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Article

Air Sampling for Early Pathogen Detection

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Abstract: This article examines air sampling as an environmental surveillance strategy for early pathogen detection and offers a preliminary comparison of air and wastewater surveillance. Air sampling involves collecting bioaerosols to analyze the microbial composition of the air, utilizing techniques like PCR and metagenomic sequencing. Air sampling appears especially promising for detecting respiratory pathogens and highly transmissible pandemic agents, as these spread primarily via aerosols and droplets. The article details various air sampling techniques, their efficiency, and practical considerations, categorizing them into filtration, impaction, impingement, electrostatic precipitation, and condensation methods. It also explores the composition of airborne microbes, emphasizing the low concentration of viral nucleic acids and the challenges this poses for metagenomic sequencing. The selection of sampling locations, including indoor environments and airplanes, is discussed as critical for effective pathogen detection. Finally, the article contrasts air and wastewater sampling, concluding that while both methods should ideally be explored as layered defenses, air sampling does not collectively appear more promising than wastewater sampling.

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What is air sampling?

Air sampling is the process of collecting particulate matter from the air with a sampling device. In identifying biological threats, this method is particularly focused on the sampling of airborne microbes, or *bioaerosols*. The collection and concentration of these bioaerosols enable the analysis of the microbial composition of the sample and its surrounding environment. Techniques such as Polymerase Chain Reaction (PCR) and metagenomic sequencing, similar to those used in wastewater biosurveillance studies, are applied for this purpose. Therefore, air sampling acts as a method of environmental surveillance, targeting materials released by humans into their environment, rather than directly sampling individuals.

Air sampling is particularly promising due to its ability to detect respiratory pathogens, which predominantly spread through the air. This is a key advantage as airborne pathogens are often considered the most likely culprits for future pandemics. Additionally, air samples tend to be less complex compared to other mediums, such as wastewater, and may contain a higher prevalence of human pathogens in relation to other microorganisms. Wastewater, on the other hand, is highly complex and teeming with a vast array of non-pathogenic organisms. These characteristics of air samples potentially make the detection of threats more tractable than in wastewater samples.

In the subsequent sections of this report, we will examine various air sampling methodologies and explore their potential as an effective tool for the early detection of biothreats. Additionally, we will provide a high-level comparison between air sampling and wastewater (WW) sampling, specifically focusing on metagenomic sequencing (MGS)-based pathogen detection.

How are air samples collected?

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To gather pathogens and other particulate matter from the air, a collection mechanism, alongside a method for directing air across or through this mechanism, is required[1]. Systems for moving air, such as fans or vacuums, are generally straightforward and seldom present significant challenges in effective air sampling. However, the successful capture of particulate matter poses a more complex challenge. This involves balancing several competing performance factors that need to be optimized together, as detailed in Table 1. An ideal collection mechanism for MGS-based surveillance would offer high flow rates, efficient capture and recovery, minimal destruction of nucleic acids (NA) in the sample, and be cost-effective. In practice, however, compromises among these aspects are often necessary[2].

Collection mechanisms can be divided into five major categories (1–3). See also Figure 1 and Appendix 1.

- **Filtration:** This method employs various physical mechanisms to capture particles of different sizes. The complexity of these mechanisms is considerable and is discussed in greater detail in this report (see page 5).
- **Impaction:** These methods gather particles that possess sufficient inertia to deviate from an airstream following a sudden directional change.
- **Impingement:** This technique involves inertial separation into a liquid medium. While liquid mediums generally facilitate easier sample recovery, they can evaporate rapidly under high flow rates.
- **Electrostatic Precipitation:** This approach charges incoming particles, which are then attracted or repelled towards a collection medium through electrostatic forces.
- **Condensation:** This method utilizes temperature variations to create water droplets around particles, making them heavy enough to fall into a collection medium.

Table 1. Key performance considerations for air sampling devices.

Parameter	Description
Flow rate	How quickly air can be moved through the device (L/min). Higher flow rates allow faster collection of material and larger/more frequent samples.
Collection efficiency	The fraction of particles passing through the sampler that is collected. Typically rated for particular size ranges, e.g., a MERV 13 filter is at least 85% efficient at capturing particles between 1 μm to 3 μm in diameter.
Recovery efficiency	The fraction of collected NA that can be recovered from the collection mechanism.
Destructiveness (viability)	The degree to which the air sampler desiccates or otherwise kills microbes such that they can no longer be cultured. Critical for culture-based surveillance, less important for sequencing-based surveillance.

[1] Passive air sampling is a branch of air sampling that depends on natural settling of particles. Methods for passive sampling include surface dust collection (e.g., vacuuming), settle plates, and adhesive tape.

[2] For example, with filter-based sampling methods, a trade-off exists between higher flow rates and increased destructiveness. The rapid air flow induces mechanical stress, leading to the destruction of microbes and damage to nucleic acids. Furthermore, while filter-based approaches can attain high capture efficiency for the small particle sizes typical of viral aerosols, this efficiency imposes restrictions on the flow rate. Similar compromises are observed across other categories of samplers, as detailed in Appendix 1.

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Destructiveness (fragmentation)	The degree to which an air sampler damages microbial NA through fragmentation or degradation, resulting in the loss of NA in the sample or shorter fragment lengths for sequencing.
Cost	Up-front and per-sample costs of buying and using the device.
Practicality	Size of sampler, ease of deployment, noise level, etc.

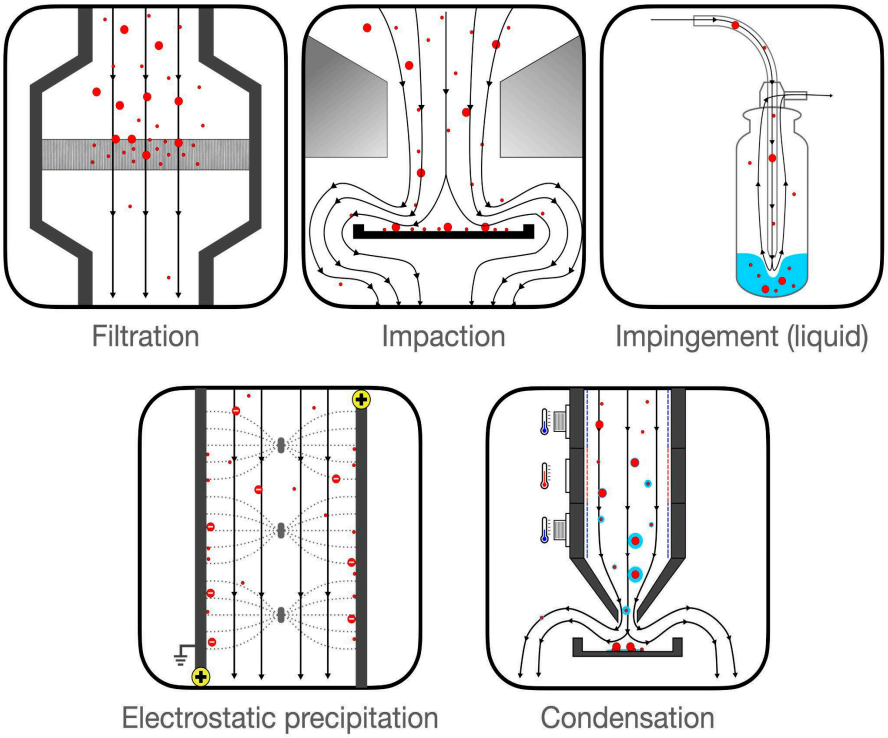

Figure 1. Collection mechanisms employed by different types of air samplers.

Certain air sampling devices incorporate multiple collection mechanisms[3]. The collection mechanism significantly impacts various key performance metrics, as outlined in Table 1, though there is also significant variation within each category.

What is in the air?

The air contains particulate matter from both biological and non-biological sources. Major biological contributors are humans, animals, plants, soil, and mold. Airborne microbes from humans and animals often originate from skin or are expelled as microbe-laden respiratory aerosols and droplets. The size of these particles varies: virus-containing particles (VCPs) are typically 0.02-0.30 μm in diameter, bacteria-containing particles (BCPs) range from 0.5-5 μm, and fungi-containing particles (FCPs) span 1-100 μm in diameter (4).

Air is generally a sparse medium for these particles. Typical indoor concentrations of VCPs and BCPs[4] are about 100 to 1000 particles per liter of air (4), while the count of individual pathogens, as measured by quantitative PCR (qPCR), often falls between 0.1 and 100 copies per liter, as shown in

[3] For example, an electrostatic precipitator might deposit particles into a liquid medium, or a condensation sampler might have an initial filter to remove larger particles.

[4] Microbes were counted using the SYBR Gold fluorescence dye to stain nucleic acid. Fluorescent particles between 0.02 and 0.50 μm were counted as VCPs and those between 0.50 and 5.00 μm as BCPs.

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Appendix 2. For context, in January 2023, Biobot reported SARS-CoV-2 levels in Boston's wastewater at approximately 1 million copies per liter, with peaks reaching around 5 million copies per liter during the Omicron surge.

Metagenomic sequencing analysis is a valuable tool for identifying the composition of airborne microbes in specific environments. Generally, the taxonomic complexity in air samples is relatively low, with most studies identifying fewer than 1000 unique taxa. In the nine metagenomic studies included in this review, bacteria and human-associated sequences were often the most abundant taxa. It was also common to find a significant proportion (over 50%) of reads or contigs without matches to any known sequences. Viruses were typically found in low relative abundances, usually under 1%, but in some cases, as high as 3.2% (5–10). Within the viral component, bacteriophages were usually the most prevalent, often accounting for over 40% of the viral reads, while human pathogens comprised a smaller percentage. Despite their lower abundance, metagenomic and qPCR studies confirm that human pathogens are detectable in air samples. Identified pathogens in air samples include SARS-CoV-2 and other common coronaviruses (11–21), influenza viruses (21–35), norovirus (36–38), various bacterial pathogens (39–42) including Tuberculosis, and even HIV (6).

What are promising locations for air sampling?

The choice of sampling location is a critical factor in the successful detection of emerging human pathogens through air sampling. Key considerations include the physical dimensions of the sampling area, the extent of external air movement, and environmental conditions such as temperature and humidity, which affect the viability, movement, and agglomeration of microbes in the air (refer to Figure 2). Additionally, the location influences the size and demographic composition of the human population contributing to the microbial content of the space, significantly impacting infection rates and shedding levels.

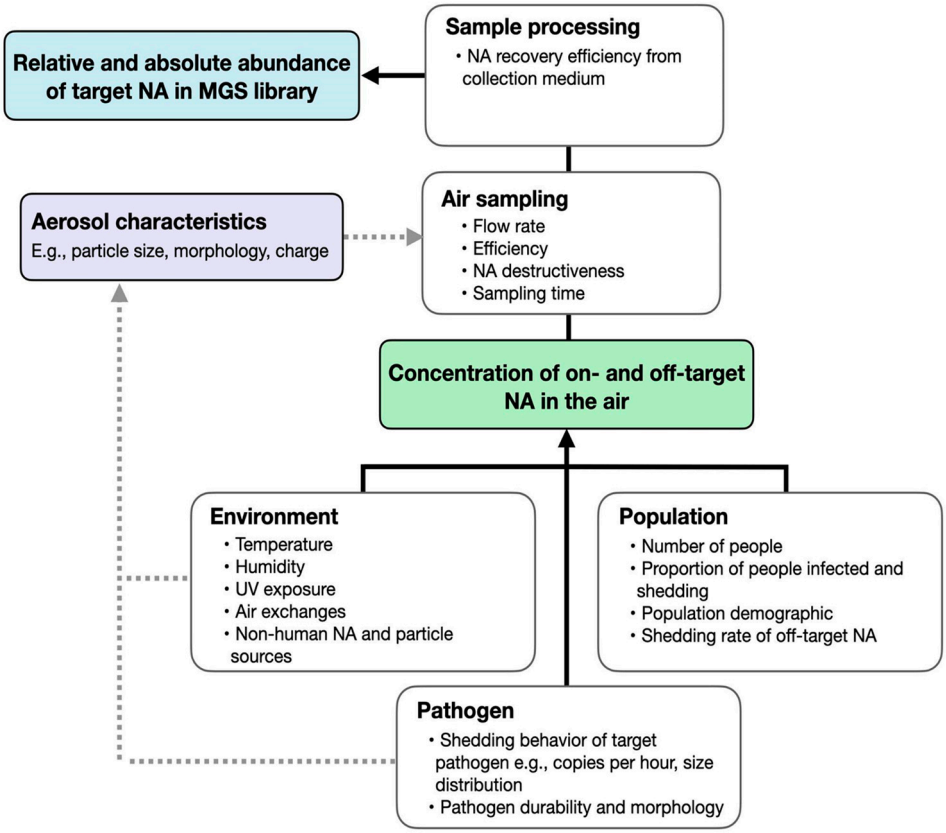

Figure 2. Factors that determine the relative abundance of a target pathogen. Environmental and population parameters are largely determined by choice of location.

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Given these factors, certain locations are more conducive to effective pathogen-agnostic biosurveillance. Indoor and in-duct environments, for instance, are generally more promising than outdoor settings due to more stable environmental conditions and higher concentrations of human pathogens (see Appendices 3 and 4). High-traffic public buildings, including hospitals, airports, and public transit stations, are particularly promising due to their large human traffic, favorable sampling demographics, and straightforward sample collection procedures.

Sampling air from individual airplanes also presents a highly promising approach. Airplane cabins, with their high density of people in confined spaces, centralized HVAC systems (Figure 3), and frequent air exchanges (13-15 changes per hour, as noted in reference 43), provide an optimal environment for sampling. Despite HEPA filters in these cabins being changed infrequently (e.g., every 18 months, according to reference 44), their accessibility and the feasibility of more frequent replacement make them valuable for collection. The combination of passenger density, a unique target population (international travelers), high air exchange rates, and the high capture efficiency of HEPA filters (over 99.97% efficiency per filter) creates ideal conditions for collecting air samples with high nucleic acid content.

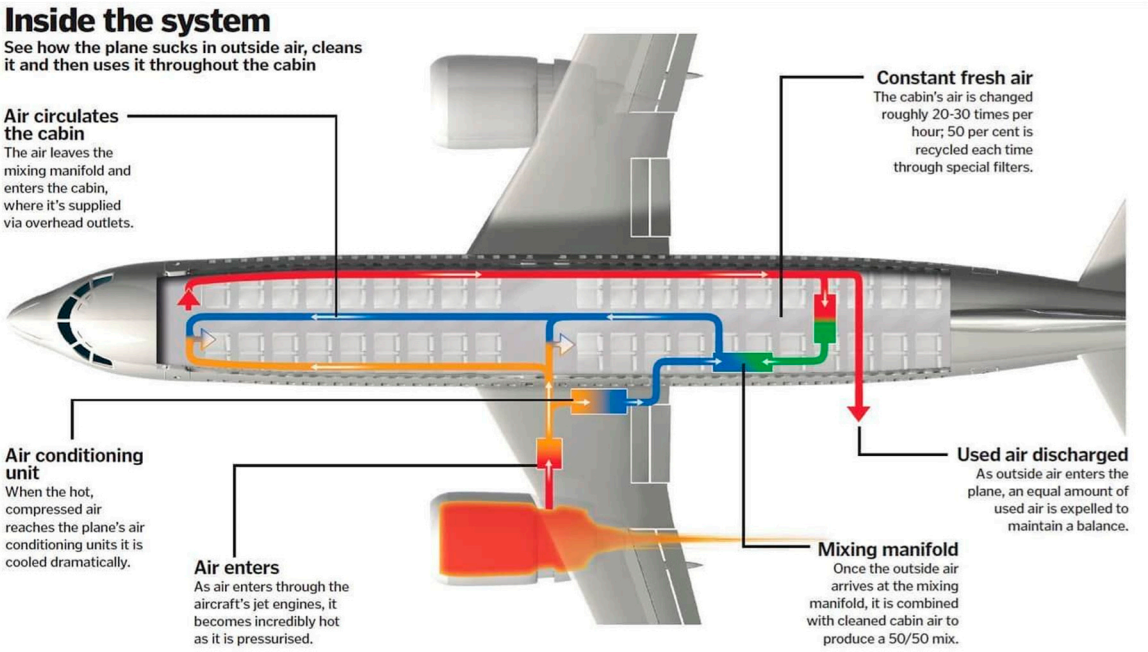

Figure 3. HVAC system aboard an airplane (source).

Conclusion: challenges and opportunities

Air sampling, as a method of environmental surveillance, offers a relatively straightfoward approach to obtaining composite samples from numerous individuals while preserving anonymity. When compared to wastewater sampling, air sampling may be more effective in detecting airborne pathogens. This is based on logical deduction (a pathogen that spreads through airborne transmission should be present in the air, hence collectible) and is supported by several studies that have successfully detected respiratory pathogens in air samples.

However, air sampling confronts significant challenges. The most prominent is the inherent sparsity of air as a medium; obtaining sufficient airborne nucleic acid (NA) for high-quality metagenomic sequencing is a formidable task[5]. This challenge is further compounded by the low concentration of viruses in the air microbiome, as existing data indicates that viral NA constitutes less than 1% of the total airborne NA, with human viruses comprising an even smaller proportion.

[5] A simple BOTEC suggests that eight hours of air sampling in an indoor environment with typical bacteria and virus concentrations at 200 L/min would yield about 300 ng of NA (Appendix 5).

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Sampling bioaerosols, especially viral bioaerosols, is complex and often involves balancing factors such as air collection rate, NA degradation, efficiency at capturing small virus-sized particles, and practical implementation issues.

Table 2 presents a comparison of air sampling to wastewater sampling. Although air sampling has its unique benefits, our analysis does not indicate that it surpasses wastewater sampling for the early detection of unknown outbreaks. While both approaches would be implemented in a layered approach, our current assessment suggests that if limited to one sampling method, wastewater sampling should be prioritized over air sampling. Nonetheless, a more detailed comparison that includes a reanalysis of metagenomic data is warranted.

Table 2. Comparison of air and wastewater sampling across several key axis.

Axes	Air sampling	Wastewater sampling
Sample collection	Technically challenging with inherent tradeoffs among desirable sampler qualities. Slow to collect NA.	Trivial at wastewater treatment plants (WWTP), though sampling at triturators or individual buildings may require custom pipe modifications. Small samples can provide large amounts of NA.
Sample complexity	Relatively low number of unique taxa (typically <1000) in indoor air, and low (<1%) abundance of viral NA from human hosts.	Uncertain, but seems to have more unique taxa and possibly a lower abundance of viral NA from human hosts.
Pathogen coverage	Well suited to detect airborne pathogens which are an important part of the threat space.	Well suited to detect fecal-oral pathogens and also many respiratory pathogens. Probably less robust across respiratory pathogens.
Catchment area	Effective surveillance of human pathogens is limited to building-level catchment areas.	Sampling from WWTPs provides city-level pathogen surveillance.
Composite traveler samples	Can monitor individual airplanes or airport terminals.	Can collect individual or aggregate airplane samples, as well as airport terminal waste.

Appendix 1: Air sampling methods

Filtration and filter-based samplers

Filter-based bioaerosol collection is a prevalent method in the field due to its simplicity, sensitivity to small particle sizes, and capacity to handle large air volumes. This technique involves using an air mover to propel air through a filter that captures particulate matter. The capturing process relies on various physical mechanisms such as interception, electrostatic attraction, and diffusion, each of which is discussed in more detail on page 5 (45). Filter and air mover can be integrated into a single device or used separately, like placing an HVAC filter in front of a vent. This flexibility allows for the collection of large air volumes, depending on the air mover's throughput capacity.

Despite its simplicity, the choice of collection device in filter-based sampling varies widely, depending on the target particles (like smog, viruses, bacteria) and subsequent analytical methods. Factors such as filter material, thickness, and porosity significantly influence recovery efficiency for

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different particle sizes and shapes, NA fragmentation and microbe viability, and power usage of the sampler (30, 45–50). Various filter materials, including polycarbonate, mixed cellulose ester, polytetrafluoroethylene (PTFE), polyvinyl chloride (PVC), nylon, gelatin, and others, have been used in bioaerosol collection. While comprehensive guidance on the optimal filter type for viral bioaerosol collection is lacking, comparative studies do exist (11, 30, 42, 46, 51). Verreault 2008 suggested that 0.3-μm PTFE filters might be the most suitable for collecting virus-laden particles of 10 to 900 nm in diameter (3).

Pros

- Filter-based samplers excel in processing large air volumes, enhancing NA collection and allowing for greater sampling frequency.
- Verreault 2008 indicated that filters are particularly effective in physically recovering nanoscale-size particles, including many viral aerosols (3). Despite often having larger pore sizes, it's typically diffusion, a Brownian motion phenomenon, that enables the collection of nanoscale particles on filters (45).

Cons

- A major limitation of many filter-based methods is NA recovery. Simple elution techniques may yield low NA recovery, whereas more intensive methods like bead beating can cause excessive fragmentation of NA. However, with sufficient sample volume, these recovery challenges might be manageable. Innovations like dissolvable or washable filters (52), along with vortexing and ultrasonic agitation techniques, offer potential solutions.
- Most filter-based samplers, except those using gelatin filters, are not conducive to preserving microbe viability, limiting their suitability for culture-based analysis (1, 3). Filters may cause structural damage such as membrane degradation (53) and increase virus desiccation, leading to infectivity loss (3). Prolonged sampling time has also been shown to exacerbate microbe viability loss (54, 55). However, for metagenomic studies, desiccation and infectivity loss are less concerning as long as the viral genomes remain intact.

Impaction-based samplers

Another widely used method for collecting bioaerosols is impaction-based sampling, which utilizes the inertia of particles to separate them from air (1). In this technique, air is drawn into the sampler through narrow openings, directed toward a solid surface, and then abruptly changes direction. Particles with sufficient inertia deviate from the airflow and adhere to the solid collection medium[6]. Common mediums include filters, agar plates for direct culturing, and glass slides or other solid surfaces for microscopy.

Impaction-based samplers offer distinctive advantages in studying air microbiomes. For instance, linking multiple impaction-based samplers together forms a "multi-stage impactor." Each stage in this setup captures progressively smaller particles, enabling size-based separation of airborne particles. This arrangement facilitates enrichment of smaller, virus-like particles and allows for assessing microbial concentration and composition at various particle sizes (3, 48, 57). Another notable feature is the ability of these samplers to measure bioaerosol concentrations over time by capturing particles on a rotating medium, typically an agar plate (3).

While impaction-based samplers are beneficial for culture-based analysis, their distinction from filter-based samplers becomes less clear when used to collect bioaerosols for sequencing via a filter. Integrating filters for NA collection with inertia-based particle separation may enrich virus particle samples, but it can also increase the complexity of the device and impose limitations on air flow rates.

Pros

- Easy separation and determination of different particle sizes, potentially allowing for enrichment of virus-laden particles.

[6] In reality, "inertia" is too simple a concept to characterize the movement of particles along streamlines. A particle's *Stokes number* provides a more rigorous explanation of its behavior (56).

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- Easy to perform culture-based analysis; airborne particles impact agar plates which can be moved directly into an incubator. However, this is not a useful feature for collecting MGS samples.
- Can determine aerosol concentrations over time with a rotating collection medium, typically a rotating agar plate.

Cons

- The sudden deceleration upon impaction can damage microbes (53,55,58).
- When used to collect NA via filters, inertia-based separation may add complexity and curb air intake rates.

Liquid-based samplers

Liquid-based samplers, often referred to as liquid impingers, use an inertial mechanism similar to impaction-based samplers for bioaerosol collection. The key difference lies in the collection medium, which is liquid rather than solid. The primary advantage of liquid mediums is the facilitation of simpler sample processing techniques and more efficient recovery of collected microbes and nucleic acids (NA). Additionally, liquid mediums help prevent microbe desiccation, which is advantageous for downstream analyses that require maintaining the infectivity of recovered particles (3, 59).

Nonetheless, common liquid-based samplers have notable drawbacks. Without periodic refilling, liquid mediums are prone to evaporation, limiting the duration of collection or necessitating reduced air intake rates to mitigate rapid evaporation. Furthermore, the evaporation of the liquid medium and the high impaction velocity of airborne particles can lead to particle re-aerosolization or damage upon impact (60, 61).

To address concerns about microbe damage and re-aerosolization, wetted-wall cyclone samplers have been developed. These devices incorporate impingement with centrifugal motion. Here, collection nozzles are angled above the liquid, creating a vortex motion during sampling that coats the container walls with a thin liquid film. Particles impact tangentially with the swirling liquid, reducing impact stress (62). Cyclone samplers are capable of higher flow rates (e.g., 1250 L/min, as noted in reference 63) compared to other liquid-based samplers and can operate for several hours, with the potential for extended duration through built-in liquid level control (1).

Pros:

- Liquid mediums allow for easier sample preparation and enhanced NA recovery efficiency.
- Least destructive collection method, preventing desiccation and improving recovery of infectious viral particles (3, 59).
- Can be used in a multi-stage format to separate and identify particle sizes.

Cons:

- High impaction velocities and liquid evaporation may compromise microbe viability for infectivity assays and cause particle re-aerosolization (60, 61).
- Some critiques point to the typically low flow rates of liquid-based samplers, although this does not take into account the capabilities of cyclone-class collectors.

Electrostatic-based samplers

Electrostatic collectors, also known as Electrostatic Precipitators (ESPs), capture airborne particles by first drawing them into the device, then imparting an electrostatic charge, and finally depositing them onto a collection medium through electrostatic attraction or repulsion (1). ESPs are versatile, compatible with various deposition mediums such as agar, liquid, and solid surfaces, which allows for the customization of the collection medium based on the requirements of downstream analysis.

ESPs offer several beneficial properties for bioaerosol sampling. Firstly, they deposit particles at significantly lower velocities than inertia-based methods, reducing the likelihood of microbe damage (1, 53). Secondly, ESPs do not require a substantial pressure drop for particle collection, as they are

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"open channel" devices[7]. This results in lower power consumption while still enabling the collection of large volumes of air. An ESP developed by the U.S. Army in the 1960s, for instance, achieved an impressive flow rate of 10,000 liters per minute (57). Despite these advantages, ESPs have seen limited investigation and commercialization compared to solid and liquid-based impaction or filter-based methods. Mainelis (2019) suggests that this may be due to the rapid advancements in other bioaerosol collection techniques (1).

A primary concern with ESPs, aside from their limited exploration and commercial availability, is their tendency to generate ozone during particle charging. The extent of this issue is unclear, but it has been raised that the ozone produced might compromise microbe integrity and viability, and in certain environments, could even pose a risk to human health due to elevated ozone levels (1, 64).

Pros

- Gentle on collected samples compared to other methods.
- Compatible with a variety of collection mediums, allowing for customization.
- Requires less power for operation.
- Capable of achieving high flow rates.

Cons

- Relatively under-investigated, possibly due to advancements in alternative collection methods.
- Generation of ozone during particle charging, which may degrade microbes and pose health risks in poorly ventilated areas (64).
- Limited availability of ESPs on the commercial market.

Condensation samplers

Condensation samplers employ a phase change from gas to liquid to capture airborne particles (34, 65–71). Fundamentally, condensation sampling involves moving air through a growth tube with varied temperature zones. This process leads to the formation of water droplets around particulate matter, which then fall onto a liquid or solid collection medium.

A notable example of condensation-based sampling is the Spot Sampler by Aerosol Devices. This device uses a three-stage growth tube for particle capture, described as follows:

1. Conditioner Stage: Air first enters the conditioner, a cool (5°C) wet wall section of the growth tube, where incoming particles acquire a thin water film.
2. Initiator Stage: The air then progresses to the initiator, where additional water vapor is introduced. Here, higher temperatures (35°C) create a supersaturated environment, encouraging larger droplet formation around particles.
3. Moderator Stage: In the moderator at 12°C, excess water vapor is removed, permitting continued droplet growth.
4. Exit: Air then exits the growth tube, with droplets impacting onto a collection medium.

Pros

Condensation samplers are highly efficient at collecting small particles. One study reported over 90% efficiency in collecting 5 nm particles (72), while another indicated the potential to capture particles as small as 2 nm (66). This implies that condensation sampling could effectively collect the smallest airborne viral particles.

- Unlike traditional liquid- and filter-based methods, condensation sampling avoids high-velocity impacts, making it a non-destructive technique ideal for preserving microbe viability and genomic integrity.

Cons

- Condensation samplers typically have low flow rates. For instance, the Spot Sampler operates at only 1.5 L/min, posing a challenge in collecting sufficient NA samples in short timeframes.

[7] A large pressure drop like that caused by a filter with a small effective pore size requires more power to push air through the pressure interface.

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- The market and research base for condensation samplers seem relatively underdeveloped, as indicated by their limited discussion in two comprehensive review papers on bioaerosol sampling (1, 3).

Appendix 2: Concentrations

See also Zhai et al. 2018 for review.

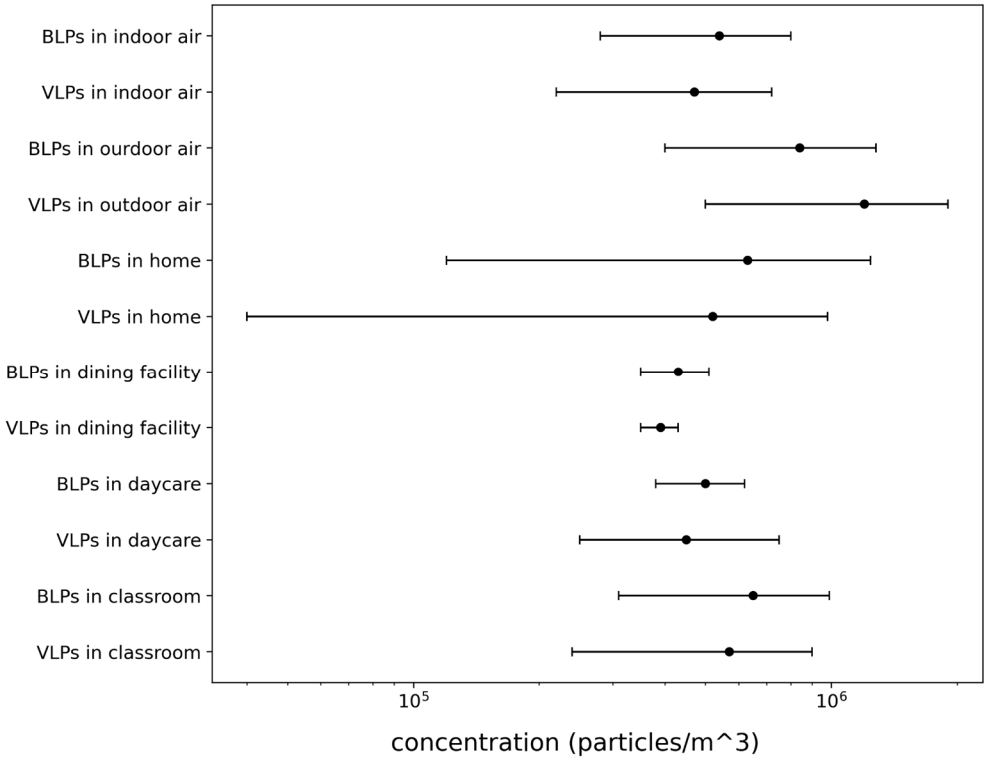

Figure A1-1. Concentration of airborne virus- and bacteria-like particles in different locations measured by Prussin et al. 2015. Microbes were counted using the SYBR Gold fluorescence dye to stain nucleic acid. Fluorescent particles between 0.02 and 0.50 μm were counted as VLPs and those between 0.50 and 5.00 μm as BLPs. Error bars represent the max-min concentrations measured in samples from the same location.

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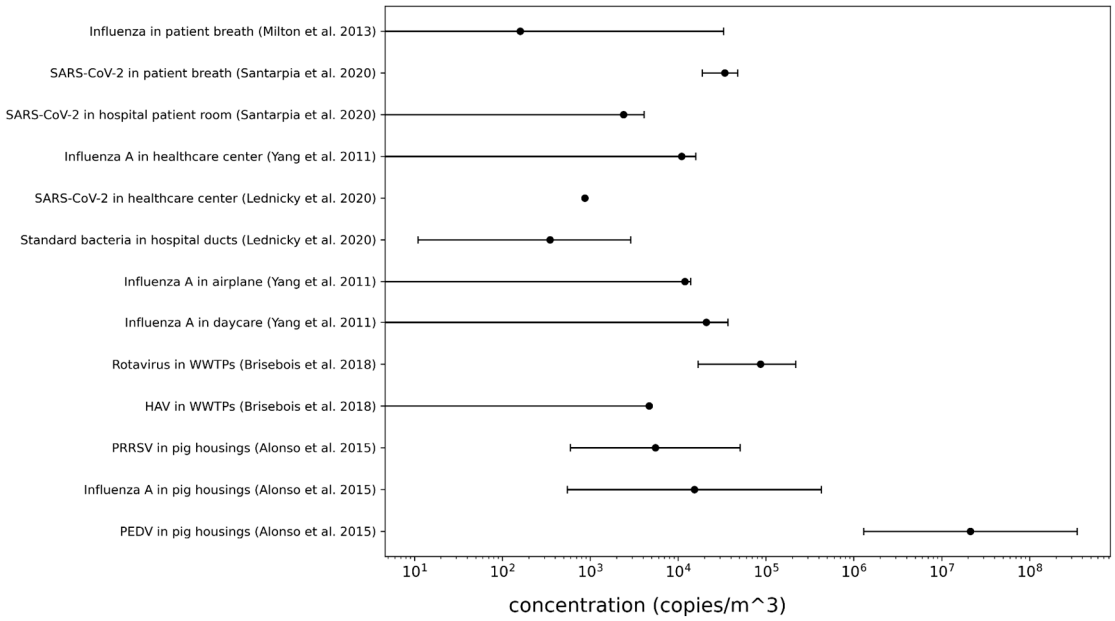

Figure A2-2. Concentration of airborne pathogens by qPCR. Error bars represent the max-min concentrations measured in samples from the same study. A concentration of 0 means that a "no detect" (nd) was also measured in this location.

Appendix 3: Outdoor sampling

Samples could be gathered outdoors in major cities and travel hubs (10, 76, 78–87). Sampling in these urban areas offers a wide geographic reach and a large population catchment. There is a possibility that if a substantial number of people in a city were infected and shedding pathogens, strategically placed high-volume air samplers might detect bioaerosols in the outdoor environment. However, the likelihood of encountering human-relevant pathogens in outdoor air is generally lower, leading to my skepticism about the effectiveness of this approach.

Moreover, outdoor sampling is particularly susceptible to environmental variables, more so than other locations. When conducting time-series detection approaches with metagenomic air sampling, it's crucial to account for environmental factors that could skew the abundance of the target pathogen. Outdoors, the presence of numerous potential confounding factors complicates this process.

Despite these challenges, various government-funded biosurveillance initiatives are focused on outdoor air sampling. It remains unclear whether these programs are specifically aimed at detecting aerial bioweapon attacks, such as anthrax-laden warheads, or if there is an unrecognized potential in outdoor air sampling that surpasses current evidence.

Notable programs:

- The SIGMA+ program from DARPA employs outdoor air collection through emergency vehicles equipped with custom air samplers and portable samplers like the pBDi.
- The U.S. government's BioWatch program initially utilized existing EPA air sampling sites and collectors, predominantly outdoor. Many of these were located near airports and other urban centers (6, 88, 89). The current sampling locations of BioWatch are not publicly available.
- The Kromek Biosequencer is an innovative system, attaching an air sampler to a vehicle, integrating automated sample preparation and long-read sequencing capabilities.

Appendix 4: In-duct sampling

Heating, ventilation, and air conditioning (HVAC) systems present a viable option for filter-based bioaerosol collection due to several factors: 1) the prevalence of existing HVAC systems with filters in many buildings, allowing for non-invasive sampling; 2) their capacity to handle large air volumes; and 3) their ability to collect particles from extensive spatial areas within buildings. Indeed,

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numerous studies have investigated microbial communities in HVAC systems, either by attaching external air sampling devices to these systems (16, 90) or by sampling directly from HVAC filters (5, 77, 91–96). Some of these studies, exploring viral diversity, have successfully identified various human pathogens, including influenza, SARS-CoV-2, retroviruses, adenovirus, and RSV, in HVAC systems (5, 16, 77, 96).

However, there are uncertainties regarding the practicality of using HVAC filters for bioaerosol sampling. Firstly, viral concentrations in HVAC filters are generally low, a characteristic common to many air samples. Additionally, there is some indication, albeit not strong, that nucleic acid (NA) concentrations within ducts may be lower than those found in-room (74, 93). Secondly, most studies on HVAC filters span several months, aligning with the typical frequency of filter replacement in these systems (every three months). This raises questions about the ability of HVAC filters to collect adequate NA over shorter time frames. While using higher efficiency filters, like MERV 12 or above, might enhance virus collection, this often isn't cost-effective in larger buildings due to increased power requirements (4).

In conclusion, I anticipate that in-room sampling methods will prove more promising than HVAC sampling. This is because in-room methods offer greater flexibility in adjusting sampling device properties, such as flow rate, and allow for more strategic selection of sampling locations.

Appendix 5: NA recovery BOTEC

Concentrations

- BLPs in indoor environment = 540 BLP/L (Prussin et al. 2015)
- VLPs in indoor environment = 470 VLP/L (Prussin et al. 2015)

Genome

- Typical bacterial genome = 5E6 bp/BLP
- Typical viral genome = 4E4 bp/VLP
- Mass of single bp = 1.08E-12 ng/bp

Air sampling

- Flow rate of air sampler = 200 L/min = 3.33 L/s (ACD 200 Bobcat air sampler)
- Sampling time = 8 hr = 28800 s

NA recovery

NA recovery rate = Flow rate × Microbe concentration × Typical genome mass

NA recovery rate = (3.33 L/s)×[(5E6 bp/BLP)×(540 BLP/L) + (4E4 bp/VLP)×(470 VLP/L)]×(1.08E-12 ng/bp)

NA recovery rate = 0.0098 ng/s

NA recovered over 8 hours = 280 ng

Assumptions

- Each VLP and BLP contains only one standard viral or bacterial genome. In reality, airborne particles often contain multiple microbes. This assumption pushes the results in the "more NA" direction.
- Microbe concentrations remain unchanged as a result of air sampling, which is probably a valid assumption in large indoor spaces, but doesn't hold in more confined environments. This assumption pushes the results in the "less NA" direction.

Relative abundance of viral NA

Relative abundance = Viral NA concentration / Total NA concentration

Relative abundance = [(4E4 bp/VLP)×(470 VLP/L)] / [(5E6 bp/BLP)×(540 BLP/L) + (4E4 bp/VLP)×(470 VLP/L)]

Relative abundance = 0.007 = 0.7%

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