Challenges in Microbial Sampling in Indoor Environments

Workshop Report Summary

A collaborative effort of the Alfred P. Sloan Foundation, Yale University and the National Institute of Standards and Technology

ALFRED P. SLOAN FOUNDATION 1934

Yale UNIVERSITY

NIST

NIST Technical Note 1737

Challenges in Microbial Sampling in the Indoor Environment

National Institute of Standards and Technology • U.S. Department of Commerce

NIST Technical Note 1737

Challenges in Microbial Sampling in the Indoor Environment

Workshop Summary Report

A collaborative effort of the Alfred P. Sloan Foundation, Yale University, and the National Institute of Standards and Technology

February 2012

U.S. Department of Commerce
John Bryson, Secretary

National Institute of Standards and Technology
Patrick D. Gallagher, Director

Acknowledgments

Thanks go to all those who participated in the *Challenges in Microbial Sampling in the Indoor Environment Workshop* held on February 14-15, 2011, at the National Institute of Standards and Technology (NIST) in Gaithersburg, MD. The presentations and discussions that took place at the workshop provided the foundation for this report. A complete list of attendees can be found in Appendix A.

Special thanks are extended to the Alfred P. Sloan Foundation for providing a grant to conduct this effort, and the workshop organizers Jordan Peccia, Yale University, and Jayne Morrow, NIST. Plenary speakers who provided their insightful views and a starting place for discussions are noted below. A complete list of speaker presentations is provided in Appendix B.

Plenary Speakers

Paula Olsiewski, Alfred P. Sloan Foundation
Jayne Morrow, National Institute of Standards and Technology
Jordan Peccia, Yale University
Andrew Persily, National Institute of Standards and Technology
Shannon Williamson, J. Craig Venter Institute
Lynn Schriml, University of Maryland
Stephen Morse, Centers for Disease Control and Prevention

Special thanks are due to the Energetics Incorporated team who organized and facilitated the workshop and assisted in preparation of a draft report. This includes Joan Pellegrino, Anand Ragunathan, and Janis Tabor.

Executive Summary

This report summarizes the results of the *Challenges in Microbial Sampling in the Indoor Environment Workshop* held on February 14-15, 2011 at the National Institute of Standards and Technology in Gaithersburg, Maryland.

As the causative agent(s) of infectious and noninfectious disease, the microbial quality of the indoor environment has a significant impact on human health. Humans spend more than 90% of their time indoors and are in intimate contact with air and surfaces. Potential exposure routes to microorganisms are diverse and include direct ingestion, inhalation, and ingestion through hand or fomite to mouth transfer [1-3]. A fundamental understanding of the microbial community in the built environment, including estimates of diversity, function, and concentrations is necessary to develop an accurate portrayal of human exposure [4]. However, characterization of indoor microbial community quality has been hindered by challenges associated with aerosol and surface sample collection methods and strategies. Known biases with sampling including sample selection and collection efficiencies limit both the accuracy of reported quantities and validity of the data. There is little work examining the direct biases in represented populations or in addressing the uncertainty accumulated through sample collection and processing for both surface and aerosol microbial communities [5]. This lack of understanding can severely impede the researcher's abilities to interpret data and quantitatively define differences in environments or treatments. Important challenges include sample extraction efficiencies, impacts of sampling on viability, introduction of contamination, and method detection limits. In the case of aerosols and surfaces, standard sampling protocols are not adequate and have not been fully validated. Furthermore, current air and surface sampling techniques traditionally coupled with culture techniques and the associated extraction and processing procedures are not optimized for modern paradigms in microbial analysis that depend heavily on genomics [6, 7].

The purpose of the *Challenges in Microbial Sampling in the Indoor Environment Workshop* was to explore current aerosol and surface sample collection methods for microbial community quality characterization. This report highlights the current state of aerosol and surface sample collection science for unknown microorganisms in complex indoor environments, identifies needed investment in fundamental research and standards development, and provides guidance based on our current understanding to inform efforts to sample aerosols and surfaces for nucleic acid-based analysis. Invited participants included representatives from academia, industry, and government.

TABLE OF CONTENTS

1. INTRODUCTION

Background

The microbial quality of the indoor environment can have a significant impact on human health. Microorganisms harbored in the spaces in which we work and live have been associated with infectious diseases, acute toxic effects, allergies and cancer. Humans spend more than 90 percent of their time indoors and are in intimate contact with the surrounding air and surfaces [8]. Potential exposure routes to microorganisms are diverse and include ingestion, inhalation, and contact transmission, either person to person or via fomite contamination[1]. A fundamental understanding of the microbial community in the built environment, including estimates of diversity, function, and concentration, is necessary to develop an accurate portrayal of human exposure and to inform the design and operation of buildings to improve human health [4, 9, 10].

Early microbiological surveys relying on culture-based detection methods provided important, but limited insight into the diversity of microflora inhabiting indoor environments [11-16]. The vast majority of microbes cannot be cultured using traditional methods [7] and the observed cultivable population may be biased by the choice of culture method (e.g. high vs. low nutrient media). In recent years, our understanding of the microbiome has broadened dramatically with the advent of DNA sequence-based phylogenetic approaches, which facilitate culture-independent characterization of the entire microbial community through direct isolation of genomic DNA from an environmental sample [7]. Studies utilizing this mostly rRNA encoding gene-based approach have revealed an astonishing diversity of microorganisms in the built environment and are helping to elucidate the ecology of healthy and impacted indoor environments [3, 17-21]. It has become clear that microbes inhabiting buildings are distinct from the outdoor populations [3] and in large part originate from humans sharing that environment [17, 19, 20]. Some commonly abundant bacterial and fungal taxa present are shown in Figure 1.1. A mix of skin-surface bacteria in indoor air, surface, bulk and dust samples is common, even if levels are relatively high. Both small scale (building characteristics)[22] and large scale factors (e.g., climate and geography) [18, 19, 21] can influence the microbial quality of the indoor environment.

Although we are gaining a better understanding of the community constituents, the overall impact of the indoor microbiome on human health is difficult to discern. Part of the challenge in determining health impacts is our limited ability to quantify exposure to potentially hazardous organisms, assess the relationship between presence of an organism and its ability to cause disease (viability) and to precisely identify which organisms present in the environment are mediating disease.

[1] A fomite is an inanimate object (e.g., cloth, mop head, bedding) or substance capable of carrying infectious organisms (such as germs or parasites) and transferring them from one individual to another.

Accurate characterization of microbial communities depends upon the ability to collect an unbiased sample from the indoor environment. However, biases in current sample collection methods (e.g., sample selection and collection efficiencies) may limit both the accuracy of reported microbial populations and the validity of the data. While there is some guidance on the selection and integration of sample collection methods into a sample collection strategy and plan [23, 24], there is little guidance for addressing the uncertainty accumulated through sample collection and processing for both surface and aerosol microbial communities. This lack of understanding can severely impede the ability of researchers and practitioners to interpret data and quantitatively define differences in environments or treatments. Furthermore, current air and surface sampling techniques traditionally coupled with culture techniques, and the associated extraction and processing procedures, are not optimized for modern microbial analysis that depend heavily on genomics [6, 7].l

Figure 1.1. Some Common Microbes in the Indoor Environment

Bacteria:

Firmicutes

Bacillus spp: Spore-forming bacteria associated mostly with soil and dust. Most are not considered to be serious pathogens.

Staphylococcus spp: Inhabitant of and shed from skin surfaces. Commonly found species indoors include *Staphylococcus epidermidis*, *S. hominis* and *S. aureus*, a potentially pathogenic species.

Actinobacteria

Including *Micrococcus, Corynebacterium,* and *Propionibacterium spp:* Bacteria from the normal shedding of skin, found in mattress dust and on surfaces in areas of higher occupant density or inadequate ventilation, and generally considered harmless.

Proteobacteria

Pseudomonas spp: May persist on wet or moist surfaces in the form of biofilms. Some species are pathogenic.

Fungi:

Dothideomycetes

Cladosporium spp: One of the most common indoor and outdoor molds. Rarely pathogenic to humans but is a common allergen.

Alternaria spp: Ubiquitous in the environment. Many species are important plant pathogens. Common allergens in humans and can cause opportunistic infections.

Eurotiomycetes

Penicillium spp: Ubiquitous in soil. Fungi that is important to both food and drug industries. Species is also a common allergen.

Aspergillus spp: In humans, may cause allergies or infections, particularly in immune compromised individuals. Some species like *A. niger* can grow in low nutrient environments (e.g. damp walls), contributing to mildew.

Important challenges in generating unbiased data from indoor surface and airborne microbial populations include the unknown sample collection and extraction efficiencies for the diversity of microbes, the impact of sample collection and transport methods on microorganism community descriptions, the introduction of contamination (whole cell contaminants and DNA), and the limited ability to quantify surface loads, the ability to produce size-resolved sampling of aerosols, and the low content captured on many aerosol samplers. In the case of aerosols and surface samples, standard sampling protocols do not exist or have not been verified to accurately describe the indoor microbiome.

Workshop Purpose and Objectives

The *Challenges in Microbial Sampling in the Indoor Environment Workshop* was held on February 14-15, 2011 at the National Institute of Standards and Technology to explore current aerosol and surface microbial sample collection techniques and identify requirements for innovation and research in the field of sample collections. The workshop was supported by a grant from the Alfred P. Sloan Foundation, and was conducted and organized through the collaborative efforts of Yale University and the National Institute of Standards and Technology.

Experts from industry, government, and academia were brought together to explore current aerosol and surface sample collection methods for microbial community quality characterization. The following questions regarding indoor microbiological sampling were discussed by workshop participants*:*

- What are the current sample collection and processing procedures available to characterize the indoor microbiome?
- What are the future requirements for monitoring and characterizing microbe communities?
- What are the challenges and limitations with current methods, and what are the gaps in existing techniques?
- What are priority issues that should be addressed to meet future requirements? What are the pathways and approaches that should be taken to develop and improve techniques to meet future needs?

While recognizing that the microbial quality of water is an important source and potential exposure route for infectious disease, the traditional focus on waterborne disease has allowed for a much greater development of water sampling strategies. Consequently this workshop focused on aerosols and surfaces in the indoor environment, as described in Figure 1.2. This report summarizes the results of workshop discussions around these questions, organized by the two primary topics of surface science and aerosols.

Figure 1.2. Workshop Topics

Surface Science – methods for sampling, collection, and characterization of microbes found on a diversity of indoor surfaces such as carpets, upholstery, wood, wall board, fiberboard, ceramics, plastics, and other nonporous surfaces. Techniques available today include vacuum or mini-vacuum devices, swabs, wipes and contact methods including adhesive tape, RODAC plates and dipslides.

Aerosols – methods for sampling, collection, and characterization of airborne microbes including allergens, toxins, bacteria and viruses. Techniques used today typically include impactors/impingers, and filtration devices.

Extensive discussions resulted in characterization of current sampling methods, followed by identification of future needs/objectives for innovation of collection techniques and new technologies. A number of existing resources of relevance to these topics were identified and are provided in Appendix C. A set of priority areas were also identified for meeting future needs. Appendix D provides detailed pathway and approach diagrams for each of the priorities. These reflect discussions from the aerosol and surface sampling groups on key challenges, recommended actions for the research community, potential applications, and possible impacts of furthering work in this area.

It is anticipated that this report will provide guidance to field researchers and field operations personnel involved in future research and development activities for aerosol and surface microbial sampling methods, and will also provide assistance to investigators in developing appropriate sampling strategies. It is not intended to be all-inclusive but rather is a snapshot of the perspectives of those experts who participated in this workshop.

2. General Information to Facilitate Collections for Building Characterization

Relevant Background on Buildings

There are two critical issues when considering the impact buildings have on the microbial populations found within them. The first is that there are many building features that impact microbial growth and transport and that there are important variations in these features among buildings. Second, given that buildings vary in their design, construction and use, building features should be measured as part of indoor environment studies of microbial population characteristics.

One of the key building features that must be considered in these studies is the boundary that separates the indoors from the outdoors, sometimes referred to as the building envelope or enclosure. The boundary provides shelter from the weather, including precipitation, and allows the interior to be more comfortable than the outdoors. However, the boundary can retain humidity, particulate matter and gaseous contaminants associated with indoor activities and materials, and can provide environments that support microbial growth. Critical building factors to consider when designing indoor sampling collection experiments include HVAC system characteristics associated with heating and cooling, humidification and dehumidification, particle filtration and air cleaning as well as wall construction and materials, furnishings, items, and activities associated with occupants. Appliances and the usage patterns of those appliances influence indoor environmental conditions, and activities such as cooking, hot water generation and indoor plumbing can all influence the dynamics of microbial populations in the indoor environment.

Evaluating key aspects of the building that influence the indoor microbial community involves determining building age, construction, foundation type, condition of the building including renovation history, HVAC system type and upgrades, and measuring outdoor air ventilation (air exchange rates). Other important parameters to investigate include indoor temperatures and the building moisture

environment, including sources of moisture, condensation sites and materials conducive to microbial growth. When sampling a facility, special attention should be paid to the nature and behavior of buildings occupants: their age, number of occupants, location in the building, and activities, including schedule of residence in the building. Relevant considerations for designing the study include how the building is maintained and cleaned, and the presence of other animals or pests. Appendix C has a number of essential building and architectural resources to assist in accounting for building parameters when designing sampling strategies and interpreting indoor environment microbial communities.

Sampling Strategies and Plans

In order to effectively characterize the built environment the samples to be collected must be determined in the context of a sampling strategy and a sampling plan. A sampling strategy includes the approach or combination of approaches used to select locations at which to collect samples and provides any relevant guidance to inform decision support and data interpretation processes. The sampling strategy is a high level or general document that guides the collection of samples for characterization (extent of contamination, differentiated zones or sectors based on use or airflow), where the samples should be collected, number of samples needed for accurate characterization and the breadth of collection methods available. It is important to note that not all indoor environments are sampled for the microbial population with a pure research interest. A list of sampling strategy resources available to the field research community is provided in Appendix C.

Indicators of disease or disease-causing organisms can also often prompt sample collection efforts. In this case sampling personnel safety and health is critical to consider when designing the sampling strategy for these scenarios. Additional guidance for the selection of personnel protective measures is found in Appendix C.

A sampling plan is an executable plan of action that addresses the sampling and analytical requirements of a specific situation and adheres to the specific sampling strategy. The sampling plan must specify the sampling approaches, methods, and analyses, as well as the number, types, and locations of samples to be collected in a given physical space. The sampling plan should account for the area under consideration, the number of samples, and the collection locations needed for statistical confidence as determined by directed and/or statistical sampling designs.

Directed sample collection utilizes an expert in the field (indoor environment engineer or microbial ecologist) to determine the suitability of the plan to meet the experimental objectives. Statistical sampling utilizes a mathematical framework to determine if the number and location of sample collection sites meets specific characterization objectives. Statistical methods such as simple random sampling, stratified sampling, systematic or grid sampling, ranked set sampling; adaptive cluster sampling, and composite sampling can all be applied based on the needed statistical approach. References for these approaches can be found in Appendix C.

3. COLLECTING MICROBES ON SURFACES

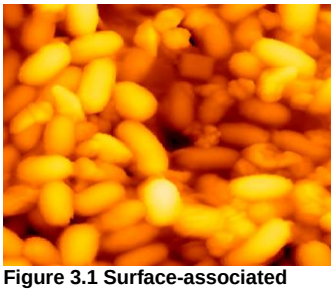


Figure 3.1 Surface-associated spores captured by Atomic Force Microscopy
Image credit: NIST/Jayne Morrow

Microorganisms found in indoor environments most often arise from human sources (continuously shed with human skin and hair) or by transport from the outdoors and once inside can settle on surfaces. Surface-associated organisms can also be re-aerosolized with human activity and building air movement from ventilation equipment and other airflows. Negative impacts to human health from indoor microbes are related to the ability of the organism to persist on surfaces resulting in potential contact transmission and the ability of the organism to degrade materials in the indoor environment generating toxic metabolic by-products.

A number of studies have demonstrated considerable persistence and prolonged viability for surface associated microbes [25-29]. Finding hundreds of thousands of bacteria per gram of dust in carpets, for example, is not uncommon.

Molds, another common group of microbes found on indoor surfaces are often sampled to characterize the source of odors or toxins (mycotoxins) in a building. The objective of mold sample collection is usually to determine whether the suspected contamination (visible stain, discoloration, etc.) is indicative of mold growth to inform remediation strategies. Molds can result in significant negative health effects in sensitive individuals (asthma and immunocompromised) if mold growth is not properly mitigated and the production of mycotoxins by some molds can lead to fatigue, nausea, headaches, and irritation to the lungs and eyes.

Surface-associated microbes can become airborne (aerosolized) through normal human activities and by airflow movement due to the building ventilation system. Health problems related to indoor microbial contamination are often associated with the inhalation of airborne particles including toxins, spores, bacteria and viruses. However, direct contact with surface contamination, contact transmission, is one of the main routes for infection transmission (Appendix C, Federal Agency Efforts and Resources, Centers for Disease Control and Prevention Healthcare Infection Control Practices Advisory Committee). [30, 31]. The particles may be consistently re-aerosolized resulting in exposures over a prolonged period of time. A discussion of aerosols and aerosol sampling is found in Chapter 4.

Current State of Science for Surface Collection Methods and Processes

Careful consideration of the downstream analysis methods is critical in the decision to apply any sample collection method. Traditionally, analytical methods for microbial characterization were largely dependent on culture techniques that required processing that preserved viability. Today, collection and analysis of microbes from surfaces is conducted using a number of different methods and for various

purposes. Measurements of surface-associated microbial populations are needed for establishing both positive and adverse impacts to human health and the environment, and for regulatory or national security purposes. Collection and analysis of surface-associated organisms can be used to ascertain environmental conditions for occupants inside buildings, and to provide data for scientific studies in a variety of fields. The users of these measurements today are as diverse as the needs and requirements for data. In the future, an even broader spectrum of stakeholders could require access to information on indoor surface microbial ecology as the breadth of the science expands and the analytical and research communities grow. Table 3.1 illustrates the various sampling processes available and in use today. These vary in accuracy and yield, and each has specific limitations and applications.

Collection Methods

Currently, sampling strategies rely on the application of relatively simple collection methods including vacuum, swabbing, wiping, contact methods and/or bulk sampling. Surface sampling by these techniques is relatively quick and inexpensive and can be analyzed with genomic methods after extensive processing. The sampling methods shown in Table 3.1 are those mostly commonly used and require little specialized equipment (except for cellulose sponge methods that require a stomacher). Contact-based methods can be immediately analyzed by culture but have limited immediate application for molecular detection (e.g. genomic techniques) aside from fluorescent *in-situ* hybridization. Wipe, swab and vacuum samples require extraction and processing for nucleic acids extraction and subsequent characterization by genomics techniques.

Post Sampling and Analysis

Table 3.1 illustrates some of the considerations for post-sampling analysis of surface samples. One aspect that has thus far been given limited attention is the preservation of the sample which may directly impact the yield and accuracy of results. Little data is available on the preferred conditions for preservation during storage and transport to prevent degradation resulting in a loss of integrity. Storage and transport conditions optimal for genomic characterization methods require additional research.

Table 3.1 Summary of Surface Sample Collection Methods

	Sampling Conditions	Organism-specific Sampling Data	Preserved Viability	Sample Suitable for Molecular Methods	Advantages/Disadvantages
Wipe Mechanism: Biological particle collection by entrapment or thermodynamic association. Typical models/materials: Woven (polyester Rayon, cotton Nonwoven (Polyester, polyester Rayon) Sponge (cellulose)	Dry wipes N/A Wetting agents for pre-moistened wipes: PBS Water Saline Ringers solution Copan SRK formula rinse solution Neutralizing Buffer (e.g. Hardy Diagnostics) Surfactant addition: +/- Triton X100, Tween 80, Tween 20	Multiple references demonstrating recovery efficiencies for a range of conditions [32-35]. Sponge collection procedures have been validated for *Bacillus anthracis* spores on nonporous surfaces[36].	Viability preservation is a function of wetting agent, transport conditions and extraction processing procedures.	Once extracted, samples can be analyzed with molecular methods. Some collection procedures have been demonstrated to provide a sample for PCR analysis specific for *Bacillus anthracis* spores and other potential pathogens [37-39].	Advantages: -Large collection area; -Demonstrated utility on porous and carpeted surfaces when vacuum sample collection is not available; -Ease of use in collection and processing; and -Relatively low cost. Disadvantages: -Potential operator contributions to collection efficiency not well studied; -Extraction from sponge requires stomacher procedure; and -Recovery efficiencies are largely dependent on collection and extraction conditions.
Swab Mechanism: Biological particle collection by entrapment or thermodynamic association. Typical models/materials: Cotton Polyester (Dacron), Rayon, Sponge (Macrofoam, SCRD, polyurethane foam), Nylon flocked, Calcium alginate	Dry swab Collection of bulk materials with dry swabs and cards or scoops. Wetting agents for pre-moistened swabs: PBS Water Saline Ringers solution Copan SRK formula rinse solution Neutralizing Buffer (e.g. Hardy Diagnostics) Surfactant addition: +/- Triton X100, Tween 80, Tween 20	Multiple references demonstrating recovery efficiencies for a range of conditions [40-42]. Multiple collection procedures have been validated for *Bacillus anthracis* spores on nonporous surfaces (Appendix C).	Viability preservation is a function of wetting agent, transport conditions and extraction processing procedures.	Once extracted, samples can be analyzed with molecular methods. Some collection procedures have been validated to provide a sample for PCR analysis specific for *Bacillus anthracis* spores and other potential pathogens[43].	Advantages: -Small localized collection areas and hard to reach places can be sampled; -Utility on a range of surface types; -Ease of use in collection and processing; and -Relatively low cost. Disadvantages: -Potential operator contributions to collection efficiency not well studied; - Small localized collection areas require many swabs to cover large areas; -Extraction is dependent on collection material, wetting agent and extraction conditions; and -Recovery efficiencies are highly variable based on collection and extraction conditions.
Vacuum Mechanism: Sampled air is passed through a	10-100 L/min vacuum air flow rates over the filter.	Collection efficiency is dependent on particle size range; very	Viability preservation is a function of vulnerability	Samples are collected on filters (Teflon or polycarbonate membranes,	Advantages: -Collects from large surface areas; -Collects from porous or carpeted surfaces;

Table 3.1 Summary of Surface Sample Collection Methods					
	Sampling Conditions	**Organism-specific Sampling Data**	**Preserved Viability**	**Sample Suitable for Molecular Methods**	**Advantages/Disadvantages**
small opening and biological particle is captured onto a filter or collection matrix. Typical models/materials: -HEPA socks (0.1 µm pore size); -3M Trace evidence collection filters (0.1 µm pore size) -Microbial-Vac (MSI)		limited data is available for a range of organisms. Efficiency drop and filters can be bypassed as collected material accumulates.	to air transport during collection, storage, transport conditions and processing procedures.	quartz fiber filters) which once extracted yield material for molecular characterization.	-Filter units are inexpensive to replace; and -Ease of use for collection. Disadvantages: -Difficult to process filters, requires units to be dismantled and/or cut to extract collected material; -High risk of cross contamination in laboratory environments during analysis; and -Filters become clogged.
Contact Techniques Adhesives, contact or RODAC plates, dipslides Mechanism: biological particles are transferred from the surface by direct contact with adhesive surface. Typical models/materials: -BD diagnostics	Collection material is applied by direct contact with a nonporous surface.	Very limited information on tape and adhesive performance at collecting a range of organisms is available. Contact plates have been extensively applied to collect organisms of interest [44] .	Viability preservation is maintained by contact plates and some adhesives.	Samples are not readily available for molecular characterization for most contact methods excluding some adhesives for which molecular methods are performed directly on the tape.	Advantages: -Inexpensive; -Direct observation of collected sample; -Preserves deposition distribution information; -Contact plates preserve viability; -Reduced chance of organism loss due to multiple processing steps; and -Ease of use. Disadvantages: -Limited collection areas; -Contaminants and other materials may mask organisms of interest; and -Requires a direct observation method or growth to evaluate.
Bulk Collection Biological particles in bulk material are removed and transported to the laboratory. Mechanism: Visible material is removed from the surface by scoop, card, scissors if carpet or upholstery, packaged and transported to the lab.	Requires visible material that is of interest to the collection.	Card collection procedures has been validated for *Bacillus anthracis* spores on nonporous surfaces [45, 46]**.**	Viability preservation is a function of storage, transport conditions and processing procedures.	Samples can be analyzed with molecular methods; extraction may or may not be required for analysis.	-Large quantity of material available for analysis; -Ease of use in collection and processing; -Relatively low cost; Disadvantages: -Visible material required; -Laboratory constraints limit quantity of material that can be collected and analyzed; and -Contaminants in bulk samples (i.e. dirty carpet) may inhibit downstream analysis methods (i.e. PCR inhibition).

Challenges and Limitations of Surface Sampling Methods and Processes

A summary of the challenges, recommended actions and approach, and the current stakeholders and resources available to accomplish the defined performance targets and subsequent impacts are summarized in Figure 3.2. The key challenge to achieving the surface sample collection methods needed for current and future applications includes a lack of standards and guidelines for collection, processing, data analysis, and reporting to provide consistency of results and enable greater comparability and utility of data across studies. For example, a number of guidelines are needed for sampling processes (e.g., collection areas, materials used in swabs, wipes and solutions). These would define proper controls and metadata requirements to enable assessment of collection process impacts on metagenomic community characterization results, ensure interoperability among systems, support consistent international and national reporting, and define a standard validation process for various methods.

A major challenge for surface sampling is the known low recovery for surface contaminant microbes historically investigated (e.g. biothreat and health care facility characterization). The efficiency of recovery for the microbiome is also vastly unknown. Low recovery can occur for various reasons, such as surface contamination interference with collection, using materials that do not attract bacteria well or retain too many of the collected bacteria excluding them from analysis, extraction liquids that do not sufficiently allow for release of the organisms from the collection material, or dissociation methods that do not adequately remove microbes from the recovery container. Another major challenge is the lack of consistency and repeatability of results, which makes comparison of results difficult. A closely related issue is the limited number of sampling procedures and methods that have been utilized and are known to produce a demonstrated set of results for a single organisms but unknown for assessing the microbiome. Additionally, sampling methods can introduce bias, and error associated with factors external to the method exist, including those associated with the operator and environmental conditions during collection. All of these factors have been addressed only in a limited way in single organism studies and are largely unknown for the microbiome.

Additionally, a lack of standards for gathering and reporting on data from the results of surface microbe sampling currently hampers the ability to compare results across different applications, to understand and compare the efficacy of various methods for indoor environments, and to allow for data sharing. Common protocols would establish requirements for data reporting, metadata, and independently reported controls are needed to effectively characterize the sampling method and enable credible comparisons of results.

Figure 3.2 Surface Sampling: Summary of Priority Topics

Standardized Guidelines for Collection of Surface Bound Biological Samples

Challenges:

There is limited data on recovery efficiency (available data indicates low recovery efficiencies) or biases of collection methods when examining the composition of the microbial community (microbiome) in the built environment. Current methods for surface sampling are optimized and validated for specific microbes and suspected contaminants on defined surfaces of interest.

Actions and Approach:

- Assemble and tap existing forums to establish a community-driven consensus for reference methods and best practices.
- Gather and analyze available data and current information about types of available collection materials (e.g., swabs and wipes) to understand how collection approaches affect a representative set of diverse microbes.
- Based on these findings, guidelines for microbiome characterization should be developed by an open forum based on standardized collection approaches already in practice and validated for known contaminants on like surfaces until methods specifically evaluated for microbiome recovery efficiency are available. In development of the guidelines, specific attention should be focused on:
  - Optimizing extraction of microbes from the sample collection device model/material
  - Optimizing DNA and RNA extraction from collected surface samples
- Draft protocols that include reference to any known recovery variables significant to the follow-on metagenomic analysis should be distributed through a web-based report, peer-reviewed literature, and eventually through guidance from relevant government agencies.

Stakeholders and Existing Resources:

- Several federal agencies, including the Environmental Protection Agency (EPA) and Centers for Disease Control and Prevention (CDC), as well as additional organizations have an interest in defined protocols from the research community including NIST, ASTM, AIHA, ACGIH, ASM, IAA, and IEST.
- Existing related resources include a large body of literature (books, journal papers, guidelines) on surface sampling of contaminants of interest from the industrial hygiene and more recently for biothreat response and a limited, but growing body of literature. Relevant resources in Appendix C include:
  - EPA Visual Sample Plan (VSP); draft environmental sampling strategy
  - *Sampling for Biological Agents in the Environment* (hardback);
  - Guideline on indoor environment sampling in healthcare facilities
  - CDC NIOSH protocols for sampling

Performance Targets and Impacts:

- Targets include:
  - Develop and disseminate guidelines, consensus standards and validated methods, where appropriate, for sample collection, extraction, subsequent processing, and data analysis. Standards and guidelines should include multiple protocols depending on the type of data and analysis desired, and the inclusions of definitive efficiencies built into the protocol to improve the accuracy, precision, and detection levels of quantitative analysis of surface bound contamination
  - Establish mechanisms for continuous feedback and evolution in guidance by the research community
- Major impacts include:
  - Increased public knowledge and awareness of the various types of methods
  - Greater comparability of studies and increased confidence in results
  - Improved health practices through better data collection and greater understanding of composition of microbial communities

Future Priorities for the Collection of Microbes on Surfaces

The future priorities identified to improve surface sampling techniques are outlined in Table 3.2 with detailed priority pathways illustrated in Appendix D. Today's surface sampling methods suffer from low recovery for a number of reasons (surface contamination, microbe retention/loss across the process, limitations of extraction methods, etc.). Optimization and validation of methods would help to address the issue of low recovery and lead to consistent, reliable methods for characterization of the microbial community in the indoor environment. Innovation of collection method and material design would eliminate some of the system's losses and enhance overall recoveries. In order for innovative techniques to emerge, comprehensive examination of collection methods/materials needs to occur to better understand how they bias the reported communities, and a representative set of diverse microbes needs to be agreed upon by the indoor microbiome research community as a control for assessing community samples.

Determination of accuracy and resolution of sample collection method performance parameters for collecting the microbiome will be needed to provide greater confidence in measurements as well as the interpretation of results and having a control microbiome is a practical place to start. Furthermore, understanding processing method bias is also critical to developing new processing methods including extraction of microbes from the sampling matrix/device and subsequent DNA and RNA extraction from solution phase cells, which requires optimization studies for metagenomic applications. Fundamental research into ecological and environmental niches will also be important for identifying and reducing known bias in collection method efficacy.

Table 3.2. Future Requirements for Surface Sampling

Improved/Advanced Collection Methods
• New designs for sampling equipment that are easily decontaminated and limit cross contamination of lab facilities • High surface coverage, low cost collection • Method for determining and separating biomass from inert surface contaminants • Increased recovery and extraction/processing efficiencies • Pressure application control or operator training to standardize collection technique • Consistency and conformity of methods
Standards and Protocols
• Standardization of procedures for collection, processing, and analysis of samples for microbiome characterization • Increase in the amount and availability of information on recovery and extraction efficiencies for nucleic acids as well as recovery of viable organisms • A representative set of diverse microbes, agreed upon by the indoor microbiome research community
Post-Sampling and Analysis
• Minimization of losses across the sample collection and extraction processing through small collection and analytical solution volumes versus large sample volumes during processing • Characterization of inhibitors in samples and/or control for and measure of inhibition of analytical methods • Better methods for culturing organisms, when culture is required

4. COLLECTING MICROBES IN AEROSOLS

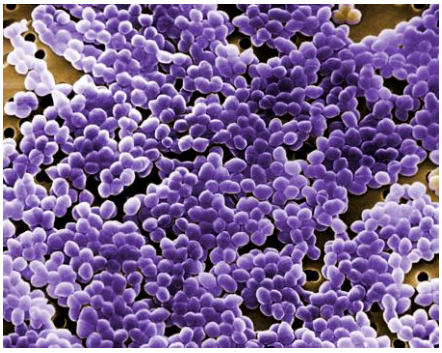
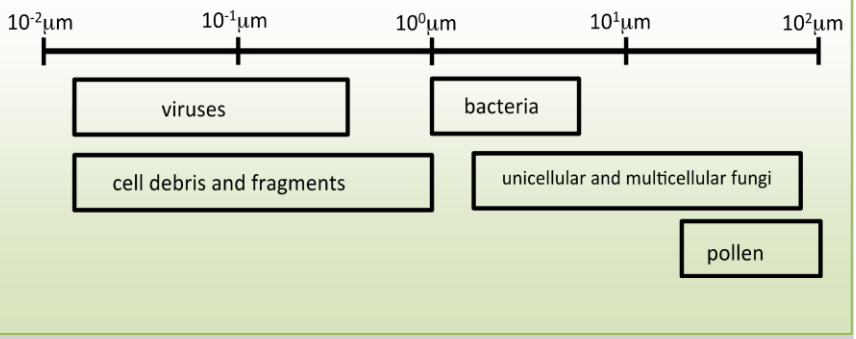


Figure 4.1. Scanning electron micrograph (SEM) depicting large numbers of Gram-positive *Enterococcus* sp. bacteria
.
Image Credit: CDC/Janice Haney Carr

The microbial fraction of particulate matter suspended in the atmosphere or indoor air is often termed "bioaerosols". These are a mixture of viable and nonviable microorganisms (e.g. pollen, algae, bacteria, fungi, yeasts) as well as other types of biomass including viruses and a wide range of antigenic compounds, such as dander, plant and insect debris, and microbial toxins. Human exposure to bioaerosols can occur through inhalation, dermal contact, and ingestion. Biological material suspended in outdoor air has important implications for climate by acting as ice nuclei and cloud condensation nuclei in the troposphere [47, 48]. The most profound impact of bioaerosols is as the causative agent of disease. A small percentage of viable bioaerosols are associated with well-known infectious human diseases, such as tuberculosis, Legionnaire's disease, and various other forms of bacterial pneumonia, influenza, measles, SARS, and gastrointestinal illness [49]. The intentional release of these pathogens and others into the air is also a large contemporary concern. Some airborne fungi, bacteria, and viruses are the causative agents of plant and animal diseases, which can have large economic and ecological, and agricultural consequences. Finally, bioaerosols have also been associated with noninfectious diseases, such as hypersensitivity pneumonitis, organic dust toxic syndrome (ODTS), allergies, and asthma [50].

The key parameter dictating the fate of and human exposure to biological aerosol in the environment is their aerodynamic diameter. Aerodynamic diameter is defined as an expression of a particle's aerodynamic behavior as if it were a perfect sphere with unit-density and diameter equal to the aerodynamic diameter. Aerodynamic diameter strongly impacts sampling efficiency, penetration, resuspension, and deposition in buildings, and deposition into human airways [51]. Figure 4.2 provides examples of the aerodynamic diameters of important classes of bioaerosols. These particles may present larger sizes in the environment due to attachment to themselves or other biotic or abiotic particles.

Figure 4.2 Typical Bioaerosol Aerodynamic Diameters



Current State of Bioaerosol Methods and Processes

The sampling methods and processes available and in use today are shown in Table 4.1 with the particle size ranges considered, ability to capture viable samples, and a short discussion of advantages and disadvantages. These methods are typically classified by their modes of collection which include either impaction onto hard surfaces or liquid impingement.

The desirable properties for biological aerosol samplers are based on the needs of post-sampling analysis method, the desire to understand the fate and transport of and exposure to the biological aerosols through size resolved measurements, and conditions dictated by the sampling environment. Often, the desired properties exist in the diversity of current aerosol samplers, but rarely in a single sampler (Table 4.1). For example, samplers that use liquid impingement to maintain viability are poorly characterized for size distribution and efficiency. Filter or impaction based samplers, on the other hand, offer better resolution of sizes and efficiencies but cannot be used if culturability or infectivity assays are to be performed. Due to the relatively lower cost, ease in decontamination, size-resolution capabilities, high flow rate, and high capture efficiency, sampling aerosols onto filters is recommended for DNA-based analysis methods. If viability or gene expression information is required, liquid impingement is more appropriate, but many of the advantages ascribed to filtration may be lost. Finally, if detailed information on size distribution is desired, an impactor is preferable due to their ability to sample multiple sizes in cascade type impactors (Table 4.1).

Table 4.1 Summary of Aerosol Collection Technologies

	Sampling Rate	Size-Resolved Sampling	Viability	Sample Suitable for Molecular Methods	Advantages/Disadvantages
Cascade impactors Mechanism: The sampling air stream makes a sharp bend and particles are stripped based on their aerodynamic diameter. Typical models/materials: -Anderson Cascade Impactor; -MOUDI cascade impactor; and -BGI 900 L/min high volume cascade impactor.	Typically 10 to 28 L/min. Some samplers allow for > 500 L/min.	Provides the best size distribution information. Different models offer between 1 and 12 stages for collecting aerosols with aerodynamic diameters from 10 nm to >18 µm.	Only at 28 L/min collection rates and requires direct sampling onto agar plates.	Stages can be covered with filters, membranes, or media plates and samples can then be extracted from these materials. The panel did not recommend use of foam as a sampling medium due to the low efficiencies associate with cell and DNA extraction.	Advantages: -Best ability to define particle size distributions; and -Models available to perform culturing. Disadvantages: -High cost per sampler, especially for high volume samplers; -Sampling inefficiencies due to particle bounce; and -Not sensitive as total sampled mass is divided among multiple stages.
Filtration Mechanism: Aerosols are	Ranges from 4 L/min and up to 1,000	Filtration samplers typically have size selective	Not recommended for viability due to high	Requires extraction from filter material, often Teflon or polycarbonate	Advantages: -High sampling rates available; -Most common and robust form of high volume sampling;

Table 4.1 Summary of Aerosol Collection Technologies					
	Sampling Rate	**Size-Resolved Sampling**	**Viability**	**Sample Suitable for Molecular Methods**	**Advantages/Disadvantages**
captured on filters by impaction or diffusional forces. Typical Models/materials: -Anderson High volume PM samplers; -SKC IMPACT samplers.	L/min.	inlets that allow for sampling 10 µm and below (PM_{10}) and 2.5 µm and below ($PM_{2.5}$) size fractions. Because of high diffusional forces, filters are efficient at sampling sizes down to the 20 nm range of viruses and microbial fragments	stresses from impaction and desiccation.	membranes, quartz fiber filters, or gelatin filters.	-Very small particles can be sampled, most efficient way to sample viruses; -Can be used as personal samplers; -low cost compared to impingers and impactors; and -Preferred method for sampling PM for regulatory compliance. Disadvantages: -No possibility for viable determination; -High volume samples are not suitable for sampling in most occupied environments; and -Limited ability to produce particle size distributions.
Liquid impingement Mechanism: Sampled air is passed through a small opening and captured into a liquid medium. Typical Models/Materials -SKC swirl impingers; -Omni 3000 high volume impinge.	14 L/min for glass impingers, new high volume models are capable of >100 liters per minute.	Very limited information on the size ranges that are collected. Efficiency drops in low volume glass impingers below aerodynamic diameters of 1 µm. High volume samplers have not been characterized for sampling efficiencies as a function of particle sizes.	Impingers are flexible since organisms are impinged into liquid media or buffer and can be used for culturing or molecular analysis.	Samples are impinged into 10 to 20 ml of liquid, which may require concentration by filtration.	Advantages: -Sample is collected into liquid and does not require extraction from a solid collection medium; and -Low cost of low flow glass impingers. Disadvantages: -Limited information on efficiencies, and the particle sizes that are sampled; -High volume impingers are high cost; -Glass impingers suffer from low sampling rate and limited sampling times due to evaporation; and -High volume impingers have complex systems for collecting the sample and rewetting surfaces, and there is large concern about effectively decontaminating the equipment.

Example Protocols for Aerosol Sampling

Due to the lack of widely accepted standardized protocols for sampling biological aerosols, this report instead presents successful protocols utilized by research groups that participated in the workshop and are currently sampling indoor environment air and apply molecular analysis to the samples collected. Tables 4.2 through 4.4 illustrate some of the protocols currently in use.

Table 4.2 Protocol from Yale University, Chemical and Environmental Engineering*

Target Analysis	Type of Environment Sampled	Sampling Equipment	Flow Rates	Aerodynamic Diameters (d_a) Collected	Sampling Media	Detection Level (# of target genes or cells)	Major Challenges
Size resolved (8 sizes) samples for quantitative PCR or PCR amplification/ amplicon sequencing.	Indoor, human-occupied settings.	Anderson nonviable eight- stage cascade impactors (New Star Environmental, Roswell, GA)	28.3 L/min. or ~3.5 L/min. for each stage	Stage 1: 0.4-0.7 Stage 2: 7-1.1 Stage 3: 1.1-2.1 Stage 4: 2.1-3.3 Stage 5: 3.3-4.7 Stage 6: 4.7-5.8 Stage 7: 5.8-9.0 Stage 8: >9.0 µm	Polycarbonate track etched filters, 0.2 µm pore size, 81 mm diameter, or Glass fiber filters, 81 mm diameter.	2,000 to 3,000 bacterial cells and 10 to 25 fungal cells. (fully accounting for filter extraction and DNA extraction efficiencies).	The major barrier is non-detect samples due to the low flow rate and limitations on sampling times in some environments.
Respirable or fine particulate matter (PM) for quantitative PCR or PCR amplification/ amplicon sequencing.	Indoor, human-occupied settings.	SKC, Personal Environmental Monitors (PM_{10} or $PM_{2.5}$) (SKC, Eighty Four, PA)	10.0 L/min for PM_{10} 4 L/min. for $PM_{2.5}$	Respirable PM: $d_a \leq$ 10 µm Fine PM: $d_a \leq$ 2.5 µm	Polycarbonate track etched filters , 0.2 µm pore size, 37 mm diameter	2,000 to 3,000 bacterial cells and 10 to 25 fungal cells. (fully accounting for filter extraction and DNA extraction efficiencies).	The major barrier is non-detect samples due to the low flow rate and limitations on sampling times in some environments.
Respirable particulate matter (PM) for quantitative PCR or PCR amplification/ amplicon sequencing.	Outdoor settings only (due to noise of the high volume samplers).	ECO-HVS3000 with PM_{10} inlet (Ecotech, Ltd, Knoxfield, VIC, Australia)	~1,000 L/min.	Respirable PM: $d_a \leq$ 10 µm	Pretreated (450°C) 20.3 cm x 25.4 cm Whatman quartz fiber filters)	2,000 to 3,000 bacterial cells and 10 to 25 fungal cells. (fully accounting for filter extraction and DNA extraction efficiencies).	The major barriers are the noise generated and the large size of the sampler, which do not allow for placement in occupied settings.

*Laboratory of J. Peccia, Yale University

Table 4.3 Protocol from Biology and the Built Environment Center, Institute of Ecology and Evolution, University of Oregon*

Target Analysis	Type of Environment Sampled	Sampling Equipment	Flow Rates	Aerodynamic Diameters (d_a) Collected	Sampling Media	Detection Level (# of target genes or cells)	Major Challenges
Total suspended particulate matter for PCR amplicon sequencing and metagenome sequencing.	Outdoor air sampling.	GAST 1023-V103 vacuum pump with 12 filters.	30 L/min for each filter	Total suspended particulates	0.22 µm pore size, 37 mm diameter cellulose nitrate filters.	$<10^6$ cells	Noise, portability.
Respirable and fine particulate matter for PCR amplicon sequencing, metagenome, and metatrans-criptome sequencing.	Outdoor air sampling.	GAST 1023-V103 vacuum pump with 12 liquid impingers (SKC BioSampler).	12.5 L/min for each impinger	0.4 - 10 µm	Impinger liquid is LifeGuard Soil Preservation Solution (MO BIO). After sampling, liquid is filtered onto 0.22 µm pore size cellulose nitrate filters for processing.	~ 5×10^7	Noise, portability, length of sampling time required to collect sufficient biomass, refilling impingers, sample storage and preservation.
Respirable and fine particulate matter for PCR amplicon sequencing,	Indoor air sampling in human-occupied spaces.	Two Welch 2425B-01 pumps connected in series to one liquid impinger (SKC BioSampler).	12.5 L/min	0.4 - 10 µm	Impinger liquid is sterile water. After sampling, liquid is filtered onto 0.22 µm pore size cellulose nitrate filters for processing.	$<10^6$ cells	Too noisy to operate in most occupied indoor spaces, refilling impingers.
Respirable and fine particulate matter for PCR amplicon sequencing,	Indoor air sampling in human-occupied spaces.	AirChek 2000 pump (SKC) with one Button Aerosol Sampler (SKC).	~4.0 L/min	< 100 µm	1.2 µm pore size, 25 mm diameter mixed cellulose ester filters.	$<10^6$ cells	Low biomass due to low flow rates, length of sampling time required to collect sufficient biomass.

*Laboratory of J. Green, U. of Oregon

Table 4.4 Protocol used by University of California, Berkeley, and University of Pittsburgh School of Medicine*

Target Analysis	Type of Environment Sampled	Sampling Equipment	Flow Rates	Aerodynamic Diameters (d_a) Collected	Sampling Media	Detection Level (# of target genes or cells)	Major Challenges
Aerosolized bacterial cells for 16S PCR amplification/ amplicon sequencing.	We used this protocol in a neonatal intensive care unit	Button Aerosol Sampler (AirChek XR5000 Sampler), SKC Inc., Eighty Four, PA	4.0 L/min for 24 hours	d_a < 100 μm	SKC Filter, Mixed cellulose ester membranes, 1.2 um pore size, 25 mm diameter filter.	# of target genes is still being determined. Preliminary results reveal sufficient DNA recovery for 16S community structure analysis.	The major barrier is non-detect samples due to the low biomass nature of neonatal intensive care units and noise disturbance if one does not utilize a muffling device.

*Laboratory of J. Banfield, U.C. Berkeley, and M. Morowitz, U. of Pittsburgh

** Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

Challenges and Limitations of Aerosol Sampling Methods and Processes

A number of challenges and limitations were identified for aerosol sampling methods. These are highlighted below. A summary of the challenges, recommended actions and approach, and the current stakeholders and resources available to accomplish the defined performance targets and subsequent impacts are summarized in Figure 4.3.

Limitations of Existing Methods

Significant issues with current bioaerosol sampling technology include the need for compact and portable sampling devices, and the significant contamination issues association with high volume liquid impingers. Regarding the application of molecular techniques, many of the current sampling techniques provide sufficient material for PCR-based analysis, but significant limitations still occur in concentrating the samples into small volumes, and collecting sufficient samples for non-PCR based analyses (metagenomics, transcriptomics, proteomics). Taken together, these methodological limitations creates challenges to the fundamental understanding of bioaerosol community structure and function in indoor and atmospheric aerosols, and for integrating sampling methods into epidemiology studies.

Standards and Protocols

A major limitation is the lack of standard protocols for aerosol sampling and sample preparation. Without standard protocols that contain information on efficiencies associated with sample collection and sample preparation, quantitative bioaerosol data lacks both accuracy and precision. Standards are necessary to provide consistency in epidemiology studies in order to compare data sets. Additionally a lack of protocols precludes the involvement of biologists and other scientific disciplines that do not have traditional training or experience in aerosol sampling and are in need of guidance.

A lack of baseline-building microbial ecology data was identified as a significant limitation to better use and interpretation of aerosols sampling studies. Published baseline information is lacking at the regional, national, and international level. Microbial ecology data sets covering diverse sample locations and types of aerosols are needed to fill this gap. Ecology data variability exacerbates this issue, by making it difficult to link data sources and construct baselines. In addition, there are limited efforts to collect data in the developing world, particularly for airborne disease, which is a significant problem for human populations globally.

Figure 4.3 Aerosols Sampling: Summary of Priority Topics

Standardized Guidelines for Sampling Biological Aerosols

Challenge:

Aerosols scientists and biologists must work together to produce standard protocols for sampling and preparing biological aerosols from the built environment. Poor communication and cross training between those with biological skills and those with aerosol science and sampling skills leads to inconsistency and a lack of comparability in resulting microbiome characterization.

Actions and Approach:

Actions that can be taken by the microbiome research community in an open forum include:

- Define the types of biological aerosols that are desired and the type of analysis that must be completed to meet the environmental and public health challenges posed by biological aerosols.
- Determine/define where reference materials are needed to establish measurement assurance of air sampler performance (e.g. sampler efficiencies) and what appropriate reference materials would be.
- Provide tutorials on aerosol physics and the aerosol sampling mechanisms/limitations relevant to commonly used equipment, working to link the well-developed science of sampling total airborne particulate matter for guidance on sampling biological material.
- Draft protocols for sampling including the following categories:
  - Total and size resolved sampling of viable aerosols
  - Total and size resolved sampling of nonviable aerosols
- Write protocols and distribute through a web-based report, through the peer-reviewed literature, and through guidance from relevant government agencies.

Stake Holders, and Existing Resources:

- Several agencies and organizations can support standardization of community adopted protocols (e.g., NIST, ASTM, AIHA, ACGIH, ASM, IAA, and ASHRAE).
- Existing related resources include a large body of literature (books, journal papers, guidelines) on aerosol sampling of total particles (i.e., PM_{10} and $PM_{2.5}$) from the aerosol science community, and a limited, but growing body of literature on efficiencies and sampling methods for biological aerosols.

Performance Targets and Impacts:

- Targets include:
  - Producing multiple protocols depending on the type of data and analysis desired
  - Inclusion of definitive efficiencies built into the protocol to improve the accuracy, precision, and detection levels of bioaerosol quantitative analysis
- Major impacts include:
  - Improvement in the quality and consistency of data produced thus enabling researchers to make meaning full comparisons across studies
  - Opening bioaerosol-based research to biologists and others with limited training in aerosol science and sampling

Future Priorities for the Collection of Bioaerosols

The future requirements identified for sampling of microbes in aerosols are shown in Table 4.2 with detailed priority pathways illustrated in Appendix D. Such improvements in sampling methods would positively impact biological aerosol research by making it easier to integrate bioaerosol sampling into occupied environments and epidemiology studies, increasing the accuracy and precision of biological aerosol measurements, and encouraging new scientists to become involved in the indoor microbiome and bioaerosol research, improve associations between human health and biological aerosol content and concentrations.

Table 4.2. Future Requirements for Bioaerosol Sampling

Improved/Advanced Collection Methods
• New designs for sampling equipment that are easily decontaminated, specifically high volume impingers • Compact, high volume, low cost, size-resolved aerosol impactors • Method for determining and separating biomass from abiotic aerosol particles • Low noise samplers for sampling in indoor environments • Automated samplers that can separate time series for personal exposure: day vs. night, and not just a composite but a profile over time related to activity • Compact and portable sample collection devices • Packaging or concentration of samples so that they are analysis-ready and non-contaminated
Standards and Protocols
• Standardization of procedures for collection, preparation, and analysis of samples for microbiome analysis • Increase in the amount and availability of information on sampler efficiencies and recovery of viable organisms
Post-Sampling and Analysis
• Addressing issue of extremely small analytical volumes versus need for large sample volumes • A clearer understand of how to removal and characterization of inhibitors in samples • Better methods for culturing organisms; some are non-existent, slow, damaged in air

Standards and protocols will be vital to ensuring comparability among results, for selection of appropriate methods, and ensuring quality control. Standards and standardized protocols are also needed for the full spectrum of the sampling process, from preparation to collection and analysis, especially for quantifying specific or general bacteria, fungi, or viruses by qPCR methods and describing appropriate experimental control.

Future advances in sampling, post-sampling and analysis are needed to keep pace with modern molecular biology methods and techniques. In particular, methods are needed to characterize viruses in

indoor and outdoor air, for bioaerosol viability and gene expression, and for high volume sampling that allows for application of modern proteomics to characterizing protein content.

Biological sampling and analysis advances must be made in concert with epidemiologists so that tools are developed to specifically target the importance of the airborne route of exposure in infectious and noninfectious disease.

Expanded capabilities for monitoring and post-exposure activities will help to provide better response to events and even prediction and/or control to mitigate events. Systems that track changes in the environment, and that can differentiate between what is the 'normal' baseline and when problems occur could help in responding to disease outbreaks or bioterrorism events. Systems that identify, monitor, and maintain the indoor 'comfort zone' could help to optimize health and safety conditions. Such systems are in development and take advantage of state of the art samplers, molecular recognition (e.g. antibodies, aptamers, nucleic acid sequences) and sensitive detection methods (e.g. surface plasmon resonance, nanowires, quantum dot detection) but currently have not shown promise in separating specific organisms in environmental aerosols that are greater than 90% nonbiological. Continued development in these automated sampling and analysis systems and eventual success in this arena could revolutionize the field by making tremendous contributions to determining airborne exposure routes, identifying events and scenarios that increase exposure to hazardous bioaerosols, decreasing the threat of bioterrorism via improved detection, and moving understanding of bioaerosol fate, transport, and exposure to a real-time transient mode, versus a time-averaged mode.

5. CROSS CUTTING ISSUES

Relationships between Surface and Aerosol Loading

There is a compelling need to understand the link between biological content in surfaces and aerosols and how they interact in the indoor environment, especially in light of the fact that epidemiology studies use surface measurements as proxy for integrated aerosol concentrations and exposure. Biocontaminants in the air can deposit on surfaces and surface contaminants often do not remain settled but are re-entrained into the air as bioaerosols. Understanding this interaction is important toward assessing and tracking the potential impacts of microbes on human health in indoor environments. Important relationships exist between surface and aerosol content, and are generally governed by the deposition of aerosol particles and resuspension theory due to human occupation and activity. This work has been carried out mostly with the measurement of total aerosols, and can be extended to biological particles if aerodynamic diameters are known (for settling and deposition). While the effect of human activity type on resuspension rates of total particles has been explored in the literature, one large limitation is the poor understanding of how surface loading impacts the rate in which particles are aerosolized. Building the critical knowledge foundation of the relationship between surface and aerosol loading is an important area for scientific research investment.

Reporting of Meta-data and Study Design

Minimum amounts of supporting data (or metadata) must be taken as an integral part of sampling in the indoor environment. These data should at a minimum include information such as room volume, occupancy level, size ranges of particles sampled (aerosol), temperature, relative humidity, evidence of moisture damage, building materials (flooring type), number of samples, sample collection locations and collection/processing methods utilized. While limited, there are some resources and studies exploring study design and building factors to consider, listed in Appendix C. Some are directly relevant to microbes in the indoor environment. Others may provide fundamental information or science that can be applied to aid in understanding microbial surface and aerosol loading relationships and for data interpretation. Better reporting of architectural and building data and sample plan is both important for a robust study design but is also necessary for study comparisons. Such links are now possible through comparison of recovered DNA sequences. Building study databases along with best practices for metadata, a current focus of the Sloan Foundation MoBeDAC program (http://mobedac.org).
Finally, in terms of physical sampling, gaining access to the building environment for sampling is in itself challenging due to limited availability, privacy concerns of residents, and liability.

Future Questions

Better characterization of microbial interactions will be needed to aid in addressing some key questions and opportunities over the longer term. These include:

- Exploring how resuspension by human occupancy influences human exposure as well as how airborne particles from human, or other origins deposit to load surfaces
- Understanding the role of microbial growth on indoor environment surface loading in flooring, ventilation ducts, filters, etc.
- Exploring the possibility of sampling and analyzing surface and air samples to determine human exposure, including the suitability of surface samples for time-averaged interpretations of exposure, aerosol sample for temporal interpretations of exposure.
- Understanding the magnitude of bias for submicron and super micron particles in surface samples as well as the size distribution of these particles.
- Exploring how the flooring acts as a reservoir of pathogenic or allergenic microorganisms.

Education, Training, and Public Awareness

The study of microbes in the built environment involves a multitude of scientific disciplines including biology, physics, aerosol science, chemistry, engineering, and public health. However, interaction and collaboration across these disciplines is very limited today. Cross-disciplinary education, training, and dedicated guidance documents or protocols are needed to advance the field and address some of these major challenges. Further, the value of research and the critical need for innovation and validation of sampling methods are not well understood by decision-makers and the public; this was identified as a major challenge to moving the field forward. The challenges associated with accurate sampling methods are often under-estimated by researchers new to the field.

Figure 5.1 illustrates the priority pathways identified to enhance education, training, and public awareness of the importance of surface and aerosol sampling of microbes. These encompass new and improved methods of training users and personnel, development of cross-disciplinary curricula, multi-disciplinary research programs and projects at universities, and greater overall communication and interaction between researchers in the various disciplines involved.

Figure 5.1 CROSS-CUTTING PRIORITY TOPIC
Education and Training

Measurement Challenge/Barrier

The study of microbes in the building environment involves biology, physics, aerosol science, and other fields—but interaction and collaboration across these disciplines is very limited. Further, the value of research and the importance of and critical need for innovation/validation of sampling methods are not well understood by decision-makers and the public.

Actions and Approach

- Establish relationships and enable comparison of methods by training users and personnel in lab and field coordination
- Develop courses that bridge disciplines
- Conduct multi-disciplinary research programs and projects at universities
- Encourage the presence of biologists at aerosol conferences and vice versa
- Communicate the need to develop and validate methods through a forum of stakeholders and researchers
- Educate the public, Congress, and other decision makers of the importance of microbial sampling; emerging infections may drive interest in particular topics

Existing Related Resources

- CDC Standards
- Healthcare Infection Control Practices Advisory Committee (HICPAC) reports
- Microbial sampling workshop reports

Stakeholders and Roles

Government	Provide programmatic support (HUD, DHHS, DOE/buildings efficiency programs)
National Laboratories	multi-disciplinary R&D collaboration with universities, technology training programs
Industry/Trade Groups	outreach, sustained support for science and R&D
Academia	Develop curricula, conduct R&D

Performance Targets/Goals

- Greater interaction among research community and decision-makers
- Greater number of trained scientists entering the workforce
- Increase in focused federal, state, local and private activities on improved sampling

Applications

- All microbial sampling applications
- Linking science of microbes in aerosols and on surfaces
- University curricula, industry or other training courses

Impacts

- Cross-disciplinary R&D
- Accelerated progress toward needed innovations and validation of methods
- Skilled workforce
- Increased awareness of impact of microbes in the indoor environment on human health

Milestones

2011

- Review existing curricula; develop plan
- Create task force on education /outreach
- Initiate national/ intl. forum to increase visibility & cooperation

2015

- Train and communicate priorities to labs/field
- Establish new permanent programs at 20 major universities
- Conduct outreach program

2020

- Continue to enhance training and education

2025+

- Innovations achieved
- Establish Center of Excellence at a major university

6. CITED REFERENCES

1. Canter, D.A., *Addressing residual risk issues at anthrax cleanups: how clean is safe?* J Toxicol Environ Health A, 2005. **68**(11-12): p. 1017-32.
2. Schram-Bijkerk, D. and B.M. Doekes G, Douwes J, Riedler J, Ublagger E, von Mutius E, Benz M, Pershagen G, Wickman M, Alfvén T, Braun-Fahrländer C, Waser M, Brunekreef B; PARSIFAL study group, *Exposure to microbial components and allergens in population studies: a comparison of two house dust collection methods applied by participants and fieldworkers.* Indoor Air, 2006. **16**(6): p. 414-25.
3. Tringe, S.G., et al., *The Airborne Metagenome in an Indoor Urban Environment.* PLoS ONE, 2008. **3**(4): p. e1862.
4. Liu, L.-J.S., et al., *Investigation of the Concentration of Bacteria and Their Cell Envelope Components in Indoor Air in Two Elementary Schools.* Journal of Air and Waste Management Association, 2000. **50**: p. 1957-1967.
5. Edmonds, J.M., *Efficient methods for large-area surface sampling of sites contaminated with pathogenic microorganisms and other hazardous agents: current state, needs, and perspectives.* Applied Microbiology and Biotechnology, 2009. **84**: p. 811-816.
6. Riesenfeld, C.S., P.D. Schloss, and J. Handelsman, *Metagenomics: Genomic Analysis of Microbial Communities.* Annual Reviews in Gentics, 2004. **38**: p. 525-552.
7. Handelsman, J., *Metagenomics: Applications of Genomics to Uncultured Microorganisms.* Microbiology and Molecular Biology Reviews, 2004. **68**(4): p. 669-685.
8. Klepeis, N.E. and W.C. Nelson, *The national human activity pattern survey (NHAPS): A resource for assessing exposure to environmental polutants.* Journal of Exposure Analysis and Environmental Epidemiology, 2001. **11**(3): p. 231-252.
9. Cabral, J.P.S., *Water Microbiology. Bacterial Pathogens and Water.* International Journal of Environmental Research and Public Health, 2010. **7**(10): p. 3657-3703.
10. Maier, R.M., et al., *The bacterial community in household dust: environmental determinants and impact on childhood asthma.* Applied and Environmental Microbiology, 2010. **76**: p. 2663-2667.
11. Andersson, A.M., et al., *Dust-borne Bacteria in Animal Sheds, Schools and Children's Day Care Centers.* Journal of Applied Microbiology, 1999. **86**: p. 622-634.
12. Scott, E., S.F. Bloomfield, and C.G. Barlow, *An Investigation of Microbial Contamination in the Home.* Journal of Hygiene, 1982. **89**: p. 279-293.
13. Weis, C.P., et al., *Secondary Aerosolization of Viable Bacillus anthracis Spores in a Contaminated US Senate Office.* JAMA, 2002. **288**(22): p. 2853-2858.
14. Womble, S.E., et al., *Prevalence and Concentrations of Culturable Airborne Fungal Spores in 86 Office Buildings from the Building Assessment Survey and Evaluation (BASE) Study.* Proceedings of Indoor Air, 1999: p. 261-266.
15. Haysom, I.W. and A.K. Sharp, *Bacterial Contamination of Domestic Kitchens over a 24-hour Period.* British Food Journal, 2005. **107**(7): p. 453-466.
16. Tsai, F.C. and J.M. Macher, *Concentrations of Airborne Culturable Bacteria in 100 Large US Office Buildings from the BASE Study.* Indoor Air, 2005. **Suppl 9**: p. 71-81.
17. Lee, L., S. Tin, and S.T. Kelley, *Culture-independent analysis of bacterial diversity in a child-care facility.* BMC Microbiology, 2007. **7**: p. 27.
18. Parkarinen, J., et al., *Predominance of Gram-Positive Bacteria in House Dust in the Low-Allergy Risk Russian Karelia.* Environmental Microbiology, 2008. **10**(12): p. 3317-3325.
19. Rintala, H., et al., *Diversity and Seasonal Dynamics in Indoor Environment.* BMC Microbiology, 2008. **8**: p. 56.
20. Taubel, M., et al., *The Occupant as a Source of House Dust Bacteria.* Journal of Allergy Clin Immunol, 2009. **124**: p. 834-840.
21. Amend, A.S., et al., *Indoor Fungal Composition is Geographically Patterned and More Diverse in Temperate Zones than in the Tropics.* Proceedings of the National Academy of Sciences, 2010. **107**(31): p. 13748-13753.

22. Sordillo, J.E., et al., *Home Characteristics as Predictors of Bacterial and Fungal Microbial Biomarkers in House Dust.* Environmental Health Perspectives, 2011. **119**: p. 189-195.
23. Emanuel, P.A., J.W. Roos, and K. Niyogi, eds. *Sampling for Biological Agents in the Environment*. 2008, ASM Press: Washington DC. 294.
24. Yang, C.S. and P.A. Heinsohn, *Sampling and Analysis of Indoor Microorganisms*. 2007, Hoboken, New Jersey: John Wiley & Sons, Inc. .
25. Noskin, G.A., et al., *Recovery of vancomycin-resistant enterococci on fingertips and environmental surfaces.* Infect Control Hosp Epidemiol, 1995. **16**: p. 577-581.
26. Scott, E. and S.F. Bloomfield, *The survival and transfer of microbial contamination via cloths, hands, and utensils.* Journal of Applied Bacteriology, 1990. **68**: p. 271-278.
27. McNeil, E., *Dissemination of microorganisms by fabrics and leather.* Development in Industrial Microbiology, 1964. **5**: p. 30-35.
28. Sanborn, W.R., *The relation of surface contamination to the transmission of disease.* Am J Public Health, 1963. **53**: p. 1278-1283.
29. Boyce, J.M., *Vancomycin-resistant enterococci: pervasive and persistent pathogens.* Infect Control Hosp Epidemiol, 1995. **16**: p. 676-679.
30. Sehulster, L. and R.Y.W. Chinn, *Guidelines for Environmental Infection Control in Health-Care Facilities: Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC)*
Morbidity and Mortality Weekly Report, 2003. **52**(RR10): p. 1-42.
31. Manangan, L.P., et al., *Infection control dogma: top 10 suspects.* Infect Control Hosp Epidemiol, 2001. **22**: p. 243-247.
32. Buttner, M.P., et al., *Evaluation of the Biological Sampling Kit (BiSKit) for Large-Area Surface Sampling.* Appl. Environ. Microbiol., 2004. **70**(12): p. 7040-7045.
33. Estill, C. and B.J. Baron PA, Hein MJ, Larsen LD, Rose L, Schaefer FW 3rd, Noble-Wang J, Hodges L, Lindquist HD, Deye GJ, Arduino MJ., *Recovery efficiency and limit of detection of aerosolized Bacillus anthracis Sterne from environmental surface samples.* Applied and Environmental Microbiology, 2009. **May 8**.
34. Valentine, N.B., et al., *Evaluation of sampling tools for environmental sampling of bacterial endospores from porous and nonporous surfaces.* Journal of Applied Microbiology, 2008. **105**(4): p. 1107-1113.
35. Vorst, K.L., E.C. Todd, and E.T. Rysert, *Improved quantitative recovery of Listeria monocytogenes from stainless steel surfaces using a one-ply composite tissue.* Journal of Food Protection, 2004. **67**: p. 2212-2217.
36. Rose, L.J., et al., *National validation study of a cellulose sponge-wipe processing method for use after sampling Bacillus anthracis spores from surfaces.* Applied and Environmental Microbiology, 2011.
37. Létant, S.E., et al., *Rapid-Viability PCR Method for Detection of Live, Virulent Bacillus anthracis in Environmental Samples.* Applied and Environmental Microbiology, 2011. **77**(18): p. 6570-6578.
38. Buttner, M.P., et al., *Determination of the Efficacy of Two Building Decontamination Strategies by Surface Sampling with Culture and Quantitative PCR Analysis.* Appl. Environ. Microbiol., 2004. **70**(8): p. 4740-4747.
39. Buttner, M.P., P. Cruz-Perez, and L.D. Stetzenbach, *Enhanced detection of surface-associated bacteria in indoor environments by quantitative PCR.* Appl Environ Microbiol, 2001. **67**(6): p. 2564-70.
40. Rose, L., et al., *Swab materials and Bacillus anthracis spore recovery from nonporous surfaces.* Emerg Infect Dis, 2004. **10**(6): p. 1023-9.
41. Moore, G. and C. Griffith, *A comparison of surface sampling methods for detecting coliforms on food contact surfaces.* Food Microbiology, 2002. **19**(1): p. 65-73.
42. Brown, G.S., et al., *Evaluation of rayon swab surface sample collection method for Bacillus spores from nonporous surfaces.* J Appl Microbiol, 2007. **103**(4): p. 1074-80.
43. Hodges, L.R., et al., *National validation study of a swab protocol for the recovery of Bacillus anthracis spores from surfaces.* Journal of Microbiological Methods, 2010. **81**(2): p. 141-146.
44. Lemmen SW, H.H., Zolldann D, Amedick G, Lütticken R, *Comparison of two sampling methods for the detection of gram-positive and gram-negative bacteria in the environment: moistened swabs versus Rodac plates.* Int J Environ Health Res, 2001. **203**(3): p. 245-8.

45. Locascio, L.E., *Standard Practice for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biological Agents from Nonporous Surfaces: Collaborative Study.* Journal of AOAC International, 2006. **89**(6).
46. E2458-10, *Standard Practices for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biothreat Agents from Nonporous Surfaces.* 2010, ASTM International.
47. Bauer, H., et al., *Airborne bacteria as cloud condensation nuclei.* Journal of Geophysical Research-Atmosphers, 2003. **108**: p. 4658.
48. Maki, L., et al., *Ice nucleation induced by Pseudomonas syringae.* Applied Microbiology, 1974. **28**: p. 456-459.
49. Peccia, J., et al., *A role for environmental engineering and science in preventing bioaerosol-related disease.* Environmental Science & Technology, 2008. **42**(3): p. 4631-4637.
50. Douwes, J., et al., *Bioaerosol Health Effects and Exposure Assessment: Progress and Prospects.* Annals of Occupational Hygiene, 2003. **47**(3): p. 187-200.
51. Nazaroff, W.W., *Indoor particle dynamics.* Indoor Air, 2004. **14**: p. 175-183.

APPENDIX A. LIST OF PARTICIPANTS

Robert M. Bowers,
University of Colorado

Ginger Lin Chew,
National Center for Environmental Health, CDC

Autumn Downey,
Biochemical Science Division, NIST

Alison Kraigsley,
Polymers Division, NIST

Janet M. Macher,
California Department of Health Services

James J. McDevitt,
Harvard School of Public Health, Harvard University

Jayne Morrow,
Biochemical Science Division, NIST

Stephen Allen Morse,
Environmental Microbiology Program, CDC

Nathan Olson,
Biochemical Science Division, NIST

Traci Pals,
Science and Technology Directorate, DHS

Jordan Peccia,
Yale University

Andrew Persily,
Energy and Environment Division, NIST

Cynthia Marie Pfannkoch,
J. Craig Venter Institute

Eugene A. Pinzer
Office of Healthy Homes and Lead Hazard Control, HUD

Laura J. Rose,
National Center for Emerging and Zoonotic Infectious Disease, CDC

Lynn M. Schriml,
University of Maryland, Genomic Science Institute

Sandra Da Silva,
Biochemical Science Division, NIST

Lindsay Vang,
Biochemical Science Division, NIST

Ann Maureen Womack,
University of Oregon

Paula Jean Olsiewski,
Alfred P. Sloan Foundation

APPENDIX B. LIST OF SPEAKERS AND PRESENTATIONS

In alphabetical order

Jayne Morrow, National Institute of Standards and Technology
A Forward Thinking Focus: What do we need to measure now, and 10 years from now?

Stephen Morse, Centers for Disease Control
Challenges in Sampling Environmental Microbes

Paula Olseiwski, Sloan Foundation
Welcome and Introduction to Sloan's Indoor Environment Microbiology Program

Jordan Peccia, Yale University
Sources and Characteristics of Indoor Microorganisms

Andrew Persily, National Institute of Standards and Technology
The Role of Buildings: What makes the Indoors, the Indoors

Lynn Schriml, University of Maryland
Impact of Sampling on How We Understand and Interpret Genomic Data from Environmental Samples

Shannon Williamson, J. Craig Venture Institute
Sampling and Sample Processing of an Aerosol Metagenome

APPENDIX C. EXISTING RESOURCES

A number of existing resources were identified that could be drawn upon to create guidelines for surface and aerosol microbe sampling and reporting.

Building and Architectural Consideration Resources

American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) documents and standards

U.S. Department of Energy

Residential Energy Consumption Survey (RECS)
http://www.eia.gov/emeu/recs/contents.html

Commercial Building Energy Consumption Survey(CBECS)
www.eia.gov/emeu/cbecs/contents.html

U.S. Census Bureau American Housing Survey
http://www.census.gov/hhes/www/housing/ahs/ahs.html

Sampling Strategy Guidance

ASTM D5792 - Standard Practice for Generation of Environmental Data Related to Waste Management Activities: Development of Data Quality Objectives

ASTM D6250 - Standard Practice for Derivation of Decision Point and Confidence Limit for Statistical Testing of Mean Concentration in Waste Management Decisions

ASTM D6311 - Standard Guide for Generation of Environmental Data Related to Waste Management Activities: Selection and Optimization of Sampling Design

U.S. Federal Bureau of Investigation (FBI) Laboratory Publication: Handbook of Forensic Services 2003

U.S. Environmental Protection Agency (U.S. EPA), Guidance for Choosing a Sampling Design for Environmental Data Collection (EPA QA/G-5S), EPA/240/R-02/005, Office of Environmental Information, Washington, DC, December 2002.

U.S. EPA, GEO - EAS 1.2.1 User's Guide, EPA/600/8-91/008, Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Las Vegas, NV, April 1991.

U.S. EPA, Guidance for the Data Quality Objectives Process, EPA QA/G-4, Quality Assurance Management Staff, Washington, DC, March 1995.

Support for Statistical Design and Sample Plan Development

ASTM D5922 - Standard Guide for Analysis of Spatial Variation in Geostatistical Site Investigations

ASTM D5923 - Standard Guide for Selection of Kriging Methods in Geostatistical Site Investigations

ASTM D5924 - Standard Guide for Selection of Simulation Approaches in Geostatistical Site Investigations

U.S. EPA Environmental Protection Agency Visual Sample Plan (VSP); draft environmental sampling strategy, http://www.frtr.gov/decisionsupport/DST_Tools/vsp.htm.

Worker Safety and Health Guidelines

U.S. EPA - Safety, Health, and Environmental Management (SHEM) Guide No. 44, Personal Protective Equipment, October 2004
U.S. EPA - Safety, Health, and Environmental Management (SHEM) Guideline No. 46, Respiratory Protection, dated October 2004
U.S. EPA - Order 1460.1, Occupational Medical Surveillance Program, June 18, 1996
U.S. NIOSH Publication No. 2009-132: Recommendations for the Selection and Use of Respirators and Protective Clothing for Protection Against Biological Agents

Hazard Controls

HCs are brief 1-2 page, user-friendly documents that describe control techniques documented to substantially reduce hazardous exposures to workers in a particular application/industry process.
http://www.cdc.gov/niosh/pubs/hc_date_desc_nopubnumbers.html

Standard Sample Collection Methods

ASTM E2458 - Standard Practices for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biothreat agents from Nonporous Surfaces
Pan-American Aerobiology Association http://www.paaa.org/StandardizedProtocols.pdf
Housing and Urban Development (HUD) dust sampling protocol
http://www.hud.gov/offices/lead/hhi/hhiresources.cfm

Additional U.S. Federal Agency Efforts and Resources

U.S. Department of Health and Human Services

- Centers for Disease Control and Prevention(CDC)
  - CDC Guidelines for Infection Control in Health Care Facilities
    http://www.cdc.gov/hicpac/pubs.html
  - Laboratory Response Network (LRN)protocols for sampling
    http://www.bt.cdc.gov/lrn/
  - CDC'S National Institute for Occupational Safety and Health (NIOSH);
    - Health Hazard Evaluations (HHEs)
      http://www.cdc.gov/niosh/hhe/hhesearch.html
    - http://www.cdc.gov/niosh/topics/indoorenv/ConstructionIEQ.html
    - National Occupational Research Agenda - Indoor Environment
      NORA Indoor Environment (IE) Team
      http://www.cdc.gov/niosh/nora/default.html
    - CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC), Guidelines for Environmental Infection Control in Health-Care Facilities
      http://www.cdc.gov/hicpac/pdf/guidelines/eic_in_HCF_03.pdf

        - NIOSH Interim Recommendations for the Cleaning and Remediation of Flood-Contaminated HVAC Systems: A Guide for Building Owners and Managers http://www.cdc.gov/niosh/topics/emres/Cleaning-Flood-HVAC.html

U.S. Environmental Protection Agency (EPA)
- Indoor Air Quality http://www.epa.gov/iaq/
- Exposure Factors Program http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20563
- Exposure Factors Handbook: 2011 Edition

U.S. Department of Labor
- Occupational Safety and Health Administration (OSHA)
- Indoor Air Quality http://www.osha.gov/SLTC/indoorairquality/index.html
- Fact sheets and Safety and Health Sheets for Legionnaires disease and molds http://www.osha.gov/SLTC/legionnairesdisease/index.html http://www.osha.gov/SLTC/molds/index.html

U.S. Department of Commerce
- National Institute of Standards and Technology
    - Building Environment Division http://www.nist.gov/el/building_environment/
    - Biochemical Science Division http://www.nist.gov/mml/biochemical/
- National Oceanic and Atmospheric Administration
    - Operational Significant Event Imagery team http://www.osei.noaa.gov/Events/Dust/

U.S. Department of Energy
- Lawrence Berkley National Laboratory http://eetd.lbl.gov/ied/sfrb/overview.html Indoor Air Quality Scientific Findings Resource Bank - Overview of IAQ

U.S. Department of Homeland Security

U.S. Department of Housing and Urban Development
- Office of Healthy Homes and Lead Hazard Control http://portal.hud.gov/hudportal/HUD?src=/program_offices/healthy_homes

U.S. Department of the Interior
- U.S. Geological Survey. Linking the Scales of Process, Observation, and Modeling of Dust Emissions
- 2004 Dust Workshop, Boulder, Colorado http://esp.cr.usgs.gov/info/dust/

U.S. National Aeronautics and Space Administration
- http://www.nasa.gov/mission_pages/station/research/experiments/SWAB.html

Relevant Organizations and Professional Societies

American Conference of Industrial Hygienists (ACGIH); coalition of government and industry hygienists, http://www.acgih.org/home.htm
American Industrial Hygiene Association (AIHA); air and dust sampling communities, http://www.aiha.org/Pages/default.aspx
American Society for Microbiology (ASM), http://www.asm.org/
American Association for Aerosol Research (AAAR) http://www.aaar.org/
American Society of Heating, Refrigerating, and Air-Conditioning Engineers, Inc. (ASHRAE)
American Association for Aerosol Research (AAAR) http://www.aaar.org/
ASTM International (formerly known as the American Society for Testing and Materials), http://www.astm.org/

Building Owners and Managers Association International (BOMA), http://www.boma.org/Pages/default.aspx
Electronic Library of Construction Occupational Safety and Health (eLOSH) Boston College Environmental Health and Safety - Maintaining Indoor Air Quality
Genomic Standards Consortium, http://gensc.wordpress.com/
International Organization for Standardization s. http://www.iso.org/iso/home.html
Institute of Environmental Sciences and Technology (IEST) http://www.iest.org/i
Microbiome of the Built Environment Data Analysis Core (MoBeDAC), http://www.microbe.net/microbiome-of-the-built-environment-data-analysis-core-mobedac/
MicroBEnet Blog http://microbenet.blogspot.com/
Sloan Foundation http://www.sloan.org/program/10

APPENDIX D. WORKSHOP PRIORITY TOPICS

SURFACE SAMPLING PRIORITY TOPIC
Low Recovery of Microbes from Surfaces

Measurement Challenge/Barrier

Because methods for sampling and recovery are not optimized and validated, there is low recovery of microbes from surfaces. As a result, the composition of the microbial indoor environment is poorly understood.

Actions and Approach

- Gather information about types of available collection materials (e.g., swabs and wipes) to understand how they affect a representative set of diverse microbes
- Validate surface sampling and recovery methods
- Optimize extraction of microbes from the sampling device
- Optimize DNA and RNA extraction from surfaces

Performance Targets/Goals)

- Develop guidelines for sample collection, extraction, and subsequent processing
- Disseminate guidelines (e.g., print, internet)
- Establish mechanisms for continuous feedback from the community

Applications

- Forensics
- Biodefense
- Public health
- Regulation
- Epidemiology
- Ecology
- Health care

Existing Related Resources

- Environmental Protection Agency (EPA) Visual Sample Plan (VSP); draft environmental sampling strategy
- *Sampling for Biological Agents in the Environment* (hardback); guideline on indoor environment sampling in healthcare facilities
- Centers for Disease Control (CDC) Laboratory Response Network (LRN); protocols for sampling

Impacts

- Increased public knowledge and awareness of the various types of methods
- Improved health practices via better data collection and greater understanding of composition of microbes

Stakeholders and Roles

Government	Program support
National Laboratories	Optimization, validation
Industry	Optimization, validation
Private Research Groups	Optimization, validation
Standards Organizations	Protocols, validation
Academia	Optimization, validation

Milestones

2011	2015	2020	2025+
• Conduct stakeholder meetings and develop roadmap	• Develop and validate guidelines; put into practice	• Update guidelines as needed	

SURFACE SAMPLING PRIORITY TOPIC
Consistency/Conformity of Methods

Measurement Challenge/Barrier

Today's surface sampling methods lack consistency and conformity, making comparison of results difficult at best. There is a lack of validated methods to provide consistency, and little consensus in the surface sampling user community on the best practices. Key, common variables need to be identified.

Actions and Approach

- Conduct large validation studies
- Develop a matrix showing breakdown of variables into fundamental components to facilitate comparison between studies
- Assemble a forum and develop community-driven consensus for reference methods and best practices
- Identify and catalogue the distinct variables within methods

Performance Targets/Goals

- Validation of consistent sampling results
- Development of a matrix of variables
- Consensus on best practices for sampling

Applications

- Microbiome characterization
- Threat characterization
- Clearance (i.e., hazard assessment to determine safe re-entry)

Existing Related Resources

- CDC'S National Institute for Occupational Safety and Health (NIOSH); validated sampling methods
- Institute of Environmental Sciences and Technology (IEST), and ASTM International, formerly known as the American Society for Testing and Materials (ASTM) (many resources)
- Interagency data call for adopted methods/standards/guidelines from beverage/food industry, carpet industry, and NASA

Impacts

- Improved consistency of results from various sampling methods
- Greater comparability of study results
- Increased confidence in results

Stakeholders and Roles

Government	Coordination
National Laboratories	Research, matrix development
Industry	Sampling technology developers provide text, validation, other inputs
Standards Organizations	Standards and protocols
Academia	Research, method validation, matrix development

Milestones

2011-12
- Identify variables in methods
- Forum initiated

2015
- Matrix developed
- Forum Developed
- Community-driven consensus →
- Validation studies →

2020

2025+

SURFACE SAMPLING PRIORITY TOPIC
Data Standards

Measurement Challenge/Barrier

Data standards and guidelines for different analysis methods are currently lacking, contributing to problems of limited comparability between sampling studies. Addressing this issue could help to provide a common basis for reporting and interpretation of results.

Actions and Approach

- Establish active communication mechanisms to develop consensus guidelines
  - ➢ Define workgroups based on applications and tasks
  - ➢ Facilitate/organize workshops
  - ➢ Leverage social networking resources and activities
- Disseminate guidelines to expand stakeholder community and improve the guideline process

Performance Targets/Goals

- Standardize data, metadata, and data visualization
- Establish core group of stakeholders

Applications

- Understanding of the complexity of indoor microbial environments
- Enhanced interpretation of outcomes
- Extension of knowledge to new applications and questions

Existing Related Resources

- Genome Sequencing Centers (GSC)
- Microbiome of the Built Environment Data Analysis Core (MoBeDAC)
- MicroBEnet Blog http://microbenet.blogspot.com/
- National Center for Biotechnology Information (NCBI)
- CDC Laboratory Response Network (LRN)

Impacts

- Greater dissemination of knowledge
- Encouragement and inspiration for the next generation of researchers and practitioners

Stakeholders and Roles

Government **National Laboratories**	programmatic support
Private Research Groups **Academia**	research, journal publishers, data contributors, large data storage facilities, software engineers and developers

Milestones

2011	2015	2020	2025+
• Stakeholder workshop series	• Standards & guidelines • Visualization of data	• Visualization tools	• Transparency of data

SURFACE SAMPLING PRIORITY TOPIC
Bias in Results

Measurement Challenge/Barrier

Known biases with sampling (e.g., sample selection and collection efficiencies) limit both the accuracy of reported microbial quantities and validity of the data. Better study designs, standardization, and improved quality in methods could all contribute to solving this problem.

Actions and Approach

- Develop proper study designs and Implementation processes (training and compliance)
- Establish good standard methods
- Improve attention to quality assurance
- Increase knowledge of environmental conditions, life ecological niches, and transport cycle of microorganism (e.g., yeast stage vs. filamentous fungi)

Performance Targets/Goals

- Valid results with understood bias
- Higher quality standardized methods

Applications

- Forensics
- Biodefense
- Public health
- Regulation
- Epidemiology
- Ecology
- Health care

Existing Related Resources

- Housing and Urban Development (HUD) dust sampling protocol
- NIOSH air sampling protocol
- American Industrial Hygiene Association (AIHA); air and dust sampling communities
- American Conference of Industrial Hygienists (ACGIH); coalition of government and industry hygienists
- Manufacturers (e.g., pump specifications, etc.)

Impacts

- Government agencies: programmatic support
- Residents/building occupants: environments for testing
- Scientific community: research and development

Stakeholders and Roles

Government **National Laboratories**	programmatic support
Public	residents/building occupants enable environments for testing
Industry **Private Research Groups** **Academia**	Research and development

Milestones

2011	2015	2020	2025+
---	• Improved standard methods (sampling) • Improved modeling	• Professional guidelines	• Widely available personal monitors and smart sensors in buildings • Healthier buildings

AEROSOLS PRIORITY TOPIC
Aerosols Sampling to Support Epidemiology*

Measurement Challenge/Barrier

Sample collection techniques are insufficient, access to environment to acquire samples is limited, and viability of microbes on surfaces and in environments can be poor. Routes of exposures are unknown for some diseases. Little work is being done to acquire baseline data, and comprehensive studies are needed. Institution Review Board (IRB) approval required for protection of human health adds to the challenges.

Actions and Approach

- Conduct pilot studies beyond known agents of disease to show associations between built environment and human health
- Create study design guidance
- Conduct sentinel studies for response to outbreaks
- Collect probability, baseline, and population distribution exposure data; process data completely; analyze archived environmental samples
- Conduct sufficiently large studies to collect representative information
  - Studies of known/suspected casual association, but also non-directed studies for unknowns
- Integrate studies, e.g., how indoor chemicals, environment, conditions impact microbes that affect people (e.g., formaldehyde, volatile organic compounds and viability, anti-genicity)

Performance Targets/Goals

- Disease-directed research to understand impacts of home environments on asthma, cancer, or other diseases
- Understand similar environments and commonalities of occupants
- Prioritize research efforts for greatest impact

Applications

- Preventive health strategies, e.g., avoiding certain indoor conditions
- Support for policies and regulation
- Bioterrorism strategies

Existing Related Resources

- Expand allergen/mold studies
- Questionnaire/survey instruments
- Genome Sequencing Centers (GSC) baseline data
- Closed environment health information
  - Space station
  - Biosphere (Arizona)
- Data from Antarctica

Stakeholders and Roles

Government National Laboratories	Regulatory: set standards, building codes that protect building environments NIH, CDC, EPA, HUD, environmental epidemiology groups: provide programmatic support
Nonprofit Organizations	APHA: Determine position on issues and research
Academia	research and collaboration across disciplines

Impacts

- Better data on agents, viability/infectivity
  - Public information and education
  - Informed public – what to avoid , safe behavior
- Improved indoor conditions
  - Better building design and operation
  - Casualty disease prevention
  - Microbial safety codes and ordinances for building owners and operators

Milestones

2011	2015	2020	2025+
	• Controlled environment studies-ensure that data is collected and analyzed	• Viability/infectivity data for treatment/ containment precautions; data on what constitutes a healthy home or building	

*Epidemiology is the scientific method used to investigate, analyze and prevent or control a health problem in a population

AEROSOLS PRIORITY TOPIC
Standard Methods and Reference Materials for Aerosols

Measurement Challenge/Barrier

Understanding how to define location and composition (i.e., dust) is a barrier for standard reference materials (SRMs) for aerosols. For standard methods, the lack of interest in the professional community and lack of a representative baseline are barriers. There are existing protocols and equipment for various purposed but these are not always commonly known, used, or widely accessible.

Actions and Approach

- Determine/define reference materials most useful for community (i.e. residential, commercial, geography, environment)
- Determine probability baseline conditions such as regional, national, or international (e.g., 80% of homes have X conditions)
- Identify different possibilities for standard methods (types of structure: home, school, commercial, analytical tools); determine how to define the standard method appropriately (not too broad or narrow)
- Devise pathways to get competing groups to accept a standard method, especially where unique methods already exist

Performance Targets/Goals

- Determining the SRM needs of the community
- Determining the most common practices/existing protocols for aerosols
- Identifying guidelines for manufacturers and specific system requirements

Applications

- Cross-linking with epidemiology
- Coordination with architects and building engineers

Existing Related Resources

- Organizations: ASTM International, AIHA, ACGIH, American Society for Microbiology (ASM), International Aerosol Association (IAA) American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE), Building Owners and Managers Association International (BOMA), architectural organizations

Stakeholders and Roles

Government National Laboratories	NIST – SRMs
Industry	Companies to develop, sell sampler systems/SRMs
Standards Organizations	ASTM-Standard methods

Impacts

- Better baseline data
- Improved basis for comparison among methods
- Greater ease and clarity of results, plus better confidence in results
- Guidance for manufacturers
- Improved collection efficiency

Milestones

2011
- Determination of SRM needs

2015
- Implementation of standard methods
- Standard technical specifications for equipment

2020

2025+

AEROSOLS PRIORITY TOPIC
Generation of Baseline Data for Aerosols

Measurement Challenge/Barrier

Baseline data for microbes in aerosols is currently inadequate due to lack of standards for collection ,a central repository for data, and limited access to data that is available. Thus, it is not clear what levels of microbes in aerosols are normal or safe. The current molecular and genomic research culture contributes to the uncertainty as it is hypothesis-driven, and may not contribute to the generation of baseline data.

Actions and Approach

- Pursue development and agreement on standards for aerosols
- Identify pathways to coordinated federal research activities (National Institute of Environmental Health Sciences, [NIEHS], CDC, EPA)
- Tap existing procedures and genomic methods (e.g., Department of Homeland Security[DHS])
- Define baseline and population of interest
- Conduct non-hypothesis driven research to generate baseline data

Performance Targets/Goals

- Populated database of microbe communities in aerosols in representative homes and other buildings

Applications

- Comparison for various aerosol sampling methods
- Context for aerosol measurements
- Epidemiology
- Environmental studies

Existing Related Resources

- GSC type organization and data structure
- CDC, NIEHS, EPA, DHS

Impacts

- Improved understanding of diseases (e.g., asthma, allergies, fibromyalgia, infectious disease)
- New tools for better building designs and interventions

Stakeholders and Roles

Government National Laboratories	Coordination and research
Nonprofit Organizations	Coordination and research
Academia	Data collection, research

Milestones

2011

- 2011 Consortia develops white paper
- 2012 Partner to develop concepts and ideas and gain access to data
- 2013 Multi-year projects begin; tap existing surveys

2015

- Data collection and database development; new techniques for fungi and viruses

2020

- Databases updated and refined

APPENDIX E. ABBREVIATIONS AND ACRONYMS

AAAR – American Association for Aerosol Research

ACGIH – American Conference of Industrial Hygienists

AIHA – American Industrial Hygiene Association

ASM – American Society for Microbiology

ASHRAE – American Society of Heating, Refrigeration and Air-Conditioning Engineers

ASTM – American Society for Testing and Materials

BOMA – Building Owners and Managers Association International

CDC – Centers for Disease Control and Prevention

DHS – U.S. Department of Homeland Security

EPA – U.S. Environmental Protection Agency

GSC – Genome Sequencing Centers

HICPAC – Healthcare Infection Control Practices Advisory Committee

HUD – U.S. Housing and Urban Development

IAA – International Aerosol Association

IEST – Institute of Environmental Sciences and Technology

IRB – Institutional Review Board

LOD – Limit of Detection

MoBeDAC – Microbiome of the Built Environment Data Analysis Core

MSQ-PCR – Multiplex Semi-Quantitative PCR

NIOSH – National Institute for Occupational Safety and Health

NCBI – National Center for Biotechnology Information

NIEHS – National Institute of Environmental Health Sciences

PCR – Polymerase Chain Reaction

APPENDIX F. GLOSSARY

PCR: polymerase chain reaction (PCR) is an *in vitro* technique to select and amplify a single or few copies of a piece of DNA across several orders of magnitude.

Amplicons: Pieces of DNA formed as the products of natural or artificial amplification events.

ELISAs: Enzyme-linked Immunosorbent Assays (ELISAs) combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily assayed enzyme. ELISAs can provide a useful measurement of specific protein, antigen or antibody concentration.

Metagenomics: The study of the metagenome, a set of DNA sequences recovered from an environmental or medical sample.

Transcriptomics: The study of the transcriptome, the complete set of RNA transcripts produced by the genome at any one time.