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A Review of Biological Agent Sampling Methods and Application to a Wide-Area Incident Scenario to Characterize Time and Resource Demands

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A Review of Biological Agent Sampling Methods and Application to a Wide-Area Incident Scenario to Characterize Time and Resource Demands

U.S. Environmental Protection Agency

Research Triangle Park, NC 27711

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DISCLAIMER

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ACRONYMS AND ABBREVIATIONS

Ba	*Bacillus anthracis*
BOTE	Bio-Response Operational Testing and Evaluation Project
CARC	Chemical-Agent-Resistant Coating
CDC	Centers for Disease Control and Prevention
CFU	Colony-Forming Unit(s)
CI	Confidence Interval
cm	Centimeter
DNA	Deoxyribonucleic Acid
DST	Decision Support Tool
DoD	U.S. Department of Defense
DOE	U.S. Department of Energy
EPA	U.S. Environmental Protection Agency
ESA	European Space Agency
FNR	False Negative Rate
ft	Feet
GIS	Geographic Information System
HEPA	High-Efficiency Particulate Air
HSPD	Homeland Security Presidential Directives
HSRP	Homeland Security Research Program
HVAC	Heating, Ventilation, and Air Conditioning
in	Inches
INL	Idaho National Laboratory
LOD	Limit of Detection
m	Meter
MCE	Mixed Cellulose Ester
MLI	Multilayer Insulation
N/U	Not Used
NASA	National Aeronautics and Space Administration
NHSRC	National Homeland Security Research Center
NPS	National Planning Scenario
ORD	Office of Research and Development
PBC	Polyester-Bonded Cloth
PBST	Phosphate-Buffered Saline including Tween
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl Chloride
RE	Recovery Efficiency
RFC	Robotic Floor Cleaner
RH	Relative Humidity
SE	Standard Error
SD	Standard Deviation
TTC	Time to Completion
UK	United Kingdom

µm	Micrometer
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases
VSP	Visual Sample Plan
WEST	Waste Estimation Support Tool

EXECUTIVE SUMMARY

A large-scale aerosol release of a persistent, disease-causing biological agent can result in contamination of a wide area, and may require significant time and resources for recovery depending on the severity of adverse health effects on the exposed population(s). Many unknowns are associated with characterization and clearance sampling during response to a wide-area (including indoor, outdoor, and underground area) biological incident. The biological agent and its characteristics, the release mechanism, amount of contaminant released, and a plethora of environmental and meteorological factors are completely separate, yet interconnected processes that greatly influence the extent and level of contamination. Similarly, decisions related to the sampling strategy (i.e., sample medium, sampling area, spacing, etc.) will affect the cost, time, amount of waste generated, and personnel (i.e., resource demand) required to characterize and clear the contaminated area. The process of understanding how these elements influence one another and contribute to the overall problem is referred to as a systems approach. To what degree sampling and, more specifically, variations in the sampling strategy interact and contribute to overall resource demand, following a wide area biological incident, is still largely unknown. To date, there have been no attempts to model characterization sampling following a wide-area biological incident.

The objectives of this study were fivefold: (1) to review available facts and information related to historical wide-area biological contamination incidents; (2) to conduct a literature review to generally assess the current state of knowledge regarding current sampling methods that may be used for characterization sampling following a wide-area biological incident, specifically focusing on *Bacillus anthracis* (*Ba*) contamination; (3) to summarize available data obtained from the literature for a predetermined set of data elements (i.e., performance metrics) for current sampling methods and technologies, (4) apply appropriate data from the literature and/or from recent operational field studies to inform an analysis of the application of wide-area sampling strategies in the context of National Planning Scenario (NPS) #2, and (5) evaluate the impacts of (a) variable sampling areas, hot spot areas, probabilities, and false negatives rates; and (b) variable resource availability; on the total estimated cost and time to complete sampling and analysis activities in a hypothetical scenario.

Three recent incidents (see Section 2) have illustrated the need for enhancement and validation of sampling methods and development of decontamination and remediation plans for a biological release. In each of those incidents, contamination occurred in a vast range of structures and sizes. The large costs associated with decontamination in these cases support the necessity of appropriate sampling methods to accurately characterize contamination and inform appropriate response efforts. No one remediation strategy is universally applicable.

A small variety of established sampling methods have been used, and are currently recommended, for many biological agents, including *Ba*. Some methods focus on small spaces: swab sampling, sponge sampling, gauze sampling, and vacuum sampling; others are more applicable to larger areas or other applications beyond the initial response stage of an incident: air sampling, aggressive air sampling, and robotic floor sampling. This study focused on gathering data on surface types sampled, recovery efficiencies, and sample area sizes for wipe, sponge, swab, and vacuum sampling. These three data elements were identified as being the most critical for the purposes of further analyzing the impacts of sampling strategy for a hypothetical wide-area incident, including impacts on cost, time, resource demand, etc. The literature revealed a diverse variety of data on surface types, recovery efficiencies, and sample area sizes. Surface types sampled included stainless steel, carpet, plastic, wood, glass, brick, wallboard, various other metals, concrete, and fabrics including cotton, rayon, and polyester. Sample areas also varied greatly depending on the source. The sampling methods reviewed and the aggregated data highlights the vast range of reported RE values for differing surface types, spore concentrations, and (in some cases) deposition methods. Additionally, the data were collected under largely controlled

conditions and there is the possibility of potential biasing of the RE values due to the individual collecting the samples or conducting the sampling. Furthermore, the identified sampling methods have been characterized on the selected surfaces, mainly focused on indoor surfaces. A wide-area biological incident may require sampling of indoor as well as outdoor surfaces and possibly underground transit systems. It is expected that the sampling in a wide-area incident may require sampling very different surfaces compared to (or in addition to) the ones evaluated in the studies above. Only a small number of surface sampling methods are well-documented with their sampling protocols. It is uncertain, if a wide-area biological incident happens, how the impacted areas would be characterized and how much resources would be required with the currently-available sampling methods.

A statistically defendable sampling plan was developed to characterize a hypothetical city following a hypothetical biological release (National Planning Scenario (NPS) #2). Realistic spatial, infrastructure, and surface media information was gathered to determine the extent of sampling and to match surface material types at each sampling location to their appropriate sampling method. Visual Sample Plan (VSP) and a custom spreadsheet were used to estimate the sampling criteria, the number of samples, and the resources needed to take those samples for outdoor, indoor, and underground environments. According to the hypothetical sampling plan, environmental characteristics, resource demand assumptions, and the assumption that *Ba* spores will remain persistent and detectable within the environment; characterization of an assumed two square mile area with a total surface area of 4.76×10^8 ft^2 using approximately 1,600 sampling personnel and 250 laboratories will cost an estimated 15.3 billion dollars in total and may take over 10 years to characterize. The total time for sampling may be up to 4 years, while the total analysis throughput is estimated to take 10 years. Although this disconnect is rather improbable, the results do lend themselves to the possibility that analysis efforts may eclipse sampling efforts. The total indoor surface area is one order of magnitude greater than the outdoor area, which is to be expected for highly-populated urban setup. Because of the increased surface area, the indoor environments represent almost 90% of the total cost and resource demand of all the environments combined.

Because of this, for wide-area biological incidents, it is recommended that a rule in/rule out approach be taken when sampling indoors. If a positive hit is discovered through preliminary sampling, the building should be considered contaminated unless the decontamination approach is costlier than further sampling to define more discrete decontamination zones. This analysis assumed that all buildings within the contaminated area would either be contaminated or need to be sampled thoroughly. Available field data or a lines of evidence approach may be useful in reducing this requirement. It is likely the requirements set forth by this environment are not practical and would likely exhaust local, state, and federal resources. Furthermore, by the time characterization has concluded (i.e., 10 years later), it is possible that the community and its economy will no longer be viable.

The process to develop a sampling strategy for wide-area biological incident response operations has up to now been poorly defined and not systematic. Additionally, the options available in terms of sampling techniques are often constrained by regulatory mandates. In situations where the sampling method is not mandated by regulation(s), then it is necessary to analyze available data concerning both sampling methods and sampling techniques before selecting the most appropriate method or technique.

Leveraging the capacity of VSP and the results of the hypothetical scenario, a predictive model for wide-area sampling strategies by way of regression modeling was developed to model the number of samples needed for the underground, outdoor, and indoor environments individually under a range of values for VSP input parameters (sampling area, hot spot area, false negative rate, and probability). The relative impact of the VSP input parameters on the resulting number(s) of samples was analyzed, and the modeling results were combined with resource demand estimates to analyze the potential impacts on total time (for sampling and analysis) and total cost impacts following a wide area biological incident. Based on the results, the total cost is a function only of the sample area size (i.e., as the area covered by each

sample collected increases, the number of samples required for a given area of interest decreases) and not on either the number of sampling teams collecting those samples nor on the number of labs available to analyze those samples. However, the amount of time needed to both collect the samples and to analyze them is a function of the number of sampling teams and analysis labs available. Once the number of samples is determined, the total cost is fixed, and is independent of the number of sampling teams and the number of labs. Once the total cost is determined based only on the number of samples required, the impact of a variable number of available sampling teams and analysis labs was evaluated.

Several needs for future research were identified based on this study. First, a lines-of-evidence approach to sampling design should be considered at the outset of the response planning phase of a wide area incident for the eventual purpose of reaching clearance goals. Second, we currently lack operational strategies to deal with such an incident, and cohesive operational information must be developed to help inform the development of those strategies. Third, the current sampling methods used to characterize a wide area would result in an overwhelmingly unrealistic number of samples that must be collected and analyzed. Existing availability of resources (sampling personnel and analytical laboratories) would not be able to handle such a large demand in even a reasonable amount of time. Different sampling methods must be developed to characterize the extent of contamination in wide areas. Those novel sampling methods must focus on larger sampling area sizes, be usable for outdoor surfaces, must require less time and personnel to collect, and thereby reduce the overall sampling and analysis burden by increasing the area characterized by each sample. Improvements to existing sampling methods with respect to recovery efficiencies would largely be inconsequential to reducing the resource demand (total cost and time to characterize) compared to improvements to sample area size (even using probabilistic sampling design approaches). Fourth, there is a lack of understanding concerning the fate and transport of spores over long periods of time. This understanding is critical when considering the amount of time potentially required to conduct characterization sampling. Spore fate and transport must be understood for effective sampling and remediation strategy development. Lastly, this study supports further consideration of a combined sampling design approach using probabilistic and non-probabilistic sampling when characterizing a wide area incident. Additional decision support tools are needed to help direct sampling efforts for wide area incidents where time and cost considerations are critical factors.

1 INTRODUCTION

This project supports the mission of the U.S. Environmental Protection Agency's (EPA) Office of Research and Development's (ORD) Homeland Security Research Program (HSRP) by providing information relevant to the decontamination of areas contaminated as a result of an act of terrorism. Under Homeland Security Presidential Directives (HSPDs) 5, 7, 8, and 10, EPA, in a coordinated effort with other federal agencies, is responsible for "developing strategies, guidelines, and plans for decontamination of…equipment, and facilities" to mitigate the risks of contamination following a biological agent contamination incident.

EPA's National Homeland Security Research Center (NHSRC) aims to help EPA address the mission of the HSRP by providing expertise and products that can be widely used to prevent, prepare for, and recover from public health and environmental emergencies arising from terrorist threats and incidents. NHSRC's mission includes providing expertise and guidance on the selection and implementation of decontamination methods and providing the scientific basis for a significant reduction in time, cost, and complexity of decontamination and waste handling activities.

A large-scale aerosol release of a persistent disease-causing biological agent can result in contamination of a wide area and may require significant time and resources for recovery depending on the severity of adverse health effects on the exposed population(s). Contamination would likely be heterogeneously deposited (i.e., light and patchy) over a wide area and might migrate to clean areas by way of resuspension and weathering over a short period (e.g., days). Although information on the agent type and possibly some of its characteristics may be available during the response, an arduous environmental sampling effort to determine the identity, concentration, viability, and approximate location of the contamination would likely follow. Such an effort would be both costly and time-consuming.

Many unknowns are associated with characterization and clearance sampling during the response to a wide-area (including indoor, outdoor, and possible underground transportation areas) biological incident. The biological agent and its characteristics, the weapon design, amount of contaminant released, and a plethora of environmental and meteorological factors are completely separate yet interconnected processes that greatly influence the extent and level of contamination. Similarly, decisions related to the sampling strategy (e.g., sample medium, sampling area, spacing, etc.) will affect the cost, time, amount of waste generated, and personnel (i.e., resource demand) required to characterize and clear the contaminated area. The process of understanding how these elements influence one another and contribute to the overall problem is referred to as a systems approach. To what degree sampling and, more specifically, variations in the sampling strategy interact and contribute to overall resource demand following a wide area biological incident is still largely unknown. To date, there have been no attempts to model characterization sampling following a wide-area biological incident.

The objectives of this study were fivefold: (1) to review available facts and information related to historical wide-area biological contamination incidents; (2) to conduct a literature review to generally assess the current state of knowledge regarding current sampling methods that may be used for characterization sampling following a wide-area biological incident, specifically focusing on *Bacillus anthracis* (*Ba*) contamination; (3) to summarize available data obtained from the literature for a predetermined set of data elements (i.e., performance metrics) for current sampling methods and technologies, (4) apply appropriate data from the literature and/or from recent operational field studies to inform an analysis of the application of wide-area sampling strategies in the context of National Planning Scenario (NPS) #2, and (5) evaluate the impacts of (a) variable sampling areas, hot spot areas,

probabilities, and false negatives rates; and (b) variable resource availability; on the total estimated cost and time to complete sampling and analysis activities in a hypothetical scenario.

Literature was aggregated using a set of predefined keywords relevant to this study (Appendix A). Each piece of literature was read, assessed, and documented based on several criteria (Appendix B). Literature deemed at least moderately relevant to meeting the objectives of this study, according to the above criteria, were then summarized and relevant data were extracted and compiled in Microsoft Excel spreadsheets.

In addition to available literature, reports from field studies, planning documents, and guidance documents were also reviewed for data and information that were relevant for the objectives of this study. The *Bio-response Operational Testing and Evaluation (BOTE) Project* was a recent collaborative effort between the Department of Homeland Security, the U.S. Environmental Protection Agency (EPA), the Department of Defense (DoD), and the Centers for Disease Control and Prevention (CDC). The BOTE Project was designed to test and evaluate the operational response to a *Ba* spore release in a moderately sized (~8,000 ft^2) building. The test building was a two-story unoccupied office building. Two of the primary objectives of the study were to "demonstrate that biological sampling and analysis methods evaluated in previous studies provide accurate characterization of *Ba* simulant concentration challenges for detection/identification purposes" and "[c]ollect and analyze the results from the decontamination study and perform a cost analysis of all aspects of the remediation approaches." [1]

Over 3,000 samples were taken during the BOTE project using a variety of sampling techniques. Surface sampling methods included cellulose sponge-stick wipes, macrofoam swabs, vacuum socks, and Versalon® Wipes. Air sampling methods included SKC BioSamplers®, Model 3314 UV-APSTM Spectrometer, Dycor XMX/2L-MIL Aerosol Collection System, and Mattson-Garvin Model 220 Slit-to-Agar Sampler. The BOTE Project represents the most current application of biological agent sampling techniques in a field application. While the sampling campaign focused solely on a single building and its immediate surroundings, this sampling campaign represented the most comprehensive study to date that has investigated the implications of sampling for a wide-area event and how the sampling plan and number of samples impacts resource requirements, cost, and time to conduct sampling, analysis, decontamination, and waste generation.

In addition to the BOTE Project report, the New York City Department of Health and Mental Hygiene's *Environmental Response and Remediation Plan for Biological Incidents* [2] was reviewed. This report delineates the operational and technical assistance required to lead a multi-agency effort to prepare for and remediate contaminated areas following an intentional or unintentional biological incident involving the release of *Ba*. The report includes: a decision-making framework, along with roles and responsibilities; operational tools and guidance for government/regulatory entities and stakeholders; technical guidance, including decontamination approaches and tactical procedures, sampling strategies and plans, and clearance criteria and procedures; and guidance for establishing technical working groups and environmental clearance committees. The plan is structured to help users employ the correct actions for assessment, decontamination, and other actions needed for a successful cleanup without regard to incident type and how many structures are involved. The plan does not focus on an incident of any particular size, but instead provides guidance that is scalable and can be applied to small-scale incidents, wide-area incidents, or regional incidents that could involve multiple city blocks, hundreds of facilities, and multiple response agencies.

This assessment report is structured in the following manner: Chapter 2 provides an overview of four historical anthrax incidents. Chapter 3 presents an overview of current sampling methods and techniques and summarizes the results of our literature review. Chapter 4 applies a combination of data identified in the literature (if relevant), relevant data from the results of the BOTE Project, relevant planning

information from the New York City Department of Health and Mental Hygiene, and best estimates for a hypothetical exercise involving a biological attack on large urban area. Chapter 5 extends the hypothetical analysis presented in Chapter 4 to evaluate and analyze a range of possible variables and their impacts on the total cost and time to complete sampling and analysis activities. Chapter 5 also introduces and discusses elements of an EPA-developed spreadsheet which allows such a resource demand and impacts analysis to be performed. Chapter 6 discusses the conclusions reached as a result of this study.

2 HISTORICAL ANTHRAX INCIDENTS

Several recent incidents have illustrated the need for enhancement and validation of sampling methods and development of decontamination and remediation plans for a biological release. These incidents include the "Amerithrax" incident in 2001, the Sverdlovsk, former Soviet Union anthrax release in 1979, and an incident leading to the cleanup of Gruinard Island, United Kingdom in 1981. Naturally occurring incidents, such as drummer-related incidents in the United Kingdom, Danbury CT, and Durham NH, were excluded from this summary of historical incidents as the extent of contamination in these was comparatively small.

2.1 Amerithrax

In October 2001—at a time when the U.S. had recently become acutely aware of its vulnerability to acts of international terrorism—a series of bioterrorism incidents involving *Ba* spores (the causative agent of anthrax) occurred. These incidents, referred to as the "Amerithrax" case, were the first known U.S. cases of anthrax-related bioterrorism and resulted in 22 cases of infection across four states and Washington, D.C., five of which were fatal [3, 4].

The *Ba* spores were sent through the mail in letters that were addressed to two U.S. senators and members of the media [3]. As the first cases of anthrax were diagnosed, the possibility of contamination led to a major disruption in the operations at various buildings including the Hart Senate Office Building in Washington, D.C., and U.S. postal facilities in the capital area and Trenton, New Jersey [5]. Approximately 42 buildings were contaminated to some degree. The mean building size (for 11 buildings for which data were available) was 23 million square feet. However, this figure may not be representative, as contamination was limited to just a few rooms for most buildings [6].

Funded through its Superfund program, EPA spent approximately $27 million to remediate (sampling, decontamination, waste management) 26 buildings in the Capitol Hill area. Of this total cost, EPA spent $25 million to hire 27 contractors and three federal and state agencies to assist in cleanup activities. The other $2 million covered EPA's personnel costs, mostly for the staff who supervised the contractors. Cleanup took three months before Capitol Hill office buildings were allowed to reopen [7].

Before the cleanup, sample collection and other analytical methods were used to detect *Ba* in postal facilities—but no validated sampling and cleanup methods were available. Thus, while testing in 286 postal facilities produced largely negative results, the accuracy of the sampling effort was unknown [3]. Without validation, sampling may have been based on false assumptions. There were many unknowns during the sampling process, such as how many samples to take and, in general, which sampling method was appropriate in a given situation.

At the time, EPA had not developed a guidance document for sampling procedures, though the U.S. Postal Service did have a sample collection plan. Site-specific decisions by EPA and the CDC were largely discretionary and based on discussions with facility members, reviews of facility floor plans, and other observations that helped inform a targeted sampling method. Unlike a probabilistic sampling design,

targeted sampling design does not provide a level of statistical confidence in sample test results. Without this statistical certainty, there could be many reasons for negative results, other than an actual absence of *Ba* spores: (1) samples not being collected from places where *Ba* spores were present, (2) the detection limit of the sampling and analysis method being greater than the actual contamination level, (3) not enough samples being collected, (4) not enough spores being recovered from the sample material during laboratory analysis, and (5) analysis of the sample extract not detecting *Ba* spores [3].

During the decontamination of congressional buildings, EPA collected both air and surface samples using three types of surface sampling methods (wet swabs and wipes for nonporous surfaces and high-efficiency particulate air [HEPA] vacuuming for porous[1] materials), and four types of air sampling. Nationwide, approximately 120,000 samples were taken at a cost of approximately 8.7 million dollars [6]. Numerous decontamination methods were used, including fumigating (vaporous hydrogen peroxide®, chlorine dioxide gas, paraformaldehyde), spray-based decontamination with liquids (Spor-klenz®, chlorine dioxide, disinfecting with Sandia foam®), and physical removal of surface-bound particulates by methods such as HEPA vacuuming [7].

In addition to a lack of standard procedures and validated sampling methods, there were logistical challenges. Insufficient laboratory analytical capacity limited the number of samples that could be collected and the speed at which samples could be processed [3, 4]. CDC's Laboratory Response Network and military laboratories such as the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) processed over 125,000 clinical specimens and 1 million environmental samples [5]. Questions surrounding the Amerithrax case remain. It was later determined that the spores used in the attacks were of the Ames strain, a unique strain used in U.S. military research. This realization led to the plausible conclusion that the attacks may not have been an act of international terrorism, but perhaps perpetrated by a well-trained and educated scientist with access to a U.S. military laboratory [4].

2.2 Sverdlovsk Anthrax Leak

In April 1979, an unknown number of anthrax-related deaths occurred in the former Soviet Union in Sverdlovsk (now Yekaterinburg). The reticent nature of the Soviet Union made a more thorough international investigation of the incident nearly impossible, leaving epidemiology as the only tool to find the source of the anthrax outbreak. One of the few sources of first-hand evidence of an anthrax outbreak comes from the handwritten notes of Dr. Faina Abramova, a doctor who treated some of the Sverdlovsk patients. The most widely accepted reports of the incident state that 96 people were infected, and 64 of these cases were fatal [4].

The Soviet authorities blamed the outbreak on the consumption of infected meat [4, 5]. Meat was collected and environmental samples were taken. However, the extent of these efforts is unknown. Local fire brigades reportedly washed trees and building exteriors in sections of the city where most cases resided. Stray dogs were shot, and some unpaved streets paved [8-10]. However, based on doctors' notes, victims suffered from symptoms that were not representative of gastrointestinal anthrax but rather what appeared to be pulmonary anthrax, a deadlier form of the disease [4]. Anthrax cases appeared up to six weeks after the incident -- exceeding the 1-10-day incubation period due to either resuspension or extended incubation time due to low dose exposure [8, 9]. Further investigations suggest that the outbreak was caused not by infected meat but by an accidental release of *Ba* spores by way of the exhaust ventilation system from a Ministry of Defense microbiological facility in Sverdlovsk [5, 11].

Debate over the source of *Ba* spores in Sverdlovsk continued for many years after the incident [5]. In 1992, a team of American scientists were allowed to review autopsy materials and other evidence from

[1] Porous surfaces are the surfaces that are permeable to water or fluid used for sampling, and nonporous surfaces are not permeable to water or fluid used for sampling.

the incident. While the KGB had confiscated many medical records during the time of the incident, based on the available evidence, the team of scientists was able to conclude that the outbreak resulted from the escape of aerosolized *Ba* spores from the Sverdlovsk microbiological facility [5]. According to epidemiological data, the contamination may have extended over 50 km and was over 1 km wide in some areas [9]. Due to the covert response to this release, very little cost or personnel information is available.

2.3 Gruinard Island

Located off the coast of Scotland, Gruinard Island was a site of biological weapon testing for Great Britain in 1942 before World War II. Tests of *Ba* bombs were carried out, and experiments on the island successfully produced anthrax among targeted sheep. The island was eventually quarantined due to the high levels of soil contamination by *Ba* spores, and Great Britain terminated testing with its offensive program between 1955 and 1965 [5]. The extent of contamination was assumed to be the entire 520-acre island [12].

Sampling was carried out annually between 1948-1968 and 1972. A survey carried out in 1979 took 125 core soil samples. After a full-scale decontamination effort, clearance sampling consisted of 78 core soil samples, 58 of which had previously indicated the highest levels before decontamination and 20 selected at random. Only three of the 58 points showed high contamination and three of the sites randomly sampled showed evidence of *Ba*. All suspect locations were subsequently decontaminated with 5% formaldehyde in 1986 [13]. Further sampling took place six months later with no positive results. Forty sheep were allowed to graze for five months for further verification [10].

The British government eventually initiated a more comprehensive cleanup of Gruinard Island in 1986. Scientists used formaldehyde that was circulated through a custom-built irrigation system that dispensed over 280 tons of formaldehyde, diluted in 2,000 tons of seawater, for approximately one year. Soil samples were tested at various distances away from the center of the *Ba* bomb explosions [10, 12]. The cost of decontamination was estimated at £500,000 or $800,000 or approximately 1.5 million dollars when accounting for inflation. It is not known to what extent sampling contributed to this total [14].

2.4 Lessons Learned

In the three historical incidents previously described, contamination occurred in a vast range of structures and sizes. The Amerithrax 2001 bioterrorism case involved large mail distribution centers and office buildings. Contamination from the Sverdlovsk leak may have extended over an industrialized urban area 50 km long and over 1 km wide. Additionally, the entire 520-acre island of Gruinard was assumed to be contaminated because of experimental weapons testing. Although information is limited on the Sverdlovsk incident, in both the Amerithrax and Gruinard Island cases, sampling for *Ba* spores informed decontamination efforts, i.e., where to focus decontamination activities and whether decontamination was effective. In the case of the Amerithrax incident, the lack of validated sampling methods led to unknown accuracy of results, which in turn, makes it difficult to fully understand whether the appropriate decontamination stringency was performed (too stringent or not stringent enough). Available information on the cost of decontamination ranged from $1.5 million dollars for Gruinard Island to $27 million dollars to decontaminate 26 buildings in the Capitol Hill area associated with the 2001 Amerithrax incident. No one remediation strategy is universally applicable, and each situation will have to be confronted in a unique manner [15]. Enhancement of sampling methods and strategies may help to improve sampling accuracy, ease of use, representativeness, and could reduce cost and time required for remediation activities.

3 OVERVIEW OF SAMPLING METHODS

A small variety of established sampling methods have been used and are currently recommended for many biological agents, including *Ba*. Some methods focus on small spaces: swab sampling, sponge sampling, gauze sampling, and vacuum sampling (swab and wipe sample processing procedures have been validated by CDC) [16]. Others are more applicable to larger areas or other applications beyond the initial response stage of an incident: air sampling, aggressive air sampling, and robotic floor sampling. As stated above, one of the objectives of the literature review was to summarize available data from the literature for a predetermined set of data elements for current and established sampling methods. Data for air sampling and robotic floor sampling technologies were also gathered. The data elements of most interest initially included all the following:

- Surface type sampled
- Recovery efficiency (RE, %)
- Cost
- Time needed to take sample
- Sample area size (sampling area per sample)
- Number of personnel required
- Necessary equipment
- Special deployment
- Safety concerns
- Waste produced
- Ease of laboratory analysis.

The initial literature search identified 212 sources as being potentially relevant for this study. These sources included many peer-reviewed and non-peer-reviewed documents, including journal articles, government reports, books, book chapters, theses, websites, magazine articles, and other studies (e.g., from the National Academies). Initial review and evaluation eliminated (or temporarily eliminated) 38 sources, leaving 174 that were further evaluated and rated against predetermined criteria (i.e., applicability, accuracy, clarity, and uncertainty and variability). Each of the 174 sources was scored against each of those criteria and the overall score was calculated. Based on the overall scores, each source was classified as "unacceptable," "low quality," "moderate quality," or "high quality." A total of 90 sources deemed at least "of moderate quality," according to the above criteria, were then summarized and relevant data were extracted and summarized in Microsoft Excel spreadsheets. A few sources were identified as being lower quality, but did contain some information relevant to this study; their data were also extracted when appropriate.

The relevant sources identified above covered one or more of the sampling methods of interest, including the currently established sampling methods. Other sources focused on one or more "wide-area" emerging sampling methods (i.e., air sampling, aggressive air sampling, robotic floor sampling) or one of the in-situ methods (i.e., point detection, standoff, passive standoff). Many of the relevant sources can be categorized as either laboratory/bench-scale/controlled studies. Table 1, below, summarizes the number of sources reviewed and the sampling methods/techniques they discuss or evaluate.

Table 1. Number of Sources That Contained Information and/or Data Relevant to Sampling Method/Technology

Current Accepted Methods	
Gauze/wipe sampling	32

Sponge sampling	15
Swab sampling	38
Vacuum sampling	25
In-Situ Detection Methods[a]	
Passive standoff	1
Point detection	2
Point detection and identification	6
Standoff	7
Other methods	17
"Wide-Area" Methods	
Air sampling	16
Robotic floor sampling	2

[a] Currently, the sensitivity and accuracy of in-situ detection methods precludes their use in response operations where contaminants are unknown. These detection devices may, however, have utility at detecting/confirming agent from a visible powder.

Not all sources reviewed included data identified as relevant for this study. For all sampling methods and technologies identified, Table 2 summarizes the number of reviewed sources that contained each of the data elements listed above.

Table 2. Number of Sources That Contained Information and/or Data Relevant to This Study

Data Element	Number of Sources
Sample area size	54
Necessary equipment	42
Ease of laboratory analysis	42
Recovery efficiency, RE (%)	40
Surface type	32
Time needed to take sample	26
Cost	7
Special deployment	7
Number of personnel	6
Safety concerns	6
Waste produced	6

After further evaluating the volume of data extracted and the percentage of coverage for each of the identified data elements in Table 2, this study was further defined to focus on the following data elements for wipe, sponge, swab, and vacuum sampling:

- Surface type
- RE (%)
- Sample area size.

These three data elements were identified as being the most critical for the purposes of further analyzing the impacts of sampling strategy for a hypothetical wide-area incident, including impacts on cost, time, resource demand, etc. The analysis itself is discussed in Chapter 4. An overall summary of the data

identified for surface type, RE, and sample area size for each sampling method appears below. General observations are also noted and discussed.

Only those sources where at least one data point was available for: (1) surface type, (2) RE, and (3) sample area size are included in the summary below. In the summary, data are presented as they were found in the reference material. For example, for references that contained multiple RE ranges for a single surface type: the summary below presents each individual range presented in the reference as opposed to combining the ranges for the single surface type. Additionally, where a single range of results for multiple surface types were found, the data are transcribed as such below.

Due to the large number of sources reviewed, the volume of data available, and the relative inconsistency in the format of the data, it is not possible to comprehensively summarize all data for the three key data elements. Variation in how the data were reported, the laboratory and/or test conditions, and the purposes for which the data were obtained and/or the study objective(s) further complicate any effort to standardize the results of the literature search. The following sections are intended to provide a general qualitative overview of the current state of the science related to the various sampling methods and technologies discussed.

For all the established sampling methods in the sections that follow, the literature reveals a diverse variety of data on surface types, recovery efficiencies, and sample area sizes. Surface types sampled included stainless steel, carpet, plastic, wood, glass, brick, wallboard, various other metals, concrete, and fabrics including cotton, rayon, and polyester. In some cases, recovery efficiencies were reported with associated errors or confidence limits and in a few cases only concentrations (measured in colony-forming units, or CFU, for an area) were available. Sample areas also varied greatly depending on the source. A summary of the data for swab sampling is presented in Table 3, wipe sampling in Tables 4 (sponge stick) and 5 (gauze), and vacuum sampling in Table 6. Sources listed in Tables 3 through 6 reported data for RE and sample area size.

3.1 Swab Sampling

Swab sampling is appropriate for small, smooth, nonporous, hard-to-reach areas, and complex surfaces less than 26 cm^2 [17]. Sampling conducted on rough or porous surfaces or over an extended area could damage the swab. A sterile solution is applied to moisten the collection medium and the swab is wiped over the sampling area in an overlapping vertical “S” pattern. After an initial pass, the swab is rotated and another “S” pattern is made horizontally. Finally, the swab is rotated again and a diagonal “S” pattern is performed over the sampling surface [16]. Of the literature reviewed, 10 sources contained identified data for surface type sampled, RE, and sample area size [18-27]. Table 3 summarizes data from those sources on surface type sampled (in alphabetic order), RE, sample area size, and number of samples (where available) for swab sampling. Note that, in most cases, RE values in the reference material were presented as a percentage; however, in a limited number of instances, RE was presented as a fractional value. Fractional values are identified via footnotes in Table 3, as well as subsequent sampling summary tables. Please refer to the list of acronyms and abbreviations at the beginning of this document for acronym definitions.

Table 3. Summary of Available Data for Swab Sampling

Source ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
Nonporous Surfaces			
[24]	Aluminum, V2A steel, multilayer insulation (MLI) foil, Kapton, Teflon	19.8–73.7	25 cm^2

Source ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
[20]	Chemical-agent-resistant coating (CARC)-painted steel	Liquid-deposited spores—cotton: 47.0, Dacron: 42.5, rayon: 43.6, macrofoam: 55.7. Aerosol-deposited spores—cotton: 51.9, Dacron: 57.6, rayon: 53.1, macrofoam: 51.5.	10 cm^2
[24]	Glass	42.5–89.1	10 cm^2
[24]	Glass	42.1–92.7	10 cm^2
[24]	Glass	68.6–73.5	5 cm^2
[20]	Glass	Liquid-deposited spores—cotton: 88.7, Dacron: 82.1, rayon: 87.5, macrofoam: 89.1. Aerosol-deposited spores—cotton: 62.4, Dacron: 64.9, rayon: 65.2, macrofoam: 61.2.	10 cm^2
[19]	Glass	52.2 ± 8.3	317 cm^2
[24]	Glass, stainless steel, polycarbonate, vinyl/vinyl tile	51.5–75.5	10 cm^2
[19]	Metal file cabinet	39.5 ± 5.3	317 cm^2
[24]	Monitor	0.5–2.7	25 cm^2
[24]	Petri dish	35.9–66.1	25 cm^2
[19]	Plastic seat	31.2 ±1.5	317 cm^2
[20]	Polycarbonate	Liquid-deposited spores—cotton: 74.9, Dacron: 83.4, rayon: 75.4, macrofoam: 88.3. Aerosol-deposited spores—cotton: 65.1, Dacron: 71.9, rayon: 68.9, macrofoam: 75.5	10 cm^2
[24]	Directly inoculated stainless steel	99.9	6.25 cm^2
[24]	Stainless steel	31.7–49.1	10 cm^2
[24]	Stainless steel	0.1–43.6	25.8 cm^2
[24]	Stainless steel	35.5–45.6	25 cm^2
[24]	Stainless steel	15.8–83.1	26 cm^2
[18]	Stainless steel	100–1,000 CFU/cm^2: 0.395 (range 0.151–0.659)[a] 10,000–100,000 CFU/cm^2: 0.429 (range 0.146–0.747)[a] Overall: 0.414 (range 0.146–0.747)[a]	25 cm^2

Source ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
[25]	Stainless steel	Nylon-flocked swab with phosphate-buffered saline including 0.02% [vol/vol] Tween 80 (PBST) extraction solution and vortexing: 45.5 ± 1.2 (SE = 2.74) Nylon-flocked swab with water extraction solution and vortexing and sonication: 49.0 ±1.9 (SE = 4.18) Cotton swab (standard National Aeronautics and Space Administration (NASA) protocol): 13.2 ± 1.2 (SE = 2.32) Recovery of *Ba* from stainless steel: Nylon-flocked-swab with PBST extraction solution and vortexing: 19.7 Nylon-flocked-swab with water extraction solution and vortexing and sonication: 19.6 Cotton swab (standard NASA protocol): 6.5	25 cm^2
[21]	Steel	3 CFU/100 cm^2: 3.4 30 CFU/100 cm^2: 6.5 200 CFU/100 cm^2: 5.0	103 cm^2
[22]	Steel	31.7–49.1	10 cm^2
[27]	Steel	Cotton Swab: Dry, no extraction: 0.5 Pre-moistened, no extraction: 4.7 Dry, vortex: 8.0 Pre-moistened, vortex: 41.7 Dry, sonication: 6.9 Pre-moistened, sonication: 13.6	4 in^2 (2×2 in)
[27]	Steel	Macrofoam Swab: Dry, no extraction: 0.7 Pre-moistened, no extraction: 6.3 Dry, vortex: 11.9 Pre-moistened, vortex: 43.6 Dry, sonication: 12.7 Pre-moistened, sonication: 17.7	4 in^2 (2×2 in)
[27]	Steel	Rayon Swab: Dry, no extraction: 0.1 Pre-moistened, no extraction: 1.0 Dry, vortex: 4.4 Pre-moistened, vortex: 11.5 Dry, sonication: 4.5 Pre-moistened, sonication: 8.5	4 in^2 (2×2 in)
[27]	Steel	Polyester Swab: Dry, no extraction: 0.1 Pre-moistened, no extraction: 2.0 Dry, vortex: 2.1 Pre-moistened, vortex: 9.9 Dry, sonication: 1.4 Pre-moistened, sonication: 11.2	4 in^2 (2×2 in)
[23]	Teflon	75.20	6,451.6 cm^2

Source ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
[20]	Vinyl tile	Liquid-deposited spores—cotton: 49.0, Dacron: 62.2, rayon: 58.3, macrofoam: 72.0. Aerosol-deposited spores—cotton: 60.3, Dacron: 68.7, rayon: 60.2, macrofoam: 67.0	10 cm^2
[19]	Vinyl tile	41.1 ± 3.5	317 cm^2
[18]	Painted wallboard	100–1,000 CFU/cm^2: 0.355 (range 0.030-0.887)[a] 10,000–100,000 CFU/cm^2: 0.456 (range 0.072-0.808)[a] Overall: 0.405 (range 0.030-0.887)[a]	25 cm^2
[19]	Wood laminate	28.6 ± 6.6	317 cm^2
Nonporous and Porous Surfaces			
[24]	Stainless steel, carpet	3.4–14.0	103 cm^2
[24]	Plastic, wood, cotton	0.2–5.5	1 cm^2
[24]	Plastic, oak wood, polyester upholstery fabric, carpet	0.6–6.6	104.04 cm^2
Porous Surfaces			
[21]	Carpet	3 CFU/100 cm^2: 12 30 CFU/100 cm^2: 14 200 CFU/100 cm^2: 12	103 cm^2
[19]	Finished concrete	0.8 ± 0.1	317 cm^2
[19]	Nylon cushion	3.9 ± 0.1	317 cm^2
[26]	Vectran fabric	Foam-tipped swab: 1.4 Nylon-flocked swab: 5.9 Puritan swab 3600: 2.4 Puritan swab 3655: 5.3 Whatman FTA card applicator: 6.6	25 cm^2

[a] RE reported as fractional values in this source.

G.F. Piepel et al. summarized 20 laboratory studies to evaluate collection, storing/transporting, processing, and analyzing samples from surfaces contaminated by *Ba*, including swab, wipe, sponge, and vacuum samples of porous and nonporous surfaces. The authors identified knowledge gaps and suggested areas for future studies. Some of the studies used methods that are out-performed by other methods, were limited in their scope, and addressed repeatability uncertainty but not reproducibility uncertainty. In their summary, Piepel et al. suggested future studies should address false negative rates (FNRs) and quantify reproducibility uncertainty, not just repeatability uncertainty [24].

A study by Estill et al. tested swab, wipe, and vacuum sampling techniques with low, medium, and high surface loadings of *Ba Sterne* spores. Sampling techniques were tested on steel and carpet. In terms of CFU/sampled area, wipes had the lowest limit of detection (LOD) on stainless steel, and swabs had the lowest LOD on carpet. The authors noted that LOD estimates should be interpreted with caution due to limitations of small sample sizes, less than ideal surface concentrations, and reaerosolization of spores during sampling. Furthermore, the recovery efficiencies and LOD values from the study should not be considered definitive as precision and confidence intervals should also be considered. Lastly, the authors

note that the results do not consider the variance introduced by the use of multiple technicians to collect samples and that precision improved with increasing surface concentration Estill, Baron [21].

Edmonds et al. studied whether differences existed in swab sampling recovery efficiencies for glass, steel painted with chemical-agent-resistant coating (CARC), polycarbonate, and vinyl tile and demonstrated that recovery of liquid-deposited spores differs significantly from recovery of dry aerosol-deposited spores in most instances [20].

Brown et al. evaluated the CDC-recommended swab surface sampling method in terms of RE and LODs from stainless steel and painted wallboard using rayon swabs. After determining a mean extraction efficiency of 75.6% with a standard deviation of 11.8%, spore material specifically prepared for aerosol suspension and inhalation was deposited on test coupons using a dry aerosol deposition system. Spores were deposited using both low and high surface loadings. Spores were collected from the coupons using rayon swabs wetted with sterile deionized water. Spores were also collected from reference coupons seeded alongside the sample coupons by placing the reference coupons into containers of buffer solution. Spores from the swabs and reference coupons were extracted via sonication. For stainless steel, the average recovery efficiency was 41.4% with a standard deviation of 16.7%. For painted wall board, the average recovery efficiency was 40.5% with a standard deviation of 23.2%. LOD was reported to be 25 CFU per sample area (25 cm^2) for both stainless steel and painted wallboard. The authors noted no statistical difference in recovery efficiencies between stainless steel and wallboard but indicated a high difference in recovery efficiency variability between the two coupon types [18].

L.R. Hodges et al. evaluated the recovery of spores by pre-moistened macrofoam swabs and vortex processing. Testing was conducted by five analysts from two laboratories. RE for all analyses ranged from 38.0% to 49.1% [22].

Buttner et al. tested a sponge (the *Speci-sponge*; Nasco, Fort Atkinson, WI) and a macrofoam swab (the *SW Kit* – currently named the Sample Collection and Recovery Device; ASD BioSystems, Danville, VA) for their sampling efficiencies. Overall efficiencies ranged from 0.7% to 52.2%, depending on the sampling method and the surface material. During laboratory trials, the swab was more efficient than the sponge by a factor of approximately 1.1 to 2.5 for five of the seven surface types. Efficiency for both methods was highest on glass surfaces and lowest for finished concrete. Test chamber trials supported laboratory results that the swab is generally more efficient than the sponge. Efficiency for both methods was highest on the metal file cabinet, vinyl tile, and wood laminate surfaces. Computer monitor screens and finished concrete had the lowest efficiencies [19].

In a study by Probst et al., ten different sampling methods were tested for their efficiency at recovering *B. atrophaeus* spores from Vectran fabric, a material used for spacecraft airbags. The most efficient method was a wipe-rinse technique with a foam-spatula protocol (13.2% efficiency). Compared with the standard wipe sampling technique used the by European Space Agency (ESA), the foam-spatula protocol performed better with an efficiency of 41.1% while the standard method had an efficiency of 13.9%. Multiple experiments were conducted with different spore concentrations and different types of *Bacillus* species and different methods of inoculating surfaces (spotting versus aerosolization) resulting in differences in recovery efficiencies. Sampling efficiency was found to depend largely on sampling material and the surface type being sampled [26].

A study by Rose et al. tested cotton, macrofoam, polyester, and rayon swabs for their efficiency in recovering *Ba* spores from nonporous 2×2-in steel coupons. Two swab preparation methods (pre-moistened and dry) were also tested. The authors found that, when vortexed, pre-moistened macrofoam and cotton swabs had the highest recovery efficiencies with mean efficiencies of 43.6% (standard deviation [SD] 11.1%) and 41.7% (SD 14.6%), respectively. Mean RE values for pre-moistened and

vortexed polyester and rayon swabs were 9.9% (SD 3.8%) and 11.5% (SD 7.9%), respectively. The authors noted that various factors can contribute to poor recovery efficiencies: sampling material, target organism to be cultured, variations in sampling surface, and differences in personnel who are collecting and processing samples. The authors add that wide variation in the results from this study demonstrates the overall low efficiency and precision of swabs used for surface sampling but add that swab sampling may sometimes be the best available method [27].

Probst et al. stated that the National Aeronautics and Space Administration's (NASA's) standard cotton swab sampling protocol has not been improved upon in decades. To determine if there is a more efficient swab type and sampling method, the authors tested two protocols for a new type of nylon-flocked swab. These novel protocols were found to recover three to four times more *B. atrophaeus* spores than the standard NASA method, with recovery efficiencies of 45.4% and 49.0% compared to a recovery efficiency of 13.2% for the standard method. The recovery efficiencies of the protocols were also relatively high for seven different *Bacillus* species, including *Ba Sterne* (a RE of 20%). Sampled surfaces include 5×5-centimeter stainless steel, carbon-fiber-reinforced plastic, roughened carbon-reinforced plastic, and Vectran fabric type A. The authors noted that variation in recovery efficiencies depended on the roughness of the sampled surface. The highest recovery efficiencies were measured from sampling on carbon-fiber-reinforced plastic and stainless steel. Certain *Bacillus* species are also sampled at a higher efficiency, possibly due to different physiochemical adhesive properties of the spores (e.g., *Ba* has an exosporium while *B. atrophaeus* does not). Other factors that may affect RE include choice of extraction method. While cotton swabs may have recovery efficiencies as high as 41.7% based on recent studies, they may have significant shortcomings. As organic material, their inherent DNA content may lead to false positives. Improved sampling techniques will contribute to efforts in both spacecraft cleanliness control and the detection of *Ba* contamination [25].

3.2 Sponge Wipe and Gauze Wipe Sampling (Wipe Sampling)

Wipe sampling is appropriate for small nonporous surfaces between 645 cm^2 and 949 cm^2 using sponge wipes or gauze wipes [17]. These CDC-developed and -recommended procedures for sponge and gauze sampling and modified procedures are described by the EPA [28]. Wet or dry samples can be collected; wipes are generally used for smooth surfaces. Wipe sampling is appropriate for determining the extent and location of contamination and for screening particular items. It is also used for determining decontamination effectiveness [16].

When collecting wet samples, a sterile solution is applied to moisten the collection medium, and the wipe is moved over the sampling area in an overlapping vertical "S" pattern. After an initial pass, the wipe is rotated, and another "S" pattern is made horizontally. Next, the wipe is rotated again, and a diagonal "S" pattern is performed over the sampling surface. Finally, as opposed to swab sampling, the surface is then wiped around the perimeter.

Seven of the sources that were reviewed contained data for surface type sampled, RE, and sample area size for sponge wipe sampling [19, 21, 24, 26, 29-33]. Table 4 summarizes data on surface type sampled, RE, and sample area size for sponge wipe sampling.

Table 4. Summary of Available Data for Sponge Wipe Sampling

Document ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
Nonporous Surfaces			
[29]	Ceramic tile	48.90	645.16 cm^2
[24]	Glass	74.3	32.49 cm^2

Document ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
[19]	Glass	47.3 ±1.0	930 cm^2
[31]	Glass	48.40	100 cm^2
[33]	Glass, clean, high relative humidity (RH)	0.333 (95% CI: 0.219–0.447; SE=0.055; SD=0.270)	100 cm^2
[33]	Glass, dirty, high RH	0.547 (95% CI:0.367–0.727; SE=0.087; SD=0.426)[a]	100 cm^2
[33]	Marble, clean, high RH	0.436 (95% CI: 0.354–0.518; SE=0.041; SD=0.318)[a]	100 cm^2
[33]	Marble, clean, low RH	0.482 (95% CI: 0.393–0.570; SE=0.044; SD=0.342)[a]	100 cm^2
[33]	Marble, dirty, high RH	0.685 (95% CI: 0.514–0.855; SE=0.085; SD=0.656)[a]	100 cm^2
[33]	Marble, dirty, low RH	0.595 (95% CI: 0.481–0.709; SE=0.057; SD=0.434)[a]	100 cm^2
[19]	Metal file cabinet	44.8 ± 0.6	930 cm^2
[29]	Plastic panel	9.8	645.16 cm^2
[24]	Stainless steel	18–31	929 cm^2
[24]	Stainless steel	46.1–77.9	645.16 cm^2
[24]	Stainless steel	24.4–32.4	645.16 cm^2
[24]	Stainless steel	32.3	645.16 cm^2
[24]	Stainless steel	26.8	645.16 cm^2
[24]	Stainless steel	36.3	645.16 cm^2
[24]	Stainless steel	26	645.16cm^2
[29]	Stainless steel	48.1	645.16 cm^2
[32]	Stainless steel	RE, % (SE): 32.4 (4.4), 24.4 (2.8), and 30.1 (2.3) for the 1-, 2-, and 4-log10 inoculum levels, respectively	645.16 cm^2
[24]	Stainless steel, ceramic tile, vinyl/vinyl tile	12.5–75.5	645.16 cm^2
[33]	Stainless steel, clean, high RH	0.18 (95% CI: 0.126–0.233; SE=0.026; SD=0.145)[a]	100 cm^2
[33]	Stainless steel, clean, low RH	0.797 (95% CI: 0.676–0.917; SE=0.059; SD=0.344)[a]	100 cm^2
[33]	Stainless steel, dirty, high RH	0.198 (95% CI: 0.146–0.249; SE=0.025; SD=0.133)[a]	100 cm^2
[33]	Stainless steel, dirty, low RH	0.968 (95% CI: 0.872–1.065; SE=0.047; SD=0.285)[a]	100 cm^2
[21]	Steel	3 CFU/100 cm^2: 31 30 CFU/100 cm^2: 22 200 CFU/100 cm^2: 18	929 cm^2
[29]	Painted Wood	25.5	645.16 cm2
[19]	Plastic seat	18.1 ± 4.2	930 cm2
[19]	Wood laminate	11.4 ± 0.7	930 cm2
[29]	Vinyl tile	25.6	645.16 cm^2

Document ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
[29]	Faux leather	30.3	645.16 cm^2
[24]	Faux leather, painted wood, plastic light over panel	4.0–76.9	645.16 cm^2
Porous Surfaces			
[24]	Carpet	21–120	929 cm^2
[21]	Carpet	3 CFU/100 cm^2: 120 30 CFU/100 cm^2: 21 200 CFU/100 cm^2: 23	929 cm^2
[19]	Finished concrete	0.7 ± 0.2	930 cm^2
[19]	Nylon cushion	11.3 ± 1.8	930 cm^2
[26]	Vectran fabric	Inoculation by spray diffuser: 3.0 Inoculation by spray gun: 18.7 Inoculation by spotting: 13.2	400 cm^2
[26]	Vectran fabric	*Ba Sterne:* 0.3 *B. atrophaeus:* 13.2 *B. megaterium* 2c1: 5.1 *B. safensis:* 0.5 *B. thuringiensis* E24: 5.4	400 cm^2
[26]	Vectran fabric	Foam-spatula protocol on Vectran fabric: 13.2 Nylon-flocked spatula protocol on Vectran fabric: 4.2 SpongeSicle (cellulose tip material) protocol on Vectran fabric: 3.4	400 cm^2

[a] RE reported as fractional values in this source.

Rose et al. reported a cellulose sponge wipe-processing protocol tested by nine laboratories. Steel coupons were inoculated with *Ba Sterne* spores, then sampled with cellulose sponges. Mean percent recovery varied by level of inoculation. The efficiencies of many sampling methods (gauze wipes, foam spatula, and BiSKit; Quicksilver Analytics, Inc., Abingdon, MD) have been evaluated but not sufficiently to provide a validated method. The authors claim that the sponge wipe-processing method was validated by this controlled study [32].

A study by Krauter et al. evaluated the sponge-wipe method in a series of tests. Factors that were evaluated were the effects of contaminant concentrations on RE, the effects of surface materials on RE, FNR, LOD, and the uncertainties of all these values. The authors stated that a nearly linear dependence was found between surface roughness (i.e., vertical deviation of surface measured in μm) and RE, with smoother surfaces having higher recovery efficiencies. FNRs corresponded with RE data, also demonstrating that smoother surfaces resulted in higher recovery efficiencies and lower FNRs. The authors also speculated that the reasons for differences between the results of this study and the results of others include the use of different surrogate spores and the lack of a mixed microbial culture. The results of the study by Krauter et al. show linear dependence of RE and FNR on surface roughness, with smoother surfaces resulting in higher mean REs and lower FNRs. The authors contend that these findings may have implications for field sampling, suggesting that field technicians might sample smooth surfaces and take quick measurements of the roughness index to estimate RE, FNR, and LOD [29].

A study by Lewandowski et al. evaluated a polyester swab, a macrofoam sponge wipe, and a foam spatula in terms of recovering *B. atrophaeus* and *Pantoea agglomerans* from 20 by 20 cm (400 cm^2) glass and

stainless steel surfaces. Aerosolized spores were used in the study because they are more representative of deposition during previous incidents than spores that are deposited via spore suspension. The coupons were divided into 10 by 10 cm (100 cm^2) quadrants, so only one quadrant on one plate was swabbed to avoid cross-contamination. Two quadrants were swabbed in experiments that tested vertical and horizontal surfaces. Using a macrofoam sponge wipe was more efficient than a polyester swab at recovering spores deposited by aerosol. Median RE for foam spatulas was 9.9% for *B. atrophaeus* spores on glass. Mean RE was 48.4%. The RE of the foam spatula was dependent on the concentration of spores aerosolized. Dilutions of samples were plated and incubated for 24 hours or for one to three days. The authors calculated RE relative to the theoretical number of spores that are deposited on a surface. They note the lack of a method to accurately measure the number of spores that settle on a surface in an experiment, but the foam spatula appears to be a reliable indicator of the level of *B. atrophaeus* aerosolized spore-contaminated surfaces [31].

Einfeld et al. discuss the evaluation and recovery of *B. atrophaeus* spores from grime-treated and clean surfaces in a controlled chamber study and outdoor surfaces including stainless steel, glass, marble, and concrete using wipe and vacuum sampling methods. Testing was conducted using both low- and high-humidity conditions, and the results show that spore recovery from grime-coated surfaces is the same as or better than spore recovery from clean surfaces. Statistically significant differences between method performance for grime-coated and clean surfaces were observed in only approximately half of the chamber tests conducted [33].

Of the literature reviewed, eight sources contained data for surface type sampled, RE, and sample area size for gauze wipe sampling [23, 24, 26, 32, 34]. Table 5 summarizes data on surface type sampled (in alphabetic order), RE, and sample area size for gauze wipe sampling.

Table 5. Summary of Available Data for Gauze Wipe Sampling

Document ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
NonPorous Surfaces			
[24]	Metal	11.3–18.5	10,000 cm^2
[24]	Monitor	2.6–4.2	25 cm^2
[34]	Painted wallboard	100–1,000 CFU/cm^2: 0.325 (range 0.081-0.574) 10,000–100,000 CFU/cm^2: 0.252 (range 0.081-0.566) Overall: 0.285 (range 0.081–0.574)	25 cm^2
[24]	Directly inoculated stainless steel	99.90	25 cm^2
[34][a]	Stainless steel	100–1,000 CFU/cm^2: 0.312 (range 0.153–0.509) 10,000–100,000 CFU/cm^2: 0.392 (range 0.167–0.674) Overall: 0.346 (range 0.153–0.674)	25 cm^2
[24]	Stainless steel	30.80	646.16 cm^2
[24]	Stainless steel, glass, marble	18.0–96.8	100 cm^2
[24]	Stainless steel, painted wallboard	25.2–39.2	25 cm^2
[23]	Teflon	90.40	1.3 m^2
Nonporous and Porous Surfaces			
[24]	Painted wallboard, stainless steel, vinyl/vinyl tile, wood	39.50	900 cm^2

Document ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
[24]	Plastic, oak wood, polyester upholstery fabric, carpet	1.4–7.9	104.04 cm^2
[24]	Plastic, wood, cotton, cloth	0.2–6.6	1 cm^2, not recorded
Porous Surfaces			
[24]	Heating, ventilation, and air condition (HVAC) filter	14.5–19.9	100 cm^2
[26]	Vectran fabric	Polyester Spec wipe 7 wipers, 115-0043 (ESA standard) from Vectran fabric: 0.5 Polyester Vectra Alphasorb TX 1050 from Vectran fabric: 0.5	400 cm^2

[a] The data presented in [34] were referenced by [32], which is also included in the bibliography presented and the end of this document.

Studies containing relevant data on gauze wipe sampling and not previously discussed in the above sections for another sampling method are summarized below.

Brown et al. evaluated extraction efficiencies, recovery efficiencies, and LODs for nonporous surface sampling (stainless steel and painted wallboard) using polyester-rayon blend wipes and the current CDC wipe collection method. The surrogate used in this study was *B. atrophaeus*. Spore material specifically prepared for aerosol suspension and inhalation was deposited on test coupons using a dry aerosol deposition system. Spores were deposited using both low and high surface loadings. Spores were collected from the coupons using sterile polyester-rayon blend gauze wipes moistened with sterile deionized water. Spores were also collected from reference coupons, seeded alongside the sample coupons, by placing the reference coupons into containers of buffer solution. Spores were extracted from the wipes and reference coupons via sonication. The average recovery efficiency for the stainless steel coupons was reported to be 34.6% with a standard deviation of 12.2%. The LOD for stainless steel was reported to be 90 CFU per unit of sample area. The average recovery efficiency for the painted wallboard coupons was reported to be 28.5% with a standard deviation of 15.2%. The LOD for painted wallboard was reported to be 105 CFU per unit of sample area. While the surface area evaluated in this study was 25 cm^2, the investigators assumed "that the number of CFU required for detection is independent of the sample surface area and primarily a function of recovery efficiency." The results of the study indicated statistically significant lower recovery efficiencies of spores using the wipe method for painted wallboard versus stainless steel, though both are considered nonporous surfaces. The authors indicated the difference in recovery efficiencies is likely due to differences in surface texture and physiochemical adhesive properties between the surface types. The authors also presented several causes for the large variability in the results for each coupon type including errors in sampling mechanism, sample collection, and sample processing. The authors also indicated that variability in surface deposition and the incomplete removal of spores from reference coupons could also contribute to overall variability in the results. [34].

A study by Sanderson et al. describes the relative effectiveness of sampling using dry swabs, wet swabs, vacuum socks, and wipes. The results are presented as median and range of CFU/cm^2, but relative recovery efficiencies could not be determined because the initial concentrations of spores on the sampled surfaces were not known. This study evaluated the results of wet and dry swab (sterile rayon—noncotton), wipe (sterile rayon gauze pad), and vacuum sock surface sampling of nonporous surfaces at a U.S. postal facility contaminated with *Ba*. The study compared detection percentage and spore concentration for the collection methods and materials used, but not collection efficiencies (since the

initial concentrations of the spores on the various surfaces were unknown). The results of the study suggest that dry swabs should not be used for *Ba* sampling: wipes are preferred for nonporous surfaces with light dust, and vacuum socks for heavily dusted surfaces [35].

Kirschner and Puleo tested polyester-bonded cloth (PBC) for its efficiency in sampling large areas. The authors determined it was most effective on surfaces less than 0.74 m^2 (8 ft^2) in area. PBC was found to have an RE of 90.4% versus 75.2% for cotton. The focus of this study is on sampling spacecraft surfaces. Advantages of the PBC over cotton swabs include the higher release of spores by the PBCs (i.e., cotton swabs tend to retain organisms), and a higher surface area sampled per unit. The bioassay of 6,451.6 cm^2 (1,000 in^2) requires 250 cotton swabs for full coverage. Alternatively, only one PBC would be needed for the same area and coverage. The maximum effective sampling area ranges between 0.74 and 1.49 m^2, but for standardization, this area should be reduced to 0.74 m^2. In terms of time needed to take samples, an experienced team can collect approximately one cotton swab per two-minute period. The same team should be able to sample an unobstructed 0.74 m^2 area in the same amount of time. While this wipe-rinse technique was designed for spacecraft hardware surfaces, its efficiency may prove useful in other settings [23].

3.3 Vacuum Sampling

As opposed to swab, wipe, and gauze sampling, vacuum collection can be used on porous surfaces as well as nonporous surfaces and where the surface may not be entirely smooth or flat. Furthermore, vacuums can be used over wider areas to collect bulk samples. Vacuum sampling uses a HEPA filter on the exhaust and a sample collection sock or cassette attached to the inlet of the vacuum collection hose. A specialized sampling vacuum, such as an Atrix Omega (Atrix International, Inc., Burnsville, MN) is used to collect samples [16].

Of the literature reviewed, five sources contained identified data for surface types sampled, RE, and sample area size for vacuum sampling [21, 24, 33, 36, 37]. Table 6 summarizes data on surface type sampled (in alphabetic order), RE, sample area size, and number of samples (where available) for vacuum sampling.

Table 6. Summary of Available Data for Vacuum Sampling

Document ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
Nonporous Surfaces			
[33]	Marble, clean, high RH	0.121 (95% CI:0.067–0.171; SE=0.012; SD=0.069)[a]	100 cm^2
[33]	Marble, clean, medium RH	0.119 (95% CI: 0.067–0.171; SE=0.056; SD=0.154)[a]	100 cm^2
[33]	Marble, dirty, high RH	0.170 (95% CI: 0.142–0.199; SE=0.014; SD=0.085)[a]	100 cm^2
[33]	Marble, dirty, medium RH	0.100 (95% CI: 0.056–0.144; SE=0.021; SD=0.123)[a]	100 cm^2
[36]	Painted wallboard	100–1000 CFU/cm^2: 0.245 (range 0.041–0.577) [a] 10,000–100,000 CFU/cm^2: 0.250 (range 0.204–0.295) [a] 100–100,000 CFU/cm^2: 0.248 (range 0.035–0.577) [a]	100 cm^2
[24]	Stainless steel	3.7–5.5	929 cm^2

Document ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
[36]	Stainless steel	100–1,000 CFU/cm^2: 0.321 (range 0.062–0.551)[a] 10,000–100,000 CFU/cm^2: 0.231 (range 0.091–0.414) [a] 100–100,000 CFU/cm^2: 0.289 (range 0.062–0.551) [a]	100 cm^2
[21]	Steel	3 CFU/100 cm^2: 5.5 30 CFU/100 cm^2: 4.7 200 CFU/100 cm2: 3.70	929 cm^2
Nonporous and Porous Surfaces			
[24]	Ceiling tile, painted wallboard, stainless steel, vinyl/vinyl tile, wood	4.4	900 cm^2
[24]	Marble, concrete	10.0–19.7	100 cm^2
[24]	Stainless steel, painted wallboard, carpet, concrete	16.4–36.1	100 cm^2
Porous Surfaces			
[37]	Carpet	Vacuum sock (slow): 25.1 Vacuum sock (fast): 15.2	2787 cm^2
[24]	Carpet	3.7–6.3	929 cm^2
[24]	Carpet	0.20–0.48	400 cm^2
[24]	Carpet	2.26–1.69	400 cm^2
[21]	Carpet	3 CFU/100 cm^2: 6.3 30 CFU/100 cm^2: 3.7 200 CFU/100 cm^2: 4.7	929 cm^2
[36]	Carpet	100–1,000 CFU/cm^2: 0.361 (range 0.057–0.650) [a] 10,000–100,000 CFU/cm^2: 0.229 (range 0.136–0.366) [a] 100–100,000 CFU/cm^2: 0.282 (range 0.057–0.650) [a]	100 cm^2
[37]	Concrete	Vacuum sock (slow): 10.2 Vacuum sock (fast): 11.7 37 mm mixed cellulose ester (MCE) filter cassette: 48.7	2787 cm^2
[36]	Concrete	100-1000 CFU/cm^2: 0.216 (range 0.011–0.627) [a] 10,000–100,000 CFU/cm^2: 0.164 (range 0.059–0.311) [a] 100–100,000 CFU/cm^2: 0.189 (range 0.011–0.627) [a]	100 cm^2
[33]	Concrete, clean, high RH	0.165 (95% CI: 0.144–0.187; SE=0.011; SD=0.082)[a]	81 cm^2 circular coupon
[33]	Concrete, dirty, high RH	0.197 (95% CI: 0.170–0.225; SE=0.014; SD=0.104)[a]	81 cm^2 circular coupon
[24]	HVAC filter	2.42	309.68 cm^2
[24]	HVAC filter	1.16–3.52	400 cm^2

Document ID	Surface Type	Recovery Efficiency (RE, %)	Sample Area Size
[37]	Upholstery	Vacuum sock (slow): 4.2 Vacuum sock (fast): 9.1 3M™ Forensic filter: 1.4	2787 cm^2

[a]RE values reported as fractional values in this source.

Calfee et al. performed a comparative evaluation of vacuum-based methods using vacuum socks, 37 mm mixed cellulose ester (MCE) filter cassettes, 37 mm polytetrafluoroethylene (PTFE) filter cassettes, and forensic evidence filters to collect samples from carpet, concrete, and upholstery [37]. For the vacuum sock filters, both a slow and fast pace were used to collect samples. The surrogate used in the study was *B. atrophaeus*. Spore material was deposited on the sampling coupons using a metered dose inhaler. Spore material was deposited simultaneously on stainless steel reference coupons which were subsequently sampled with pre-moistened wipes. The wipe recovery results from the stainless steel coupons were used to normalize the recoveries of the vacuum based sampling methods. The 37 mm MCE method resulted in significantly higher relative recoveries than the other studied methods when sampling concrete and upholstery. For the concrete coupons, the 37 mm MCE filter had a relative recovery of 1.242 as compared to the next highest value, 0.33 for the forensic filter. For the upholstery coupons, the 37 mm MCE filter had a relative recovery of 0.350 as compared to the next highest value, 0.232 for the vacuum sock used at a fast pace. On carpet, the vacuum sock used at a slow pace had a significantly higher relative recovery of 0.641 as compared to all other studied methods, except the 37 mm MCE filter with a relative recovery of 0.474. The authors speculated that the MCE filter demonstrated higher recovery efficiencies because it likely has fewer spores tightly bound to the collection media as compared to the vacuum sock and forensic filter. The authors provided the time required for sampling and analysis for each method and concluded that sample collection speed and ease of laboratory analysis should be considered, in addition to recovery efficiency, in selecting a sampling method [37].

Another study by Brown et al. evaluated vacuum filter sock sampling in terms of recovery efficiency and LODs from nonporous surfaces (stainless steel and painted wallboard) and porous surfaces (carpet and bare concrete) [36]. The surrogate used in this study was *B. atrophaeus*. Spore material specifically prepared for aerosol suspension and inhalation was deposited on test coupons using a dry aerosol deposition system. Spores were deposited using both low and high surface loadings. Spores were collected from the coupons using 100% polyethylene vacuum filter socks. Spores were also collected from reference coupons, seeded alongside the sample coupons, by placing the reference coupons into containers of buffer solution. Spores were extracted from the vacuum filter socks and reference coupons via sonication. The average recovery efficiency for the stainless steel coupons was reported to be 28.9% with a standard deviation of 13.8%. The average recovery efficiency for painted wallboard was reported to be 24.8% with a standard deviation of 14.5%, the average recovery efficiency for carpet and bare concrete was reported to be 28.2% and 18.9%, with standard deviations of 13.4% and 14.1%, respectively. The LOD for stainless steel and carpet was reported to be 105 CFU per unit of sample area. The LOD for painted wallboard was reported at 120 CFU per unit of sample; bare concrete was 160 CFU per unit of sample area. The authors noted a high variability in recovery efficiency between all the surface types and discussed sources of potential error. The authors indicated that no statistically significant difference was detected when comparing recovery efficiencies between porous and nonporous surfaces, indicating this was a departure for normal trends for other sampling methods such as swabs or wipes [36].

3.4 Air Sampling

Air sampling methods are not always available for the initial first response or characterization of biological contamination but may be appropriate for helping determine the existence of contamination in the air or rapidly identify contaminated indoor areas. To perform air sampling, a sampling pump is used

to draw air through dry filters, impingers, or impactors [16]. Dry filter sampling is generally used for biological agent detection and filter types include gelatin, MCE inert, and Teflon filters.

Data from three sources were identified as being relevant, primarily for RE and sample rate. RE for air sampling is simply the RE from the media after collection and does not include capture/collection efficiency. These data are summarized in Table 7.

In one study, water-soluble tape was used in place of swabs to measure reaerosolization of spores from polyvinyl chloride (PVC) floor and carpet in an environmental chamber. The study also showed water-soluble tape is a viable replacement for swabs [38]. Another study measured filter materials and extraction methods that are best suited for environmental sampling of *Ba* using *B. subtilis var. niger* endospores (also known as *B. globigii*) as a surrogate. MCE and PTFE filters combined with vortexing and shaker extraction yielded the best performance for the filter collection and extraction of *B. globigii* spores [39].

Table 7. Summary of Available Data for Air Sampling

Document ID	Recovery Efficiency (RE, %)	Sample Rate
[38]	99.999[a]	50 liters/min of air flow rate
[39][b]	Physical collection efficiency by filter type: MCE (3 µm): 97.6; polytetrafluoroethylene (1 µm): 94.2; polytetrafluoroethylene (3 µm): 63.6; gelatin (3 µm): 97.9; gelatin (3 µm): 94.3; polycarbonate (3 µm): 61.4	4 liters/min of air flow rate for inhalable aerosol samplers

[a] The source presents the results of a study to determine reaerosolization of spores from flooring surfaces. However, the authors indicate the sampling efficiency of the apparatus used in the study has previously been shown to be 99.999%for *B. atrophaeus* spores. The original source is cited as Parks SR, Bennett AM, Speight SE, Benbough JE. 1996. An assessment of the Sartorius MD8 microbiological air sampler. J. Appl. Bacteriol. 80: 529–534. http://dx.doi.org/10.1111/j.1365-2672.1996.tb03252.x, last accessed March 2017.
[b] The data presented in Document ID 38 were referenced by Document ID 2, which is also included in the bibliography presented at the end of this document.

3.5 Aggressive Air Sampling

Aggressive air sampling has been used to confirm a negative finding of lead dust and asbestos contamination, either as part of a public health investigation, during clearance after decontamination for an area known to be contaminated, or at Superfund sites with where air is a pathway of exposures. While air sampling has been used for asbestos and *Ba*, there is limited information on sensitivity and impacts from environmental conditions [40]. Aggressive air sampling involves (1) vigorous agitation of surfaces in a space (using leaf blowers, for example) to aerosolize particles and (2) use of high-volume air samplers to acquire and concentrate aerosolized materials for analysis [16].

3.6 Robotic Floor Sampling

One innovative sampling technique is the use of robotic floor cleaners (RFCs). These devices may be used as a force multiplier and an alternative to exposing personnel to contaminated areas. A recent EPA study evaluated three vacuum-based cleaning robots, one wipe-based robot, and one wet vacuum-based robot for their sampling efficiency on nonporous surfaces (laminate and tile). EPA also evaluated the vacuum-based RFCs on carpet to assess efficiency on a nonporous surface. The results showed that sampling via RFC is a viable option—in some cases, the RFCs were as effective as currently recommended methodologies. The sampling efficacy of a wet-mop RFC was comparable to the currently used wet-wipe method on nonporous surfaces with low spore loadings. Vacuum RFCs performed

comparably to vacuum sock sampling. Overall, these technologies show promise for future sampling. The investigators compared RFC test results to currently used surface sampling methods (vacuum sock for carpet and sponge wipe for laminate). The results showed that the average sampling efficacies for three vacuum based RFCs on carpet were 26%, 162%, and 92% of vacuum sock sampling efficacy, respectively. On laminate, five RFCs including vacuum, wet mop, and wet vacuum, average sampling efficacies were 8%, 11%, 2%, 62%, and 32% of sponge wipe sampling efficacy, respectively. The authors concluded that some robotic cleaners were as efficient as the currently used surface sampling methods for *B. atrophaeus* spores on these surfaces [41].

A subsequent study evaluated two RFCs to explore their effectiveness at collecting samples widely dispersed in a small area (approximately 4.5 square meters) and located in a small hot spot covering 2% of the overall area within that same small area. A vacuum-based robot was used to assess effectiveness on a carpet surface while a wipe-based robot was used on a laminate floor surface. The effectiveness was compared to the effectiveness of the currently-used sampling methods, including vacuum sock and sponge wipe. This study helped demonstrate that cleaning robots have various benefits for wide-area sampling over the currently used sampling methods: fewer samples (i.e., one per deployment), composite samples (fewer samples requiring analysis), higher chance to detect hotspots, and less risk of personnel exposure [42]. Data from these two studies are summarized in Table 8 below. The first study [41] reported recovery efficiencies relative to sponge wipe and vacuum sampling recovery efficiencies and the second absolute spore concentrations (CFU/area).

Table 8. Summary of Available Data for Robotic Floor Sampling

Source ID	Surface Type	Spore Loading Concentration	Recovery	Sample Area Size
Nonporous Surfaces				
[41]	Laminate	~1x10^6 on 30.5 x 30.5 cm	Comparative Recovery[a] Robot R1: 8.1 Robot R2: 10.9 Robot R3: 2.4 Robot R4: 61.7 Robot R5: 31.9	71.5 cm^2
[42]	Floor laminate	Hotspot scenario: 10^4 CFU/cm^2 (but only loaded onto approximately 2% of the entire area of the room) Widely dispersed scenario: 10^{-1} CFU/cm^2	Hotspot scenario: 1.2×10^7 and 2.1×10^6 CFU Widely dispersed scenario: 2.8×10^3 and 4.9×10^2 CFU	Entire test surface: 4.4 m^2; inoculated area: 1.8 m^2

Source ID	Surface Type	Spore Loading Concentration	Recovery	Sample Area Size
Porous Surfaces				
[41]	Carpet	~1x10^6 on 30.5 x 30.5 cm	Comparative Recovery[b] Robot R1: 25.8 Robot R2: 161.5 Robot R3: 91.9	71.5 cm^2
[42]	Carpet	Hotspot scenario: 10^4 CFU/cm^2 (but only loaded onto about 2% of the entire area of the room) Widely dispersed scenario: 10 CFU/cm^2	Hotspot scenario: 1.4×10^5 and 5.8×10^5 CFU Widely dispersed scenario: 5.0×10^3 and 3.2×10^2 CFU	Entire test surface: 4.4 m^2; inoculated area: 1.8 m^2

[a] Source data presented are recovery efficiencies as compared to a sponge wipe sampling method on laminate surface.
[b] Source data presented are recovery efficiencies as compared to a vacuum sock sampling method on carpet surface.

3.7 Summary of Sampling Methods and Data

The sampling methods reviewed and the aggregated data detailed above highlight the vast range of reported RE values for differing surface types, spore concentrations, and (in some cases) deposition methods. Additionally, the data were collected under largely controlled conditions and there is the possibility of potential biasing of the RE values due to the individual collecting the samples or conducting the sampling. Furthermore, the identified sampling methods have been characterized on the selected surfaces, mainly focused on indoor surfaces. A wide-area biological incident may require sampling of indoor as well as outdoor surfaces and possibly underground transit systems. It is expected that the sampling in a wide-area incident may require sampling very different surfaces compared to (or in addition to) the ones evaluated in the studies above. Only a small number of surface sampling methods are well-documented with their sampling protocols. It is uncertain, if a wide-area biological incident happens, how the impacted areas would be characterized and how much resources would be required with the currently-available sampling methods.

Some of the major gaps in our knowledge of sampling methods and capabilities to conduct wide-area sampling currently include;

- Prediction of sampling performance (RE) in real-world settings based on laboratory studies
- Lack of method performance data (RE) for many/most outdoor surface types
- Prediction of analytical method accuracy and sensitivity in real-world settings, particularly outdoor samples
- Detailed field-use protocols for all sampling methods are not available
- Inconsistent laboratory test parameters and conditions, resulting in inability to adequately compare sampling method performance across studies

4 WIDE-AREA INCIDENT HYPOTHETICAL SCENARIO

4.1 Introduction

For this scenario, a statistically defendable sampling plan was developed, based on a hypothetical city. Realistic spatial, infrastructure, and surface media information was gathered to determine the extent of sampling and to match surface material types at each sampling location to the appropriate sampling

method. The most appropriate sampling method for each surface material was chosen based upon the findings of the sampling method review (Chapter 3). The resulting data, along with data gathered during the literature survey portion of this study (Chapter 2) were loaded into a custom spreadsheet that estimated the total resource demand required for characterization. Sections below describe the methodology, inputs, and results of this study.

4.2 Methodology

4.2.1 Hypothetical Scenario

For this study, NPS #2 was used to simulate a biological attack by way of aerosolized *Ba* spores. According to NPS #2, a truck, concealing an improvised spraying device, covertly aerosolizes approximately 100 liters of a wet-fill *Ba* spore slurry (10^9 CFU/mL) into a densely packed urban area. Approximately 1%, or 10^{12} CFU, of *Ba* spores are dispersed across two square miles.

To bind the event to some spatial context, a plume from a prior wide-area biological exercise was used [43]. The plume served as a reference point for surface and infrastructure characteristics for a city of comparable size and for plotting sampling points. The area of interest is further described in the following sections.

4.2.2 Assumptions

4.2.2.1 Environment

This study used a hypothetical city: a very large, densely populated urban area with a number of high-rise buildings and a highly developed underground transportation system. A tangible city was used to derive the spatial, infrastructure, and surface media information, using a combination of EPA and geographic information system (GIS) tools and estimates. This study also used a plume derived from an earlier biological exercise to define the area of contamination. The outputs from this model were converted to a shapefile (i.e., polygon) for use in GIS software. Using the GIS software, a half-mile buffer was applied to the plume to represent an area of uncertainty. Using the oval-shaped polygon, surface area and characteristics were extrapolated from intersecting outdoor, indoor, and underground geospatial data. The total estimated surface area for the outdoor, indoor, and underground environments is approximately 2, 15, and 0.5 square miles, respectively. Figure 1 shows a to-scale comparison of total ground/floor surface area across the three environments. Note that the indoor surface area is approximately nine times greater than the outdoor surface area, which would likely be the case for most densely populated urban areas. The following sections describe the spatial and temporal boundaries for the outdoor, indoor, and underground areas.

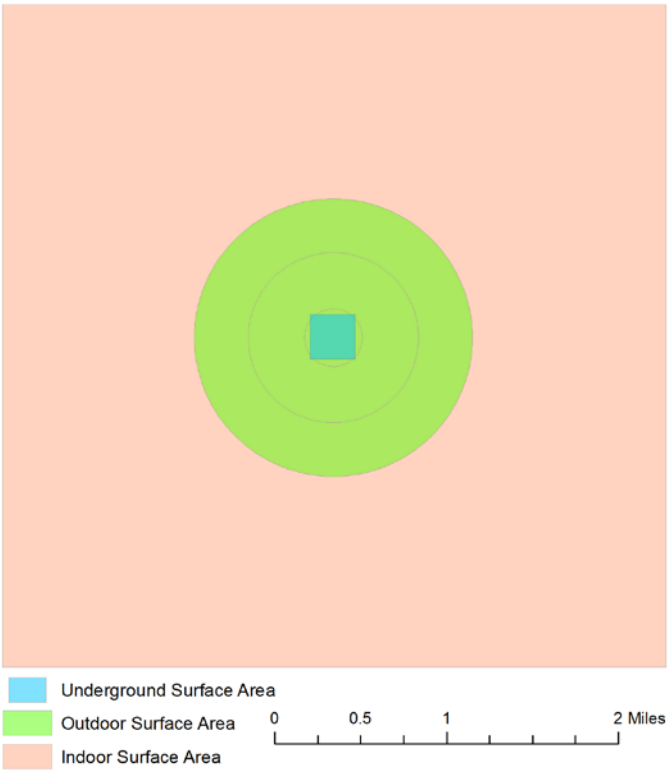

Figure 1. Comparison of Surface Areas

4.2.2.1.1 Outdoor

Table 9 shows the inputs used to represent the surface area between buildings and the total outdoor surface area. These values were obtained using EPA's Waste Estimation Support Tool (WEST) [44].

Table 9. Outdoor Surface Area

Surface Area	**Total (m^2)**
Building footprints (estimated roof area)	2.93×10^7
Surface area between buildings	2.82×10^7
Total outdoor surface area (excluding vertical surfaces)	5.75×10^7

4.2.2.1.2 Indoor

Table 10 shows the inputs used to represent the building footprint (i.e., first floor surface area) and the total indoor floor surface area. These values were generated using EPA's Incident Waste Assessment & Tonnage Estimator (I-WASTE) [45] and Waste Estimation Support Tool (WEST) [44].

Table 10. Indoor Surface Area

Surface Area	**Total (m^2)**
Building footprint	2.93×10^7
Total indoor floor surface area	4.16×10^8

4.2.2.1.3 Underground Transit System

Table 11 shows the inputs used to represent the surface area for the underground transit system. These values represent a best guess estimate based on publicly available datasets for a city of similar size.[2]

Table 11. Underground Surface Distribution

Surface Area	Total (m^2)
Track area	1.03×10^5
Station area	7.53×10^4
Total area	1.78×10^5

4.2.2.2 Sampling Methods According to Surface Type

The characteristics of the surface being sampled determine the sampling method. Collection efficiencies can vary greatly across material types. To better model the resource requirements associated with each sampling medium, the distribution of surface types for all three environments were estimated using WEST, I-WASTE, and empirical observations for outdoor, indoor, and underground surfaces, respectively. Each surface type was assigned an appropriate sampling medium. The number of samples attributed to each sample medium was calculated based on the proportion of the surface being sampled. Sampling methods for each surface type were assigned based on guidance in EPA's Comprehensive Biological Tactical Guidebook [16].

For this study, bulk sampling was assigned to surfaces such as trees and grass. Nonporous surfaces were assigned a sponge wipe sampling technique because the sponge wipe method has been validated by CDC and is applicable to the surface types identified in I-WASTE and WEST. Porous surfaces such as carpet were assigned the vacuum sampling technique [16]. It is acknowledged that air techniques, such as high-volume air samplers, may have utility in large outdoor release operations. However, air sampling methods have yet to be well characterized for outdoor sampling of *Ba*. Accordingly, was not considered in the current analysis. In addition to sampling, laboratory throughput was considered and estimated according to sample media type. The following sections describe the processes used to determine surface medium and sample type assignment.

4.2.2.2.1 Outdoor Surface Characteristics

The distribution of outdoor surfaces was determined using WEST. WEST uses a built-in image classification algorithm that identifies surface types for the purposes of estimating waste streams following radiological incidents. This algorithm outputs an estimated distribution for outdoor surface media using aerial imagery [44]. Aerial imagery from a city analogous to the hypothetical city described in section 4.2.2.1 (i.e., a large, densely populated metropolitan area) was used to derive outdoor surface media that are commonly found in cities of similar size. The results are shown in Table 12. Although nonporous surfaces requiring the use of the sponge wipe sample type may also constitute a significant portion of the outdoor surface types, WEST cannot identify these surface types. As a result, only soil and vacuum sample types were assigned to outdoor surfaces.

[2] See https://www.gc.cuny.edu/Page-Elements/Academics-Research-Centers-Initiatives/Centers-and-Institutes/Center-for-Urban-Research/CUNY-Mapping-Service/Projects/NYC-Subway-and-Bus-data-in-GIS-format. (last accessed March 2017). The shapefiles were loaded into GIS and were used to estimate the dimensions and surface area of the length of subway tunnel that intersected with the plume.

Table 12. Outdoor Sample Medium to Surface Type Assignment

Surface Type	Surface Distribution	Sample Type
Trees	5%	Soil sample (at base of tree)
Grass	1%	Soil sample
Buildings	51%	Vacuum
Asphalt	22%	Vacuum
Concrete	21%	Vacuum

4.2.2.2.2 Indoor Surface Characteristics

For indoor surfaces, I-WASTE was used to estimate the distribution of indoor floor material based on the number of buildings, square footage, and occupancy distribution. Due to the sheer number of indoor materials that I-WASTE outputs, surface media were broken down according to porous and nonporous materials. The results are shown in Table 13.

Table 13. Indoor Sample Medium to Surface Type Assignment

Surface Type	Surface Distribution	Sample Type
Porous (i.e., carpet)	90%	Vacuum
Nonporous (i.e., marble, ceramic tiles)	10%	Sponge Wipe

4.2.2.2.3 Underground Transit System

Methods for estimating underground surfaces are less explored than the other surfaces. Therefore, surface estimates for underground transit systems were based on empirical observations. Concrete is assumed to account for a large portion of the surface media, with the trains contributing a relatively small amount of metal and carpet surfaces. The distribution of surfaces expected to be present in underground transit systems are shown in Table 14.

Table 14. Underground Transit System Sample Medium to Surface Type Assignment

Surface Type	Surface Distribution	Sample Type
Concrete	90%	Vacuum
Metal	9%	Sponge Wipe
Carpet	1%	Vacuum

4.2.2.3 Resources

Resource demand based on time, cost, personnel, throughput, and waste were studied to better understand the implications of the prescribed sampling plan. Each of these factors is defined below, along with its assigned value.

4.2.2.3.1 Time

The amount of time required to return an area to normalcy following a biological incident is critical. If the chosen sampling strategy requires an arduous number of samples, the characterization process may take years or more. For this study, time is defined as the duration needed to collect an individual sample. This value is based on the type of sample medium. The inputs used for this study were derived from the BOTE study [1] and are shown in Table 15.

Table 15. Sampling Time per Sample Medium

Sample Medium	Time	Unit[a]
Soil sample	0.07	team hours/sample
Sponge/gauze/wipe	0.08	team hours/sample
Vacuum	0.13	team hours/sample

[a] "Team hours" is the total number of hours it takes a single team to collect the sample. A single team consists of three people.

4.2.2.3.2 Cost

Barring the social and political impacts of a biological incident, cost is one of the primary drivers of recovery. Cost is a direct derivative of the sampling plan, number of samples, and personnel. There is very little costing information specific to wide-area characterization, especially for wide-area incidents. Table 16 shows the assumptions for the sample medium costs, excluding sample collection labor.

Table 16. Cost per Sample Medium

Sample Medium	Cost	Unit
Soil sample	$25	$/sample
Sponge/gauze/wipe	$20	$/sample
Vacuum	$29	$/sample

4.2.2.3.3 Personnel

For wide area incidents, the number of personnel participating in characterization may decrease its duration; however, the number of skilled personnel available for sampling will be limited. For each of the three environments, the total number of available response personnel is based on planning documentation for a city of similar size [2]. Personnel numbers are shown in Table 17.

Table 17. Available Response Personnel per Environment and Sample Medium[3]

Sample Medium	Teams	Personnel/Team	Total Personnel
Outdoor			
Soil sample	50	3	150
Sponge/gauze/wipe	100	3	300
Vacuum	N/U	N/U	N/U
Indoor			
Soil sample	N/U	N/U	N/U
Sponge/gauze/wipe	150	3	450
Vacuum	150	3	450
Underground			
Soil sample	N/U	N/U	N/U

[3] The authors acknowledge that the sampling teams would not be limited to collecting one type of sample, but were arranged in this manner for evaluation purposes. The number of personnel assigned to a particular sampling medium does affect the Time to completion (TTC) but does not affect the overall labor costs. Due effort should be made to distribute personnel so that TTC is somewhat equal for all sampling media.

Sponge/gauze/wipe	50	3	150
Vacuum	25	3	75

N/U – Not used

4.2.2.3.4 Throughput

Laboratory throughput is defined as the total amount of time needed to process all samples. Throughput capacity varies depending on the sampling medium, analysis method used, equipment availability, laboratory personnel, and laboratory operating time. Single laboratory throughput information used for this study was modeled after data provided by the Interim Consequence Management Guidance for a Wide-Area Biological Attack document and the New York City Department of Health and Mental Hygiene Environmental Response and Remediation Plan for Biological Incidents [2, 46]. The total, factor based, single-laboratory throughput for each sample medium used in this study is shown in Table 18. For this study, the total number of laboratories available for analysis was assumed to be 250 based on EPA input.

Table 18. Individual Laboratory Throughput Factors per Sample Medium

Sample Medium	Individual Laboratory Throughput	Unit
Soil sample	10	samples/day
Sponge/gauze/wipe	100	samples/day
Vacuum	10	samples/day

4.2.2.3.5 Waste

Waste is generated immediately in an emergency response. Therefore, it is important to plan for waste early on. Most waste is commonly associated with decontamination. However, sampling should also be considered as an implicating factor. Waste from sampling is undoubtedly the least studied of all the above factors. The waste inputs used for this study were based on information collected from the BOTE study [1] and are shown below in Table 19.

Table 19. Waste Generated per Sampling Medium

Sample Medium	Waste	Unit
Soil sample	1	lb/sample
Sponge/gauze/wipe	0.31	lb/sample
Vacuum	0.31	lb/sample

4.2.3 Sampling Strategy

During the early phase of the incident, the boundary (i.e., plume) of the contamination can be estimated using intelligence, dispersion modeling, best guess estimates based on epidemiological data, or a combination of all sources. Screening by way of environmental sampling is used to determine the identity, concentration, viability, and approximate location of the contamination [46]. Once the evidence suggests a wide-area release of an infectious biological agent has occurred, characterization sampling should begin. Characterization is defined as an initial estimate of the environmental extent, concentration, and characteristics of a given contaminant. Its ultimate purpose is to determine whether, and to what extent, decontamination is needed. From this information, a decontamination strategy can be derived. A software tool such as DOE's Visual Sample Plan (VSP) can be used to ensure that a statistically robust sampling plan is developed. VSP is a decision support tool for determining the number and location of

environmental samples based on a variety of sampling goals. Sampling clearance criteria, methods, and geographical locations are typically defined by the sampling plan well before characterization takes place [47, 48]. The resulting information can be used to develop a sampling plan for use by decision-makers and help define the problem so that sampling objectives can be determined for characterization, decontamination configuration, and clearance [49].

For the purposes of this study, VSP was used to develop a sampling strategy. The polygons (i.e., shapefiles) used to describe the outdoor, indoor, and underground scenarios in the above analysis were also used as sampling maps in VSP. Within VSP, there are several sampling strategies (sampling goals) that can be used for characterization sampling. Given the likelihood of the contaminant being deposited in patchy spots across a wide area, the "locate a hot spot" goal was used for all three environments. This goal is used to statistically determine the probability of finding a hot spot of a given size and shape. Tables 17, 18, and 19 further describe the VSP hot spot sampling design; associated statistical assumptions; and general guidelines for estimating the number of samples for the outdoor, indoor, and underground environments.

The inputs to VSP include the following[4]:

- **Sampling Area**: The area of the location being sampled for each sample collected;
- **Hot Spot Area**: The local contiguous area that has concentrations that exceed a threshold value;
- **Probability**: Probability of a sampling location being collocated with a hot spot; and
- **False Negative Rate**: Probability each contaminated sample will not be detected.

As previously discussed, Krauter etal. demonstrated that FNRs corresponded with RE data where smoother surfaces resulted in higher recovery efficiencies and lower FNR [29]. Furthermore, the developers of VSP note the following:

> *Standard statistical formulas assume that the overall FNR = 0 when calculating 1) the number of samples required to achieve the desired confidence for a characterization or clearance sampling goal, and 2) the uncertainty and confidence associated with a characterization or clearance decision using a specific sampling approach implemented following a contamination incident. When FNR = 0, the formulas account only for the uncertainty in results associated with the specific type of statistical or hybrid sampling approach being used. However, the overall FNR is affected by anything in the sampling process that might yield a false negative, including (i) the RE of a sampling method (e.g., swab, wipe, or vacuum).*[5]

For the purposes of our analysis, we are assuming that the RE is a function of FNR given that VSP aggregates anything that affects the sampling process into the FNR.

The choice of inputs is somewhat arbitrary, and in no way reflects EPA policy or even likely strategies for a real biological incident. However, the choice of inputs does represent a conservative approach to sample strategy development for a wide area biological incident and is based on currently accepted clearance levels, common sampling strategies, and available technologies: a first guess at a sampling strategy that might be used based on expertise of EPA response personnel. Many factors can change and/or reduce the inputs as well as the number of estimated samples (e.g., on-site conditions, learning through sampling

[4] See http://vsp.pnnl.gov/docs/PNNL-23211.pdf. (last accessed March 2017)
[5] See http://www.pnnl.gov/main/publications/external/technical_reports/PNNL-20910.pdf, p.2. (last accessed March, 2017)

results, multiple lines of evidence approaches[6], etc.). This analysis does, however, provide an understanding of how these variables interact and their impacts on the overall resource demand with regards to a systems approach. Therefore, the results of this study are applicable to almost any sampling strategy and can help optimize the response.

Table 20. Summary of Sampling Inputs for the Outdoor Environment

Input	Value
Probability of detection	95%
Grid pattern	Square
Area of hot spot[7]	10 ft^2
Total area to sample	5.75×10^7 ft^2
Spacing between samples	3 meters
Optimum number of samples (VSP output)	3.63×10^6

Table 21. Summary of Sampling Inputs for the Indoor Environment

Input	Value
Probability of detection	95%
Grid pattern	Square
Area of hot spot	10 ft^2
Total area to sample	4.16×10^8 ft^2
Spacing between samples	3 meters
Optimum number of samples (VSP output)	2.63×10^7

Table 22. Summary of Sampling Inputs for the Underground Transit System Environment

Input	Value
Probability of detection	95%
Grid pattern	Square
Area of hot spot	10 ft^2
Total area to sample	1.92×10^6 ft^2
Spacing between samples	3 meters
Optimum number of samples (VSP output)	1.21×10^5

[6] According to DOE's *Multiple Lines of Evidence*, " 'lines of evidence' is the ability to identify factors that are influencing the possible contamination of a given decision area and then convert this knowledge into probabilities estimating the likelihood of each area still being unacceptable" [50].

[7] A 10 ft^2 hot spot area was selected based on sampling criteria as defined by the BOTE exercise and best judgment estimates.

4.3 Results

Working with the inputs described above, VSP and a custom spreadsheet were used to estimate the sampling criteria, the number of samples, and the resources needed to take those samples. The results are reported by environment type (i.e., outdoor, indoor, and underground) in the following sections.

4.3.1 Outdoor

The results for the outdoor area are shown in Tables 23 and 24. The total number of samples for an area of 5.75×10^7 ft^2 with a hot spot area of 10 ft^2 is approximately 3,630,000. Based on the total number of samples and the prescribed sampling medium, it may cost approximately 1.9 billion dollars and require 1.4 years to characterize.

Table 23. Outdoor Samples Sampling Results

Total Samples	Sample Type	Number of Samples	Sampling Cost ($)	Labor Cost ($)	Sampling Time (team hours)
3,630,000	Soil sample	219,370	5,484,260	6,449,489	15,356
	Sponge/gauze/wipe	N/U	N/U	N/U	N/U
	Vacuum	3,410,630	98,908,259	181,922,984	433,150

Table 24. Outdoor Samples Laboratory Analysis Results

Total Samples	Sample Type	Number of Samples	Analysis Cost ($)	Labor Cost ($)	Analysis Time (laboratory hours)
3,630,000	Soil sample	219,370	57,913,781	29,615,002	175,496
	Sponge/gauze/wipe	N/U	N/U	N/U	N/U
	Vacuum	3,410,630	982,261,329	515,005,072	2,728,504

4.3.2 Indoor

The results for the indoor area are shown in Tables 25 and 26. The total number of samples for an area of 4.16×10^8 ft^2 with a hot spot area of 10 ft^2 is approximately 26,300,000. Based on the total number of samples and the prescribed sampling medium, characterization may cost approximately 13.4 billion dollars and require 8.7 years.

Table 25. Indoor Sampling Results

Total Samples	Sample Type	Number of Samples	Sampling Cost ($)	Labor Cost ($)	Sampling Time (team hours)
26,300,000	Soil sample	N/U	N/U	N/U	N/U
	Sponge/gauze/wipe	2,630,000	52,600,000	90,577,200	215,660
	Vacuum	23,670,000	686,430,000	1,262,557,800	3,006,090

Table 26. Indoor Analysis Results

Total Samples	Sample Type	Number of Samples	Analysis Cost ($)	Labor Cost ($)	Analysis Time (laboratory hours)
26,300,000	Soil sample	N/U	N/U	N/U	N/U
	Sponge/gauze/wipe	2,630,000	628,570,000	310,340,000	210,400
	Vacuum	23,670,000	6,816,960,000	3,574,170,000	18,936,000

4.3.3 Underground Transit System

The results for the underground transit system area are shown in Tables 27 and 28. The total number of samples for an area of 1.92×10^6 ft^2 with a hot spot area of 10 ft^2 is about 121,000. Based on the total number of samples and the prescribed sampling medium, characterization may cost about 1.4 million dollars and require 34 days.

Table 27. Underground Transit System Sampling Results

Total Samples	Sample Type	Number of Samples	Sampling Cost ($)	Labor Cost ($)	Sampling Time (team hours)
121,000	Soil sample	N/U	N/U	N/U	N/U
	Sponge/gauze/wipe	10,890	217,800	375,052	893
	Vacuum	110,110	3,193,190	5,873,267	13,984

Table 28. Underground Transit System Analysis Results

Total Samples	Sample Type	Number of Samples	Analysis Cost ($)	Labor Cost ($)	Analysis Time (laboratory hours)
121,000	Soil sample	N/U	N/U	N/U	N/U
	Sponge/gauze/wipe	10,890	2,602,710	1,285,020	871
	Vacuum	110,110	31,711,680	16,626,610	88,088

4.4 Observations

Based on the results of the hypothetical scenario described above, the following observations and conclusions can be made:

- According to the prescribed sampling plan, environmental characteristics, and resource demand assumptions, characterization with a total surface area of 4.76×10^8 ft^2, will cost an estimated 15.3 billion dollars in total and may take over 10 years to characterize.
- For all three environments combined, the total time for sampling may be up to 4 years, while the total analysis throughput is estimated to take 10 years. Although this disconnect is rather improbable, the

results do lend themselves to the possibility that analysis efforts may eclipse sampling efforts. In practice, the actual sampling rate (e.g., samples collected per day, etc.) will likely be dictated by laboratory throughput in part due to sample hold time limitations.

- The total indoor surface area is one order of magnitude greater than the outdoor area, which is to be expected for a highly-populated urban setup. Because of the increased surface area, the indoor environments represent almost 90% of the total cost and resource demand of all the environments combined. Because of this, for wide-area biological incidents, it is recommended that a rule in/rule out approach be taken when sampling indoors. If a positive hit is discovered through preliminary sampling, the building should be considered contaminated unless the decontamination approach is costlier than further sampling to define more discrete decontamination zones. This analysis assumed that all buildings within the contaminated area would either be contaminated or need to be sampled thoroughly. Available field data or a lines of evidence approach may be useful in reducing this requirement
- It is likely the requirements set forth by this environment are not practical and would likely exhaust local, state, and federal resources. Furthermore, by the time characterization has concluded (i.e., 10 years later), it is possible that the community and its economy will no longer be viable.

5 WIDE-AREA INCIDENT ANALYSIS

5.1 Introduction

The process to develop a sampling strategy for wide-area biological incident response operations has up to now been poorly defined and not systematic. Additionally, the options available in terms of sampling techniques are often constrained by regulatory mandates. In situations where the sampling method is not mandated by regulation(s), it is then necessary to analyze available data concerning both sampling methods and sampling techniques before selecting the most appropriate method or technique [51].

The purpose of this chapter is fourfold: 1) to leverage the capacity of VSP outputs to build a predictive model for wide-area sampling strategies by way of regression modeling, and 2) using the hypothetical scenario described in Section 4.2.1, model the number of samples needed for the underground, outdoor, and indoor environments individually under a range of values for VSP input parameters (sampling area, hot spot area, false negative rate, and probability); 3) evaluate the relative impact of the VSP input parameters on the resulting number(s) of samples; and 4) use the modeling results (for the number of samples required) in conjunction with resource demand estimates (based on a review of the literature and the BOTE study) to analyze the potential impacts on total time (for sampling and analysis) and total cost impacts following a wide area biological incident. The results of this chapter and the accompanying developed spreadsheet tool will assist responders in evaluating the impacts of multiple variables associated with a range of sampling techniques, and help inform the selection of an appropriate sampling technique based on an informed evaluation of those variables according to the project's goals and requirements.

5.2 Sampling Models

The following sections describe the approach used in developing several models for determining the most appropriate number of samples based on a given sampling area, hot spot area, probability, and false negative rate for all three environments (i.e., underground, outdoor, and indoor), using data generated by VSP.

5.2.1 VSP Modeling

A preliminary assessment of the ability of VSP to model wide area incidents showed instability due to software memory limitations. Because of this, regression modeling was used to expand the limitations set forth by VSP. The equations derived by regression modeling can be used to predict the number of samples using a simple spreadsheet without interacting with VSP. Additionally, since the results are generated and stored within a spreadsheet, a more extensive analysis of the data can be performed. Furthermore, by using this approach, the relative impact that the predictors (i.e., sampling area, hot spot area, probability, false negative rate) have on the target variable (i.e., number of samples) can be analyzed.

The data needed for the regression analysis were generated by automating VSP. An Automation (i.e., OLE Automation) method was used to remotely access and manipulate VSP using VBscript (i.e., Visual Basic Scripting Edition). The input parameters (Table 29) are sampling area (SA), hot spot area, probability, and FNR, and their associated range of inputs referenced by VSP is shown in Table 30. Two data sets were derived from VSP for each environment; a separate dataset for a range of false negative rates and another for a range of sampling areas (VSP does not allow sampling area and false negative rate to be varied simultaneously).

Table 29. Input Parameters Used for VSP Modeling

VAP Parameter	Meaning
Sampling Area (SA)	The area of the location being sampled
Hot Spot Area	The local contiguous area that has concentrations that exceed a threshold value
Probability	Probability of a sampling location being collocated with a hot spot
False Negative Rate	Probability each contaminated sample will not be detected

Table 30. Range of Values Used for VSP Input Parameters

Variable	Scenario	False Negative Rate (%)	Sampling Area[a] (ft^2)	Probability (%)	Hotspot Area (ft^2)
SA	Indoor	-	50-1,000	50-99	50-1,000
SA	Outdoor	-	50-1,000	50-99	50-1,000
SA	Underground	-	10-1,000	50-99	10-1,000
FNR	Indoor	1-8	-	50-95	55-1,000
FNR	Underground	1-10	-	50-99	4-1,000

[a] The sampling area value represents the area of the location being sampled on a per sample basis. For example, a value of 50 ft^2 for a vacuum indicates that the vacuum nozzle is passed over 50 ft^2 for every sample collected. For a fixed area of interest, as the sampling area increases, the number of samples decreases.

The resulting data sets from VSP were each analyzed using Eureqa[8], totaling six datasets comprised of over five million data points. Eureqa is a genetic programming-based symbolic regression mathematical software tool for detecting equations and hidden mathematical relationships in scientific data. Eureqa identified and ranked regression models that best corresponded with the input data. Equations that were

[8] www.nutonian.com

most representative of the training set or highest relevance to the VSP results are discussed in the following section.

5.2.2 Regression Modeling Results

Equations 1-5 show the regression models for false negatives and assume no false negatives for the underground, outdoor, and indoor environments, respectively. Limitations of VSP prevented the generation of sufficient data to perform a regression modeling analysis for the outdoor environment with false negatives. These equations were identified by Eureqa to have the highest relevance to the VSP results. Tables 31-35 show the model performance statistics for those equations.

$$S = 5960*FNR/(HA*\sin(2.384 + P)) + (2.467e7 + 41.67*P^3 + 2.079e5 *\sin(2.384 + P) + 1.812*FNR*P^3 + 1.9e4*P*\cos(41.67*P))/HA \quad \textbf{Eq.1}$$

where S = number of samples, dimensionless
SA = sampling area, ft^2
FNR = false negative rate, %
HA = hot spot area, ft^2
P = probability, %

Table 31. Model Statistics with False Negatives in the Indoor Environment

Mean Absolute Error	981.87148
Mean Square Error	4523080
R^2 Goodness of Fit	0.99981571
Correlation Coefficient	0.99991905
Maximum Error	32765.938

$$S = 308.9*\text{sqrt}(SA) + -1683*P/SA + 1.086e6*P/SA^2 + 2.659e7 /(HS*0.9335 + SA*\text{sqrt}(SA - 26.93)) - 5513 - \tan(308.9*\text{sqrt}(SA)) - 4.685*SA \quad \textbf{Eq.2}$$

Where, S = number of samples, dimensionless
SA = sampling area, ft^2
HA = hot spot area, ft^2
P = probability, %

Table 32. Model Statistics with no False Negatives in the Indoor Environment

Mean Absolute Error	77.149587
Mean Square Error	53011.12
R^2 Goodness of Fit	0.99965262
Correlation Coefficient	0.99982948
Maximum Error	5121.8342

$$S = 0.05987*SA + 5.448e4/(SA - 24.77) + 4.378e7*P/(2.828e4 + 150.5 *HA + SA^2 + 4.239*SA*HA + P*SA^2) - 99.24 \quad \textbf{Eq.3}$$

Table 33. Model Statistics with no False Negatives in the Outdoor Environment

Mean Absolute Error	12.133013
Mean Square Error	1864.24
R^2 Goodness of Fit	0.9993602
Correlation Coefficient	0.99968813
Maximum Error	1510.3916

$S = 3.541 + -6.385e4/SA^2 + 1.484e5*sqrt(P/(SA^4 + 7.232*HA*SA^2)) + 2.232e5*SA^2/(SA^4 + 7.232*HA*SA^2) - 0.003847*SA$ **Eq.4**

Table 34. Model Statistics with no False Negatives in the Underground Environment

Mean Absolute Error	3.7782199
Mean Square Error	242.60252
R^2 Goodness of Fit	0.99746774
Correlation Coefficient	0.99882424
Maximum Error	842.55387

$S = (1.906e4*Probability + 1.345e4*FNR + 0.1514*exp(0.1547*P + 0.1162*FNR) - FNR*P*tan(1.095*P))/HA$ **Eq.5**

Table 35. Model Statistics with False Negatives in the Underground Environment

Mean Absolute Error	111.86019
Mean Square Error	595210.75
R^2 Goodness of Fit	0.99967441
Correlation Coefficient	0.99983964
Maximum Error	79767.245

5.2.2.1 Variable Sensitivity

Eureqa features the ability to show how changes in each variable affect the generated models. For the purposes of this section, sensitivity is defined as the relative impact that a predictor has on the target variable (i.e., number of samples). The variable sensitivity of the selected best fit models is discussed in the following sections.

5.2.2.1.1 No False Negatives Errors

When varying sampling area, hot spot area, and probability, the sampling area has a relatively higher impact on the number of samples versus hot spot area and probability for all three environments (i.e., underground, outdoor, and indoor). Although this phenomenon remains constant across all three locations, the significance of this impact decreases with increasing surface area. Tables 36, 37, and 38 show the variable sensitivity for the underground outdoor and indoor environments with no false negatives, respectively.

Table 36. Variable Sensitivity with no False Negatives in the Underground Environment Model

Variable	Sensitivity[9]	% Positive[10]	Positive Magnitude[11]	% Negative[12]	Negative Magnitude[13]
Sampling Area	4.9421	0%	0	100%	4.9421
Hotspot Area	0.51549	0%	0	100%	0.51549
Probability	0.073084	100%	0.073084	0%	0

Table 37. Variable Sensitivity with no False Negatives in the Outdoor Environment Model

Variable	Sensitivity	% Positive	Positive Magnitude	% Negative	Negative Magnitude
Sampling Area	2.7538	0%	0	100%	2.7538
Hotspot Area	0.094395	0%	0	100%	0.094395
Probability	0.032768	100%	0.032768	0%	0

Table 38. Variable Sensitivity with no False Negatives in the Indoor Environment Model

Variable	Sensitivity	% Positive	Positive Magnitude	% Negative	Negative Magnitude
Sampling Area	2.7736	3%	0.011193	97%	2.8636
Hotspot Area	0.094389	0%	0	100%	0.094389
Probability	0.032678	100%	0.032678	0%	0

5.2.2.1.2 With False Negatives Errors

Conversely, when varying the false negative rate, hot spot area, and probability for the underground and indoor environments, the hot spot area has a higher relative impact (17.085) on the number of samples versus probability (0.38083) and false negative rate (0.070417) for the underground scenario (i.e., smaller environment). For the indoor environment (i.e., larger environment) the hot spot area also has a higher relative impact (2.03) on the number of samples versus probability (0.35668) and false negative rate (0.059827). These inverse outcomes (compared to the results where there were no false negatives) may be due to the increase in the area of interest, percentage (probability) versus a set range of integers (i.e., hotspot size), and the statistical method used to account for false negatives. Notably, *the false negative rate has relatively low impact on the number of samples*. However, the models were less sensitive to these variables as the area of interest increased. Tables 39 and 40 show the variable sensitivity for the underground outdoor, and indoor environments with false negatives, respectively.

[9] The relative impact within this model that a variable has on the target variable.
[10] The likelihood that increasing this variable will increase the target variable.
[11] When increases in this variable lead to increases in the target variable, this is generally how big the positive impact is.
[12] The likelihood that increasing this variable will decrease the target variable.
[13] When increases in this variable lead to decreases in the target variable, this is generally how big the negative impact is.

Table 39. Variable Sensitivity when Accounting for False Negatives in the Underground Environment Model

Variable	Sensitivity	% Positive	Positive Magnitude	% Negative	Negative Magnitude
Hotspot Area	17.085	0%	0	100%	17.085
Probability	0.38083	100%	0.38083	0%	0
False Negative Rate	0.070417	100%	0.070417	0%	0

Table 40. Variable Sensitivity when Accounting for False Negatives in the Indoor Environment Model

Variable	Sensitivity	% Positive	Positive Magnitude	% Negative	Negative Magnitude
Hot Spot Area	2.03	0%	0	100%	2.03
Probability	0.35668	100%	0.35668	0%	0
False Negative Rate	0.059827	100%	0.059827	0%	0

5.3 Resource Demands

As discussed above, when comparing the impacts of (1) varying false negative rates on the number of samples, and (2) varying the sampling area on the number of samples, variations in the sampling area had a greater relative impact on the required number of samples than did variations in the false negative rate. To evaluate the additional impacts that resource demands might have on the total time to complete sampling and analysis and on the total cost, a Microsoft Excel spreadsheet was developed. The spreadsheet combines the regression equations generated as described above for the number of samples (based only on a variable sampling area) with a variety of different numerical factors for various resources that would be needed following an event. These inputs and their associated "default" factors are listed in Table 41.

Table 41. Table of Factors Used in Resource Demand Estimation Spreadsheet

Component		Default Value	Unit	Basis
Sampling Plan				
Probability		95	%	EPA
Hotspot Area		10	ft^2	EPA
Sample Area Size		***1***	ft^2	EPA
Sampling				
Number of Available Teams for Sampling		***500***	teams	Estimated
Personnel per Sampling Team		3	persons/team	Assumed
Time to Collect Samples	Soil Sample	0.07	team hours/sample	Estimated
	Sponge Sample	0.08	team hours/sample	BOTE; Adjusted Time Per Sample, pg. H-34 [1]
	Gauze/Wipe Sample	0.08	team hours/sample	Estimated
	Vacuum Sample	0.13	team hours/sample	BOTE; Adjusted Time Per Sample, pg. H-34 [1]
Sampling Team Hours per Shift		5	team hours/shift	Assumed
Sampling Team Shifts per Day		1	team shifts/day	Calculated
Sampling Hours per Day		2500.0	sampling hours/day	Calculated
Sampling Personnel Hours per Day		7500.0	person hours/day	Calculated
Sampling Team Labor Cost		420.00	$/hour/team	BOTE; Team Makeup, pg. H-33 [1]
Sampling Personnel Labor Cost		140.00	$/hour/person	Calculated
Sampling Material Cost	Soil Sample	25.00	$/sample	Estimated
	Sponge Sample	20.00	$/sample	BOTE; Cost Equations, pg. H-6 [1]
	Gauze/Wipe Sample	20.00	$/sample	Estimated
	Vacuum Sample	29.00	$/sample	BOTE; Cost Equations, pg. H-6 [1]
Analysis				
Number of Available Laboratories for Analysis		***250***	laboratories	EPA

Component		Default Value	Unit	Basis
Time to Analyze Samples	Soil Sample	10	samples/day	EPA and Estimated based on IBRD + NYC
	Sponge Sample	100	samples/day	EPA and Estimated based on IBRD + NYC
	Gauze/Wipe Sample	100	samples/day	EPA and Estimated based on IBRD + NYC
	Vacuum Sample	10	samples/day	EPA and Estimated based on IBRD + NYC
Analysis Laboratory Hours per Day		24	hours/day/ laboratory	Assumed
Analysis Hours per Day		6000.0	analysis hours/day	Based on a network of 250 laboratories
Analysis Labor Cost	Soil Sample	135.00	$/sample	Estimated
	Sponge Sample	118.00	$/sample	BOTE; Analytical Costs, pg. H-5 [1]
	Gauze/Wipe Sample	118.00	$/sample	Estimated
	Vacuum Sample	151.00	$/sample	BOTE; Analytical Costs, pg. H-5 [1]
Analysis Material Cost	Soil Sample	264.00	$/sample	Estimated
	Sponge Sample	239.00	$/sample	BOTE; Analytical Costs, pg. H-5 [1]
	Gauze/Wipe Sample	239.00	$/sample	Estimated
	Vacuum Sample	288.00	$/sample	BOTE; Analytical Costs, pg. H-5 [1]

The spreadsheet calculates/estimates the values listed in Table 42 (primary outputs shown in **bold**):

Table 42. Resource Demand Estimation Spreadsheet Outputs

Total Number of Samples
Total Required Sampling Time (team h)
Time to Complete Sampling (days)
Total Sampling Labor Cost ($)
Total Sampling Material Cost ($)
Total Required Analysis Time (laboratory h)
Time to Complete Analyses (days)
Total Analysis Labor Cost ($)
Total Analysis Material Cost ($)
Total Cost ($)
Total Time to Completion (days)
Limiting Time Factor

There are three separate regression equations for the number of samples based on sampling area, hot spot area, and probability (one each for the indoor, outdoor, and underground environments). Furthermore, different sampling methods/techniques are assumed for each of the different environment types based on the types of surfaces in each environment (see Section 4). The total number of samples for each environment type is distributed according to the percentage of the surfaces in each environment for which a different sampling technique might be used. For example, if 364 samples are needed for the indoor environment, and 10% of the indoor surfaces will be sampled using sponge sampling and 90% will be vacuumed, then there will be 36 sponge samples and 328 vacuum samples. This procedure is repeated for the number of samples for each of the three environment types, and the resource demands are calculated for each sampling technique in each environment type. Those resource demand estimates for each environment type are then totaled giving the primary outputs listed in Table 41 above.

5.3.1 Resource Demands Analysis

As Table 41 shows, default values were selected for the resource demand factors based largely on existing data and information from EPA reports and field studies, assumptions, and educated estimates. The three main variables evaluated for this analysis were: (1) sample area size, (2) number of available sampling teams, and (3) number of available analysis laboratories. Sampling area was varied across the narrowest range of values used as inputs to the VSP modeling runs for the three environment types, the number of available sampling teams was varied from 100 to 500 teams, and the number of available analysis laboratories was varied from 100 to 500 laboratories.

5.3.2 Resource Demands Results

Assuming all variables are fixed except for the sample area size, number of sampling teams, and number of laboratories, the results of varying those three remaining variables on total cost and number of days required for sampling and number of days required for analysis are presented in Tables 43 and 44, respectively.

Table 43. Effect of Varying the Sample Area Size and Number of Sampling Teams on Total Cost and Time to Complete Sampling

	Sample Area Size = 50 ft^2		Sample Area Size = 500 ft^2		Sample Area Size = 1,000 ft^2	
Sampling Teams	**Total Cost ($)**	**Sampling Days**	**Total Cost ($)**	**Sampling Days**	**Total Cost ($)**	**Sampling Days**
100	81,470,347	39	928,441	0.45	217,320	0.10
200	81,470,347	20	928,441	0.22	217,320	0.052
300	81,470,347	13	928,441	0.15	217,320	0.035
400	81,470,347	9.8	928,441	0.11	217,320	0.026
500	81,470,347	7.8	928,441	0.089	217,320	0.021

Table 44. Effect of Varying the Sample Area Size and Number of Analysis Laboratories on Total Cost and Time to Complete Analyses

	Sample Area Size = 50 ft^2		Sample Area Size = 500 ft^2		Sample Area Size = 1,000 ft^2	
Analysis Laboratories	**Total Cost ($)**	**Analysis Days**	**Total Cost ($)**	**Analysis Days**	**Total Cost ($)**	**Analysis Days**
100	81,470,347	49	928,441	0.56	217,320	0.13
200	81,470,347	24	928,441	0.28	217,320	0.065
300	81,470,347	16	928,441	0.19	217,320	0.044
400	81,470,347	12	928,441	0.14	217,320	0.033
500	81,470,347	9.8	928,441	0.11	217,320	0.026

Based on the results presented in Tables 43 and 44, the total cost is a function only of the sample area size (i.e., as the area covered by each sample collected increases, the number of samples required for a given area of interest decrease) and not on either the number of sampling teams collecting those samples nor on the number of laboratories available to analyze those samples. However, the amount of time needed to both collect the samples and to analyze them is a function of the number of sampling teams available and the number of analysis laboratories available. Once the number of samples is determined, the total cost is fixed, and is independent of the number of sampling teams and the number of laboratories. For a range of sample area sizes from 50 to 1,000 ft^2, Figure 2 illustrates the impact on both total cost and the total time for both sampling and analysis combined, but reflects the limiting time value (i.e., if sampling takes 20 days and analysis takes 30 days, then the total time to completion is 30 days).

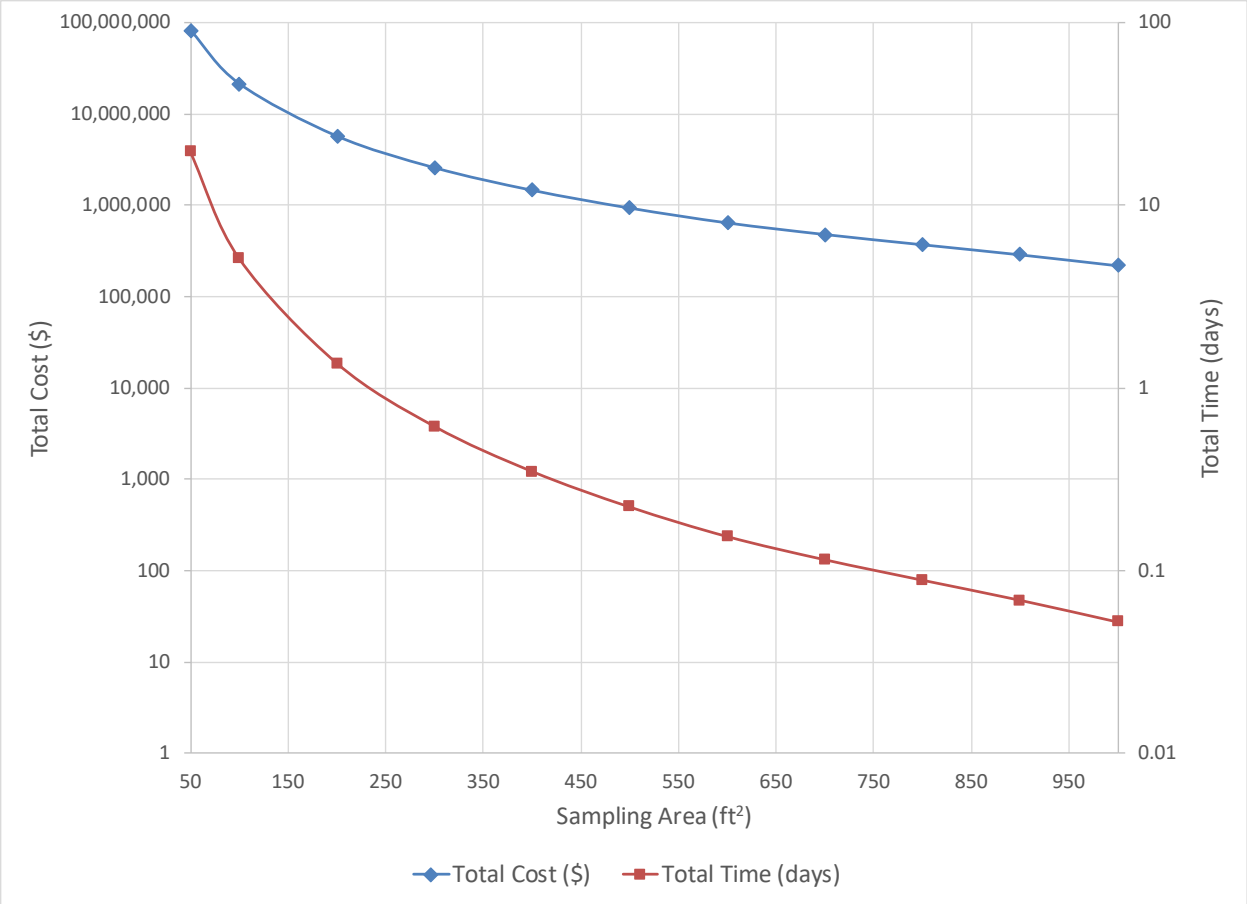

Figure 2. Total Cost and Total Time for Sampling and Analysis as a Function of Sample Area Size

Once the total cost is determined based only on the number of samples required, the impact of a variable number of available sampling teams and analysis laboratories was evaluated. Figures 3 through 5 present the total times to complete sampling and analyses based on the available number of sampling teams and the number of available analysis laboratories for three increments of sample area sizes, 50 ft^2, 500 ft^2, and 1,000 ft^2, respectively.

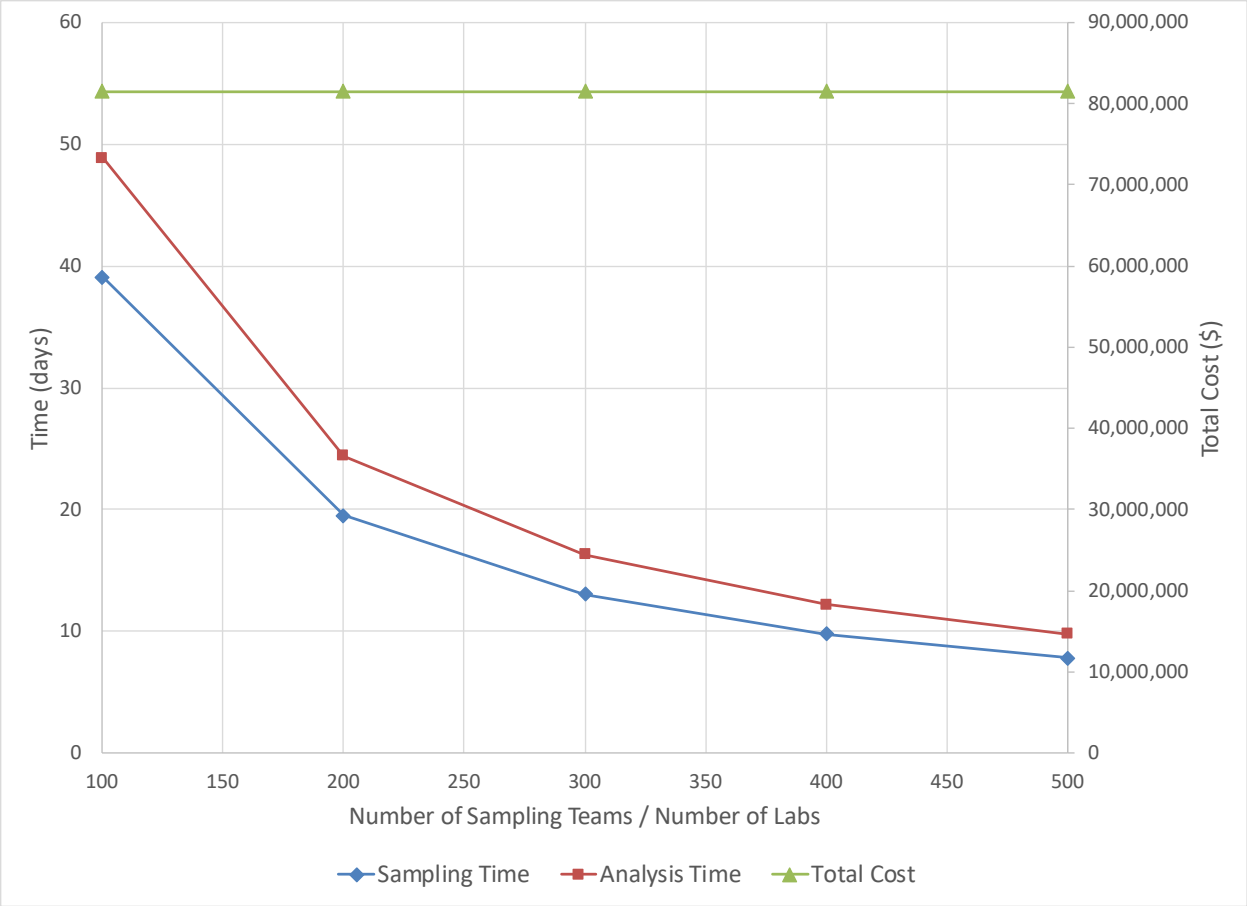

Figure 3. Sampling and Analysis Time as a Function of Number of Available Resources at 50 ft^2 Sample Area Size

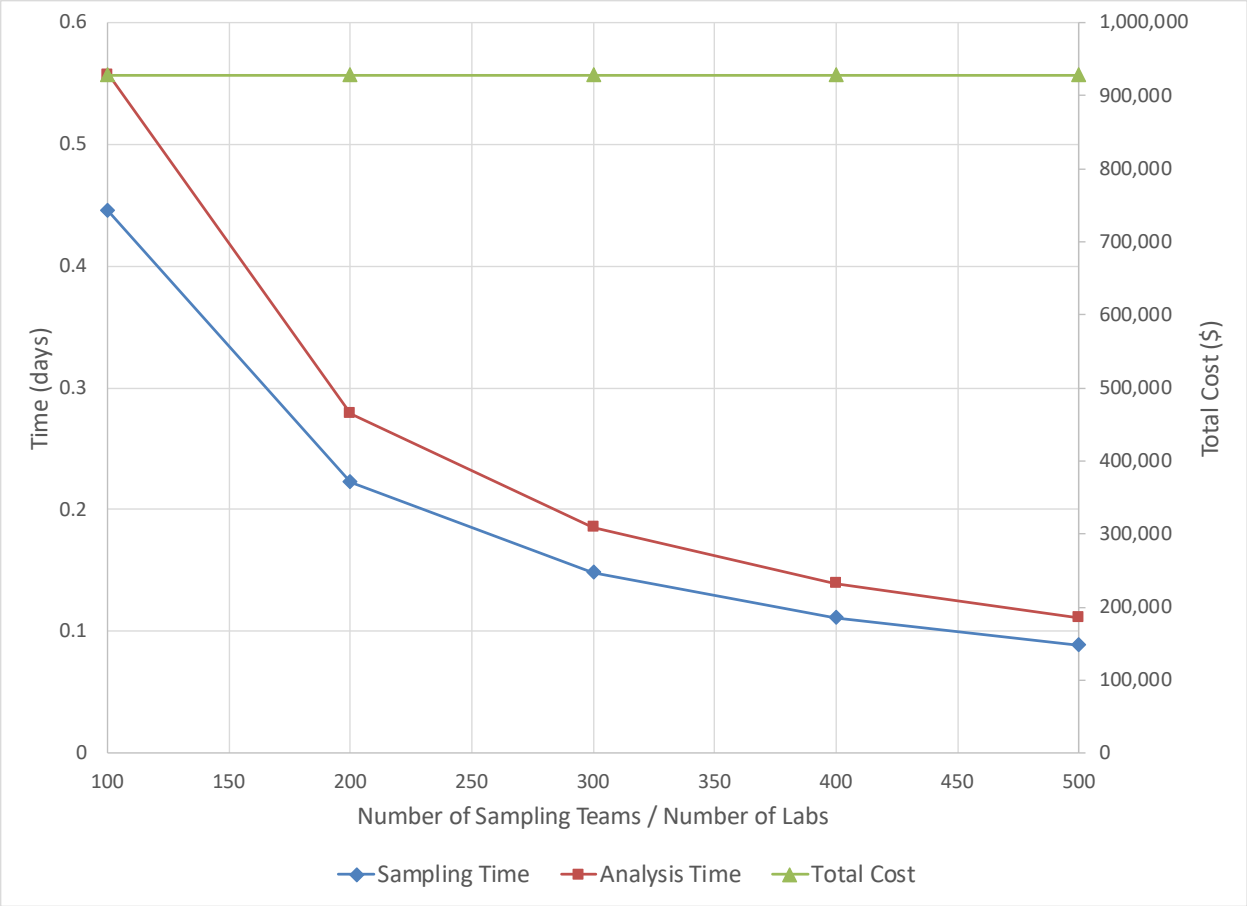

Figure 4. Sampling and Analysis Time as a Function of Number of Available Resources at 500 ft^2 Sample Area Size

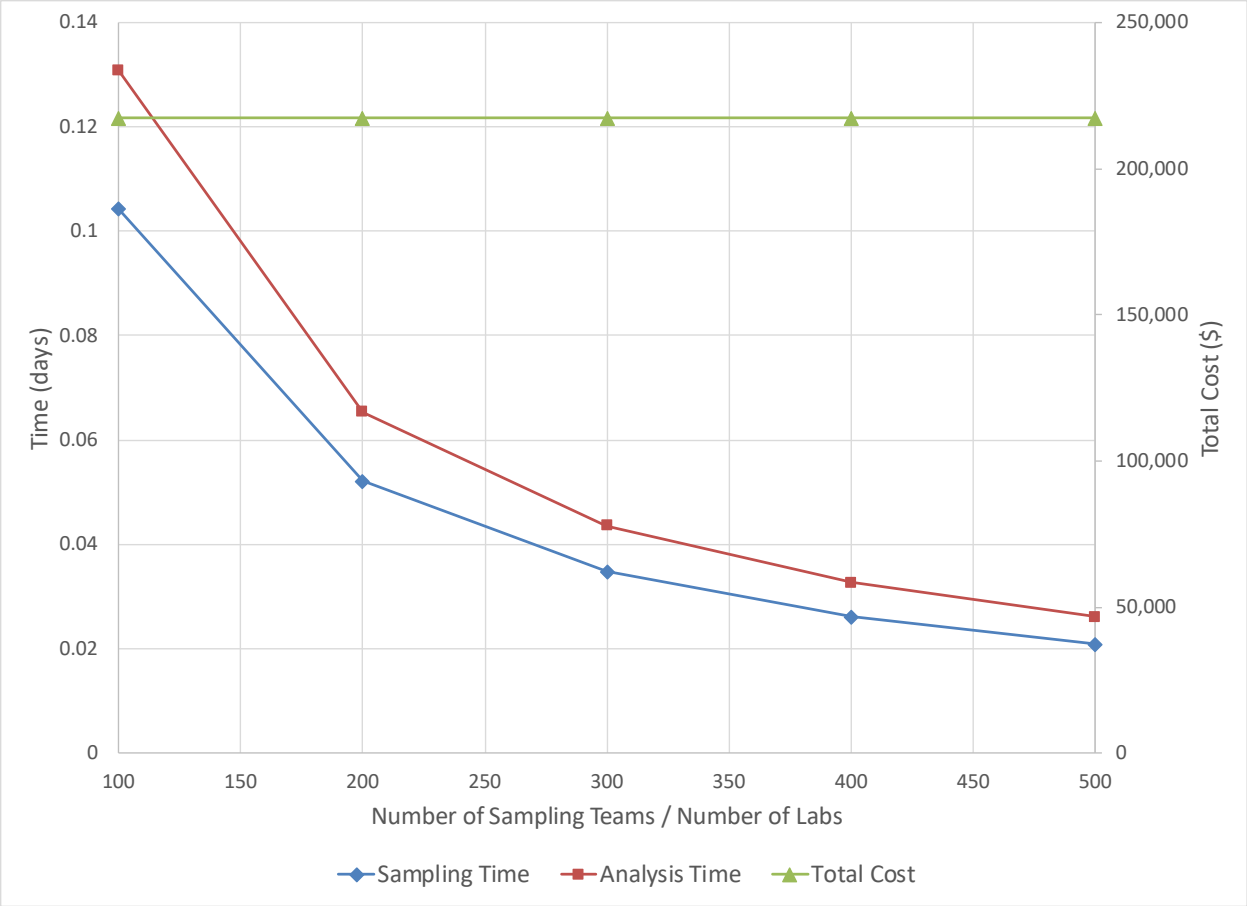

Figure 5. Sampling and Analysis Time as a Function of Number of Available Resources at 1,000 ft^2 Sample Area Size

6 OBSERVATIONS AND RECOMMENDATIONS

Several observations can be made based on the results of the above analysis of a sampling campaign following a hypothetical wide-area incident that can potentially impact indoor, outdoor, and underground transportation areas. Primarily, the study demonstrated that a wide area incident response will require extended time and resources if a probabilistic sampling approach is utilized with high probability requirements (i.e., >90%) and currently available sampling methods.

The current study used an accepted and commonly used tool (VSP) for developing a probabilistic sampling design and followed accepted guidance for sampling strategy/methods. VSP may have some limitations when applied to a wide-area sampling design. These limitations include: memory limitations when running large scenarios, and uncertainty as to how VSP calculates total numbers of samples, especially for indiscriminate surface areas. Further, EPA uses different terminology than VSP. Many of the VSP inputs and the terminology used (e.g., false negative rate, probability, etc.) are used inconsistently within the operational response community, and the primary users of VSP may have a relatively limited understanding of the statistical significance of the VSP inputs, the VSP model, and probabilistic design in general. Many, if not the majority, of bench-scale studies reviewed for this report use different terminology (e.g., recovery efficiency), and there seems to be a lack of understanding of the

correlation or relationship between parameters quantified in a laboratory and operationally significant parameters used as inputs for sampling design.

Additionally, variation in recovery efficiency (and thus detection probability) by surface material types (e.g., porous, nonporous, soil) are not accounted for in VSP which thus cannot be evaluated in the context of wide area characterization. Additional information must be gathered on the distribution of surface types within the study area before appropriate sampling methods can be prescribed. Other widely used EPA tools such WEST and IWASTE and other GIS tools can be useful for wide area sample plan development by assisting responders in determining or estimating the presence and distribution of surface material types for both structures and open areas.

Further, due to the large area of potential contamination, the variety of indoor and outdoor surface types, and uncertain and variable environmental conditions, probabilistic sampling design (and statistical design in general) may not be appropriate for a wide area incident and likely would not be used for characterization of a wide area incident. In addition, the current study used a plume derived from an earlier biological exercise to define the area of contamination; however, recognition of a covert release through illness would likely required a different strategy and could result in a much larger initial area of interest.

Considering the observations noted above, several needs have been identified. First, a lines of evidence approach to sampling design should be considered at the outset of the response planning phase of a wide area incident for the eventual purpose of reaching clearance goals. Second, we currently lack operational strategies to deal with such an incident, and cohesive operational information must be developed to help inform the development of those strategies.

In addition, the current sampling methods used to characterize a wide area would result in an overwhelmingly unrealistic number of samples that must be collected and analyzed. Existing availability of resources (sampling personnel and analytical laboratories) would not be able to handle such a large demand in even a reasonable amount of time. Different sampling methods must be developed to characterize the extent of contamination in wide areas. Those novel sampling methods must focus on larger sampling area sizes, be usable for outdoor surfaces, and must require less time and personnel to collect samples. For example, “native air samplers,” such as HVAC filters, bus/train/car cabin air filters, and ambient air quality samples may be used as potential sampling resources. As the above analysis has demonstrated, improvements to existing sampling methods with respect to recovery efficiencies would largely be inconsequential to reducing the resource demand (total cost and time to characterize) compared to improvements to sample area size (even using probabilistic sampling design approaches).

Also, there is a lack of understanding concerning the fate and transport of spores over long periods of time. This understanding is critical when considering the amount of time potentially required to conduct characterization sampling. Spore fate and transport must be understood for effective sampling and remediation strategy development. Lastly, this study supports further consideration of a combined sampling design approach using probabilistic and non-probabilistic sampling when characterizing a wide area incident. Additional decision support tools are needed to help direct sampling efforts for wide area incidents where time and cost considerations are critical factors.

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APPENDIX A. Literature Search Source Criteria and Keywords

Criteria for Sources of Information

- Information should come from sources that are considered recognized, reputable, and credible
- Information sources may include nationally and internationally recognized scientific, technical, or response organizations
- Information can come from written text, publications, reports, subject-matter experts, and internet sites
- Information sources can include (but are not limited to):
    - Peer-reviewed journals, scientific manuals, and other scientific publications
    - Federal, state, and local agency web sites or publications
    - University web sites or publications
    - Professional societies and organizations web sites or publications
    - Recognized international scientific/environmental organizations
    - International government web sites and publications
    - Military web sites and publications
    - Industry providers of equipment and materials (i.e., vendors)
    - Conference proceedings.

Literature Search Strategy and Keywords

("Environmental Monitoring" OR "Environmental Microbiology" OR "Sample collection" OR "Sample recovery" OR "Sample processing" OR Sampling OR "Sampling techniques" OR "Sampling procedure" OR "Sample preparation" OR ("Environmental sample" OR "Environmental sampling") OR ("Surface sample" OR "Surface sampling") OR ("Particle sample" OR "Particle sampling") OR ("Gross sample" OR "Gross sampling") OR ("Air sample" OR "Air sampling") OR ("Soil sample" OR "Soil sampling") OR ("Composite sample" OR "Composite sampling") OR ("Grab sample" OR "Grab sampling") OR (PCR OR "Polymerase chain reaction") OR "Biological detection" OR "Limit of detection" OR Detector* OR ("Detection methods" OR "Detection method") OR "Field detection")

AND

(("Biological agent" OR "biological agents") OR ("Bio-agent" OR "bio-agents") OR ("Bioagent" OR "bioagents") OR "Biological weapons" OR "Bacterial aerosol" OR Bioaerosol* OR "Biological aerosol" OR "Spores" OR "Bacillus anthracis" OR "Bacillus anthracis Ames" OR "Bacillus anthracis Sterne" OR "Bacillus cereus" OR "Bacillus megaterium" OR "Bacillus atrophaeus" OR "Bacillus subtilis" OR "Bacillus thuringiensis" OR "Geobacillus stearothermophilus")

AND

("Biological incident response" OR Bioterrorism OR "Biohazard release" OR "Biological warfare" OR "Field demonstration" OR "Field exercise" OR "Site characterization" OR "Site clearance" OR simulation)

To search PubMed, the following MeSH search segment was added:

(("Bacillus anthracis/isolation and purification" OR "Spores, Bacterial/isolation and purification” OR "Spores/isolation and purification") AND ("Environmental Monitoring/instrumentation" OR "Environmental Monitoring/methods"))

APPENDIX B. Literature Review Scoring Criteria

Process for Conducting Reviews

After identifying and locating relevant articles and other information from appropriate sources using predefined keywords (see Appendix A). The reviewers read, assessed, and documented pertinent articles. To standardize this process, a *Literature Assessment Questionnaire* was used to document the overall quality of literature. Each article was evaluated using a Likert scale (i.e., (1) Poor – (5) Excellent) based on four criteria: applicability and utility, accuracy, clarity and completeness, uncertainty and variability, soundness, and evaluation and review. Articles were scored according to the presence of these criteria. Table 1 shows the rubric for tallying articles.

Table B-1. Rubric for Tallying Articles

Overall Rating	Description
25—30	High quality article. Article shall be recorded and summarized accordingly
19—24	Moderately high quality article. Article shall be recorded and summarized accordingly
10—18	Lower quality article but with some useful information. Article shall be recorded and summarized accordingly
<10	Unacceptable/Do not use

Articles that score greater than or equal to 10 are deemed at least moderately relevant and shall be recorded and summarized accordingly; however, articles scoring less than 10 shall be discarded. For each source deemed as at least moderately relevant, the reviewer shall conduct an article summary. EPA shall receive a synthesized report containing the main points presented by all of the pertinent articles reviewed.

Literature Assessment Questionnaire

Relevant articles were defined as those crucial to answering research questions pertaining to current sampling methods for wide-area biological incidents. The reviewers considered the following criteria:

- ***Applicability and Utility:*** The extent to which the work not only addresses the area of inquiry under consideration but also contributes to its understanding; it is germane to the issue at hand. The extent to which the information is relevant for the intended use

- ***Accuracy:*** The extent to which data are consistent with accepted knowledge in the field or, if not, the new or varying data are explained within the work. The degree to which data fit within the context of the literature and are intellectually honest and authentic.

- ***Clarity and Completeness:*** The degree of clarity and completeness with which the data, assumptions, methods, QA, and analyses employed to generate the information are documented.

- ***Uncertainty and Variability:*** The extent to which variability and uncertainty (quantitative and qualitative) related to results, procedures, measures, methods, or models are evaluated and characterized.

- ***Soundness***: The extent to which the scientific and technical procedures, measures, methods, or models employed to generate the information is reasonable for, and consistent with, the intended application.

- ***Evaluation and Review:*** The extent of independent verification, validation, and peer review of the information or of the procedures, measures, methods, or models.

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