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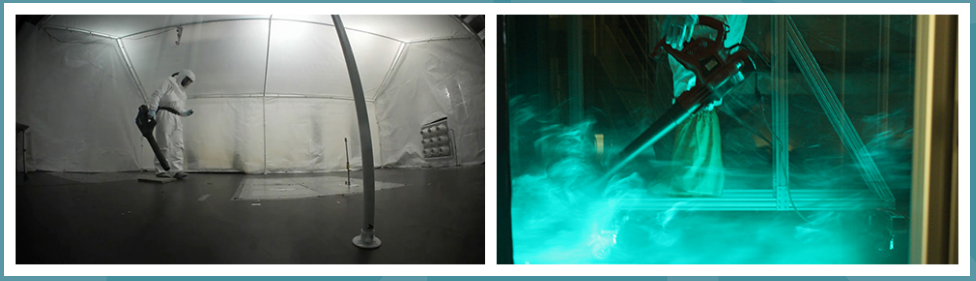
Analysis of Aggressive Air Sampling for *Bacillus anthracis* Indoors



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Analysis of Aggressive Air Sampling for *Bacillus anthracis* Indoors

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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.

Gregory Sayles, Director

Center for Environmental Solutions and Emergency Response

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Acronyms and Abbreviations

ft^2	square foot/feet
ft^3	cubic foot/feet
ft^3/min	cubic foot/feet of air per minute
in	inch(es)
µm	micron(s) or micrometer(s)
AAS	Aggressive Air Sampling
Ba	*Bacillus anthracis*
Be	Beryllium
Bg	*Bacillus atrophaeus subspecies globigii*
BOTE	Bio-response Operational Testing and Evaluation Project
Ca	Calcium
CBRN	Chemical, Biological, Radiological, and Nuclear
CESER	Center for Environmental Solutions and Emergency Response
CFR	Code of Federal Regulations
Cu	Copper
DFU	Dry Filter Unit
DHS	U.S. Department of Homeland Security
DOE	U.S. Department of Energy
EPA	U.S. Environmental Protection Agency
Fe	Iron
FRM	Federal Reference Method
GAO	U.S. Government Accountability Office
HEPA	High Efficiency Particulate Air
HSMMD	Homeland Security and Materials Management Division (EPA)
HSRP	Homeland Security Research Program
LLNL	Lawrence Livermore National Laboratory
MCE	Mixed Cellulose Ester
NAM	Negative Air Machine
NIOSH	National Institute for Occupational Safety and Health

NRC	National Research Council
NRT	National Response Team
OLEM	Office of Land and Emergency Management (EPA)
ORD	Office of Research and Development (EPA)
OTD	Operational Test Demonstration
PAPR	Powered-air Purifying Respirator
PPE	Personal Protective Equipment
PSU	Portable Sampling Unit
RH	Relative Humidity
TSP	Total Suspended Particulates
UTR	Underground Transport Restoration

Executive Summary

This project supports EPA's Homeland Security Research Program (HSRP) and the Center for Environmental Solutions and Emergency Response Homeland and Materials Management Division's strategic goals as described in detail in the Homeland Security Strategic Research Action Plan (US EPA, 2020). This work is pertinent to Long-Term Goal 2, which states, "The Office of Land and Emergency Management (OLEM) and other clients use HSRP products and expertise to improve the capability to respond to terrorist attacks affecting buildings and the outdoor environments."

The primary objectives of this project research effort were to analyze the potential for application of an aggressive air sampling (AAS) technique for measurement of spores resulting from a dissemination of a biological agent, *Bacillus anthracis* (*Ba*), in an indoor environment and provide approaches for application. This goal was accomplished first by a review of lessons learned from published studies on the application of AAS for asbestos clearance sampling, sampling for biological spores or other particulates, and in the post-remediation sampling of facilities contaminated with *Ba*. Secondly, a first principles model was developed for AAS indoors to reveal the critical parameters that determine the efficacy of its application. This model was then used to examine the recovery efficiencies and costs of AAS compared to a standard reference method of microvacuum surface sampling. Finally, optimal conditions for AAS indoors were outlined, and gaps in research inhibiting the implementation of the sampling technique for *Ba* were identified.

Lessons learned from asbestos, beryllium, and spore sampling publications included information regarding site preparation, personal protective equipment, supplies required, implementation, and sample analysis. The derived model for AAS showed that settling can have a significant impact on sampling efficiency, so design of sampling rate versus enclosed volume is paramount, and sampling should be long enough for three times the enclosed volume of air to be pulled through the sampler (three air exchanges). With careful attention to sampling design, sample collection efficiencies of >90% of particles in the air can be achieved. The main factor impacting sample recovery is the ability of the aggressive air technique to resuspend particles from the surfaces.

From a cost perspective, as the area of interest increases to the size of a room or office floor, AAS cost becomes comparable and even less than the cost of microvacuum surface sampling, even if the surface sampling incorporates only 1% of the available surface area. Considering the sample recovery and limit of detection, if the room size is greater than 200 square feet (ft^2) and the resuspension fraction is greater than 0.1%, AAS has an equivalent or better total sample recovery and limit of detection compared to microvacuuming sampling, assuming a uniform dispersal of material in the air. With lessons learned from previous experiments and information gathered from the model and cost analysis, optimal conditions for indoor AAS were determined. These conditions include: 1) areas that can be isolated so that particles are not transported out of the area of interest; 2) areas with minimal dust and dirt; 3) areas of sufficient space for a decontamination line; 4) large enough surface area of interest so that contamination would result in detectable material from AAS; and 5) sufficient access to power outlets for all necessary AAS

equipment.

Research gaps that still need to be filled before a definitive determination of the effectiveness of AAS can be made include a systematic investigation into the range of resuspension factors for spores from indoor surfaces, experiments using a range of deposition techniques and loading scenarios, and experiments in a range of space sizes, layouts, and temperatures/humidities.

1.0 Introduction

An intentional release of a pathogenic biological agent in an urban area may require characterization of contaminated areas as well as decontamination and subsequent clearance of decontaminated areas for reoccupation. The U.S. Environmental Protection Agency (EPA) has the responsibility to remediate such biological contamination to protect human health and the environment. This research supports the mission of the Homeland Security and Materials Management Division (HSMMD) within EPA's Center for Environmental Solutions and Emergency Response (CESER) by providing information pertinent to the characterization and decontamination of areas contaminated through an act of terrorism.

In 2001, letters containing *Bacillus anthracis* (*Ba*) were mailed to various locations throughout the United States, contaminating several buildings and causing anthrax illness and death. Following the U.S. "anthrax" attacks in 2001, the organizations responsible for the response and remediation began to develop methods to sample and treat areas contaminated by *Ba*. The initial and residual contamination from the *Ba* spores was difficult to detect, identify, and decontaminate efficiently and quickly. In addition, the affected parties incurred significant costs to decontaminate buildings and equipment suspected of having been contaminated. Government reports and inquiries organized by the US Government Accountability Office (GAO) indicated that *Ba* sampling and decontamination methods were not standardized or validated and that biological agent location and characterization efforts were deficient (US GAO 2005). Federal agencies made recommendations for standardizing and validating procedures for characterizing biological agent contamination. Further, they made follow-on recommendations for effectively clearing buildings and associated areas by using efficient decontamination measures. Since 2001, significant advances have been made in addressing responses to *Ba* releases.

The vast complexity of both porous and nonporous man-made and natural surfaces has led to a myriad of sampling techniques that had to be developed for effective indoor surface sampling. Nonporous surfaces such as metal and glass were used to develop moist swabbing surface sampling techniques (US CDC 2012). Complex porous surfaces such as concrete, asphalt, carpet, upholstery, and sod have seen either the development of vacuum-based technologies (concrete, asphalt, carpet, and upholstery) or collection of material and liquid extraction (sod, foliage). These techniques collect samples from an area on the order of one square foot (ft^2) that, for a wide area outdoor release, generates an enormous and unfeasible number of probabilistic samples to be collected and analyzed at an exorbitant cost (US EPA 2013a). Analysis of these surface sampling costs has led to investigation of innovative methods to reduce the number of samples and time/manpower investment required for characterization and clearance.

One method that is being explored has historically been part of the indoor asbestos remediation process (US EPA 2013a), namely, aggressive air sampling (AAS). Briefly, the AAS method involves forced aerosolization of particles from a surface using a leaf blower, collection of aerosolized particles by air sampling onto filters, followed by quantitative analysis of collected samples for the target contaminant. To fully realize the potential of AAS as a sampling technique compared to standard surface sampling, it is necessary to fully flesh out the physical parameters

governing the AAS process as well as the costs associated with deployment. Additionally, it is important to research and understand how AAS has been deployed and utilized in the past.

The objectives of the research presented in this report were to:

1. Understand how AAS has historically been used for
   - asbestos clearance sampling
   - sampling for biological spores or other particulates
   - in the post-remediation sampling of facilities contaminated with *Ba.*
2. Develop a model to determine the critical parameters for AAS
3. Analyze and compare the costs associated with surface sampling and AAS
4. Analyze the theoretical particle recoveries associated with surface sampling and AAS
5. Provide approaches for conducting AAS indoors for *Ba*
6. Identify gaps in knowledge regarding AAS for *Ba*.

2.0 Literature Review

An extensive literature search was conducted to gather results from implementation of AAS in research settings or actual clearance sampling for spores, asbestos, or other particulates. The specific focus of the literature review was to collect technical information, operational information and lessons learned to aid in developing guidelines for AAS. This section summarizes the significant findings of the literature review related to AAS, is organized by target particle type, and presents lessons learned from bacterial spore sampling events.

2.1 Asbestos Clearance Sampling

2.1.1 Guidance for Asbestos Clearance Sampling

AAS was developed as a clearance sampling technique following indoor asbestos abatement. Such clearance sampling is mandated following asbestos abatement in schools as specified in Appendix A to Subpart E of 40 Code of Federal Regulations (CFR) Part 763 (USNARA CFR 2003). The regulation and subsequent EPA publications provide general guidance on when and how to conduct AAS for asbestos remediation (US EPA 1985a, US EPA 1985b).

To briefly summarize, the guidance for asbestos clearance sampling includes the following:

- Final clearance sampling should be conducted after primary containment barriers have been removed (but barriers over windows, doors, and air passageways remain), the area is thoroughly dry and has passed visual inspection;
- Negative air filtration units used during asbestos abatement should remain on during AAS;
- Before sampling begins, forced air equipment should be used on all surfaces (floor, walls, ceiling, and other surfaces), taking at least 5 minutes per 1,000 ft^2 floor area;
- Mixing fan(s) should be used after the forced air, pointed toward the ceiling on slow speed, with one 20-inch (in) diameter pedestal fan per 10,000 cubic feet (ft^3) of space;
- Sampling locations should be random to provide unbiased and representative samples;
- Minimum sampling time should be calculated to collect an air volume sufficient to ensure that the minimum quantitation limits for the analysis method are achieved.

The guidance provided requires knowledgeable decision makers to define the parameters for each clearance sampling scenario. These parameters include the sample analysis method; areas for clearance sampling; mixing fan type, number, and locations; sampler type, flow rate, and duration; and sampling locations.

There is a process described by EPA for declaring asbestos abatement complete and releasing the abatement area for use (US EPA 1985a). A flow chart provided in the EPA report "Measuring Airborne Asbestos Following an Abatement Action" is also shown in Figure 1.

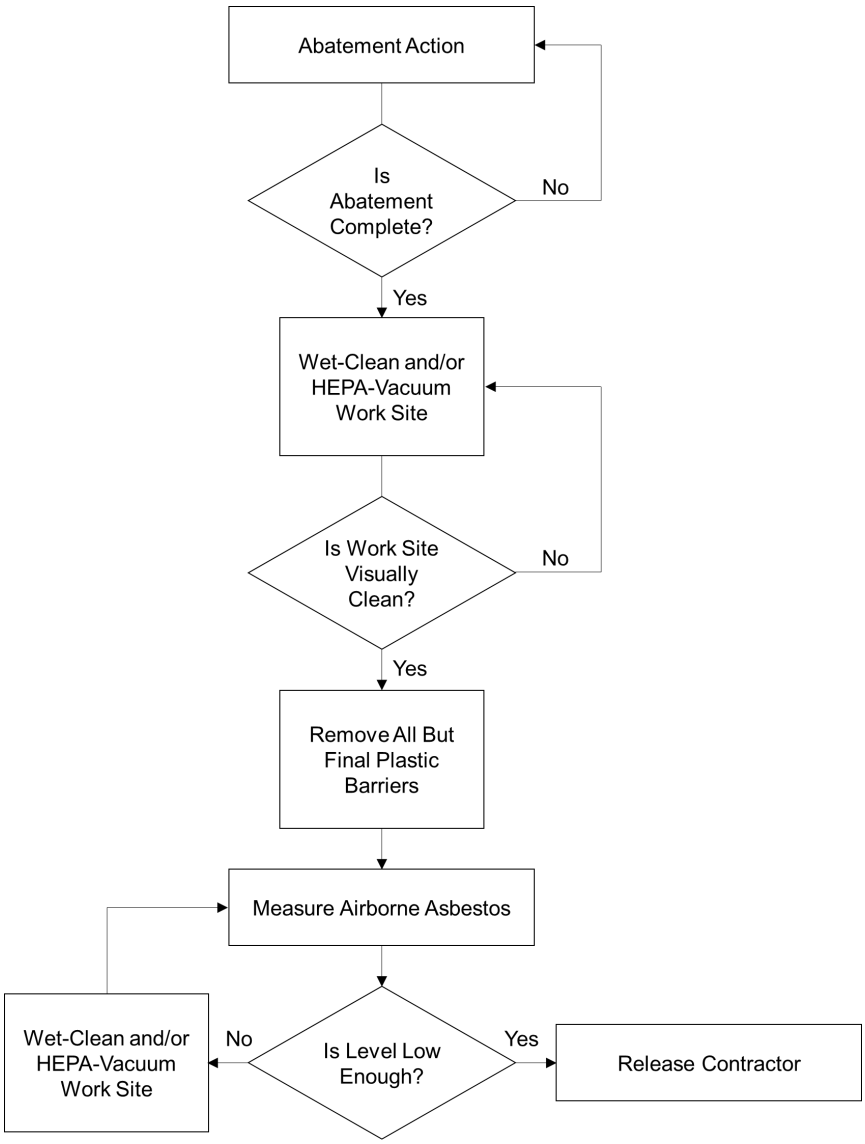

Figure 1: The process for releasing an asbestos abatement contractor (US EPA 1985a)
NOTE: HEPA = High Efficiency Particulate Air

2.1.2 Application of Asbestos Clearance Sampling

The actual field implementation of AAS, however, does not always conform to the guidance. For example, Kominsky et al. (Kominsky 1989) observed final cleaning and clearance sampling at 20 asbestos abatement sites in New Jersey schools. Of those 20 sites, one failed to use AAS, 14 failed to meet the forced air recommendation of at least five minutes per 1000 ft^2, 15 sites failed to use the required number of mixing fans for the room volume (eight of those sites used no mixing fans), and the recommended pedestal-type fans were used at only three sites. While the personnel observed by Kominsky et al. failed to follow many of the recommendations and even some requirements of the EPA regulation (USNARA CFR 2020), other reports of this type were not found in the literature to indicate whether this problem was widespread.

EPA (US EPA 2003) and Tang et al. (Tang 2004) described the cleanup of contamination (including asbestos) and clearance sampling of residences in lower Manhattan following the September 11, 2001, attack on the World Trade Center. In this effort, both AAS and a modified-AAS technique were used in some spaces, where the modified technique did not use a leaf blower prior to sampling but did use mixing fans during sampling. When AAS and modified-AAS were used in the same residence, the modified-AAS was conducted several days after AAS with no cleaning activities between. EPA reported that they "did not find a measurable difference in the use of the modified or aggressive air disturbance technique." However, there were some instances where a residence was not cleared based on the AAS results but was cleared based on the modified-AAS results (US EPA 2003).

2.2 Beryllium (Be) Clearance Sampling

Presentations of the Beryllium Health and Safety Committee at Lawrence Livermore National Laboratory (LLNL 2010, LLNL 2011) described the clearance sampling of a large (approximately 8,500 ft^2 or 187,000 ft^3) facility contaminated with beryllium (Be) following the AAS guidance for asbestos clearance sampling. The area for AAS was one large high-ceiling space with no dividing walls. The setup included first starting 65 filter-based personal air samplers, then 32 mixing fans, and forced air disturbance with 1-horsepower leaf blowers. The first round of AAS was conducted in the facility with visible dust and debris, resulting in the failure of more than half of the air samplers due to filter overloading during the sampling period. The facility was cleaned of visible dust and debris though the cleaning methods were not specified. Then, a second round of AAS was conducted where all 65 air samplers operated for the entire sampling period. Air samples were analyzed for Be, calcium (Ca), copper (Cu), and iron (Fe), and surface wipe sampling was conducted, and the surface wipe samples were analyzed for Be only. The mean air concentration of each analyte in the second round was significantly less than the air concentration measured in the first round, with the unitless ratio of round 1 to round 2 mean concentrations as follows: Be = 7, Ca = 7, Cu = 6, and Fe = 25. Mean wipe sample results were not reported (only minimum and maximum). However, the maximum surface concentration detected in round 1 wipe sampling was nine times the concentration detected in round 2 wipe sampling. The minimum reported for each round was the limit of detection.

The author concluded that:

- Thorough mixing of the large space was achieved;
- AAS could distinguish between contaminated (first round) and clean (second round);
- AAS provided insight on the health protection of surface load limits.

2.3 Spore Sampling

2.3.1 Planning and Guidance Documents

Organizations and government agencies such as the U.S. Department of Homeland Security (DHS), U.S. Department of Energy (DOE), the National Research Council, the National

Response Team (NRT; comprised of representatives from 16 federal agencies), and local municipalities have developed documents to guide remediation of a potential biological contamination (NRT 2005, Kirvel 2010, Carlsen et al. 2012, NRC 2005, Raber et al. 2011). While some of the publications provided more detailed clearance sampling guidance than others, the guidance presented in these documents is generally consistent. In these documents, AAS was recommended for indoor clearance sampling with a clearance goal of no spore growth on all post-remediation samples.

The NRT (NRT 2005) recommended AAS for post-decontamination sampling, modeled on the EPA guidance for clearing facilities for re-occupancy following asbestos abatement. The NRT guidance stressed the importance of consulting with the analytical laboratory in development of the sampling plan to determine the capabilities and analytical processes (NRT 2005). The Seattle Urban Area Consequence Management Guidance (Kirvel 2010) recommended AAS supplemented by surface samples for indoor clearance sampling.

Summary of AAS Implementation Guidance

Guidance for response and remediation planning includes establishing an independent group of subject-matter experts (such as a Technical Working Group) to provide advice and recommendations, analyze processes and data, and make a final recommendation on whether the facility should be reopened (Carlsen 2012). These experts can be consulted on any number of issues related to the decontamination and clearance effort, including the sampling protocol.

The DHS-funded report prepared by Lawrence Livermore National Laboratories (LLNL) (Carlsen 2012) states that "NIOSH [National Institute for Occupational Safety and Health] has determined that a minimum of two room volumes of air should be collected in a given room to maximize the likelihood of capturing contamination in the samplers." However, a reference is not given for that statement. The provided recommendations related to AAS include (Carlsen 2012):

- Isolate and seal areas in the same manner as for prevention of fumigant release;
- Place air samplers at various heights and spaced closely enough so that particles are likely to encounter a sampler (different from asbestos AAS guidance);
- Use personal samplers for workers conducting AAS;
- Use fans to create turbulence;
- Use "hand-held fans and blow air across all accessible surfaces while the air samplers operate";
- Address the use of negative-air machine (NAM) on a case-by-case basis (different from asbestos AAS guidance).

The National Research Council (NRC 2005) provided examples of high flow rate air samplers that could be used for AAS indoors, including SpinCon (InnovaPrep LLC, Drexel, MO), Universal Air Sampler (Applied Physics, Inc., Monte Vista, CO), and Dry-Filter Unit (DFU; Lockheed Martin Integrated Technology LLC, Gaithersburg, MD) and examples of size-selective samplers [Graseby-Anderson Mark III Cascade Impactor (Andersen Instruments, Smyrna, GA)

and Sioutas Cascade Impactor (SKC, Inc., Eighty Four, PA). NRT (NRT 2005)] and recommends using an air sampling method that "maximizes the likelihood of detecting contamination" and presents a list of sampling methods for *Ba* with advantages, disadvantages, and recommendations for the best application of the method. Relevant air sampling methods are described, and the best applications relevant to AAS include (NRT 2005):

- Gelatin filter (low volume) – personal sampling for workers conducting AAS;
- Single stage impactor with agar plate – post-decontamination sampling in small-volume areas (requires many samples);
- Dry filter unit (DFU) – post-decontamination sampling as a supplement to surface samples.

Although NRT (NRT 2005) recommended using gelatin filters for personal sampling, the effective use of gelatin filters is restricted to short sampling periods, and polycarbonate filters are recommended for personal bioaerosol samplers (Wang et al. 2015). Gelatin filters dry out at high temperature, low humidity, and with increased sampling time and flow rate, which leads to lower detected concentrations.

2.3.2 Surrogate Studies

Research studies are routinely conducted using non-pathogenic surrogates for *Ba* to allow researchers to better understand the behavior, fate, and transport of spores. One such study, the Bio-response Operational Testing and Evaluation (BOTE) project, was a multi-agency effort to evaluate biological incident response (US EPA 2013a) using *Bacillus atrophaeus* subspecies *globigii* (*Bg*) spores. As part of BOTE, post-decontamination AAS was conducted in one room of a building that had been contaminated with *Bg* spores and subsequently decontaminated. AAS sampling and operating conditions were as follows:

- Air samplers: XMX/2L-MIL (Dycor Technologies Ltd., Edmonton, AB, Canada) and Mattson-Garvin slit-to-agar samplers (Barramundi Corp., Homosassa Springs, FL);
- Three-hour sampling duration with collection media changed every hour;
- Total sampled volume approximately three times the room volume;
- One mixing fan in the center of the room, directed toward the ceiling;
- Leaf blower operation: ≥ 45° from surface, as close to surface as possible, sweeping side-to-side, agitated all horizontal surfaces at least 15 minutes, agitated all vertical surfaces and ceiling at least 5 minutes (20 minutes total for 210 ft^2 room);
- Used iPads as electronic field notebooks inside sealed Ziplock bags (to allow decontamination).

The room where AAS was conducted was sealed with plastic sheeting but was not kept under negative pressure because the NAM flow rate was considered too high for ventilating a small room. Therefore, areas outside the containment were contaminated during AAS.

AAS studies conducted by EPA under controlled laboratory conditions (US EPA 2013b) using a high-volume air sampler (FRM PM-10, Thermo Fisher Scientific, Inc., Pittsburgh, PA) showed that one air change in the test room was sufficient to collect 95% of the total spores collected

during AAS, as the second and third air change samples contained close to background levels. These tests were conducted with *Bg* spores as the surrogate for *Ba*, and the leaf blower operation was very similar to the BOTE study.

In September 2016, EPA conducted a field evaluation of composite sampling methods during the Operational Technology Demonstration (OTD), part of the DHS-funded Underground Transport Restoration (UTR) program. This post-decontamination AAS exercise (US EPA 2017) was conducted in a mock subway tunnel at Fort A.P. Hill in Bowling Green, VA, that had been contaminated with *Bg* and subsequently decontaminated. Air sampling was conducted using DFU samplers, Bioaerosol Button personal samplers (SKC, Inc., Eighty-Four, PA), and a household-style furnace filter (Filtrete™ MPR 2800, 3M, St. Paul, MN) installed as a prefilter on the NAM inlet for particle capture. The tunnel where the sampling exercise was conducted contained a considerable amount of dirt and debris, which created problems with overloading of the DFU filters, resulting in extreme drops in flow rate during AAS. The NAM prefilter was evaluated for high-flow rate capture of particles. However, this method did not work in the field due to duct collapse between the NAM unit (located outside the containment area) and the rigid ductwork connected to the prefilter.

2.3.3 Bacillus anthracis Sampling

Remediation and clearance of buildings that were contaminated with *Ba* following the 2001 anthrax attacks was a process that evolved quickly as federal agencies worked to determine the source and extent of contamination.

The American Media, Inc., building in Boca Raton, FL, was confirmed to be contaminated with *Ba* via surface sampling and was fumigated with chlorine dioxide gas (Lippy 2016). AAS was implemented as part of the clearance sampling protocol with target volume sampled three times the building volume (three air exchanges) and target clearance level of no culturable spores. DFUs were used and found to be durable and flexible: able to sample both in a room and above a drop ceiling. TSP high-volume air samplers (Tisch Environmental, Inc., Cleves, OH) were also used and had the benefit of higher sampling flow rate compared to the DFU samplers.

2.4 Lessons Learned

The primary sources for lessons learned from field exercises of AAS for surrogate spores are the BOTE project (US EPA 2013a) and the EPA subway tunnel sampling exercise (US EPA 2017). Lessons learned from clearance sampling of the sites that had been contaminated with *Ba* in 2001 provide valuable insight into the actual problems encountered in this situation. The lessons learned found during the literature review relevant to AAS for *Ba* or surrogate spores are summarized in Table 1.

Table 1. Summary of Lessons Learned from Spore Sampling

Category	Recommendation	Reference
Site preparation	Perform AAS only after surface samples are negative.	Lippy 2016
	Seal off area and maintain under negative pressure.	Wang et al 2015, US EPA 2013b, Lippy 2016
	If possible, clean area of excess dust and debris prior to beginning AAS to prevent overloading of filters or develop multistage filtration.	Wang et al 2015, US EPA 2013b
	Dirty spaces can also contain other biological background contamination that can interfere with bacterial culture analyses, particularly if soil is present.	EPA 2013b
	Sample outside containment area to verify that contamination is not spread by AAS operations.	Wang et al 2015
	Use multiple types of air samplers if at all possible.	EPA 2013b, Lippy 2016
Personal protective equipment (PPE)	Powered-air purifying respirators (PAPRs) are easier on operators in the containment area than standard full-face respirators.	US EPA 2013b
	Glove change-out needs careful attention to prevent cross-contamination.	US EPA 2013b
	Anticipate environmental conditions and plan to keep operators cool in PPE.	US EPA 2013b
	Hearing protection is needed by everyone in the containment area while leaf blowers are operated.	US EPA 2013b
Supplies	Have charged backup batteries ready for all battery-powered equipment.	US EPA 2013b
	Carefully pack and label all supplies in clear containers.	US EPA 2013b
	Use electronic tablets sealed in clear plastic bags for data entry and easy decontamination.	Wang et al 2015, US EPA 2013b
	If electronic tablets are not used, use laminated sheets and permanent pen to record data. Take digital photographs of data sheets for backup prior to decontamination.	US EPA 2013b
	Provide shoulder straps for the leaf blowers for ease of operation.	US EPA 2013b
	If NAM is used as a sampling device, place inside hot zone and use only non-collapsible duct on NAM inlet.	US EPA 2013b
	DFU samplers have proven useful, flexible, and durable.	Hinds and Lambert 2011
	Prepare and pre-label sampling kits.	Wang et al 2015, US EPA 2013b
	For sample storage, have refrigeration or coolers with temperature data loggers to verify storage conditions.	US EPA 2013b
Implementation	Cord management needs to be planned so that no one trips on power cords, particularly leaf blowers.	US EPA 2013b
	Have a timekeeper or visible timer to help leaf blower operators manage time.	US EPA 2013b
	If the area is visibly dusty during AAS, the sampler flowrates will need to be checked frequently, and filters may need to be changed.	US EPA 2013b
	Plan breaks for operators and provide medical monitoring.	US EPA 2013b
Analysis	Analyze 100% of sample collected to maximize sensitivity.	Lippy 2016
	Use neutralizing agent (for decontamination agent) before analyses.	Lippy 2016

3.0 AAS Model

A theoretical mechanistic model was developed to determine the critical parameters necessary to design and implement an AAS system for an indoor environment. The model was used to determine sampling duration, amount of material collected, and the amount of material lost to the AAS system. This, in turn, was used to establish a cost and sampling efficiency analysis for using a large area AAS sampling system compared to surface vacuum sampling methods for a spore-contaminated environment indoors. However, the cost and sampling efficiency analysis could be used to establish when AAS is useful in any enclosed area sampling scenario both indoors and outdoors. All components used in the equations of the model are given in Table 2 with their description and dimensions.

Table 2. Model Components

Unit	Description	Dimension
$C(d)$	instantaneous concentration of particles in volume per diameter	number/length^3
$C_a(d)$	concentration of particles entering volume from outside per diameter	number/length^3
$C_{AAS}(d_i, t_{AAS})$	concentration of particles in volume at the end of AAS	number/length^3
C_0	initial particle concentration in volume of air	number/length^3
$\delta C(d)$	small change in particle concentration per diameter	number/length^3
δt	small change in time	time
d	particle diameter	length
$L(d)$	flow equivalent particle settling per diameter	length^3/time
Q	rate of air entering and exiting volume	length^3/time
$S_{AAS}(d)$	rate of particles entering volume of air due to AAS per diameter	number/time
t	absolute time	time
t_0	time at the beginning of AAS	time
t_{AAS}	time at the end of AAS	time
T_{AAS}	total particles collected during AAS	number
t_{final}	time at the end of sampling	time
$T_{sampling}$	total particles collected after AAS	number
V	volume	length^3
$v(d)$	Stokes' particle settling velocity per diameter	length/time

The model development began with a basic setup of an enclosed space of volume V that has a filtered exhaust system for sampling particulate material and a duct to allow air from outside the enclosed system to enter the volume. This setup is different from those implemented in the past as the exhaust system here is used for negative pressure and sampling instead of a separate sampler and a negative air machine (US EPA 2013a), simplifying the calculation without loss of information. Particle matter is present in the volume and may be either an aerosol or deposited on surfaces inside the volume and resuspended. This first equation summarizes the change of material in the air of a given room volume.

$$\boldsymbol{V\,\delta C(d) = QC_a(d)\,\delta t + S_{\mathrm{AAS}}(d)\,\delta t - \big(Q + L(d)\big)C(d)\,\delta t} \tag{1}$$

The left side of Equation 1 is the volume V (length^3) times the change in aerosol particle number concentration as a function of particle diameter d (length), $\delta C(d)$ (number/length^3). The left side of the equation defines the instantaneous change in number of particles in the air volume at a given time. The right side of Equation 1 is comprised of the multiple ways in which particles will enter or leave the volume given an isobaric exchange of air. The first term $QC_a(d)\,\delta t$ represents infiltration of material from the air entering the chamber to maintain a constant pressure, where Q is the rate of air entering the space (length^3/time) and is equivalent to the rate of air exiting the space (isobaric exchange), $C_a(d)$ is the particle concentration (number/volume) as a function of particle diameter of the air entering the space, and δt is a very short change in time. The second term on the right $S_{\mathrm{AAS}}(d)\,\delta t$ represents the emission of particles from the surfaces and in this case, it is specified as resuspension of particles from the AAS procedure. $S_{\mathrm{AAS}}(d)$ is the rate of particles entering the air (number/time) from AAS. This factor is treated as a constant in time in this model and is dependent on the AAS surface agitation rate (length^2/time), particle density on the sampling surface (number/length^2), and the resuspension fraction of particles as a function of diameter. In a real-life scenario, the surface particle concentration may not be constant along the area, the person conducting AAS may not cover the area of interest at a constant rate, and if the surface materials are heterogeneous across the floor of the enclosed space, the resuspension fraction may have a dimensional dependence. Therefore, particles may not be emitted from the surface at a constant rate. The final term on the right side of the equation $(Q + L(d))C(d)\,\delta t$ describes particle removal from the air by two mechanisms, exfiltration (exhaust) and settling. Here, Q (length^3/time) is the rate of air exiting the space which is equivalent to the rate of air entering the space as stated previously, $L(d)$ (length^3/time) is the airflow equivalent rate at which particles settle out of the air as a function of diameter, $C(d)$ (number/length^3) is the instantaneous concentration of particles in the air as a function of diameter, and δt is as described previously. This assumes that there is only one unit filtering the air in the volume such as a negative air machine (NAM). If, however, a sampler is deployed with the NAM or an array of samplers is deployed, additional terms would be added to the parentheses to reflect the air filtration rate of each unit.

The airflow equivalent particle settling rate $L(d)$ is derived by multiplying the Stokes' settling velocity of each particle as a function of diameter $v(d)$ (length/time) by the area of the floor of the enclosed space, A (length^2) to obtain an effective volumetric flow rate with dimensions identical to Q (length^3/time). This expression is not exact but is an approximation as the current

derivation assumes perfect mixing at all times (i.e., particle concentration ***C(d)*** is uniform across the volume, and settling necessarily gives a concentration gradient with a lower concentration at the top of the volume as particles fall to the floor). Further investigation regarding the role of settling and wall loss of particles would require numerical simulations. However, this estimation will provide an approximation of the relative magnitudes of ***Q*** and ***L(d)*** and at what point one is the dominant process over the other. If the volume does involve constant mixing, ***L(d)*** becomes zero and can be eliminated from the equation, assuming mixing occurs at a velocity much greater than the particle Stokes' settling velocity.

Using standard algebraic rearrangement and integration over time, we arrive at the following equation (Equation 2) for air particle concentration as a function of time and diameter.

$$\boldsymbol{C(d,t) = \frac{S_{AAS}(d)+Q\,C_a}{L(d)+Q}\left(1 - e^{-\frac{L(d)+Q}{V}(t-t_0)}\right) + C_0 e^{-\frac{L(d)+Q}{V}(t-t_0)}} \tag{2}$$

During implementation of the AAS method, there are two distinct phases of the AAS technique. The first phase consists of sampling while conducting the actual aggressive air resuspension activity (e.g., leaf blowing), and the second is the air clearance phase where no resuspension activity is occurring. During AAS, the air is being sampled while particles are being resuspended and $\boldsymbol{S_{AAS}(d)} > 0$. The particles are continually being mixed by air disturbance from AAS so $\boldsymbol{L(d)} = 0$, we assume no particles are infiltrating the volume from outside $\boldsymbol{C_a} = 0$, and the initial air concentration of particles is zero $\boldsymbol{C_0} = 0$. In the second phase, no particles are being resuspended and $\boldsymbol{S_{AAS}(d)} = 0$. There is no active mixing so particles can settle $\boldsymbol{L(d)} > 0$, and the initial concentration C_0 is equal to the particle concentration at the end of AAS.

This leads to two equations for particle concentration that can be multiplied by the sampling rate ***Q*** (or if multiple samplers are used, the individual sampler flow rate) and integrated once more to find the total number of particles collected as a function of particle diameter during AAS ($\mathbf{T_{AAS}}$) and after AAS ($\mathbf{T}_{\text{sampling}}$). These two equations are summed over all relevant particle sizes to give the total number of particles collected over all diameters.

$$\mathbf{T_{AAS}} = \sum_{i=1}^{\infty} \mathbf{S_{AAS}}(d_i)\ \mathbf{t_{AAS}} - \frac{\mathbf{S_{AAS}}(d_i)\,\mathbf{V}}{\mathbf{Q}}\left(1 - e^{-\frac{Q}{V}(t_{AAS})}\right) \tag{3}$$

and

$$\mathbf{T_{sampling}} = \sum_{i=1}^{\infty} \frac{V\,Q}{L(d_i)+Q}\, C_{AAS}(d_i, t_{AAS})\left(1 - e^{-\frac{L(d_i)+Q}{V}(t_{final}-t_{AAS})}\right) \tag{4}$$

In the second equation $\mathbf{C_{AAS}}\,(\boldsymbol{d_i}, \mathbf{t_{AAS}})$ (number/volume) represents the particle concentration at the end of AAS, $\mathbf{t_{final}}$ is the absolute time from $\boldsymbol{t_0} = 0$ until the end of sampling and $\mathbf{t}_{AAS}$ is the absolute time from $\mathbf{t_0} = 0$ to the end of AAS with $\mathbf{t_{final}}$ always greater than or equal to $\mathbf{t_{AAS}}$. The index **i** in Equations 4 and 5 above denotes a discrete particle diameter. The full derivation of the above equations can be found in an attached PDF entitled "Derivation of Equations Governing Aggressive Air Sampling (AAS) in a Volume." The equation for $\mathbf{T_{sampling}}$ shows a very

important relationship between the effective settling flow rate *L* and the air removal rate, **Q**. These parameters essentially act in parallel to remove particles from the air. However, as *L* increases, the number of particles collected on a sampler decreases. It is then necessary to examine the effective settling flow rates to determine when ***L*** becomes a significant fraction of ***Q*** for a particular AAS configuration. As an example, Figure 1 shows the ratio of the effective settling flow rate of particles ranging from 1 to 10 microns (µm) in aerodynamic diameter to that of a negative air machine pulling at 500 cubic feet per minute (ft^3/min) in enclosed spaces with between 100 ft^2 and 1000 ft^2 of floor space.

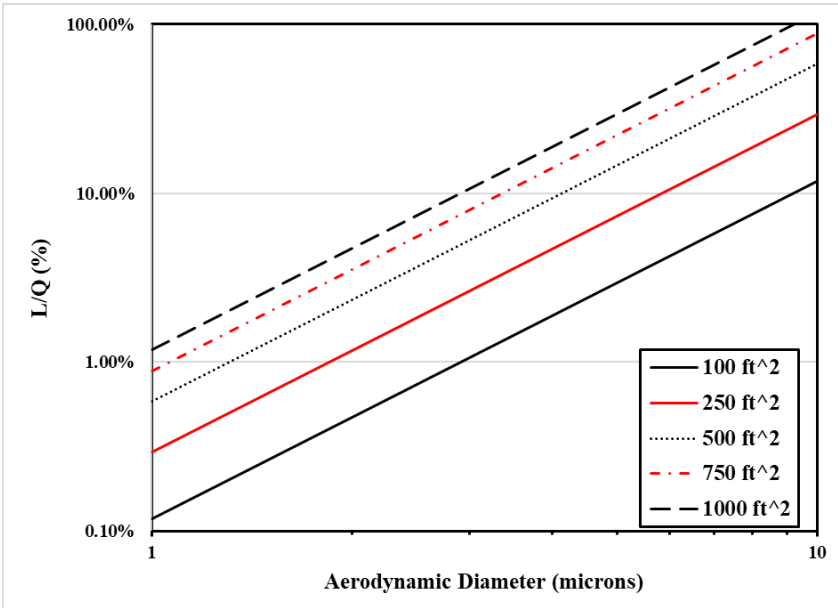

Figure 2: L/Q versus Aerodynamic Diameter

Figure 2 shows that for a 1000 ft^2 floor space, 3-µm particles are settling at 10% of the rate they are being removed through the negative air machine. However, for a smaller (200 ft^2) floor space, 6-µm particles and below settle at less than 10% of their removal rate through the NAM. As stated above, these rates are a function of aerodynamic diameter or, in simpler terms, particles with the density of water. If, however, particles are of higher density than water (such as silica), the settling rate will be more than twice as fast and 3-µm diameter particles will settle at 20% the rate of removal for a 1000 ft^2 floor space. Figure 3 shows the effect particle settling has on the percent of resuspended particles collected over the entire AAS process connecting equations 3 and 4. This figure varies the ratio of L to Q and is plotted as function of air exchanges t * Q/V to eliminate the need to specify a particular volume and air sampling rate. One air exchange, t = V/Q, defines the amount of time required to sample a volume of air equivalent to the volume of the enclosed space. As the figure shows, when L is 10% of Q, the number of available particles collected at three air exchanges is 88% and when L is 50% of Q, 73% of the available particles are collected with a maximum of approximately 75% collected when t = infinity. Therefore, active mixing should be implemented if the AAS parameters during a specific implementation suggest that L approaches 10% of Q.

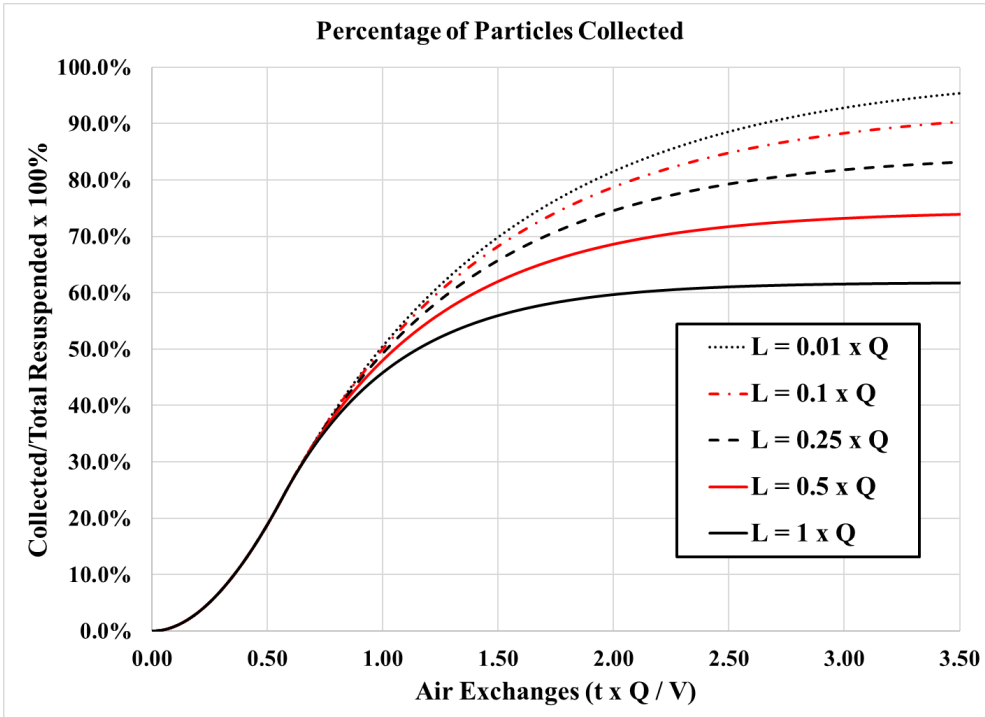

Figure 3: Collection Percentage as a Function of Air Exchanges

Another critical piece of information that can be determined from the above equations is sampling duration required to clear the volume of the particles resuspended from AAS. Equation 5 (below) gives the particle concentration in the volume as a function of time after AAS.

$$\mathbf{C_{sampling}(d,t) = C_{AAS}(d,t_{AAS})e^{-\frac{L(d)+Q}{V}(t-t_{AAS})}} \quad \textbf{(5)}$$

If we divide both sides of the equation by the initial concentration of particles in the air after AAS (C_{AAS}), we find that the concentration ratio falls as a function of $e^{-1/A * t}$ where $A = V/(L(d) + Q)$. A is therefore the amount of time to sample a volume of air equivalent to the volume of enclosed space with particle settling included as a way of removing particles (i.e., one air exchange). Figure 4 shows a plot of concentration as a function of t/A. The particle concentration falls to 10% of its initial value when the amount of time after AAS is 2.3 air exchanges. For example, a 2000 ft^3 enclosed space with an air flow rate (Q) of 400 ft^3/min and minimal particle settling L<<Q requires 5 minutes to exchange the air one time and would require 11.5 minutes of sampling time to reduce the air concentration to 10% of the initial value. When t = 3A, the particle concentration is 5% of the initial value, and an increase in sampling time beyond that leads to a relatively small reduction in particle air concentration that would not contribute significantly to the overall sampled particle count.

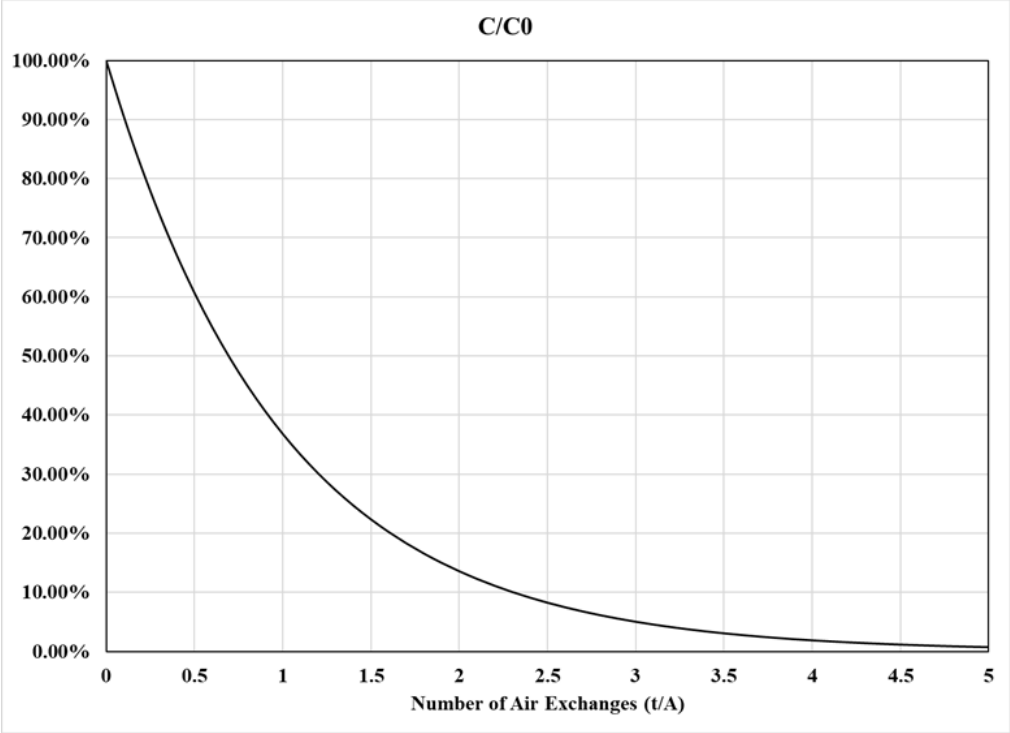

Figure 4: Concentration Decay as a Function of Air Exchanges

3.1 AAS versus Microvacuum Cost Comparison

Costs associated with 37-mm cassette microvacuum sampling a given area for *Ba* contamination were calculated based on surface sampling costs derived by the Bio-response Operational Testing and Evaluation (BOTE) Project (US EPA 2013a). Though 37-mm cassette filters specifically were not used in the BOTE Project for surface sampling, they were used inside air collection systems. Therefore, material costs could be derived. Field personnel and analysis cost would be similar for all surface sampling techniques. Each cost (based on the year 2010) associated with field personnel hours for each sample based on a variety of expertise and a three-person team ($420/hour/team), analysis materials cost per sample ($288), vacuum materials cost per sample ($29), and analysis team labor cost per sample ($155) were derived directly from field experience during a simulated release of biological agents and implementation of vacuum surface sampling methods. In addition, personnel at the BOTE Project conducted a brief analysis of AAS deployment for clearance sampling and determined certain specific costs associated with AAS deployment. Laboratory processing costs for vacuum surface sampling methods and AAS are identical as filter extraction is a similar process. However, AAS teams are comprised of more specialized workers, and the team costs were determined to be $454/hour. The material costs for AAS can vary and depend on the sampler used. If a NAM is used for sampling, the base cost of an Omni 2200c NAM is approximately $1500 (www.grainger.com). If the internal HEPA filter of the NAM is used for sampling, no additional cost of modification of the NAM to hold a sampling filter would be required. Entry and exit costs, prep time, and personnel

decontamination time are not figured into the cost comparison here as they are identical for either sampling method.

Microvacuuming durations per sample were calculated from recommended vacuum sampling time and area (1 ft^2 in 5 minutes). AAS sampling time was dependent on the volume of the space (floor area x 10-ft height) and the NAM sampling rate (500 ft^3/min) to provide three air exchanges, and the AAS area sampling rate of 100 ft^2/min (5). The AAS sampling time can vary from seven total minutes for a 100-ft^2 floor space (1-minute AAS + 7 minutes air sampling) to 70 total minutes (10 minutes AAS + 60 minutes air sampling) for 1000-ft^2 floor area. The number of samples generated by vacuuming is dependent on the percentage of the space sampled and was varied between 100% and 1% for the cost analysis. The number of AAS samples was one since a single NAM can be used to sample all spaces in containment. Figure 5 shows the results of the cost analysis for sampling various sized spaces with a 37-mm cassette microvacuum sample (A Vac-U-Go Pump SKC, Int.: part# 228-9605 and a 37-mm mixed cellulose ester (MCE) cassette filter) and AAS.

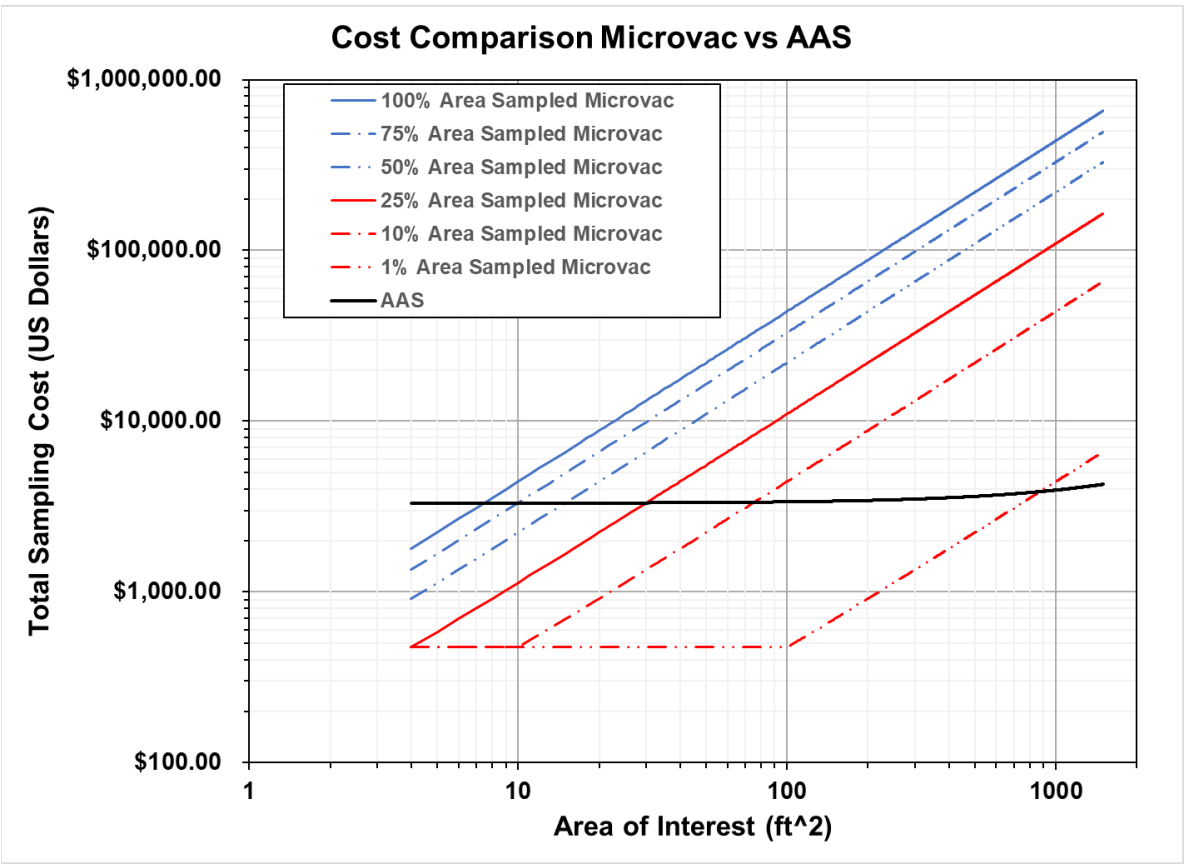

Figure 5: Cost Comparison of Microvacuum Sampling versus AAS

As Figure 5 shows, the cost of conducting AAS indoors is relatively constant compared to surface sampling, and the cost of vacuum sampling begins below the cost of AAS for small areas of interest because AAS requires a significant startup cost due to the NAM. However, as the area

of interest approaches the size of a single room (100 ft^2), total costs of microvacuuming, except 1% sampling area, are greater than the total costs of AAS. The increase in cost of vacuuming is because, as the area of interest goes up, the number of probabilistic samples increases, as does the time required to sample. However, for indoor deployment of AAS, the number of samples remains fixed, and only the required sampling time changes. As you approach the square footage of a small home (1000 ft^2), however, AAS becomes increasingly comparable to microvacuum sampling for 1% of the area sampled. The flat portion of the vacuum sampling cost curves is based on the percentage of the area sampled being lower than the standard sample size of 1 ft^2, and the minimum number of samples is one. We can conclude here that AAS could be significantly cost-beneficial, approximately an order of magnitude cheaper, when sampling large surface areas as compared to microvacuum surface sampling methods. As an addition to the cost, the time for sampling becomes significant for microvacuuming over large areas. At 10% sampling of a 1000 ft^2 area, the total man hours involved in collecting samples only would be ~8.3 hours compared to 1.2 for AAS, making sampling of large spaces impractical for microvacuuming.

4.0 Sampling and Collection Efficiency

The collection efficiency of AAS is determined by four main parameters: the settling of material after it is resuspended (L), the speed with which material is collected on the sampling filter (Q), the enclosed volume of the space (V), and the amount of material ejected into the volume (S_{AAS}) * t (the resuspension rate x time). The L, Q, and V values are determined by the specific geometries associated with the design of AAS deployment containment and the size distribution of material to be sampled. The resuspension rate itself is defined by the particle contaminant level (# of particles/ft^2), the AAS surface sampling rate (ft^2/min), and the fraction of particles resuspended from the surface or resuspension fraction. It is this last number that is the defining portion of AAS efficiency calculations. All other parameters can be changed by experimental design or are part of the primary purpose of the sampling itself, i.e., calculation of particle contaminant level. Experiments with microvacuum sampling discussed in the previous section have determined that for an array of porous materials, the recovery efficiency is on the order of 25% (Calfee et al. 2013). As an example, for every 100 particles in a one square foot sampling area, 25 particles are collected, extracted, and counted from the microvacuum filter material, giving a baseline for comparison with some theoretical AAS collection scenarios and determining the limits of where AAS would be less efficient than a single microvacuum sample. This effort contrasted with the cost estimates derived in the previous section will provide tools to help decision makers implement the most effective means for sampling. The basic parameters for the AAS collection estimation are a ceiling height of 10 ft, a sampling rate of 500 ft^3/min, a 2-µm aerodynamic diameter particle, an AAS blowing flow rate of 100 ft^2/min, and an assumption of evenly distributed particles across the surface area.

Figure 6 shows the number of particles collected by a single AAS sample collection and a single microvacuum sample as a function of surface concentration, when the theoretical resuspension fraction is 1.0% for four different surface areas covered by AAS (100 ft^2, 200 ft^2, 300 ft^2, and 400 ft^2) and a single microvacuum sample collected over 1 ft^2. As the figure shows, AAS collects more particles than microvacuuming for every surface area and has a lower surface area coverage limit of detection as it encounters significantly more particles.

Figure 6: Particle Collection at 1.0% Resuspension Fraction

Figure 7 shows the AAS sample collection if the resuspension fraction is 0.1%, which is a low estimate of actual resuspension fractions reported in the literature and determined empirically by EPA. As the figure shows, the 25% collection efficiency of the microvacuum becomes nearly identical to the collection efficiency of AAS at 200 ft^2 and 300 ft^2 sampling areas and within a factor of two of the 400 ft^2 sampling area. Therefore, in this instance, deployment decisions could be based strictly on cost and areas of interest.

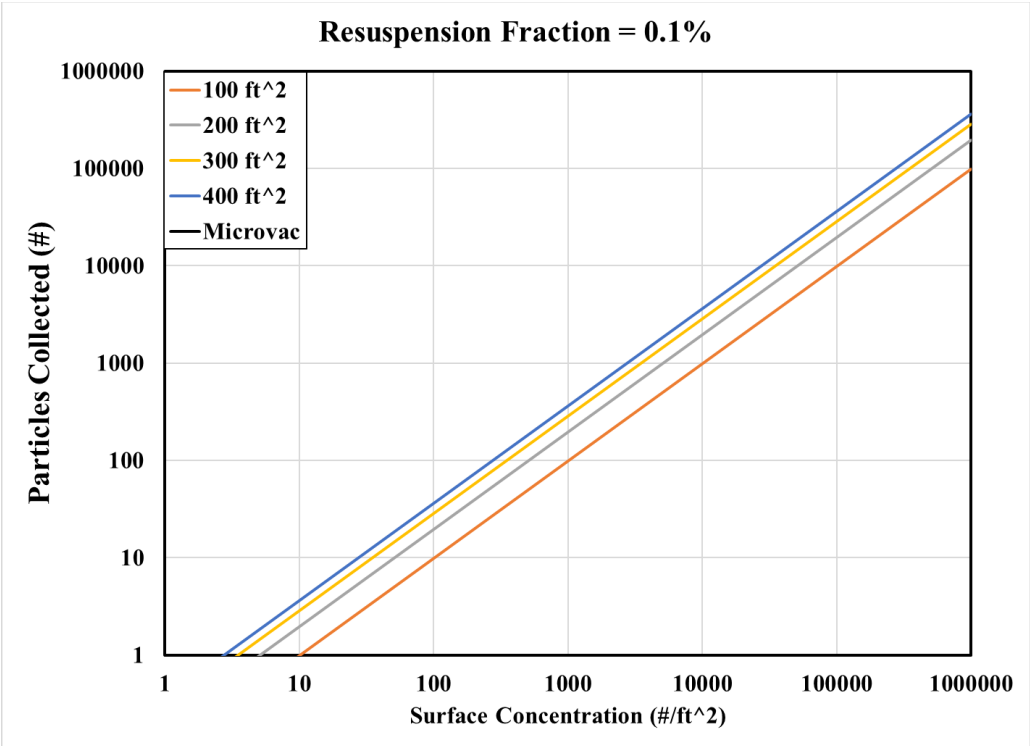

Figure 7: Particle Collection at 0.1% Resuspension

Figures 8a and 8b show the collection comparison of both 0.01% and 0.001% resuspension fractions.

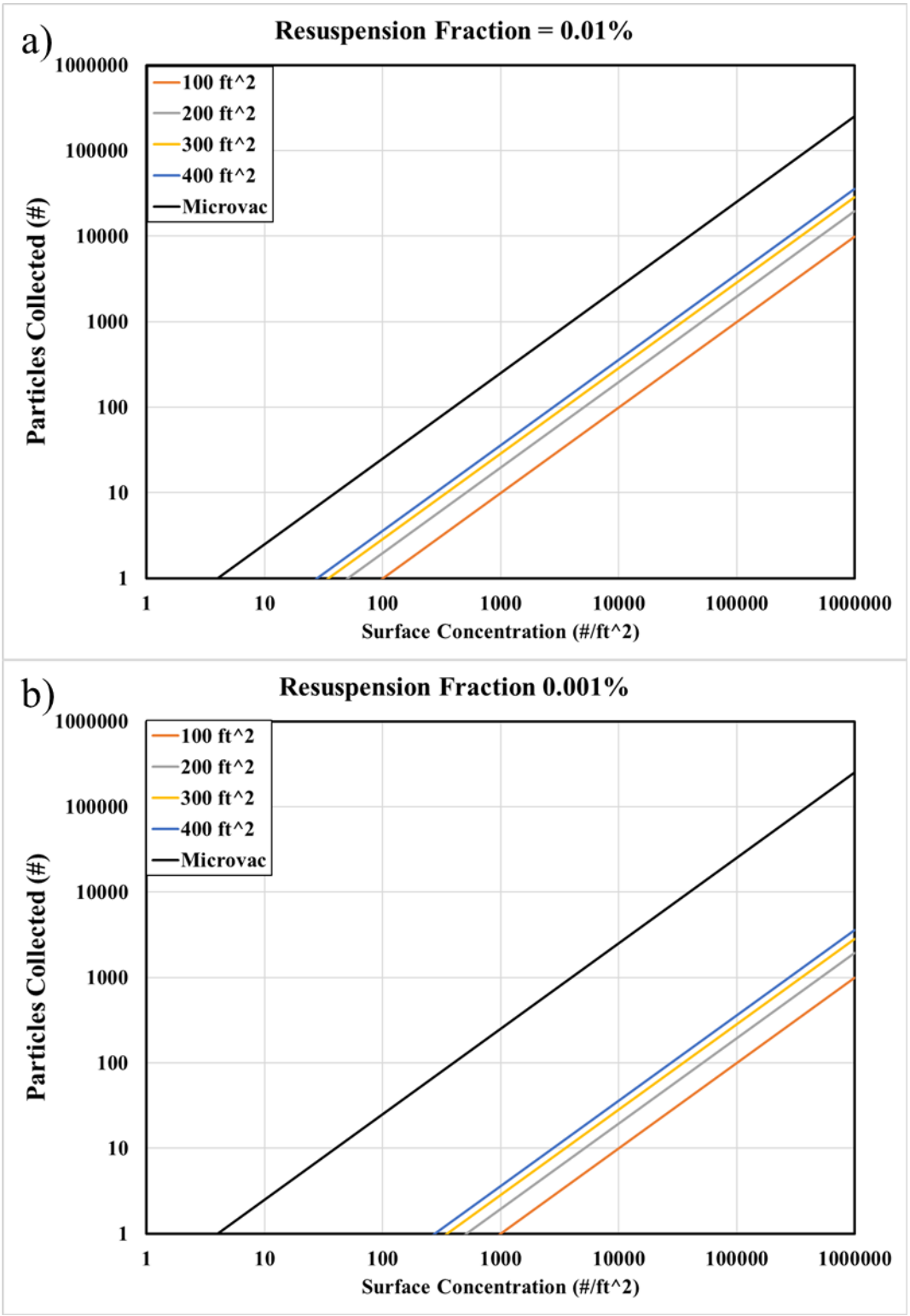

Figure 8: Particle Collection at a) 0.01% Resuspension and b) 0.001% Resuspension

Figures 8a and 8b show that the microvacuum is always more efficient than AAS, and the

particle concentration must be significant for AAS to collect particles at a resuspension rate of 0.001%. It is evident that for any decision to be made about implementation of AAS, it is critical that the order of magnitude of resuspension fraction using AAS on a given surface must be determined. Resuspension fractions in literature of *Bacillus* spores from different materials have varied greatly, and there have not been significant efforts placed into measuring the actual resuspension fraction of spores using the AAS technique specifically from indoor materials. Thus, experiments using AAS on a variety of materials are of vital importance to inform the best path forward for sampling extent of contamination or characterization as well as clearance.

5.0 Optimal Conditions for AAS

AAS may be a useful tool for sampling in a wide-area biological release scenario, but AAS is not an appropriate sampling method for all scenarios. Outdoor areas may not be appropriate for AAS unless the area of interest can be sealed or contained, as the particles removed from the surface will not likely dwell in the area of the sampler, and it would be impossible to determine the likelihood of a false negative result. Sampling in outdoor areas can use an activity-based sampling procedure such as those used for asbestos (US EPA 2008, US EPA ERT). For indoor AAS, optimal site specifications for implementation to determine the presence of biological contamination such as *Ba* include the following criteria:

- The area must be isolated, sealed, and placed under negative pressure using a HEPA-filtered exhaust blower (e.g., NAM);
- There is sufficient space to set up a personnel decontamination line outside the contaminated area in the contamination reduction zone;
- There is sufficient surface area so that contamination and subsequent resuspension/collection during AAS would result in a concentration above the AAS detection limit;
- The area has minimum dirt and debris that could overload sample filters or contain significant microbial background contamination, or the sampler can separate large particles from the desired contaminant via cyclonic air movement;
- There are sufficient power outlets for leaf blowers, air samplers, and fans (or battery-powered equipment is available with a plan for battery replacement and charging);
- Because resuspension of contaminants is likely limited by high relative humidity (RH), it is recommended that AAS be conducted under low RH conditions, if possible.

The number of personnel, air samplers, mixing fans, leaf blowers, etc., depends on the size of the space to be sampled. A large space will require an adequate sampler volumetric air flow to sample the space in a reasonable time if settling will be an issue, $L>0.1Q$ and V/Q must be of a level such that a sampling duration of three air exchanges falls within a reasonable sampling duration (i.e., less than an hour). The sampling duration may be limited by the appropriate time for sampling personnel in Level C (air purifying respirator, chemical resistant clothing, chemical resistant gloves and boots.) or above PPE.

In a situation where the sampler and the exhaust (negative pressure) system are two separate components of AAS, a competition between the sampler and the exhaust system may occur. Therefore, a very small space such as an office might require a much smaller HEPA-filtered exhaust blower. The BOTE project report noted that the NAM flowrate was too high to provide exhaust in the room where AAS was conducted (US EPA 2013a) because the NAM flow rate was significantly faster than the sampler, and the particles were evacuated from containment prior to being sampled by the separate air samplers. If the NAM is the source of sampling flow as well as exhaust flow, then this is not an issue.

6.0 Approaches for *Bacillus anthracis* AAS

Prior to prescribing and designing an AAS approach to determine if viable *Ba* spores are present in a particular environment, knowledge about the site, environmental conditions, and contaminant characteristics is necessary. Subject-matter experts (such as a Technical Working Group) should be consulted in the planning stage to provide recommendations on the number and location of air samplers, mixing fans, and HEPA-filtered exhaust blower(s). However, some specific recommendations can be made for AAS operating parameters based on data from prior laboratory experiments and field operations.

Higher shear forces applied to a surface have been demonstrated to result in increased reaerosolization of particles (US EPA 2014). The leaf blower parameters recommended to achieve maximum particle reaerosolization while conducting AAS are:

- Use of electric leaf blowers with the highest power rating (minimum of 1 horsepower);
- Operate the leaf blower with the nozzle as close to the surface as possible;
- Hold the leaf blower at an angle of 45° to the surface being agitated;
- Move the leaf blower nozzle across the surface in a side-to-side sweeping motion;
- Agitate all horizontal surfaces from at least two different directions;
- Agitate all vertical surfaces and the ceiling (if it can be reached);
- Agitate all accessible surfaces in this manner for a minimum of 10 minutes per 1,000 ft^2 of surface area.

A space that is comprised of several smaller rooms might need to be considered differently from one contiguous space. In deciding on the equipment configuration for AAS, each room within the larger sealed space may need to be approached as if it were separate from the others. This approach would require a minimum of one mixing fan and one sampler to be deployed in each room. In the case of an office building with many small rooms or offices, the overall result would be a much higher density of mixing fans and samplers per unit volume compared to a gymnasium or open industrial facility of the same volume. However, if the mixing fans and AAS could be oriented such that material is directed to a space that connects the smaller rooms, it is possible that a single NAM could be used to sample the entirety of the space as shown in the AAS model in Section 3.

Mixing fans are employed during AAS to keep aerosolized particles airborne so that they are more likely to be collected by an air sampler. The asbestos clearance sampling framework requires one 20-in diameter fan per 10,000 ft^3, operated on low speed and directed toward the ceiling. This fan size and density should be the minimum deployed in a space to provide adequate mixing. The mixing fan deployment should be planned to maximize mixing in the

specific site. The clearance sampling for beryllium conducted at LLNL (US EPA 2003, Tang et al. 2004) provides a good example of optimizing mixing in a large contiguous space. Fan operation should begin concurrently with surface agitation and air sampling.

The volume of air sampled during AAS should be equivalent to at least three air exchanges in the space to collect as many reaerosolized particles as possible. Lower flow air samplers should be spread evenly throughout the space and placed near the ground, but they should not be collocated with mixing fans. Higher flow air samplers that can provide three air exchanges in a space quickly should still be placed low to the ground to maximize capture of larger particles. For a given room volume and number/type of air samplers, the duration of sampling can be calculated by dividing the room volume by the total flowrate of all samplers combined. For example, a space with a volume of 50,000 ft^3 and 20 DFU samplers (approximately 30 ft^3/min flow rate each) would require an approximate sampling time of 4.2 hours to achieve three air exchanges. To decrease the sampling time, additional DFUs or high-volume air samplers should be used. Alternatively, a prefilter could be fitted to one or more NAMs already deployed in the space to increase the total sampling rate; two NAMs of flow rate 500 ft^3/min each would reduce the sampling time to 2.5 hours, for the scenario given above. A filter processing protocol would need to be established for the particular filter type deployed to ensure reliable results. Additionally, analytical laboratories that conduct culture or other analysis for sampling filters would need to be consulted about the sampling matrix ahead of time to ensure their acceptance.

Selection of air samplers for a biological response event will depend heavily on what is available at the time. DFU and other high-volume particulate air samplers such as the FRM PM-10 sampler or PSU sampler (HI-Q Environmental Products, San Diego, CA) are appropriate for AAS deployment because they have high volumetric sampling flow rates, supplies are not difficult to obtain, and filters are generally easy for analytical laboratories to handle. NAMs are also readily attainable. However, they may require verification of the sampling efficiency prior to field deployment. Personal bioaerosol samplers such as the Bioaerosol Button Sampler (SKC Inc, Eighty-four, PA) are suitable for personal sampling of those conducting AAS. Other air samplers with lower sampling flow rates can be used for supplemental sampling, but they are not recommended as the only sampling method.

Factors that must be considered in planning AAS for *Ba*, in addition to the site specifications and AAS parameters, include, but are not limited to:

- Adequate power, extension cords, and cord reels are needed for all equipment;
    - NAMs or other HEPA-filtered exhaust blowers
    - Electric leaf blowers
    - Fans
    - Air samplers.
- Adequate space for the personnel decontamination line;
- Adequate personnel are needed to conduct AAS;

    - 1-2 operators for each leaf blower
    - 3-person team(s) for sample deployment and collection ("dirty", "clean", data recorder)
    - Backup personnel for minimizing heat stress from PPE
    - Health and safety personnel for monitoring operators and ensuring proper PPE use
    - Decontamination line personnel.
- Appropriate PPE is needed for all personnel (NIOSH 2009);
    - Chemical Biological Radiological Nuclear (CBRN) full facepiece PAPR with CBRN cartridge
    - Level C protective ensemble
    - Hearing protection (during leaf blower operation).
- Sufficient time should be allowed for all AAS procedures to be followed, including setup, sampling and equipment removal.
    - Time required will depend on the size of the site and the number of personnel available.
- Air sampling should also be performed outside the containment area where AAS is being conducted to evaluate adequacy of containment.
- Tablets or other electronic devices are highly recommended for electronic data recording and documentation of sampling locations. A tablet can be used while in a clear sealed plastic bag to allow easy decontamination.
- Sampling kits should be prepared ahead of time for AAS
    - Prepare off-site (in an uncontaminated and isolated area);
    - Pre-label all containers and bags with sample locations; and.
    - Mark sample type and location on each kit in large, bold, easy-to-read font.
- Refrigeration or coolers with ice or ice packs are needed at the end of the decontamination line for storage and transport of samples.
    - Place a temperature data logger in each cooler for verification of storage and transport conditions for samples.
- The analytical laboratory should be consulted on issues such as:
    - capacity of the laboratory;
    - the sampling media (e.g., filters);
    - sample containment;

- sample storage and transport conditions
- addition of any agent to neutralize decontamination chemicals.

Provide sample identification codes to the analytical laboratory prior to delivering the samples to facilitate rapid processing of samples.

7.0 Conclusions

AAS has been used for multiple chemical and biological contaminants, including asbestos, beryllium, and bacterial spores (*Ba* or surrogates). AAS should be considered a complementary sampling option to traditional surface sampling techniques, but as previously shown, AAS has several distinct advantages over surface sampling when comparing area of coverage and cost. To fully understand the advantages and limitations of AAS indoors, however, there are several research gaps that must be explored further.

As shown previously in Section 3, the system for AAS can be designed so that >90% of particles in the air can be sampled. Therefore, the only limitation to the effectiveness of AAS is the amount of material resuspended from the surface via the aggressive air technique. This factor is determined by the density of particles on the surface and the fraction of those particles from a given surface resuspended due to the aggressive air, the resuspension fraction. In effect, the density of particles on the surface is what AAS is set to determine, so the resuspension fraction of the material from the surface must be known to a minimum of an order of magnitude. Understanding of this fraction for *Ba* from indoor surfaces is therefore paramount to estimating the efficacy of AAS. To date, no systematic study of resuspension fractions of *Ba* or representative surrogates from indoor surfaces using aggressive air velocities has been conducted. Recovery efficiencies for AAS from laminate, carpet, and hardwood of *Bacillus globigii* (a surrogate for *Ba*) using high volume samplers have been measured (EPA 2013b). However, deconvolution of the recovery efficiencies to the actual number of spores resuspended was not conducted. Thus, inefficiencies such as wall losses were not included in the measurements, and the recovery efficiencies determined are specific to the experimental setup. For an understanding of the application to a larger system or other materials, fundamental measurements of resuspension fractions from materials found indoors with varying dust loads and humidities must be conducted using air speeds specific to AAS. Systematic field investigations are also needed to assess AAS for targeted sampling approaches. Future studies should seek to determine the application of AAS to varied dissemination scenarios (e.g., hot-spot, low levels over wide area, wet deposition, dry deposition, etc.), environmental conditions (e.g., humidity, building area, and layout), and applications (e.g., characterization or clearance sampling).

8.0 Bibliography

Calfee, M.W.; Rose, L.J.; Morse, S.; Mattorano, D.; Clayton, M.; Touati, A.; Griffin-Gatchalian, N.; Slone, C.; and McSweeney, N. **2013**. "Comparative evaluation of vacuum- based surface sampling methods for collection of *Bacillus* spores". *J. Microbiol Methods* 95(3), 389-396

Carlsen, T.; Raber, E.; Macqueen, D.; Berg, L.; Coty, J.; Brown, G.; Fish, C.; Folks, K.; Mancieri, S.; Canter, D.; and Kempter, J. **2012**. "Remediation Guidance for Major Airports after a Bioterrorist Attack". LLNL-TR-562207. Lawrence Livermore National Laboratory. Livermore, CA.

Hinds, B. and Lambert, L. **2011**. "Interagency Biological Restoration Demonstration (IBRD): CXT Support". DTRA FTR-10-029. Defense Threat Reduction Agency. Kirtland AFB, NM.

Kirvel, R.D. **2010**. "Summary of the Seattle Urban Area Consequence Management Guidance for a Wide-Area Biological Attack". LLNL-TR-455571. Lawrence Livermore National Laboratory. Livermore, CA.

Kominsky, J.R.; Freyberg, R.W.; Brownlee, J.A.; Lucas Jr., J. H.; and Gerber, D.R. **1989**. "Observational Study of Final Cleaning and AHERA Clearance Sampling at Asbestos-Abatement Sites in New Jersey". EPA/600/2-89/047. US Environmental Protection Agency, Washington, DC.

Lawrence Livermore National Laboratory (LLNL) **2010** "Validating Aggressive Air Sampling for Beryllium Clearance", presentation from Beryllium Health and Safety Committee Meeting, Lawrence Livermore National Laboratory, November 4, 2010.

Lawrence Livermore National Laboratory (LLNL) **2011** "Beryllium Aggressive Air Sampling", Beryllium Health and Safety Committee Meeting April 7, 2011, Panel Discussion Presentation.

Lippy, B. **2016** "Bioterrorism and Anthrax Attacks: Lessons Learned at the AMI Building. National Institute of Environmental Health Worker Training Program September 20, 2016. Accessed online July 14, 2020 at https://www.niehs.nih.gov/about/events/pastmtg/hazmat/assets/2016/wtp_fall_16_lippy_508.pdf.

National Institute for Occupational Safety and Health (NIOSH). **2009**. "Recommendations for the Selection and Use of Respirators and Protective Clothing for Protection Against Biological Agents" DHHS (NIOSH) Publication Number 2009-132. Accessed online July 14, 2020 at https://www.cdc.gov/niosh/docs/2009-132/default.html.

National Research Council (NRC). **2005**. "Reopening Public Facilities After a Biological Attack a Decision Making Framework". Washington, DC: National Academies Press.

National Response Team (NRT). **2005**. National Response Team Technical Assistance for Anthrax Response Interim-Final Draft. Accessed online July 14, 2020 at https://yosemite.epa.gov/sab/sabproduct.nsf/17A8EBA9CAA030468525758D006D7A1B/$File/HSAC+Anthrax+TAD+2005+4-21-09+Meeting.pdf.

Raber, E; Hibbard, W.J.; and Greenwalt, R. **2011**. "The National Framework and Consequence Management Guidance Following a Biological Attack." *Biosecur Bioterror*. 9(3), 271-279.

Tang, K.M.; Nace Jr., C.G.; Maddaloni, M.A.; Harkay, J.D.; Graham Jr., D.R.; Koustas, R.N.; Rotola, J.D.; LaPosta, D.; and Callahan, K.C. **2004**. "Evaluation of Vacuum and Wet-Wipe Methods for Removal of World Trade Center Dust from Indoor Environments." *J Air Waste Manag Assoc*. 54(10), 1293-1298.

U.S. Center for Disease Control and Prevention (US CDC), **2012** The National Institute for Occupational Safety and Health Emergency Response Resources. "Surface Sampling Procedures for *Bacillus anthracis* Spores from Smooth, Nonporous Surfaces." Available at http://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html (accessed July 14, 2020)

U.S. Environmental Protection Agency (US EPA). **1985a**. Measuring Airborne Asbestos Following an Abatement Action. EPA 600/4-85-049. Office of Pesticides and Toxic Substances. Washington, DC.

U.S. Environmental Protection Agency (US EPA). **1985b**. Guidance for Controlling Asbestos-containing Materials in Buildings. EPA 560/5-85-024. Office of Pesticides and Toxic Substances. Washington, DC.

U.S. Environmental Protection Agency (US EPA). **2003**. Interim Final WTC Residential Confirmation Cleaning Study, Volume 1. Region 2. New York City Response and Recovery Operations. New York, NY.

U.S. Environmental Protection Agency (US EPA). **2008**. Framework for Investigating Asbestos-Contaminated Superfund Sites. OSWER Directive #9200.0-68. Office of Solid Waste and Emergency Response. Washington, DC.

U.S. Environmental Protection Agency (US EPA). **2013a**. Bio-response Operational Testing and Evaluation (BOTE) Project Phase 1: Decontamination Assessment. EPA 600/R-13/168. Office of Research and Development. Washington, DC.

U.S. Environmental Protection Agency (US EPA). **2013b**. Systematic Evaluation of Aggressive Air Sampling for *Bacillus anthracis* Spores. EPA 600/R-13/068. Office of Research and Development. Washington, DC.

U.S. Environmental Protection Agency (US EPA). **2014**. Determination of the Difference in Reaerosolization of Spores off Outdoor Materials. EPA 600/R-14/259. Office of Research and Development. Washington, DC.

U.S. Environmental Protection Agency (US EPA). **2017**. Field Application of Emerging Composite Sampling Methods. EPA/600/R-17/212. Office of Research and Development. Washington, DC.

U.S. Environmental Protection Agency (US EPA). **2020**. Homeland Security: Strategic Research Action Plan 2019–2022. EPA 601K20002. Office of Research and Development. Washington, DC.

U.S. Environmental Protection Agency (US EPA). ERT Helpful Hints for Activity-Based Sampling for Asbestos in Air. Accessed online July 14, 2020 at https://semspub.epa.gov/work/HQ/175325.pdf.

U.S. Government Accountability Office (US GAO). **2005**: Anthrax Detection: Agencies Need to Validate Sampling Activities in Order to Increase Confidence in Negative Results. Washington DC: GAO, GAO-05-251 Accessed online July 29, 2020 https://www.gao.gov/products/GAO-05-251

U.S. National Archives and Records Administration Code of Federal Regulations (US NARA CFR). **2003**: Protection of Environment, Title 40 Subpart E, Sec 763. Accessed online July, 29 2020 https://www.ecfr.gov/cgi-bin/text-idx?SID=5e303552ea6bd32c01baeb4362b79b04&mc=true&node=pt40.34.763&rgn=div5

Wang, C-H; Chen, BT; Han, B-C; Liu, AC-Y; Hung, P-C; Chen, C-Y; and Chao, HJ. **2015**. Field Evaluation of Personal Sampling Methods for Multiple Bioaerosols. *PLOS ONE* 10(3): e0120308

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