EPA/600/R-23/204 | September 2023 I www.epa.gov/research

United States Environmental Protection Agency

Development of an Activity-based Air Sampling (ABS) Strategy/Protocol for Use Outdoors, Including US Coast Guard Installations

Assessment and Evaluation Report

Office of Research and Development
Center For Environmental Solutions and Emergency Response

This page is intentionally left blank

EPA 600/R23/204 I September 2023 I www.epa.gov/research

Development of an Activity-based Air Sampling (ABS) Strategy/Protocol for Use Outdoors, Including US Coast Guard Installations

Assessment and Evaluation Report

John Archer, MS, CIH

Homeland Security and Materials Management Division

Center for Environmental Solutions and Emergency Response

Office of Research and Development

U.S. Environmental Protection Agency

Research Triangle Park, NC 27711

Disclaimer

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development's Center for Environmental Solutions and Emergency Response (Homeland Security and Materials Management Division), directed and managed this research through Contract No. EP-C-15-008 with Jacobs Technology, Inc. The research described in this study has been funded wholly or in part by the U.S. Department of Homeland Security (DHS) Science and Technology Directorate (S&T) under an interagency agreement (EPA No. RW-070-95937001 and DHS S&T IA# 70RSAT18KPM000084).

This report has been peer and administratively reviewed and approved for publication as an EPA document. This report does not necessarily reflect the views of the EPA. No official endorsement should be inferred. This report includes photographs of commercially available products. The photographs are included for the purpose of illustration only. Any mention of trade names, manufacturers or products does not imply an endorsement by the United States Government or the EPA. EPA and its employees do not endorse any commercial products, services, or enterprises.

Questions concerning this report or its application should be addressed to the following individual:

John Archer, MS, CIH
Homeland Security and Materials Management Division
Center for Environmental Solutions and Emergency Response
U.S. Environmental Protection Agency (MD-E343-06)
Office of Research and Development
109 T.W. Alexander Drive
P.O. Box 12055
Research Triangle Park, NC 27711
Telephone No.: (919) 541-1151
Fax No.: (919) 541-0496
E-mail Address: archer.john@epa.gov

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The Center for Environmental Solutions and Emergency Response (CESER) within the Office of Research and Development (ORD) conducts applied, stakeholder-driven research and provides responsive technical support to help solve the Nation’s environmental challenges. The Center’s research focuses on innovative approaches to address environmental challenges associated with the built environment. We develop technologies and decision-support tools to help safeguard public water systems and groundwater, guide sustainable materials management, remediate sites from traditional contamination sources and emerging environmental stressors, and address potential threats from terrorism and natural disasters. CESER collaborates with both public and private sector partners to foster technologies that improve the effectiveness and reduce the cost of compliance, while anticipating emerging problems. We provide technical support to EPA regions and programs, states, tribal nations, and federal partners, and serve as the interagency liaison for EPA in homeland security research and technology. The Center is a leader in providing scientific solutions to protect human health and the environment.

This report summarizes novel air sampling research conducted for the multi-agency Analysis for Coastal Operational Resiliency (AnCOR) program which sought to develop and demonstrate capabilities and strategic guidelines to prepare the US for a wide-area release of a biological agent, including mitigation of impacts to United States Coast Guard (USCG) facilities and assets. The intent of this research was to develop novel air sampling methods for outdoors to complement traditional surface sampling methods. Specifically, an air sampling technique was developed that incorporates aggressive air sampling (AAS) and activity-based sampling (ABS) into a feasible effort to be used for characterization and/or clearance sampling following a biological agent release. The initial version of the sampling system utilized a tent-based containment with high volume air sampling. Based on several limitations of this system, a subsequent mobile sampler version was developed that incorporated the same aggressive air system, high volume air sampling and containment into a mobile cart sampler. This novel sampler can provide another tool for responders to use following a wide-area release and inform decision makers of exposure risk.

Gregory Sayles, Director

Center for Environmental Solutions and Emergency Response

Acknowledgments

The principal investigator from the Office of Research and Development's Center for Environmental Solutions and Emergency Response (CESER), Homeland Security and Materials Management Division (HSMMD) directed this effort with support of EPA and interagency project teams. Special thanks to the HSMMD Research Triangle Park (RTP) Microbiological Laboratory (BioLab) staff for all of their support with microbiological sample analysis. Contributions of the following individuals were a valued asset throughout this effort:

U.S. EPA Principal Investigator
John Archer, CESER/HSMMD/Disaster Characterization Branch (DCB)

U.S. EPA Technical Reviewers
Anne Mikelonis, CESER/HSMMD
Timothy Boe, CESER/HSMMD

External Technical Reviewers

Emile Benard, USCG Atlantic Strike Team
Andrew Imler, Oxbow EHS Solutions

U.S. EPA Research Team

M. Worth Calfee, CESER/HSMMD
Sang Don Lee, CESER/HSMMD
Sarah Taft, CESER/Immediate Office (IO)
Elise Jakabhazy, OEM/CMAT
Jessica Duffy, EPA R3
Philip Solinski, EPA ERT East

Jacobs Technology, Inc.

D. Adam Hook
Robert Yaga
William Schoppman
Jerome Gilberry

DHS S&T Project Manager
Andrea Wiggins, DHS S&T Office of Mission Capability and Support (MCS)
Donald Bansleben, DHS S&T MCS, Ret.

U.S. EPA Quality Assurance Reviewer
Ramona Sherman, CESER/HSMMD

Table of Contents

Figures

Tables

Acronyms and Abbreviations

AAS	aggressive air sampling
AACeSS	Activity-based Aggressive-air Contained Sampling System
ABS	activity based sampling
ADA	aerosol deposition apparatus
ANOVA	analysis of variance
AnCOR	Analysis for Coastal Operational Resiliency
APS	Aerodynamic Particle Sizer
ATF	Aerosol Test Facility
AWT	aerosol wind tunnel
BioLab	CESER/HSMMD Microbiology Laboratory
Ba	*Bacillus anthracis*
Bg	*Bacillus atrophaeus subspecies globigii*
BSL	*Biosafety Level*
Btk	*Bacillus thuringiensis subsp. kurstaki*
CDC	Centers for Disease Control and Prevention
CESER	Center for Environmental Solutions and Emergency Response
CFU	colony forming unit(s)
CMAD	Consequence Management Advisory Division
CMAT	Consequence Management Advisory Team
COC	chain of custody
CV	coefficient of variation
°C	degrees Celsius
DI	deionized
$D_{sampler}$	sampler diameter
$D_{sampling}$	sampling diameter
DQI	data quality indicator

DQO	data quality indicator
EPA	U.S. Environmental Protection Agency
FMF	Fluid Modeling Facility
fpm	feet per minute
ft	foot/feet
ft^2	square foot/feet
ft^3	cubic foot/feet
GPS	global positioning system
h	hour(s)
HASP	Health and Safety Plan
HEPA	high-efficiency particulate air
HETS	Human Exposure Test Section
HSMMD	Homeland Security and Materials Management Division
HSPD	Homeland Security Presidential Directive
HSRP	Homeland Security Research Program
ID	inner diameter
ID	identification
in	inch(es)
L	liter(s)
lb(s)	pounds
LOD	limit of detection
MCS	Office of Mission Capability and Support
MDI	metered-dose inhaler
mg	milligram(s)
mL	milliliter(s)
µL	microliter(s)
µg	microgram(s)

µm	micrometer(s)
min	minute(s)
mph	miles per hour
NAM	negative air machine
NIST	National Institute of Standards and Technology
OEM	Office of Emergency Management
ORD	Office of Research and Development
OSHA	Occupational Safety and Health Administration
PBST	phosphate-buffered saline with 0.05 % TWEEN 20
PPE	personal protective equipment
psi	pounds per square inch
PTFE	polytetrafluoroethylene
Q	volumetric flow rate
QA	quality assurance
QAM	quality assurance manager
QAPP	quality assurance project plan
QC	quality control
QR	quick read
RH	relative humidity
RMC	reference material coupon
rpm	rotation(s) per minute
RTP	Research Triangle Park, NC
s	second(s)
SO	safety officer
SOP	standard operating procedure
SE	standard error
SPORE	Scientific Program on Reaerosolization and Exposure

SS	stainless steel
Stdev	standard deviation
STS	sampler test section
t	sampling time
T	temperature
TO	task order
TOCOR	Task Order Contract Office Representative
TOM	Task Order Manager
TPU	thermoplastic polyurethane
TSA	trypticase soy agar
UC	unified command
$U_{sampler}$	sampling velocity
USCG	United States Coast Guard
WAD	wide area demonstration

Executive Summary

The Analysis for Coastal Operational Resiliency (AnCOR) program is an interagency effort involving the Environmental Protection Agency (EPA), Department of Homeland Security Science and Technology Directorate (DHS S&T), and the United States Coast Guard (USCG). The overall purpose of this multiagency program is to develop and demonstrate capabilities and strategic guidelines to prepare the U.S. for a wide-area release of a biological agent, including mitigating impacts to USCG facilities and assets. These capabilities included sampling and analysis, decontamination, and waste management. One of the AnCOR sampling subtasks was to develop novel sampling methods for outdoors following a wide-area biological agent release to inform mitigation and consequence management decisions and alleviate some of the extensive labor and time constraints when sampling over a large complex outdoor area.

This report summarizes one specific portion of the overall AnCOR program (Subtask 1.4) which was to develop an activity-based air sampling protocol for use in outdoor areas. This novel sampling concept combines aggressive air sampling (AAS) and activity-based sampling (ABS) into a feasible effort that could be conducted post release to characterize areas of contamination or potentially support clearance of areas for reuse following remediation. AAS is a sampling technique used for clearance following indoor asbestos remediation that uses a leaf blower (aggressive air) to resuspend materials from surfaces for filter capture and analysis. ABS is a sampling technique that consists of simulating outdoor human activities while collecting personal breathing zone samples during those activities to estimate inhalation exposure risk to contaminants such as asbestos from contaminated soils. Much research has been conducted on the sampling of indoor surfaces following a biological agent release. However, a release outdoors over a wide area presents many challenges for traditional surface-based sampling, namely the diverse outdoor surfaces and potential large area of contamination to be sampled. As such, novel or nontraditional sampling may provide an alternative to alleviate some of these challenges. Additionally, air sampling may help inform decision makers on the risk posed by outdoor contamination as compared to surface sampling alone. The Activity-based Aggressive-air Contained Sampling System (AACeSS) was developed to help fill these research and sampling gaps. Traditional surface sampling methods using probabilistic schemes may lead to an inordinate number of samples, time, and resources to characterize the potential widespread contamination from complex outdoor surfaces. AACeSS may provide for responders another tool that can sample over a much larger area as well as provide a measure of the potential inhalation risk from a biological agent such as *Bacillus anthracis.*

Similar to aggressive-air sampling conducted following asbestos remediation indoors and activity-based sampling conducted by responders for outdoor areas or soils contaminated with asbestos, the first concept was to utilize aggressive mechanical means to resuspend the biological agent from surfaces, capture the agent onto high volume air filters, and then determine concentrations of resuspended agent. Because it would not be prudent to spread contamination from the aggressive air generation, the first version of AACeSS (AACeSS V1) was a high-volume sampling tent system that provided containment during sampling. While the tent-based sampling system was shown to efficiently resuspend and collect surrogate spores, it did have several drawbacks, including lack of mobility and wall losses. The tent walls collected considerable quantities of spores in addition to the high-volume filter sampler which presented challenges for decontamination and reuse or waste management.

Subsequent field tests for AACeSS V1 also demonstrated an important operational limitation in that tents are susceptible to high wind and need to be rigidly attached to the ground, further limiting the utility of tents to be used to sample multiple zones at once. The applicability of a rigidly attached tent sealed to prevent escape of biological agent may be more applicable to contaminated vehicles and USCG vessels with complex geometries that can be feasibly tented. All things considered, AACeSS V1 does provide a potential sampling tool for establishing some measure of inhalation risk for biological agents through secondary resuspension from outdoor or vehicle/vessel surfaces, but its utility as a tent-based system using commercially-available tents may be limited.

Due to some of the operational limitations of AACeSS V1, we determined that further development of AACeSS should include a reduction in sampling volume to maximize sampling efficiency, decrease wall losses, and provide more portability for sampling complex outdoor surfaces. Designs for a mobile sampling system using the same resuspension mechanism (leaf blower) and filter sampling yet providing containment were undertaken. Following scoping tests, a second, mobile sampling version of AACeSS (AACeSS V2) was developed for future deployment in the AnCOR 2022 field demonstration and use during a real-world event. This second version of AACeSS is included in Appendix A. Future testing and development of V2 will be reported on in future publications. Finally, lessons learned during the field test of AACeSS V1 (tent version) led to development of a sampling protocol for the tent-based AACeSS V1 system which can be found in Appendix B.

1.0 Introduction

1.1 Background

The United States Environmental Protection Agency (EPA) is designated as a coordinating Agency, under the National Response Framework, to prepare for, respond to, and recover from a threat to public health, welfare, and/or the environment caused by actual or potential oil and hazardous materials incidents. Hazardous materials include chemical, biological, and radiological substances, whether accidentally or intentionally released. Under Homeland Security Presidential Directive (HSPD) 10, the U.S. Department of Homeland Security (DHS) is tasked to coordinate with other appropriate Federal departments and agencies, to develop comprehensive plans which, "provide for seamless, coordinated Federal, state, local, and international responses to a biological attack." As part of these plans, the EPA, in a coordinated effort with DHS, is responsible for "developing strategies, guidelines, and plans for decontamination of persons, equipment, and facilities" to mitigate the risks of contamination following a biological weapons attack.

In the event of a biological weapons attack, determining the location of the contaminants and the potential of resuspension of the agent materials is of great interest to the EPA, DHS and the United States Coast Guard (USCG). After release, a wide range of surfaces would likely be contaminated. The EPA, as well as the USCG, has the general responsibility for protecting human health and the environment, which would include biological agent exposure from secondary emission of materials from these areas. EPA could be requested or required to mitigate, provide consequence management, and decontaminate the area of concern. To that end, the EPA's Center for Environmental Solutions and Emergency Response (CESER), Homeland Security and Materials Division (HSMMD) has been developing methods to sample areas contaminated with *Bacillus anthracis (Ba)* spores and assess resuspension risk from a broad array of surfaces.

This research involving biological agents (e.g., *Ba*) is based on significant work that has been conducted in the past on methods to determine the risk involved in occupying outdoor areas previously determined to contain some level of asbestos contamination. The EPA has developed activity-based sampling (ABS) methods for assessing the resuspension of asbestos fibers from outdoor surfaces into the breathing zone and determining if there is an inhalation risk from the resuspended asbestos fibers (EPA 2008). One component of ABS is aggressive air sampling (AAS) where a leaf blower is used as an extreme form of surface agitation to resuspend asbestos into the breathing zone and the air is sampled. AAS helps to provide clearance criteria for proper utilization and occupancy of the previously contaminated area. Prior work has also been conducted by the EPA to evaluate using AAS technology for *Ba* on indoor and subway surfaces under controlled conditions with some success (EPA 2013, EPA 2017). The sampling of biological agents from outdoor surfaces, materials, and vessels used by the USCG poses challenges due to variations in humidity, surface coatings, and surface histories, such as surface oxidation and salt accumulation, which can vary significantly between vessels and coastal installations (i.e., base, station, post, etc.). Resuspension of *Ba* spores from surfaces specific to USCG in the humidity extremes expected on those installations have not been thoroughly evaluated in the past. To determine a strategy for deployment of AAS at a

USCG installation, it is important to understand under what conditions the technology is applicable. In addition, application of AAS in an open environment presents difficulties in sampling the resuspended material as air flow or wind has the potential to move aerosolized material away from the air sampler bringing into question the sampling efficacy in evaluating the worst-case scenario exposure risk. Additionally, resuspending biological particles in the open outdoor environment may pose hazards to those in or near the contaminated area. To address these concerns, a contained sampling system to prevent resuspended particles from escaping during sampling is necessary.

1.2 Objectives

The purpose of this research, conducted under the Analysis for Coastal Operational Resiliency (AnCOR) program was to determine if an AAS technology could be successfully deployed at a USCG installation or other outdoor environment. Additionally, a strategy/protocol needed to be developed for implementation of this sampling technology. As a response to this research need, an Activity-based Aggressive-air Contained Sampling System (AACeSS) was designed. The primary objectives of this work were to test AACeSS on outdoor surfaces representative of USCG installations to determine if the aggressive sampling system promotes the resuspension of *Ba* surrogate spores from surfaces and efficiently captures them in the contained sampling system. This system was evaluated under laboratory-controlled conditions and field-tested to identify the scenarios where its deployment is most effective. Additionally, we analyzed potential modifications that could enhance its operability, as well as the efficiency of sampling and collection.

2.0 Experimental Approach

This study involved measuring resuspension (under varied environmental conditions) of surrogate bacterial spores deposited onto representative outdoor surfaces typically found at USCG installations. As the use of the actual biothreat agent, *Ba,* for resuspension studies in a laboratory or field setting must be conducted under Biosafety Level 3 (BSL 3), appropriate surrogates were used. See Section 3.5 for details on the selected bacterial spore surrogate. Previous experiments conducted at the EPA under the Scientific Program on Reaerosolization and Exposure (SPORE) demonstrated that *Bacillus thuringiensis subsp. kurstaki* (*Btk*) is a suitable *Ba* surrogate for reaerosolization studies (EPA, 2014). However, for field experiments the presence of *Btk* in the environment and inability to distinguish it via culture from background flora, makes *Btk* unsuitable for field testing and another surrogate is preferred. *Bacillus atrophaeus subspecies globigii* (*Bg*) has a long history of use in the biodefense community as a simulant for anthrax (Gibbons et al. 2011), and this simulant has been used frequently in EPA-related field studies where *Btk* is unsuitable (EPA 2017).

To assess the field use of AACeSS, the ability of the system to resuspend material from a surface and effectively collect the reaerosolized material must be determined. To assess the ability of the system to resuspend and collect material, sampling efficiencies (SEs) were calculated using the following equation:

$$SE = \frac{P_c}{P_d}$$

where P_c is the number of colony forming units (CFUs) collected after a sampling event, and P_d is the number of CFUs deposited onto the surface. This equation is valid as long as the total volume of air is sampled or the percentage of air sampled is sufficiently high (i.e., 90% or greater to ensure effective filter capture and minimization of wall losses/settling) to be below the sampling variation. For AACeSS, the total particles captured is governed by a dilution ventilation equation (Talty 2005). Neglecting settling and assuming good mixing throughout the enclosed volume, the percentage of particles captured by the high-volume sampler is given by:

$$\left(1 - e^{-\frac{Q}{V}t}\right) \times 100\%$$

where Q is the volume flow rate of the AACeSS high-volume sampler, V is the volume of the sampling space, and t is the sampling time. Therefore, the sampling time was chosen so that >95% of particles were captured on the filter.

In addition, personal breathing zone samples were collected to represent potential personal exposure during the AACeSS sampling event.

Due to the variety of outdoor surface materials located in a USCG installation and the difficulty of conducting large scale field experiments, laboratory-based studies needed to be conducted first on a variety of materials to help determine the applicability of AACeSS. The test materials were chosen to represent materials found outdoors at USCG installations. These test materials included concrete and asphalt – found on the ground – as well as materials generally found on

smaller USCG vessels. The vessel materials chosen were marine grade aluminum, marine grade aluminum covered in a nonslip/skid resistant tape, and broadside boat rubber. The materials were new and clean without the presence of dust or debris that would be characteristic of materials found outside. Larger dust particles provide an easier opportunity for material to resuspend due to either collisions or settling onto the particles themselves. Therefore, clean non-weathered surfaces in the laboratory likely represent the worst-case scenario for the application of an aggressive air sampling technique (EPA 2014).

Relative humidity (RH) can also be a major factor in the ability of particles to resuspend in that higher RH inhibits resuspension. Extremes in RH (<30% and >80%) can have an order of magnitude difference in particle resuspension depending on what surface the particles are deposited (Kim et al. 2016). Thus, it is also necessary to vary RH in the laboratory testing at some extrema that may be encountered on USCG installations. Though laboratory tests provide some insight into the ability of an aggressive air sampling technique to detect surface contamination and inhalation risk, they do not adequately represent all difficulties encountered during a field-testing event or the resuspension from a dirty weathered outdoor surface. To establish the effectiveness of the AACeSS system and develop any necessary modifications to the sampling protocol, we ultimately decided that in addition to the laboratory evaluation, conducting a field test was also necessary. This field test would allow for the evaluation of particle resuspension and collection in an outdoor environment, as well as an assessment of any operational factors that may limit the use of AACeSS to ensure that AACeSS can operate effectively under real-world conditions.

The general experimental approach used to meet the project objectives is described below:

1. **Laboratory evaluation of AACeSS performance using representative USCG materials.** To evaluate the performance of AACeSS in a laboratory setting the tent-based system was deployed inside a large temperature- and RH-controlled wind tunnel. The system provided clean, HEPA-filtered background air at a wide range of temperatures and relative humidities. Prior to testing, the wind tunnel surfaces were thoroughly cleaned with a 10% bleach solution to remove any background spores.

    a. To test the performance of an aggressive air sampling technique inside the AACeSS system for an array of USCG materials, 14” x 14” squares of material were placed on the floor of the system, inoculated with ~1 x 10^8 CFU (6.9 x 10^5 CFU/square inches (in^2)) of *Bg*, and aggressive air sampling was conducted. Resuspended material was captured onto the high-volume air sampler filter. The amount of material captured was compared to the amount deposited to determine sampling efficiencies.

    b. The variation of resuspension due to relative humidity was determined by varying the RH from 30% to 80% and comparing the sampling efficiencies of each material. Each 14” x 14” square was allowed to equilibrate to the RH condition overnight prior to testing.

c. The assessment of the effectiveness of AACeSS to provide a measure of potential inhalation risk was tested by measuring the amount of material (spores) captured by personal air samplers worn by the technician conducting aggressive air sampling.

d. One square foot (ft^2) sections of the AACeSS tent walls were surface sampled with sponge sticks to estimate the amount of material resuspended but lost to the walls to help determine capture efficiency of the high volume sampler as well as contamination of the system.

2. **Field deployment of AACeSS.** To evaluate the deployment of AACeSS in a field environment, the testing procedure is shown below:

    a. A team deployed three AACeSS tents and three surface sampling tents on a concrete pad outside an EPA facility.

    b. Aggressive air background sampling was conducted inside each AACeSS tent to determine if any *Bg* was present prior to inoculation.

    c. A single 1 ft^2 concrete area was inoculated inside each tent with ~1 x 10^5 CFU (6.9 x 10^2 CFU/in^2) of *Bg*.

    d. Aggressive air sampling was conducted the following day. High volume filter samples were collected as well as personal air samples inside the tent during the entire sampling process.

    e. After sampling, wet vacuums were used to sample the initial inoculated area as well as the rest of the “non-inoculated” tent floor to estimate the amount of material remaining in the 1 ft^2 inoculation zone as well as the amount of material that spread throughout the concrete surface inside the tent footprint. One ft^2 sponge stick samples were also collected on the tent walls to estimate spore wall loss.

In the three surface sampling tents, the inoculated area was sampled by wet vacuum to determine the wet vacuum recovery efficiency from the undisturbed concrete surface to compare with the AACeSS tent samples. This wet vacuum method has been demonstrated by EPA (Mikelonis et al. 2020) to provide similar recoveries to sponge stick sampling on outdoor concrete surfaces and can sample larger surface areas.

3.0 Experimental Materials and Methods

This section describes AACeSS design and implementation, test materials, laboratory environmental chambers, the test surrogate, deposition method, sampling methods, and outdoor field space used to achieve the project objectives.

3.1 AACeSS Version 1 – Laboratory and Field Tests

The initial version of AACeSS is a sampling device that uses a self-contained modular enclosure and filtered air capture system for resuspending and subsequently capturing resuspended bioaerosols from surfaces contained within the enclosure. AACeSS consists of an enclosure, one or more high volume air samplers, and an aggressive air resuspension method such as a leaf blower. It can be used for sampling outdoor hard surfaces such as asphalt and concrete or more complex surfaces such as dirt, gravel or vegetative surfaces or marine vessels/vehicles that can be contained within its enclosure volume (i.e., tented). A schematic wireframe of the AACeSS setup is shown in Figure 3-1. For these experiments, the initial version of AACeSS consisted of an outdoor car storage tent, a high-volume air sampler comprised of nine felt filters mounted onto a modified negative air machine (NAM) with filter holders mounted on the inlet face (Figure 3-2) (Omni-Aire 2200C, Omnitec Design, Inc., Mukilteo, WA), and a technician operating a leaf blower (Kobalt KHB 400B, Lowes Companies Inc., Mooresville, NC).

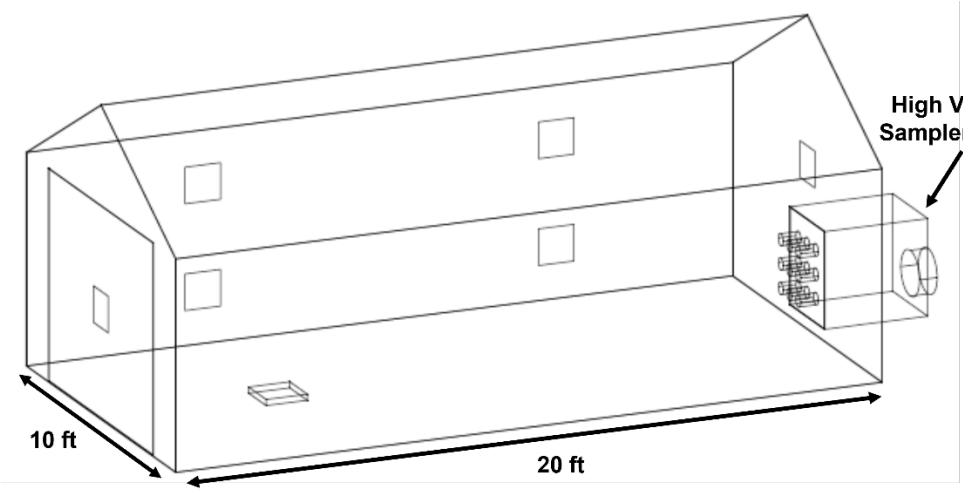


Figure 3-1. Schematic wireframe of AACeSS Version 1

Two tent models were used for these experiments, and both types had a nominal base dimension of 10 ft x 20 ft. The tent (Caravan Canopy Carport Model 22006200010 and 12000211010, Caravan Global US, La Mirada, CA) used for the laboratory experiments conducted within an environmental chamber (wind tunnel) had floor dimensions of 10 ft by 20 ft and a sloped roof with average height of 7.2 ft, providing an internal volume of 1440 cubic feet (ft^3). The tent was constructed of heat-sealed polyethylene material. This tent was chosen to fit in the environmental chamber and was easy to assemble. The tents (Autoshelter #62680,

Shelterlogic Group Inc., Watertown, CT) used for the outdoor field test had a sloped roof, an actual internal base dimension of 9 ft 7 in x 19 ft 7 in, and an internal volume of 1236 ft^3. The tent material was triple-layer UV-protected polyethylene. This tent was chosen to withstand wind in the field, be similar in size to the tent used in the laboratory testing and have excess material on the lower walls or skirt that could be covered with sandbags to help seal the tent from particle exfiltration. Both tents had zippered end flaps that could be closed.

The high-volume air sampler labeled in Figure 3-1 is shown in more detail in Figure 3-2 (a) and was comprised of a negative air machine with a custom faceplate to mount 9 holders containing felt filters (EQXSCIEN-001, Superior Felt and Filtration, Ingleside, IL, USA). Figure 3-2 (b) shows the back end of the felt filter holders – developed for previous resuspension experiments (EPA 2014) – that are inserted into the flanges of the NAM plate. The average flow rate through each filter face was 28.96 ft^3/minute (min) with a total flow rate for the high-volume sampler of ~260 ft^3/min. Therefore, for 20 minutes of sampling, using the equation from Section 2.0, ~97.3% of aerosol material was captured within the tent used in the laboratory testing and ~98.5% was captured within the tent for the field test. The NAM contained a high-efficiency particulate air (HEPA) filter to capture any material that managed to penetrate completely through the felt filters, so it would not be exhausted back into the tent space.

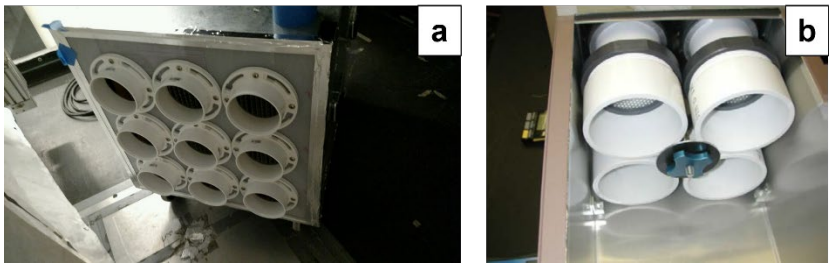


Figure 3-2. (a) Negative air machine with filter mounting faceplate and (b) filter holders

Figure 3-3 shows the location of the NAM at the end of the AACeSS tent. A hole just smaller than the face of the NAM was cut into the end of the tent, and the connection was sealed with high adhesive tape. The location of the NAM filter faceplate was centered on the short tent face horizontally to create a more direct path to the filters and cut to accommodate the height of the NAM while on casters and minimize further modifications.

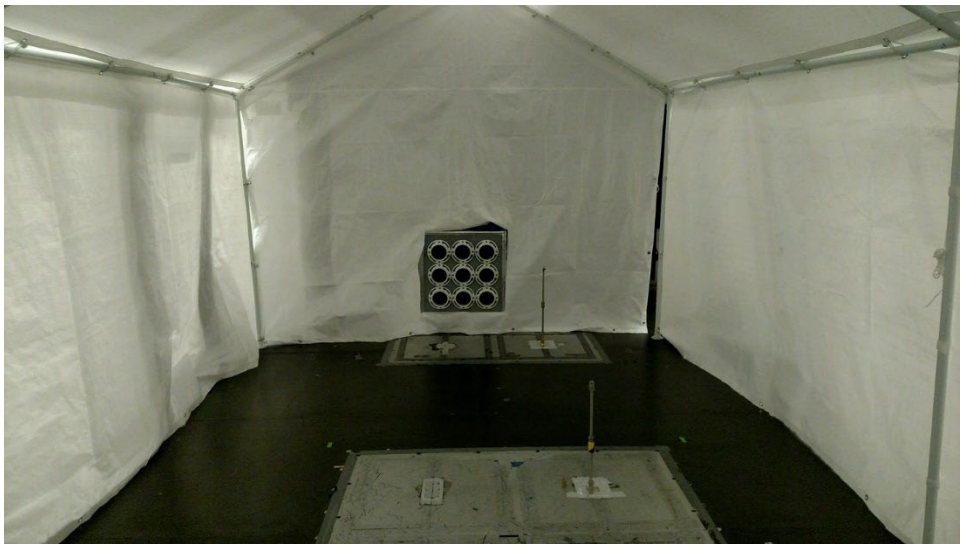


Figure 3-3. Negative air machine AACeSS location

Figure 3-4 shows a top view of AACeSS, the walking path of the sampling technician, and the position of the inoculated area/coupon within the tent. Aggressive air sampling in AACeSS had two main phases – an active resuspension phase and a passive sampling phase. The active AAS phase took place along three 3ft 4in lanes across the 10ft width of the tent. Starting on the farthest corner from the high-volume sampler on the side that faces the entrance door to the wind tunnel environmental enclosure, the technician blew toward the sampler with the leaf blower using a side-to-side sweeping motion while walking forward for 20 seconds (s) until reaching the tent face with the high-volume sampler. The technician then turned around and walked away from the sampler while leaf blowing for another 20 s until returning to the original position. The technician then moved to the center lane and repeated this pattern. Finally, the technician moved to the third lane and repeated the pattern once again for a total active AAS time of 2 min with a total surface coverage rate of 100 ft^2/min. The passive phase duration was 18 min. During this phase, all samplers were operating (personal and high volume); however, no particles from the surface were being added to the air allowing the high-volume samplers to clear the tent volume of nearly all particles. The total sampling time for the AAS was 20 min. This duration was chosen to ensure incorporation of three air exchanges inside the tent space. In general, sampling volume, surface areas, and number of personnel should be chosen so that the entire aggressive air sampling procedure should last no more than 30 min in duration to minimize the time workers are in personal protective equipment (PPE) (Level C or higher) to minimize heat stress, especially when sampling in hot outdoor settings.

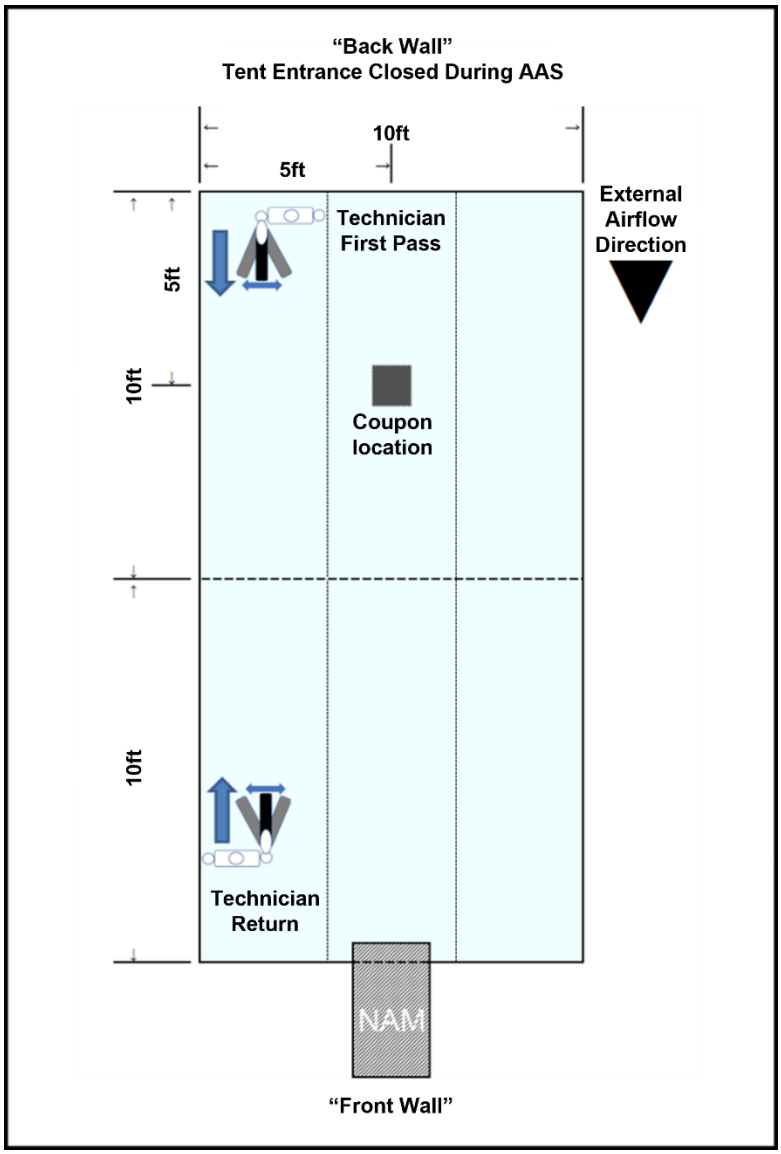


Figure 3-4. Top view of AACeSS with sampling pattern and hot spot

3.2 Laboratory Environmental Systems

To achieve specific environmental conditions for material coupon storage/conditioning and resuspension testing, the EPA RTP facility has multiple environmental chambers to ensure controlled conditions and containment of particles. These chambers include an environmental test/conditioning chamber (shown in Figure 3-5) used for storage and conditioning of test material coupons and the EPA's recirculating Aerosol Wind Tunnel (AWT, shown in Figure 3-6) used to house the AACeSS tent (Section 3.1) for the laboratory-based testing. The AWT has a large volume section referred to as the human exposure test section (HETS). Its floor dimensions are 30 ft x 12 ft (L x W) and its ceiling height is 10 ft which allows it to fully house

the AACeSS tent and high-volume sampler with room for technicians to maneuver around the system. The recirculation of the tunnel allows for constant control of temperature and humidity while additionally providing an airflow to disperse any potential particles that are able to exfiltrate the system. Both containment systems are high-efficiency particulate air (HEPA)-filtered and are at negative pressure relative to the surrounding laboratory spaces. Both systems were temperature (T)- and RH-controlled. All set conditions were monitored throughout the experiments via a calibrated probe (VWR 35519-041, VWR International, Radnor, PA), HOBO Micro Station data logger (Onset Computer Corp., Bourne, MA), and Humicap HMT330 (Vaisala Inc., Louisville, CO).

Figure 3-5. T- and RH-Controlled environmental storage chamber

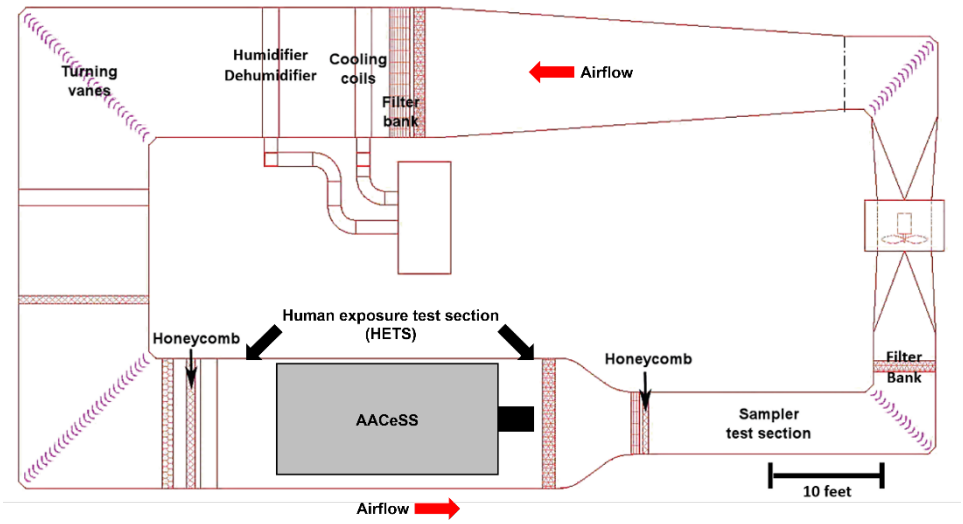


Figure 3-6. Plan view of the EPA Aerosol Wind Tunnel with AACeSS

3.3 Laboratory Test Materials

The representative materials chosen for the laboratory tests were drawn from a subset of materials generally found outdoors on USCG installations such as hard ground surfaces and materials found on vessels. The hard ground surfaces chosen for testing were concrete and asphalt. Vessel materials were marine grade aluminum, aluminum coated with skid-resistant tape, and aliphatic thermoplastic polyurethane (TPU) boat rubber. The concrete and asphalt coupons were constructed of dimensions 14 in x 14 in x ~2 in and fabricated in bulk at the EPA RTP facility to ensure uniformity of materials. Concrete coupons were produced in custom fabricated molds using QUIKRETE sand/topping mix (Product # 1103, The QUIKRETE Companies, Atlanta, GA) according to manufacturer specifications. The coupons were allowed to cure for at least 5 days at indoor ambient temperature and RH covered by plastic. The concrete coupons were removed from the molds and allowed to continue curing in an environmental chamber at 20 degrees Celsius (°C) and 30% RH for a minimum of two weeks before use. Asphalt coupons – shown in Figure 3-7 (a) – were prepared using pills from the North Carolina Department of Transportation Asphalt Laboratory of the Materials and Tests Unit (Raleigh, NC). Multiple cylindrical pills were heated to approximately 138 °C for 3–5 h, homogenized (Mud Monster Model #G06160, Goldblatt Industries, Saddle Brook, NJ and 9-Amp 1/2-in Keyed Corded Drill Model # DW130V, DEWALT, Baltimore, MD), and proportioned into custom fabricated coupon molds by a mass that corresponded to the same average density as the cylindrical pills. The asphalt was then pressed using a hydraulic Arbor press (Model LP-500, Devin Mfg. Inc., Arcade, NY) and 1,814 kg of applied pressure. The coupons were allowed to cool to room temperature before removal from the molds.

Aluminum coupons were created by attaching 14 in x 14 in sheets of marine-grade aluminum (Type 5086, Dillon Supply Inc., Raleigh, NC) to 14 in x 14 in x 0.75 in thick plywood with spray adhesive (Super 77™, 3M™, Maplewood, MN). Skid resistant tape (3M Safety Walk 17440, McMaster-Carr, Douglasville, GA – shown in Figure 3-7 (b) – and TPU boat rubber (TPU 2051, E2 Technical Textiles, Hillside, NJ) surface coupons were created identically to the aluminum coupons; however, the skid-resistant tape was attached directly to marine grade aluminum prior to attaching to the plywood backing, and the boat rubber was attached directly to the plywood with adhesive spray. Loose particles were removed from the concrete, asphalt, and skid-resistant surfaces by spraying with an air nozzle at 30 pounds per square inch (psi) compressed house air prior to particle seeding/inoculation. Aluminum and rubber coupons were wiped clean with a lint-free cloth and methanol prior to particle seeding/inoculation.

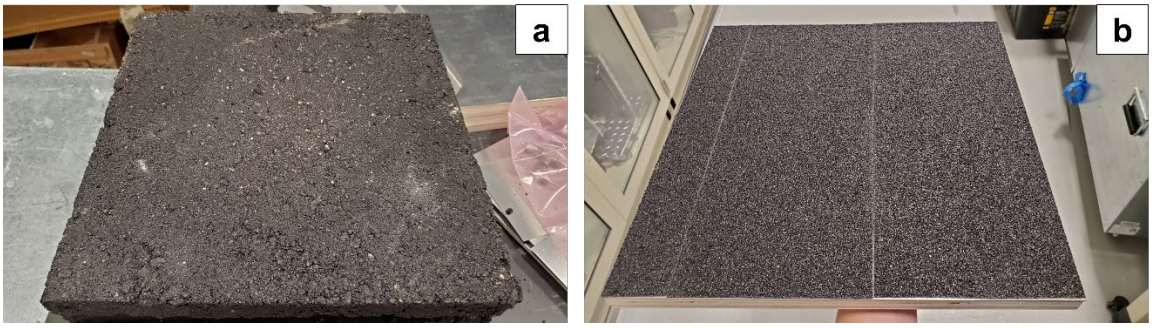


Figure 3-7. a) Pressed asphalt coupon b) Skid-resistant tape on aluminum

3.4 Field Test Surface and Location

The field test site for this work was located at the EPA RTP campus [Fluid Modeling Facility (FMF)] in Durham, NC. This EPA facility is a leased facility that includes a wind tunnel research facility and the EPA storage warehouse. Features on this site included buildings, an asphalt-paved parking lot/roadway, gravel/dirt areas, grassy areas, and concrete pad surfaces as shown in Figure 3-8 and Figure 3-9. Figure 3-8 shows a total aerial view of the FMF site. Figure 3-9 shows a zoomed aerial view of the test area with dimensions in feet. The test area was a concrete slab of approximately 100 ft x 100 ft (10,000 ft^2) and was located directly behind the FMF building. The slab was unshielded from the environment, and there was no effort to remove naturally deposited material for the test areas. This material included natural fine particulate matter (e.g., dirt and pollen), as well as small twigs and pine needles. However, the coverage of the concrete pad with twigs and pine needle was sparse and care was taken to minimize large debris in the inoculated areas.

Figure 3-8. Aerial view of EPA Warehouse/FMF Facility in Durham, NC

Figure 3-9. EPA Warehouse/FMF Facility in RTP, NC (Zoomed aerial view of concrete test pad)

3.5 Test Surrogate Preparation and Deposition

For both the laboratory testing of AACeSS and the outdoor field test, *Bacillus atrophaeus subspecies globigii* (*Bg*) spores were used as the nonpathogenic surrogate for *Ba* spores. The *Bg* was obtained from Dugway Proving Ground (Dugway, UT) as a dry powder of lyophilized spores. *Bg* spores were prepared by cultivating *Bacillus atrophaeus* in tryptic soy broth (Difco, Detroit, MI) containing 3 milligrams (mg)/liter $MnSO_4$ (Fisher Scientific, Pittsburgh, PA). After 80 to 90% sporulation, the spore suspension was centrifuged to obtain a spore suspension containing approximately 20% solids. The spore material was dry blended with Aerosil R812 fumed silica particles (Degussa, Frankfurt am Main, Germany) at 80% dry spore material to 20% silica and jet milled to a uniform particle size. The powdered spore preparations were loaded into metered-dose inhalers (MDIs) at the EPA with volumes corresponding to doses of 10^8 colony forming units (CFU)/puff for the laboratory tests or 10^5 CFU/puff for the field tests. Each MDI canister contained *Bg* spores suspended in ethanol and HFA-134 propellant (1,1,1,2-tetrafluoroethane) sufficient to deliver +200 charges of 50 microliters (µL) per discharge. The number of discharges was tracked to ensure discharges from a single MDI remained below 200. The weight change of the MDI was measured after every inoculation. The expected weight change of each actuation was 50 micrograms (µg) ± 10 µg. If the total weight of the MDI was below 8.5 g before the start of the series of inoculations, the MDI was retired. For the depositions, the MDIs were attached onto an aerosol deposition apparatus (ADA), shown in Figure 3-10, and actuated with a single puff. The ADA had a deposition dimension of 12 in x 12

in and a total base area of 14 in x 14 in. The larger area is due to a 1-in lip that contains a gasket to help seal the ADA to the substrate. The MDI + ADA combination has been used extensively for sampling and decontamination studies at the EPA. (EPA 2012, Calfee et al. 2013, EPA 2017, EPA 2018,)

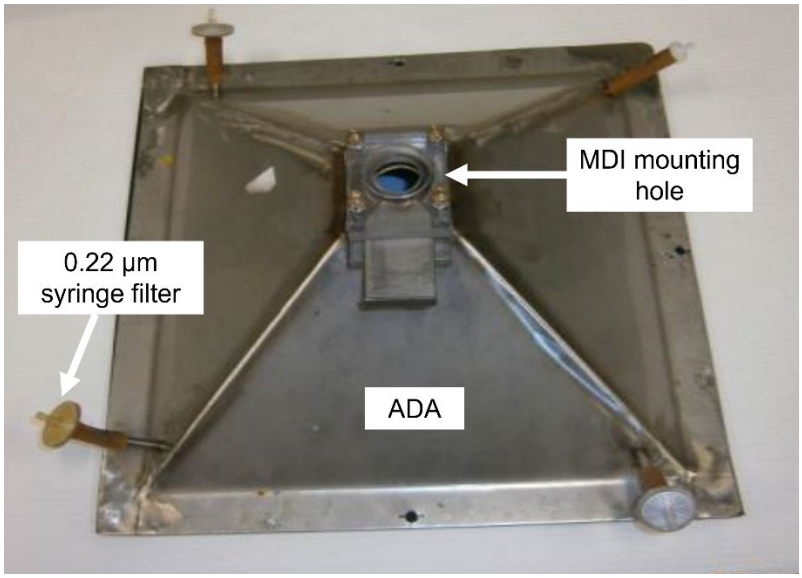


Figure 3-10. Aerosol deposition apparatus (ADA)

3.6 Personal Air Sampling

The personal air samplers for collecting breathing zone samples during the AAS procedures consisted of a 25 mm, 3.0 micrometer (µm) pore size polytetrafluoroethylene (PTFE) filter (FSLW02500, Millipore Sigma, Burlington MA), an SKC Button Sampler filter holder (Cat. No. 225-360, SKC, Inc. Covington, GA), Tygon sample tubing, and personal sampling pump (PN 224-PCXR8, SKC, Inc.) calibrated to a 4 liters (L)/min air flow using a DryCal Defender 520 field flow calibrator (Mesa Labs, Butler, NJ). SKC Button samplers have been evaluated in the past with 3.0-µm pore PTFE filters for *Bacillus* bioaerosol inhalation studies and were shown to sample efficiently and minimize pressure drop on the sampling pumps (Grinshpun et al. 2017). Figure 3-11 (a) shows the SKC Button Sampler assembly, and (b) shows the breathing zone location of the Button Samplers and waist location of the pumps on a technician's PPE. The Samplers in Figure 3-11 (b) are shown covered by red caps to prevent contamination prior to sampling. Two Button Samplers (duplicate) were deployed in the breathing zone for each AACeSS resuspension test to ensure quality data and account for sampling variation.

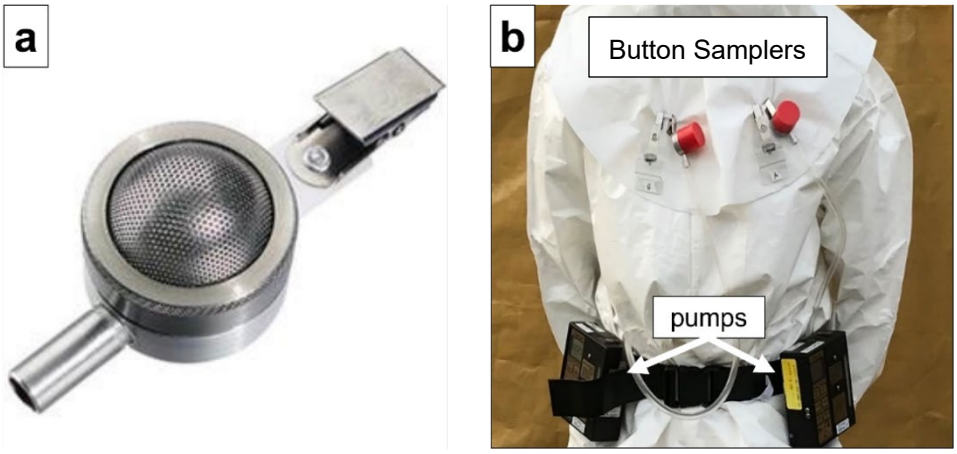


Figure 3-11. a) SKC Button Sampler assembly, b) Sampler and pump position on AACeSS technician

3.7 Sponge Stick Sampling

Sponge-stick sampling was conducted with every resuspension test in two main areas. The first was a measurement of positive control coupons for every deposition. In this instance for every deposition (both for the field and for laboratory measurement testing), a 14 in x 14 in stainless-steel coupon was also deposited with the inoculum and sampled with a sponge stick (3M Sponge Stick SSL-10NB, St. Paul, MN) in accordance with the Centers for Disease Control and Prevention (CDC)-published procedure (CDC 2012). In addition, sponge-stick sampling was conducted on the inside of the AACeSS tent walls in 12 in x 12 in sections also using the same procedure. For the wall samples in the laboratory-based experiments, the areas were decontaminated with pH-amended bleach (8:1:1 ratio of water, >5% household bleach, and 5% acetic acid), rinsed with water, and dried using ethanol prior to each test run since only one tent

was used for all testing and required a reset. The locations of the sampling areas were as follows:

- The first area was positioned in the center of the width of the wall containing the high-volume NAM filter array with the bottom of the sampling area 1 ft above the top of the filter array.
- The 20-ft sides of the tent contained 4 sampling areas each.
- Along the length of each of the 20-ft walls, two sampling areas were aligned vertically 5 ft from the nearest corner to the high-volume sampler, and two were aligned vertically 5 ft from the farthest corner.
- The centers of the sampling areas were horizontally aligned at 1.5 ft from the floor and 4.5 ft from the floor. This design located a sampling area in the center of the 4 quadrants of the 20-ft wall sections.
- A final sampling area was located in the center of the farthest wall from the high-volume sampler for a total of 10 wall samples.

These placements can be seen in Figure 3-1 as vertical squares on the walls with the nearest wall wipe locations removed for clarity. The intention of these wall samples was not to give a full quantitative representation of the particle loss to the walls but a relative estimate of spore loss in orders of magnitude.

3.8 Wet-vacuum Sampling

Wet-vacuum sampling during the AACeSS field test was conducted using the method and procedures developed by the EPA in prior deployments of wet vacuums for *Bg* sampling on concrete (EPA 2018). For concrete, the recovery of deposited spores via MDI and ADA has been shown to be on the order of 33% ± 17% (EPA 2018). The sampling technique was used in three areas for the field test:

- The first wet-vacuum sample was collected in a tent separated from each AACeSS tent, allowing for collection of the surface sample while minimizing cross contamination from the AACeSS samples. These 1 ft x 1 ft samples over the deposition or inoculated areas (hot spots) served as the baseline for the wet vacuums to help establish their spore recovery percentage on that surface for that particular sampling day, as surface variation and change in environmental conditions can affect spore recovery.
- The second sampling area was the concrete surface area under the AACeSS tent footprint after AAS had been conducted. The second sampling was conducted in two parts; the first was sampling the 199 ft^2 of space surrounding the initial deposition zone inside the tent (1 ft^2) to determine how much material was spread around the area under the tent. The second part of the AACeSS tent sampling was done directly on the 1 ft^2 deposition zone (hot spot) to measure the residual material left after AAS and provide corroboration for quantities collected from the air samples.
- The third sampling zone was a 1-ft-wide sample (~200 ft^2) of the ground surrounding the perimeter of one of the AACeSS tents. Each wet-vacuum sampling area used a single unused wet vacuum. Hoover Max Extract Cleaners (F7425-900 SteamVac Dual V with SpinScrub Hand Tool, Hoover, Glenwillow, OH) were prepared for sampling according to

the specified procedure (EPA 2018) The Hoover Max Extract has a clean water tank and a dirty water tank (Figure 3-12). Two liters of sterile 0.05% Tween solution prepared with deionized water were transferred to the clean tank.

Figure 3-12. HooverMax Extract wet vacuum.

Prior to the start of wet-vacuum sampling, the tank of each wet vacuum was sponge-stick sampled to serve as a sterility check. The wet vacuums were operated with both Rinse and Power Scrub modes turned on. Hot spots were directly sampled (1 ft x 1 ft) with little overlap of outside areas (any area not directly inoculated). Full tent (199 ft^2) samples were operated parallel to the 10-ft-width of the tent. The initial vacuum stroke was with the liquid dispensing trigger on, followed by two vacuum-only strokes covering the same area. The wet vacuum was moved over to cover 50% new area as well as 50% of the area just covered. A new initial vacuum stroke began with the liquid dispensing trigger on. Sampling proceeded in this manner, one wet stroke followed by two dry strokes, until the total area was covered. An observer was assigned to monitor the wet vacuum sampling from outside the tent with the door open to ensure that the operators were conducting the sampling according to the prescribed procedure. If a wet vacuum malfunctioned, this vacuum was immediately replaced by a backup wet vacuum. Immediately following the completion of the wet-vacuum test (while the effluent is homogenized) a 1-L aliquot was transferred to a sterile Nalgene (Nalgene Nunc International Corp., Rochester, NY) bottle and transported to the HSMMD RTP Microbiology Laboratory (Biolab) for microbiological analysis.

3.9 Sample Processing and Enumeration

Bg spores that were collected onto filter media (high-volume filters/personal air sampler PTFE filters) or sponge sticks had to be extracted into a liquid medium before additional processing could occur. All processing and enumeration of samples occurred at the Biolab. The high-volume air sampler filters were grouped into sets of 3 filters by row of the 3 x 3 filter array and placed into Stomacher 400 bags (58976-957, VWR International, LLC, Radnor, PA). Likewise, sponges were aseptically removed from their sticks and placed into individual stomacher bags.

Then, 90 milliliters (mL) of phosphate buffered saline with 0.05% Tween 20 (PBST) was added to the stomacher bags and they were processed using a Seward Model 400 circulator (Seward Laboratory Systems, Inc., Port Saint Lucie, FL) for 2 minutes at 260 revolutions per minute. The eluent was then removed from the bags and sonicated for 15 minutes. The PTFE filters were removed from their filter holders and placed into 50-ml conical tubes containing 10 mL of PBST. These tubes were vortexed for 2 minutes at 10-second bursts followed by ultrasonic agitation for 15 minutes. Following extraction, all liquid samples, including the liquid from the wet vacuum, were either directly assayed by growth on nutrient agar plates using a spiral plater (Autoplate spiraling system, Advanced Instruments Inc., Norwood, MA) in triplicate or if the samples presented a high degree of background flora and debris required additional processing before growth and enumeration. This high debris analytical method is described in a previous study (EPA 2017). Briefly, liquid extracts were heat-treated at 80 °C for 20 min and centrifuged to pellet the debris. The supernatant was filter-plated, and the pelleted debris was resuspended in 10 mL of PBST and spread-plated in 1-mL aliquots. All plates were incubated at 35 ± 2 °C for 16-24 h and enumerated either by a QCount colony counter (Advanced Instruments Inc.) or directly counted by a laboratory technician in the case of filter and spread plating. Data from the Biolab are reported as colony forming units per mL (CFU/mL) along with the volume of extraction fluid (for filters and sponge sticks) or the volume of fluid collected by the wet vacuum. The CFU/mL value was multiplied by the volume to obtain the total number of CFU collected with the sampling method.

4.0 Laboratory AACeSS Testing

This section discusses the test matrix and approach for the laboratory testing of the AACeSS system on USCG materials.

4.1 Laboratory Test Matrix

As outlined in Section 2.0, the objective of the laboratory-based AACeSS testing was to assess the effectiveness of the system in handling USCG materials across a range of RH levels, thereby determining the suitability of AACeSS for use at USCG installations. To achieve testing of the system effectiveness, we selected representative materials commonly found at USCG installations and subjected these materials to the AACeSS system, evaluating its performance under extreme environmental RH conditions. The test matrix for the laboratory tests is shown in Table 4-1. The matrix consists of triplicate runs of materials at two different RH values, 30% and 80% RH. The 30% value was deemed to be representative of the average winter relative humidity, and 80% was representative of the average summer humidity at USCG installations. The temperature of each test was kept constant at 20 °C. The target spore loading was kept constant at $1x10^8$ CFU/ft^2 for all the tests. This level of contamination was chosen so that at even extremely low resuspension percentages (~0.001%), *Bg* could be detected reliably on the high-volume filters. Each day of testing consisted of a background test to establish the cleanliness of the system and tent at the chosen RH, a deposition check to estimate the load of the inoculated coupon, and a test run of AACeSS at the set RH. Each test day generated two high-volume air measurements (one background and one test run), four personal air samples (two background and two test runs), 20 wall samples (10 background and 10 test runs), and one deposition check.

Table 4-1. USCG material test matrix

Material	Spore Load (CFU/ft^2)	Condition	Replicates
Marine Aluminum	1 x 10^8	30% RH	3
	1 x 10^8	80% RH	3
Skid Resistant Coating	1 x 10^8	30% RH	3
	1 x 10^8	80% RH	3
Boat Rubber	1 x 10^8	30% RH	3
	1 x 10^8	80% RH	3
Concrete	1 x 10^8	30% RH	3
	1 x 10^8	80% RH	3
Asphalt	1 x 10^8	30% RH	3
	1 x 10^8	80% RH	3

4.2 Laboratory AACeSS Testing Procedure

Prior to testing, the AACeSS sampling components were decontaminated with pH-amended bleach, rinsed with deionized (DI) water, wiped with ethanol to remove water, and allowed to dry. The floor of the wind tunnel was mopped with pH-amended bleach, allowed to dry and mopped clean with DI water at the end of every testing day. The 1-ft^2 portions of the tent wall slated for surface sampling were marked with tape and decontaminated with pH-amended bleach, DI water, and ethanol. As stated in Section 4.1, each sampling day consisted of:

1. Deposition onto a material coupon;
2. Deposition positive control;
3. Background AACeSS test with personal air samples, a high-volume air sample, and wall samples;
4. AACeSS test run with personal air samples, a high-volume air sample, and wall samples; and
5. Postrun decontamination

The material coupon slated for testing was allowed to equilibrate under the test conditions for a minimum of 24 hours (h) prior to testing (within the wind tunnel HETS or separate environmental chamber). At the beginning of a test day, the material coupon was inoculated outside the AACeSS tent but within the HETS to prevent contamination and was allowed to sit while covered by the ADA pyramid during the background testing phase. The stainless-steel positive control was also inoculated immediately next to the material coupon and was covered by the ADA until sampled. The background tests followed inoculation. These tests were conducted to

demonstrate that the decontamination procedure between samples was effective. A technician (referred to as the helper) placed the high-volume sampler filter holders onto the NAM and measured the flow rate through each filter. Then another technician (referred to as the sampler) donned two SKC Button Samplers with sampling pumps – one on each shoulder in the breathing zone – and prepared to conduct the AAS procedure. The AAS procedure was conducted in the following steps:

1. Helper: Turns on personal sampling pumps on sampler, seals tent, and turns on NAM.
2. Sampler: Begins AAS with the leaf blower following pattern in Figure 3-4 at a rate of 20 s per pass (~2 min total).
3. Sampler: Finishes active AAS and waits additional 18 min for tent clearance by the NAM.
4. Helper: After 20 min, turns off the NAM and enters tent.
5. Helper: Turns off personal sampler pumps and caps samplers.

After the helper caps the personal samplers, the units are placed into individual labeled bags for processing. Following personal sampler collection, the NAM filter holders are collected by row and placed into bags for processing. Finally, the wall samples are taken. The tent is then set up for material sampling by placing the ADA-covered test material into the tent in the location shown in Figure 3-1. Immediately prior to the inoculated material testing the positive control is sponge-stick sampled. Once the positive control is sampled, the NAM flow is measured, and the ADA is removed from the inoculated material. AACeSS sampling follows the identical procedure conducted during background testing; however, the material is recovered with the ADA when the helper enters the tent to prevent additional particle resuspension. All filters are then processed according to Section 3.9 and transferred to the Biolab. Finally, all components and the HETS floor are decontaminated by hospital disinfectant towels (Dispatch Wipes with bleach) – ~0.65% sodium hypochlorite – (The Clorox Company, Oakland, CA), followed by ethanol, and water.

5.0 AACeSS Field Test

This section discusses the test matrix and sampling procedures for the field deployment of AACeSS to test sampling capabilities and operational considerations for an actual deployment.

5.1 Field Test Matrix

The full test matrix for the outdoor AACeSS field test is shown in Table 5-1. The testing consisted of two main sampling phases: background and hot-spot sampling. The background (first phase) was performed to determine if any residual *Bg* existed on the concrete, and the second phase was intended to sample low levels (1 x 10^5 CFU) of newly deposited *Bg* spores. Each AACeSS test generated nine felt filters grouped into three stomacher bags, 10 wall sponge sticks, two personal air filters, and three liquid samples from the wet vacuums (hot spot remainder, tent floor surrounding hot spot, tent perimeter). For the surface sampling tests, one liquid wet-vacuum sample was collected per surface hot-spot test. The surface sampling was done in three separate tents so that the sampling could be done in separate portions of the concrete pad to provide a large variation in potential surface conditions. For AACeSS, the background sampling was conducted in three separate tents. These tents were then replaced with three new tents over the same location to ensure subsequent samples were collected from the same surface (Table 5-1). Hot-spot inoculation of the AACeSS sampling tent and surface sampling tent was conducted in pairs with a positive control deposition onto a 14 in x 14 in stainless-steel square between. As an example, Tent 4 was inoculated, followed by a stainless-steel coupon, and then Tent 7 was inoculated.

Table 5-1. The two-phase field test matrix and sample log

Background Sampling (Phase 1)				
	AAS Filters	**Wall Sample**	**Personal Sampler**	**Wet Vacuum**
Tent 1	3 groups - 3 filters	10 Sponges	2 Filters	
Tent 2	3 groups - 3 filters	10 Sponges	2 Filters	
Tent 3	3 groups - 3 filters	10 Sponges	2 Filters	
Tent 4				1 Liquid Sample
Tent 5				1 Liquid Sample
Tent 6				1 Liquid Sample
Hot-spot Sampling (Phase 2)				
	AAS Filters	**Wall Sample**	**Personal Sampler**	**Wet Vacuum**
Tent 4				1 Liquid Sample
Tent 5				1 Liquid Sample
Tent 6				1 Liquid Sample
Tent 7	3 groups - 3 filters	10 Sponges	2 Filters	2 Liquid Sample
Tent 8	3 groups - 3 filters	10 Sponges	2 Filters	2 Liquid Sample
Tent 9	3 groups - 3 filters	10 Sponges	2 Filters	3 Liquid Sample

5.2 Field Test Sampling Procedures

As stated previously, the purpose of the field test was to evaluate AACeSS in a field setting on an outdoor surface similar to the surfaces that might be found on USCG installations (e.g., concrete). Three background evaluations of previously uncontaminated areas were conducted followed by three hot-spot evaluations. *Bg* was directly inoculated via the MDI/ADA procedure onto the concrete pad and contained within the 10 ft x 20 ft-tents to minimize aerosol release outside the enclosed tent areas. The depositions were broken down as follows:

1. (3) hot-spot depositions for AACeSS, each area 1 ft x 1 ft with an inoculation loading of 1 x 10^5 CFU each; and

2. (3) hot-spot depositions for surface sampling in tents adjacent to AACeSS each 1 ft x 1 ft with an inoculation loading of 1 x 10^5 CFU.

The “hot zone” for the field site encompassed all contained areas inoculated with spores (inside tents and immediate surrounding space). To reduce cross contamination, individuals entering the “hot zones” wore booties (Innovative Haus, Inc. Shelton, CT) while inside the tent and removed booties and any other personal protective equipment when exiting, then stepped into a container of 1:10 household – ~7.5% – bleach (The Clorox Company) to water for decontamination of shoes/booties so that the areas surrounding the tent could be considered a “warm zone” where limited spore transport would be present. The layout of the tents on the concrete pad can be seen in Figure 5-1. The unlabeled green tent was the sample processing tent where bagged sample containers were kept prior to transport to the Biolab at the end of each test day.

Figure 5-1. Schematic of field tent locations

Field testing was conducted in two phases: background sampling and inoculated “hot-spot” sampling. The general timeline breakdown of the background sampling is shown in Figure 5-2 and spanned a period of two days. The first day was set aside for initial setup of all tents and equipment with flow checks of all samplers and pumps. The second day encompassed all hot-spot inoculation, active surface and AACeSS sampling, and sample processing for hot spot samples.

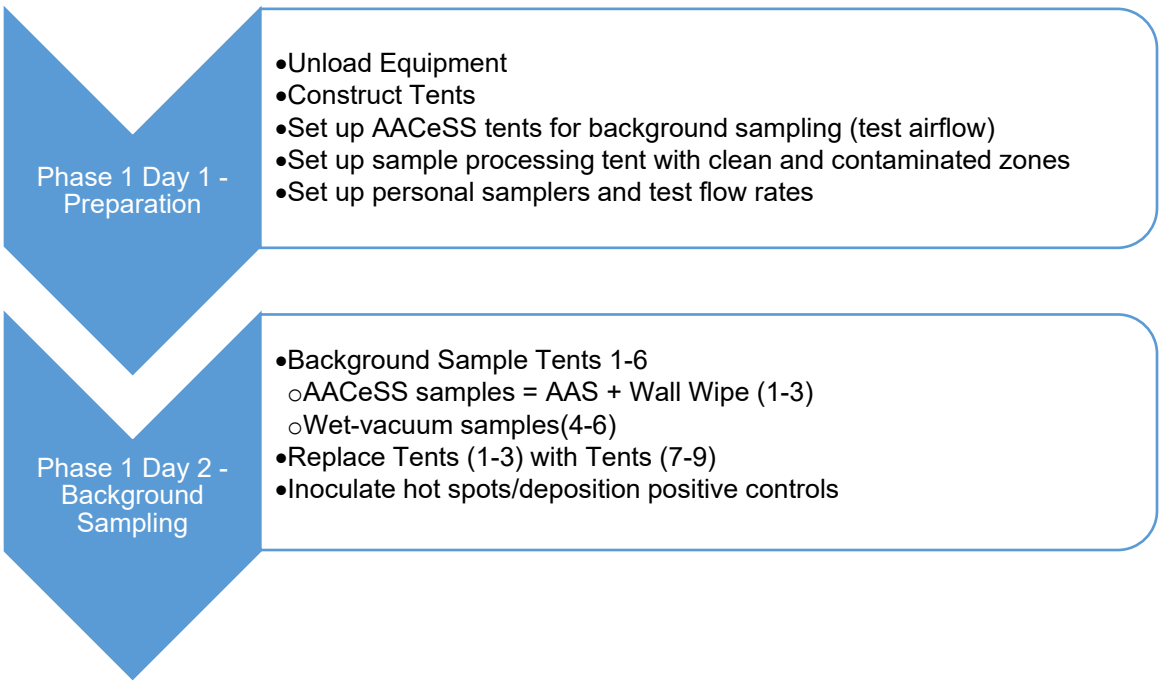


Figure 5-2. General background sampling scheme and timeline

After completing the background sampling, the next two days were set aside for hot-spot AACeSS and surface sampling and demobilization and cleanup on the final day. The wet vacuum surface hot-spot recovery sampling in the surface sampling tents 4-6 was carried out in parallel to the AACeSS sampling (tents 1-3/7-9) for both the background and inoculation sampling with teams operating independently. Wet vacuuming was conducted according to the processes described in Section 3.8. The process for AACeSS background sampling was identical to the laboratory process described in Section 4.2 since there was no additional wet-vacuum sampling to be conducted. However, the ground was not decontaminated prior to sampling as in the laboratory tests. After all background samples were collected, tents 1-3 were moved and replaced with new tents labeled 7-9. As shown in Figure 5-3, the tents were labeled with painter’s tape, and the perimeter skirts were weighted down to ensure containment and prevent material escape. Figure 5-4 shows the hot-spot placement in each tent covered by an ADA. The positive control coupons remained covered with the ADAs overnight like the hot spots and were sampled on the day of AACeSS sampling and wet-vacuum sampling. Temperature

and RH were recorded at the time of deposition. All background samples were transported to the Biolab after inoculations were completed.

Figure 5-3. AACeSS Tent 9 prepared for AAS

Figure 5-4. Hot spot covered with ADA in AACeSS tent

The day after background sampling and inoculation was completed, hot-spot sampling was conducted. Figure 5-5 shows the general timeline for the hot spot field test of AACeSS. The timeline was compressed compared to the background sampling as tent setup was the major portion of the first day of phase one. All filter setup and flow testing were conducted early in the morning with hot-spot sampling following.

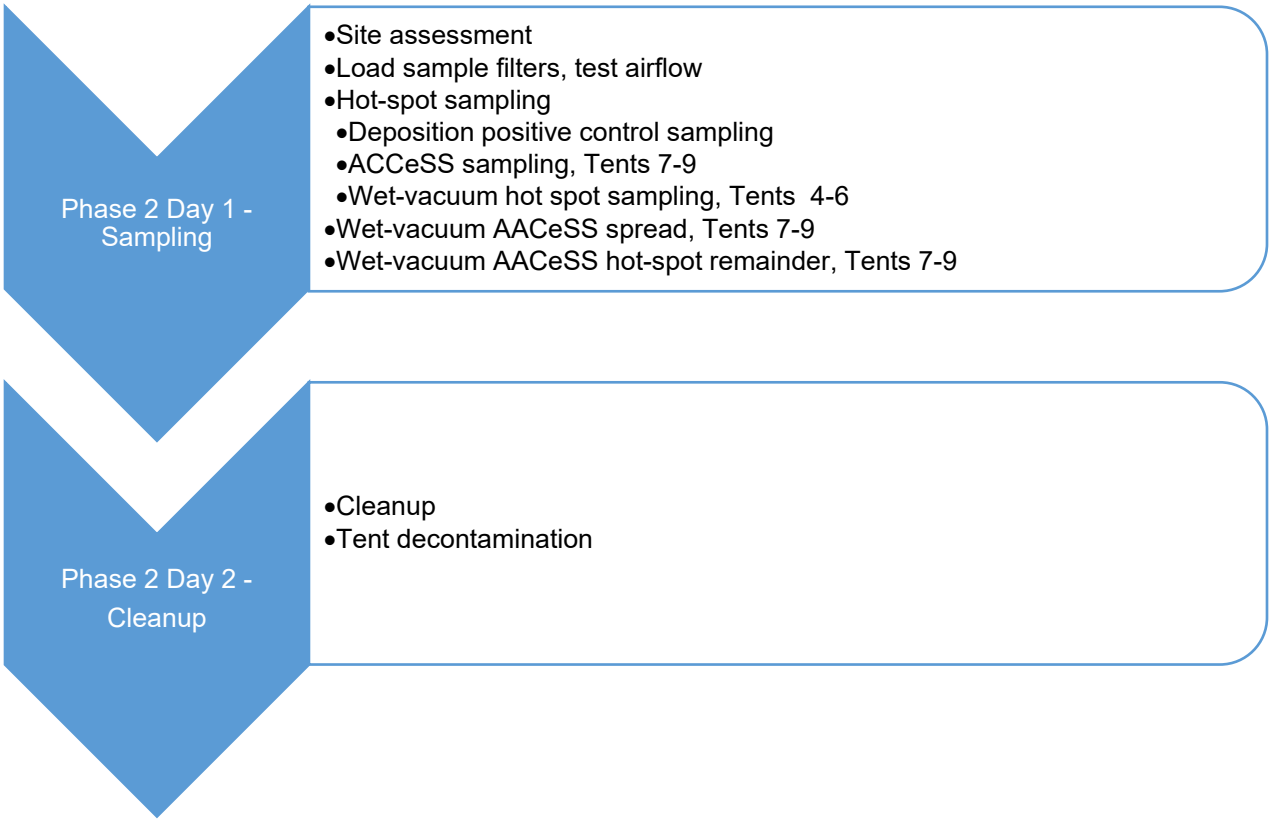


Figure 5-5. General hot-spot sampling scheme and timeline hot spot

The procedure for AACeSS hot-spot sampling in the field differed slightly from the procedure for laboratory testing in that wet-vacuum sampling occurred in the tent prior to wall sampling. Each AACeSS tent-sampling team consisted of an AACeSS sampler, an AACeSS helper, a wet-vacuum sampler, and a wet-vacuum helper. All AACeSS personal samplers and NAM filters were installed, and airflow measurements were collected at the beginning of the test day. In addition, all wall sampling areas were marked at the beginning of the test day. The full sampling procedure is as follows.

1. AACeSS Sampler dons PPE and two personal samplers on each shoulder.
2. AACeSS helper dons booties.
3. AACeSS sampler steps into tent with a new, clean leaf blower.

4. AACeSS helper accompanies sampler into tent and removes protective covers on the personal samplers.
5. AACeSS helper turns on personal sampling pumps, removes the ADA cover from the hot spot, exits tent and closes the tent opening.
6. AACeSS helper walks around and turns on the NAM high-volume sampler.
7. Once the NAM is turned on, the AACeSS helper verbally signals the AACeSS sampler to begin.
8. AACeSS sampler turns on the leaf blower, starts a 2-min timer and performs the aggressive air sampling resuspension procedure described in Sections 3.1 and 4.2.
9. Once the 2-min resuspension procedure is completed, the AACeSS sampler turns off the leaf blower and sits in a corner of the tent on a stool for the 18-min air sampling portion to be completed. The AACeSS helper starts an 18-min timer after hearing the leaf blower stop.
10. Once the 18 min has elapsed, the AACeSS helper turns off the NAM, dons gloves and booties, opens the tent and enters. The AACeSS sampler stops the personal sampling pumps.
11. The AACeSS helper places protective caps over the personal samplers, removes them from the tubing connecting them to the sampling pumps and places them in plastic bags for transport to the sample collection tent.
12. The AACeSS sampler steps out of the tent and takes off their PPE.
13. The AACeSS helper collects the NAM filters in bags and hands the personal sampler filter holder bags and NAM filter bags to the AACeSS sampler outside the tent before exiting and removing gloves and booties.
14. The wet-vacuum sampler dons booties, then enters the AACeSS tent with a wet vacuum and samples the 199 ft^2 floor area surrounding the inoculated hot spot.
15. Once the area is sampled, the wet-vacuum sampler hands the wet vacuum to the wet-vacuum helper outside the tent.
16. The wet vacuum helper hands a new wet vacuum to the sampler, and the sampler samples the 1-ft^2-hot-spot area.
17. Once the sampling is completed, the wet vacuum sampler hands the wet vacuum to the helper outside the tent, exits the tent, and removes booties.
18. The wet vacuum sampling of the tent is then completed, and the wet vacuum team moves to aliquot the sampled liquid as described in Section 3.8.

19. The AACeSS team (sampler and helper) dons booties and gloves and reenters the AACeSS tent to collect wall sponge stick samples.

20. This sequence completes all sampling procedures for Tents 7 and 8. For Tent 9, a 1-ft-wide perimeter of the ground directly outside the tent is wet-vacuum sampled after wall sampling is completed, and the AACeSS team leaves the tent.

A set of 10 NAM filters and two personal samplers are delivered to the sample tent and a team aseptically removes the filters from the holders and places the filters in their respective processing containers as described in Section 3.9. The wet vacuum hot-spot samples in Tents 1-3 and the positive control sponges are all collected during the first 20-min AACeSS sample. At the end of the sampling day, all samples are placed into secondary containment and transported to the Biolab for analysis with appropriate chain of custody forms. the following day following procedures outlined in Section 3.9. Liquid samples are stored at 4 °C overnight to prevent sample degradation. Filter samples are stable and do not require refrigeration. The day after testing, all tents are sprayed down with pH-amended bleach and broken down. All tables, stools, and leaf blowers are wiped down with Dispatch Wipes (The Clorox Company).

6.0 Results and Discussion

In this study, a specially designed sampler (AACeSS), designed and built by the EPA aerosol test facility team, was tested on representative outdoor surface materials found on USCG installations (both ground and vessel) to determine if the system resuspended surrogate spores for *Ba* and could be used to assess inhalation risk. This testing was accomplished by inoculating a variety of clean materials (concrete, asphalt, marine aluminum, skid resistant-coated aluminum, and boat rubber) with the surrogate spore *Bg* and testing AACeSS on those materials in a laboratory setting at two extreme (low and high) RH values of 30% and 80% to establish the lower bounds of resuspension and material collection. Following the laboratory testing, the system was deployed in a field scenario to help determine its performance in a natural setting with background flora. Logistical and operational difficulties were also recorded to help determine any modifications that could improve the portability or inform the limitations of the use of AACeSS. The results of the laboratory experiments are presented in this section, followed by the results of the field test.

6.1 AACeSS Laboratory-based Testing

This section focuses on results from performance testing of AACeSS in a laboratory setting under controlled conditions. ACCeSS was tested under varied RH conditions and used multiple representative outdoor surfaces, including surfaces found at a USCG installation. Details on these experiments conducted in the wind tunnel are included in this section.

6.1.1 AACeSS High-volume Spore Recovery

The general approach to assessing the performance of AACeSS was first to measure the sampling efficiency of the system from different outdoor surface materials representative of USCG installations at different relative humidities. For all experiments, background testing showed no residual *Bg* on any of the high-volume sampler tests before inoculated-material testing. Figure 6-1 shows the average of three triplicate tests of each inoculated material at both 30% and 80% RH. All material coupons were conditioned at the RH to be tested for a minimum of 24 h. The error bars shown are calculated from propagation of the triplicate plating standard deviation of the AAS filter recovery combined with the triplicate plating of the reference coupon for each day. These errors are then propagated through the average of the replicate sampling efficiencies according to methods from Bevington (2003).

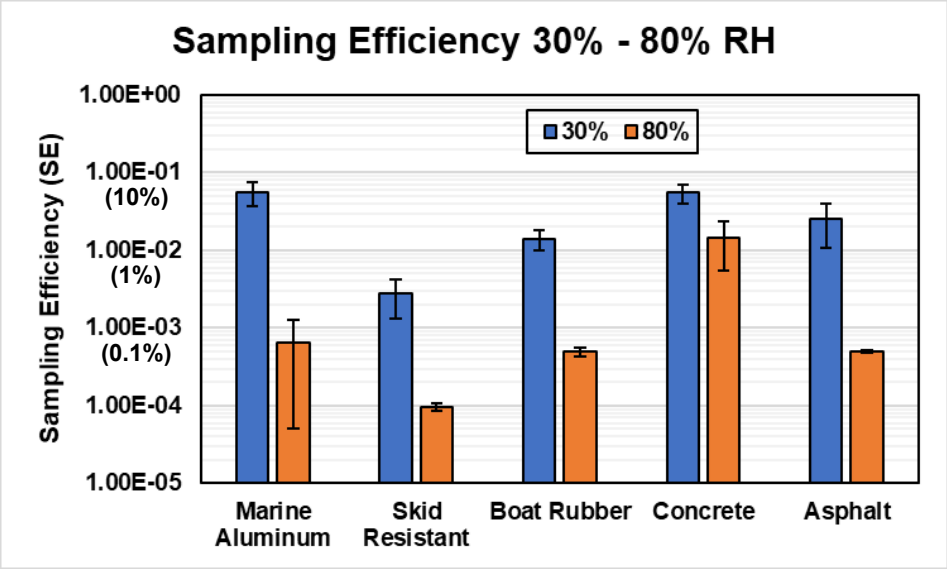


Figure 6-1: AACeSS sampling efficiency at 30% and 80% RH

For the 30% RH testing, the sampling efficiencies for all materials other than the skid-resistant coating were above 1% (1.5 x 10^6 CFU recovered). Considering the average surface deposition of ~1.5 x 10^8 CFU and low RH (30%), marine aluminum, boat rubber, concrete and asphalt all had average collections above 1.5 x 10^6 CFU *Bg* on the high-volume samplers as part of the AACeSS tent system. Concrete and marine aluminum had the highest sampling efficiencies at ~5% (~7.5 x 10^6 CFU recovered) and the skid-resistant coating had the lowest at ~0.3% (~4.5 x 10^5 CFU recovered). The limit of detection for these tests from the high-volume sampler was 22 CFU giving a lower detection limit of sampling efficiency of 1.5 x 10^{-7} (0.00001%). Concrete was expected to have one of the highest collection efficiencies. This is due to the aggregate nature of the cement and incomplete binding of materials. This incomplete binding causes concrete to continue to shed or generate small aerosol particles over time even after a significant shear stress is used to remove loose material (Penkala 2018). These concrete particles may have been a carrier for *Bg* or may have tumbled and collided with *Bg* on the surface, resuspending the spores through collisions. The marine aluminum appeared to be smooth, leading to the potential assumption that the sampling efficiency would be relatively low compared to concrete. However, the marine aluminum likely has a rough surface on the microscale – on the order of the size of the *Bg* spore. This roughness is known to lower particle adhesion forces, making the particles easier to resuspend (Miwa 2020). The low sampling efficiency of the skid-resistant tape could be due to potential porosity of the tape itself, creating low regions for the spores to settle into or the fact that the engineered material likely has a high surface energy by design, creating higher adhesion and friction.

For reference, traditional surface sampling methods have reported sampling efficiencies or recoveries of surrogate spores up to 30% from stainless steel with sponge sticks, and 49% from 37-mm microvacuum cassette sampling on concrete (Calfee 2013). However, a sampling study on actual “non-clean” outdoor surfaces reported much lower recoveries of 1.7% and 6.7% from sponge stick sampling on asphalt and concrete, respectively (Mikelonis 2020). These values were higher than those using 37-mm microvacuum cassette sampling. While sampling efficiency

with the AACeSS tent-based sampling system is approximately an order of magnitude lower than those reported in the literature from traditional surface sampling under controlled conditions, the AACeSS system was designed to capture resuspended spores to provide an understanding of potential inhalation exposure from spores on surfaces. It is expected that sampling directly from surfaces would result in higher recoveries, however, surface sampling methods do not provide a measure of inhalation exposure risk. Thus, AACeSS was intended to be a complementary sampling tool in a wide-area release scenario.

All replicate test data were subjected to a Shapiro-Wilk test for normality prior to conducting the single factor analysis of variance (ANOVA) test, which was conducted for all 30% RH data. All 30% RH replicates were shown to have an approximately normal distribution with a significance level of 0.05, with the caveat that only three replicates results in a low statistical power. The single factor ANOVA test showed a statistically significant difference between the surface materials with a p-value < 0.05 (0.0012). Using a Tukey Statistic for comparison showed that the average sampling efficiencies from the boat rubber and skid-resistant material differed significantly from marine aluminum and concrete but not asphalt or between themselves. No statistical difference was seen between marine aluminum, concrete, and asphalt. Marine aluminum, boat rubber, and concrete had the lowest coefficients of variation (CVs) within each replicate (<33%). The asphalt and the skid-resistant tape had the highest level of variance from test run to run with CVs of 57% and 52%, respectively. This increase in variance between test runs is likely due to the materials being combinations of other materials of different surface properties. The asphalt is made of stones compressed into form, creating a nonuniform surface on which spores can land, creating a higher variance run-to-run if the spores favor one surface over the other on any given deposition. Similarly, the skid-resistant tape has a high roughness mineral coating and a binder.

Figure 6-1 also shows the effect of increasing the environmental humidity to 80% on sampling efficiency of AACeSS. The figure shows distinct decreases in sampling efficiency for all materials, although the decrease for concrete appears to be less significant than the others.

Table 6-1 shows results of a Welch's t-test at the 95th percentile for each material comparing the average sampling efficiency (SE) for 30% and 80% humidity and the percent difference. Percent difference is calculated by the following formula.

$$\frac{(30\%\ SE - 80\%\ SE)}{30\%\ SE} \times 100\%$$

Table 6-1. Percent difference in SE for 30% and 80% RH

Welch's t-test 95th Percentile				
Material	**30% SE**	**80% SE**	**% Difference**	**p-value**
Concrete	5.50E-02	1.40E-02	75%	0.027
Asphalt	2.50E-02	5.00E-04	98%	0.097
Boat Rubber	1.40E-02	5.00E-04	96%	0.028
Marine Aluminum	5.60E-02	3.60E-03	94%	0.038
Skid Resistant	2.80E-03	9.61E-05	97%	0.085

For a Welch's t-test to demonstrate a significant difference between the two group means at the 95th percentile, the p-value must be less than 0.05. For our tests, even though the highest percent differences (between 30% and 80% RH) calculated were for the asphalt and skid-resistant tape – 98% and 97%, respectively – the Welch's t-test does not meet the 95th percent mark, as the p-value is not less than 0.05, likely due to the initial variance in the 30% RH sampling efficiency replicate data and the low number of replicates. Though not statistically significant, the effect is noticeable between the high and low RH values. Concrete appears to be affected least by an increase in RH, possibly due to the small dust particle effect as stated for the 30% RH data. If material sheds from the concrete itself or dust particles are larger than the spores, they will be less likely to be affected by capillary adhesion and easier to shear from the surface (Kim et al. 2016, Kweon et al. 2011, Hinds 1999). The lowest sampling efficiency calculated at 80% was that of the skid-resistant tape at 9.61 x 10^{-5}. If we compare this to the theoretical sampling efficiency detection limit of 1.5 x 10^{-7}, we can see that in this worst case AACeSS sampling of resuspended spores is two orders of magnitude above the limit of detection (LOD). In effect, for newly applied nonweathered skid-resistant tape, the number of surface spores required to be detected at a 9.61 x 10^{-5} sampling efficiency would be >2.3 x 10^{5} CFU, spread over the entirety of the AACeSS sampling area. If the sampling area were 10 ft^2 similar to the outside floor surface of a small vessel, the detectable surface concentration would be 2.3 x 10^{4} CFU/ft^2. As stated previously, this situation may be similar to a worst case scenario. Materials subject to the natural environment will likely be covered in small particles like pollen, dust, or in the case of many USCG installations, settled sea salt minerals. These particles will likely increase resuspension from surfaces, thereby increasing the sampling efficiency of AACeSS. It should be noted, though, that the additional debris/particles could include higher background organisms which could present challenges with analysis. In addition, surfaces with a natural high surface energy will attract materials to lower the average surface energy further reducing adhesive forces. Concrete at 30% RH might be the best indicator for real world sampling efficiency as it does continually generate small particles due to shedding and shear stress. At a sampling efficiency of 0.055 (5.5%), it would theoretically require >400 CFU to be deposited over the entire sampling area or 40 CFU/ft^2 over a 10-ft^2-space on average.

6.1.2 Activity-based Personal Sampling Using Button Samplers

To assess the potential exposure of the technician to resuspended spores during the AACeSS sampling, two personal Button Samplers were deployed in the technician's breathing zone

described in Section 3.6 and were operational throughout the entire sampling process. The two samples were averaged for each experiment, and then the triplicate experiments were averaged. Average capture/recovery for each material and relative humidity condition are shown in Figure 6-2. The personal sampling results show a very similar effect of relative humidity as the high volume sampling with high humidity, resulting in lower exposures.

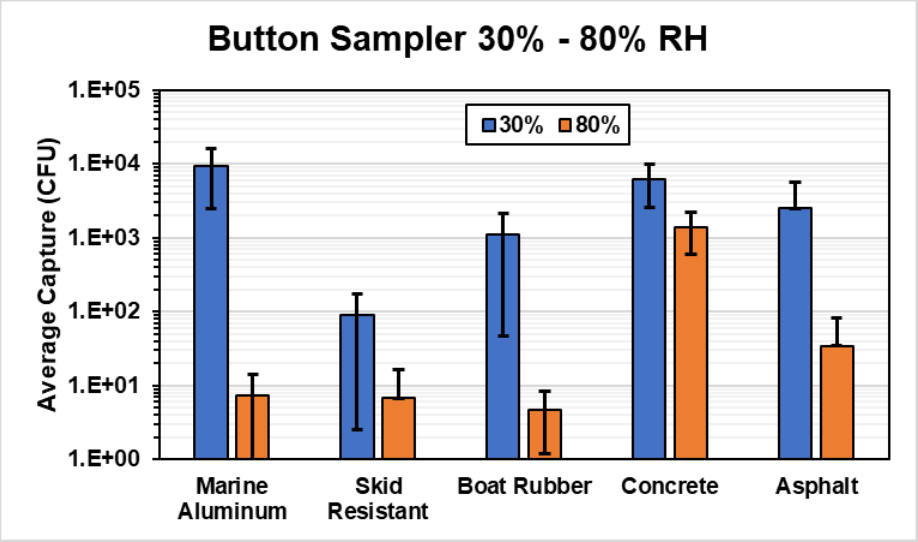


Figure 6-2. Button Sampler CFU capture

Since the Button Sampler results were similar between high and low humidity for the high volume AACeSS sampler, the two sampler types were plotted in Figure 6-3 to determine if the high-volume sampler recoveries could be a predictor of potential breathing zone concentration. Due to the large concentration range of spore collection, the $\log_{10}$ was taken of the collected CFU for both the high-volume and the personal Button-Sampler results. As can be seen in the figure, there is a significant positive correlation between the AACeSS high volume sampler collection and the personal Button Sampler collection. The Pearson correlation coefficient for all of $\log_{10}$ data combined (30% and 80%) is $r = 0.946$ with a p-value of <0.001, meaning there is a strong positive linear correlation between the logs of the corresponding samples. Therefore, it does appear that there is a predictive power of the high volume sampler for technician personal exposure, as a linear fit to the paired log-log values gives an R^2 value of 0.8851. However, any negative value generated by the fit would correspond to less than one CFU, which is not physically possible.

Finally, though there does appear to be predictive power of the high-volume sampler for technician personal exposure in our experimental setup, this observation cannot be generalized to all scenarios encountered in the field. Specifically, our experiments used a single contaminated “hot spot” in the middle of the test area, which may differ from a real-world scenario in which surface contamination may be more widespread.

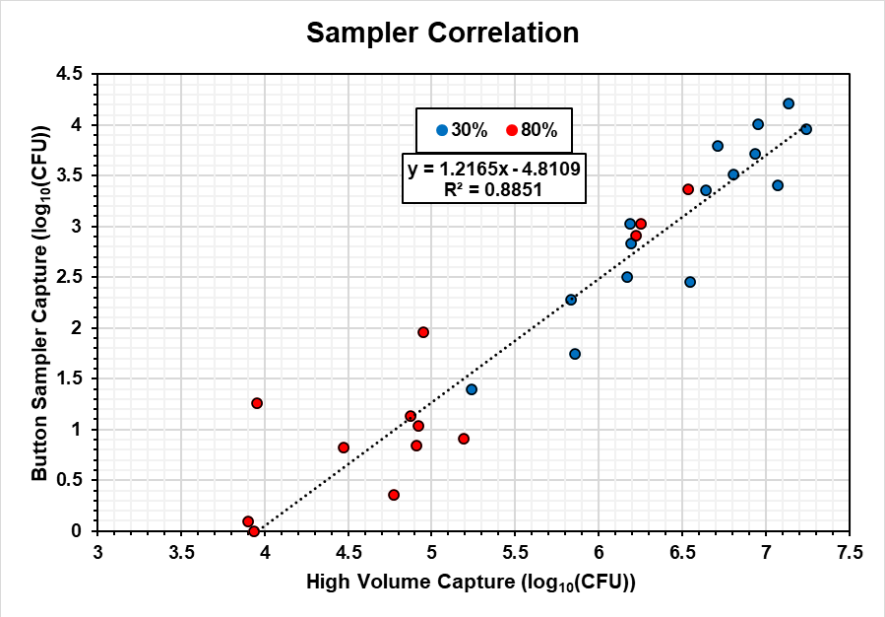


Figure 6-3. Log-log plot for personal Button Sampler versus High-volume

6.1.3 Tent Wall Samples

After AACeSS testing was completed and filters were collected, ten 1-ft^2-wall samples were taken via sponge sticks. These wall sample results were used to estimate the number of particles deposited onto the tent walls. After analysis of all wall samples, we determined that no tent wall position was more likely to collect CFU than any other (i.e., CFU were not collected more readily on the NAM than the side wall). Therefore, we determined that the wall collection estimates could be made by averaging the CFU collected for all ten of the 1-ft^2 sponge-stick areas and multiplying by the surface area of the tent walls – neglecting the tent ceiling and floor. CVs for all tent wall averages were nearly 100%, with the average CV being 101% for the 30% RH tests and 169% for the 80% RH tests. The CVs greater than 100% demonstrate the high variability in tent wall losses within and between the RH conditions. The inconsistency in wall loss measurements is caused by multiple factors including the static charge on the tent materials. Due to the high variability in these measurements, wall surface deposition measurements do not allow the wall losses to be estimated reliably. By comparison, the high-volume sampler collection variability was lower and was not impacted by the high wall loss variability. Figure 6-4 shows the number of CFU collected by the NAM high volume sampler versus the estimated number of spores collected on the tent walls grouped by RH condition and plotted on a log-log scale. Error bars shown on the figure represent the calculated standard deviation of the wall sample averages. Data points with only positive error bars demonstrated a CV above 100%. As a result, the negative error bars fall below zero and are not shown on the log-log plot. The figure shows a significant positive correlation between the high-volume air sampler and the estimation of the CFU collected on the tent wall surfaces. The higher resuspension fraction at 30% RH also resulted in a higher CFU collection on the walls. In this

case, linearity on a log-log scale does not necessarily suggest a nonlinearity in the relationship as it appears to be nearly 1:1.

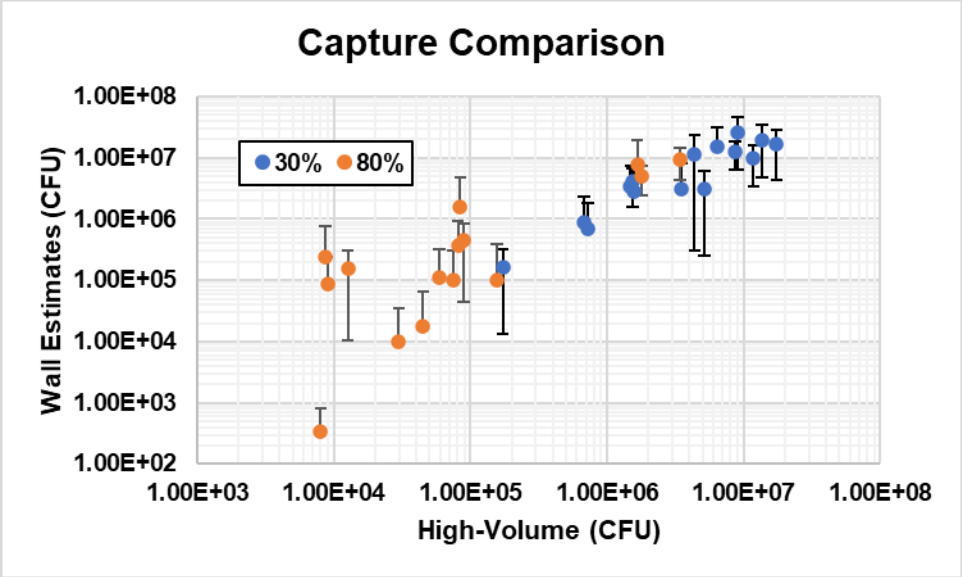


Figure 6-4. Capture comparison for high-volume versus wall

Figure 6-5 shows the high-volume sampler collection versus the ratio of the high-volume sampler to the estimated wall capture.

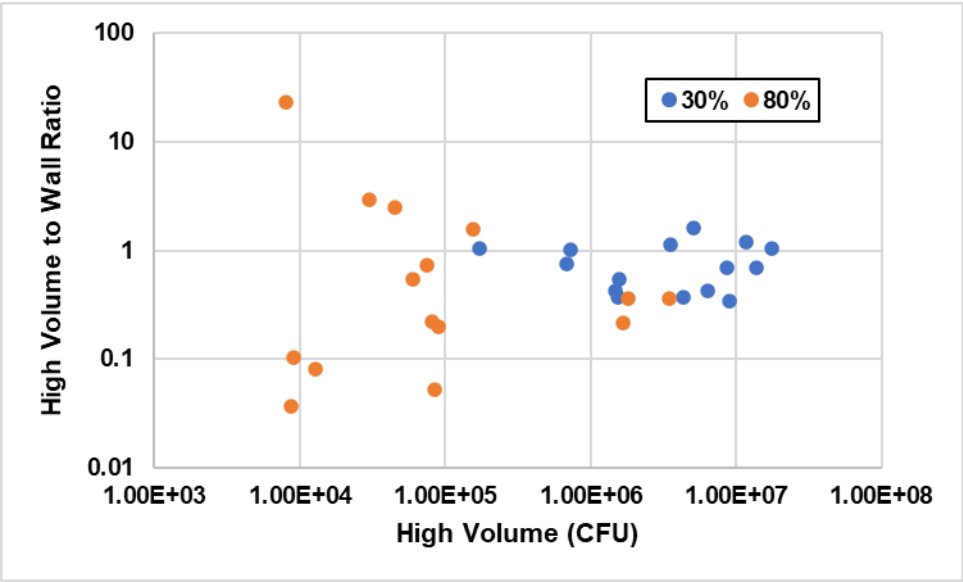


Figure 6-5. Ratio of high-volume sample to wall capture

The average ratio for 30% RH was 0.78 with a standard deviation of 0.38. For the lower resuspension 80% RH tests, the ratio was 2.2 on average with a standard deviation of 5.8, showing that at higher RH and low resuspension the wall deposition is inconsistent, possibly due to a static charge on the tent walls that would be present at lower humidities but be dissipated at higher humidities (Elovitz 1999). The static charge at lower RH would generate an electric field potentially attracting particles that approached the tent wall. Inconsistent wall deposition may also simply be due to the random nature of wall collisions, and at lower spore concentrations, there are simply fewer wall collisions, increasing the likelihood of outliers. The three orange 80% humidity data points grouped with the 30% blue data points in Figure 6-6 correspond to the concrete surface tests, and the correlation is noticeable, leading to a possible conclusion that the consistency of the lower RH data is most likely due to the higher particle concentration and increased wall-collision frequency. In either case, high RH or low RH, wall collection can be significant and should be considered when determining the overall AACeSS concentrations.

6.2 Tent-based AACeSS Field Test Results

The field deployment of the tent-based AACeSS took place over 3 days in the week of March 16, 2020. The first day (March 18th) consisted of unloading all equipment, construction of 10 tents, the setup of sampling filters, and the mounting of NAMs to the sampling tents. Tent construction was accomplished by 10 people in teams of two. Each tent took between 30 min to an hour to construct fully. Tents were weighted with sand in buckets to help prevent movement due to wind as shown in Figure 5-3. March 19th consisted of all background sampling and 1-ft^2 hot-spot inoculations. The average RH measured during all sampling and inoculations was 45%. This value remained relatively consistent throughout both sampling days. Total sampling time for the wet vacuums over each 1-ft^2 background was 20 s. Consisting of AAS, filter collection, and wall sampling, the total AACeSS sampling time was approximately 40 min to complete a single sample inside the tent. AACeSS high volume samplers captured no CFU during any of the background tests. Two wet vacuum backgrounds were zero; however, a third wet vacuum returned a background count of 2.56 x 10^3 (2560) CFU. The sterility sponge stick of the wet-vacuum area immediately prior to sampling also showed the presence of residual *Bg* in the recovery tank, suggesting an incomplete decontamination of the wet vacuum from previous use. Two background wall samples also contained *Bg*. One background sample on the left side of Tent 1 contained 360 CFU and the background sample above the NAM in Tent 2 contained 460 CFU. The origin of the background spores in these two samples is unclear. The tents were newly opened, and spores had not been released into any tent at the time of sampling, indicating the potential for naturally occurring *Bg* in the region or potential cross contamination in sample processing (though the lack of *Bg* present on the high-volume samplers suggests the latter). Hot-spot sampling was conducted on March 20th. Initial observation of the test site concluded that Tent 6 used for wet-vacuum hot-spot sampling had completely blown away. However, the ADA covering the hot spot remained in place, and the hot spot was still sampled. No other tent appeared to be displaced. Figure 6-6 shows the average recovered CFU for wet vacuuming and AACeSS.

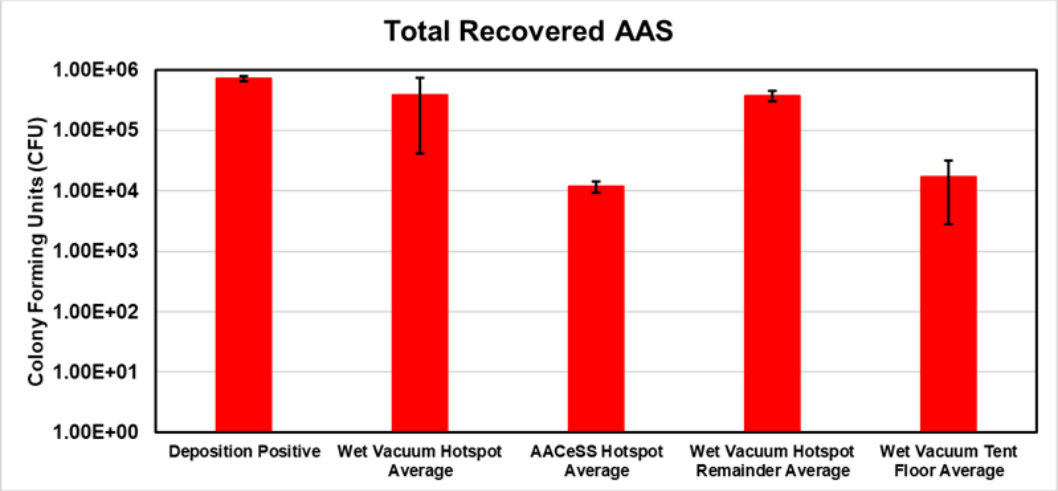


Figure 6-6. CFU recovered from hot-spot sampling

The deposition positive controls had an average deposition of 7.18 x 10^5 CFU with a CV of 9%. The wet vacuum hot spot surface sampling had significant variation with an average recovery of 3.92 x 10^5 CFU and a CV of 90%, possibly due to a lower than expected deposition in Tent 5 as the number of CFU sampled was 7 x 10^4 CFU. All other measurements of hot spots and hot-spot remainders had high repeatability with CVs below 22% across the sampling tents. AACeSS sampling of the tents recovered an average of 1.2 x 10^4 CFU on the high-volume samplers. The wet-vacuum sampling of the remainder left on the AACeSS hot spot recovered an average of 3.78 x 10^5 CFU and vacuuming of the entire tent floor recovered an average of 2.56 x 10^4 CFU though the CV of these measurements was ~84%, demonstrating that AAS not only resuspends material but also redistributes material across the sampling area. Table 6-2 shows the ratio of CFU recovered (%) from AACeSS and wet vacuuming versus the deposition positive control.

Table 6-2. Recovery ratios of wet vacuum and AACeSS

AACeSS Ratios	Ratio %	Error
AACeSS Hot spot/Depo	1.65%	0.39%
Wet Vacuum Hot spot/Depo	54.70%	49.25%
Wet Vacuum Remainder/Depo	52.61%	11.15%
Wet Vacuum Tent Floor/Depo	2.42%	2.04%

Depo = Deposition Positive Control

The AACeSS hot spot and wet-vacuum hot-spot ratios can be considered as calculations of sampling efficiencies similar to the laboratory testing. In general, the remainder would not be considered a quality measurement of sampling efficiency since the surface was disturbed by AAS and potentially from walking through the hot spot prior to additional sampling, and the true magnitude of material removed from the hot spot is unclear. However, both the remainder and tent floor spread measurements are indicators of the magnitude of removal and can be used to assess the amount of material exiting the AACeSS tent system. In this case, high-volume samplers clearly collect some of the material that was removed from the hot spot from the leaf

blower AAS; however, a significant portion of the material removed was spread along the ground. The material that remained on the hot spot was of the order of magnitude as the amount deposited. The wet vacuuming around the perimeter of sampling Tent 9 recovered no CFU of *Bg,* demonstrating that spores did not escape the tent and deposit directly around the concrete surface perimeter. It is still possible, however, that some spores exited the tent system and were transported away from AACeSS. Combined with the non-detects from perimeter dry filter units (DFUs), this transport of spores may be an indicator that the sealing of the tent skirt was sufficient. DFUs were placed around the perimeter of the tent within 2 ft of the tent skirt (one per tent) to monitor for breakthrough in the tent system during the AACeSS sample collection using leaf blowers.

Figure 6-7 shows the sampling efficiency of AACeSS during the field test compared to the laboratory testing of concrete since the field test was conducted on a concrete slab. The laboratory test concrete surface was a “clean” surface that had not been subjected to outdoor conditions including ambient particulate matter deposition.

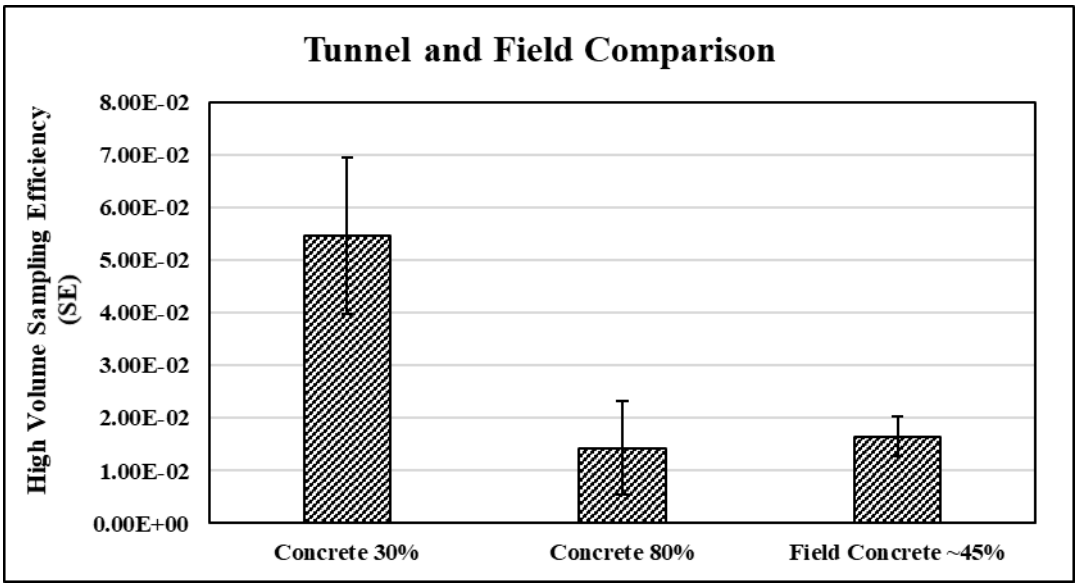


Figure 6-7. Tunnel and field sampling efficiencies

The figure shows that the results of the field testing of the aggressive air sampling technique on concrete was similar to the results of the laboratory tests. A Welch’s t-test shows the field sampling efficiency to be not statistically different from the 80% RH laboratory testing but statistically significantly different from the 30% RH laboratory testing. These similarities are possibly due to capillary condensation occuring on the spore/concrete interface after 30% RH but before reaching 45% RH. Another explanation is that natural deposition of particulate matter on the concrete slab from weather and the surrounding environment or wear over time changed the outdoor concrete surface properties. For the field test the LOD was estimated to be 54 spores for the high-volume samplers due to the necessity of using the high debris analytical technique, which has less resolution due to dirty samples. Therefore, for the outdoor AACeSS sampling efficiency, the system could detect >3500 CFU over the entire tent sampling area. If the spores were deposited over the entire ground area of the tent, ~200 ft^2, that would

correspond to a surface coverage of 17.5 CFU/ft^2 For comparison, 37-mm cassette-microvacuum sampling has an LOD of 4.4 CFU in a single 1-ft^2 sample The wet-vacuum LOD can vary, depending on how much debris is collected and the amount of sampling fluid used with a range of 12.2 to ~40 CFU in our field experiments.

Table 6-3 shows the average spores collected (CFU) on the two duplicate technician Button Samplers during each tent test relative to the average spores collected on the high-volume sampler. As demonstrated in the laboratory tests and in Figure 6-3, low spore recoveries from the high-volume sampler correlate well to low recoveries from the personal Button Samplers. Using the linear fit from Figure 6-3 and converting back from log CFU values to CFU, the equation predicts between ~1-2 CFU collected on the Button Samplers during each of the tests from the high volume sampler recovery. While this value is lower than Button Sampler collections during our experiments, it is within the same order of magnitude.

Table 6-3. Field personal Button Sampler versus high-volume sampler

Personal Button/High-volume Sample Comparison		
	Average Button CFU	**High-volume CFU**
Tent 7	4.12	1.24E+04
Tent 8	7.85	1.41E+04
Tent 9	4.86	9.05E+03

Table 6-4 shows the estimates of total spores (CFU) collected onto the tent walls during AAS. As with the laboratory experiments, the CV for the estimates are extremely large due to certain tent wall samples collecting no spores. However, the estimates for Tents 7 and 8 do match closely with the amount of material collected on the high volume samplers; however, the estimate for Tent 9 was an order of magnitude lower. The estimates for wall sampling, though, should still be treated as highly unreliable and not used in a field deployment of AACeSS as quality data or critical measurements, especially at low contamination levels. The wall samples do show, however, that the walls of the tents can be a deposition area for spores after AAS and need to be included in any decontamination and/or waste disposal plan, also showing the difficulty in reusing a tent for additional sampling, as decontamination would be required before moving, and cross-contamination from location to location would be highly likely.

Table 6-4. Field tent wall sampling estimates

	High Volume Collection (CFU)	**Wall Estimate (CFU)**	**Wall Estimate Error (CFU)**	**Wall Estimate CV (%)**
Tent 7	1.24E+04	1.02E+04	2.21E+04	218%
Tent 8	1.41E+04	4.06E+04	8.95E+04	220%
Tent 9	9.05E+03	6.84E+02	1.39E+03	203%

6.3 Conclusions and Future Work

AACeSS was developed to fill a critical sampling-related capability gap following a wide-area release of a biological agent. Following such an event, sampling would be necessary both to characterize the area of contamination and clear areas for reoccupancy following remediation. However, traditional surface sampling using probabilistic schemes that have been used for indoor responses may lead to an inordinate number of samples, as well as quantity of time and resources to characterize the potential widespread contamination from complex outdoor surfaces. AACeSS provides another tool for responders that can sample over a much larger area as well as provide a measure of the potential exposure risk from a biological agent such as *Bacillus anthracis.* AACeSS testing from USCG relevant surfaces provides information as to environmental conditions that would be ideal for sampling outdoor areas and materials contaminated with a biological agent. The laboratory and field tests that were conducted provided a worst-case scenario for the sampling, as experiments demonstrated AACeSS V1 can detect a contamination level of 2×10^5 spores inside the contained tented area (200 ft^2). As the sampling surface area increases, this minimal surface contamination for detection would be

lower. Sampling efficiency of the system is affected by high relative humidity to some degree as resuspension decreases with increasing RH. However, the potential for inhalation exposure would also be reduced under such conditions due to less resuspension. Therefore, AACeSS sampling is a good indicator of the exposure risk of secondary biological particulate resuspension (bioaerosol) from surfaces if relatively high spore surface contamination is present. Outdoor surfaces that are “dirty” with native particles, pollen, debris, etc., will likely increase the resuspension and capture of biological agents but decrease the sampling limit of detection due to reduced extraction efficiency.

The AACeSS high volume tent sampling was shown to potentially be a predictor of technician breathing zone exposure during the sampling event from a hot-spot contamination event. However, additional work should be conducted to determine if the correlation holds when sampling from a widespread and uniform area of contamination. Wall sampling during both laboratory and field tests showed significant transport from a surface hot spot to the tent walls during aggressive air sampling, potentially on the order of the number of particles sampled on the high volume filters, possibly indicating that wall loss reduces the high volume sampling efficiency and as the containment area increases, the high-volume sampling rate should scale accordingly to decrease the time particles remain suspended in the tent system and subsequently impact on the tent walls. In addition, sampling protocols utilizing AACeSS need to include a significant decontamination strategy for the tent, especially if the tent will be reused in future sampling events. It is also likely that AACeSS tents could not be reused in a single sampling day. The tent would likely be treated as site waste due to difficulty in decontamination. Furthermore, since the technician sampler is operating the leaf blower within the sampling space, biological agent migration to PPE increases the potential for cross contamination and inhalation hazard for the sampler. Appropriate levels of PPE with respiratory protection (Level C at a minimum) in accordance with the Health and Safety Plan (HASP) are necessary during sampling.

Field tests for AACeSS V1 demonstrated an important operational consideration in that tents are susceptible to high wind and need to be rigidly attached to the ground, further limiting the utility of tents to be used to sample multiple zones at once. Though no transport of material outside the tents was detected by the DFUs, anecdotal observations noted that tent skirts did slightly billow and aerosol escape was a potential concern. The applicability of a rigidly attached tent sealed to prevent material escape may be most applicable to contaminated vehicles and USCG vessels with complex geometries that can be tented. Another potential application would be to tent large areas of complex geometries and multiple materials where a single traditional sampling protocol would not be feasible. AACeSS does provide the only current sampling tool for assessing the inhalation risk for biological agents through resuspension and air sampling collection from outdoor or vehicle/vessel surfaces.

These initial laboratory and field tests demonstrated that future development of AACeSS should include a reduction of sampling volume to maximize sampling efficiency and decrease the wall surface area to simplify decontamination and minimize turnaround time. A reduction in system size with a manageable weight would also increase the mobility of the system. A mobile system with a small sampling containment box, a left blower mounted to the inside, and a high volume sampler would allow the resuspension and collection of biological agent in a sampler that could

be used to sample large outdoor areas. From these lessons learned, a second mobile sampling version of AACeSS was developed during this study for future deployment in a wide-area demonstration and during a real-world event. Appendix A describes the design of the sampling system and preliminary field testing for the mobile AACeSS cart. Future testing and development will be reported in future publications. Finally, lessons learned during the field test of AACeSS (tent version) led to development of a sampling protocol for the tent-based AACeSS system (Appendix B).

7.0 Quality Assurance (QA) and Quality Control (QC)

To maintain quality assurance/quality control (QA/QC), laboratory work was conducted under the approved category B/Applied Research Quality Assurance Project Plan (QAPP), Development of an Activity-based Air Sampling (ABS) Strategy/Protocol for use at US Coast Guard Installations QAPP-4J20-008.1, and the field test was conducted under the approved category B QAPP, Field Test for Activity-Based Aggressive-Air Contained Sampling System (AACeSS) QAPP-4J20-009.0.

To ensure the integrity of samples and to maintain a timely and traceable transfer of samples, an established and proven chain of custody (CoC) process was followed for all collected samples in the lab or field. A CoC record accompanied all 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 for transfer of custody of a group of samples from one individual or laboratory to another. The same CoC process was used for samples collected in the lab and field as samples were carefully packed and hand carried between the lab/field to the BioLab for analysis.

7.1 Equipment Calibration

Before beginning experiments, all of the following monitoring equipment was checked against either a primary or a secondary standard to ensure that the monitoring equipment was operating within acceptance criteria:

- A Rotronic (Hauppauge, NY) model HT205 probe was used to monitor and control temperature and relative humidity inside the B155A test chamber.
- A Pacer rotary vane anemometer (Model D410, Pacer Instruments, Keene, NH) was used to test the high-volume filter flow rate.
- A National Institute of Standards and Technology (NIST)-traceable temperature probe was used to record temperatures in the incubators and environmental chamber.
- A Humicap HMT330 (Vaisala Inc.) provides temperature and humidity measurements inside the AWT.
- VWR 35519-041 temperature and humidity probe (VWR International) was used to measure temperature and humidity of the sod as well as spot check the tunnel and environmental chamber.
- A “K” style three-axis Research Series sonic anemometer SATR/3K (Applied Technologies, Inc., Longmont, CO) was used to measure flow (velocity) inside the AWT.
- Class A volumetric glassware was used where possible.
- Ohaus GA200D (Ohaus Corporation, Parsippany, NJ) or Sartorius ME 5-F (Sartorius AG, Goettingen, Germany) microbalances will be used to measure weight of MDI.
- Smart Weight Digital Heavy-Duty Shipping and Postal Scale, 200 kg capacity, was used to weigh asphalt mix for asphalt coupons.

- Lab Oven Reliance 1024 (STERI S Corp., Mentor, OH), located in room B367 on the EPARTP campus.

All equipment used for critical measurements in the project was maintained and verified as being certified, calibrated, or having calibration validated by the EPA Metrology Laboratory once per year, or immediately following an event that could cause damage (e.g., power surge) or an equipment modification according to operation manual specifications and/or previous investigations.

7.2 Quality Assurance/Quality Control Checks

The Quality Assurance/Quality Control (QA/QC) checks were performed following the guidelines set forth in EPA 815-B-04-001 and EPA 841-B-96-003. (EPA 2004, EPA 1996) A list of QA/QC checks can be found in Table 7-1. For each deposition, one positive control and one negative control were performed. The sample for the positive control was sampled from a separately deposited stainless-steel (SS) coupon. A negative control (blank) of the PBST that was used was analyzed via standard plating methods. In addition, background samples were collected before every test. The background test was run and collected onto filters just as the test runs were but with a sanitized test surface. QA/QC checks and calibrations of all equipment served to establish quality data and fulfill the necessary data quality indicators (DQIs).

Table 7-1. QA/QC Checks and DQIs

QC Sample	Information Provided	Frequency	Acceptance Criteria	Corrective Action
Procedural blank (sample matrix without biological agent)	Controls for sterility of materials and methods used in the procedure	1 per sample matrix	No observed CFU	Reject results of samples of the same order of magnitude
Blank plating of microbiological supplies	Controls for sterility of supplies used in dilution plating	3 of each supply per plating event	No observed growth following incubation	Sterilize or dispose of contamination source Replate samples
Blank TSA* sterility control (plate incubated but not inoculated)	Controls for sterility of plates	Each plate is incubated for 18–24 h	No observed growth following incubation	All plates are incubated prior to use; all contaminated plates will be discarded as source of contamination identified, if possible
Procedural blank samples	Contamination level present during sampling	1 per sample matrix	Nondetect	Clean up environment Sterilize sampling materials before use
Replicates of microbiological dilution plates	Repeatability of results	3 per dilution	Counts > 30 are reportable; standard deviation must be < 50%; Grubbs outlier test or equivalent; TNTC* will be diluted and replated	Replate sample

QC Sample	Information Provided	Frequency	Acceptance Criteria	Corrective Action
MDI positive control (aluminum deposition reference disk)	Initial contamination level on the coupons; evidence of the amount of *Bg/Btk* introduced in hot spots; shows plate's ability to support growth	1 per deposition	Target loading CFU per sample with a standard deviation of < 0.5 log; no evidence of MDI decay during inoculation event target 10^7 CFU per discharge	Outside target range: discuss potential impact on results with TOCOR*; correct loading procedure for next test and repeat depending on decided impact Outlier: evaluate stability of MDIs
Field blank (unexposed sample matrix transported with samples)	Contamination due to handling	1 per sample matrix	Nondetect	Clean up environment Identify contamination route
Check weighing of Laboratory Balance/Scale with Check Weights	Ensures that the balance is measuring accurately and precisely	1 per day of measuring	Check each balance/scale for acceptable balance range for each check weight	Contact Metrology Laboratory for new calibration and certification of balance
Chamber Temperature/RH	Experiments were performed under the desired conditions	Continuous	Temperature ± 2 °C RH ± 5 %	Stop sampling and correct as necessary.
Background samples (sample matrix without biological inoculation)	Understand the field level of contamination	3 field samples per sample type	This value is a direct measure; therefore, level is always accepted.	Reject results of samples on the same order of magnitude

TSA = Trypticase Soy Agar

TOCOR = Task Order Contracting Office Representative

7.3 Data Quality Objectives

The precision and accuracy goals have been established for each measurement parameter based on: (1) scientific requirements needed to achieve the primary objectives, (2) knowledge of the measurement system, (3) in-house experience with the sampling and measurement methods, and (4) other similar research studies. Data quality objectives (DQOs) for each major measurement parameter are listed in Table 7-2.

Table 7-2. DQOs for Critical Measurements

Critical Measurement	Measurement Device	Accuracy/Precision
Plated volume	Spiral plater	50% relative standard deviation among the triplicate platings
CFU/plate	QCount	Check of spiral plater template that is within 1.82×10^4 to 2.30×10^4.

MDI Actuation Weight Change	Laboratory Balance	The expected weight change after each actuation is 50 µg ± 10 µg.
Time	NIST-Calibrated Timer	±1 min/30 days
Personal Pump Sampling Rate	DryCal Defender 520	Within 5% of 4 L/min
Asphalt Weight	Laboratory Scale	The expected weight should be ± 100 grams based upon the initial pill density.

Substantial effort was expended to ensure that samples and measured parameters were representative of the media and conditions being measured. All data were calculated and reported in units that were consistent with similar measurements from other organizations to allow for comparability of data among organizations. DQOs for precision and accuracy were based on prior knowledge of the measurement system employed and method verification studies, which include the use of replicate samples and duplicate analyses. During data analysis for this project, the DQOs were observed and met in every instance. Definitions of DQOs are given below.

Accuracy: the degree of agreement of measurements (or an average of measurements) with an accepted reference or true value. Accuracy is a measure of the bias or systematic error in a system. Accuracies of each measurement technique were established by measurement of laboratory standards. Automated colony counting software was checked to laboratory standards for each counting run. All environmental measurement instruments were calibrated prior to experimentation. Variation in colony counting checked against standards fell well within the precision variation, thus the measurements were deemed accurate and representative.

Precision: a measure of mutual agreement among individual measurements of the same property, usually under prescribed similar conditions. Precision is best expressed in terms of the standard deviation. Various measurements of precision exist depending on the prescribed similar condition. The precision goals for this project were replicate CFU triplicate counts within 30%.

References

U.S. Environmental Protection Agency (EPA), 2008. Framework for Investigation of Asbestos-Contaminated Superfund Sites, OSWER Directive #9200.0-68

U.S Environmental Protection Agency (EPA) Standard Operating Procedure (SOP) 2084: Activity Based Sampling for Asbestos, 2007 https://clu-in.org/download/ert/2084-R00.pdf. Washington, DC. Accessed on 8/9/2023.

U.S. Environmental Protection Agency (EPA), 2013. Systematic Evaluation of Aggressive Air Sampling for *Bacillus anthracis* Spores, EPA/600/R-13/068. Research Triangle Park, NC 27711

U.S. Environmental Protection Agency (EPA). 2014 Determination of the Difference in Reaerosolization of Spores off Outdoor Materials, EPA/600/R-14/259 Research Triangle Park, NC 27711

U.S. Environmental Protection Agency (EPA), 2017. Field Application of Emerging Composite Sampling Methods, EPA/600/R-17/212. Research Triangle Park, NC 27711

Gibbons HS, Broomall SM, McNew LA, Daligault H, Chapman C, Bruce D, et al., Genomic Signatures of Strain Selection and Enhancement in *Bacillus atrophaeus* var. *globigii*, a Historical Biowarfare Simulant. PLoS ONE 2011, 6 (3), e17836. https://doi.org/10.1371/journal.pone.0017836.

Talty, JT, Industrial Hygiene Engineering. 2005, Jaico Publishing House

Kim, Y, Wellum, G, Mello, K, Strawhecker, K E, Thoms, R, Giaya, A, and Wyslouzil, B E. Effects of relative humidity and particle and surface properties on particle resuspension rates, Aerosol Science and Technology 2016, 50:4, 339-352

Mikelonis, AM, Abdel-Hady, A, Aslett, D, Ratliff, K, Touati A, Archer J, Serre S, Mickelsen L, Taft S, Calfee MW. Comparison of surface sampling methods for an extended duration outdoor biological contamination study. Environmental Monitoring and Assessment 192, 455 (2020). https://doi.org/10.1007/s10661-020-08434-8

Calfee, M W, Lee, S D, Ryan, S P. A Rapid and Repeatable Method to Deposit Bioaerosols on Material Surfaces. Journal of Microbiological Methods 2013, 92:3, 375-380

U.S. Environmental Protection Agency (EPA), 2018. Evaluation of the Removal and Inactivation of *Bacillus* Spores on Outdoor Surfaces Using a Small-Scale Street Sweeper, EPA/600/R-18/271. Research Triangle Park, NC 27711

U.S. Environmental Protection Agency (EPA). 2012. Assessment of Liquid and Physical Decontamination Methods for Environmental Surfaces Contaminated with Bacterial Spores: Development and Evaluation of the Decontamination Procedural Steps, EPA/R-12/025 Research Triangle Park, NC 27711

Grinshpun, SA, Weber, AM, Yermakov, M, Indugula, R, Elmashae, Y, Reponen, T, Rose, L. Evaluation of Personal Inhalable Aerosol Samplers with Different Filters for Use During Anthrax Responses, Journal of Occupational and Environmental Hygiene 2017, 14:8, 585-595

Centers for Disease Control and Prevention (CDC). 2012. Surface Sampling Procedures for *Bacillus anthracis* Spores from Smooth, Nonporous Surfaces. Accessed on March 3, 2017. On-line Address: https://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html

U.S. Environmental Protection Agency (EPA) 2018. Evaluation of Commercial Wet Vacuums of *Bacillus* Spore Sampling on Surfaces. EPA/600/R-18/158. Research Triangle Park, NC 27711

Penkala, M, Ogrodnik, P, Rogula-Kozlowska, W. Particulate Matter from Road Surface Abrasion as a Problem of Non-Exhaust Emission Control, Environments 2018, 5(1), 9

Miwa, T, Miya, G, Kanno, S. Effect of Surface Roughness on Small Particle Adhesion Forces Evaluated by Atomic Force Microscopy, Japanese Journal of Applied Physics 2020, 59: 076504

Calfee MW, Rose LJ, Morse S, Mattorano D, Clayton M, Touati A, Griffin-Gatchalian N, Slone C and McSweeney N. Comparative Evaluation of Vacuum-based Surface Sampling Methods for Collection of *Bacillus* Spores. 2013. Journal of Microbiological Methods 95(3): 389–396.

Kweon, H, Yiacoumi, S, Tsouris, C., Friction and Adhesion Forces of *Bacilllus thuringiensis* Spores on Planar Surfaces in Atmospheric Systems, Langmuir 2011, 27(24): 14975-14981

Hinds, W C. Aerosol Technology: Properties, Behavior and Measurement of Airborne Particles, 2nd ed.; John Wiley & Sons: New York. 1999

Elovitz, KM. Understanding What Humidity Does and Why, ASHRAE Journal 1999, 41(4): 84

Bevington, PR, Robinson, DK. Data Reduction and Error Analysis for Physical Sciences 3rd ed.; McGraw Hill: Boston 2003

Appendices

Appendix A: AACeSS Mobile Version 2 (V2) and Field Scouting Tests

A.1 AACeSS Mobile V2 Description

This appendix presents the redesign for a mobile version of AACeSS based on the initial tent-based version field tests and includes preliminary scouting tests of *Bg* spore recovery in a field environment. The lessons learned from the initial AACeSS laboratory and field tests described previously in this report led to the new mobile design for AACeSS encompassing a smaller footprint, high mobility, a self-contained resuspension and sampling system, and an easily decontaminated system or inexpensive swappable components for subsequent samples. Figure A-1 shows the constructed mobile prototype AACeSS version 2 (V2).

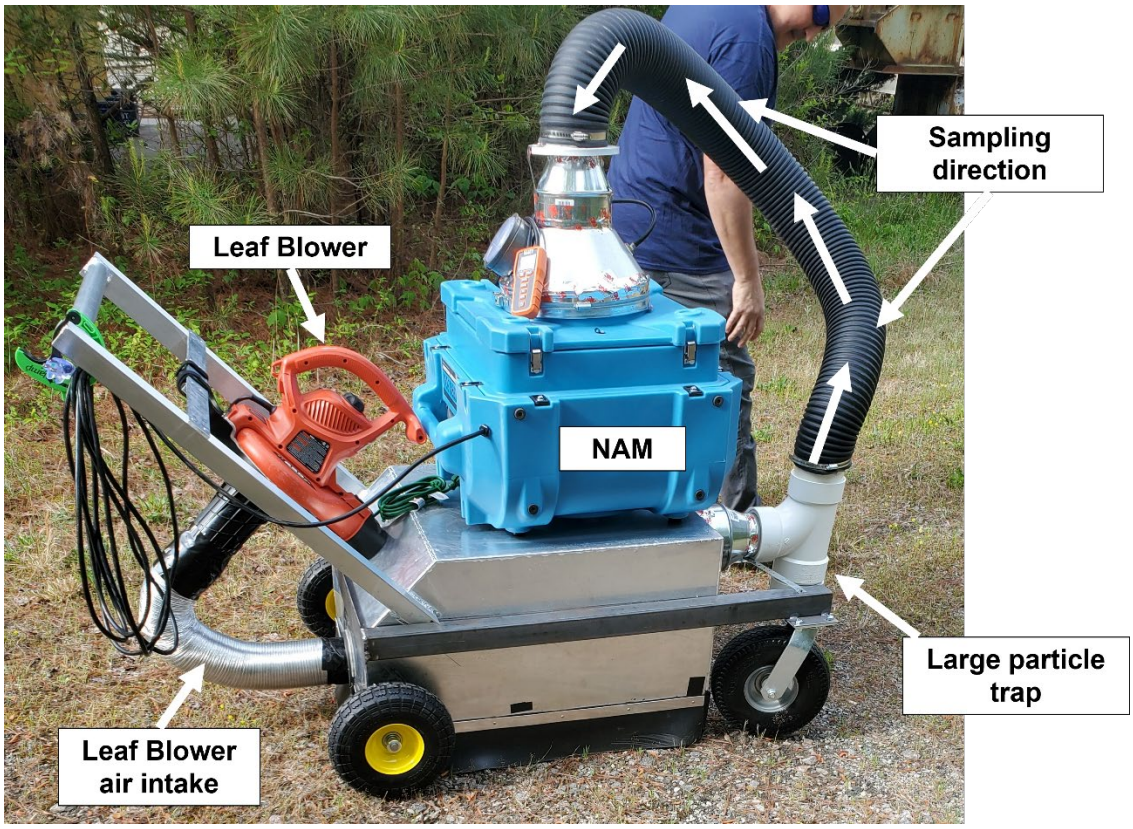


Figure A-1. AACeSS Version 2 with labels

AACeSS V2 is constructed from sheet aluminum welded into a box of approximately 5-ft^3 inner volume. The approximate dimensions of the containment box are 2 ft W x 2.5 ft L x 1.5 ft H with a 45° notch or interface cut out for mounting of a leaf blower. The leaf blower is a model BV6000 with a maximum velocity of 250 miles per hour (mph) at 400 cubic feet per minute (cfm) (Stanley Black and Decker, New Britain, CT) and is directly mounted to the box at an angle of 45° to the ground with the output nozzle ~4 in above the ground. The air intake of the leaf blower is connected to the box so that the supply air is sourced from inside the box, which

creates a recirculated loop, allowing for the negative pressure generated by the air intake to balance the positive pressure generated by the leaf blower's output and minimizing the pressure differential between the inside of the box and outside. A compact negative air machine (NAM) F284 DefendAir HEPA 500 (Dri-Eaz Products, Inc., Burlington, WA) is mounted on top of the system with the air intake pointed upward. The NAM is capable of a maximum 500 CFM sampling rate and creates negative pressure inside the system so that minimal particles escape during resuspension and provides aerosol/particle capture. The NAM is connected to the front of the sampling box through a polyvinyl chloride (PVC) t-section and is used as an impactor or settling chamber to allow larger heavy particles/debris to collide with the inner PVC wall and fall into a removable particle collector for separate analysis. A specially designed stainless-steel filter plate was used to mount the felt filters described in Section 3.1 of this report for resuspended aerosol sampling. Figure A-2 shows the filters and filter mounting plate inside the NAM.

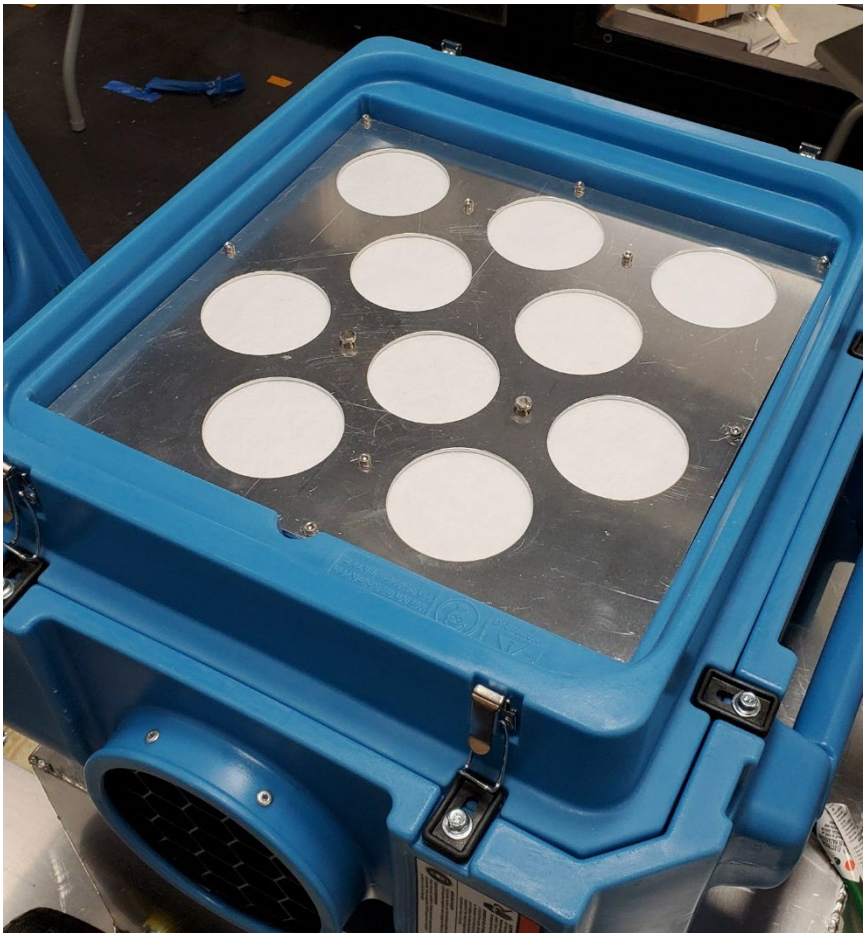


Figure A-2. Felt filters and filter plate inside the NAM

The top section of the NAM is secured into place with butterfly clamps when closed and presses the filter plate into a foam seal. Similar to the NAM described in the tent-based AACeSS, the small NAM is fitted with a HEPA filter to prevent particles not captured by the felt filters from being released into the environment. A rubber skirt is fitted around the bottom of the sampling

system so that it can move over rough or uneven terrain during sampling and retain contact with the ground to prevent aerosol escape during the resuspension/sampling process. Figure A-3 shows the underside of the AACeSS system.

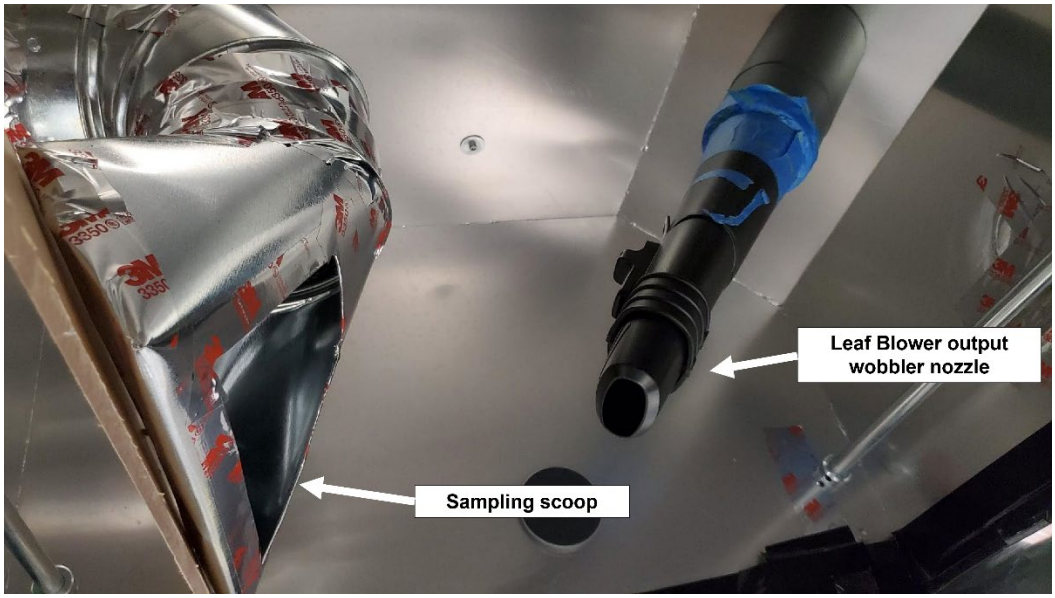


Figure A-3. AACeSS V2 underside

The output of the leaf blower is fitted with a “wobbler nozzle” (PN 90525002, Stanley Black and Decker) providing a sweeping motion across the entire width of the system similar to the manual side-to-side sweep used in traditional leaf blowing and the method used for the tent-based AACeSS. A sampling scoop was fitted to the NAM intake port at the front of the system to allow resuspended material a larger opening to the sampling zone and prevent impact on the front rubber skirt or system walls.

The cost breakdown of the AACeSS construction encompasses the two main components: the sampling mechanism (resuspension/capture equipment) and the overall construction materials. The leaf blower cost is determined by brand and is on average less than $100. Requirements are that the leaf blower has a plumbable air intake and an output velocity above 200 mph. The NAM should be of a form factor similar to the one listed previously and shown in Figure A-2, have a minimum 500 cfm flow, be fitted with a HEPA filter, be able to be retrofitted with a fabricated filter plate, and be able to be easily connected to the sampling system with commercial off-the-shelf ductwork. Costs of these NAMs are currently between $500-$1,200. Thus, the ACCeSS sampling mechanism total cost is between $600 - $1,300. The construction of the sampling volume or box itself is highly dependent on region and material cost at the time of construction. For the current system, which is built with 1/8” aluminum with a square steel hollow tube support structure and various small components such a wheels, casters, and tubing, the cost of construction was approximately $1,000. Therefore, the total cost of the complete AACeSS sampling system should be less than $2,300.

A.2 Field Scouting Test

A field scouting test of AACeSS V2 was conducted on the same concrete test location as the tent-based field test described in Section 5.0. The test occurred on April 20 and April 21, 2022, surface spore inoculation (*Bg*) occurring on the 20th and sampling occurring on the 21st. This test followed the same analysis procedures for the felt filters as described in Section 3.9 and the same QA/QC standards with DQOs discussed in Section 7.0. However, instead of compositing three felt filters in each stomacher bag for culture analysis, this field test resulted in two sets of five felt filters per stomacher bag. In addition, in preparation for an upcoming wide area demonstration (WAD) field demonstration where a liquid inoculation method was going to be used, a liquid deposition technique was also chosen for this field test. Briefly, a handheld electrostatic sprayer was used to deposit 100 mL of deionized water containing 5 x 10^4 CFU/mL of *Bg* over a 3 ft x 3 ft square area on the concrete surface. The liquid well of a Ryobi model PSP02k (Ryobi Inc., Fuchu, Hiroshima, Japan) electrostatic sprayer was filled with 1 L of inoculum and spread over the inoculation zone as shown in Figure A-4.

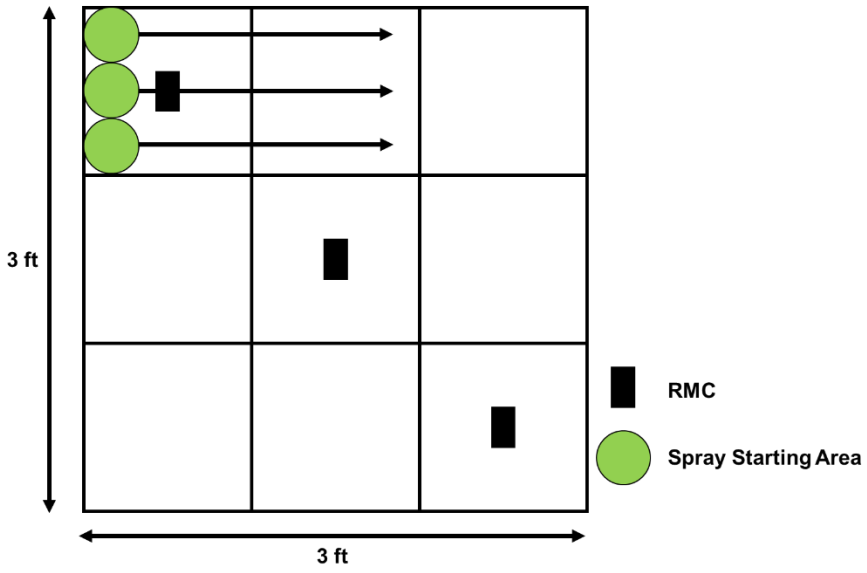


Figure A-4. Field surface liquid inoculation

In the figure, the circular areas are where the spray starts and the black rectangles are 2 in x 1 in reference material coupons (RMCs) used to check and estimate the level of inoculation. Since the spray area was circular, it could not perfectly cover the entire square inoculation zone without overlap. However, the difference in spray area from the total inoculation zone was less than 3%. The RMCs were allowed to sit in the inoculation zone overnight so that any environmental disturbance of the inoculation zone and potential removal/inactivation of spores would be mimicked on the RMCs. Each RMC was collected on the sampling day (next day), placed into a conical tube with PBST, and processed identically to personal sampling filters described in this report under Section 3.9. For each 3 x 3 ft^2 area, the three RMC samples were averaged to determine the CFU/ft^2 of each total deposition zone. Then, that number was multiplied by the 9-ft^2 area to determine the total CFU coverage. For the scouting field test, a single 9-ft^2 deposition zone was inoculated in three total 10 ft x 20 ft sampling areas separate

from the inoculated zones detailed in Section 5.0. Figure A-5 shows a marked deposition zone including the triplicate RMCs being inoculated with the electrostatic sprayer.

Figure A-5. Liquid Field Inoculation with RMCs

Humidity at the time of inoculation was 35% and at the time of sampling was 40%. Figure A-6 shows the sampling path for a 10 ft x 20 ft (200 ft^2) sampling area.

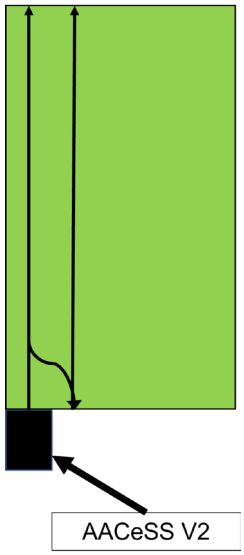


Figure A-6. AACeSS V2 field sampling pattern

The procedure for AACeSS V2 sampling was as follows. The AACeSS sampling cart was moved to just outside the sampling area and placed with its left edge to the left edge of the sampling zone. The cart was loaded with a filter sampling plate with 10 felt filters, and the

sampler lid/duct was replaced. The NAM was then turned on first followed by the leaf blower, to ensure proper pressurization under the sampling cart. The sampler started a stopwatch affixed to the handle of the cart and proceeded to move forward at a comfortable walking speed between 2 and 4 ft/s. After reaching the end of the sampling zone between 5 to 10 seconds later, the sampler then pulled the sampling cart back along the same path until approximately 3 feet away from the opposite end. At this point the sampler maneuvers the cart to the right, without lifting, the full width of the sampler to begin the next pass along the length of the sampling zone. This pattern is repeated until the entire width of the sampling zone is covered. On the final reverse path, the AACeSS system was pulled/pushed the full length of the sampling zone without shifting to the right and stopped at the right corner. The blower is turned off first followed by the NAM, again to maintain negative pressure in the sampler. After the NAM is turned off, the system is removed from the sampling area, the sampling plate is removed from the NAM, and the filters are removed from the plate for processing. The filters are split into two sets of five and placed into two stomacher bags for extraction. Finally, the NAM was reset by removing any large particles collected in crevices with a portable HEPA vacuum (PN 0882-20, Milwaukee Tool, Brookfield, WI) and wiping down direct impact zones with Dispatch (The Clorox Company) bleach wipes followed by a water spray to remove bleach residual. Filter plates were cleaned in a similar fashion with Dispatch wipes and water before reloading with felt filters for another test run. Figure A-7 shows a sampler operating the system over an inoculated concrete zone demarcated by buckets.

Figure A-7. Operation of AACeSS V2 on concrete

Table A-1 shows the estimated number of spores (CFU) deposited in each sampling zone with the standard deviation (Stdev) and coefficient of variation (CV) calculated from the average of the three RMCs.

Table A-1. AACeSS V2 Field Scouting Deposition

Sampling Zone	Estimated CFU Deposited	Stdev	CV
1	4.54E+06	2.25E+06	50%
2	6.00E+06	1.54E+06	26%
3	5.77E+06	1.37E+06	24%

The table shows that each inoculation spore count was close to the targeted 5 x 10^6 CFU inoculation value, and standard deviations for the triplicate RMCs per zone were at or below 50% of the estimate with two of the three sampling zone depositions falling below 30%. Figure A-8 gives the AACeSS V2 sampling results from the three zones sampled in the scouting test.

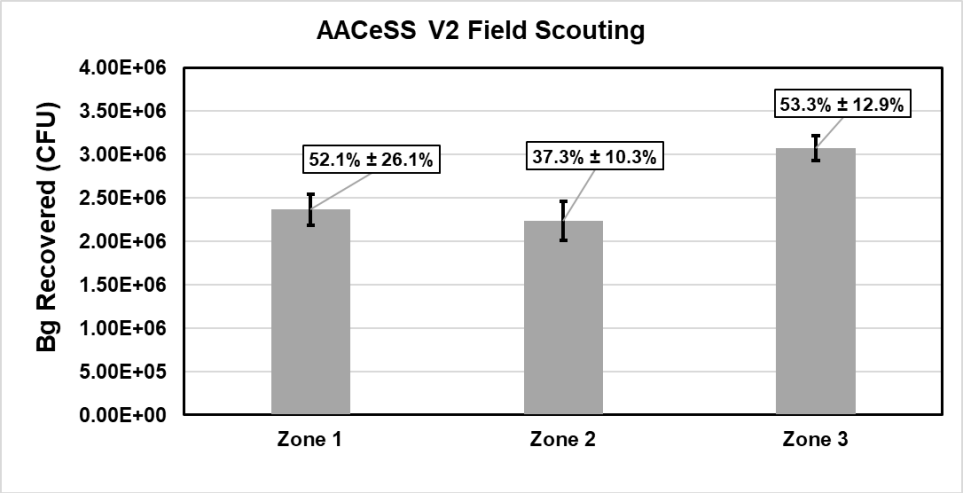


Figure A-8. AACeSS V2 field scouting sampling results

As shown in the figure, the total *Bg* recovered is calculated by summing the *Bg* recovered from the two sets of five filters from each test run. The error bars are calculated by propagating the standard deviations of the group's triplicate culture platings through the sum using methods described in Bevington et al. (Bevington 2003). The number of spores (CFU) recovered was between 2 x 10^6 and 3.5 x 10^6 CFU. In addition, the data call outs on the figure list the estimated sampling efficiency of the system for each zone. As can be seen, the sampling efficiencies are all above 37% with the maximum being 53.3% of the deposited spores recovered. The ± error is calculated from standard error propagation from the errors of the deposition and the *Bg* recovered. (Bevington 2003) For Zones 2 and 3, the error is at or below 30% of the calculated sampling efficiency. The error for Zone 1 is approximately 50% of the calculated efficiency is due to the high variance in the RMC data for that deposition/inoculation. Comparing these sampling efficiencies to the efficiencies calculated in Section 6.2 for the tent-based AACeSS field test, it is evident that for this limited comparison from same surface, AACeSS V2 collects a significantly higher percentage of the spores present on the concrete surface. Given that a liquid deposition was used in the AACeSS V2, this comparison is even more significant, even though the sample size is small. Additionally, the added mobility of the cart-based sampling system makes it considerably more deployable over a variety of outdoor surfaces.

Appendix B: AACeSS Field Sampling Protocols – V1 and V2

PROCEDURE FOR *BACILLUS ANTHRACIS* FIELD SAMPLING USING TENT-BASED AACeSS WITH PERSONAL AIR SAMPLING (AACeSS V1)

MATERIALS:

Sampling Kit

Quantity	Item Name
2	Sterile Nunc 50-mL conical tubes (339652, Thermo Fisher Scientific, Waltham, MA)
2	4.5” x 7.5" Twirl’em small bags with flat wire enclosures (1186T33, Thomas Scientific, Swedesboro, NJ)
3	400 mL Stomacher bag (Seward Laboratory Systems, Inc., Port Saint Lucie, FL: BA6141/CLR)
2	Individually wrapped disposable forceps (NC9642907 Thermo Fisher Scientific) or equivalent
9	AACeSS felt filters 5-micron pore polyester felt 4” round (1-005-72 4” Die Cut Superior Felt and Filtration, McHenry, IL)
2	Button Sampler 25 mm PTFE filters – Millipore Fluoropore PTFE 3.0 µm Pore Size Membrane Filters (FSLW02500, MilliporeSigma, Burlington, MA)
5	Unique labels (5520, Avery Dennison, Mentor, OH) with quick read (QR) code (AACeSS_A, AACeSS_B, and AACeSS_C, for stomacher bags and Button_A and Button_B for conical tubes)
2	1 gallon Ziploc storage overpack bag (94602, SC Johnson, Racine, WI or equivalent)
3	12 in x 12 in 4-Mil antistatic resealable bags (S-1327 Uline, Pleasant Prairie, WI)

Tools/Equipment

Quantity	Item Name
1	AACeSS Tent 10 ft x 20 ft (Caravan Canopy Carport Model 22006200010 and 12000211010, Caravan Global US, La Mirada, CA or equivalent)
1	High Volume Sampler (Modified OmniAire 2200C, OmniTec, Mukilteo, WA)
1 (If No Field Power Available)	5000 W Portable Generator 6000 W startup amps (CMXGGAS030731 Stanley Black & Decker or equivalent)
1	100 ft Outdoor 15A Heavy Duty Extension Cord marked in 20-ft sections (S-13800 Uline or equivalent)
9	AACESS Sampling Filter Holders (Custom)
1	>120 mph battery powered leaf blower (EGO Power+ LB6151, Lowes Inc., Morrisville, NC)
2	Button Sampler housing assemblies with red caps (SKC 225-360, SKC Inc., Eighty Four, PA)
2	Sampling Pump Universal PCXR8 (SKC 224-PCXR8, SKC Inc.)
2	Tygon tubing - 3 ft - 1/4 in ID (SKC 225-13-4A, SKC Inc.)
1	Folding table for sample processing
2 minimum	Clear backpacks (Eastsport, EST193971BJBLK, North Brunswick, NJ) for supplies 1 per team member
2	Stopwatches

Button Sampler Calibration Kit

Quantity	Item Name
1	Button Sampler Calibration Adapter (SKC 225-361, SKC Inc.)
1	Pump Calibration Unit >5 L/min (DryCal Defender 510, Mesa Laboratories Inc., Lakewood, CO or equivalent)
1	Button Sampler housing assemblies with red caps (SKC 225-360, SKC Inc.)
1	Button Sampler 25-mm PTFE filters – Millipore Fluoropore PTFE 3.0 µm Pore Size Membrane Filters (FSLW02500, Millipore Sigma)
2	Tygon tubing - 3 ft - 1/4 in inner diameter (ID) (SKC 225-13-4A, SKC Inc.)

Consumables

Quantity	Item Name
20-30	Disposable nitrile gloves (SciMart, Box Elder, SD) (sized for samplers) 10 per sampler team member
1	Germicidal Disinfecting wipes (69150 Dispatch Disinfectant Towels with Bleach ~0.65% hypochlorite concentration, The Clorox Company or equivalent)
1	Permanent Marker
1	Light-colored Masking Tape

A. Preliminary Steps

Warning

THERE IS POTENTIAL RISK FROM WORKING AROUND *BACILLUS ANTHRACIS* SPORES. TO MINIMIZE THE RISK OF INHALATION EXPOSURE DURING SAMPLING OF POTENTIALLY CONTAMINATED AREAS, APPROPRIATE PROTECTIVE MEASURES MUST BE FOLLOWED. IT IS RECOMMENDED THAT THESE SAMPLING PROTOCOLS BE INCLUDED IN A COMPREHENSIVE HEALTH AND SAFETY PLAN (HASP) PRIOR TO INITIATION OF SAMPLING ACTIVITIES.

TO REDUCE THE RISK, PROPER PERSONAL PROTECTIVE EQUIPMENT (PPE) MUST BE USED WHEN WORKING IN A POTENTIALLY CONTAMINATED ENVIRONMENT. PPE INCLUDES PROPER RESPIRATORY PROTECTION, PROTECTIVE GARMENTS, AND GLOVES. CONSULT WITH THE SITE SAFETY OFFICER FOR PROPER PPE SELECTION.

When mandated by the Safety Officer (SO) under Unified Command (UC) or other incident management system, PPE, including respiratory protection, must be employed under Occupational Safety and Health Administration (OSHA) standards and regulations found in 29 CFR Part 1910 Subpart I (1910.132—1910.138) or the equivalent construction standards (29 CFR Part 1926). These standards require training on the proper selection, use, removal, and disposal of PPE; a medical evaluation and fit testing must be conducted before the use of any respiratory protection and annually thereafter.

Contact the Safety Officer for PPE requirements, and for selecting applicable sampling procedures, stations, and routes.

B. Sampling

Warning

ALL TEAM MEMBERS MUST WEAR APPROPRIATE PPE. FOLLOWING DECONTAMINATION PROCEDURES, PPE MUST BE REMOVED AND DISPOSED OF ACCORDING TO THE HASP.

Caution

TO PREVENT SPREAD OF CONTAMINATION, DO NOT PERFORM AACeSS SAMPLING IN THE VICINTY OF WATER INTAKES, ENVIRONMENTALLY SENSITIVE AREAS, OR IN AREAS WHERE THE PUBLIC COULD BE AFFECTED. UNDERSTANDING OF WIND DIRECTION IS ALSO IMPORTANT. CONSULT WITH THE SAFETY OFFICER FOR POTENTIAL DRIFT OF MATERIAL DURING SAMPLING BEFORE DETERMINING SAMPLING ZONES. ALL SAMPLING PERSONNEL SHOULD BE TRAINED IN THE USE OF THE AACeSS SYSTEM AND BE ABLE TO LIFT 50 pounds (LB).

1. Identify sampling location and parameters

This sampling procedure is for sampling of biological agents on outdoor surfaces inside a closed 10 ft x 20 ft tent using an aggressive air sampling (AAS) technique with a modified negative air machine (NAM) as a high-volume air sampler. This procedure can be generalized to be used for any enclosed tent/volume with a boat/vehicle or other materials that have a vertical component as long as the surfaces can be accessed within the closed volume. Ideally sampling times should be kept to 30 minutes or less; therefore, the high-volume sampler and tent air volume combination should be chosen so that three air exchanges can be achieved in 30 minutes or less. The minimum ratio of high-volume sampler flow rate to tent air volume for a 30-minute sample is 1/10. For example, a 2000 ft^3 volume requires a minimum high-volume sampling rate of 200 cfm and a 30-minute sampling time. A higher sampling flow rate can be chosen to lower the necessary sampling time. The required sampling time is simply three divided by the flow rate to volume ratio (e.g., a 400 cfm sampler used in a 2000 ft^3 volume requires 15 minutes of sampling time. For a boat/vehicle enclosed in the sampling tent, the air volume should be estimated by taking the actual volume of the tent and subtracting an estimated volume of the boat/vehicle (length x width x height).

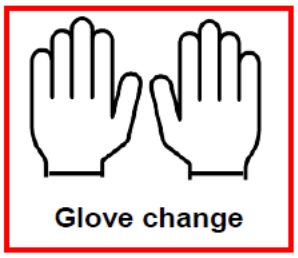
At a minimum, a two-person sampling team must be used. The team should consist of a sampler (person who will actively conduct the sampling with the AACeSS system) and a support person (person who will be responsible for taking notes, labeling, starting and stopping the NAM, filter sample processing, and other sampling procedures). Ideally a third person (sample processor) would be present to perform note-taking and filter sample processing while the support person would aid the sampler.

This protocol assumes that the sampling area has been prepared before the sampling event and the tent plus high-volume sampler have been constructed and installed inside the contaminated outdoor area (exclusion zone) prior to samplers entering.

Use of Personal Button Samplers: This procedure includes the potential use of personal Button Samplers for deployment on the AACeSS sampling team. If Button Samplers are not used, ignore sections or steps below that discuss the Button Sampler. If Button Samplers are deployed across all sampling teams in a response, defer to the specific Button sampling protocol dictated by the UC, Operations Section Chief, and/or Safety Officer.

Note

Clean outer nitrile gloves should be worn for the collection of each new sample and while processing each sample to prevent cross-contamination. Points at which gloves MUST be changed during the sampling procedure are indicated using the “Glove change” graphic below. However, gloves should be changed any time a glove integrity is compromised (i.e., small hole or tear or a known contact with a potentially contaminated surface occurs).



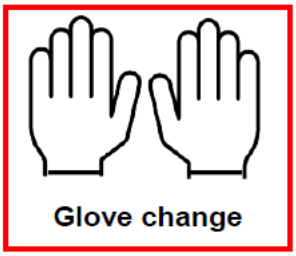
2.1 Sampling Preparation

2.1.1 Sampling Kit Preparation

Sampling kits can be prepared in advance and stored in a clean dry environment.

A sampling kit for each AACeSS plus Button sample will be prepared in a laboratory setting using the following procedure:

1. Don nitrile gloves.



2. Assemble the AACeSS collection kit
    - Place 9 4” felt filters into a Ziploc bag.
    - Label 3 Stomacher bags with the unique sample IDs plus QR code for AACeSS_A, AACeSS_B, and AACeSS_C (consistent with an approved Sampling and Analysis Plan).
    - Place one wrapped disposable forceps with stomacher bags and felt filters.
    - Place 3 12” x 12” bags with the felt filters and with a sharpie write AACeSS_A/AACeSS_B/AACeSS_C on the bags, one per bag.
3. Assemble the Button Sampler collection kit
    - Label 1 conical 50 mL conical tubes with the unique sample IDs plus QR code for Button_A and Button_B (consistent with an approved Sampling and Analysis Plan).
    - With a tweezer, remove paper separator between PTFE filters and place one filter in each conical tube.
    - Place 2 Twirl’em bags with the conical tubes.
    - Place 2 wrapped disposable forceps with Twirl’em bags.
4. Place all materials for the AACeSS collection kit and the Button Sampler collection kit into the second Ziploc bag.

5. Store Ziploc bag with both collection kits in a clean, dry environment.

2.1.2 Preentry Procedures

AACeSS filter preparation

1. Don nitrile gloves.
2. Remove 9 filter holder assemblies and set them on a clean table.
3. Remove the bag with the felt filters from the sampling collection kit.
4. With the small end facing down, unscrew the retaining ring and separate the two halves
5. Remove the backing screen.
6. Place one felt filter into the filter holder on top of the gasket and ensure it is centered.
7. Place the backing screen onto the filter.
8. Screw on the retaining ring until snug.
9. Repeat for all 9 filter holders.
10. Stack the loaded filter holders in groups of 3 and place 1 stack in each of the 12” x 12” bags.

Button Sampler Preparation

1. Don nitrile gloves.
2. Remove the red cap from a sterile Button Sampler.
3. Unscrew and remove the inlet of the sampler and remove the PTFE O-ring.
4. Open one of the conical tubes from the sampling kit and open the cap.
5. Remove the PTFE filter from the conical tube using sterile tweezers and place on top of the support screen inside the sampler.
6. Place the PTFE O-ring on top of the filter, replace inlet, and gently tighten to just past finger tight. Note: overtightening can damage filter.
7. Replace red cap.
8. Attach Tygon tubing onto the outlet of the Button Sampler.
9. Repeat for the second Button Sampler.
10. Place capped Button Sampler assemblies with attached tubing into the gallon bag.

Button Sampler Pump Calibration

Each Button sampler pump comes with a unique ID tag attached to it. In addition, a piece of tape should be attached to each pump with a label Button_A or Button_B for quick identification in the field. The calibration kit includes: an adapter, a Button Sampler assembly containing a PTFE filter, 2 lengths of ¼ in ID Tygon tubing, pump adjustment tool, and a pump calibration unit.

For each pump to be calibrated, record unique ID and Button_A/Button_B label into the field notebook.

1. Connect the pump to be calibrated to the prepared testing Button Sampler assembly with one piece of the Tygon tubing.
2. Connect the calibration adapter to the Button Sampler assembly.

3. Connect the second length of Tygon tubing to the adapter and to the low pressure/pull side of the calibration unit.
4. Turn on the calibration unit and allow to warm up and set to continuous measurement. If using a rotameter, this step can be skipped.
5. Turn on the pump to be calibrated and observe the flow rate.
6. Adjust the pump until the flow rate is 4 ± 0.2 L/min.
7. Record final measured flow rate in field notebook.
8. Repeat for the next pump to be calibrated.

After pumps are calibrated, place the sampling kit and pumps into the clear backpack carried by the sampler. Place all tools necessary for the sampler into the backpack carried by the support person. All team members should be double gloved before entry.

3. Field Sampling Procedure

3.1 Field Setup

Figure 1 shows the general orientation of the components of a field sampling event and the sampling pattern. The components include the AACeSS tent, NAM connection, and the filter processing table. The tent should be constructed on level ground and have weights placed around the skirt to minimize resuspended material exiting the enclosure during the aggressive air leaf blowing. The NAM should be completely sealed to the front wall. If a generator is needed to power the NAM, the generator should be placed ~50 ft behind the NAM on the “front wall” side of the tent.

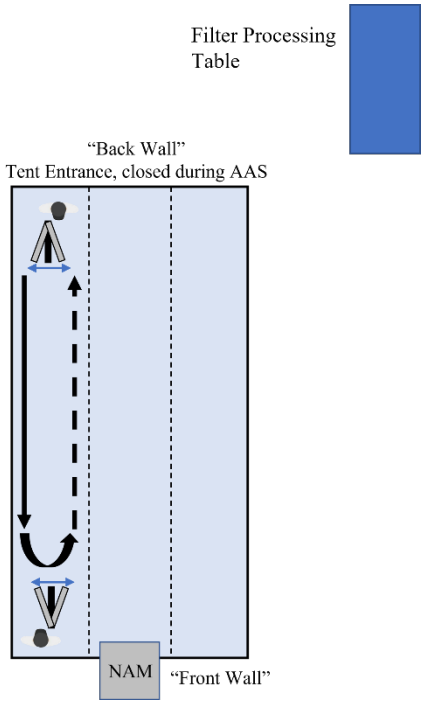


Figure B-1. AACeSS field setup and sampling pattern

1. The team members will start with double nitrile gloves for setup.

2. The backpack containing the sampling kit will be placed on the filter processing table.
3. Nitrile gloves, dispatch wipes and the filter plate cover can be placed on the filter processing table.
4. The support person will plug the NAM into the power source. If the generator is used, then the support person will start the generator.
5. The sample support person will remove the Button Sampler pumps from the sampling backpack and hand the one marked Button_A to the sampler.
6. The team members will attach their respective pumps to their respirator belts.
7. The sampler support person will the remove a Button Sampler assembly with a tube from the sampler backpack, attach the tube to the sampling pump, and attach the Button Sampler to the PPE of the sampler at approximately shoulder height.
8. The support person will remove the second Button Sampler assembly with tube from the sampling backpack, hand it to the sampler, and the sampler will make the tube connection to the pump and PPE of the support person.
9. The sample support person will record the sample ID numbers and location in the sampling log.

Note

Barcoded and automated linking of sampling kit ID and sample location may be utilized instead of manual recording.

10. The sampler support person will remove the red caps from the Button Samplers and place into the sampling backpack.
11. Each team member will turn on their sampling pumps or ask for assistance from the other team member. Team members will check and ensure the internal rotameter bulb is showing flow through the pump of the other team member.
12. The sampler will enter the tent, and the support person will stand outside the tent entrance.
13. The support person will hand the sampler the bag containing AACeSS_A filter holders.
14. The sampler will install the filter holders on the top row of the NAM and hand the bag back to the support person through the entrance.
15. This process will be repeated for AACeSS_B in the middle row of the NAM and AACeSS_C on the bottom row.

Note

Once the sampler has entered the tent area, they will not exit the tent until sampling is complete.

16. The support person will then hand the sampler the leaf blower.

3.2 Sampling Procedure

1. As shown in Figure 1, the sampling area should be broken into sections across the shortest width of the tent. These sections should be no wider than 3.5 ft.
2. The support person and sampler will synchronize their stopwatches and the support person will close the tent.
3. When the stopwatch reads 2 minutes, the support person will start the NAM, and the sampler will begin aggressive air sampling following the pattern shown in Figure 1.
4. The sampler will hold the leaf blower in a relaxed position at approximately 30 degrees as close to the surface to be sampled as is comfortable.
5. While walking forward toward the NAM, the sampler will slowly sweep the leaf blower side to side to cover a width of approximately 3-3.5 ft.
6. The walking rate should be relaxed, approximately a step (1 - 1.5 ft) per second.

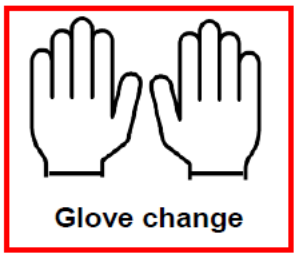
Note

If only materials or vehicles/vessels are to be sampled and the ground is unimportant the aggressive air sampling can be concentrated on the materials of interest. The first pass with the leaf blower should always be directed towards the NAM/high volume sampler, and the leaf blower should be swept over the entire surface of the material.

7. Once the sampler has reached the front wall, they will turn around and walk back toward the back wall performing the same sweeping motion.
8. Once the sampler reaches the back wall on the return pass, they will move over to the next section and perform the down and back sampling pattern again on the new section.
9. The sampler will repeat this until the full footprint of the tent is covered or all materials of interest have been sampled whichever comes first.
10. After the sampler has completed the aggressive air sampling, they will slowly walk around the space with the leaf blower on pointed upward to mix the air until the stopwatch reads 32 min.
11. At 32 min, the sampler will stop the leaf blower, and the support person will stop the NAM and turn off the generator.
12. The sampler will then open the tent but remain inside.
13. The sampler and support person will then stop their Button Sampler pumps.

3.3 Filter Collection Procedure

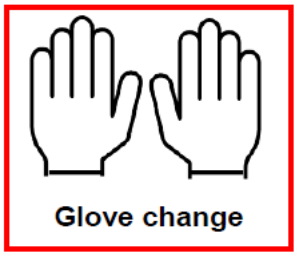
1. The sampling team will don new gloves.



2. The support person will retrieve the red caps covering their Button Sampler and then cover the sampler's Button Sampler through the tent entrance without entering the tent.
3. The support person will take the sampling kit from the backpack and lay each conical tube with a twirl'em bag.
4. They will then remove their Button Sampler with Tygon tube and lay it next to the conical tube labeled Button_B.
5. The support person will then retrieve the 12" x 12" bags and carry them to the sampler.
6. The support person will hand the bag labeled AACeSS_A to the sampler.
7. The sampler will retrieve the 3 filter holders from the top row of the NAM face, stack them, and place them in the bag.
8. The sampler will hand the bag to the support person.
9. The support person will hand the bag labeled AACeSS_B to the sampler and place AACeSS_A on the filter processing table.
10. The sampler will collect the filter holders from the middle row and place in the bag and hand the bag to the support person.
11. The process will be repeated for AACeSS_C and the bottom row of filter holders.
12. The support person will then remove the Button Sampler with Tygon tube from the sampler and place it on the processing table next to the conical tube labeled Button_A.
13. The sampler will then exit the tent with the leaf blower.

3.4 Button Sampler Processing

1. The support person will remove two of the forceps packs from the sampling kit, open the packs, and place one next to each Button sampler with the opening facing them such that they can remove the forceps.
2. The support person will wipe the area below the Button Samplers with a dispatch wipe and will loosen the top of the Button Samplers so that they can be lifted off.
3. The support person will don a new pair of nitrile gloves.

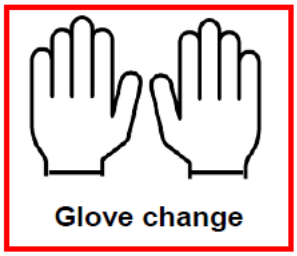


4. The support person will collect the conical tube labeled Button_A, remove the cap, set it top-down on the dispatch wiped area, and hold the conical tube in one hand.
5. With the other hand, the support person will remove the Button Sampler top exposing the PTFE ring/filter and will set the top down next to the Button Sampler.

6. The support person will take the forceps from the package, remove the PTFE retaining ring, set it into the Button Sampler top, remove the PTFE filter from the Sampler and place it into the conical tube.
7. Then, the support person will cap the conical tube and place it into a Twirl'em bag.
8. The support person will repeat steps 4-7 for Button_B.
9. Once both Button Samplers are processed, the support person wipes both Twirl'em bags with Dispatch wipes and places the bags in the sampling kit Ziplock bag.
10. The support person then reassembles the Button Samplers and sets them aside for decontamination.

3.5 AACeSS Filter Processing

1. The sampler will remove the filter holders from the bag labeled AACeSS_A, set them on the filter processing table with the small end facing upwards and loosen the retaining ring on each holder and lift off the top, exposing the 3 filters.
2. The sampler will then don new nitrile gloves.



3. The support person will remove the stomacher bags labeled AACeSS_A and the remaining forceps from the sampling kit and lay them next to the kit.
4. The support person will open the forceps and the stomacher bag labeled AACeSS_A.
5. Using the forceps, the support person will remove the gasket from the top of the filters and set them aside.
6. The sampler will then stack the 3 filters on top of each other and place the stack in the AACeSS_A stomacher bag and squeeze the air out of the stomacher bag.
7. The sampler will repeat steps 1-6 for each of the remaining filter holders (AACeSS_B and AACeSS_C), matching the filter holder labels to the corresponding stomacher bags and reusing the forceps.
8. Once all three stomacher bags are filled with filters, the sampler will wipe the stomacher bags with a dispatch wipe and place them in the sampling kit Ziploc bag.
9. The sampler will then reassemble the filter holders and put them back into their corresponding 12" x 12" Uline bags.

3.6 Field Exit

1. Once all filter processing is complete the support person will wipe the sampling kit Ziploc with a Dispatch wipe, will compress it to remove as much air as possible, and place it into the sampler backpack.
2. The sampler will collect the leaf blower, extension cord, and filter holders and deliver them to the designated AACeSS decontamination area.

3. The Button Sampler assemblies with Tygon tubing will be placed into the support backpack with any tools that were used.
4. The support person will use a Dispatch wipe to clean the filter processing table for the next sampling event if one will occur.
5. The sampler will wipe the generator pull handle and movement handle with a dispatch wipe.
6. If no additional AACeSS sampling will occur, the table will be delivered to the AACeSS decontamination area as well as the generator.
7. The sampling team will then exit the field through a decontamination line following standard protocols.
8. After decontamination, the team will collect the Button Sampler pumps and return them to the pump calibration area.
9. Using the flow measuring device utilized for the pump calibration, each pump flow will be measured and recorded in the log book next to its unique ID and pre deployment sampling flow.

PROCEDURE FOR *BACILLUS ANTHRACIS* FIELD SAMPLING USING AACeSS MOBILE CART WITH PERSONAL AIR SAMPLING (AACeSS V2)

Sampling Kit

Quantity	Item Name
2	Sterile Nunc 50-mL conical tubes (339652, Thermo Fisher Scientific, Waltham, MA)
2	4.5” x 7.5" Twirl’em, small bags with flat wire enclosures (1186T33, Thomas Scientific, Swedesboro, NJ)
3	400-mL Stomacher bags (Seward Laboratory Systems, Inc., Port Saint Lucie, FL: BA6141/CLR)
3	Individually wrapped disposable forceps (NC9642907 Thermo Fisher Scientific) or equivalent
10	AACeSS felt filters 5 micron pore polyester felt 4” round (1-005-72 4” Die Cut Superior Felt and Filtration, McHenry, IL)
2	Button Sampler 25-mm PTFE filters – Millipore Fluoropore PTFE 3.0 µm Pore Size Membrane Filters (FSLW02500, MilliporeSigma, Burlington, MA)
4	Unique labels with QR code (AACeSS_A, AACeSS_B, and Material for stomacher bags and Button_A and Button_B for conical tubes)
2	1 gallon Ziploc storage overpack bag (94602, SC Johnson, Racine, WI or equivalent)

Tools/Equipment

Quantity	Item Name
1	AACeSS Sampling Cart with leaf blower
1	AACeSS Sampling Filter Holder Plate with plate cover (Custom)
1 (If No Field Power Available)	5000 W Portable Generator 6000 W startup amps (CMXGGAS030731 Stanley Black & Decker, New Britain, CT or equivalent)
2	Button Sampler housing assemblies with red caps (SKC 225-360, SKC Inc., Eighty Four, PA)
2	Sampling Pump Universal PCXR8 (SKC 224-PCXR8, SKC Inc.)
2	Tygon tubing - 3 ft - 1/4 in ID (SKC 225-13-4A, SKC Inc.)
1	Button Sampler Calibration Adapter (SKC 225-361, SKC Inc.)
1	100-ft Outdoor 15 A Heavy Duty Extension Cord marked in 20-ft sections (S-13800 Uline, Pleasant Prairie, WI or equivalent)
1	6” slip joint pliers (STHT84401 Stanley Black and Decker, New Britain, CT or equivalent)
1	Folding table for sample processing
2 minimum	Clear backpacks (Eastsport, EST193971BJBLK, North Brunswick, NJ) for supplies, 1 per team member
1	Can of white water-based athletic field marking paint (206043 RUST-OLEUM, Vernon Hills, IL or equivalent)

Button Sampler Calibration Kit

Quantity	Item Name
1	Button Sampler Calibration Adapter (SKC 225-361, SKC Inc.)
1	Pump Calibration Unit >5 L/min (DryCal Defender 510, Mesa Laboratories Inc., Lakewood, CO or equivalent)
1	Button Sampler housing assemblies with red caps (SKC 225-360, SKC Inc.)
1	Button Sampler 25-mm PTFE filters – Millipore Fluoropore PTFE 3.0 µm Pore Size Membrane Filters (FSLW02500, MilliporeSigma, Burlington, MA)
2	Tygon tubing - 3 ft - 1/4 in ID (SKC 225-13-4A, SKC Inc.)

Consumables

Quantity	Item Name
20-30	Disposable nitrile gloves (SciMart, Box Elder, SD) (sized for samplers) 10 per sampler team member
1	Disinfecting wipes (69150 Dispatch Disinfectant Towels with Bleach, The Clorox Company, Oakland, CA or equivalent)
1	Permanent Marker
1	Light-colored Masking Tape

A. Preliminary Steps

Warning

THERE IS POTENTIAL RISK FROM WORKING AROUND *BACILLUS ANTHRACIS* SPORES. TO MINIMIZE THE RISK OF INHALATION EXPOSURE DURING SAMPLING OF POTENTIALLY CONTAMINATED AREAS, APPROPRIATE PROTECTIVE MEASURES MUST BE FOLLOWED. IT IS RECOMMENDED THAT THESE SAMPLING PROTOCOLS BE INCLUDED IN A COMPREHENSIVE HEALTH AND SAFETY PLAN PRIOR TO INITIATION OF SAMPLING ACTIVITIES.

TO REDUCE THE RISK, PROPER PERSONAL PROTECTIVE EQUIPMENT (PPE) MUST BE USED WHEN WORKING IN A POTENTIALLY CONTAMINATED ENVIRONMENT. PPE INCLUDES PROPER RESPIRATORY PROTECTION, PROTECTIVE GARMENTS, AND GLOVES. CONSULT WITH THE SITE SAFETY OFFICER FOR PROPER PPE SELECTION.

When mandated by the Safety Officer (SO) as part of a Unified Command (UC) or other incident management system, PPE, including respiratory protection, must be employed under Occupational Safety and Health Administration (OSHA) standards and regulations found in 29 CFR Part 1910 Subpart I (1910.132—1910.138) or the equivalent construction standards (29 CFR Part 1926). These standards require training on the proper selection, use, removal, and disposal of PPE; a medical evaluation and fit test must be conducted before the use of any respiratory protection and annually thereafter.

Contact the Unified Command (UC), Operations Section Chief, and/or Safety Officer for PPE requirements, and for selecting applicable sampling procedures, stations, and routes.

B. Sampling

Warning

ALL TEAM MEMBERS MUST WEAR APPROPRIATE PPE. FOLLOWING DECONTAMINATION PROCEDURES, PPE MUST BE REMOVED AND DISPOSED OF ACCORDING TO THE HEALTH AND SAFETY PLAN (HASP).

Caution

TO PREVENT SPREAD OF CONTAMINATION, DO NOT PERFORM AACeSS SAMPLING IN THE VICINTY OF WATER INTAKES, ENVIRONMENTALLY SENSITIVE AREAS, OR IN AREAS WHERE THE PUBLIC COULD BE AFFECTED. UNDERSTANDING OF WIND DIRECTION IS ALSO IMPORTANT. CONSULT WITH THE SAFETY OFFICER FOR POTENTIAL DRIFT OF MATERIAL DURING SAMPLING BEFORE DETERMINING

SAMPLING ZONES. ALL SAMPLING PERSONEL SHOULD BE TRAINED IN THE USE OF THE AACESS SYSTEM AND BE ABLE TO LIFT 50 pounds (LB).

2. Identify sampling location and parameters

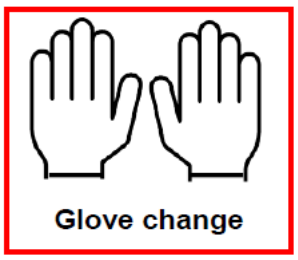
This procedure is intended for sampling of a minimum 400 ft^2 rectilinear section of ground, nominally square in shape. However, the procedure can be scaled to any size determined as necessary and within the capability of the sampling system. Ground debris in addition to the potential contamination will load the filters and eventually prevent the system from sampling further. Hard surfaces such as concrete and asphalt will tend toward having a larger surface sampling capability than surfaces with loose debris such as grass and gravel. Before deployment, determine the most important 400 ft^2 zone of interest to sample and any connected areas to sample if the surface allows. When sampling, target the initial 400 ft^2 area, assess the filter load by checking the pressure measurement on the AACeSS system and continue to the next sampling zone if possible. The limit of pressure drop of the NAM is **~3.2 in H_2O.**

At a minimum, a two-person team must be used. The team will consist of a sampler (person who will actively conduct the sampling with the AACESS system, i.e. the “dirty person”) and a support person (person who will be responsible for taking notes, labeling, providing assistance with AACeSS power cord control, filter sample processing, and other sampling procedures, i.e., “clean person”). Ideally, a third person (sample processor) would be present to perform note taking and filter sample processing while the support person would provide assistance to the sampler.

Button Sampler Note: This procedure includes the potential use of Button Samplers if they are deployed for the AACeSS sampling team. If Button Samplers are not used, ignore sections or steps that discuss the Button Sampler. If Button Samplers are deployed across all sampling teams in a response, defer to the specific Button sampling protocol dictated by the UC, Operations Section Chief, and/or Safety Officer

Note

Clean outer nitrile gloves should be worn for the collection of each new sample and while processing each sample to prevent cross contamination. Points at which gloves MUST be changed during the sampling procedure are indicated using the “Glove change” graphic below. However, gloves should be changed any time glove integrity is compromised, i.e., small hole or tear or a known contact with a potentially contaminated surface occurs.



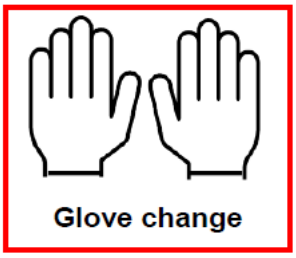
2.1 Sampling Preparation

2.1.1 Sampling Kit Preparation

Sampling kits can be prepared in advance and stored in a clean dry environment.

A sampling kit for each AACeSS plus 2 Button samples will be prepared in a laboratory setting using the following procedure:

6. Don nitrile gloves.



7. Assemble the AACESS collection kit
    - Place 10 4” felt filters into a Ziploc bag.
    - Label 3 Stomacher bags with the unique sample IDs plus QR code for AACESS_A, AACESS_B, and Material (consistent with an approved Sampling and Analysis Plan).
    - Place one wrapped disposable forceps with stomacher bags and felt filters.
8. Assemble the Button Sampler collection kit
    - Label 2 conical 50-mL conical tubes with the unique sample IDs plus QR code for Button_A and Button_B (consistent with an approved Sampling and Analysis Plan).
    - With a tweezer, remove paper separator between PTFE filters and place one filter in each conical tube.
    - Place 2 Twirl’em bags with the conical tubes.
    - Place 2 wrapped disposable forceps with Twirl’em bags.
9. Place all materials for the AACeSS collection kit and the Button Sampler collection kit into the second Ziploc bag.
10. Store Ziploc bag with both collection kits in a clean dry environment

2.1.2 Preentry Procedures

AACeSS cart preparation

11. Don Nitrile gloves.
12. Visually inspect the cart skirt for defects or separation from the housing that would allow aerosolized material to escape.

13. Assemble the large particle separator and attach to the cart.
14. On a clean table, remove the thumb screws from the two halves of the filter holder assembly.
15. Separate the two halves and inspect all rubber gaskets to ensure proper seating into the retaining grooves.
16. Remove the bag with the felt filters from the sample collection kit.
17. Place one felt filter into each filter holder circle and ensure each is centered.
18. Place the top of the filter holder plate onto the felt filters and secure with the thumb screws, ensuring that lateral movement is kept to a minimum to prevent displacement of the felt filters.
19. Open the top of the Negative Air Machine (NAM) and place onto the holder.
20. Place the filter holder containing the filters into the NAM and press down to ensure it is seated onto the gasket. The filter properly seated in the NAM is shown in figure 1.

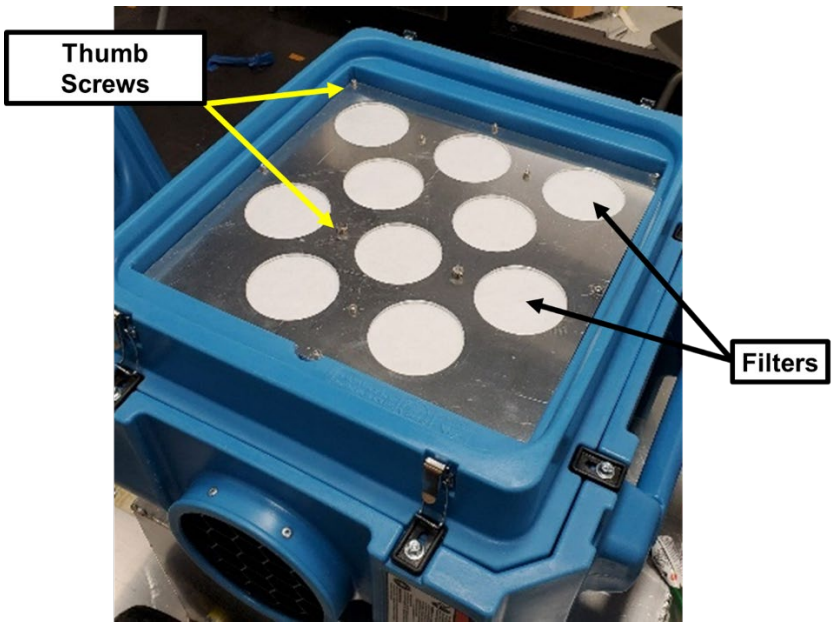


Figure B-2. Felt filter holder seated in NAM

21. Replace the top of the NAM and attach connections to the large particle separator.
22. Ensure the leaf blower is plugged into the NAM, and the NAM is connected to the extension cord.
23. Run the extension cord over the extension cord pole and secure the wrapped cord to the extension cord hook.

Button Sampler Preparation

11. Don nitrile gloves.
12. Remove the red cap from a sterile Button Sampler.

13. Unscrew and remove the inlet of the sampler and remove the PTFE O-ring.
14. Remove one of the conical tubes from the sampling kit and open the cap.
15. Remove the PTFE filter from the conical tube using sterile tweezers and place on top of the support screen inside the sampler.
16. Place the PTFE O-ring on top of the filter, replace inlet, and gently tighten to just past finger tight. Note: overtightening can damage filter.
17. Replace red cap.
18. Attach Tygon tubing onto the outlet of the Button Sampler.
19. Repeat for the second Button sampler.
20. Place capped Button Sampler assemblies with attached tubing into the gallon bag.

Button Sampler Pump Calibration

Each Button Sampler pump comes with a unique ID tag attached to it. In addition, a piece of tape should be attached to each pump with a label Button_A or Button_B for quick identification in the field. The calibration kit includes: an adapter, a Button Sampler assembly containing a PTFE filter, 2 lengths of ¼ in ID Tygon tubing, pump adjustment tool, and a pump calibration unit.

For each pump to be calibrated, record unique ID and Button_A/Button_B label into the field notebook.

9. Connect the pump to be calibrated to the prepared testing Button Sampler assembly with one of the Tygon tubes.
10. Connect the calibration adapter to the Button Sampler assembly.
11. Connect the second length of Tygon tubing to the adapter and to the low pressure/pull side of the calibration unit.
12. Turn on the calibration unit and allow to warm up and set to continuous measurement. If using a rotameter, this step can be skipped.
13. Turn on the pump to be calibrated and observe the flow rate.
14. Adjust the pump until it is 4 ± 0.2 L/min.
15. Record final measured flow rate in field notebook.
16. Repeat for the next pump to be calibrated.

After pumps are calibrated, place the sampling kit and pumps into the clear backpack carried by the sampler. Place all tools necessary for the sampler into the backpack carried by the support person. All team members should be double-gloved before entry.

3. Field Sampling Procedure

3.1 Field Setup

Figure 2 shows the general orientation of the components of a field sampling event. These include the AACeSS sampling cart, the generator if used, and the filter processing table. The orientation of the generator and filter processing table are so that the AACeSS cart is pushed away from them and pulled towards them. The generator should be placed so that the cart can move across the full length and width of the sampling area, for a 20 ft x 20 ft square and a 100 ft extension cord; this is <50 ft away.

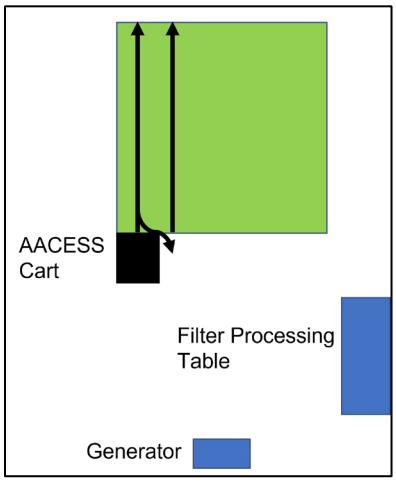


Figure B-3. Field sampling layout and sampling pattern

17. The team members will start with double nitrile gloves for setup.
18. The team members will mark the corners of the sampling area with the white field paint.
19. The team members will set up the equipment as shown in Figure 2. Nitrile gloves, Dispatch wipes and the filter plate cover can be placed on the filter processing table.

Note

The orientation of the equipment will depend on the comfort of the sampling team and the terrain to be sampled. If necessary, the cart may be operated right to left across the sampling area instead of left to right as shown in Figure 2. In which case, the filter processing table should be placed on the opposite side.

Caution

Operating the cart on a slope risks tipping the cart. If the sampling area is on a hill the cart should be located at the bottom of the slope and operated by pushing up the slope and slowly allowing the cart to roll back down. Always maintain two hands on the cart during operation.

20. After setup of equipment, each member of the sampling team will don a new clean pair of gloves.

21. The sample support person will remove the Button Sampler pumps from the sampler backpack and hand the one marked Button_A to the sampler.
22. The team members will attach their respective pumps to their respirator belts.
23. The sampler support person will the remove a Button Sampler assembly with a tube from the sampler backpack, attach the tube to the sampling pump, and attach the Button Sampler to the PPE of the sampler at approximately shoulder height.
24. The support person will remove the second Button Sampler assembly with tube from the sampler backpack, hand it to the sampler, and the sampler will make the tube connection to the pump and PPE of the support person.
25. The sample support person will record the sample ID numbers and location in the sampling log.

Note

Barcoded and automated linking of sampling kit ID and sample location may be utilized instead of manual recording.

3.2 Sampling Procedure

1. If the generator is used the sampler will pull- start the generator.
2. If utilizing a Global Positioning System (GPS) tracker, the sampler will start the tracker.
3. The sampler support person will remove the red caps from the Button Samplers and place into the sampler's backpack.
4. Each team member will turn on their sampling pumps or ask for assistance from the other team member. Team members will check and ensure the internal rotameter bulb is showing flow through the pump of the other team member.
5. The sampling support person will then remove the extension cord from the hook on the cart and unravel the extension cord toward the power source (generator if used) while keeping the cord taut with the cart to minimize ground contact.
6. The sampling support person will plug the extension cord into the power source while retaining tension, then move back toward the cart collecting the cord while moving.
7. The sample support person should be located directly behind the sampler and cart approximately 20 ft away, holding the extension cord at one of the 20-ft markers.
8. Once the sample support person is in place, the sampler will start the NAM, count ~5 seconds for the NAM to fully engage, start the leaf blower, and check the reading on the pressure gauge (should be ~1-2 in H_2O).

Note

If the pressure reading on the gauge is zero, there is a leak or the NAM is not operating correctly. If the pressure is >3.5 in. H_2O, no air is moving through the filters and sampling cannot continue.

9. If the pressure reads in the correct range, the sampler will start the stopwatch and begin sampling in the pattern shown in Figure 2.
10. The sampler will move forward, pushing the cart at a walking rate of 1 step per second at a relaxed step length (~2 ft/s).
11. After 9 seconds, the sampler will begin to observe the sampler support person through the cart rearview mirror for indication of 20 ft of sampling.
12. The sampler support person will allow the extension cord to release while keeping tension to prevent the cord from making contact with the ground. Once the sampler support person sees the next 20-ft mark on the extension cord pass through their hands, they will alert the sampler by 3 quick tugs of the extension cord and waving.
13. The sampler will then pull the cart backward while the support person recollects the extension cord.
14. Once the sampler reaches the back of the sampling area, the sampler will angle the cart to move over the width of the cart (2 ft) and realign for the next pass.
15. The process repeats until the cart has traversed the entire width and length of the sampling area in both push and pull directions or if the pressure measurement of the NAM reaches **3.2 in H_2O,** whichever comes first.
16. The sampler will then stop the leaf blower, followed by stopping the NAM.
17. If the sampling must stop prior to sampling the entire area of interest, estimate the percentage of the area sampled to the nearest quarter and note in the field notebook or electronic record.
18. The sample support person collects the extension cord, unplugs it from the power source, and attaches it the cart extension cord hook all while minimizing the cord contacting ground.
19. The sampler stops the GPS tracker, if utilized.
20. The team members will turn off their Button Sampler pumps.
21. Button Sampler pumps will remain on each team member and follow the team member through the decontamination line.

3.3 Filter Collection Procedure

The filter processing will utilize the filter processing table. The Button Samplers and sampling kit will be confined to one-half of the table, and the filter holder plate will be confined to the other half to minimize cross contamination as shown in Figure 3.

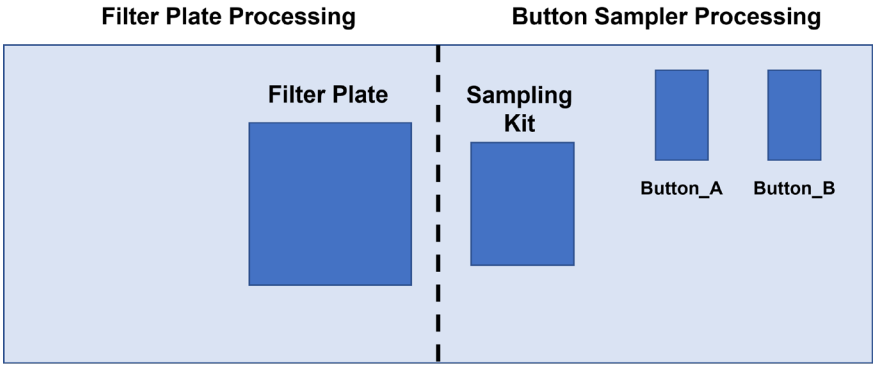


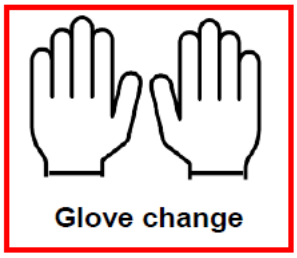
Figure B-4. Filter processing table

1. The support person will retrieve the red caps from the sampler backpack and cover the Button Samplers.
2. The sampler will collect the pliers from the support backpack.
3. The support person will then retrieve the sampling kit from the sampler backpack and take the kit to the filter processing table and lay out each conical tube with a Twirl'em bag on one side as shown in Figure 3.
4. They will then remove their Button Sampler with Tygon tube and lay it next to the conical tube labeled Button_B.
5. The support person will then retrieve the AACeSS filter plate cover and deliver it to the sampler.
6. While the support person is retrieving the filter plate cover, the sampler will remove the large particle separator connections from the NAM and remove the NAM cover to expose the filter plate.
7. The sampler will remove the thumb screws from the filter plate holder loosening each with the pliers first, to prevent tearing of protective gloves.
8. Once the thumb screws are removed, the sampler will place the plate cover on the filter plate and secure it with thumbscrews in the four corners.
9. The support person will then remove the Button Sampler with Tygon tube from the sampler and place it on the processing table next to the conical tube labeled Button_A.
10. The sampler will carry the covered filter plate holder and remaining thumbscrews to the filter processing table and set in on the side opposite of the Button Samplers, as shown in Figure 3.

3.3 Button Sampler Processing

Refer to Figure 5 for an exploded view of the button sampler components.

11. The support person will remove two of the forceps packs from the sampling kit, open the packs, and place one next to each Button Sampler with the opening facing them so that they can remove the forceps.
12. The support person will wipe the area below the Button Samplers with a Dispatch wipe and will loosen the top of the Button Samplers so that they can be lifted off.
13. The support person will don a new pair of nitrile gloves.



14. The support person will collect the conical tube labeled Button_A, remove the cap, set it top down on the Dispatch-wiped area, and hold the conical tube in one hand.

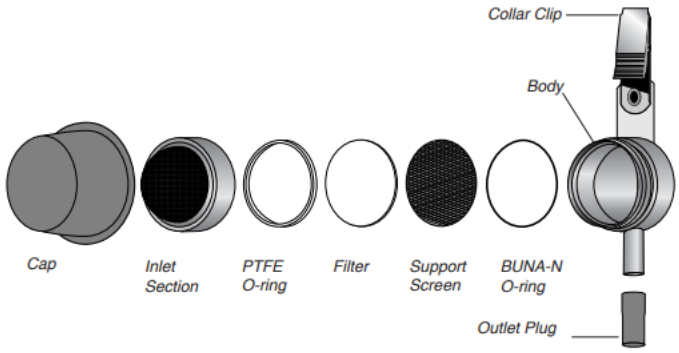


Figure B-5. Button Sampler components

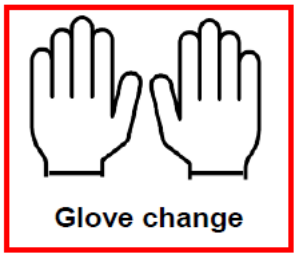
15. With the other hand, the support person will remove the Button Sampler top exposing the PTFE ring/filter and will set the top down next to the Button Sampler.
16. The support person will take the forceps from the package, remove the PTFE retaining ring, set it into the Button Sampler top, remove the PTFE filter from the sampler and place it into the conical tube.
17. Then, the support person will cap the conical tube and place it into a Twirl'em bag.
18. The support person will repeat steps 4-7 for Button_B
19. Once both Button Samplers are processed, the support person wipes both Twirl'em bags with Dispatch wipes and places them in the sampling kit Ziploc bag.
20. The support person then reassembles the Button Samplers and sets them aside for decontamination.

3.4 Large Particle Separator Material Collection

Figure B-6. Grass and debris caught by the separator.

1. **Collection Requires Two Team Members**
2. The sampler will remove the separator from the cart and remove its lid.

3. If no significant material is present loosely in the bottom of the separator, no material collection needs to occur.
4. The team will don new nitrile gloves.



5. The support person will open the stomacher bag labeled ‘Material’.
6. The sampler will assess if the collected material can be poured into the stomacher bag or if, in the case of significant amounts of grass, part of the grass needs to be collected by hand.
7. The sampler will collect any large material by hand as necessary and place it into the opened stomacher bag.
8. The sampler will then lift the separator and begin to tip using the inlet as a pour spout.
9. The support person will place the stomacher bag under the separator inlet to collect the loose material being poured out by the sampler.
10. Once pouring is complete, the support person will close the stomacher bag, wipe the outside with a dispatch wipe, and place the stomacher bag into the sampling kit Ziploc bag.
11. The sampler will reassemble the particle separator and reattach it to the AACeSS cart.

3.5 AACeSS Filter Plate Processing

10. The support person will remove the filter plate cover by unscrewing the 4 corner thumbscrews and setting them with the other thumbscrews.
11. The support person will then remove the filter retaining lid and set it to the side away from the sampling kit.
12. The support person will then don new nitrile gloves.

13. The support person will remove the stomacher bags labeled AACeSS_A and AACeSS_B and the remaining forceps from the sampling kit and lay them next to it.

14. The support person will open the forceps and the stomacher bag labeled AACeSS_A.
15. Using the forceps, the support person will stack 5 filters on top of each other while attempting to retain any loose material sitting on top of the filters.
16. The support person will then use the forceps to place the 5 stacked filters into the AACeSS_A stomacher bag, will squeeze the air out of the stomacher bag to make it as thin as possible and will close the stomacher bag.
17. Using the same forceps, the support person will stack the remaining 5 filters and place them in stomacher bag AACeSS B, will squeeze the air out of the stomacher bag to make it as thin as possible and will close the stomacher bag.
18. Once both stomacher bags are filled with filters and any loose material on the filters, the support person will wipe the stomacher bags with a Dispatch wipe and place them in the sampling kit Ziploc bag.
19. The sampler will then reassemble the filter holder with the thumbscrews and place it with the AACeSS cart.

3.6 Field Exit

10. Once all filter processing and material collection is complete, the support person will wipe the sampling kit Ziploc bag with a Dispatch wipe, will compress the bag to remove as much air as possible, and place it into the sampler backpack.
11. The sampler will collect all AACeSS cart components, extension cord, and filter plate assembly/cover and deliver them to the designated AACeSS decontamination area.
12. The Button Sampler assemblies with Tygon tubing will be placed into the support backpack with any tools that were used.
13. The support person will use a Dispatch wipe to clean the filter processing table for the next sampling event if one will occur.
14. The sampler will wipe the generator pull handle and movement handle with a Dispatch wipe.
15. If no additional AACeSS sampling will occur, the table will be delivered to the AACeSS decontamination area as well as the generator.
16. The sampling team will then exit the field through a decontamination line following standard protocols.
17. After decontamination, the team will collect the Button Sampler pumps and return them to the pump calibration area.
18. Using the flow measuring device utilized for the pump calibration, each pump flow will be measured and recorded in the log book next to its unique ID and pre deployment sampling flow.