Aerosol Science and Technology

ISSN: 0278-6826 (Print) 1521-7388 (Online) Journal homepage: https://www.tandfonline.com/loi/uast20

Field sampling of indoor bioaerosols

Jennie Cox, Hamza Mbareche, William G. Lindsley & Caroline Duchaine

To cite this article: Jennie Cox, Hamza Mbareche, William G. Lindsley & Caroline Duchaine (2020) Field sampling of indoor bioaerosols, Aerosol Science and Technology, 54:5, 572-584, DOI: 10.1080/02786826.2019.1688759

To link to this article: https://doi.org/10.1080/02786826.2019.1688759

Accepted author version posted online: 05 Nov 2019.
Published online: 21 Nov 2019.

AEROSOL SCIENCE AND TECHNOLOGY
2020, VOL. 54, NO. 5, 572–584
https://doi.org/10.1080/02786826.2019.1688759

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

Field sampling of indoor bioaerosols

Jennie Cox[a], Hamza Mbareche[b], William G. Lindsley[c], and Caroline Duchaine[b]

[a]Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio, USA; [b]Department of Biochemistry, Microbiology, and Bioinformatics, Université Laval, Québec, Canada; [c]National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, West Virginia, USA

ABSTRACT

Because bioaerosols are related to adverse health effects in exposed humans and indoor environments represent a unique framework of exposure, concerns about indoor bioaerosols have risen over recent years. One of the major issues in indoor bioaerosol research is the lack of standardization in the methodology, from air sampling strategies and sample treatment to the analytical methods applied. The main characteristics to consider in the choice of indoor sampling methods for bioaerosols are the sampler performance, the representativeness of the sampling, and the concordance with the analytical methods to be used. The selection of bioaerosol collection methods is directly dependent on the analytical methods, which are chosen to answer specific questions raised while designing a study for exposure assessment. In this review, the authors present current practices in the analytical methods and the sampling strategies, with specificity for each type of microbe (fungi, bacteria, archaea and viruses). In addition, common problems and errors to be avoided are discussed. Based on this work, recommendations are made for future efforts towards the development of viable bioaerosol samplers, standards for bioaerosol exposure limits, and making association studies to optimize the use of the big data provided by high-throughput sequencing methods.

ARTICLE HISTORY
Received 26 June 2019
Accepted 22 October 2019

EDITOR
Tiina Reponen

Introduction

Indoor bioaerosols

Bioaerosols are airborne particles that originate from biological sources including bacteria, viruses, fungi, protozoa, plants, and animals. These ubiquitous particles can include a variety of living and non-living entities, and may be single or grouped organisms or spores, fragments of organisms, or residues or products of organisms like endotoxins or mycotoxins. Particle sizes can range in size from tens of nanometers to more than 100 µm and can vary with relative humidity. Indoor environments include homes, office buildings, schools, factories, agricultural facilities, aircraft, subways, buses and other indoor locations. Indoor bioaerosols have been the topic of a substantial body of research in recent years, primarily because of their health effects on humans, and they have been the subjects of numerous reviews addressing topics such as bioaerosol sources, exposure-response relationships, disease transmission, and sampling and detection methods (Mbareche et al. 2017; Mirskaya and Agranovski 2018; Mubareka et al. 2019; Walser et al. 2015). The purpose of this review is to provide a brief overview of techniques for studying indoor bioaerosols, identify some common problems and misconceptions, and discuss future research needs for methods to better understand indoor bioaerosols.

Sources of indoor bioaerosols

Indoor bioaerosols can be generated from various natural and anthropogenic sources. Microbes can grow on a variety of items such as food, wood, paper, textiles, and damp construction materials, and can become airborne in liquid or dry particles. Because of their small size and mass, they are easily transported from one place to another, and persist in the air for long periods of time (Brown and Hovmøller 2002). The sources of indoor bioaerosols include outdoor air, building materials, furnishings, human occupants (coughing, sneezing, talking, walking, etc.), animals, plants, and organic wastes (Prussin and Marr 2015). In some specific cases, such as indoor locations near farms, waste treatment facilities and composting sites, the transport of outdoor bioaerosols into the indoor

CONTACT Jennie Cox Jennie.Cox@uc.edu University of Cincinnati, Department of Environmental Health, P.O. Box 670056. Cincinnati, OH, 45267-0056, USA.

environment is of particular concern (Douglas et al. 2018; Pearson et al. 2015).

General guidelines for indoor bioaerosol sampling

No single sampling or analytical method is appropriate for all indoor bioaerosols. Bioaerosol behavior is strongly coupled to particle size (Nazaroff 2016), and thus sample collection is dependent on the size selectivity of the sampler. Each study will have a specific type of sampling equipment, number and location of samples, volume of air to be sampled, and, if culturing, type of culture medium and incubation conditions. Quality control decisions are also method-specific such as determination of an acceptable sample and procedures of identification, counting, and data analysis (Macher, Chatigny and Burge 1995).

Conditions at each sampling location should always be recorded, including the location within the room (potentially with a drawn figure), air movement (for example, if the heating, ventilation and air conditioning [HVAC] was on or off), air exchange rates, distance from air supply vents, occupancy and activities occurring during sampling. Additional information such as temperature, relative humidity and carbon dioxide levels are also beneficial to record. Environmental information is particularly helpful when studying the air microbiota because it allows variations in microbial diversity to be linked to specific environmental conditions during sampling. This linkage will lead to a better understanding of the impact of environmental conditions on the microbial dynamics of bioaerosols.

The sampling principles for biological and non-biological aerosols are the same, but minimizing damage and ensuring the survival and integrity of microorganisms is required when subsequent analyses include the determination of the culturable or infectious portion of the bioaerosol. In such cases, minimizing the microbial stress during sampling (such as desiccation, shear forces, or high-speed impaction that could affect the cell integrity), and immediate care of samples is often necessary to maintain the cultivability of microbes. When molecular methods are applied, maintaining the viability is not mandatory, but maintaining nucleic acid integrity is essential, especially for RNA viruses that can rapidly degrade during sampling. For that reason, more aerosol sampling options are available when molecular analytical approaches will be used. A selection of commercially available samplers for bioaerosol collection can be seen in Table 1 (Lindsley et al. 2017). A further discussion on bioaerosol sampling strategies and challenges is also presented in this special issue (Mainelis 2019).

Methods for indoor bioaerosol collection and analysis

Many factors affect the indoor air composition including the presence of bioaerosol sources (plants, animals, humans, damaged materials), building conditions, and air exchange with the outdoors. The choice of analytical methods depends upon the agents in bioaerosols that need to be measured and the purpose of study. Bioaerosols in indoor air have been principally characterized by the presence and quantification of endotoxins, mycotoxins, microbial volatile organic compounds (MVOC's), $(1 \rightarrow 3)$ β-D-glucan, and microorganisms (bacteria, archaea, fungi, and viruses).

Culture-based methods are widely used to characterize indoor microbial communities. Culture-based methods determine if the microorganisms are viable and thus capable of growth and infection. Bacteria and fungi are often collected directly onto culture plates or impinged into liquid media that is then applied to plates. However, culture can be difficult and time-consuming, and, by definition, culture-based methods only detect microorganisms that are culturable. A large majority of microorganisms currently cannot be cultured by routine laboratory methods (DeLong and Pace 2001), and many microorganisms, including many pathogenic bacteria, can enter a viable but non-culturable state in which they cannot be readily cultured in the lab but can still cause illness (Li et al. 2014). In addition, the non-viable, non-cultivable portion of bioaerosols can still be harmful to exposed persons since several health issues related to bioaerosol exposure are not linked to microorganism viability or infectious potential.

Molecular methods offer a broader view of the microbial diversity using amplicon-based sequencing or metagenomics. Other molecular techniques such as metatranscriptomics can identify transcript DNA from a given sample and provide an explanation of aerosol changes in the microbiota community and gene expression under different environmental factors (Coulon and Colbeck 2017). This could be particularly helpful when understanding the influence of the built environment on gene expression and the implications for human health (Hegarty, Dannemiller and Peccia 2018). In addition, the general biomass or the concentration of a specific microbe of interest (e.g., *Legionella pneumophila*) can be determined by quantitative polymerase

Table 1. Common commercially-available samplers for bioaerosol collection, adapted from Lindsley et al. (2017).

Filter Samplers	
Manufacturer/ Distributor	*Sampler Name*
Bi-Air	Bi-Air Filter Cassette
Burkard	High Throughput 'Jet' Spore and Particle Sampler
Dycor	CSU-1 Low Volume Air Sampler
EMD Millipore	Various filter and membrane media
GE (Whatman)	Various filter and membrane media
InnovaPrep	ACD-200 Bobcat Air Sampler
Pall Corp.	Various filter and membrane media
Research International	SASS® 3100 Dry Air Sampler
Sartorius	AirPort MD8 Air Sampler
SKC	Button Aerosol Sampler
Zefon	Various filter and membrane media

Multi-stage Impactors	
Manufacturer/Distributor	*Sampler Name*
MSP Corporation	MOUDI™ Impactor/ Nano/Mini
Thermo Scientific	Andersen Cascade Impactor

Single-stage Impactors	
Manufacturer/ Distributor	*Sampler Name*
A.P. Buck	BioAire™/BioSlide™ /Bio-Culture™
Aquaria	MICROFLOW Microbiological Air Sampler
Barramundi	Mattson-Garvin Model 220 Air Sampler (240V is Model 270)
Beijing SENNON	Handy Microbial Air Sampler®
	Microbial Air Sampler (Universal®, Professional®, airIDEAL®)
bioMérieux	airIDEAL® 3P™ Traceability/ Samp'air™ Air Sampler
Bioscience International	SAS Duo 360/SAS Isolator/SAS Super 100
Burkard	24-Hour Recording Volumetric Spore Trap
	Continuous Recording Air Sampler
	Personal Volumetric Air Sampler
	Portable Air Sampler for Agar Plates
	Recording Air Sampler
	Seven-Day Recording Volumetric Spore Trap
Climet	CI-90/CI-95/CI-99 Airborne Microbial Sampler
Dycor	Dycor Slit Sampler

Single-stage Impactors	
Manufacturer/ Distributor	*Sampler Name*
EMD Millipore	MAS-100 Air Sampler
	RCS® Microbial Air Sampler
Environmental Monitoring Systems	Allergenco MK-III/ Allergenco-D
	BioSIS Slit Impaction Air Sampler
	Micro5/cyclex-d Cassettes
	E6 Single-Stage Bioaerosol Impaction Sampler
F.W. Parrett	MicroBio MB1/MB2 Air Sampler
Particle Measuring Systems	Air Trace®/BioCapt™/ MiniCapt™
Sartorius	AirPort MD8 Air Sampler
SKC	BioStage® Standard Single-Stage Viable Cascade Impactor
	VersaTrap® Spore Trap Cassette
Thermo Scientific	IUL Basic Air/Spin Air Air Sampler
Veltek	SMA MicroPortable® Air Sampler
Zefon International	Air-O-Cell®/Via-Cell® Sampling Cassette
	A-6 Bioaerosol Impactor

Cyclones	
Manufacturer/ Distributor	*Sampler Name*
Burkard	Cyclone Sampler for Airborne Particles
	Cyclone Sampler for Field Operation
Evogen	Sceptor DryClone™
FLIR Systems	C100 Modular Tactical Collector

Impingers	
Ace Glass	AGI-30 Impinger
	Greenburg-Smith Impinger
	Midget Impinger

Wetted-Surface Bioaerosol Samplers	
Manufacturer/ Distributor	*Sampler Name*
Bertin	Coriolis® μ Microbial Air Sampler
	Coriolis® RECON Portable Air Sampler
Bioscience International	SAS Cyclone Air Sampler
Evogen	Sceptor SpinCon™ Advanced Air Sampler
FLIR Systems	Fido® B1 (BioCapture® 650) Portable Air Sampler
InnovaPrep	SpinCon® II Advanced Air Sampler
InnovaTek	BioGuardian® Air Sampler

Real-Time Bioaerosol Monitors	
Manufacturer/ Distributor	*Sampler Name*
Bioscience International	SAS-PCR Pathogenic Microorganisms Air Sampler
Droplet Measurement Technologies	Wideband Integrated Bioaerosol Sensor (WIBS)
Dycor	C-FLAPS Biological Detection System
Environics	ENVI BioScout™
FLIR Systems	Fido® B2 Instantaneous Biological Aerosol Detector
Particle Measuring Systems	BioLaz™ Real-Time Microbial Monitor

(*Continued*)

Table 1. Continued.

Cyclones		Wetted-Surface Bioaerosol Samplers		Real-Time Bioaerosol Monitors	
Manufacturer/ Distributor	*Sampler Name*	*Manufacturer/ Distributor*	*Sampler Name*	*Manufacturer/ Distributor*	*Sampler Name*
Burkard	Multistage Liquid Impinger	Research International	BioHawk® 8-Channel Collector/ Bioidentifier	Research International	BioHawk® 8-Channel Collector/ Bioidentifier
	XMX/102 High Volume Bioaerosol Sampling System		SASS® 2300/ SASS® 2400 Low-Volume Wetted-Wall Air Sampler		TacBio™ Biological Aerosol Detector
Dycor	XMX/2L-MIL Bioaerosol Sampler – Military	SKC	BioSampler®	TSI	BIOTRAK® Real-Time Viable Particle Counter 9510-BD
	XMX-CV Microbial Air Sampler – Civilian	Tecora	CIP10-M personal bioaerosol sampler		Fluorescence Aerosol Particle Sensor (FLAPS) 3317 (FLAPS III)™

chain reaction (qPCR) (Sánchez-Parra, Núñez and Moreno 2019). Other approaches include ATP-based bioluminescence, which provides a less expensive way of quantifying the biomass content of a bioaerosol, and propidium monoazide (PMA) staining, which allows the specific PCR amplification of targeted genes extracted from intact cells only (Bonifait et al. 2015; Bonifait et al. 2014).

Lately, culture-based methods have gained more popularity due to culturomics, which combines high-throughput culture with 16S rRNA amplification and sequencing or matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) for the identification of previously unidentified colonies. Studies applying culturomics allowed the identification of new colonies of the gut microbiota, previously undetected by culture-independent methods, and the method was later also used with bioaerosols (Azhar et al. 2017). For this and other reasons (e.g., viability, phenotypic and genotypic characteristics), both culture-based and culture-independent methods are used to provide a fuller image of indoor bioaerosols. Assays and enumeration of bioaerosols are further discussed in a separate paper within this special issue (King et al. 2019).

Passive bioaerosol samples can be collected using methods such as settling plates or electrostatic dust collectors (Frankel et al. 2012; Noss et al. 2010). Passive sampling results cannot be easily related to airborne concentrations because the sample air volume is undetermined, but passive sampling can be useful in situations where the settling of microorganisms is the primary concern, such as the contamination of wounds during surgery or food in a production line (Friberg, Friberg and Burman 1999; Haig et al. 2016b; Smith et al. 2018).

One study has suggested that assessing the microbial diversity of bioaerosols with filter-based high-flow rate air samplers may allow a higher diversity coverage than liquid-based high-flow rate air samplers when applying bacterial and fungal amplicon-based high-throughput sequencing approaches (Lemieux et al. 2019; Mbareche et al. 2018). This is possibly because the collection media in the liquid cyclonic impactors may be subject to evaporation depending on the environment and the relative humidity. This liquid loss may cause an imbalance in the microbial diversity represented in bioaerosol samples.

Bioaerosol analysis standardization has been a hot topic for the last decade, and with the rapid development in analytical method possibilities, this is becoming more urgent. Several technical organizations and standard-setting bodies have subcommittees and working groups that are focused on bioaerosols. ASTM International has a subcommittee on microorganisms, and both the International Organization for Standardization (ISO) and the European Committee for Standardization (CEN) have bioaerosol working groups. All three of these groups have published standards or technical specifications related to bioaerosols, most notably fungi. There is a need to support and encourage reproducibility and continuity within bioaerosol research. Ultimately, such initiatives could lead to a more efficient interpretation of bioaerosol study data. A further discussion on biological aerosol reference standards is also presented in this special issue (Eversole et al. 2019).

Indoor fungi

Indoor sampling of fungal aerosols often is performed to better gain an understanding of the level of exposure when there is suspected or known fungal growth in the indoor environment. When indoor fungal exposure is being assessed, building characteristics such as water damage and/or moldy odor are often

recorded (Adhikari et al. 2010). Fungal species have different growth requirements, habitats where they are likely found, and health effects on individuals.

Methods

Most bioaerosol data of indoor fungal sampling have been collected utilizing culturing methods or microscopy (Nazaroff 2016). Results on culturable fungi only reflect viable organisms that were successfully sampled and grown on the selected media. Not all fungal species can easily be cultured, some grow more slowly than others, and competition can occur when multiple species are present. Non-viable spore traps are commonly used to collect airborne fungi for analysis by microscopy. This is labor intensive, needs to be performed by a specialist, and identification can be limited to spore types or groups of genera. However, microscopy can yield reliable results and be combined with staining to highlight features such as metabolic competence.

Quantitative PCR is suitable for quantification of total fungal DNA using universal probes and primers, or for species-specific quantification (Haugland et al. 2004). The Environmental Relative Moldiness Index (ERMI) includes qPCR of 36 specific mold species based on settled dust sampling in homes across the United States. This method can also be utilized with indoor air sampling (Cox et al. 2017). The ERMI has advantages over traditional culturing, since it is not dependent on viability of the organisms, and over high-thoughput sequencing, as the ERMI has established protocols including species-specific primers and probes. The main disadvantage, however, is that ERMI only evaluates 26 known species that are associated with water damage, and 10 species associated with the outdoor environment (Vesper et al. 2007). Other species that could be informative of the fungal diversity are not included.

Devices

Fungal aerosol samples can be collected with impactors, impingers, cyclones or filters (Haig et al. 2016a; Lindsley et al. 2017; Macher 1999). When sampling for total fungal spores, slit-to-slide samplers are commonly used for microscopic analysis. Andersen cascade impactors collect directly onto culture plates, eliminating some losses that can occur in processing. Passive bioaerosol samples can be collected using the settle plate method or electrostatic dust collectors. Settled dust has been collected as a means of identifying bioaerosols in buildings, especially allergens and mold. However, settled dust can also contain particles from non-aerosol sources such as shoes, skin flakes, and clothing fibers, which can make interpretation difficult. Settled dust has been collected using vacuums, swabs, wipes, adhesive tape, and contact plates (Cox et al. 2017; Reponen et al. 2011).

Indoor location and conditions

For evaluation of fungal contamination in a building, the American Conference of Governmental Industrial Hygienists (ACGIH) recommends that sampling be conducted in an expected high exposure area, an expected low exposure area, and an outdoor area adjacent to the air intake for the building (Macher 1999). Additional outdoor sites could include near and/or far potential sources of outdoor bioaerosols. When assessing a ventilation system for fungal contamination, the investigator should take samples at different times during the unit's cycle, including when it has been turned off and when it has resumed after being off.

Typically, indoor and outdoor air samples are collected simultaneously, and the general assumption is that indoor concentrations should be lower than the outdoor concentrations. Some species of fungus are known to be abundant indoors, like *Aspergillus*, while others are rarely found indoors, like most basidiomycetes (National Research Council 2005). Overall, however, most indoor fungi originate from the outdoor air and are present in lower concentrations indoors than outdoors (Burge et al. 2000). If the indoor fungal levels are higher than the outdoor levels, this could indicate an indoor fungal source (Rao, Burge and Chang 1996). Human activity has been shown to result in significantly higher concentrations of airborne spores (Lehtonen, Reponen and Nevalainen 1993; Scheff et al. 2000). In Buttner and Stetzenbach (1993), surface sampling of a carpet revealed moderate to heavy contamination despite relatively low airborne counts when there was no human activity. As mentioned previously, conditions during sampling can impact the concentration of fungal spores and should be recorded. For example, the level of fungal spores in a room may increase if the relative humidity rises and causes condensation that promotes active growth. The activity in the room and conditions during sampling need to be considered when evaluating the quality of the indoor air and when making comparisons with other studies or recommended values.

Indoor bacteria

Humans are a common source of indoor airborne bacteria. Human occupancy of a classroom has been

shown to greatly increase bacterial bioaerosols (Hospodsky et al. 2012), and human-associated bacteria were shown to be twice as abundant in indoor air compared to outdoor air (Meadow et al. 2014). Animals can be important sources of indoor airborne bacteria, particularly in indoor agricultural environments (Blais Lecours et al. 2012; Just et al. 2011; Wathes 1995). Other sources, such as humidifiers, water sprays and aerosol-generating medical procedures can increase the risk of exposure to pathogens in indoor air (Sattar 2016). For example, Legionellae bacteria can grow in building water systems and become aerosolized by aeration systems and water sprays, while the flushing of toilets can aerosolize bacteria contained in feces such as *Clostridium difficile* (Best, Sandoe and Wilcox 2012; Springston and Yocavitch 2017). Indoor bioaerosol sampling for bacteria is often performed because of health concerns from exposure, particularly in locations such as healthcare facilities where the risks of disease transmission are heightened (Stockwell et al. 2019).

Methods

Culture-based methods are useful for determining if airborne bacteria are capable of causing an infection, or when studying the performance of indoor air disinfection techniques such as ultraviolet germicidal irradiation. However, non-culturable and non-viable bacterial aerosols can carry endotoxins and other toxic components which can have adverse health effects and therefore are still of concern. For these reasons, both culture-based and culture-independent methods are needed, and bioaerosol sampling techniques for both are widely used (Lindsley et al. 2017).

Devices

For culture-based analysis, airborne indoor bacteria are most commonly collected using impingers and impactors (Lindsley et al. 2017). Filter collection is less common because desiccation of the bacteria can greatly diminish their viability unless the bacteria are hardy, such as *Bacillus* spores (Dybwad, Skogan and Blatny 2014; Jensen et al. 1992; Macher and First 1984). Culture-independent methods of analyzing indoor bacterial aerosols avoid the need to preserve bacterial viability, which allows the use of a wider range of aerosol collection techniques. For culture-independent analysis, the collection of bacterial aerosols with filters is common (Lindsley et al. 2017). Some investigators have explored the analysis of building HVAC filters as a simple method of collecting samples from high air volumes over extended times (Haaland and Siegel 2017).

Indoor archaea

Archaea are ubiquitous microbes in a vast range of environments including soils, oceans, and human and animal skin and gastrointestinal tracts. No archaea are presently known to be human pathogens, but this may change as more is understood about these microorganisms (Lurie-Weinberger and Gophna 2015). Archaea are understudied in bioaerosols, and their presence in indoor air and factors affecting their abundance are not well characterized. Exposure to archaea is known to induce a full immune response in a murine model of airway exposure (Blais Lecours et al. 2011).

Methods

The great diversity of the archaeal domain was revealed by culture-independent techniques that allowed the detection and identification of a considerable number of uncultured archaea with unknown culture conditions (Bahram et al. 2018). Quantitative PCR can be applied to measure the total archaeal counts in indoor environments (Just et al. 2013; Nehmé et al. 2009). Amplicon-based high-throughput sequencing methods usually target the prokaryotic 16S rRNA gene to study archaeal diversity. Several primers are available for amplification of bacterial and archaeal 16S rRNA genes. However, these primers are more suited for bacteria as they fail to amplify the broad spectrum of archaeal diversity (Eloe-Fadrosh et al. 2016). Recently, specific primers targeting different sub-regions of the 16S rRNA gene than the ones normally used for bacteria offered a better coverage of the archaeal diversity (Bahram et al. 2018). Therefore, indoor studies designed for the characterization of archaea should consider the recommended set of primers.

Devices

The choice of the indoor sampling strategy for archaea follows the same rules as bacteria. The comparison of three samplers (IOM sampler with a gelatin membrane, NIOSH two-stage cyclone, and the liquid impinger Coriolis) gave comparable results in terms of archaeal gene copy number per cubic meter of air in dairy farms (Blais Lecours et al. 2012). This

indicates that impingers can be used for aerosol sampling, and the sample then divided for both culture-based and culture-independent analysis of archaea.

Indoor viruses

Indoor airborne viruses are most often studied because of concerns about infectious disease transmission. Many human pathogenic viruses, such as measles, influenza and norovirus, can spread through the indoor air when they are expelled by infected people or when they are aerosolized by medical procedures, flushing of toilets, and other means. The potential airborne transmission of viruses such as influenza is of particular concern because of the constant threat of a global pandemic. Thus, studies of indoor viral bioaerosols are often focused on examining the prevalence of pathogens in a specific indoor setting (such as a healthcare facility) or on the effectiveness of various infection control measures.

Bacteriophages are viruses that infect bacteria rather than eukaryotic cells. Because bacteriophages do not infect humans, they are safer to work with than pathogenic viruses, and bacteriophages are easier to culture since they grow in bacteria. For these reasons, bacteriophages such as MS2 are often used as surrogates for pathogenic viruses in studies of indoor viral bioaerosols (Turgeon et al. 2014; Verreault et al. 2015). Airborne bacteriophages also can be a problem in indoor industrial environments in which bacteria are used, such as plants making dairy products (Verreault et al. 2011). The methods and issues described here for viral aerosol collection also apply to bacteriophages.

Methods

As with bacterial aerosols, the methods used to collect and analyze airborne viruses can be broadly divided into culture-based and culture-independent methods, and many of the same considerations apply (Lindsley et al. 2017; Verreault, Moineau and Duchaine 2008). Culture-based methods require preserving the viability of an airborne virus during and after bioaerosol collection, which is more difficult than preserving the viability of bacteria or fungi. In addition, because viruses are parasites and require host cells in order to reproduce, viral assays are considerably more complex and difficult than bacterial or fungal assays, and many viruses currently cannot be cultured. PCR and other culture-independent methods are more widely used than culture-based methods, but they do not determine if the airborne virus is potentially infectious or not, which is often the question of greatest interest.

Devices

The impinger is the most commonly used aerosol sampler to collect airborne viruses for culture-based analysis (Verreault, Moineau and Duchaine 2008). The liquid collection media in an impinger preserves the viability of sensitive viruses much better than dry collection, and this factor usually outweighs the drawbacks of impingers. Culture-independent methods of virus detection allow the use of a broader range of aerosol sampling equipment. Filter and cyclone-based aerosol samplers are often used to collect viral bioaerosols for culture-independent analysis because of their simplicity and because they are effective at collecting aerosol particles of all sizes (Lindsley et al. 2017). A recently-developed aerosol sampler using a condensation-based growth tube collector is reported to collect airborne viruses with minimal damage, which helps maintain viability (Pan et al. 2016).

Common problems and misconceptions with indoor bioaerosols

Bioaerosol concentrations are not the same everywhere in a room

It is not uncommon to see indoor bioaerosol studies in which only a few, or even just one, sampling location is monitored within an indoor space. This can be necessary for many reasons, including time, cost and equipment limitations. However, investigators do not always appreciate how much bioaerosol concentrations can vary within a building, or even within a room, especially if the source of the bioaerosols is within the space (Crawford et al. 2009; Morey 2007). For example, if a person has a viral respiratory infection, the concentration of airborne virus from that person tends to be highest in their immediate vicinity and decrease rapidly as the sampling location moves further away (Jones and Brosseau 2015). Other factors such as building airflow patterns and operation of the HVAC system also can substantially affect bioaerosol distributions (Luongo et al. 2016). To fully characterize an indoor bioaerosol, sampling locations should be carefully thought out and samples should be collected in as many locations as feasible. If the variations in bioaerosol concentrations in space are not of interest or if the bioaerosol concentrations are low, the samples can be pooled to get an average concentration, which reduces the cost of analysis.

Bioaerosol concentrations change over time

In addition to spatial variations, large temporal variations in bioaerosol concentrations are common over time scales

ranging from seconds to months, particularly if the bioaerosol source is intermittent or seasonal (LeBouf, Yesse and Rossner 2008; Lindsley et al. 2010). Thus, sampling at multiple time points or for long durations is frequently needed to fully understand the dynamics of the bioaerosols in an indoor space (Emerson et al. 2017). Indoor bioaerosol samples are often collected for short time periods (typically 15-30 min for viable impactors and impingers), which only provides the average bioaerosol concentration during the collection time. However, the bioaerosol exposure for an individual is a function of both the bioaerosol concentration and the exposure time. Fluctuations in bioaerosol concentrations can greatly affect the overall exposure, and this can be missed by limiting sampling to a few short-term collections.

The size of a microorganism does not necessarily indicate the size of the bioaerosol

Bioaerosols often are not produced by the aerosolization of individual microorganisms, but rather by the aerosolization of agglomerations or of solutions containing many components besides the microorganisms themselves. Thus, airborne microorganisms are frequently part of a larger mixture of material, and the size of the bioaerosol particles can be much larger than the size of the native microorganisms (Eduard et al. 1990). For example, the influenza virus has a diameter of about 100 nm, but influenza is normally shed in droplets that contain salts, proteins and cellular debris in addition to the virus, and thus airborne influenza virus usually is primarily found in particles greater than 1 µm (Lindsley et al. 2010).

Filter pore size does not indicate the size of the aerosol particle that will be collected

The manufacturer's specifications of an aerosol filter often include a "pore size" or "equivalent pore diameter". It is important to understand that the filter pore size does not indicate the minimum aerosol particle size that will be collected by a filter. Unlike in liquid filtration, aerosol filters collect particles primarily through impaction, electrostatic capture, interception, and diffusion, and most aerosol filters will efficiently collect aerosol particles that are much smaller than the nominal pore size. This is important because filters with smaller pore sizes typically have a higher air flow resistance, which reduces the maximum air flowrate, decreases the running time for a battery-powered pump, and increases the pump noise level. Investigators also occasionally err in assuming that they can use a filter with a given pore size as a size-selective aerosol filter, which emphatically is not the case. A more detailed discussion of the collection of aerosol particles with filters and the role of filter pore size can be found in Lindsley (2016), which is available on-line.

Static aerosol sampling does not necessarily indicate personal aerosol exposure

Static (area) aerosol sampling provides the bioaerosol concentration at a specific location, while personal aerosol sampling measures the bioaerosol concentrations around a person as they go about their tasks. Static sampling has several advantages; it can measure aerosol production from specific sources, it does not encumber people with aerosol samplers, it allows for more data collection when the number of people or the time they spend in a particular location is limited, and it is necessary when using samplers and pumps that cannot be worn. However, static sampling does not always provide a reliable indication of the personal aerosol exposure that people experience, particularly if the person is close to the aerosol source (Cherrie et al. 2011; Kissell and Sacks 2002). Thus, both types of sampling may be necessary to fully characterize the exposure of people to indoor bioaerosols (Toivola et al. 2002). The recent ISO standard EN 689, for example, requires that personal sampling devices be used instead of static sampling whenever possible for workplace exposure measurement (ISO 2018).

Fungal cell recovery from liquid aerosol samplers can be affected by centrifugation

Concentrating aerosol samples before nucleic acid extraction is necessary to obtain detectable amounts of DNA or RNA before applying culture-independent approaches. The concentration is usually achieved by centrifuging liquid samples and resuspending the pellets in a smaller volume. This concentration method causes the loss of fungal cells, possibly due to their known different behavior in a liquid-air surface caused by hydrophobicity and polarity at the cell membrane (Gregory 1957; Mbareche et al. 2019; Wösten et al. 1999). Filtration offers higher yields qualitatively and quantitatively for a better description of the fungal exposure of indoor aerosol studies (Mbareche et al. 2019). Filtration should be applied in studies where liquid-based analyses are expected no matter what aerosol sampling regime is used. For example, studies involving bulk dust or settled dust sampling (vacuuming or electrostatic dust collection) that include resuspension of dust into a liquid will have the same concentration challenge.

Future needs

Better viable bioaerosol samplers and sampling methods

The viability of airborne microorganisms is often a critical parameter; for example, only viable microorganisms can cause an infection. However, as noted earlier, efficiently collecting airborne microorganisms while preserving their viability is fraught with difficulty, especially for viruses. Improved collection systems and methods are needed to better understand the viability of airborne microorganisms and the risks that they pose.

Standards for acceptable bioaerosol exposure limits

Standards and recommendations for acceptable exposure limits for airborne fungi and bacteria are limited, and standards do not exist for viruses, protozoa, or archaea. Eduard (2009) suggested an exposure limit of 10^5 fungal spores/m^3 of air, but this is limited to fungal spores that are not pathogenic and do not produce mycotoxins. The Health Council of the Netherlands has proposed a limit of 90 units/m^3 for endotoxin based on acute effects on lung function (Health Council of the Netherlands 2010). The Federal Institute for Occupational Safety and Health in Germany established a workplace limit of 50,000 CFU/m^3 for mesophilic fungi (BAuA 2013). Exposure limits for bioaerosols have been discussed recently in reviews by Walser et al. (2015), Eduard et al. (2012), Pearson et al. (2015).

Exposure limits are difficult to establish because, although it is widely agreed that exposure to various bioaerosols can lead to adverse health outcomes, it is very difficult to define a safe exposure level. This lack of guidance places tremendous limitations on the practical applications of bioaerosol measurements. For example, suppose a sampling study shows that airborne viruses or bacteria are present at a certain level in a healthcare facility. This information may be useful if it shows that high-risk bioaerosols are present or absent, or that concentrations are very high in certain locations or during certain medical procedures. However, most locations in the facility will have some level of bioaerosols, and the practical use of this information is limited because it is unclear how much risk this presents to patients and staff. Unfortunately, establishing acceptable exposure limits for bioaerosols is extremely challenging because bioaerosols are often complex mixtures of microorganisms and other materials, most microorganisms and their components have not been well-characterized, and the health effects of bioaerosol components can vary substantially from person to person.

Standardization for bioaerosol sampling protocols

It would be beneficial to achieve a standard for bioaerosol sampling protocols so that sampling and analysis can be performed consistently. Organizations such as ISO, CEN and ASTM International have created standards and recommendations for sampling for bioaerosols, primarily for airborne fungi (ASTM 2014; CEN 2011; ISO 2008). However, such standards are often not used by researchers, especially as new technologies are brought into use that are not covered by the standards. Several studies have attempted to standardize and compare sampling protocols by evaluating the relative efficiencies of selected sampling methods for the retrieval of fungal aerosols (Buttner and Stetzenbach 1993; Eduarda and Heederik 1998; Nieto-Caballero et al. 2019). Unfortunately, it is difficult to compare results between studies utilizing different methods, and even if the same methods are used, investigators can reach different conclusions. Interpreting mold aerosol sampling results to decide if an indoor source exists is not always straightforward. Investigators tend to develop their own set of criteria relying on their personal judgement because there is no consensus for analyzing results. When evaluating the level of agreement among practitioners of indoor air quality in their evaluation of sampling data for airborne mold, Johnson et al. (2008) found there was only weak overall agreement and substantial inter-evaluator differences. The development and more widespread use of standardizing protocols would allow studies to be compared more easily. However, this remains challenging as each study has different environmental settings, aims, and access to resources.

Better use of the big data generated from High-Throughput sequencing

The avalanche of information accumulated from the high-throughput sequencing approaches should be used for future association and epidemiological studies to take full advantage of the results. Presently, this information is used to identify as many microbes as possible. The idea is to push this information further to create associations, in the long term, between the core microbiome of a specific indoor environment, the identified microbial markers, and the health outcomes observed on the type of population exposed. Practically, this can be resource intensive, but can take bioaerosol studies a step further in the determination of standards for acceptable bioaerosol exposure limits.

Concluding remarks

The study of indoor air has received significant attention in the last few decades because of the recognition of major health impacts and severe disease outbreaks due to exposure to airborne pathogens. This recognition has also led to the rise of the development, assessment, and application of methods to decontaminate indoor air. Despite the recognized significance of indoor air as an important factor affecting the health of exposed individuals, major gaps exist in indoor bioaerosol characterization and standardization. The authors hope that this review will serve as a guideline that encourages reproducibility and that it will help inform field sampling of indoor bioaerosols and analytical methods for future bioaerosol research.

Acknowledgments

The authors would like to thank Shanna Ratnesar-Shumate and J. Alex Huffman for organizing this issue following suggestions from the AAAR Bioaerosol Working Group and Bioaerosol Standardization Workshop at the International Aerosol Conference in St Louis, Missouri in September 2018.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease control and Prevention (CDC). Mention of any company or product does not constitute endorsement by NIOSH, CDC.

Funding

The authors also acknowledge Natural Sciences and Engineering Research Council of Canada (RGPIN-2014-05900) and Fond de Recherche du Québec, Nature et Technologie and the Institut Robert-Sauvé en Santé et en Sécurité du Travail.

ORCID

Jennie Cox http://orcid.org/0000-0002-7951-2687
Hamza Mbareche http://orcid.org/0000-0002-4169-5203
William G. Lindsley http://orcid.org/0000-0003-0720-5829
Caroline Duchaine http://orcid.org/0000-0002-9912-0349

References

Adhikari, A., J. S. Lewis, T. Reponen, E. C. DeGrasse, L. F. Grimsley, G. L. Chew, Y. Iossifova, and S. A. Grinshpun. 2010. Exposure matrices of endotoxin,(1→3)-β-D-glucan, fungi, and dust mite allergens in flood-affected homes of New Orleans. *Science Total Environment* 408 (22): 5489–5498. doi:10.1016/j.scitotenv.2010.07.087.

ASTM. 2014. Standard practice for collection of total airborne fungal structures via inertial impaction methodology. ASTM d7788-14. *4*, ASTM International, West Conshohocken, PA.

Azhar, E. I., A. Papadioti, F. Bibi, A. M. Ashshi, D. Raoult, and E. Angelakis. 2017. *Pseudomonas saudimassiliensis*' sp. nov. a new bacterial species isolated from air samples in the urban environment of Makkah, Saudi Arabia. *New Microbes New Infections* 16 :43–44. doi:10.1016/j.nmni.2016.12.021.

Bahram, M., S. Anslan, F. Hildebrand, P. Bork, and L. Tedersoo. 2018. Newly designed 16S rRNA metabarcoding primers amplify diverse and novel archaeal taxa from the environment. *Environmental Microbiology Reports* 11 (4):487–494. doi:10.1111/1758-2229.12684.

BAuA. 2013. Technische Regel für Biologische Arbeitsstoffe 214 (Technical Rules for Biological Agents 214), Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (Federal Institute for Occupational Safety and Health). http://www.baua.de/de/Themenvon-A-Z/Biologische-Arbeitsstoffe/TRBA/TRBA-405.html.

Best, E., J. Sandoe, and M. Wilcox. 2012. Potential for aerosolization of Clostridium difficile after flushing toilets: The role of toilet lids in reducing environmental contamination risk. *Journal Hospital Infection* 80 (1):1–5. doi:10.1016/j.jhin.2011.08.010.

Blais Lecours, P., C. Duchaine, M. Taillefer, C. Tremblay, M. Veillette, Y. Cormier, and D. Marsolais. 2011. Immunogenic properties of archaeal species found in bioaerosols. *PLoS One* 6 (8):e23326. doi:10.1371/journal.pone.0023326.

Blais Lecours, P., M. Veillette, D. Marsolais, and C. Duchaine. 2012. Characterization of bioaerosols from dairy barns: Reconstructing the puzzle of occupational respiratory diseases by using molecular approaches. *Applied and Environmental Microbiology* 78 (9): 3242–3248. doi:10.1128/AEM.07661-11.

Bonifait, L., R. Charlebois, A. Vimont, N. Turgeon, M. Veillette, Y. Longtin, J. Jean, and C. Duchaine. 2015. Detection and quantification of airborne norovirus during outbreaks in healthcare facilities. *Clinical Infectious Diseases* 61 (3):299–304. doi:10.1093/cid/civ321.

Bonifait, L., M. Veillette, V. Létourneau, D. Grenier, and C. Duchaine. 2014. Detection of *Streptococcus suis* in bioaerosols of swine confinement buildings. *Applied and Environmental Microbiology* 80 (11):3296–3304. doi:10.1128/AEM.04167-13.

Brown, J. K., and M. S. Hovmøller. 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* 297 (5581):537–541. doi:10.1126/science.1072678.

Burge, H. A., D. L. Pierson, T. O. Groves, K. F. Strawn, and S. K. Mishra. 2000. Dynamics of airborne fungal populations in a large office building. *Current Microbiology* 40 (1):10–16. doi:10.1007/s002849910003.

Buttner, M. P., and L. D. Stetzenbach. 1993. Monitoring airborne fungal spores in an experimental indoor environment to evaluate sampling methods and the effects of human activity on air sampling. *Applied and Environmental Microbiology* 59 (1):219–226.

CEN. 2011. Ambient air quality - Measurement of bioaerosols - Part 1: Determination of moulds using filter

sampling systems and culture-based analyses, in Cen/TS 16115-1. 2011, European Committee for Standardization, Brussels, Belgium.

Cherrie, J. W., L. Maccalman, W. Fransman, E. Tielemans, M. Tischer, and M. Van Tongeren. 2011. Revisiting the effect of room size and general ventilation on the relationship between near- and far-field air concentrations. *Annals of Occupational Hygiene* 55 :1006–1015. doi:10.1093/annhyg/mer092.

Coulon, F., and I. Colbeck. 2017. RAMBIE, rapid monitoring of bioaerosols in urban, agricultural and industrial environments, NERC. *Impact* 2017 (11):12–14. doi:10.21820/23987073.2017.11.12.

Cox, J., R. Indugula, S. Vesper, Z. Zhu, R. Jandarov, and T. Reponen. 2017. Comparison of indoor air sampling and dust collection methods for fungal exposure assessment using quantitative PCR. *Environmental Science: Processes Impacts* 19 :1312–1319. doi:10.1039/C7EM00257B.

Crawford, C., T. Reponen, T. Lee, Y. Iossifova, L. Levin, A. Adhikari, and S. A. Grinshpun. 2009. Temporal and spatial variation of indoor and outdoor airborne fungal spores, pollen, and (1→ 3)-β-D-glucan. *Aerobiologia* 25 (3):147–158. doi:10.1007/s10453-009-9120-z.

DeLong, E. F., and N. R. Pace. 2001. Environmental diversity of bacteria and archaea. *Systematic Biology* 50 (4): 470–478. doi:10.1080/106351501750435040.

Douglas, P., S. Robertson, R. Gay, A. L. Hansell, and T. W. Gant. 2018. A systematic review of the public health risks of bioaerosols from intensive farming. *International Journal of Hygiene and Environmental Health* 221(2): 134–173. doi:10.1016/j.ijheh.2017.10.019.

Dybwad, M., G. Skogan, and J. M. Blatny. 2014. Comparative testing and evaluation of nine different air samplers: End-to-end sampling efficiencies as specific performance measurements for bioaerosol applications. *Aerosol Science and Technology* 48 (3):282–295. doi:10.1080/02786826.2013.871501.

Eduarda, W. 2009. Fungal spores: A critical review of the toxicological and epidemiological evidence as a basis for occupational exposure limit setting. *Critical Reviews in Toxicology* 39 :799–864.

Eduarda, W., and D. Heederik. 1998. Methods for quantitative assessment of airborne levels of noninfectious microorganisms in highly contaminated work environments. *American Industrial Hygiene Association Journal* 59 (2): 113–127. doi:10.1080/15428119891010370.

Eduard, W., D. Heederik, C. Duchaine, and B. J. Green. 2012. Bioaerosol exposure assessment in the workplace: The past, present and recent advances. *Journal Environmental Monitoring* 14 (2):334–339. doi:10.1039/c2em10717a.

Eduard, W., J. Lacey, K. Karlsson, U. Palmgren, G. Strom, and G. Blomquist. 1990. Evaluation of methods for enumerating microorganisms in filter samples from highly contaminated occupational environments. *American Industrial Hygiene Association Journal* 51 (8):427–436. doi:10.1202/0002-8894(1990)051<0427:EOMFEM>2.0.CO;2.

Eloe-Fadrosh, E. A., N. N. Ivanova, T. Woyke, and N. C. Kyrpides. 2016. Metagenomics uncovers gaps in amplicon-based detection of microbial diversity. *Nature Microbiology* 1 (4):15032. doi:10.1038/nmicrobiol.2015.32.

Emerson, J., P. Keady, N. Clements, E. Morgan, J. Awerbuch, S. Miller, and N. Fierer. 2017. High temporal variability in airborne bacterial diversity and abundance inside single-family residences. *Indoor Air* 27 (3): 576–586. doi:10.1111/ina.12347.

Eversole, J., M. Hart, C. Scotto, D. Mcpherson, Z. Minter, J. Tucker, J. Kesavan, and H.-B. Lin. 2019. Toward biological aerosol reference standards. *Aerosol Science and Technology*. In Review.

Frankel, M., M. Timm, E. Hansen, and A. Madsen. 2012. Comparison of sampling methods for the assessment of indoor microbial exposure. *Indoor Air* 22 (5):405–414. doi:10.1111/j.1600-0668.2012.00770.x.

Friberg, B., S. Friberg, and L. G. Burman. 1999. Inconsistent correlation between aerobic bacterial surface and air counts in operating rooms with ultra clean laminar air flows: Proposal of a new bacteriological standard for surface contamination. *Journal of Hospital Infection* 42 (4): 287–293. doi:10.1053/jhin.1998.0598.

Gregory, P. 1957. Electrostatic charges on spores of fungi in air. *Nature* 180 (4581):330. doi:10.1038/180330a0.

Haaland, D., and J. A. Siegel. 2017. Quantitative filter forensics for indoor particle sampling. *Indoor Air* 27 (2): 364–376. doi:10.1111/ina.12319.

Haig, C. W., W. G. Mackay, J. T. Walker, and C. Williams. 2016. Bioaerosol sampling: sampling mechanisms, bioefficiency and field studies. *Journal of Hospital Infection* 93 (3):242–255.

Haig, C., W. Mackay, J. Walker, and C. Williams. 2016. Bioaerosol sampling: Sampling mechanisms, bioefficiency and field studies. *Journal of Hospital Infection* 93 (3): 242–255. doi:10.1016/j.jhin.2016.03.017.

Haugland, R. A., M. Varma, L. J. Wymer, and S. J. Vesper. 2004. Quantitative PCR analysis of selected aspergillus, penicillium and paecilomyces species. *Systematic and Applied Microbiology* 27 (2):198–210. doi:10.1078/072320204322881826.

Health Council of the Netherlands. 2010. Endotoxins—Health-Based Recommended Occupational Exposure Limit, No. 2010/04OSH, Health Council of the Netherlands, The Hague. https://www.healthcouncil.nl/documents/advisory-reports/2010/07/15/endotoxins-health-based-recommended-occupational-exposure-limit.

Hegarty, B., K. Dannemiller, and J. Peccia. 2018. Gene expression of indoor fungal communities under damp building conditions: implications for human health. *Indoor Air* 28(4):548–558. doi:10.1111/ina.12459.

Hospodsky, D., J. Qian, W. W. Nazaroff, N. Yamamoto, K. Bibby, H. Rismani-Yazdi, and J. Peccia. 2012. Human occupancy as a source of indoor airborne bacteria. *PloS One* 7 (4):e34867. doi:10.1371/journal.pone.0034867.

ISO. 2008. Indoor air — Part 16: Detection and enumeration of moulds — Sampling by filtration, in *ISO 16000-16:2008(en)*.

ISO. 2018. Workplace exposure - Measurement of exposure by inhalation to chemical agents - Strategy for testing compliance with occupational exposure limit values, Standard: CSN EN 689, Brussels, Belgium.

Jensen, P. A., W. F. Todd, G. N. Davis, and P. V. Scarpino. 1992. Evaluation of eight bioaerosol samplers challenged with aerosols of free bacteria. *American Industrial*

Hygiene Association Journal 53 (10):660–667. doi:10.1202/0002-8894(1992)053<0660:EOEBSC>2.0.CO;2.

Johnson, D., D. Thompson, R. Clinkenbeard, and J. Redus. 2008. Professional judgment and the interpretation of viable mold air sampling data. *Journal Occupational Environmental Hygiene* 5 (10):656–663. doi:10.1080/15459620802310796.

Jones, R. M., and L. M. Brosseau. 2015. Aerosol transmission of infectious disease. *Journal of Occupational and Environmental Medicine* 57 (5):501–508. doi:10.1097/JOM.0000000000000448.

Just, N., P. Blais Lecours, M. Marcoux-Voiselle, S. Kirychuk, M. Veillette, B. Singh, and C. Duchaine. 2013. Archaeal characterization of bioaerosols from cage-housed and floor-housed poultry operations. *Canadian j. Microbiology* 59 (1):46–50. doi:10.1139/cjm-2012-0305.

Just, N., S. Kirychuk, Y. Gilbert, V. Letourneau, M. Veillette, B. Singh, and C. Duchaine. 2011. Bacterial diversity characterization of bioaerosols from cage-housed and floor-housed poultry operations. *Environmental Research* 4 :492–498. doi:10.1016/j.envres.2011.01.009.

King, M. P., A. Joseph, G. Fearing, T. Ramos, Baig, B. Smith, and A. Koustova. 2019. Assays and enumeration of bioaerosols: Historical approaches to modern practices. *Aerosol Science and Technology*. In Review.

Kissell, F. N., and H. K. Sacks. 2002. Inaccuracy of area sampling for measuring the dust exposure of mining machine operators in coal mines. *Mining Engineering* 54 :33–39.

LeBouf, R., L. Yesse, and A. Rossner. 2008. Seasonal and diurnal variability in airborne mold from an indoor residential environment in Northern New York. *Journal of the Air & Waste Management Association* 58 (5):684–692. doi:10.3155/1047-3289.58.5.684.

Lehtonen, M., T. Reponen, and A. Nevalainen. 1993. Everyday activities and variation of fungal spore concentrations in indoor air. *International Biodeterioration & Biodegradation* 31: 25–39. doi:10.1016/0964-8305(93)90012-Q.

Lemieux, J., M. Veillette, H. Mbareche, and C. Duchaine. 2019. Re-aerosolization in liquid-based air samplers induces bias in bacterial diversity. *Aerosol Science and Technology* 53 :1244–1260.

Li, L.,. N. Mendis, H. Trigui, J. D. Oliver, and S. P. Faucher. 2014. The importance of the viable but non-culturable state in human bacterial pathogens. *Frontiers in Microbiology* 5 :258. doi:10.3389/fmicb.2014.00258.

Lindsley, W. G. 2016. Filter pore size and aerosol sample collection. In *NIOSH manual of analytical methods*, eds. Ashley, K. and P. F. O'Connor, FB1–14. Cincinnati, OH: National Institute for Occupational Safety and Health.

Lindsley, W. G., F. M. Blachere, K. A. Davis, T. A. Pearce, M. A. Fisher, R. Khakoo, S. M. Davis, M. E. Rogers, R. E. Thewlis, J. A. Posada, et al. 2010. Distribution of airborne influenza virus and respiratory syncytial virus in an urgent care medical clinic. *Clinical Infectious Diseases* 50 (5):693–698.

Lindsley, W. G., B. J. Green, F. M. Blachere, S. B. Martin, B. F. Law, P. A. Jensen, and M. P. Schafer. 2017. Sampling and characterization of bioaerosols. In *NIOSH manual of analytical methods*, eds. Ashley, K. and P. F. O'Connor, BA1–115. Cincinnati, OH: National Institute for Occupational Safety and Health Cincinnati.

Lindsley, W. G., B. J. Green, F. M. Blachere, S. B. Martin, B. F. Law, P. A. Jensen, and M. P. Schafer. 2017. Sampling and characterization of bioaerosols. In *NIOSH manual of analytical methods (NMAM)*, eds. Ashley, K. and P. F. O'Connor, BA1–115. Cincinnati, OH: National Institute for Occupational Safety and Health Cincinnati.

Luongo, J. C., K. P. Fennelly, J. A. Keen, Z. J. Zhai, B. W. Jones, and S. L. Miller. 2016. Role of mechanical ventilation in the airborne transmission of infectious agents in buildings. *Indoor Air* 26 (5):666–678. doi:10.1111/ina.12267.

Lurie-Weinberger, M. N., and U. Gophna. 2015. Archaea in and on the human body: Health implications and future directions. *PLoS Pathogens* 11 (6):e1004833. doi:10.1371/journal.ppat.1004833.

Macher, J. M., M. A. Chatigny, H. A. Burge, 1995. Sampling airborne microorganisms and aeroallergens. In *Air sampling instruments for evaluations of atmospheric contaminants*, eds. B. S. Cohen and S. V. Hering, 589–617. 8th ed. Cincinnati, OH: American Conference of Governmental Industrial Hygienists, Inc.

Macher, J. 1999. *Bioaerosols: Assessment and control*. Cincinnati, OH: American Conference of Governmental Industrial Hygienists (ACGIH).

Macher, J. M., and M. W. First. 1984. Personal air samplers for measuring occupational exposures to biological hazards. *American Industrial Hygiene Association Journal* 45 (2): 76–83. doi:10.1202/0002-8894(1984)045<0076:PASFMO>2.3.CO;2.

Mainelis, G. 2019. Bioaerosol sampling: Classical approaches, advances, and perspectives. *Aerosol Science and Technology* 1–24. doi:10.1080/02786826.2019.1671950.

Mbareche, H., E. Brisebois, M. Veillette, and C. Duchaine. 2017. Bioaerosol sampling and detection methods based on molecular approaches: No pain no gain. *Science of the Total Environment* 599 :2095–2104. doi:10.1016/j.scitotenv.2017.05.076.

Mbareche, H., M. Veillette, G. J. Bilodeau, and C. Duchaine. 2018. Bioaerosol sampler choice should consider efficiency and ability of samplers to cover microbial diversity. *Applied and Environmental Microbiology* 84 :e01589–01518.

Mbareche, H., M. Veillette, W. Teertstra, W. Kegel, G. J. Bilodeau, H. A. Wösten, and C. Duchaine. 2019. Fungal cells recovery from air samples: A tale of loss and gain. *Applied and Environmental Microbiology: AEM* 85 :e02941–02918

Meadow, J. F., A. E. Altrichter, S. W. Kembel, J. Kline, G. Mhuireach, M. Moriyama, D. Northcutt, T. K. O'Connor, A. M. Womack, G. Z. Brown, et al. 2014. Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. *Indoor Air* 24 (1):41–48. doi:10.1111/ina.12047.

Mirskaya, E., and I. E. Agranovski. 2018. Sources and mechanisms of bioaerosol generation in occupational environments. *Critical Reviews Microbiology* 44 (6):739–758. doi:10.1080/1040841X.2018.1508125.

Morey, P. R. 2007. Microbiological sampling strategies in indoor environments. In *Sampling and Analysis of Indoor Microorganisms*, 51–74.

Mubareka, S., N. Groulx, E. Savory, T. Cutts, S. Theriault, J. A. Scott, C. J. Roy, N. Turgeon, E. Bryce, G. Astrakianakis, et al. 2019. Bioaerosols and transmission, a diverse and growing community of practice. *Frontiers Public Health* 7 :1–7. doi:10.3389/fpubh.2019.00023.

National Research Council. 2005. Chapter 3 Indoor and outdoor bioaerosol backgrounds and sampling strategies in *Sensor systems for biological agent attacks: Protecting buildings and military bases*, 1–45. The National Academies Press, Washington, DC.

Nazaroff, W. W. 2016. Indoor bioaerosol dynamics. *Indoor Air* 26 (1):61–78. doi:10.1111/ina.12174.

Nehmé, B., Y. Gilbert, V. Létourneau, R. J. Forster, M. Veillette, R. Villemur, and C. Duchaine. 2009. Culture-independent characterization of archaeal biodiversity in swine confinement building bioaerosols. *Applied and Environmental Microbiology* 75: 5445–5450. doi:10.1128/AEM.00726-09.

Nieto-Caballero, M., N. Savage, P. Keady, and M. Hernandez. 2019. High fidelity recovery of airborne microbial genetic materials by direct condensation capture into genomic preservatives. *Journal Microbiological Methods* 157: 1–3. doi:10.1016/j.mimet.2018.12.010.

Noss, I., G. Doekes, I. Sander, D. J. Heederik, P. S. Thorne, and I. M. Wouters. 2010. Passive airborne dust sampling with the electrostatic dustfall collector: optimization of storage and extraction procedures for endotoxin and glucan measurement. *Annals of Occupational Hygiene* 54 :651–658. doi:10.1093/annhyg/meq026.

Pan, M., A. Eiguren-Fernandez, H. Hsieh, N. Afshar-Mohajer, S. Hering, J. Lednicky, Z. Hugh Fan, and C. Y. Wu. 2016. Efficient collection of viable virus aerosol through laminar-flow, water-based condensational particle growth. *Journal of Applied Microbiology* 120 (3): 805–815. doi:10.1111/jam.13051.

Pearson, C., E. Littlewood, P. Douglas, S. Robertson, T. W. Gant, and A. L. Hansell. 2015. Exposures and health outcomes in relation to bioaerosol emissions from composting facilities: A systematic review of occupational and community studies. *Journal of Toxicology Environmental Health, Part B* 18 (1):43–69. doi:10.1080/10937404.2015.1009961.

Prussin, A. J., and L. C. Marr. 2015. Sources of airborne microorganisms in the built environment. *Microbiome* 3 (1):78. doi:10.1186/s40168-015-0144-z.

Rao, C. Y., H. A. Burge, and J. C. Chang. 1996. Review of quantitative standards and guidelines for fungi in indoor air. *Journal of the Air & Waste Management Association* 46 :899–908. doi:10.1080/10473289.1996.10467526.

Reponen, T., S. Vesper, L. Levin, E. Johansson, P. Ryan, J. Burkle, S. A. Grinshpun, S. Zheng, D. I. Bernstein, J. Lockey, et al. 2011. High environmental relative moldiness index during infancy as a predictor of asthma at 7 years of age. *Annals of Allergy, Asthma & Immunology* 107 (2):120–126. doi:10.1016/j.anai.2011.04.018.

Sánchez-Parra, B., A. Núñez, and D. A. Moreno. 2019. Preventing legionellosis outbreaks by a quick detection of airborne Legionella pneumophila. *Environmental Research* 171 :546–549. doi:10.1016/j.envres.2019.01.032.

Sattar, S. A. 2016. Indoor air as a vehicle for human pathogens: Introduction, objectives, and expectation of outcome. *American Journal of Infection Control* 44 (9): S95–S101. doi:10.1016/j.ajic.2016.06.010.

Scheff, P. A., V. K. Paulius, L. Curtis, and L. M. Conroy. 2000. Indoor air quality in a Middle school, part II: Development of emission factors for particulate matter and bioaerosols. *Applied Occupational and Environmental Hygiene* 15 (11): 835–842. doi:10.1080/10473220050175715.

Smith, J., C. E. Adams, M. F. King, C. J. Noakes, C. Robertson, and S. J. Dancer. 2018. Is there an association between airborne and surface microbes in the critical care environment? *Journal of Hospital Infection* 100 (3): e123–e129. doi:10.1016/j.jhin.2018.04.003.

Springston, J. P., and L. Yocavitch. 2017. Existence and control of legionella bacteria in building water systems: A review. *Journal of Occupational Environmental Hygiene* 14 (2):124–134. doi:10.1080/15459624.2016.1229481.

Stockwell, R. E., E. L. Ballard, P. O'Rourke, L. D. Knibbs, L. Morawska, and S. C. Bell. 2019. Indoor hospital air and the impact of ventilation on bioaerosols: A systematic review. *Journal of Hospital Infection* 103 (2):175–184. doi:10.1016/j.jhin.2019.06.016.

Toivola, M., S. Alm, T. Reponen, S. Kolari, and A. Nevalainen. 2002. Personal exposures and microenvironmental concentrations of particles and bioaerosols. *Journal of Environmental Monitoring* 4 (1):166–174. doi:10.1039/b108682k.

Turgeon, N., M. J. Toulouse, B. Martel, S. Moineau, and C. Duchaine. 2014. Comparison of five bacteriophages as models for viral aerosol studies. *Applied and Environmental Microbiology* 80 (14):4242–4250. doi:10.1128/AEM.00767-14.

Verreault, D., L. Gendron, G. M. Rousseau, M. Veillette, D. Masse, W. G. Lindsley, S. Moineau, and C. Duchaine. 2011. Detection of airborne lactococcal bacteriophages in cheese manufacturing plants. *Applied and Environmental Microbiology* 77 (2):491–497. doi:10.1128/AEM.01391-10.

Verreault, D., M. Marcoux-Voiselle, N. Turgeon, S. Moineau, and C. Duchaine. 2015. Resistance of aerosolized bacterial viruses to relative humidity and temperature. *Applied and Environmental Microbiology* 81 (20): 7305–7311. doi:10.1128/AEM.02484-15.

Verreault, D., S. Moineau, and C. Duchaine. 2008. Methods for sampling of airborne viruses. *Microbiology and Molecular Biology Reviews* 72 (3):413–444. doi:10.1128/MMBR.00002-08.

Vesper, S., C. McKinstry, R. Haugland, L. Wymer, K. Bradham, P. Ashley, D. Cox, G. Dewalt, and W. Friedman. 2007. Development of an environmental relative moldiness index for US homes. *Journal of Occupational and Environmental Medicine* 49 (8): 829–833. doi:10.1097/JOM.0b013e3181255e98.

Walser, S. M., D. G. Gerstner, B. Brenner, J. Bünger, T. Eikmann, B. Janssen, S. Kolb, A. Kolk, D. Nowak, M. Raulf, et al. 2015. Evaluation of exposure–response relationships for health effects of microbial bioaerosols–a systematic review. *International Journal of Hygiene and Environmental Health* 218 (7):577–589. doi:10.1016/j.ijheh.2015.07.004.

Wathes, C. 1995. Bioaerosols in animal houses. In *Bioaerosols handbook*, eds. C. S. Cox and C. Wathes, 547–577. Boca Raton, FL, USA: Lewis Publishers.

Wösten, H. A., M.-A. van Wetter, L. G. Lugones, H. C. van der Mei, H. J. Busscher, and J. G. Wessels. 1999. How a fungus escapes the water to grow into the air. *Current Biology* 9 (2):85–88. doi:10.1016/S0960-9822(99)80019-0.