Test Plan

for

The Evaluation of Carpet Steam/Heat Cleaners as Biological Sampling Device

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14. ABSTRACT
Additional approaches for large area remediation of biological spores from porous surfaces are needed. This project covered research activities performed to evaluate current models of carpet steam cleaners based on their utility as a sampling device in extracting Bacillus anthracis surrogate, B. globigii, spores off contaminated commercial grade carpets. The Informatin Sheet provides a brief description of the project and contact data to request additional information.

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WARRP, Sample Collection, Bacillus anthracis, Carpet Cleaners, Test Plan

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The Evaluation of Carpet Steam/Heat Cleaners as Biological Sampling Devices

Background and Rationale

In 2001, five envelopes containing a significant amount of anthrax-causing spores of *Bacillus anthracis* was intentionally transported through the U.S. Postal Services, and over a short time period resulted in the deaths of five individuals, infection of 17 others, and contamination of 35 postal facilities, commercial mailrooms, and buildings. This attack and its effects demonstrated the consequence of bioterrorism on a limited scale. A congressional inquiry and Governmental Accounting Office (GAO) investigation (2005) identified two main concerns with the response to this event, one related to a reliance on sampling specific locations with high probability of finding spores, and the other regarding the methods used in the sampling and analysis process, which were not validated. The latter GAO concern seriously challenges the negative sampling results and decision process used to establish and substantiate adequate clearance of anthrax spores. Since 2005, a number of Interagency programs have been established to further examine and enhance capabilities for sampling, decontaminating, and clearing biologically contaminated areas, including the Interagency Biological Restoration Demonstration (IBRD); Wide Area Recovery and Resiliency Program (WARRP); Validated Sampling Protocol (VSP); and Transatlantic Collaborative Biological Resiliency Demonstration (TaCBRD). These programs are primarily led by the Department of Homeland Security (DHS) and the Department of Defense (DoD), with significant collaboration with a number of other Federal agencies, such as US Environmental Protection Agency (EPA), and Health and Human Services' Assistant Secretary for Preparedness and Response (ASPR) and Centers for Disease Control (CDC).

The threat of a wide-area release of biological warfare agent, such as *B. anthracis* spores, is valid, and the consequences of such a release are perceived to be very challenging and daunting in terms of managing health consequences and environmental cleanup. Two of the biggest challenges facing a wide-area release is recognizing the overlap between response and recovery phases, and managing the scale of restoration activities. One of the first steps in restoration and recovery following such a release is to delineate the zone of buildings contaminated with *B. anthracis* spores based on quantitative sampling. Three common methods currently used for the collection of biological samples to establish extent of surface contamination are: vacuum socks, swabbing, and swiping. A summary of these methods is provided in Table 1.

The vacuum sock is the only method of the three that is suitable for sampling from porous and large area surfaces. Unfortunately, the biological capture efficiencies from porous surfaces using this method are very poor (10-20%; Ryan, 2011). Therefore, additional approaches for sampling off the porous surfaces are highly desirable, with alternative methods possibly improving wide area restoration activities in two areas: (1) Increased biological capture efficiencies, and (2) Increased availability of sampling methods and resources to support rapid recovery and cleanup following wide-area release and contamination.

Table 1. Common Methods for Biological Sampling of Contaminated Surfaces

Method	Surface Type	Surface Characteristics	Validated?	Capture Efficiencies (%)
Swab	Nonporous	Smooth, small non-porous glass and metal, and hard to reach smooth surfaces	Yes (Hodges *et al*., 2010)	15.8 – 55.0
Wipe	Nonporous	Larger size smooth, nonporous glass and metal surfaces	Yes (Rose *et al*., 2011)	24.4 – 32.4
Vacuum Sock	Porous	Ventilation ducts, carpet, fabric, brick, cinder block, asphalt	No	10-20 (Ryan, 2011)

Approaches to reduce the source contamination on a wider scale are expected to expedite the recovery efforts. Reducing the contamination levels form surfaces using commercial off-the-shelf (COTS) approaches, such as household wet/dry vacuuming, commercial cleaners, and industrial-level cleaners may augment rapid recovery efforts by reducing the time and cost of cleanup through application of low-tech decon options, and reducing the level of personal protective equipment (PPE). Two objectives of this study therefore are:

a. Can household or commercial cleaning methods, such as wet/dry vacuuming, provide an adequate approach for biological sampling off porous surfaces?
b. Which of the three tested cleaning options represents the most effective sampling device and provides greatest reduction in the contamination levels by capturing spores into collection reservoir?

The proposed study will evaluate current models of carpet steam cleaners based on their utility as a sampling device, and efficiency in extracting *B. anthracis* surrogate, *B. globigii* spores off contaminated commercial-grade carpets. This is an important study as it will provide data to support the possible use of such devices for sampling and help meet the challenge of biological contamination reduction off the porous surfaces. In the event of a wide-area release, such devices, if applicable and suitable, can be recruited for contamination reduction on a wider scale.

Test Description

Three types of steam carpet cleaners will be tested: industrial grade, commercial grade, and a portable residential unit. The general approach for this project will be a quantitative assessment of the number of spores recovered from the carpet sections through analysis of rinsate collection. Additional controls will be used to determine the number of spores remaining on the carpet following the process, as well as approximate the number of spores re-aerosolized

during the vacuuming process. Reference coupons will be used to determine the inoculation density on the carpet following the inoculation process, and limited air samples will be taken to qualitatively assess re-aerosolization. Although it is unrealistic to expect to account for all the deposited spores, this proposed multi-faceted approach will allow for a thorough quantitative analysis of the recovery efficiency by each of the three cleaners.

A brief description of the testing is as follows. A preparation of fluidized *B. globigii* spores will be aerosolized onto a large carpet sample (8x10-ft) and allowed to settle over-night. Reference coupons placed on carpet will be extracted to assess deposition density. A carpet steam cleaner will be used to recover the deposited spores from the carpet surface. The rinsate collected during this process will be analyzed to determine the number of spores collected. In addition, an attempt will be made to cut core samples from the carpet to determine the quantity of spores remaining on the carpet after the process. Four replicate carpet samples will be included for each cleaner type.

Regarding choice of inoculation method for this test - Some of the differences seen with the various sampling techniques used in the current literature might be explained by the use of different inoculation methods in the testing protocols. Four techniques are commonly used for inoculation of spores onto testing surfaces: aqueous suspension deposition, aqueous fine mist deposition using a nebulizer, puff deposition using metered dose inhaler (organic propellant as a carrier), and aerosol deposition of milled dry spores. Aerosol deposition of dry milled spores most closely resembles the incident of 2001; therefore it will be the method of choice for our proposed testing.

Test Site and Testing Facilities

Testing will be conducted at Edgewood Chemical Biological Center by the BioDefense Branch and Aerosol Sciences Branch of the Research & Technology Directorate. Aerosol disposition, steam cleaner extraction, and sample collection will be performed in building E5951. Coupon extraction, sample analysis, and quantitative assessment will be performed in lab 307 located in the McNamara building E3150.

The BioDefense Branch BSL-1 and BSL-2 laboratory facilities are fully equipped to handle a broad range of work that requires the safe use and storage of surrogates and pathogens. Since its initial certification in 2001, the facility has functioned as both a research and a technology development laboratory in support of DoD chemical and biological (CB) programs. The BDB and its personnel have demonstrated the capability for storing and safely handling a variety of BW agents and their surrogates.

Aerosol deposition of spores will be conducted in a 64 m^3 BSL 1+ chamber located in the Aerosol Sciences Building E5951, Figure 1. Temperature and relative humidity of the chamber can be set using a computer and will be maintained at 75$\pm$5 ^oF and 35$\pm$5% RH. Power receptacles inside the chamber are also controlled by this computer. HEPA filters are installed at the inlet to filter air entering the chamber to achieve very low background particle concentrations

in the chamber. Similarly, HEPA filters are installed at the exhaust port to filter all particles leaving the chamber.

Figure 1 – Aerosol Deposition Chamber in Building E5951

Test Technologies - Carpet Steam/Heat Cleaners

There are numerous varieties of carpet steam cleaners available for use in residences and commercial facilities. Portable residential units are those which may be purchased at hardware or home stores. Commercial and Industrial units are typically stocked by specialty companies. The key differences between residential units and commercial/industrial grade units are the temperature and pressure levels which tend to be higher for the latter. All cleaners to be tested have the capability to heat the aqueous solution being used. However, it is worth noting that none of these units produce true steam.

The industrial steam cleaner to be used in this work will be the TM Sapphire 370ss unit. This is a truck mounted carpet cleaning machine with a 20 horsepower gasoline engine. The unit will be mounted into a 6 x 12 ft. cargo trailer and includes a vacuum blower, solution pump, heat exchangers, and a 90 gallon waste tank. The unit allows complete temperature control up to 250 ^{o}F.

The commercial grade steam cleaner unit will be the XTREME POWER XPH-9650 carpet extractor manufactured by Daimer. It can be operated at up to 500 psi and the cleaning solution can be heated up to 210 ºF. It has a 15 gallon recovery tank in which the rinsate is collected.

The residential carpet cleaner to be used for this testing will be the Bissell ProHeat 2X CleanShot 9500. Unlike many residential units, this model includes a built-in heater that increases the temperature of hot tap water by as much as 25 ºF. The unit has a 1 gallon tank for rinsate collection.

Table 2. Summary of Vacuum Cleaners Tested in This Study

Cleaner Type	Temperature (ºF)	Pressure (psi)	Waste Tank (L)	Vacuum Flow (CFM)	Cost ($)	Comments
Industrial (TM Sapphire 370ss)	250	1500	340	317	30,000[a]	Can be trailer or truck mounted
Commercial (XTREME Power XPH-9650) by Daimer Industry	210	500	56	200	5000	
Residential (Bissell ProHeat CleanShot 9500)	25 (above the ambient)	N.A.	3.7	N.A.	300	

N.A. – not available

a. The cleaning unit is 25K, but additional 5K is for a trailer

Carpet Specifications

Shaw carpeting, Viking style, will be used for the work outlined here within. This carpeting is representative of typical flooring found in office spaces and is 100% level loop olefin with a polypropylene backing. The carpet sections to be used for each run will be 8 ft. x 10 ft and placed on plywood board to allow coring small carpet pieces after vacuuming. For each run, a new carpet section will be used, and with each technology four replicate carpet sections will be used.

Test Inoculum

The fluidized milled *B. globigii* spores to be used for this project will be procured from Dugway Proving Grounds. A specifications sheet with certificate of analysis will document QA/QC of the spore preparation upon receipt. Spores will be prepared by milling the preparation until particles consist of single spores and then adding fluidizer. Before testing, a known mass of powder (20 mg) will be suspended in 10 mL volume of 0.01% Tween-80, and enumerated by serial dilution and plating on TSA plates. Three separate enumerations will be performed to determine the number of viable spores per gram of dry powder. All plating will be done in duplicate for each dilution.

Aerosol Deposition of Spores

The fluidized spores will be aerosolized onto the carpet using a two-fluid pneumatic sonic nozzle, Figure 2. A brief description of this nozzle follows. One nozzle is connected to the compressed air that exits out through a small annular opening. The other nozzle is connected to the powder to be aerosolized. The low pressure created in the exit region due to the air flow causes powder to be pulled through an axial tube at a very low feed rate due to the Bernoulli Effect. The desired air to powder mass ration is 80-100:1.

For each test, one 8 x 10 ft. carpet section will be contaminated with 1×10^7 spores per sq ft or 8×10^8 spores a total surface area of 80 sq-ft. In order to deliver the intended concentration, it is anticipated that each run will require deposition of approximately $1.6 \times 10^9 – 2.0 \times 10^9$ total spores in the chamber. It is expected that some spores will remain in the nozzle, and some will be lost due to extra chamber floor space not covered by the carpet sections.

Aerosols will be generated inside the chamber. The chamber air will be mixed by fans after and/or during the aerosol generation to achieve a uniform aerosol concentration in the chamber. Previous tests showed that mixing the aerosol in the chamber for 1 min is adequate to achieve a uniform aerosol concentration. After the aerosol deposition process, fans will be turned off and the spores will be allowed to settle overnight. The following day, prior to sample collection, a bleach solution will be used to remove the spores from all chamber floor surfaces not covered by the carpet section.

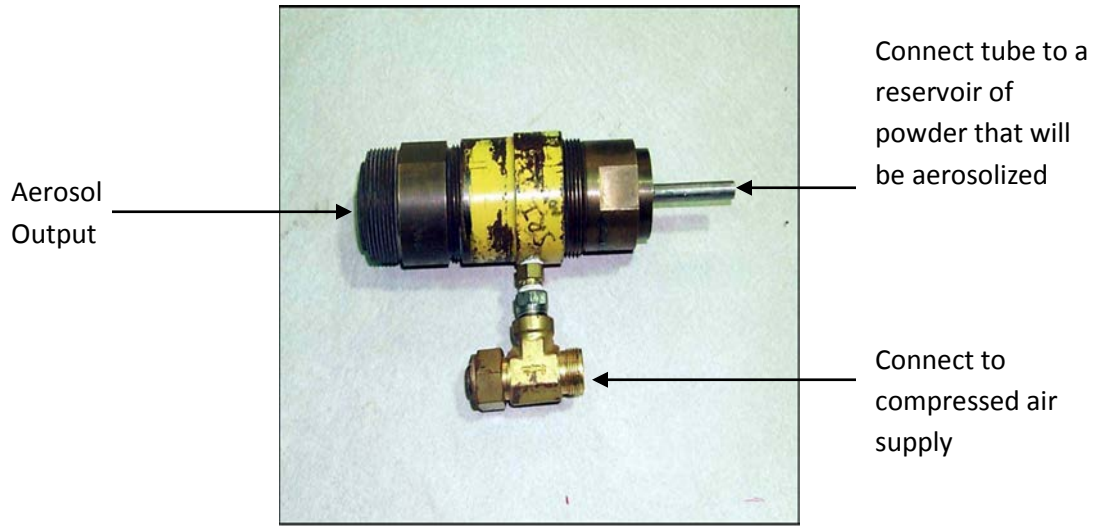
The aerosol chamber is cleaned between runs by exhausting the chamber air through the HEPA filters, and by pumping HEPA-filtered air into the chamber. The maximum amount of airflow that can be exhausted from the chamber by the exhaust pump is approximately 2×10^4 L/min. There is also a small recirculation system that removes air from the chamber, passes it through a HEPA filter, and delivers it back to the chamber. This system is useful when the aerosol concentration in the chamber needs to be reduced incrementally. In addition, the chamber walls and floor will be wiped down with 10% bleach in between runs. If air sampling shows significant number of spores, the chamber will be decontaminated with vapor hydrogen peroxide (VHP).

Spore Extraction Process

One of three technologies will be used for each test run, and each technology will be tested in a total of 4 runs. Therefore, the testing will require 12 total test runs. The surfactant (Tween-80) will be added to the carpet cleaner reservoir of the technology being tested at a final concentration of 0.01% prior to the start of the run.

For the spore extraction procedure, the carpet cleaner will be run over the entire surface of the carpet section twice. The cleaners will be used as per manufacturer's recommendations. The surfactant will first be sprayed onto the carpet surface, then the wand or cleaning unit will be used in a left to right pattern to extract the spores from the full length of the carpet. This procedure will be repeated at a 90º angle to the first pass. Care will be taken to ensure that the steam cleaner operator or other personnel do not step onto the carpet during the test runs. This will help to prevent unwanted cross contamination during the process.

Figure 2 – Sonic Nozzle used for the Aerosol Generation



Rinsate Sample Analysis

Maximal rinsate volume collected with each of the three types of cleaners is 3000-L (industrial), 56-L (commercial), and 3.78-L (household). However, actual volume used for collection will be determined in the baseline study, e.g. 300-L (industrial), 30-L (commercial), and 3.7-L (household). $1/00^{th}$ volume of the collected rinsate will be heat-treated, and analyzed by direct plating and filtering in three batches and dilution plating. Aliquots will be filtered by vacuum filtration onto 0.2-µ Nalgene filter units. The filters will be transferred to tryptic soy agar plates (TSA) and plates will be incubated at 37 ºC overnight. Colony forming units will be counted the following day using Q count instrument. Additionally, 1-mL rinsate will be analyzed by plating 100-µL aliquots on ten TSA plates. If the number of CFU exceeds 200 on a

filter, the samples will be serially diluting 1:10 up to 10^{-3}. Each dilution tube will contain 0.01% Tween-80 solution. One 100-µL of each dilution, in addition to the undiluted sample, will be plated in duplicate onto TSA plates. Plates will be incubated overnight at 37 ºC and colony forming units will be counted the following day using a Q count.

Reference Coupons

Each test run will include 25 reference coupons. These coupons will be used to determine the quantity of spores deposited during each run. Five out of the 25 coupons will be glass since these coupons have shown consistent high spore recovery in past studies. The other 20 reference coupons will be carpet material. Each coupon will be 2 x 5 cm in size.

Carpet reference coupons will be placed in each 4 sq ft section (see figure 3). Five glass coupons will be co-located with carpet reference coupons. Coupons will be placed on the carpet section to be tested prior to the aerosol deposition procedure. Following the overnight settling period, the coupons will be removed and placed into 50 mL conical tubes containing 20 mL 0.01% Tween-80 extraction solution.

The reference coupons will be extracted using a 10 min sonication and 2 min vortexing procedure. The samples will then be serially diluted and plated on duplicate plates as previously described (Rastogi *et al*., 2009 and 2010).

Core Samples (Pending Further Discussion)

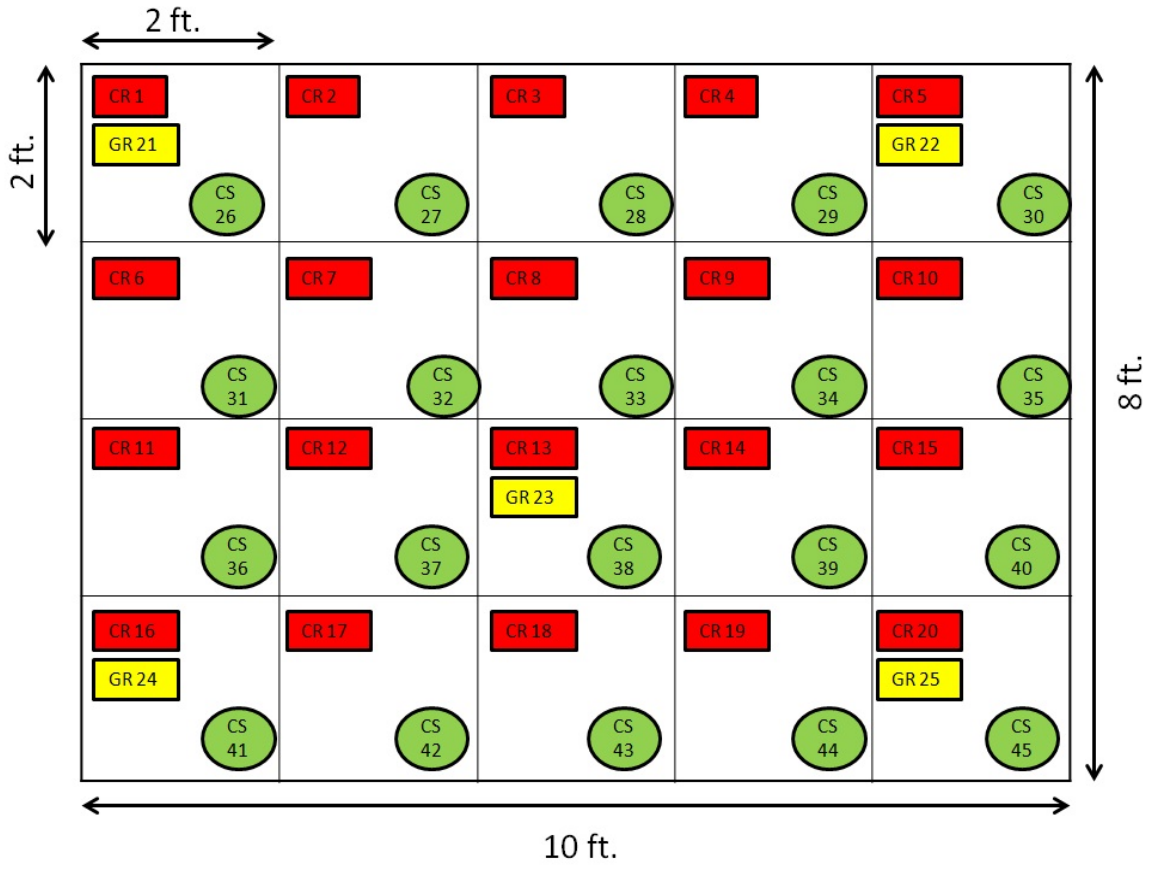
In order to estimate the quantity of spores which are not recovered using the carpet extractor, core samples will be taken from the carpet section used for each test. A total of 20 core samples will be taken from each carpet tested. A hollow punch and hammer or a hole saw will be used to obtain these samples after the carpet extractor process has been completed with the steam cleaner. Once obtained, the core sample will be placed in a 50 mL conical tube containing 20 mL 0.01% Tween-80. The samples will be processed using the same procedure as was used for the reference coupons.

**Note- The size of the core samples is yet to be determined. This determination will be based on the feasibility of obtaining the core samples.*

Air Sampling

Since it is anticipated that spores will re-aerosolize during the sampling process, glass filters for sampling will be placed at 2, 4, and 6-ft height and analyzed for spore numbers. Glass fiber filters, 47 mm in size, will be placed in proper position after the overnight spore settling period but before the carpet extraction process. After the carpet extraction process is completed, the filters will each be placed in a 50 mL tube containing 0.01% Tween-80 and processed in the same manner as the reference coupons and core samples. The spore numbers will be quantified by dilution plating.

Figure 3. Sampling Grid (CR = Carpet Reference Samples; GR = Glass Reference Samples; and CS = Carpet Core Samples)



Samples Collected during each Test Run

The test matrix for each run will include: 20 carpet reference samples, 5 glass reference coupons, and 3 rinsate analysis samples. In addition, 20 carpet core samples (if possible) will also be analyzed. The test matrix is detailed in Table 1 below.

Table 3. Sample Designations Detailed in the Testing Matrix

Carpet Reference Coupons	Glass Reference Coupons	Carpet Core Samples	Rinsate Samples	Air Samples
CR 1	GR 21	CS 26	RS 46	AS 49
CR 2	GR 22	CS 27	RS 47	AS 50
CR 3	GR 23	CS 28	RS 48	AS 51
CR 4	GR 24	CS 29		
CR 5	GR 25	CS 30		
CR 6		CS 31		
CR 7		CS 32		
CR 8		CS 33		
CR 9		CS 34		
CR 10		CS 35		
CR 11		CS 36		
CR 12		CS 37		
CR 13		CS 38		
CR 14		CS 39		
CR 15		CS 40		
CR 16		CS 41		
CR 17		CS 42		
CR 18		CS 43		
CR 19		CS 44		
CR 20		CS 45		

Data Reduction

Primary data used for quantitative estimations will be collected from two sources, reference coupons and the cleaner rinsate. In addition, air samples on glass filters will be collected for semi-quantitative estimation of re-aerosolization. Both types of reference coupons, five glass and 20 carpet will be extracted in 20 mL of extraction buffer. Carpet and glass reference coupons are included to estimate maximal deposition density. Maximal rinsate volume collected with each of the three types of cleaners is 3000-L (industrial), 56-L (commercial), and

3.78-L (household). However, actual volume used for collection will be determined in the baseline study, e.g. 300-L (industrial), 30-L (commercial), and 3.7-L (household).

Data reduction will include calculating total viable spores recovered.

1. Reference Coupons (10-sq-cm) - Total colony-forming units (CFU) recovered per reference coupon will be calculated using the following equation - Average CFU/plate x dilution factor x 10 (since 100 µL was plated) x extract volume (20 mL). Average density and standard deviation from 20 carpet reference coupons will be calculated. Since the total area equals 80-sq-ft (or 72000-sq-cm), the log(CFU) will be multiplied with a factor of 7400. Recovery efficiency from carpet reference coupons will be calculated by comparing log(CFU) recovered from reference carpet coupons relative to recovery from the glass surface.

2. Rinsate Sample – A 100-µL aliquot will be plated onto 10 plates. In addition, $1/100^{th}$ volume collected from each technology will be filtered in three batches and viable spores enumerated by dilution plating. Total CFU will be calculated using the following equation – Mean CFU/mL (from 10 plates) x Total rinsate volume (mL). If no colonies on these plates, total number of viable spores will be calculated by Mean CFU on filter x 100.

3. Air Sampling – This measurement is not quantitative. The intent of making these measurements is to confirm the re-aerosolization prediction during sampling process and to confirm the height to which these spores aerosolize, since glass filters will be placed at 2, 4, and 6 feet height.

4. Sampling Efficacy – Efficacy of each of the three technologies will be calculated by: log(CFU) collected in rinsate/log(CFU) inoculation density x 100. Averages and statistical variability will be calculated by using data from four replicate runs. The data can also be used for calculating effectiveness of one or more of the tested technologies in reducing contamination levels.

   Total spores deposited over the 8 x 10 ft carpet section can be calculated by averaging the number of spores recovered from the glass reference coupons and multiplying by a factor of 7400. This number was derived by dividing the total square footage of the carpet sections by the square footage of the reference coupons.

Data Quality Objectives

The Data Quality Objectives (DQO) defines the critical measurements performed to address the stated objectives and specify tolerable potential errors associated with the proposed

sampling study. The following measurements were deemed to be critical to accomplish project objectives:

CFU counts
Rinsate volume

The Data Quality Indicators (DQI) listed in Table 4 is specific criteria used to quantify how well the collected data meet the DQOs.

Table 4. DQIs for Critical Measurements

Measured Parameter	Analysis Method	Accuracy	Detection	Completeness Goals
CFU counts	Counting using QCount (image recorded	+/- 5% of CFU count	1 CFU	100%
Rinsate volume	Visual (volumetric)	+/- 10%	1 mL	100%

Table 5. Acceptance Criteria for Critical Measurements

Measured Parameter	Target Value	Precision RSD (%)
Reference Coupons	1-300 CFUs per plate	50% (replicate plates)
Rinsate volume	3.5-L	25%

Training Requirements

Biological Safety Training
Effective Use of Engineering controls
BSAT Physical Security Training
Security Training
Hazardous Waste Tracking System Training
Special Medial Waste Training
Security Awareness Training
OPSEC Training
Chemical Hygiene Plan Training
Bloodborne Pathogen Training

Safety and Environmental Protection Requirements for Test

All personnel performing the work will be familiar with the standard laboratory techniques and equipment operation required for use during this project. Any additional specialized training and certification for the use of the equipment/instruments will be identified

and provided by each vendor on site. Any personnel required to work in the BSL-3 Lab will be trained appropriately, as described in our standard operating procedure.

Quality Control Requirements for Test

The tests will be conducted in accordance with standard good laboratory practices. The BioDefense Branch has a quality assurance system in place. A number of internal operating procedures listed below will be followed relating to carrying out this project.

IOPs

Document Number	Document Title
H2001.00	Issuance, Distribution and Control of Internal Operating Procedures and Controlled Documents
3019.01	Operation of the Vortex Mixer
H2006.00	Management Responsibility and Review of Controlled Documents
3003.01	Operation and Maintenance of the Spiral Biotech Q-Count
2013.00	Documentation Practices and Procedures for Completing Laboratory Notebooks
3104.00	Operation of Consolidated Stills Steromaster MK Autoclave
H3001.00	Initial Use, Operation, and Cleaning of Temperature Controlled Units
3002.02	Weigh Balances
3008.01	Performance Evaluation of Mechanical Action Pipettes
H2021.00	Procedure for Documenting and Approving Deviations
4003.02	Media and Reagents Prep, Sterilization, storage
4101.00	Preparation of TSA culture media
5202.00	Plating and Enumeration of Bacteria on Solid Media

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