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Passive Bioaerosol Samplers: A Complementary Tool for Bioaerosol Research. A Review

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Abstract

Bioaerosols consist of airborne particles of biological origin. They play an important role in our environment and may cause negative health effects. The presence of biological aerosol is typically determined using active samplers. While passive bioaerosol samplers are used much less frequently in bioaerosol investigations, they offer certain advantages, such as simple design, low cost, and long sampling duration. This review discusses different types of passive bioaerosol samplers, including their collection mechanisms, advantages and disadvantages, applicability in different sampling environments, and available sample elution and analysis methods. Most passive samplers are based on gravitational settling and electrostatic capture mechanism or their combination. We discuss the agar settle plate, dustfall collector, Personal Aeroallergen Sampler (PAAS), and settling filters among the gravity-based samplers. The described electrostatics-based samplers include electrostatic dust cloths (EDC) and Rutgers Electrostatic Passive Sampler (REPS). In addition, the review also discusses passive opportunity samplers using preexisting airflow, such as filters in HVAC systems. Overall, passive bioaerosol sampling technologies are inexpensive, easy to operate, and can continuously sample for days and even weeks which is not easily accomplished by active sampling devices. Although passive sampling devices are usually treated as qualitative tools, they still provide information about bioaerosol presence and diversity, especially over longer time scales. Overall, this review suggests that the use of passive bioaerosol samplers alongside active collection devices can aid researchers in developing a more comprehensive understanding of biological presence and dynamics, especially over extended time scales and multiple locations.

Keywords

Bioaerosol sampler; passive sampling; gravitational settling; electrostatic forces; opportunity sampling

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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1. Introduction

Bioaerosols, or biological aerosols, consist of airborne particulate matter of biological origin and include bacteria, fungi, archaea, viruses, pollen, and their fragments and byproducts such as endotoxins, mycotoxins, DNA, and others (Baron et al., 2011; Cox & Wathes, 1995; Ghosh et al., 2015; Lindsley et al., 2017). There is an increasing awareness of the importance of airborne microbiota, including its role in causing negative health effects, importance in cloud formation and atmospheric processes, and its existence and function in the airborne environment as an ecological niche (Smets et al., 2016). Furthermore, human exposure to bioaerosols is of concern because of their ability to cause or exacerbate asthma, allergies, toxicity, cardiovascular problems, and other negative health effects (Cox & Wathes, 1995). The SARS-CoV-2 virus is arguably the best-known current example of an infectious bioaerosol due to the transmission of COVID-19 via airborne exposure route (Baboli et al., 2021; Delikhoon et al., 2021; Liu et al., 2020; Morawska & Cao, 2020; Passos et al., 2021; Samet et al., 2021). Therefore, there is a need to sample and analyze bioaerosols in order to understand their presence in various environments and their impact on human health.

There are numerous methods and devices to sample bioaerosols, and they can be classified into two primary categories: passive and active methods and samplers (Ghosh et al., 2015; Hinds, 1999; Mainelis, 2019; Wight, 1994). Active samplers utilize a pump or other air mover to draw air at a certain flow rate and deposit particles onto the chosen collection medium. In addition, pre-conditioning steps in the air streams of active samplers can also be utilized to select particles of a certain size or grow particles hygroscopically (Hinds, 1999; Wight, 1994). Since the collected air volume is known, active samplers allow quantitative bioaerosol determination (Hinds, 1999; Yates et al., 2016). On the other hand, passive samplers do not utilize pumps or air movers to collect samples but rely on gravity, electrostatic forces, their combination, or other natural phenomena to deposit particles on a collection medium. Some commonly referenced active bioaerosol samplers include the Andersen Cascade Impactor (Thermo Fisher Scientific, Franklin, MA), the National Institute of Occupational Safety and Health (NIOSH) cyclone sampler (Tisch Environmental, Cleves, OH), the SKC Biosampler (SKC, Inc., Eighty Four, PA), the Coriolis μ air sampler (Bertin Technologies, Montigny-le-Bretonneux, France), and the Institute of Medicine (IOM) sampler (SKC, Inc.). A brief comparison of general differences between passive and active bioaerosol samplers is provided in Table 1.

Passive sampling has long been a staple in gas sampling, including for personal and environmental monitoring (Namie nik et al., 2005). Since gasses have known diffusion rates, passive gas samplers yield quantitative data. On the other hand, passive airborne particle samplers, including passive bioaerosol samplers, are typically considered qualitative measurement tools because the collected air volume is unknown, and particle flux by diffusion is relatively weak and varies depending on particles size. That prevents determining the bioaerosol concentration as only the captured bioaerosol quantity is determined but not air volume. At the same time, since bioaerosol behavior and transport are affected by electrostatic forces, thermal gradients, and turbulent dispersion (Baron et al.,

2011; Cox & Wathes, 1995), these phenomena can be utilized as opportunities to enhance particle capture. Turbulent dispersion is the random motion of unstable or turbulent air flow, which may impact particle behavior and is a major factor in dry deposition (Cox & Wathes, 1995; Farmer et al., 2021). Despite the mentioned differences, both active and passive devices can be used in bioaerosol projects and investigations, either separately or together, depending on a project's specific needs.

Long-term sampling using active devices can be more expensive and labor-intensive than passive sampling (Therkorn, Thomas, Scheinbeim, et al., 2017). Active samplers require attention by trained operators, e.g., to measure the sampling flow rate before and after sampling or to ensure adherence to the set sampling flow rate. Also, compared with passive devices, active samplers generally require a more frequent replacement of collection media to prevent losses through evaporation and desiccation of media, reduction in sample viability, and sampler overloading (Haig et al., 2016; Hinds, 1999). Active samplers also require access to a power source such as an electrical outlet or battery, creating constraints for long-term sampling activities, especially in remote areas. Active samplers can also be noisy and heavy due to the operation of the air mover. Furthermore, the high impaction and impingement velocities in many active samplers can cause stress to sampled microorganisms, which could reduce their viability and culturability, especially for sensitive species (Dungan, 2010; Haig et al., 2016). This can produce a sample bias favoring hardier microorganisms and under detection of sensitive species when analyzing samples for viability and culturability.

By contrast, passive samplers provide a less stressful means of particle capture because bioaerosols are collected by natural phenomena, such as gravity, naturally-present electrostatic forces, or turbulent dispersion. The electrostatic collection mechanism in passive samplers does not involve particle charging via corona or similar means, thereby avoiding the potentially negative effects of ozone production. Since there are minimal power and personnel requirement, passive bioaerosol samplers can be used for long-term sampling and help elucidate temporal bioaerosol community trends, which may not be as easily described by short-term active sampling procedures. Passive bioaerosol samplers can also provide an affordable way to achieve massively distributed simultaneous sampling to identify spatial variability within an environment. When applied alongside active samplers, passive bioaerosol tools can complement and provide additional data that would not be feasible by active means alone.

Given the increased interest in bioaerosol sampling and the potential of passive samplers as a complementary tool in bioaerosol investigations, this paper aims to provide an overview of the available passive bioaerosol sampling techniques, including their advantages, limitations, and methods of sample analysis. A description of the methodology of this review is included in the Supplemental Material.

2. Passive Bioaerosol Sampling Methods

Passive sampler concepts described in the literature are listed in Table 2. Such samplers could be primarily categorized by their collection mechanism, namely gravitational settling,

electrostatic attraction, or their combination. Although dust accumulation is not a passive sampler in a strict sense, researchers have also used it as a quick and inexpensive surrogate for estimating previously suspended particles (Cox et al., 2017; Emerson et al., 2015; Würtz et al., 2005). In addition, we will discuss a few sampling methods that do not contain a power source or an air mover but instead utilize existing air streams to collect an airborne sample. As such, they could also be considered passive samplers.

2.1. Passive samplers based on gravitational settling

Gravitational settling is the most commonly used method to passively collect airborne particulate matter, including bioaerosols, onto a collection medium for analysis. Passive sampling tools that utilize gravitational settling, such as settle plates or filters, collect predominantly larger particles (>5 μ m) due to their high settling velocities (Cox & Wathes, 1995; Therkorn, Thomas, Calderón, et al., 2017; Yates et al., 2016). Such particles may include microorganisms attached to airborne particles or microorganism agglomerates (Lighthart, 1997; Mainelis, 2019; Stetzenbach et al., 2004). Therefore, the settling method might under-sample smaller particles (<5 μ m) that have lower settling velocities, such as free bacteria and viruses. However, Brownian motion is also strong for <100 nm biological particles (e.g., free viruses), which could contribute to the capture of nano-sized bioaerosols, such as free viruses or microorganism fragments and byproducts (Cox & Wathes, 1995; Verreault et al., 2008).

Perhaps the earliest report on the existence of bioaerosols (e.g., microorganisms in the air) and demonstration of passive bioaerosol sampling was Louis Pasteur's famous swan-neck flask experiments (Lee, 2011; Pasteur, 1862) even though it was not the original intent of the experiment. Dr. Pasteur intended to disprove spontaneous generation as the cause of putrefaction. He used curved or swan-necked flasks with heat-sterilized nutrient broth that acted as passive samplers to collect and detect airborne bacteria. Dr. Pasteur used two sets of flasks: one as designed, i.e., with the "swan-neck" intact and allowing the air, but not particles, to enter the flask; and the second flask, where the curved neck was removed, and airborne material was allowed to settle into the flask. Since the intact "swan-neck" flask did not allow airborne particles to settle onto the nutrient broth, the liquid was protected from the bacteria and their subsequent growth; on the other hand, bacterial growth was observed in the second group, where airborne particles were allowed to settle into the growth medium (Lee, 2011). With this experiment, Dr. Pasteur not only disproved spontaneous generation as the cause of gravitational settling to capture them.

Agar settle plates are commonly used to measure culturable bioaerosols in different environments (Haig et al., 2016; Mainelis, 2019; Rautemaa et al., 2006; Rocha-Melongo et al., 2019; Sandle, 2015). This method collects bioaerosols directly onto an agar medium via settling and, once the plates are incubated, enables the growth of culturable microorganisms directly on the collection medium. Direct sampling onto culture media eliminates the need for post-sampling processing. The plates are then incubated for several days to allow the formation of colonies (e.g., Colony Forming Units [CFU]) for detection and counting of captured microorganisms. However, only the cells that grow on the used medium and

specific incubation conditions will form colonies and be counted. Therefore, applying culture-only sampling techniques does not represent viable but not culturable (VNBC) or total bioaerosol particles in the sample (Lindsley et al., 2017). The needed incubation time and selective growth also apply to active sampling methods that use culture-based methods for sample analysis. Furthermore, the agar medium desiccates over time, limiting sampling time with agar settle plates to four hours or less (Sandle, 2015). This maximum time is recommended by both the United States Food and Drug Association and the European Union (Center for Drug Evaluation Research et al., 2004; European Union, 2008). Despite these limitations, the agar settle plates are often used in operating rooms, clean rooms, meat processing facilities, drug manufacturing, spacecraft assembly, and other sensitive environments, where there is a need for a simple marker of biological air contamination (Adams & Dancer, 2020; Asefa et al., 2009; Haig et al., 2016; Pasquarella et al., 2019; Pasquarella et al., 2012).

Filters also are a relatively simple and readily available medium for passive collection of bioaerosols (Haig et al., 2016; Näsman et al., 1999; Therkorn, Thomas, Scheinbeim, et al., 2017). Filters can be placed on a surface and exposed to open air in a selected environment. However, one limitation of filters is that their light weight can allow the filters to bow, thus reducing the collection surface. Therefore, the filters used for passive sampling should also be secured to prevent their disturbance or even loss due to air currents. The Einstein-Lioy Sampler allowing for passive collection of airborne particles onto up to four secured filters is one such design (Einstein et al., 2012). Although this sampler has not been reported to be used for bioaerosol sampling, the device has been demonstrated to work for other particulate matter, and it can be easily adapted for bioaerosol applications.

Filters used in passive sampling are of the same type and materials as used in active sampling, namely polytetrafluoroethylene (PTFE), mixed cellulose ester (MCE), or gelatin filters. The main difference is the collection mechanism: passive sampling does not use a pump to draw air through the filter medium. Instead, it relies on gravitational settling to capture biological and other particles onto the exposed filter surface. Samples from settle filters can be eluted or otherwise analyzed by similar methods as filters used in active sampling applications. Filters used in passive sampling usually collect less particle mass during sampling than filters in active sampling; however, biological particles captured on filters during active sampling are also exposed to stress from impaction and desiccation, which can reduce microorganism viability and culturability (Cox & Wathes, 1995).

The dustfall collector described by Wurtz and colleagues (2005) is a passive dust sampler utilizing an aluminum-lined box with a mesh cover. Adams and colleagues later modified this design and collected dust into an empty Petri dish (2015). Other studies have also used open Petri dish samplers for collecting bioaerosol such as pet allergen (Karlsson, Hedrén, et al., 2002; Karlsson, Renstrom, et al., 2002; Noss et al., 2010). Petri dish and dustfall collectors, similar to agar settle plates and filters, use existing supplies for convenient long-term sampling. After sampling, the accumulated dust is gathered by vacuum (Würtz et al., 2005) or swab (Adams, Tian, et al., 2015; Docherty et al., 2018; Emerson et al., 2015; Mhuireach et al., 2016; Sylvain et al., 2019) before elution into a liquid for analysis. A

recent study incorporated 5 mL of liquid in the open Petri dish so that the bioaerosol settles directly into the liquid media for convenient sample analysis (Baboli et al., 2021).

The Durham Spore Trap is another gravity-based method used to collect bioaerosols outdoors, including pollen and spores [50] and bacteria (Serrano-Silva & Calderón-Ezquerro, 2018). The Durham Spore Trap collects bioaerosol samples onto adhesive or oil-coated slides suspended on support columns between two round stainless-steel plates.

The lightweight Remote Airborne Microbial Passive (RAMP) sampling system utilizes an air balloon to rise to target altitudes to sample bioaerosol for DNA sequencing (Spring et al., 2018). The sampler consists of 16 gel-coated square Petri dishes and covers housed in a sealed box that can be opened and closed remotely once the system has reached the desired sampling altitude. This sampling system has been demonstrated to sample at specific altitudes with proof of concept demonstration at 150 m (Spring et al., 2018). Here, samples were eluted from the Petri dishes using flocked swabs, and the DNA was extracted into a buffered solution for analysis by a bead-beating procedure. However, the sampler has not yet been compared to other sampler technologies.

The Personal Aeroallergen Sampler (PAAS) is a personal, passive sampler that was developed by Yamamoto and colleagues to measure individual exposures to airborne allergens, such as dust mite fecal pellets, pollen, and fungi that are part of particles >10 μ m in diameter (2006). The device works similarly to other gravity-based passive samplers by collecting samples primarily through settling. PAAS is designed to be worn around the neck with the sample substrate in a person's breathing zone (Yamamoto et al., 2012; Yamamoto et al., 2006; Yamamoto et al., 2011). However, the device reportedly works less effectively in areas of low particle concentration (<10³ particles/m³), especially when the particle aerodynamic diameter is less than 5 μ m (Yamamoto et al., 2011). This finding is similar to the common limitations when gravitational settling is used as a primary collection mechanism.

Each of the gravity-based passive samplers discussed above collects bioaerosol particles by allowing them to settle onto the collection substrate. However, this method usually requires long sampling times to accumulate enough biological material for analysis and preferentially samples larger particles.

2.2. Passive samplers based on electrostatic forces

Some passive bioaerosol samplers also utilize electrostatic attraction to collect electrostatically charged airborne particulate matter in addition to their capture by gravitational settling. This mechanism has been incorporated into passive samplers relatively recently. Because freshly aerosolized particles, including bioaerosols, carry an electrostatic charge (Cox & Wathes, 1995; Delort & Amato, 2017; Therkorn, Thomas, Calderón, et al., 2017; Yao & Mainelis, 2006), passive devices that utilize electrostatic collection in addition to gravitational settling may have an advantage over passive samplers that utilize gravitational settling only in terms of captured biological material amount. Examples of this approach are the Electrostatic Dustfall collector (EDC) (Adams, Tian, et al., 2015; Cozen et al., 2008; Madsen et al., 2019; Madsen et al., 2012; Noss et al., 2008; Viegas et al.,

2018) and the Rutgers Electrostatic Passive Sampler (REPS) (Therkorn, Thomas, Calderón, et al., 2017; Therkorn, Thomas, Scheinbeim, et al., 2017). These devices use charged or polarized materials to create electrostatic fields that attract bioaerosols carrying a static charge. They also collect particles by gravitational settling, just like traditional gravity-based passive samplers.

Electrostatic dust cloths used in EDCs are made from electret fibers (Brita Kilburg-Basnyat et al., 2016). These materials are commonly available as household products for brands, such as Swiffer®, Lysol®, and Pledge® (Adams, Tian, et al., 2015; Kristono et al., 2018). The EDC was first described by Noss et al. as a "low-cost electrostatic dustfall collector," which utilized four EDC cloth pieces mounted onto a polypropylene folder for indoor sampling (Brita Kilburg-Basnyat et al., 2016; 2008).

REPS uses a permanently polarized poly(vinylidene) fluoride (PVDF) film (Therkorn, Thomas, Calderón, et al., 2017) as its collection substrate. The PVDF material is uniaxially oriented during manufacturing, achieving permanent orientation of internal electrical dipoles. This orientation is maintained until the material's Curie temperature (167 °C) is exceeded (Therkorn, Thomas, Calderón, et al., 2017). (The Curie temperature is the temperature at which a material loses its ferroelectric properties and becomes paraelectric (Schneider & Kirschner, 2000).) REPS is constructed by winding the PVDF film into a film holder in a spiral configuration causing the film sides with opposite polarization to face each other, thus creating an electrostatic field between them. The overlapping PVDF film layers are positioned 2.25 mm apart, which was an optimal distance to capture particles by electrostatic attraction (Therkorn, Thomas, Calderón, et al., 2017). The sampler was demonstrated to have an equivalent sampling flow rate (defined below) of 2.6 L/min for culturable bacteria in an outdoor field study, which is similar to several active personal samplers (Therkorn, Thomas, Scheinbeim, et al., 2017). Since REPS is designed for typical indoor and outdoor sampling, with temperatures much lower than 167 °C, it will maintain its electrostatic collection mechanism. Even the hottest air temperature ever recorded, 58.0 °C in 1922 in El Azizia, Libya (Mildrexler et al., 2006), is substantially below the film's Curie temperature.

2.3. Dust: proxy measures of bioaerosol

The dust accumulated in indoor environments is often used as a convenient proxy to examine the bioaerosol presence in the past. For example, dust samples could be collected using surface wipes, swabs, vacuum sampling, and other means (Adams, Tian, et al., 2015; Ghosh et al., 2015; Noris et al., 2011; Würtz et al., 2005). These devices often utilize a template to sample a predetermined surface area.

Surface wipes or swabs and vacuum samples offer a convenient means of collecting household dust for analysis (Brooks et al., 2018; Cozen et al., 2008; Noss et al., 2008; Park et al., 2011). The use of dust samples enables rapid, one-time collection of samples. This can reduce the length and number of visits to collect enough material and space required to deploy active samplers. However, the efficiency of collecting particles onto wipes or swabs is operator-dependent because of the difference in applied pressure and wiping patterns. Thus, adherence to the sampling protocol and wiping the entire predetermined surface area

is also important. The use of vacuum cleaners offers the ability to collect dust samples not only from the hard surfaces but also from the carpeted areas. (Cozen et al., 2008; Noss et al., 2008).

These dust collection approaches are an indirect method of sampling bioaerosols indoors. They provide a much quicker means of collecting a sample than conventional passive techniques; however, the age of dust is generally unknown, and it can vary across samples (even within the same room) because dust accumulation depends on human and pet activities (including cleaning) as well as surface type and building or environmental conditions (Adams et al., 2013; Bi et al., 2018). In addition, surface samples may collect not only bioaerosols from the settled dust but also the microorganisms inhabiting the surface from which the sample was taken, i.e., microorganisms from a local biofilm, thus biasing bioaerosol analysis.

2.4. Diffusion-based nonpolar compound sampler

The Fresh Air Clip is a passive personal sampler originally designed to sample volatile organic compounds (VOCs) (Lin et al., 2020), but it was recently adapted for sampling the SARS-CoV-2 aerosols (Angel et al., 2022). The Fresh Air Clip uses a polydimethylsiloxane (PDMS) sorbent material to trap airborne nonpolar compounds, such as lipid enveloped viruses that pass through the collection substrate due to diffusion (Angel et al., 2022). This sampler's ability to capture lipid-enveloped viruses was demonstrated in a rotating drum chamber and a small field demonstration in different community settings (Angel et al., 2022). The community study detected SARS-CoV-2 RNA in a restaurant and homeless shelter but not in healthcare facilities or other community locations. However, given the recent adaptation of Fresh Air Clip technology, there is still limited knowledge about its applications or comparability to other bioaerosol samplers.

2.5. Special cases: passive opportunity samplers using existing air flows

We classified devices and approaches that utilize existing sources of air movement, such as ventilation systems, as passive opportunity samplers. Since no additional air mover is needed, such devices could be considered passive samplers. For example, filters inside Heating Ventilation and Air Conditioning (HVAC) systems have been used as bioaerosol samplers (Emerson et al., 2015; Forthomme et al., 2014; Noris et al., 2011). This method has an advantage because airflow and air volume are known or could be determined (Ackelsberg et al., 2011). In this aspect, this bioaerosol collection mechanism is closer to active bioaerosol sampling. Similarly, vehicle air cabin filters have been used for bioaerosol sampling to determine the regional presence of antibiotic resistance genes in bacteria (George et al., 2021). Furthermore, maintenance records of filters (when available) allow estimating the age of collected dust (Emerson et al., 2015). However, the HVAC ducts can also be a reservoir for microorganism growth, impacting sample results and accuracy (Hassan Al-Abdalall et al., 2020).

The Nasal Air Sampler (NAS) is a personal sampler that clips into the nasal passage and uses the nasal breathing airflow to capture particles onto an adhesive surface (Graham et al., 2000). The Nasal Air Sampler allows direct measurement of inhalation exposures but

could be biased if the user breathes through the mouth. Inhalation rates can be estimated using different pulmonary function tests, respiratory sensors, and references, such as the Environmental Protection Agency's (EPA) Exposure Factors Handbook (Fan et al., 2018; U.S. EPA, 2008, 2011). The sampler is able to capture particles of ~5 μ m in size and greater with 50% efficiency (Graham et al., 2000).

The NAS has been used in inhaled allergen and fungal exposure studies alongside active personal samplers or dust samples collected by floor vacuum (Gore et al., 2015; Gore et al., 2006; Graham et al., 2000; Mitakakis et al., 2000; Poulos et al., 2002). However, the intranasal design has shown limits to its potential applications. One study reported that some child subjects could not wear the NAS device (Mitakakis et al., 2000) due to difficulty breathing. The NAS was also limited to less than two hours of sampling times for adult subjects to minimize sampler overload and subject discomfort (Gore et al., 2006; Graham et al., 2000; Mitakakis et al., 2000). While this sampler uses the active air flow due to inhalation, the inhaled volume can only be estimated based on sample duration and a person's exertion level.

The Infectious Aerosol Capture Mask (IACM) was introduced to minimize the spread of infectious aerosol by hospitalized COVID-19 patients and applied to measure exhaled SARS-CoV-2 aerosols from infected persons (Santarpia et al., 2021). The IACM is a modified oxygen delivery mask with an attached filter cartridge that can be attached to a hospital vacuum outlet or other vacuum pump operated at 1 SCFM (standard cubic feet per minute, or 28.3 L/min). The mask has been demonstrated to minimize the spread of infectious bioaerosol and used to measure aerosols generated by infected individuals (Santarpia et al., 2021).

Another unique passive sampler was used by the National Aeronautics and Space Administration (NASA) for sampling particulate matter present on the International Space Station (ISS). Here, this passive aerosol sampler was placed over the air intake of the ventilation system (Haines et al., 2019; Meyer, 2017, 2018). The sampler consisted of five drawers, each containing an individual aluminum block with double-sided carbon tape as the collection medium. Each drawer could be independently opened or closed (Haines et al., 2019). This sampler was designed to be compact and allow secure transport to and from the ISS (Meyer, 2017). This device utilized the forced air movement through the air intake of the ventilation system to capture particles by impaction onto the collection substrate. From the ISS air, this device was able to collect particulate matter, including bacterial and fungal particles, that were later analyzed by qPCR (Haines et al., 2019)

3. Discussion

3.1. Metrics used to describe passive sampling results

The metrics used to quantify airborne bioaerosol concentrations when using active sampling methods, such as $\#/m^3$ or CFU/m³, are not directly applicable for passive samples. One alternative for passive samplers is to quantify the amount of bioaerosol that has settled per surface area per time, such as $\#/cm^2/min$ (Rocha-Melogno et al., 2020) or, if using settle plates, then CFU/plate/h or CFU/m²/h could be used.

Given the numerous ways that passive settle plates are used, the Index of Microbial Air contamination (IMA) was suggested to standardize settle plate protocols. The Index recommends a common standard to measure bioaerosol presence using Petri dishes in a 1/1/1 scheme (Pasquarella et al., 2000). This scheme recommends that open Petri dishes be placed 1 meter off the ground, 1 meter away from walls or other large obstructions, and samples collected for 1 hour (Montagna et al., 2019; Napoli et al., 2012; Pasquarella et al., 2000; Pasquarella et al., 2012; Scaltriti et al., 2007; Setlhare et al., 2014). Sample concentrations are reported as CFU/plate or CFU/ $(m^2 \text{ or } dm^2 \text{ or } cm^2)/h$. The IMA has been used to determine bioaerosol presence in operating rooms or other hospital environments, food processing, industrial plants, libraries, museums, and residences (Pasquarella et al., 2000; Pasquarella et al., 2012). Pasquarella and colleagues describe the IMA recommendations for different risk environments based on settle plates using the 1/1/1scheme (Pasquarella et al., 2000). These recommendations included five class and index ranges, including "0-5 very good, 6-25 good, 26-50 fair, 51-75 poor, and 76 very poor (Pasquarella et al., 2000)." For example, the very good category (0-5) ranged from 0-9 CFU/dm²/h and was suggested for applications with very high risk, such as ultra-clean rooms or operating rooms. At the other extreme of the index, concentrations of 125 CFU/dm²/h were labeled very poor (IMA 76) and unacceptable for any areas requiring infection control or contamination.

Some researchers have used Omelianskii's formula to estimate airborne culturable bioaerosol concentration based on observed CFU in settle plates (Awad & Mawla, 2012; Bogomolova & Kirtsideli, 2009; Borrego et al., 2010; Cernei et al., 2013; Hayleeyesus et al., 2015; Ilies et al., 2018; Li et al., 2020; Viani et al., 2020):

$$N = 5a \cdot 10^4 (bt)^{-1} \tag{1}$$

where N is culturable bioaerosol concentration (CFU/ m^3), a is number of colonies per Petri dish, b is dish area (cm²), and t is collection time (min).

The Omelianskii formula assumes that a 100 cm² Petri dish exposed to air for 5 minutes will capture microorganisms present in 10 m³ of air via settling onto the plate's surface (Omelianskiĭ, 1922, 1940). The described process is highly unlikely as it would require particles present in a 1 km tall column to settle onto the 100 cm² Petri dish in just 5 minutes; or, in other terms, particles from 10 m^3 of air must settle onto the dish in 5 minutes (2000) L /min). Even a pollen particle of 30 μ m would need ~10 hours in calm conditions to settle 1 km distance. Other research has also acknowledged that the Omelianskii formula will tend to overestimate bioaerosol concentrations (Awad & Mawla, 2012; Viani et al., 2020). While the origin of this formula is uncertain, Omelianskii himself also acknowledged that active air sampling was needed for a more accurate determination of microorganisms in the air (Omelianskii, 1922, 1940). This formula provides an interesting historical context into the timeline of bioaerosol research; however, it is obvious that it does not accurately convert the bioaerosol amount captured by a settling-based passive sampler into a corresponding airborne concentration. Therefore, the amount of bioaerosol captured by a settling-based passive sampler should be converted to airborne microorganism concentration using only the Omelianskii formula as it leads to a major overestimate of airborne concentration.

On the other hand, when an active sample is collocated with a passive sampling device, an equivalent sampling flow rate (Q_{eq} , L/min) of the passive device could be estimated (Manibusan & Mainelis, 2021; Therkorn, Thomas, Scheinbeim, et al., 2017; Yamamoto et al., 2011):

$$Q_{eq} = \frac{N_p}{C_a \cdot t_p} \tag{2}$$

where Q_{eq} = equivalent sampling flow rate (L/min), N_p = bioaerosol metric determined by the passive sampler (number of particles or CFU), C_a = concentration determined by the collocated active reference sampler (number of particles/L or CFU/L), and t_p = sampling time for the passive sampler (min). A passive sampler's equivalent sampling flow rate could then be used to convert the observed bioaerosol quantity into airborne concentration. Yamamoto et al. (2011) also related Q_{eq} to particle deposition velocities:

$$Q_{eq} = AV_d \tag{3}$$

where Q_{eq} is the equivalent sampling flow rate (mL/min), A is the effective particle deposition area of a passive sampler (cm²), and V_d is the deposition velocity of a particle (cm/min). Based on Equation 3, Q_{eq} is directly related to particle gravitational settling velocity, is a function of particle mass and size (squared) (Cox & Wathes, 1995; Yamamoto et al., 2011),

$$V_d = \frac{\rho d^2 g c}{18\eta} \tag{4}$$

where ρ is the particle density (g/cm³), d is particle diameter (cm), g is the gravitational acceleration (cm/s²), C is the Cunningham slip correction factor, and η is the air viscosity (g/ (cm·s)). Therefore, the equivalent sampling flow rate and the efficiency of a passive sampler will be impacted by the size of the bioaerosol of concern. However, the determination of equivalent flow rate based on gravitational deposition alone does not account for additional factors such as electrostatic attraction, which aid in collecting bioaerosol in electrostatics-based passive samplers.

The use of equivalent sampling flow rates for bioaerosol samplers is a relatively new approach, and more research is needed to confirm the utility of Q_{eq} for different passive devices. The available data also suggested that the Q_{eq} might depend on the sampling environment and conditions (Manibusan & Mainelis, 2018, 2021; Yamamoto et al., 2011).

One study reported quantifying bioaerosol concentration using gravitational settling plates (Mainelis & Rivera, 2006). However, this study used a closed settling chamber design, which provided a known air volume from which microorganisms had settled (Mainelis & Rivera, 2006).

3.1.1. Collocated passive and active sampler studies—Collocated active and passive sampler comparisons have been conducted in previous studies. These studies have

demonstrated the performance of passive samplers and identified the limitations of the devices.

Napoli and colleagues compared settle plates and a Surface Air System Sampler (SAS, International PBI, Milan, Italy) operating at 180 L/min in operating rooms. Settle plates used the IMA scheme while SAS collected 500 L air samples in 100L intervals 12 minutes apart over one hour. The analysis included culturable bacteria and fungi. The test found a strong correlation between the passive and active sampling methods. The authors indicated the benefit of passive sampling to determine likely contamination on surfaces during surgery, whereas active sampling provided information on inhalable viable particle concentrations.

The PAAS was compared alongside an IOM sampler, an active, filter-based, personal sampler (Yamamoto et al., 2006). The study collected samples ranging from 5 hours to 6 days and found a strong correlation (r = 0.69 to 0.95) between the bioaerosol collected by PAAS and IOM based on microscopy.

Another PAAS study compared the device to the NIOSH Bioaerosol Sampler (Yamamoto et al., 2011). Samples were analyzed for fungi by quantitative polymerase chain reaction (qPCR). The study also found relatively strong correlations (r= 0.48 to 0.76) between sampler concentrations, which varied by qPCR primer groups. Using a collocated active sampler allowed for direct comparison of fungal cells collected by both methods and allowed for the determination of equivalent (or effective) sampling flow rates for the PAAS. For example, PAAS sampling of *Alternaria alternata* resulted in an equivalent flow rate of 0.032 L/min, but *Epicoccum nigrum* resulted in 0.066 L/min.

EDCs have also been used in collocated studies with active samplers. One study compared endotoxin concentrations from EDCs and the active Button Aerosol Sampler (SKC, Inc.) with glass fiber filters in farm homes (B. Kilburg-Basnyat et al., 2016). EDCs were deployed over a 7-day sampling period, while collocated Button samplers were replaced every 24-hours. The study found strong correlations (r = 0.7) between the concentrations determined by the two samplers.

REPS was investigated in a 10-day outdoor field sampling campaign which also included collocated Button Aerosol Samplers, settling filters, and agar settle plates (Therkorn, Thomas, Scheinbeim, et al., 2017). The study found that REPS had equivalent sampling flow rates of 2.6 L/min for culturable bacteria when compared against the collocated Button sampler operating at 4 L/min.

These studies illustrate the versatility of passive samplers in effectively collecting different types of bioaerosol, and their equivalent sampling rate can be comparable to those of active samplers.

3.2. Considerations when using passive samplers

3.2.1. Sampling environment—Bioaerosol samples are collected in different environments, both indoors and outdoors, including homes, schools, offices, hospitals, wastewater treatment sites, agricultural areas, landfills, and other locations (Anderson et

al., 2016; Brooks et al., 2018; Faridi et al., 2015; Mbareche et al., 2018; Mui et al., 2017; Pearson et al., 2015; Rendon et al., 2017). In addition, many indoor air sampling projects also include simultaneous outdoor measurements to determine the contribution of the outdoor environment to the presence of indoor airborne particles, including bioaerosols (Adams, Bhangar, et al., 2015; Adams et al., 2013; Chen & Zhao, 2011; Faridi et al., 2015). Therefore, passive bioaerosol samplers should also be useable and functional in various environments.

Agar settle plates, settling filters, and the dustfall collector have been used in indoor and outdoor environments (Adams, Tian, et al., 2015; Docherty et al., 2018; Einstein et al., 2012; Ghosh et al., 2015; Haig et al., 2016; Mhuireach et al., 2016; Näsman et al., 1999; Rendon et al., 2017; Therkorn, Thomas, Scheinbeim, et al., 2017; Würtz et al., 2005). The electrostatic dust cloths are designed for indoor sampling, and none of the referenced studies described their application in outdoor environments (Adams, Tian, et al., 2015; Kilburg-Basnyat et al., 2016; Madsen et al., 2012; Noss et al., 2008; Viegas et al., 2018). PAAS is designed as a personal sampler to be worn in an individual's breathing zone in various environments, including indoors and outdoors (Yamamoto et al., 2006; Yamamoto et al., 2015; Yamamoto et al., 2010; Yamamoto et al., 2011). REPS has been operated in outdoor and indoor environments and in a controlled experimental chamber (Manibusan & Mainelis, 2021; Metaxatos et al., 2022; Therkorn, Thomas, Calderón, et al., 2017; Therkorn, Thomas, Scheinbeim, et al., 2017). The RAMP system has been specifically designed for outdoor, high-altitude sampling using a balloon as a carrying system and, therefore, is not applicable for indoor sampling (Spring et al., 2018).

Overall, most passive samplers can be used in both indoor and outdoor environments. However, when sampling outdoors, similarly to active samplers, passive samplers should be adequately shielded from the elements and/or secured to minimize disturbance from wind and precipitation.

3.2.2. Sampling duration—Sampling duration is an especially important consideration for passive samplers because they usually require longer sampling times than active samplers to collect enough material needed to determine bioaerosols. At the same time, the longer sampling times also offer a potential opportunity to gain information about long-term bioaerosol presence and trends. In many cases, the needed sampling time is a "guestimate" as the air volume from which the microorganisms are captured cannot be determined with certainty (Adams, Tian, et al., 2015; Haig et al., 2016; Würtz et al., 2005). At the same time, agar settle plates cannot be deployed for extended periods due to the desiccation of the medium (Therkorn, Thomas, Scheinbeim, et al., 2017). NAS has been reported to collect a sufficient sample quantity for analysis during 10 to 20 minutes of sampling (Graham et al., 2000). EDCs are recommended to sample no longer than 14 days as the EDC materials have been demonstrated to lose their charge and thereby collect with less efficiency over extended sampling durations (Brita Kilburg-Basnyat et al., 2016). REPS has been demonstrated to collect culturable samples for up to 21 days in indoor and outdoor settings (Manibusan & Mainelis, 2021). It was used for 6–10 days to capture airborne biological material outdoors for microorganism diversity study (Metaxatos et al., 2022). Surface dust samples can be readily and rapidly collected; however, the sample age is often unknown (Würtz et al.,

2005). Similar challenges occur when using HVAC or cabin filters of unknown filter age. On the other hand, the dustfall collector provides an option to collect settled dust with known sample age and has been used to collect for several weeks and even up to 140 days (Adams, Tian, et al., 2015; Würtz et al., 2005).

3.2.3. Physical collection efficiency—As previously discussed, when gravitational settling is used as the primary collection mechanism, it preferentially samples particles larger than 5 μ m in diameter (Ghosh et al., 2015; Therkorn, Thomas, Scheinbeim, et al., 2017). As such, gravity-based samplers may undersample smaller particle fractions. Electrostatic attraction aids in collecting smaller particles, such as free bacteria and viruses, which would be underrepresented by gravity-based collection means alone (Miksch et al., 2009). Thus, the use of electrostatic attraction in passive samplers might therefore improve physical collection efficiency overall and especially for smaller particles.

The relationship between settling velocity and collection efficiency of passive samplers has been well established and modeled when sampling overall aerosol (K. Lai & Nazaroff, 2000; Wagner & Leith, 2001a, 2001b, 2001c; Wagner & Macher, 2003), but the relationship has been less thoroughly examined for bioaerosols (Yamamoto et al., 2006; Yamamoto et al., 2011). As described in section 3.1, the settling of particles onto a collection surface is largely determined by their deposition velocity, thus contributing to a sampling bias toward larger particles and agglomerates.

The EDC has also been demonstrated to collect less efficiently when exposed to higher temperatures which may be due to loss of charge in the electrostatic cloth (Brita Kilburg-Basnyat et al., 2016). This can reduce the benefit of electrostatic collection for EDCs when sampling outdoors during warmer months or other similar conditions. However, we have not seen any reports on how humidity can impact the collection efficiency of EDCs.

REPS has been successfully tested in an extended 10-day outdoor campaign with reported collection efficiencies higher (~29%) than that of a collocated passive PTFE filter (~4%) when compared against an active filter-based sampler (Therkorn, Thomas, Scheinbeim, et al., 2017). During this study, the mean temperature ranged from $2^{\circ}C \pm 4^{\circ}C$ to $9^{\circ}C \pm 5^{\circ}C$ between tests, and relative humidity ranged from 20% to 99%, illustrating a range of conditions when REPS could effectively perform (Therkorn, Thomas, Scheinbeim, et al., 2017). The REPS sampler was also demonstrated to successfully sample culturable airborne bacteria and fungi for different durations, up to 21 days, indoors and outdoors in Fall and Spring (Manibusan & Mainelis, 2021).

3.2.4. Bioefficiency—In general, a bioaerosol sampler's physical collection efficiency, i.e., its ability to capture particles, is determined by its design and operating conditions but could also be affected by environmental parameters and conditions, such as humidity and wind speed (Cox & Wathes, 1995). However, bioaerosol sampling also requires the consideration of bioefficiency. Bioefficiency, or biological sampling efficiency, pertains to a sampler's ability to maintain the collected sample's culturability, viability, biological integrity, or other properties allowing its determination, quantification, and identification (Haig et al., 2016; Yates et al., 2016). Active sampling methods are known to impose stress

on microorganisms through impaction, impingement, or desiccation during sampling(Haig et al., 2016). These stressors are not present in passive sampling devices, thus improving the preservation of viability and culturability of samples (Haig et al., 2016; Mainelis et al., 2002; Mbareche et al., 2018; Näsman et al., 1999). Therefore, one can argue that bioaerosol samples obtained by passive collection methods better represent the culturable or viable bioaerosol state due to lower sampling stress. Based on results from REPS, comparison of different sample durations for as long as 21 days, CFU recovery was not dependent on sample length (Manibusan & Mainelis, 2021).

3.2.5. Sample elution or other pre-processing—Sample recovery can be affected in several ways during sample collection and processing. Aside from the stress during sampling, the elution of a sample into a liquid medium and any additional sample processing should minimize the stress on microorganisms, potential contamination, and sample losses. Similarly, the elution from collection media should ideally have maximum recovery. Agar settle plates do not require additional processing before analysis for culturable bioaerosols. As with other culture-based approaches to bioaerosol analysis, agar settle plates provide a relatively simple and affordable method of determining the culturable fraction of the microorganisms in an air sample. However, as mentioned, only a fraction of viable microorganisms are also culturable, and a specific organism's culturability will vary depending on the growth medium type, environmental conditions, and interactions with other organisms (Cox & Wathes, 1995). Many passive and active bioaerosol samplers generally require some elution procedure to prepare the sample for analysis, except liquid or agar-based samplers. Therefore, the ease and efficiency of sample elution, the ability to analyze a sample in addition to bioefficiency should be important considerations in selecting a bioaerosol sampler.

EDCs require processing using Tween detergents and multiple extractions in a stomacher or orbital shaker to efficiently remove particles for quantitative PCR (qPCR) analysis (Adams, Tian, et al., 2015; Madsen et al., 2012; Viegas et al., 2018). Despite these multiple steps, EDCs have a modest elution efficiency (51% for bacteria and 58% for fungi) (Adams, Tian, et al., 2015; Madsen et al., 2012; Therkorn, Thomas, Calderón, et al., 2017), limiting the research conclusions drawn from the analysis of EDC samples. In addition, the use of Tween detergents may reduce the cell membrane integrity of microorganisms, thus limiting the EDC sampler's applicability for culture or other viability analysis methods (Therkorn, Thomas, Calderón, et al., 2017; Zhen et al., 2013).

REPS analysis involves the elution of particles captured on the film into the water using a combination of vortexing and sonication (Therkorn, Thomas, Calderón, et al., 2017; Therkorn, Thomas, Scheinbeim, et al., 2017). When challenged with airborne bacteria in chamber studies and by use of spiked samples, REPS was reported to have an efficient sample recovery and elution efficiency (~100% recovery of microorganisms) compared to both filters (~80%) and EDCs (~63%) (Therkorn, Thomas, Calderón, et al., 2017). However, the REPS elution protocol requires 35 mL of liquid to cover the entire assembled sampler, resulting in a dilute sample and reducing bioaerosol detection, especially in clean environments or after shorter sampling durations (Therkorn, Thomas, Scheinbeim, et al., 2017). Sample concentrators offer ways to concentrate dilute samples and could improve the

detection limit of REPS samples; however, they have certain drawbacks, including sample loss and reduction in culturability (Oh et al., 2020).

Dustfall collectors can require multiple swabs to collect the settled particles from the Petri dishes for analysis; collection swabs can then be eluted into a buffer solution (Adams, Tian, et al., 2015). RAMP samples have also been processed by swabbing for DNA-based analysis similar to dustfall collectors (Spring et al., 2018).

3.2.6. The versatility of passive samplers for use with different analysis

methods—Bioaerosol samples can be analyzed using multiple methodologies, including culture, adenosine triphosphate (ATP) analysis, microscopy, polymerase chain reaction (PCR), sequencing, and others. The adoption of molecular techniques for bioaerosol analysis is becoming more common because of its declining cost, speed, and expanded available techniques for next-generation sequencing technology (Ghosh et al., 2015; Mbareche et al., 2017; Mbareche et al., 2018). At the same time, the few available bioaerosol guidelines are designed based on culture-based methods (Kim et al., 2018; Lindsley et al., 2017; Mbareche et al., 2019). Therefore, there should be some consideration for both culture-based as well as non-culture analysis of bioaerosols.

Passive filter sampling can be combined with multiple analysis methods, including by their direct press onto a culture medium to grow colony forming units or by their elution into liquid, which is amenable to various analysis methods (Näsman et al., 1999; Therkorn, Thomas, Scheinbeim, et al., 2017; Xu et al., 2013). The REPS design allows its efficient transport and sample elution into liquid media (Therkorn, Thomas, Scheinbeim, et al., 2017), and its samples have been analyzed by microscopy, ATP activity, culture, and sequencing techniques (Manibusan & Mainelis, 2019, 2021; Metaxatos et al., 2022; Therkorn, Thomas, Calderón, et al., 2017; Therkorn, Thomas, Scheinbeim, et al., 2017; Therkorn, Thomas, Scheinbeim, et al., 2017; Metaxatos et al., 2017). EDCs, Dustfall collectors, and swabs can also be eluted into liquid media for analysis by microscopy, culture, or next-generation sequencing techniques (Adams, Tian, et al., 2015; Madsen et al., 2012; Therkorn, Thomas, Calderón, et al., 2018). Overall, most passive samplers can be eluted into a liquid media for multiple analyses; however, there is currently no uniform method for elution or analysis of bioaerosol samples. The same could be said about the elution and analysis methods for active sampling approaches.

3.2.7. Other considerations: cost, time, and sample distribution—The selection of a bioaerosol sampler and analysis methods depends on a research question. Passive bioaerosol samplers are useful for long-term sampling campaigns and provide an affordable means to measure bioaerosol concentration and potential exposures over periods as long as several weeks. There are few, if any, active samplers that could be deployed for such durations without any involvement of technical personnel during sampling. However, as already discussed, the use of passive bioaerosol samplers might be challenging during short sampling times, e.g., minutes and hours, as they usually capture less biological material than active samplers in the same environment. That should be considered when selecting a sampler and its integration into a project.

Passive samplers generally have a lower cost compared to active devices. In fact, many of them are disposable, as they utilize materials already commonly available for other bioaerosol and aerosol sampling applications (e.g., Petri dishes, filters). In addition, the absence of power supply requirements and minimal technician time during sampling further reduce overall sampling cost. These features allow for the affordable deployment of multiple passive samplers that could be spatially distributed throughout a large area to achieve a massively distributed bioaerosol sampling.

The passively collected sample, especially over an extended time, might provide a more comprehensive picture of bioaerosols in a particular environment than the short-term samples collected by active samplers. In fact, a long-term integrated sample is less affected by short-term variability in bioaerosol concentration and composition and offers a better representation of average bioaerosol concentration. Furthermore, passive samplers may better preserve microorganism culturability and viability by avoiding the stress imposed by active devices, especially for sensitive bioaerosol species, which offers another advantage of passive sampling.

3.3. Passive sampling applications for SARS-CoV-2

Passive sampling has been applied for SARS-CoV-2 sampling in different studies. As previously discussed, the IACM (Santarpia et al., 2021) and Fresh Air Clip (Angel et al., 2022) were developed or adapted in response to the COVID-19 pandemic. Other existing passive samplers have also been applied for SARS-CoV-2 sampling, such as filters (Liu et al., 2020; Passos et al., 2021), empty Petri dish (dustfall collector) (Hermesch et al., 2020), Petri dish filled with liquid media (Baboli et al., 2021), surfaces with accumulated particles (Horve et al., 2021; Nannu Shankar et al., 2022; Pan et al.; Pena et al., 2021), and HVAC filters (Pan et al.). Baboli and colleagues found lower rates of positive SARS-CoV-2 samples when using passive sampling compared to the detection rate of a collocated active glass impinger operated for 30 minutes (Baboli et al., 2021). However, Pan and others effectively used both swabs and HVAC filters to detect SARS-CoV-2 in isolation dormitories (Pan et al., 2022). In addition, Pena and colleagues argue that both active and passive samplers should be used in SARS-CoV-2 detection (Pena et al., 2021). These studies demonstrate the applicability of passive devices for SARS-CoV-2 sampling and eventual detection.

4. Conclusions

When choosing a bioaerosol sampler, one should consider multiple factors, including physical and biological efficiency, sample recovery, the bioaerosol of concern, planned analysis methods, sampling environment(s), as well as sampling duration, convenience, and cost. These decisions should tie into the overall research question, including the best and most efficient use of resources. While most projects use active bioaerosol samplers, passive sampling methodology could serve as a valuable complementary tool, especially if a long sampling duration is needed or when the project could benefit from multiple samples distributed over wide areas. Although passive samplers based on gravitational settling methods have limitations regarding the collected amount of material per time and bias toward larger particles, they have useful applications that can inform researchers of the

Page 18

overall bioaerosol community over extended sampling periods. In addition, passive samplers that incorporate electrostatic attraction may help overcome size fraction limitations observed in samplers that rely on gravity only. In addition, the natural phenomena used for bioaerosol collection by passive devices can minimize cell damage during the sampling process.

Although the actual volume of air sampled by passive devices is unknown, passive samplers can still indicate bioaerosol presence and microorganism types. The amount of collected material can still be reported in quantifiable terms based on the sampler's collection area and time (e.g., #/cm²/h) or, once calibrated against active devices, as an equivalent sampling flow rate (e.g., L/min). The affordability of passive samplers also allows for the deployment of units across a study area over extended periods to determine spatial distributions of bioaerosol, which can be cost-prohibitive using a similar number of samplers and time periods with active methods alone. In summary, passive bioaerosol samplers can provide complementary information to expand our understanding of bioaerosol presence and composition in different environments. This may be particularly beneficial in determining the presence and viability of stress-sensitive species over time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

• This review discusses different types of passive bioaerosol samplers.

- Advantages, limitations, and analysis methods of passive samplers are discussed.
- Quantification of passive samples is considered.
- Latest developments in passive sampling, including for SARS-CoV-2 is described.

Table 1.

Comparison of main features of passive and active bioaerosol samplers.

Feature	Passive Samplers	Active Samplers
Known flow rate	No (work is ongoing to establish an equivalent flowrate)	Yes
Known sample volume	No	Yes
Airborne concentration determination	Estimate only	Yes
Sampling duration	Hours to weeks	Minutes to weeks
Primary collection mechanisms	Gravitational settling, electrostatic forces, diffusion, turbulent dispersion, opportunity-based sampling	Filtration, impaction, impingement, cyclonic forces, electrostatic forces
Power requirements	None	Pump and electrostatic components, if ESP is used
Weight	Lightweight and portable	Heavier due to addition of sampling media (e.g., agar) and a pump
Operator attention requirements	Deployment and retrieval	Deployment and retrieval, flowrate determination and maintenance, media replenishment for liquidbased samplers, possible replacement of collection media
Cost	Sampler	Sampler, pump, power supply

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Table 2.

Passive Bioaerosol Samplers

Sampler	Description and References	Advantages	Limitations
Gravity-based Samplers			
Agar Settle Plates	100mm agar media plates are left in the sampling environment for up to 4 hours to capture settling organisms (Markel et al., 2018; Sandle, 2015).	Settle plates offer a low-cost and quick method of analysis of culturable organisms. This method has been frequently cited in the literature.	Limited sampling duration due to desiccation of the media. Determines only the culturable bioaerosol fraction.
Settling Filters	It can include any type of filter used for bioaerosol or general particulate matter sampling (Johnston et al., 1978; Näsman et al., 1999).	Affordable, long-term sampling. Filters are commonly available in aerosol sampling labs or are easily obtained.	No consistent sampling protocols or list of materials. Sampler needs to be secured to prevent filter warping or disturbance from the wind.
Einstein-Lioy Sampler	The Einstein-Lioy Sampler allows sampling on up to four filters simultaneously in a filter holder while minimizing filter disturbance by wind or other elements (Einstein et al., 2012).	Secures 37mm filter samplers (with an exposed 25mm diameter) for convenient long term-sampling using the preferred filter material of choice.	Although it has been reported for passive sampling for particulate matter studies, this device has not been reported for bioaerosol measurement.
Dustfall collector or Petri dish	The dustfall collector allows sample collection in a box or sterile Petri dish (Adams, Tian, et al., 2015; Noss et al., 2010; Würtz et al., 2005).	Sterile container and timeline for dust samples. A more versatile alternative to agar plates.	It may require multiple swabs to gather all dust from the sampler.
Durham-type Passive Spore Trap	The Durham trap protects prepared slides for long-term outdoor sampling in different field conditions (Durham, 1946; Serrano-Silva & Calderón- Ezquerro, 2018).	Secures prepared slide samplers for convenient long-term sampling for microscopic analysis.	The sampler was designed specifically for slide analysis by microscopy, limiting analysis methods.
Gravity-based Samplers			
Remote Airborne Microbial Passive (RAMP) Sampler	The RAMP system is designed for sampling onto gel-coated square Petri dishes for bioaerosol at altitudes with a balloon (Spring et al., 2018).	Lightweight design allows for high-altitude sample collection.	Due to design considerations, no reference sampler was included in testing as a comparison.
Personal Aeroallergen Sampler (PAAS)	The PAAS is designed as a personal sampler to be worn around the neck near a person's breathing zone to capture an individual's personal exposure (Yamamoto et al., 2006; Yamamoto et al., 2011).	A passive personal sampler to directly measure personal exposures.	Low sensitivity at low bioaerosol concentrations. Similar to all gravity-based samplers, particle sizes under 5 pm are undersampled.
Electrostatic Collection			
Electrostatic Dustfall Collectors (EDCs)	Utilizes electrostatic materials to aid in the collection. Commercially available as Swiffer® or Pledge® Grab-It TM (Böhlandt et al., 2016; Cox et al., 2017; Cozen et al., 2008; Brita Kilburg-Basnyat et al., 2018; Matson et al., 2012; Noss et al., 2008; Viegas et al., 2018).	Sampled bioaerosol can be determined using a large suite of analysis methods. In addition, electrostatic properties may aid in attracting smaller particles than gravity alone.	Difficult to elute particles from the collection material. May lose charge over time.
Rutgers Electrostatic Passive Sampler (REPS)	Permanently polarized PVDF film is wound into a 3D printed film holder to create an electrostatic field between layers to capture bioaerosol particles (Metaxatos et al., 2022; Therkorn, Thomas, Calderón, et al., 2017; Therkorn, Thomas, Scheinbeim, et al., 2017).	The electrostatic attraction may aid in capturing more particles than gravity alone. Convenient transport and elution in standard sterile conical centrifuge tubes.	Currently requires sample into a large volume of liquid (35 mL), which increases the detection limit.
Special Cases: Passive O	Special Cases: Passive Opportunity Samplers Using Existing Air Streams		

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Nasal Air Sampler The sampler is clipped into th (NAS) (NAS) capture particles onto an adh. (NAS) HVAC or Other Use of existing filtration syst et al., 2011; Bonetta et al., 21 Existing Air Filters Use of existing filtration syst et al., 2011; Bonetta et al., 21 Stanley et al., 2008). Stanley et al., 2008). Infectious Aerosol Modified oxygen delivery me spitent spitent spitent for a hospital vacuum for a hospital vacuum spitent for a hospital vacuum spitent for a hospital vacuum for a hospital	e nasal passage and uses nasal breathing to esive surface (Graham et al., 2000). ems to collect bioaerosol samples (Ackelsberg 10; Goyal et al., 2011; Noris et al., 2011; ste with an attached filter cartridge that can be not the The 1ACM is used to minimize astrood	Allows direct measurement of inhalation exposures. Airflow and air volume are known or can be	Does not measure exposures due to mouth breathing.
ters sol IACM)	ems to collect bioaerosol samples (Ackelsberg 010; Goyal et al., 2011; Noris et al., 2011; sk with an attached filter cartridge that can be	Airflow and air volume are known or can be	.0
		determined.	Information on sample age depends on the accuracy of maintenance records. Unmaintained HVAC systems can be a source of microorganisms, which can impose sample bias.
		The mask has been demonstrated as a tool for both minimizing the spread of infectious bioaerosol and measurement of aerosols generated by infected individuals.	Information is limited to source strength post-exposure and disease development.
ISS Passive Aerosol Developed for sample Sampler Station (ISS) by plac sampler included fiv collection medium. (Developed for sampling particulate matter present on the International Space Station (ISS) by placing device over the ventilation system intake. The sampler included five aluminum blocks with double-sided carbon tape as the collection medium. (Haines et al., 2019; Meyer, 2017, 2018).	This sampler was designed to be compact and allow secure transport to and from the ISS. This device utilized the forced air movement through the air intake of the ventilation system to capture particles by impaction onto the collection substrate.	Specifically designed for passive sampling applications in microgravity environments.
Related Dust Collection Methods			
Vacuum, surface A vacuum, cloth, or swabs, or wipes often using a templat et al., 2018; Cox et a	A vacuum, cloth, or swab can be used to collect settled dust from surfaces, often using a template defined surface area (Adams & Dancer, 2020; Aktas et al., 2018; Cox et al., 2017; Magyar et al., 2018; Viegas et al., 2020).	Affordable, quick sampling method for historical settled particles which does not require extensive collection time.	Does not provide the age of the collected dust sample. Collection efficiency may vary between surface materials and applied wiping/swabbing pressure during collection.
Diffusion-based nonpolar compound sampler			
Fresh Air Clip Uses a polydimethylsiloxane such as lipid enveloped virus	(PDMS) pad to capture nonpolar compounds particles (Angel et al., 2022; Lin et al., 2020).	Demonstrated ability to capture measurable quantities of viral particles in chamber and field tests.	Currently, there is limited knowledge about its applications to different bioaerosol types.