coupon was placed in the middle of an AFSD testing chamber and a sterilized AFSD was placed on the bottom left corner of the coupon. The cleaning start button was pressed and the lid to the AFSD testing chamber was closed. Air sampling was initiated simultaneously with the onset of AFSD sampling process and the sampling duration was monitored. After the blank sampling, *B. atrophaeus* spore-inoculated test coupons (one coupon per an AFSD testing chamber) were sampled with AFSD. The inoculated coupons were placed in an individual AFSD testing chamber, taking care not to touch the inoculated surface. After the completion of sampling, the AFSD were powered off, removed from the testing chamber one at a time, and disassembled for retrieval of the sample per Table 2-2. The AFSD sampling duration was recorded and included the total time that each AFSD was sampling actively.

Table 2-2. Components from AFSD extracted for analysis

AFSD	Components extracted for sample	Treatment
R1 – R3	Collection Bin and Filter	Inlet sealed with Sterilized Parafilm during transport
R4	Mop cloth	Aseptically placed in sterile sample bag.
R5	Retrieved liquid from “Dirty” tank	Tank triple rinsed with sterile PBST, packed in leak-proof jar
	Filter	Aseptically placed in sterile sample bag.

The AFSD components were placed in a sterilized plastic bag. Each bag was then secondarily contained in another bag and transported to the on-site Microbiology Laboratory for processing. Filters from air sampling units were aseptically removed and placed in a sterile plastic bag or specimen cup for analysis in the Microbiology Laboratory.

2.3.5 Comparative Surface Sampling Methods

To evaluate the collection performance of the AFSD, AFSD recoveries were compared to recoveries obtained by currently-used surface sampling methods. Control coupons of carpet, tile, and laminate were sampled using currently-used surface sampling methods.[9, 11, 15, 16, 20] Laminate and tile surfaces were sampled with a sponge wipe sampling method and carpet surfaces with a vacuum sock method. An area of 34 cm x 34 cm was sampled with the sponge wipe, delineated with a sterile stainless steel template placed over the target area. Sponge wipe samples, described in MOP 3165, were collected

using the following 5 steps: (1) using one flat side of the sponge wipe, the surface was sampled using horizontal S-strokes, covering the entire template area; (2) the sponge wipe was then flipped over to the opposite flat side to sample the surface in a vertical S-stroke pattern, covering the entire template area; (3) using the narrow edges of the sponge wipe, the surface was sampled using the same S-strokes but applied diagonally across the template, (4) rotating the sponge to use the opposite side at the midway point of the coupon; and (5) the tip of the sponge wipe was then used to sample the perimeter of the sampling area. The sampling method is described in detail in the study by Rose et al.[16]

Vacuum socks are the currently-used method for sampling porous surfaces, and were therefore used as the comparative method for collection of spores from carpet surfaces. During vacuum sampling, a 34 cm x 34 cm sterile stainless steel template and a sterile sock/nozzle attachment were used to collect the sample. The nozzle was lightly pressed against the coupon surface while holding the nozzle at a 45 degree angle to the sampled surface. The samples were collected using horizontal and vertical S-strokes. This method, described in MOP 3145, is a modified version of the method detailed in the study by Brown et al.[11].

Wipe sampling was done on non-porous surfaces that had undergone AFSD sampling. These samples were collected as a quality control check to validate inoculation had occurred. Gauze wipe sampling, described in MOP 6567 was performed on the entire 71 cm x 71 cm surface. This method is better adaptable to large surface area sampling than sponge wipe samplers; the sponges are pre-moistened with a fixed volume of liquid and can become dry if sampling a large area. Checks were done to verify sterility using swab samples collected according to MOP 3135. All relevant MOPs are included in the appendix.

2.3.6 Aerosol Sampling

ViaCell® bio-aerosol cassette samples were collected according to MOP 3155 for 20 minutes beginning with the start of AFSD sampling to evaluate the potential for re-aerosolization. A flow rate of 15 liters per minute (lpm) was used and measured by a calibrated dry gas meter.

2.3.7 Sample Extraction and Spore Recovery

Sponge wipe (PN SSL10NB, 3M Inc., St. Paul, MN) samples were extracted by stomaching (1 minute, 260 RPM) in 90 ml of PBST using a Seward® Model 400 circulator (Seward® Laboratory Systems, Inc, Port Saint Lucie, FL). Vacuum sock samples were extracted by first wetting the collection (white) portion of the filter by dipping in PBST, then cutting it with sterile scissors (vertically then horizontally) into small pieces (approx. 1 cm x 4 cm). As the filter was fractioned, the resulting pieces were allowed to fall into a 120 ml sterile specimen cup (Starplex Scientific LeakBuster Specimen Containers - Fisher Scientific #14-375-459) containing 20 ml sterile PBST. The cups were then agitated (30 minutes, 300 RPM, ambient temperature) using an orbital platform shaker incubator (Lab-Line, Model 3625). Spores collected by R4 were recovered from the mopping cloth by stomaching the cloth (2 minutes, 230 RPM) in 133 ml PBST using a Seward® Model 400 circulator.

Two extraction procedures were required for R1, R2, and R3, as collected spores could have partitioned to either the collection bin or the filter. Recovery from the filters of R1 proceeded by placing both filters (each AFSD is equipped with two filters) into a 120 mL specimen cup (Starplex Scientific Inc., Cleveland, TN, PN 3008-3TN), adding 90 mL PBST, and then agitating (30 minutes, 300 RPM) on an orbital platform

shaker (Lab-Line, Model 3625). Recovery from the filters of R2, and R3 proceeded by placing each filter into two 14 cm x 23 cm sterile sample bags (Fisher Scientific, P/N 01-002-53), one inside the other for double containment. 180 ml of sterile PBST was then added to the innermost bag, and the samples were agitated (30 minutes, 300 RPM) on an orbital platform shaker (Lab-Line, Model 3625). Spore recovery from the particle bins of R1, R2, and R3 was accomplished by placing the bins into double 25 cm x 38 cm sterile sample bags, aseptically adding 180 ml of PBST to each bag containing the bin, and then agitating (30 minutes, 300 RPM, ambient temperature) on an orbital platform shaker incubator (Lab-Line, Model 3625).

The wet vacuum AFSD (R5) also required two extraction procedures, one procedure for the liquid fraction and liquid collection reservoir and one procedure for the filter. First, the original 60 mL sterile PBST R5-collected liquid was retrieved from the "dirty tank" using a 100 ml sterile serological pipette. The reservoir was then rinsed twice with 60 ml PBST, and the three fractions were combined (for a total of 180 ml). The filters from R5 were extracted with the same procedures used for filters from R2 and R3. The resulting liquid extracts from all AFSD and all fractions were then each concentrated by centrifugation where briefly, each sample was retrieved from its respective extraction bag or cup, and dispensed equally into four 50 ml conical tubes (~45 ml for each tube). The samples were then centrifuged (3500 x g, 15 min, 4°C) to sediment the collected spores. All but 5 ml of the supernatant was carefully removed using a 50 ml sterile serological pipette. Each spore pellet was resuspended in the remaining 5 ml by three cycles of alternating vortex mixing (30 seconds) and sonication (30 seconds, 40 kHz, Branson Model 8510).

Following resuspension, the four fractions per sample were recombined into one ~20 ml sample extract. All sample extracts (AFSD, vacuum sock, and sponge wipe) were then subjected to a series of ten-fold dilutions, as necessary, by adding 0.1 ml of the sample to 0.9 ml of PBST using a micropipette. Appropriate dilutions were spread in triplicate (0.1 ml each) onto trypticase soy agar (BD™; Becton, Dickinson, and Company; Franklin Lakes, NJ) plates and incubated at 35 ± 2^{o}C. Plates were visually examined and CFUs were enumerated after approximately 18 hours. The sampling results were determined by averaging the observed CFUs from triplicate plates (subsamples), multiplying by the inverse of the dilution factor, dividing by the volume plated (typically 0.1 ml), and multiplying by the total volume of the sample extract. Mean recovery (CFUs) for each device and material type was determined.

2.4 Test Matrix

The test plan consisted of two tasks that were completed sequentially. The first task (tests 1-8 listed in Table 2-3) consisted of evaluating and characterizing the five AFSD types on two different surfaces. Three AFSD types were used for carpet, (R1, R2, and R3), and all five for laminate flooring. The target spore surface loadings for this series of tests ranged between 5 x 10^5 and 5 x 10^6 CFU per ft^2. Based on the results of Tests 1 through 8 in Table 2-3, two AFSD (R2 and R4) were chosen for further evaluation at additional inoculum levels and materials (tests 11-O1 through 17-O2). Each test in Table 2-3 included one blank AFSD surface sample coupon, three inoculated AFSD sample coupons, four positive control wipe sample coupons, four ViaCell® samples collected during AFSD operation, as well as field blank samples for vacuum socks, aerosol samples, and sponge and wipe sample kits.

Table 2-3. Test matrix for small coupon tests

Test Number	AFSD	Number of AFSD	Material Type	Target Spore Loading (CFU per cm^2)
1	R1	4	Carpet	5×10^2 to 5×10^3
2	R2			
3	R3			
4	R1		Laminate	
5	R2			
6	R3			
7	R4			
8	R5			
11-01	R2		Carpet	2×10^5
13-01			Laminate	2×10^1
13-02	R4			
14-O2				5×10^{-1}
15-01	R2		Tile	1×10^6
15-02	R4			
16-O2				2×10^1
17-O2				5×10^{-1}

The second task was designed to further evaluate two AFSD types (R2 and R4) on a larger spatial scale. The testing was conducted inside the COMMANDER, and utilized a total test area equal to 1.8 m x 2.5 m (Figure 2-2). Subsections of the floor were inoculated under aerosol deposition apparatus (ADAs). This task consisted of four tests for each material (carpet and laminate) that were conducted in duplicate, as listed in Table 2-4.

COMMANDER was fitted with pre-sterilized coupons as shown in Figure 1. The set-up consisted of two 16.5 cm x 16.5 cm coupons, four 11 cm x 11 cm coupons, and a single 5.5 cm x 5.5 cm coupon in the center. The spore inoculation was performed at the center of the room (for Hot Spot inoculation) or throughout the whole area (for Wide Area inoculation). For the wide area release, the two 16.5 cm x 16.5 cm coupons and the center coupon were inoculated.

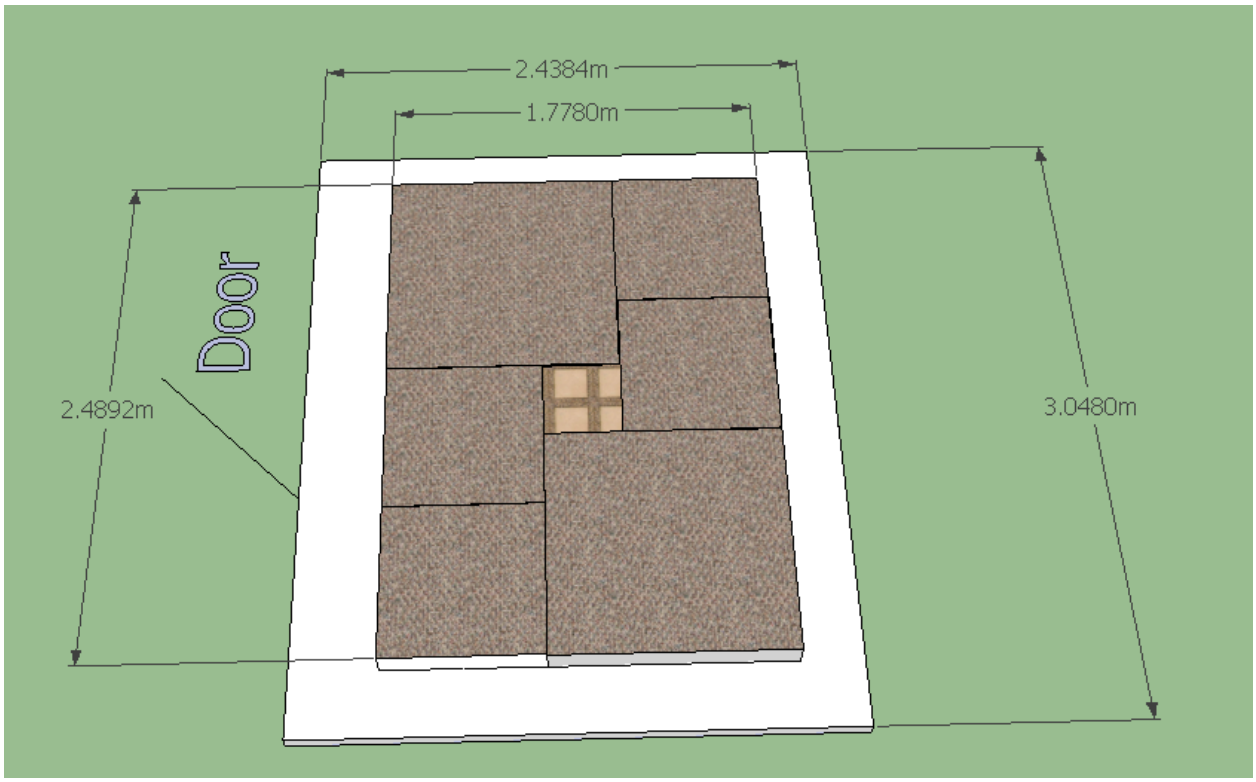

Figure 2-2. COMMANDER floor testing setup

Table 2-4. Test matrix for large-scale testing

Test Number	Inoculation Area	Number of AFSD	Material Type	AFSD type	Target Spore Loading (CFU per cm^2)	Comparative Surface Sampling Method
18	Hot Spot	1	Carpet	R2	2 x 10^4 CFU	Vacuum sock
19	Hot Spot	1			2 x 10^4 CFU	Vacuum sock
20	Wide Area	1			2 x 10^1 CFU	Vacuum sock
21	Wide Area	1			2 x 10^1 CFU	Vacuum sock
22	Hot Spot	1	Laminate	R4	2 x 10^4 CFU	Sponge wipe
23	Hot Spot	1			2 x 10^4 CFU	Sponge wipe
24	Wide Area	1			2 x 10^{-1} CFU	Sponge wipe
25	Wide Area	1			2 x 10^{-1} CFU	Sponge wipe

2.4.1 Test Facility Sampling Procedures

2.4.1.1 Sampling/Monitoring Points

Table 2-5 lists the samples collected for each test.

Table 2-5. Frequency of sampling monitoring events

Sample Type	Sample Number	Purpose
Test AFSD	3 per test condition	To determine the number of viable spores recovered from the AFSD
Negative control AFSD	1 per test	To determine extent of cross-contamination
Positive control coupon – vacuum sock sample	3 per test condition	To determine the number of viable spores recovered by conventional methods
Positive control coupon – sponge wipe sample	3 per test condition	To determine the number of viable spores recovered by conventional methods
Reference coupon – sponge wipe sample	a set of 4 stainless steel coupons inoculated at the beginning, middle, and end of test coupon inoculations	To provide the best estimate of the number of viable spores deposited onto the material test coupons
Laboratory blank coupons	3 sterile coupons	To demonstrate sterility of coupons and extraction materials.
Biocontaminant Laboratory material blanks	3 per material	To demonstrate sterility of extraction and plating materials
Aerosol Samples	1 per AFSD	To determine the extent of resuspended spores during operation
Aerosol sample during blank	1 per test	To determine extent of cross-contamination
RH/Temp	Logged every 10 seconds	To determine environmental conditions during AFSD operation

Table 2-6 lists the critical and non-critical measurements for each sample.

Table 2-6. Critical and non-critical measurements

Sample Type	Critical Measurements	Non-critical Measurement
Test AFSD	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature, operation time
Negative control AFSD	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature, operation time
Positive control coupon – vacuum sock sample	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Positive control coupon – sponge wipe sample	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Reference coupon – sponge wipe sample	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Laboratory blank coupons	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Biocontaminant Laboratory material blanks	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Aerosol Samples	Plated volume, incubation temperature, extracted volume, volume of air sampled, CFU	Ambient temperature during air sampling, duration of air sampling
Aerosol sample during blank	Plated volume, incubation temperature, extracted volume, volume of air sampled, CFU	Ambient temperature during air sampling, duration of air sampling
RH/Temp		RH and temperature during AFSD operation

2.5 Sampling Handling and Custody

2.5.1 Prevention of Cross-contamination of Sampling/Monitoring Equipment

Several management controls were instituted 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). Specific procedures were put in place in the effort to prevent cross-contamination among the samples. Adequate cleaning of all common materials and equipment was critical in preventing cross-contamination.

There were three primary activities for each test in the experimental matrix. These activities were preparation of the coupons, sampling, and analysis. The AFSD were sterilized prior to use with VHP® (250 ppmv vaporous hydrogen peroxide for four hours). Specific management controls for each of the three following activities are described below.

- Negative control coupons were present for each test. Growth on these coupons would indicate contamination during inoculation or sample collection activities.

- Swabs were used to sample coupon surfaces prior to inoculation. Growth of these swab samples would indicate the failure of the sterilization methods. While some swabs did indicate contamination,

no systematic changes to sterilization protocols were required since the level of contamination was insignificant compared to the test inoculum amounts.

- Only one AFSD was handled at a time.

- The AFSD was sealed with sterilized parafilm and placed in sample bags immediately following use.

General aseptic laboratory technique was followed and is embedded in the standard operating procedures (SOPs) and MOPs used by the on-site Biocontaminant Laboratory to recover and analyze samples. The SOPs and MOPs document the aseptic technique employed to prevent cross-contamination. Additionally, the order of analysis was always as follows: (1) all blank coupons; (2) all test coupons; and (3) all positive control coupons.

2.5.2 Sample Identification

Each coupon was identified by a unique sample number. The sampling team maintained an explicit laboratory log which included records of each unique sample number and its associated test number, inoculant amount, any preconditioning and treatment specifics, and the date treated. The sample codes eased written identification. Once the coupons were transferred to the on-site Biocontaminant Laboratory for microbiological analysis, each sample was additionally identified by replicate plate (Petri dish) number and dilution. Table 2-7 specifies the sample identification (e.g., 28-4-C1-O1). The Biocontaminant Laboratory also included on each plate the date it was placed in the incubator.

Swabs collected as sterility checks were identified by the code 29-[Test Number]-SW-[unique area code]. The swabs were collected according to MOP3135 from coupons and inoculation materials prior to inoculation.

2.5.3 Sample Custody

Careful coordination with the on-site Biocontaminant Laboratory was required to achieve successful transfer of uncompromised samples in a timely manner for analysis. Test schedules were confirmed with the Biocontaminant Laboratory prior to the start of each test. To ensure the integrity of samples and to maintain a timely and traceable transfer of samples, established and well-documented chain of custody (COC) procedures are mandatory. Accurate records were maintained whenever samples were created, transferred, stored, analyzed, or destroyed. The primary objective of these procedures was to create an accurate written record that can be used to trace the possession of the sample from the moment of its creation through the reporting of the results. A sample was in custody in any one of the following states:

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

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

Table 2-7. Coupon sample coding

Coupon Identification: 28-T-(X)MM-SS		
Category	**Example Code**	
T	1	Test Number
(X) MM (Material)	X	Procedural Blank
	C#	Carpet, where # is replicate (1-3)
	L#	Laminate Flooring, where # is replicate (1-3)
	S#	Stainless Steel (for QC purposes), where # is replicate (1-4)
	T#	T, where number is replicate (1-3)
	FB	Field Blank
SS (Sample Type)	R#	As purchased AFSD, where # is for Type 1, type 2, type 3, type 4 or type 5
	O#	Optimized AFSD, # for selected AFSD and optimization
	P	Sponge wipe Sample
	HS	Vacuum sock sample
	A#	Aerosol sample, where # is replicate (1-3)
Microbiology Lab Plate Identification 28-T-(X)MM-SS -Rd		
25-T-(X)M-SS	As above	
R (Replicate)	R	A – C
d (Dilution)	1	0 to 4, for 10E0 to 10E4

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

- A COC record accompanied the samples. When turning over possession of samples, the transferor and recipient signed, dated, and noted the time on the record sheet. This record sheet allowed transfer of custody of a group of samples from the testing laboratories to the on-site Biocontaminant Laboratory.

- If the custodian had not been assigned, the laboratory operator had the responsibility of packaging the samples for transport. Samples were carefully packed and hand-carried between on-site laboratories. The COC record showing the identity of the contents accompanied all packages.

2.5.4 Sample Preservation

Following transfer to the on-site Biocontaminant Laboratory, all samples were stored at 4 ± 2 °C until analyzed. All samples were allowed to equilibrate at room temperature for one hour prior to analysis.

2.5.5 Sample Holding Times

After sample collection for a single test was complete, all biological samples were immediately transported to the on-site Biocontaminant Laboratory, with appropriate COC form(s). Samples were stored no longer than five days before the primary analysis. Typical hold times, prior to analyses, for most biological samples was ≤ 2 days.

2.5.6 Sample Archiving

All samples and diluted samples were archived for two weeks following completion of analysis. This time allowed for review of the data to be performed to determine if any re-plating of selected samples was required. Samples were archived by maintaining the primary extract at 4 ± 2 °C in sealed 50 mL conical tubes.

3 Results and Discussion

3.1 Inoculation and Recovery

Stainless steel coupons were used to verify the magnitude and repeatability of spore loadings for every inoculation event. A total of 32 stainless steel coupons were inoculated and sampled using the sponge wipe method. The recovery results showed the average loading level of each inoculation event ranged from 9.12 x10^5 through 1.61 x 10^7 CFUs between tests. The coefficients of variation of spore inoculations were between 8 and 63%. The sampling efficiency from stainless steel using the sponge wipe method is approximately 48% according to the study by Krauter et al.[15] Therefore, using this crude assessment, it can be assumed that the inoculated spores were between 10^6 and 10^7 CFUs per coupon.

The recoveries from blank coupon sampling using AFSD were all negative (0 CFUs) except R1 and R5 from laminate. For these samples, viable spores were recovered; however, the recoveries were below the quantification limit (less than 30 CFUs per plate). The air sampling results from blank coupons were negative for all blank tests. The recoveries from blank tests were minimal compared to the test spore loadings (10^6 – 10^7 CFUs) and no further action was required.

3.2 Extraction Efficiency

As described in the previous section, the extraction efficiency was determined for each AFSD prior to conducting surface recovery tests since extraction efficiency may affect the overall sampling efficacy. Recovery from the AFSD depends on two distinct properties: the efficiency of the AFSD to collect the sample (spores) and the efficiency of the method to then remove and quantify the sample from the device. In order to focus on the AFSD, rather than to optimize recovery efficiency from each AFSD, extraction techniques were the same for all AFSD as much as possible, given that each had unique filter and bin designs. These data are summarized in Table 3-1.

Table 3-1. AFSD extraction efficiency test results

AFSD	Extracted Parts	Extraction Method	Average Extraction Efficiency (%)	Test sample size
R1	Filter, dust bin	Orbital shaking	65 ± 14	3
R2	Filter, dust bin	Orbital shaking	57 ± 8	3
R3	Filter, dust bin	Orbital shaking	67 ± 4	3
R4	Wipe cloth	Stomaching	49 ± 7	3
R5	Tank	Rinsing	90 ± 7	3

The efficiency was calculated by normalizing the recovery (CFUs) from AFSD parts by the number of spores spiked onto the parts. The results showed the extraction efficiency was approximately 50 – 90 %. The extraction efficiencies for vacuum units were within 10% difference. The maximum difference between R4 and R5 was approximately 40%. The recovery efficiency test was conducted with a liquid

spore inoculum on clean, unused AFSD parts. While the extraction efficiency during surface sampling tests may be different due to the complexity of the collected sample matrix, the similarities of the extraction recovery suggests that results from the AFSD may be directly compared.

3.3 Sampling Efficiency

The sampling efficiency of the AFSD is a measure of the spores recovered from a contaminated material surface by the AFSD as compared to the spores recovered by traditional methods. The sampling efficiencies of AFSD were compared to the sponge wipe method for laminate coupons and the vacuum sock method for carpet coupons. The AFSD sampling comparative recovery (CR) was calculated using Equation 1.

$$CR\ (\%) = \frac{average\ recovery\ from\ robot\ (CFUs)}{average\ recovery\ from\ comparative\ surface\ sampling\ method\ (CFUs)} \times 100 \quad \text{eq (1)}$$

Results are based on the total recovery (CFUs) from an AFSD, in some cases determined as the sum of the recoveries (CFUs) from two or more parts.

3.3.1 Scoping Studies

Laminate Surfaces: Initially, laminate coupons were tested with five AFSD to compare surface sampling recoveries among all AFSD types. The test results from laminate surfaces are summarized in Table 3-2 and Figure 3-2. The highest CR was achieved by R4 (62%) among the five AFSD types tested. The second highest CR was achieved by R5 (32%). The vacuum units (R1, R2, and R3) demonstrated CRs equal to or less than 10%. Low CRs from vacuum units were expected since previous sampling studies have shown that the wet wipe or the sponge wipe method on nonporous surfaces has higher recovery efficiency than vacuum-based methods. The sampling process by R4 was similar to the wet wipe or the sponge wipe method because R4 used a PBST-wetted cloth in conjunction with a rubbing action on the surface. The size of cloth used by R4 for cleaning was approximately 25 cm x 10 cm and, therefore, resulted in a 10 times larger contact area than that of the sponge wipe. The sampling efficiency of R4 was found to be lower than that of the sponge wipe method; however, the sampling efficiency of the sponge wipe may depend not only on the wipe size, but also on applied pressure, actual surface contact area, residence, and other parameters pertinent to the given sampled surface.

The relatively lower CR of the R5 units compared to the R4 units is due to the fact that the latter unit collects the spores directly by the wetted wipe while the former unit sample surfaces by first releasing clean PBST onto the surface followed by scrubbing and wiping the surface. The dispensed PBST liquid, along with any particles contained in the liquid, is recollected by the unit from the surface and stored in the “dirty tank”. The spore recovery by R5 is mainly dependent on recollection of the dispensed liquid. It is expected that the recollection of liquid may vary significantly depending on the surface morphology sampled. Lower sampling efficiencies would be expected when sampling coarse or irregular surfaces.

Table 3-2. Summary of laminate surface sampling comparative recovery using AFSD

AFSD	Average Sampling Duration (sec)	Mean Recovery from AFSD (CFUs x10^5)[a]	Mean Recovery from Sponge Wipe (CFUs x10^5)[a]	CR (%)
R1	422	1.7 ± 1.5	21 ± 11	8.1
R2	76	0.16 ± 0.07	1.4 ± 1.1	11
R3	540	1.3[b] ± 0.7	53 ± 30	2.5
R4	130	19 ± 4.6	31 ± 6	62
R5	545	21 ± 17	64 ± 2	32

a. Mean recovery ± standard deviation
b. One R3 unit stopped after approximately 10 seconds of operation. Data are calculated with duplicate sample results.

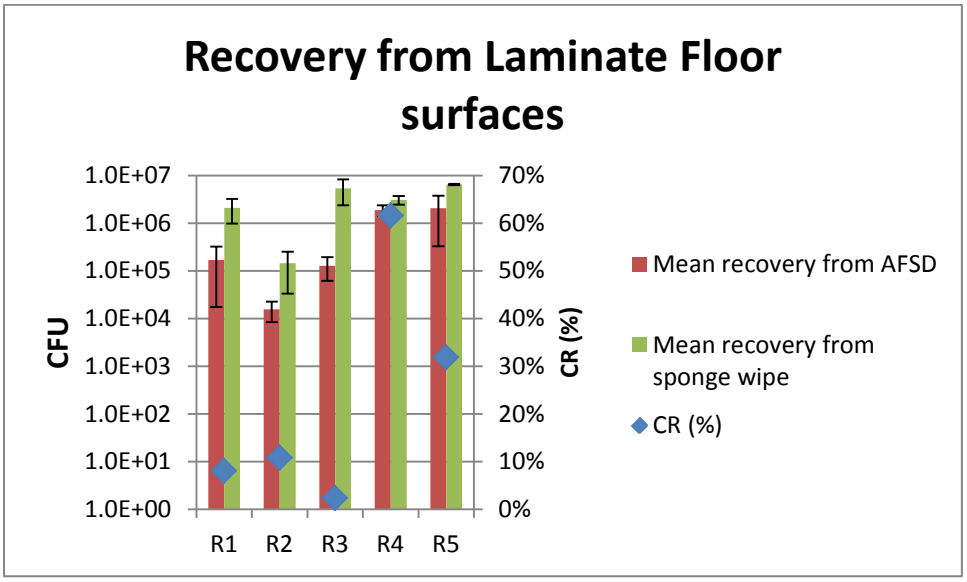

Figure 3-2: Recovery from Laminate Floor by AFSD Type

The sampling duration (the total time that each AFSD was sampling actively) was shortest for R2 (mean of 76 seconds) and longest for R5 (mean of 545 seconds). R3 was operated for approximately the same time as R5. R4 was also the most efficient unit in terms of the number of spores collected per unit area and time.

Table 3-3. Aerosol recoveries during laminate tests

AFSD	Recovery from Air Filters (CFUs)	% CFU from sponge wipe	Beater Bar
R1	67	0.0032%	Yes
R2	81	0.0566%	Yes
R3	4	0.0001%	No
R4	127	0.0041%	No
R5*	607	0.0094%	Yes

*These data were collected from ViaCell cassettes which were past the expiration date.

Aerosol sample results observed during sampling are listed in Table 3-3. The CFU/sample values have been reported because all samples were operated for the same length of time (20 minutes) and the same approximate flow rate (15 lpm). It is unknown when the CFUs are resuspended, but it is much more likely that the concentration in the chamber air is much higher at the beginning of the AFSD operation than at the end. The results are further confounded by the total number of CFU present on the coupon, which varied by nearly two orders of magnitude. The results showed a small but detectable spore re-aerosolization, slightly higher from R4 and R5 sampling tests, and relatively high (in comparison to coupon CFU) for R2. The re-aerosolization from R1 and R2 was presumably due to the presence of surface agitation devices (brush or a beater bar) on these units. Spore re-aerosolization from R3 was minimal compared to R1 and R2, likely due to its lack of a surface agitation tool. Though R5 uses a wet method for collection, R5 also has a surface agitation tool, and a filter that was designed for collection of large liquid particles rather than aerosolized spores.

Tile surfaces: Two AFSD types, R4 and R2, were tested for sampling efficiency on tile surfaces. These were designed as follow-up tests to the laminate series to determine if different hard material surfaces affected efficiency. Only AFSD with demonstrated efficiency were chosen for this extended study. Table 3-4 summarizes the results. Limits of detection were investigated for R4 by lowering the inoculation.

Table 3-4. Summary of tile surface sampling comparative recovery using AFSD

AFSD	Average sampling duration (sec)	Mean recovery from AFSD (CFUs)[a]	Mean recovery from sponge wipe (CFUs)	CR (%)
R2	65	$1.68 \times 10^5 \pm 2.05 \times 10^5$	$7.25 \times 10^6 \pm 1.53 \times 10^6$	2%
R4	209	$1.97 \times 10^6 \pm 4.04 \times 10^5$	$1.31 \times 10^7 \pm 1.46 \times 10^7$	15%
R4	170	$1.44 \times 10^4 \pm 9.76 \times 10^3$	$1.62 \times 10^4 \pm 2.60 \times 10^3$	89%
R4	206	$9.12 \times 10^2 \pm 6.10 \times 10^2$	$6.41 \times 10^2 \pm 5.10 \times 10^2$	142%

[a]Mean CFUs + one standard deviation

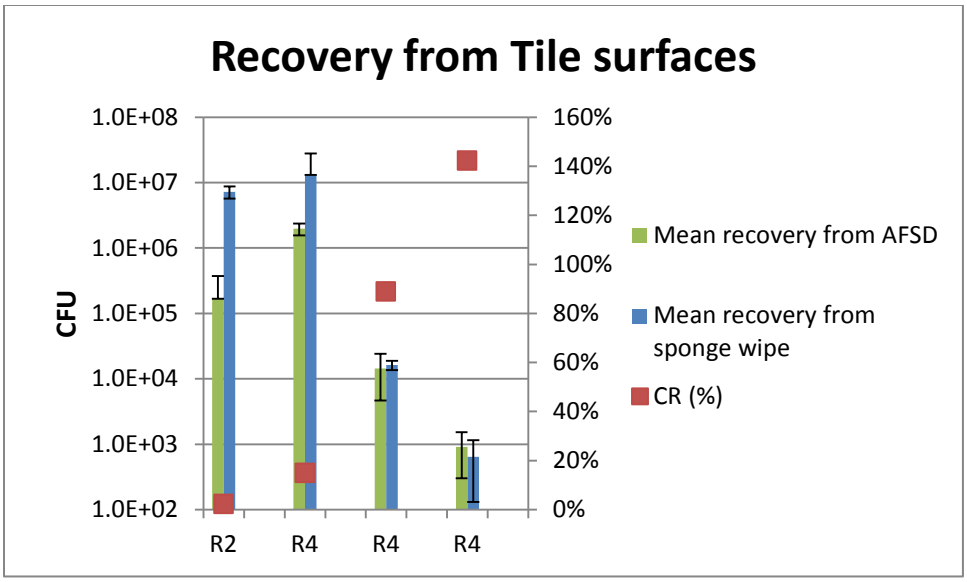

Figure 3-3: Recovery from tile surfaces by AFSD type

There was an inverse relationship of CR to initial surface spore loading. This suggests that R4 can be used for detection of relatively low spore loadings on non-porous surfaces.

Recoveries from aerosol samples are listed in Table 3-5.

Table 3-5. Recoveries from aerosol samples collected during tile tests

AFSD	CFU Present (Stainless Steel)	Mean Air filter (CFUs)	% CFU from sponge wipe	Beater Bar
R2	5.83×10^6	6239	0.086%	Yes
R4	9.96×10^6	76	0.001%	No
R4	1.57×10^4	<1	0.003%	No
R4	2.80×10^2	<1	0.087%	No

Re-aerosolization from R2 operation was much higher from tile than from laminate surfaces. This suggests that the condition and type of the floor surface may be a larger factor in re-aerosolization than the type of AFSD used within the current test conditions.

Carpet surfaces: Three AFSD types (R1, R2 and R3) were tested for sampling efficiency on carpet coupon surfaces. R4 and R5 were not tested on carpet surfaces because of their recommended usage only on hard surfaces, according to the instruction manuals. Similar to the laminate surface tests, comparative recovery efficiencies for carpet sampling were determined by comparison of AFSD recoveries to that of the vacuum sock sampling method. The test results are summarized in Table 3-6

and Figure 3-4. The highest average CR was achieved with R2 (161%). This unit was also the most effective AFSD type per surface area and time. R3, a vacuum only unit, demonstrated AFSD sampling results similar to that of the vacuum sock method. R1 showed the lowest CR among three AFSD. It is not clear why R1, which was equipped with an agitating brush bar, showed a lower CR than R2 or R3. One reason might be that a rotating brush bar, such as that on R1, may not be effective for resuspending sparsely distributed micron-size spores on surfaces. The brush is likely more effective for collection of fibrous materials such as animal hair. However, a rotating flexible beater, such as that on R2, may come in contact with a larger portion of the surface and therefore more effectively dislodge spores. There may be other reasons to explain the low CR from R1, such as vacuum power, sampling speed, sampling coverage area, etc. However, determining the effect of these variables was not part of the study objective. One thing to note is that two R3 units failed during testing. It is questionable whether R3 is reliable enough for incident field sampling following an actual incident.

Table 3-6. Summary of carpet surface sampling comparative recovery using AFSD

AFSD	Average sampling duration (sec)	Mean recovery from AFSD (CFUs x10^5)	Mean recovery from vacuum sock (CFUs x10^5)	CR (%)
R1	423	1.3 ± 0.4	5.2 ± 2.7	26
R2	81	1.0 ± 0.2	0.6 ± 0.2	161
R3	422	2.4 ± 0.4	2.6 ± 0.6	92

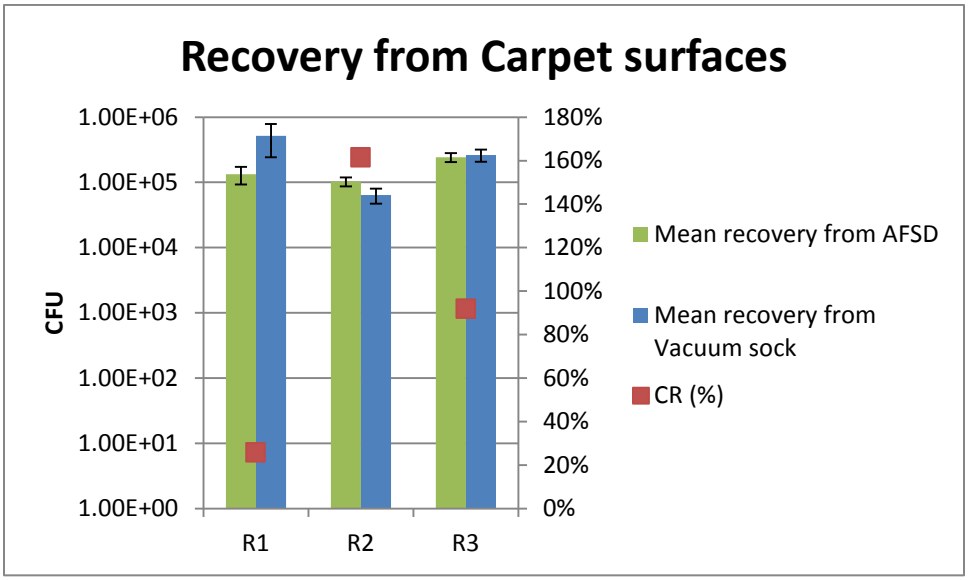

Figure 3-4: Recovery from carpet surfaces by AFSD type

The data from the air filter analyses during carpet sampling tests are shown in Table 3-7, and suggest that re-aerosolization is likely for any AFSD.

Table 3-7. Recoveries from aerosol samples during carpet tests

AFSD	Recovery from Air Filters (CFUs)	% CFU from sponge wipe	Beater Bar
R1	507	0.098%	Yes
R2	273	0.429%	Yes
R3	760	0.288%	No

Table 3-8 shows an indication of where within AFSD the spores were partitioned. The reservoir was difficult to seal, and would be problematic to ship because of this. In nearly all cases, analysis of only the filter would allow detection of the spores, while lowering demand on laboratory and shipping. Only R1 came with a HEPA filter, which should improve collection efficiency, while an aftermarket HEPA filter is available for R2. The filter for R5 was not designed for dry particulates, but rather for liquid, so it is not surprising that R5 demonstrated such low recovered from the filter.

Table 3-8. Partitioning of recovered spores in AFSD

AFSD	Material Type	Filter recovery	Bin recovery	% on filter
R1	Carpet	8.66×10^4	4.03×10^4	65%
	Laminate	4.20×10^4	1.27×10^5	25%
R2	Carpet	5.28×10^4	5.00×10^4	51%
	Laminate	5.36×10^3	1.02×10^4	34%
	Carpet	9.42×10^1	8.19×10^1	54%
	Laminate	1.77×10^1	3.44×10^1	34%
	Tile	1.15×10^4	1.56×10^5	7%
R3	Carpet	2.16×10^5	2.63×10^4	89%
	Laminate	9.51×10^4	3.20×10^4	75%
R5	Laminate	4.76×10^3	2.05×10^6	0%

3.4 Scenario-based Evaluation

Following the tests outlined in Table 2-3 and discussed in Section 3.3.1; two AFSD, R2 and R4, were chosen for further evaluation with a larger sample area; R2 for evaluation on carpet coupons, and R4 for evaluation on laminate coupons. Duplicate tests were performed with each of these AFSD. Results of the hot spot tests are summarized in Table 3-9.

Table 3-9. Results from "Hot Spot" testing

AFSD	AFSD Sampling area (ft^2)	Mean Recovery from AFSD (CFU/sample)	Surface Type and (Sampling Method)	Mean recovery from surface CFU/cm^2	CR (%)
R4	47.6	2.09×10^6	Laminate (sponge wipe)	$1.85 \times 10^3 \pm 1.52 \times 10^3$	121
R4	47.6	1.23×10^7	Laminate (sponge wipe)	$1.71 \times 10^4 \pm 4.20 \times 10^3$	78
R2	47.6	5.82×10^5	Carpet (vacuum sock)	$2.00 \times 10^3 \pm 1.81 \times 10^3$	31
R2	47.6	1.41×10^5	Carpet (vacuum sock)	$1.75 \times 10^3 \pm 6.52 \times 10^2$	9

A single inoculated coupon was placed in the middle of the larger sample area for these "hot spot" tests. For all of these tests, the AFSD successfully sampled the inoculated section within the large floor and recovered spores from that section. The recoveries from AFSD were higher from laminate floors than from carpeted floors, as was the case for the scoping tests. When the R2 AFSD samples spores, it sequesters them in the filter and bin, while the R4 AFSD keeps the spores on the mop in contact with the floor. In theory then, the efficacy of an R4 AFSD could be inversely proportional to the area it samples.

The results from wide contamination tests are shown in Table 3-10. For these tests, lower inoculums were used on a wider area of floor surface. Total CFU present was estimated by multiplying the total number of inoculated coupons by the recovery (CFUs) from control coupons.

Table 3-10. Results from wide area "release" testing

AFSD	Surface Type	Mean Recovery from Materials (CFU/929 cm^2)	Inoculated area (929 cm^2)	Estimated Total CFU present	AFSD Recovery (CFU)	CR (%)
R4	Laminate	$4.51 \times 10^2 \pm 4.53 \times 10^2$	19	8.57×10^3	2.79×10^3	33%
R4	Laminate	$6.6 \times 10^1 \pm 3.8 \times 10^1$	19	1.26×10^3	4.90×10^2	39%
R2	Carpet	9.55×10^2 5.18×10^2	19	1.81×10^4	5.00×10^3	28%
R2	Carpet	$3.95 \times 10^2 \pm 2.70 \times 10^2$	19	7.50×10^3	1.60×10^3	21%
R2	Carpet	$2.27 \times 10^2 \pm 4.2 \times 10^1$	19	4.31×10^3	3.16×10^2	7%
R2	Carpet	$1.5 \times 10^2 \pm 9 \times 10^1$	19	2.84×10^3	5.59×10^3	197%

The test plan required collecting samples after AFSD operation from areas of test coupons not originally inoculated. These samples were used to determine if the AFSD transferred spores from the contaminated (inoculated) areas to areas not previously contaminated (i.e., cross-contamination). The percent of spores transferred to sterile areas by the AFSD is the ratio of the estimated recovery from the non-inoculated areas to the initial spore loading recovered from the inoculated areas using the respective comparative sampling methods. These results are shown in Table 3-11.

Table 3-11. Spread of spores by AFSD

Dispersal	AFSD	Surface Type	Pre-Test Recovery on Hot Spot	Post-Test Recovery on Hot Spot	Avg. Post-Test Recovery on non-inoculated areas	Avg. Post-Test Recovery on non-inoculated areas	Spores transferred to sterile areas by AFSD
			CFU/929 cm^2			Total CFU	%
Hot Spot	R2	Carpet	1.86×10^6	5.83×10^5	2.03×10^2	9.69×10^3	0.5%
Hot Spot	R2	Carpet	1.63×10^6	4.84×10^5	2.09×10^2	9.96×10^3	0.0%
Wide Area	R2	Carpet	955	7	4	208	21.8%
Wide Area	R2	Carpet	227	29	4	175	77.2%
Hot Spot	R4	Laminate	1.72×10^6	4.71×10^4	3.33×10^3	1.59×10^5	9.2%
Hot Spot	R4	Laminate	1.59×10^7	3.17×10^5	6.68×10^4	3.18×10^6	20.0%
Wide Area	R4	Laminate	451	31	13	606	7.1%
Wide Area	R4	Laminate	66	31	3	152	12.0%

These results are completely anticipated and can be used to great advantage in a field response. Any area from which an AFSD proved positive for bacterial spores could then be characterized with traditional surface sampling methods, yielding a greater chance of detection using methods accepted by the response community. Collection of point surface samples within the hot zone by personnel could then provide additional information, such as concentration gradients, which may help characterize original distribution history. It is expected that an AFSD which encountered a hot spot towards the end of its operation would cross-contaminate a smaller area, thereby introducing a bit of randomness in interpretation. Regardless, further research is needed to determine the consequences of contamination redistribution by the samplers.

4 Quality Assurance

This project was performed under an approved Category III Quality Assurance Project Plan titled *Development of Automated Floor Sampling Device for Bacillus anthracis Spores (May 2012)* (available upon request).

4.1 Sampling, Monitoring, and Analysis Equipment Calibration

There were standard operating procedures for the maintenance and calibration of all laboratory equipment. All equipment was verified as being certified calibrated or having the calibration validated by EPA's 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. Sampling and monitoring equipment calibration frequency

Equipment	Calibration/Certification	Expected Tolerance
Meter box	Volume of gas is compared to NIST-traceable dry gas meter annually	± 2 %
RH sensor	Compare to 3 calibration salts once a week.	± 5 %
Stopwatch	Compare against NIST Official U.S. time at http://nist.time.gov/timezone.cgi?Eastern/d/-5/java once every 30 days.	±1 min/30 days
Clock	Compare to office U.S. Time @ time.gov every 30 days.	±1 min/30 days

Table 4-2. Analysis equipment calibration frequency

Equipment	Calibration Frequency	Calibration Method	Responsible Party	Acceptance Criteria
Pipettes	Annually	Gravimetric	External Contractor	±1% target value
Incubator thermometers	Annually	Compared to NIST-traceable thermometer	ARCADIS Metrology Laboratory	± 0.2 °C
Scale	Before each use	Compared to Class S weights	ARCADIS	± 0.01% target

4.2 Data Quality Objectives

The primary objective of this project was to determine the efficacy of various AFSD to collect biological samples from contaminated floor surfaces. This section discusses the Quality Assurance/Quality Control (QA/QC) checks (Section 4.3) and Acceptance Criteria for Critical Measurements (Section 4.4) considered critical to accomplishing the project objectives.

The Quality Assurance Project Plan (QAPP) in place for this testing was followed with several deviations, many of which were documented in the relevant sections of this report. Deviations included:

- Some samples from Test 01 ruptured primary containment during analysis. Samples were recoverable due to secondary containment.
- Stainless steel control samples from Test 03 were inadvertently combined.
- Some Test 6 AFSD did not operate.
- The Test 7 blank AFSD recovery (CFUs) was high due to contamination
- Test 08B ViaCell Cassettes were used past the expiration date.
- Test 13 O2 AFSD became wedged on the coupon. This was likely due to a splinter on the edge of the coupon which snagged the mop cloth.
- Test 14 O2 had only two positive control coupons. The third, which showed no detect, was considered an outlier and removed from the data set.
- Two MDIs were used for Test 15 O2, and seem to be different, however not all stainless steel controls were collected for both MDIs.
- Some samples required heat shock to enumerate due to contamination from another organism.
- One tile coupon for Test 16 O2 broke in half during installation. This did not seem to affect AFSD operation or recovery.
- The Test 20 AFSD required manipulation from a stuck brush error.
- Test 20b was included despite a high lab blank value.

4.3 QA/QC Checks

Uniformity of the test materials was a critical attribute to assuring reliable test results. Uniformity was maintained by obtaining a large enough quantity of material that multiple material sections and coupons could be constructed with presumably uniform characteristics. Samples and test chemicals were maintained to ensure their integrity. Samples were stored away from standards or other samples which could cross-contaminate them.

Supplies and consumables were acquired from reputable sources and were NIST-traceable when possible. Supplies and consumables were examined for evidence of tampering or damage upon receipt and prior to use, as appropriate. Supplies and consumables showing evidence of tampering or damage were not used. All examinations were documented and supplies were appropriately labeled. Project personnel checked supplies and consumables prior to use to verify that they met specified task quality objectives and did not exceed expiration dates.

Quantitative standards do not exist for biological agents. Quantitative determinations of organisms in this investigation did not involve the use of analytical measurement devices. Rather, the CFU were enumerated manually and recorded. Critical QC checks are shown in Table 4-3. The acceptance criteria were set at the most stringent level that could be routinely achieved and are consistent with the data quality objectives described in Section 4.4. Positive controls and procedural blanks were included along with the test samples in the experiments so that well-controlled quantitative values were obtained. Background checks were also included as part of the standard protocol. Replicate coupons were included for each set of test conditions when possible. Qualified, trained and experienced personnel using SOPs/MOPs ensure data collection consistency. When necessary, training sessions were conducted by knowledgeable parties, and in-house practice runs were used to gain expertise and proficiency prior to initiating the research.

4.4 Acceptance Criteria for Critical Measurements

Critical measurements (CM) are used to determine and assess the stated objectives and specify tolerable levels of potential errors associated with simulating the prescribed decontamination environments. The following measurements were deemed to be critical to accomplish part or all of the project objectives:

- enumeration of spores from traditionally surface sampling methods
- enumeration of spores from AFSD samples

The Data Quality Indicators (DQIs) listed in Table 4-4 are specific criteria used to quantify how well the collected data met the DQOs. Failure to provide a measurement method or device that meets these goals results in the rejection of results derived from the CM. For instance, if the plated volume of a sample is not known (i.e., is not 100% complete), then that sample is invalid

Table 4-3. QA/QC sample acceptance criteria

Sample Type	Purpose	Acceptance Criteria	Corrective Actions	Frequency
Negative Aerosol Background Samples	Determine extent of cross-contamination in COMMANDER and from each sampling technique	None	If CFU detected, discuss potential impact on results with EPA WAM. Repeat test if necessary after identifying and removing source of contamination	1 per sample per sampling technique per test
Negative coupon control sample	Determine extent of cross-contamination in COMMANDER	None	Values on test coupons of the same order of magnitude will be considered to have resulted from cross-contamination	3 per test
Field Blank	Verify the process of moving coupons does not introduce contamination	No detectable spores	Determine source of contamination and remove	1 per sampling type
Laboratory Materials	Verify the sterility of materials used to analyze viable spore count	No detectable spores or some if Dahman says it is okay.	Determine source of contamination and remove	1-3 per material per test
Blank Tryptic Soy Agar Sterility Control (plate incubated, but not inoculated)	Controls for sterility of plates	No observed growth following incubation	All plates are incubated prior to use, so any contaminated ones will be discarded	Each plate
Reference Control Coupons (also puffing control)	Used to determine the extent of inoculation on the coupon.	Target CFU, ±0.5 log Target varies per test. First set must be within 0.5 log of second set	Outside target range: discuss potential impact on results with EPA WAM; correct loading procedure for next test and repeat depending on decided impact	4 per test
Puffing Control Coupons (also positive control)	Used to determine drift in the MDI	The recovered (CFUs) from the first set of positive controls must be within 0.5 log of the second set of positive controls	Reject results and repeat test	
Biological Samples	Controls for outliers in colony growth	CFU counts between 30-300	Replate or filter plate if CFU outside criteria	Each sample

Table 4-4. Critical measurement acceptance criteria

Critical Measurement	Measurement Device	Accuracy	Precision	Detection Limit	Completeness
Plated Volume	Pipette	± 2%	± 1%	NA	100%
CFU/Plate	Visual Inspection	± 10% (between 2 counters)	± 5	1 CFU	100%
ViaCell® Total Volume	Dry gas meter	± 10%	± 5%	0.002 ft^3	100%

Plated volume critical measurement goals were met. All pipettes are calibrated yearly by an outside contractor (Calibrate, Inc.).

Plates were quantitatively analyzed (CFU/plate) using a visual inspection method. For each set of results (per test), a second count was performed on 25 percent of the plates with significant data (data found to be between 30-300 CFU). All second counts were found to be within 10 percent of the original count.

There are many QA/QC checks used to validate microbiological measurements. These checks include samples which demonstrate the ability of the NHSRC Biocontaminant Laboratory to culture the test organism, as well as to demonstrate that materials used in this effort do not themselves contain spores. The checks include:

- Negative control coupons: sterile coupons sampled alongside inoculated coupons
- Laboratory blank coupons: sterile coupons not removed from NHSRC Biocontaminant Laboratory
- Laboratory material coupons: includes all materials, individually, used by the NHSRC Biocontaminant Laboratory in sample analysis
- Positive control coupons: coupons inoculated but not subjected to AFSD operation
- Inoculation control coupons: stainless steel coupons puffed at beginning, and end of each inoculation campaign, not subjected to AFSD operation, to assess the stability of the puffer during the inoculation operation.

The Vaisala RH meters were calibrated weekly and were within the factory specifications during each AFSD operation.

4.5 Data Quality Audits

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

4.6 QA/QC Reporting

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

5 Summary and Recommendations

The initial scoping tests consisted of testing three vacuum-based AFSD (R1, R2 and R3) and one wipe- and one wet vacuum-based AFSD (R4 and R5, respectively) for sampling efficiency on a non-porous surface (laminate). These tests showed that CRs for laminate surfaces were higher for the wet wipe- and wet vacuum-based AFSD than the vacuum-based AFSD that were tested. The sampling process used by the wet wipe-based AFSD is similar to the currently established wet wipe surface sampling method since both methods use a PBST-wetted cloth in conjunction with a rubbing action on the surface. Low CRs from vacuum units were expected since previous sampling studies have shown that the surface sampling using the wet wipe or sponge wipe method on nonporous surfaces has higher recovery efficiency than vacuum-based methods.

Similar to the laminate surface tests, CRs for porous material (carpet) sampling were determined by comparison of the recoveries of three vacuum-based AFSD to that of the vacuum sock sampling method. The test results showed that the recoveries from AFSD were on the same order or greater than the vacuum sock sampling method. The differences in the test results among the three vacuum-based AFSD may be related to the unique design of each AFSD and operating conditions.

Aerosol recoveries of spores observed during sampling for all five types of AFSD and surface material types showed that small, but detectable, spore re-aerosolization can occur. The observed relative differences in the level of spore re-aerosolization for each AFSD/material combination may be due to the presence of surface agitation devices (brush or a beater bar) on these units, and the type of AFSD sampling scheme (vacuum-based versus wet-wipe sampling).

Two top performers (R2 and R4) from the scoping tests were evaluated further in a more complicated environment. The results from this test demonstrated that the AFSD were capable of sampling a hot spot placed in the middle of the large area. For all of these tests, the AFSD successfully sampled the inoculated section within the large floor and recovered spores from that section. Further, minimal contamination of the non-inoculated adjacent surfaces was observed. The same type of AFSD used on lower inoculated wide areas, showed comparable results to the more established comparative surface sampling methods. These results are of a great importance in a field response to localize “hot spots” and “secondary contamination” that may help design targeted decontamination strategies. Moreover, this AFSD approach may possibly enable assessment of the contamination spatial distribution.

In addition to wide area sampling, these AFSD could be deployed to areas where human sampling is difficult, such as inside HVAC ductwork and in highly contaminated areas (hot zones). Extending the use of these devices for sampling of other biological, chemical, or radiological agents may also be pursued. However, for real world application, these AFSD need further evaluation on larger spatial scales, with an extended set of surface types, dissemination types (contamination scenario), surface loadings (contamination surface concentration), and environmental conditions (relative humidity variation, exposure duration, etc.).

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Appendix A: Miscellaneous Operating Procedures (MOPs)

MOP 3135	Procedure for Sample Collection using BactiSwab™ Collection and Transport Systems
MOP 3144	Procedure for Wipe Sampling of Coupons
MOP 3145	Procedure for HEPA Vacuum Sampling of Large and Small Coupons
MOP 3150-All	Procedure for Fabrication of 14” x 14”, 28” x 28”, and 42” x 42” Material Coupons
MOP 3155	Procedure for Via-Cell Air Sampling
MOP 3165	Sponge Sample Collection Protocol
MOP 6535a:	Serial Dilution: Spread Plate Procedure to Quantify Viable Bacterial Spores
MOP 6587:	Retrieval and Processing of Biological Samples Collected by the iRobot Scooba Robot Vacuum
MOP 6588:	Retrieval and Processing of Biological Samples Collected by the Mint Automatic Floor Cleaner
MOP 6589:	Retrieval and Processing of Biological Samples Collected by the iRobot Roomba
MOP 6590	Retrieval and Processing of Biological Samples Collected by the P3 International P4920
MOP 6592	Retrieval and Processing of Biological Samples Collected by the Neato

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