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Review of Field Sampling Technologies for Characterizing Bioaerosols in Compact Spaces

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Final Report for October 2018 to December 2019

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14. ABSTRACT

Bioaerosol sampling and characterization is a challenge in industrial hygiene, both in the civilian realm and for the Air Force. This literature and technology review reports several bioaerosol collection technologies that are currently commercially available (and possibly modifiable) or in development. Fifteen commercially available options were evaluated by a set of selection criteria to provide a comprehensive comparison and recommendation. Technologies under development were not scored in the same manner but were appraised for future utility. The selection criteria were based on parameters such as size ranges of bioaerosols that could be collected, biological agent survivability, collection time, collection quantity, collection efficiency, sampling flow rates, device portability and device ruggedness. Technologies were selected based on criteria required to be effective samplers in real-world AF-relevant environments where space is limited and personal sampling is desirable. Technologies were down-selected based on the criteria evaluation. There were six commercial off-the-shelf technologies down-selected, including a high volume cyclone impinger (Coriolis Recon, Bertin Instruments), a handheld high volume rotating impactor (BioCapture 650, FLIR Systems), a two-stage high volume impactor (SASS 4100, Research International), a compact high volume dry cyclone sampler (Coriolis Compact, Bertin Instruments), a high-volume membrane filter impaction device (MD8 AirPort Sampler, Sartorius), and a cassette filter with sampling pump (available from various manufacturers). There were two technologies under development that are recommended for further investigation, including a personal electrostatic bioaerosol sampler (PEBS) and the Rutgers Electrostatic Passive sampler (REPS). All recommended samplers have advantages and disadvantages and require further experimental validation for use in specific AF applications.

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1.0 EXECUTIVE SUMMARY

Bioaerosol sampling is of interest in many Air Force applications, such as mitigating cross-infection during aeromedical transport, ensuring decontamination of exposed aircraft, and detecting biological warfare agents. For many of these applications, there is interest in a robust portable sampler that can be operated in small spaces, such as aircraft cockpits and fuel cells, and also be used for breathing zone sampling. The standard instrument used by Bioenvironmental Engineers (BEE) in the Air Force is the XMX-2L/MIL (Dycor Technologies). With its high flowrate (~500 LPM) and mass flow concentrator system, this unit is an effective area sampler and can concentrate dilute bioaerosols (~1-10 µm in size) into a sample with sufficient biomass for analysis (Black, 2011). An extension of this technology is the C-FLAPS (XMX unit integrated with real-time Fluorescent Laser Aerodynamic Particle Sizer) (Black, 2011). The drawbacks to the XMX-2L/MIL is that it is heavy (37 lbs) and has a large footprint (1.5 ft x 1.9 ft x 1 ft), and requires 110V AC or 220V AC power. This report reviews several alternative COTS options and developing novel technologies with strengths and weaknesses described, so that the best bioaerosol sampling instrument can be easily selected based on the requirements for specific applications.

The ideal sampler for use in small spaces would be compact, lightweight, and capture the broadest range of bioaerosols (e.g. fungi, bacteria, viruses) while maintaining sample viability to allow for a thorough characterization of the exposure environment. After extensive literature review on bioaerosol sampling technologies, a list of criteria for rugged and portable bioaerosol samplers was devised for evaluation (Table 1). Fifteen COTS technologies (Tables 2-3) and four technologies under development (Table 4) were reviewed according to the following criteria: Product Development Status, Collection Technology, Sample Recovery, Collection Time, Flow Rates, Particle Size Collection Capabilities, Operating Humidity, Operating Temperature, Consumables, Dimensions, Weight, Power Source, and Automation. Out of the fifteen technologies evaluated by these criteria, six COTS technologies and two technologies under development were down-selected as the top recommendations.

The first two COTS technologies down-selected were the high volume cyclone impinger (Coriolis Recon, Bertin Instruments) and compact high volume dry cyclone sampler (Coriolis Compact, Bertin Instruments). The Coriolis Recon's advantage is its ruggedness and high efficiency to collect 0.5-10 µm size particles. The Coriolis Micro and Compact have similar sampling capabilities but are less ruggedized; however, the Coriolis Compact is ideal for moderately high-flow sampling (50 LPM) in smaller or enclosed spaces.

The third COTS technology down-selected was the BioCapture 650 (LAURUS Systems), which is a rotating impactor which deposits samples onto a solid impactor surface and rinsed with media for sample storage (Enderby, 2012). The BioCapture 650 samples particles 0.5-10 µm at 200 LPM. Compared to the other technologies, however, it is not only handheld but has some limited real-time biological agent detection capabilities with the manufacturer's Hand-Held Assays (HHA). This unit has already been used in harsh environments (e.g. sandy, dusty), is operational at 15,000 ft altitude, and has successfully sampled a variety of bioaerosols, including viruses and anthrax.

The fourth COTS technology down-selected was a two-stage high volume dry filter impactor, the SASS 4100 (Research International). This system is relatively portable (13 lbs, and 1.2 ft x 0.8 ft

diameter) and ruggedized. Despite being small, it uses an extremely high flowrate (4000 L/min) and a HEPA filter and electret filter for bioaerosol capture. A unit-specific particle extractor is supplied by the manufacturers for use with the electret filter. Since the electret filter attracts and retain particles electrostatically, collection of particles < 1 µm is enhanced.

The fifth COTS technology down-selected was a high-volume membrane filter impaction device (MD8 AirPort Sampler, Sartorius). This instrument is handheld and has been used in simulated flight environments, and in rugged outdoor environments for bacteria and fungi. Since it utilizes a porous gelatin membrane filter, it has a high collection efficiency, confirmed with test bioaerosol particles (*Bacillus* spores and a bacteriophage virus). Its collection versatility and handheld form factor make it attractive for operational environments.

The sixth and final COTS technology down-selected was a cassette filter with sampling pump (available from various manufacturers). This is a straightforward technology that can be used for personal sampling. However, it suffers from challenges related to bioagent survivability on dry filters and low collection volume. Several researchers and regulating bodies have had success using the Button Aerosol Sampler (SKC, Inc.) with various filter media, including gelatin, polytetrafluoroethylene, and polycarbonate. This active filtration technique has been shown to effectively collect bioaerosols with a very low footprint.

There were two technologies under development that are recommended for further investigation, including a personal electrostatic bioaerosol sampler (PEBS) and the Rutgers Electrostatic Passive sampler (REPS) (Han et al., 2018) and a passive sampling device, the Rutgers Electrostatic Passive Sampler (REPS) (Therkorn et al., 2017). The PEBS is attractive due to its portability and low flowrate, which is ideal for personal sampling or sampling in enclosed spaces. Additionally, its electrostatic sampling mechanism is highly efficient for particles less than 200 nm in diameter, smaller than the COTS technologies' lower limit for efficient collection. The REPS unit’s sampling mechanism is a polarized ferroelectric polymer film which electrostatically collects particles ranging in sizes from 14 nm to 5 µm, which is promising for airborne viruses or proteins, notoriously difficult bioaerosols to sample due to their size (Therkorn, et al., 2017). While more development would be necessary, these unique technologies are promising for novel low-flow personal sampling.

2.0 BACKGROUND

Bioaerosols are aerosols containing biological matter (e.g. pollen, fungi spores, bacteria, viruses and protein toxins) and are ubiquitous in both indoor and outdoor environments (Heikkinen et al., 2005). Bioaerosol sizes span a wide range, from 0.01 to 100 µm (Ghosh et al., 2015). Bioaerosols are classified by aerodynamic diameter, which is used to describe their behavior, specifically aerodynamic transport and deposition. A particle’s aerodynamic diameter is defined as the diameter of an equivalent spherical particle of unit density (1 g/cm^3) with the same terminal settling velocity (Hinds, 1999). Thus, two particles with the same aerodynamic diameter will have the same transport and deposition (and thus collection) properties even if they have different chemical and morphological characteristics.

Bioaerosol sampling is used to collect biological material for offline characterization of concentration, viability, culturability, and biodiversity. The aerodynamic properties of aerosols

drive the principles of operation for bioaerosol sampler (Lindsley et al., 2017). s. There are several different principles of operation for bioaerosol sampling that have been developed based on particle transport mechanisms: sedimentation, filtration, impaction, cyclonic separation, liquid impingement, electrostatic precipitation, and condensation-based particle growth and impaction (Heikkinen et al., 2005; Ghosh et al., 2015).

Generally, all these mechanisms except sedimentation and sometimes electrostatic are active, meaning a pump is used to draw a known volume of air through the sampler (Therkorn et al., 2017). In contrast, passive sampling methods rely on gravitational settling and/or sedimentation (Heikkinen et al., 2005). Passive sampling is limited in application since this method provides no information of airborne bioaerosol concentration because the volume of air sampled is unknown.

Bioaerosol collectors can be classified by the cut-off diameter, defined as the aerodynamic diameter above which particles are ideally captured with 100% efficiency and below which particles are not collected and pass through the device. Since not all collectors have the ideal cut-off curve, in practice, this cut-off diameter, also called d50, marks a benchmark collection efficiency of 50% for that diameter (Hinds, 1999). Thus, different samplers with different cut-off diameters will be size-selective when sampling bioaerosols. Different samplers' cut-off diameters are constrained by the collection mechanism used.

Filtration-based aerosol samplers are commonly used to collect aerosol particles (including bioaerosols) because of their ubiquity, low cost, and high collection efficiencies for a wide range of sizes (Heikkinen et al. 2005, Hinds, 1999). Air sampling filter media are available in several materials, including micro-cellulose ester (MCE), glass fiber (GF), polycarbonate (PC) and polytetrafluoroethylene (PTFE) depending on the contaminant of interest and the requirements of the analytical technique (Lindsley et al., 2017). While some studies have had success with different filter types (mainly PC and PTFE) and custom extraction protocols for specific filters, porous gelatin filters are a special class reserved for bioaerosol sampling (commercially available from SKC, Inc). These filters can maintain agent viability and be melted or dissolved into the extraction medium agents for further analysis. However, a distinct disadvantage unique to gelatin filters is susceptibility to dissolution in fields with high temperature and relative humidity, as well as desiccation effects if sampling times are too long (>15-30 min).

Impaction collection mechanisms use a vacuum pump to draw air through nozzles towards an impaction surface where particles are separated from the air stream by their inertia. The incoming air stream exits the impactor nozzle perpendicular to the impaction surface but is directed to flow parallel to the impaction surface. As the airstream makes this 90° turn, particles of a certain size with a higher inertia will fall out of the airstream onto the impaction surface. A cascade impactor is comprised of several of these stages stacked in series, separating the particles in an airstream into size fractions based on each stage's aerodynamic cut-off diameter. For impaction-driven samplers, the collection surface is often comprised of agar on a Petri plate that can be directly transferred to an incubator for later analysis. Most impactors with a perforated sampling face require a positive hole correction factor to correct for coincidence if two bioaerosol particles pass through the same hole before hitting the agar surface (Lindsley et al., 2017). Some limitations of impactors are that the collection agar can desiccate during longer sampling runs, reducing sample viability (Moon et al., 2009). If a different collection surface is used, particle bounce can occur, resulting in sample loss. For particulate aerosols this can be remedied with an oil to trap particles and some bioaerosols may be able to be similarly trapped in mineral oil (SKC, Inc.).

Similarly, a cyclone sampler consists of a circular chamber with the aerosol stream entering through one or more tangential nozzles where the particle is deposited on the sampler wall as the air stream curves around inside the chamber. An advantage this has over impactor samplers is that cyclones are less prone to particle bounce and can collect larger quantities of material (Lindsley et al., 2017). Additionally, cyclones may also provide a gentler collection than impactors, which improves the recovery of viable organisms being collected.

In impingement style collectors, the body of the impinger is filled with a collection liquid, and the aerosol stream flows down through a nozzle and enters the liquid at a high velocity. The aerosol particles are collected when they collide with the bottom of the collection vessel or disperse into the liquid (Lindsley et al., 2017). This collection method is advantageous because many microorganisms can lose their viability if they are collected onto dry solid surfaces or filters because of impact damage and desiccation (Verreault et al., 2008). A distinct disadvantage to impingers is that the impaction of the airflow onto a liquid surface results in bubbling, foaming, and agitation which can be detrimental to some microorganisms (Lindsley et al., 2017). However, additives to the collection liquid such as proteins, antifoam, or antifreeze, can be used to minimize injury to biological agents, aid in the resuscitation of bacterial cells, prevent foaming and loss of the collection fluid (Dungan and Leytem, 2016).

Cyclone collectors use centrifugal force to spin larger particles out of an air stream. Wet-walled surface cyclonic bioaerosol samplers incorporate the technologies of cyclones and impingers, where bioaerosols are collected on the wall of a cyclone wetted with collection media. This technique greatly reduces the agitation, bubbling, and possible reaerosolization of the collected agent (Lindsley et al., 2017). These samplers recirculate the collection fluid, but still require replenishment over time to replace evaporative losses. This extends the collection time available and allows the concentration of the aerosol from a large volume of air into a relatively small volume of liquid, ideal for pathogens that may be present in very low concentrations (Lindsley et al., 2017). These types of samplers have been shown to collect particles with aerodynamic diameters as low as 0.3 μm (Kesavan et al., 2010). However, it is important to note that when conducting long-term collection runs with these devices, particles that remain in the collection liquid for extended periods of time may undergo spore germination and cell amplification resulting in a false positive result indicating a much higher concentration than may actually be present in the sampling environment.

Electrostatic precipitation is another principle that can be used to collect bioaerosols that is a more efficient for sampling submicron particles compared to the other methods described (Heikkinen et al., 2005; Therkorn 2017; Han 2018). Electrostatic precipitation utilizes either particles' inherent surface charge or a charge induced onto particles as they pass through the sampler to deposit onto a collection surface. Commercially available instruments for electrostatic collection of aerosols include the NanoAerosol Sampler (TSI, Inc.) and the Partector (Naneos), a real-time electrometer for particle counting with an optional electrostatic precipitation feature. The developing technologies designed for bioaerosols are discussed later in this report, although certain challenges arise when targeting bioaerosols using electrostatics, specifically ozone generation during sampling, which can be injurious to bioaerosols (Therkorn 2017, Han 2018).

Another sampling technique typically used to characterize ultrafine particulate aerosols but can be transitioned to bioaerosol sampling is condensation-based particle growth and impaction. Aerosolized particles are drawn through the sampler inlet into a chamber supersaturated with a growth medium (historically isopropanol, but water is also becoming more common) where each

particle acts as a nucleation site for the supersaturated vapor. For particulate analyzing instruments, the particle growth is controlled and each particle is counted by a laser optics system downstream within the sampling instrument, and thus accurate real-time counts for submicron particles can be recorded. These instruments do not typically have a collection capability. However, this has become an area of interest with one commercially available sampler exclusively for bioaerosol collection mediated by condensation particle growth and impaction of the enlarged particles (as discussed later in this report). There are also samplers that use these technology in development.

There are several options for offline analysis that affect how biological agents should be preserved upon collection. The wide range in bioaerosol agents, aerodynamic sizes, and offline analysis options makes bioaerosol sampling challenging, and all of these considerations are important when choosing a bioaerosol sampler. Thus, it is key to understand the types of bioaerosols present in the various environments being sampled and decide which data is most important to measure (e.g. total concentration, viable concentration) and to choose a sampler that best preserves the target bioaerosol with a sufficiently high sample collection efficiency for the analytical technique of interest.

Analytical techniques post-sampling include but are not limited to microscopy, biochemical and immunological assays, incubation and culture, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequencing, and/or bulk chemical analysis (Heikkinen et al., 2005). Most of these techniques must be performed offline in a laboratory environment. To date, the only commercially available real-time exception is the MinION (Oxford Nanopaore Technologies) for sequencing (determining the primary structure of DNA, RNA, proteins, or other biologic material) which provides rapid agent identification. Like bioaerosol samplers, each analysis method is optimized for a specific analytical endpoint that provides different information.

Microscopy can be used for enumeration and limited identification of collected biological agents (Heikkinen et al., 2005). Cell staining can also be used to identify certain structures of cells for further characterization, e.g. Gram staining used on bacteria for classification. Light microscopy is limited to larger particles, such as pollen, bacteria, or spores. More powerful microscopy, such as scanning electron microscopy or transmission electron microscopy are necessary to identify morphology of smaller particles. For an example, viruses, being on the nanoscale, would be visible only with transmission electron microscope. Air sampling filters and passively sampled glass slides can be imaged directly using light microscopy or scanning electron microscopy. A primary advantage of microscopy-based methods is that the biological material does not have to be viable; therefore, sample collection does not need to be as strict and careful. Flow cytometry is an additional method that can be used for enumeration of total microbial cells (Gales et al., 2015, Yasmeen et al., 2020).

Biochemical and immunological assays can also be used to analyze bioaerosol samples (Heikkinen et al., 2005). The limulus amebocyte lysate assay is a biological assay used for endotoxin analysis of Gram-negative bacteria (King et al., 2020). Adenosine triphosphate bioluminescence has been used to quantify the biomass in a sample (King et al., 2020). Immunoassays can also quantify biological composition of a sample using specific antigen and antibody reactions in order to quantify its specific or bulk proteins (King et al., 2020). These assays are therefore highly specific and sensitive. Some common types of immunoassay include radioimmunoassay, fluorescent immunoassay, enzyme immunoassay, and enzyme-based halogen immunoassay (King et al., 2020).

Culture-based methods are some of the more common ways to analyze bioaerosol samples. Several samplers (e.g. SKC BioStage and Cantium Scientific MicroBio MB2) collect directly into an agar plate which are required for culture-based analysis. Samples collected in a liquid or onto a dry filter following appropriate post-processing can be plated for culture as well. Under the correct growth conditions for the microorganism of interest, samples can be analyzed, and results can be traced back to understand the airborne concentration. Microorganisms can be characterized for viability (metabolically active) and culturability (ability to reproduce). In some cases, viable microorganisms can become non-cultivable due to sampling stressors (Heikkinen et al., 2005). Culture-based methods are better suited for sampling applications targeting a bacterial or fungal microorganism rather than a broad scan to identify all possible species.

The identity and biodiversity of a collected bioaerosol can be determined using sequencing and polymerase chain reaction (PCR). These techniques do not require viable or cultivable organisms and are useful for a wide range of biological agents (Heikkinen et. al, 2005; Mbareche et al., 2017). Sequencing is performed by utilizing genomic DNA that is isolated from an unknown sample and matched with a specific microorganism based on available libraries of genomic DNA from known microorganisms. Conventional PCR can be used to confirm the presence of a microorganism. Quantitative PCR (qPCR) can be used to quantify and approximate the concentration of a microorganism. Molecular methods such as sequencing or PCR are best for identification and not for determining factors like proliferation rates, which may be of interest for potentially infectious agents. Disadvantages to molecular methods are that protocols are difficult to standardize across laboratories, samplers, and individual project needs. Furthermore, sampling must be gentle enough to allow for DNA or RNA isolation downstream, robust enough to collect enough biomass for comprehensive analysis, which generally requires shorter sampling times and wet collection methods (Mbareche et al., 2017).

Chemical analysis is typically done by high-performance liquid chromatography or gas chromatography mass spectrometry and does not require viable samples. Targets of chemical assays for bioaerosol samples include compounds in bacterial or fungal cell walls, compounds specific to Gram-negative or Gram-positive bacteria, and aerosolized toxins and volatile metabolites. If coupled with cultivation methods, chemical analysis of cellular fatty acids can aid identification of microorganisms.

In summary, different post-sampling analytical methods will complement different sampling principles. Generally, physical collection efficiency (i.e., how representative the true airborne concentration and size distribution) and biological efficiency (how well is the sample preserved in terms of viability, culturability, integrity) must be balanced when choosing a sampler and analytical method. Understanding how sampling, collection, and analytical limitations interact is crucial in devising a sampling strategy that will effectively characterize the exposure. Choosing the correct bioaerosol sampler for the desired endpoint analysis is a significant step in any bioaerosol exposure assessment.

3.0 METHODS

Bioaerosol sampling technologies were evaluated for the following set of criteria (Table 1). These technologies were evaluated the application of field sampling for the widest range of biological agents in a small space, such as a cockpit. For this application, the instrument should be ruggedized, portable, have a small footprint, run on battery and collect a large volume to

increase the limit of detection. COTS sampling technologies were scored on a scale from 1 to 5, with 1 being the least useful (fulfills less criteria) to 5 being the most useful (fulfills more criteria). After an extensive literature review, seventeen technologies, with four of those being novel technologies, were down-selected for further exploration.

Table 1. Criteria and Descriptions

Criterion	Description
Product Status	Commercially available or novel (in development)
Collection Technology	Mechanism used to collect bioaerosols
Sample Recovery	Substrate or resuspension medium; determines possible offline analysis
Collection Time	Recommended sampling time
Flow Rates	Flow rate with collection time determines volume of air sampled
Particle Sizes Collected	Sizes of bioaerosols that are collected; determines application
Operating Humidity	Determines effect of environmental conditions on sampler performance
Operating Temperature	Determines effect of environmental conditions on sampler performance
Consumables	Related to long-term cost and possible ease-of-use
Dimensions	Determines portability
Weight	Determines portability
Power Source	Plug-in or battery-operated; rechargeable batteries or consumable
Automation	Sampler be programmed with user-specific sampling protocols

Additional criteria of interest include collection efficiency, sample recovery efficiency, viability of collected sample, and device ruggedization and cost. These criteria were not included in comparison tables because the data were not always readily available.

4.0 RESULTS

4.1 Commercial off the Shelf (COTS) Technology Ranking

COTS technologies were evaluated based on the criteria presented in Table 1. The instruments that received the highest score (5/5) included the Coriolis Recon, Coriolis Compact, SASS 2300, 2100 and 4100, BioCapture 650, and MD8 Airport Portable Air Sampler (Table 2). A full description of each technology is included in sections 4.1.1 – 4.1.4. The remaining instruments that were reviewed are shown in Table 3. A full description of each technology is included in sections 4.1.5 – 4.1.12.

Table 2. Highest-ranked COTS Bioaerosol Samplers Reviewed

Device	Coriolis RECON	Coriolis Compact	SASS 2300	SASS 3100	SASS 4100	BioCapture 650	MD8 Airport Portable Sampler
Score	5	5	5	5	5	5	5
Collection Technology	wet cyclone	dry cyclone	wet-walled cyclone	dry filter	two-stage filter	rotating impactor	filter and impactor
Sample Recovery	collection liquid	suspend into liquid	collection liquid	liquid with particle extractor	liquid with particle extractor	suspend into liquid	suspend into liquid
Collection Time	10 min to 6 h	10 min to 8 h	adjustable; days	adjustable; days	adjustable; days	5, 15, 30, 60 min	12 s to 4.5 h
Flow Rate (LPM)	600	50	325	50 – 300	primary: 4000 secondary: 265	200	30 – 125
Particle Collection Size (µm)	0.5 – 10	0.5 – 10	0.5 – 10	0.5 – 10	0.5 – 10	0.5 – 10	> 0.65
Operating Humidity (%)	not specified	10 – 90	non-condensing	all weather; optional rain shield	no limitations	up to 95 non-condensing	up to 85
Operating Temperature (˚C)	5 – 49	5 – 45	5 – 66	-40 – 70	-40 – 60	2 – 43	< 30
Consumables	cyclone vials	cyclone vials	sample vial	electret filter	electret filter	disposable cartridge	gelatin filters & BACTair sampling heads
Dimensions (in)	14.4 x 8.7 x 12.0	10.0 x 5.3 x 5.1	7.2 x 8.4 x 13.5	5.9 x 6.7 x 7.9	15 high x 10 diameter	7.0 x 14.0 x 6.0	11.8 x 5.3 x 6.5
Weight (lbs)	22	2.6	12.6	4.0	13.9	7.5	5.5
Power Source (battery life)	rechargeable lithium-ion battery (1-6 h)	rechargeable lithium-ion battery (8 h)	primary battery (> 20 h)	primary (> 24 h) & rechargeable battery (> 20 h)	compatible with car battery or AC supply	rechargeable lithium-ion battery (2 h)	rechargeable NiMH (4 h)
Automation	remote trigger	user-defined sampling protocols	user-defined protocols, optional wireless control	user-defined protocols; optional wireless control	user-defined protocols; optional wireless control	optional fully autonomous	none

Table 3. Remaining COTS Bioaerosol Samplers Reviewed

Device	Filter Cassette or Button Sampler with Sampling Pump	RCS High Flow Touch Microbial Air Sampler	SKC Biosampler	SKC BioStage Impactor	MicroBio MB2	BioSpot 300-P	DFU-1000 / 2000	XMX/2lMIL
Score	4	4	4	4	4	3	3	3
Collection Technology	active filter	centrifugal impactor	impinger	impactor	impactor	condensation particle growth + impinger	filter	impactor and impinger
Sample Recovery	suspend into liquid	Agar plates	collection liquid	Agar plates	Agar plates	collection liquid	suspend into liquid	suspend into liquid
Collection Time	adjustable; hours	not specified	up to 8 h	5 min to 4 h	12 s to 4.5 h	not specified	up to 12 h	not specified
Flow Rate (LPM)	1 – 5	100	12.5	28.3	100	8	1000	560
Particle Collection Size (µm)	filter-dependent	$\geq$ 1.2	0.01 – 10	$\geq$ 0.65	1.5 – 10	0.005 – 10	$\geq$ 0.1	1.0 – 10
Operating Humidity (%)	variable	20-80	0-95 (based on pump)	0-95 (based on pump)	Not reported	Not reported	Not reported	Not reported
Operating Temperature (˚C)	variable	5-40	0-40 (based on pump)	0-40 (based on pump)	Not reported	0-40	Not reported	0-50
Consumables	filters	Agar plates	collection liquid	Agar plates	Agar plates	collection liquid; wicks; Petri dishes	filters	Collection liquid, filters, tubes
Dimensions (in)	variable	4.6 (diameter) x 2 x 1.7	2 (diameter) x 6	4 (diameter)	7.7 x 3.9 x4.3	30 x 19 x 14.5	13x13x15	13 x 18 x 23
Weight (lbs)	1 – 2	3.3	~16 (with pump)	~6 (with pump)	1.65	65	42	38
Power Source (battery life)	variable	rechargeable lithium-ion battery	AC supply	AC supply; rechargeable battery (4 hr)	Rechargeable NiMH (variable)	AC supply	AC supply	compatible with car battery or AC supply
Automation	variable	User-defined or preset sampling protocols	None	none	Preset sampling protocols	none	none	none

4.1.1 Coriolis Recon and Compact

The Coriolis Recon is a wet cyclone collector capable of collecting bioaerosols ranging from 0.5 to 10 μm in diameter into a ~20 mL liquid sample for downstream analysis of biological material (Alam et al., 2012). Wet-walled cyclones and liquid impingers that use swirling liquid have the additional advantage of maintaining the viability of cells that can be killed by impaction onto solid surfaces (West and Kimber, 2015). Carvalho and colleagues found that the collection efficiency was about 50% for particles with aerodynamic diameters of about 0.5 μm, and the collection efficiency was about 92% for 10 μm particles (Carvalho et al., 2008). The Recon is portable (lithium-ion rechargeable battery), light (22 lbs), and has been ruggedized. Additionally, it has been designed to collect low and high concentrations of airborne biological pathogens with an adjustable airflow rate of up to 600 L/min. Furthermore, sampling time can be set for 5, 10, or 15 minutes or programmed for a long-time collection period of up to 6 hours (Bertin Instruments, 2016; Global CBRN Detector Market Survey, 2018).

The Coriolis Recon was successful in collecting and concentrating botulinum neurotoxins (BoNTs), *Clostridium perfringens* epsilon toxin (ETX), staphylococcal enterotoxin B (SEB), Shiga toxin (STX), and plant toxin ricin that were aerosolized by a nebulizer (Alam et al., 2012). These protein toxins are involved in numerous diseases and are considered potential agents for bioterrorism and biowarfare. The Coriolis Recon does not have a built-in detection unit; however, the collected liquid sample is compatible with any type of molecular downstream applications such as PCR, immunoassays, or any other detection methodology (Bertin Instruments, 2016).

A sister unit to the Recon is the newer (October 2019) Coriolis Compact. Similar to the Recon, this unit is cyclonic but uses dry collection. The dry sample is reconstituted into the sampling liquid of choice. Similar to the Recon, the Compact collects particles 0.5-10 μm in size. This system is rated for indoor or outdoor use and has a maximum flowrate of 50 L/min. Similar to the Recon, the Compact can sample autonomously for several hours, has a lithium ion battery, and samples can be analyzed using culture-based methods, next-generation sequencing methods, or PCR techniques. This unit is much smaller than the Recon, weighing only 4.4 lbs. Given its footprint, the Compact is attractive for uses in small spaces and is also the least expensive of the Coriolis units.

4.1.2 SASS 2300, 3100 and 4100+

The next three devices are different models of the Smart Air Sampling System (SASS) that was developed by Research International: the SASS 2300, SASS 3100 and the SASS 4100 Plus. First, the SASS 2300 is a highly efficient, portable multi-stage wetted-wall cyclone sampler that extracts particulates and water-soluble chemical vapors from air and transfers them to a liquid phase for later detection and analysis. This device can collect airborne pathogens, particulates, bacteria, and spores and employ extremely long sampling protocols that can last almost two days to sample low aerosol concentrations. Furthermore, single or multiple units can be controlled wirelessly, and users can upload their own customized sampling protocols into the units. To prevent cross-over contamination from one sample run to the next, the SASS 2300 has a rinse protocol. Additionally, the SASS 2300 can operate in temperatures near 0°C and up to 66°C. The SASS 2300 weights 12.5 lbs. Therefore, the device is highly automated, user-friendly, and can

be easily carried by a single person and may operate unattended for extended periods of time which is ideal for an Airman to use in an operational environment.

The SASS 2300 has been successfully used to collect and detect the airborne viral pathogens that cause Exotic Newcastle disease and hoof-and-mouth disease, as well as some strains of the avian flu virus (Hietala et al., 2005). The capability of efficiently collecting viral pathogens (approximately 150-300 nm) and maintaining their viability for downstream detection is very attractive. Furthermore, in comparison to four other liquid-based sampling devices for monitoring cultivable, viable, and total *Legionella pneumophila* (which can cause Legionnaire's disease or nonpneumonic Pontiac fever) around cooling towers, the SASS 2300 scored the highest regarding detection rates (Chang and Hung, 2012).

The next SASS device is the model 3100, also developed by Research International, which operates by using a proprietary electret dry filter media with a high-efficiency centrifugal fan. It has an adjustable sampling rate from 50 to 350 L/min controlled by an onboard microprocessor that can be programmed for different types of fieldwork using a bundled PC software package. The proprietary component of this device is the disposable snap-on filter disc element that is made of a felt-like polymer. Each fiber in the disc has an electric field frozen into it via a proprietary process which induces a charge in aerosols passing through the filter and provides a capture mechanism much more effective than impaction and up to 50 times more efficient than conventional glass or cellulosic filters (Kesavan et al., 2010). This standard 44 mm diameter electret filter samples at a maximum rate of 300 L/min and has a collection efficiency of 50% at an aerosol particle diameter of 0.5 μm. A second HEPA-style electret filter that is physically interchangeable has 95%+ collection efficiency for particles greater than 0.3 μm in diameter. Dybwad et al. (2012) used this device to characterize the airborne bacteria in an underground subway station where it was successful in the collection of a total of 37 different genera of bacteria.

One potential downfall associated with the SASS 3100 is that biological particles are collected on a dry filter disc. If the sample is left alone on the collection disc, then these particles can lose viability over time due to desiccation (Lindsley et al., 2017). In response to this, Research International has developed a particle extractor that allows the operator to extract the collected sample into a liquid form for prolonged viability and back-end analysis, called the SASS 3010. This extraction process is simple and takes 1-2 minutes.

Like the SASS 2300 model, the SASS 3100 has the same customizable, programmable, and remote operation capabilities thus making it a highly automated and field-deployable bioaerosol sampler. Additionally, both SASS models have a 20+ hour rechargeable battery that allows for a quick battery swap in challenging situations. The 3100 model, unlike the 2300 model, has the additional ability to also collect radioactive samples.

Finally, the SASS 4100 Plus model is a commercially available device; however, its technology is still patent pending. The 4100 Plus is a two-stage filter-based aerosol collection device that uses the same proprietary electret dry filter media as the SASS 3100. This unit can collect airborne pathogens, virus-sized particulates, bacteria, and spores. The SASS 4100 Plus processes over 4000 L/min of ambient air that is continuously sampled into a primary air stream. Particulates in this air stream are transferred to a much smaller secondary air stream using centrifugal and virtual impaction principles. Particles are then routed into the secondary flow by forcing primary circuit air to circulate through specially shaped channels where centrifugal force

and particle momentum isolate and concentrate the particles. This increases the aerosol concentrations in the secondary flow 4-15 times higher than the incoming air and therefore, the two-stage sampler amplifies and slows down the captured ambient aerosol particles prior to their collection.

Like the 3100 model, this dry sampling method has several favorable attributes. Firstly, the primary fan rotor is the only moving part, therefore, maintenance on this system is minimal. It has a wide operating temperature range of -40°C to 70°C. Moreover, sampled air volume is maximized by using low airflow velocities and a bulk electret filter media thus improving collection statistics and organism viability (Tradeways, 2009). The electret filter is mounted in a compact, easily disengaged holder located under the SASS 4100's baseplate. To further increase organism viability, the manufacturer recommends using the SASS 3010 particle extractor like the one previously mentioned for the SASS 3100.

Like the SASS 3100, the filter collection efficiency for the HEPA-style filter is more than 95% for particles with a diameter of or above 0.3 µm and for the bioaerosol filter is has a cutoff diameter (collection efficiency of 50%) of 0.5 µm. The unit is field ready weighing only 6.4 lbs and comes with extendable quick-detach tripod legs for easy surface mounting. Additionally, the tripod legs allow for the circumferential sampling section to be located from about 0.6 m to 1.43 m above the tripod mounting surface. One disadvantage is that the unit operates on two primary batteries as opposed to a rechargeable batteries, therefore slightly increasing consumable costs and potentially hindering field use if the operator does not have back-up batteries.

4.1.3 BioCapture 650

The BioCapture 650 is a small handheld device that actively collects particles with a rotating impactor/impeller onto an impactor plate from which a water-based collection fluid washes the sample into a collection vial. Air can be exhausted separately from the liquid that is optimized for minimum evaporation but a maximum flow rate (Edgewood Chemical Biological Center, n.d.). The BioCapture 650 has the capability to collect particles from 0.5 to 10 µm. The BioCapture is also rated for harsh environmental conditions that are representative of possible field settings. It can be operated in temperatures of 2 - 43^{o}C and in 5 - 95% relative humidity (non-condensing). The equipment is powered by a rechargeable lithium-ion battery that has a two-hour life with a full charge.

The BioCapture is operated by a single button. It was initially designed to be used by first responders in the event of a biological threat or chemical attack. The ability to operate by one button makes it very simple to use when the responder is wearing Level A or MOPP-4 (highest level of Mission Oriented Protective Posture gear) personal protective equipment (PPE). MOPP-4 PPE is used when the risk is the greatest, such as when unidentified chemical or biological agents are present. Since these suits are bulky and hard to maneuver when worn, the ease of use with a device is extremely important, making the BioCapture an attractive option.

A research comparison study of multiple sampling devices discusses the BioCapture 650 in its collection of *Bacillus thuringiensis* subspecies *Kurstaki* (BTK) and male-specific coliphage 2 (MS2) as a surrogate for viruses. The authors showed that the device ranked higher in total operational performance collecting over a median total count of $3.0x10^{7}$ CFU/L of BTK and over $1.0x10^{8}$ PFU/L for MS2 when the collection time was shorter; however, the total count of MS2

decreased with a higher sampling time (Enderby et al., 2012). The study concluded that the BioCapture 650's high sample time options can present unfavorable for the collection of MS2 and viral agents due to a possible loss of aerosolized virus particles. It is still a valid option for use, but modifications should be considered for viral collection.
Another unique feature of the device is the ability to directly use the solution that contains the sample to be applied to detection devices such as Hand Held Assays (HHA) via a test strip port. It is important to note that detection capabilities are not the main priority, however, this feature in addition to other characteristics sets this technology apart. For field use, this device would be all-encompassing, time saving, and provides rapid detection of agents.

4.1.4 MD8 AirPort Sampler

The MD8 Airport Sampler is a portable handheld bioaerosol sampler produced by Sartorius. Its sampling mechanism is membrane filtration and impaction, typically onto 80 mm, 3 µm pore size gelatin filters or BACTair bacterial culture media plates (both also available from Sartorius). This sampler can draw air at 30, 40, 50, or 125 LPM to recommended sample volumes of 25, 50, 100, 250, 500, 700, or 1000 L. It has a nominal d50 of 0.65 µm and has a 99% efficiency for collection of *Bacillus sub. Niger* spores and coliphage T3 with the gelatin filter (Sartorius). This sampler also has a rechargeable battery and weighs only 5.5 lbs, proving its portability.

As discussed previously in the Background section, gelatin filters are vulnerable to high relative humidity (> 85%), high temperatures (> 30 °C), and high sampling times. The built-in flowrates and sampling volumes for the MD8 result in a maximum collection time of 33.3 minutes (30 LPM, 1000 L of air) to control sampling time. The gelatin filters can be directly cultured on agar after sampling and incubated for microbial growth, enumeration, and further analysis. Recently, the manufacturers have also published a protocol to perform PCR on MD8 gelatin filter samples (Scherwing and Patzelt, 2020). However, the BACTair plates are like the MB2's aluminum sampling heads with 400 sampling holes, but unlike those sampling heads are attached to the culture media plate. The advantage of this approach is that sterilizing sampling heads is no longer necessary and whole sample plates can be changed out as needed in the field. The manufacturers tested the BACTair plates with aerosolized *S. epidermidis, B. subtilis,* and *P. citrinum,* demonstrating higher microorganism recovery and less susceptibility to desiccation effects compared to a slit-to-agar sampler, a sieve impactor, and a centrifugation sampler (Sartorius).

Several research studies have been published characterizing the MD8 sampler or using it in bioaerosol field sampling work. Engelhart et al. tested the MD8 against aerosolized thermotolerant fungi (*Aspergillus fumigatus*) in a naturally ventilated laboratory alongside a Merck MAS-100 for comparison (Engelhart et al., 2007). Both samplers had good agreement for how many microorganisms were captured and successfully cultured (the MD8 sample was plated from the gelatin filter, whereas the MAS-100 sampled onto an agar plate analyzed directly), showing their usefulness for fungal sampling over extended sampling periods (12 days). Similarly, Lewandowski et al. (2013) evaluated the MD8 for detection of *Bacillus atrophaeus* endospores in a controlled chamber simulating indoor air conditions. The MD8 was compared against the BioSampler, Anderson viable impactor, and MAS-100. The authors found that the AirPort MD8 was the most efficient of the three samplers for collecting culturable spores from small and large volumes (0.1-1 m^3), and the MD8 sampler was able to detect *Bacillus atrophaeus* endospores at the low concentration of spores used (<10^3 CFU/m^3), demonstrating a good

detection limit. The MD8 has also been used for virus detection at poultry farms (Jonges et al., 2015). The authors used the MD8 with 8 um pore size cellulose nitrate filters and sampled for virus for 20 minutes at 50 LPM, showing that the sampler filter can be versatile. Both the MD8 filters and low-volume PTFE filters were tested for influenza and endotoxin recovery. No virus isolates were recovered from the MD8 samples, but endotoxin was detected on some, corroborating positive endotoxin PTFE filter samples.

Finally, the MD8 was used in a study simulating an air cabin environment (Grün et al., 2008). This study also investigated parameters such as air cabin pressure and their health effects (measured via oxygen saturation in peripheral blood flow) on volunteers after a 7-hour flight simulation. The MD8 was used to sample for fungal and bacterial contamination alongside Grimm Portable Dust Monitors measuring particulate matter as part of an overall air quality assessment. Measurements taken throughout the flight indicated that pre- and post-baseline (30 minutes each) CFU/m^3 for bacteria and fungi were the same. During cruise, bacteria counts decreased but increased later during the cruise; fungal count was the opposite. This study shows that the MD8 can collect microorganisms in relevant aircraft environments and detect changes throughout the flight.

4.1.5 Air Sampling Filters

Air sampling for bioaerosols with traditional 25 or 37-mm filters is still a feasible low-flow option in a compact space and for personal sampling. Literature suggests that specialized filter-based samplers will be advantageous over a traditional sampling cassette in certain conditions, i.e. the Button sampler (Wang et al., 2001; Wang et al., 2015). Filter cassette sampling was shown to be the most efficient collection method of airborne *C. difficile* spores compared to an impinger and a slit-to-agar system (Cooper et al., 2019). While culture-based analysis is not always the most effective or comprehensive post-sampling analytical technique, depending on the bioaerosol, filter samples can still be used for molecular approaches. However, extraction procedures should be carefully characterized with appropriate microbial surrogates, since Wang et al. demonstrated that although recovery efficiency of PC filters reached ~97% for their target microorganism, the culturability was only 43% for different spores (Wang et al., 2001). Similarly, as stated previously, extraction procedures should be gentle enough to maintain RNA and DNA integrity, if not 100% viability and/or culturability.

Several COTS filter media have been thoroughly studied by various research groups. Gelatin filters can dissolve directly into agar for culture-based analysis but have the drawbacks of desiccating if flowrates or sampling times are too high or dissolving in high humidity environments (Cooper et al., 2019). However, gelatin filters in Button samplers particularly have been studied and used for effective bioaerosol capture in several studies (Wu et al., 2010; Burton, et al., 2007; Burton et al., 2007). MCE filters were used for colony growth after enough absorption of agar media (Cooper et al., 2019) but were also successfully used to sample and extract *Bacillus globigii* spores in a laboratory experiment (Burton et al., 2005). In Cooper's 2019 study, however, a specific COTS chromogenic agar tailored to culturing *C. difficile* spores was used for CFU enumeration from all samplers, so Cooper's culture method may not be applicable for all microorganisms. PC filters are also useful since black filters can be used for fluorescent microscopy and have been successfully used for extraction (Wang et al., 2001).

Wang et al. studied the effects of sampling time (2 min; 8 h), selection of personal filter sampler (Button Inhalable Aerosol Sampler; 37-mm filter cassette), and relative humidity (30%, 85%) were examined on black 0.2 µm PC filters. Challenge aerosols included *Penicillium melinii* and *Aspergillus versicolor* fungal spores and *Bacillus subtilis* bacterial spores, produced and sampled in a controlled laboratory experiment (Wang et al., 2001). The extraction efficiencies of PC filters were as high 96-98%, for the most efficient extraction method tested. Microscopy and culture-based colony count were the offline analyses used and for both samplers and fungi relative culturability decreased over time for both relative humidity conditions, indicating that relative humidity is an important factor when bioaerosol sampling. Overall, the authors concluded that PC filters can achieve a high extraction efficiency; fungal spores can survive longer sampling times and are more effectively sampled this way for culture; higher relative humidifies generally increased culturability; and the Button sampler outperformed the filter cassette for *B. subtilis* spores at high relative humidity.

Burton et al. examined PC (1 and 3 µm pore size), PTFE (0.3, 0.5, 1, 3 µm pore size), and gelatin (3 µm pore size) filter performance in a controlled laboratory environment against bacterial, viral, sodium chloride and polystyrene bead latex (PSL) bead aerosols (Burton et al., 2007). While almost all tested filters had high (>90%) physical collection efficiencies for the bacteria and PSL beads, only the 0.3, 1, and 3 µm PTFE and gelatin filters had > 90% physical collection efficiencies for the viral MS2 aerosol. Additionally, the 3 µm and 0.3 µm PTFE filters outperformed the PC filters for sodium chloride particles ranging 10-1000 nm. Overall the authors recommended the PTFE filters and found success using them with the Button sampler for longer sampling times in a previous work (Burton et al., 2005). However, no extraction processes for biological analysis were performed in this study, and other researchers' work showed that PTFE filters were difficult to extract from due their hydrophobicity, compared to PC filters (Wang et al., 2015).

Wang et al.'s 2015 study examined several types of filters (PC, gelatin, and PTFE) and personal samplers (Institute of Medicine (IOM) Inhalable Dust Sampler, Button Inhalable Aerosol Sampler, and National Institute for Occupational Safety and Health (NIOSH) Personal Bioaerosol Cyclone Sampler, and a traditional 37-mm filter cassette sampler). In their study, PC was the most efficient for collection, both in their results and literature review. Each extraction solution was split for culture-based enumeration of culturable microbes and for microscopy for total bacterial count. The PC filters revealed slightly more culturable bacterial species, but the gelatin filters recovered slightly more fungal taxa. The authors acknowledge that filter pore size plays a role given inconsistent result with similar studies evaluating the same filter types (Burton et al., 2007). Regarding samplers, the IOM and Button samplers recovered bacterial taxa and culturable fungi the most, and the Button sampler recovered the highest concentration of total culturable bacteria. While the Button sampler generally outperformed the other samplers in terms of highest recovered concentrations and biodiversity in all cases sampled, the authors recommended the IOM sampler for high dust environments due to its lower flowrate and thus resistance to clogging. This work did not compare to actual bioaerosol concentrations or investigate molecular methods, but the findings for relative efficiencies between samplers and filter types are still helpful.

Gales et al. (2015) used polyethersulfone (PES) filters in an Electrical Low-Pressure Impactor (Dekati) to sample of bacterial cells in compost bioaerosols. PES membrane filters are hydrophilic membranes with low protein binding and has been used for removal of fine particles,

bacteria, and fungi in aqueous applications. In this study, a glycerol coating was applied to prevent particle bounce off these filters and the Electrical Low-Pressure Impactor was operated without charging the incoming aerosol depositing on each filter. The extraction method was more involved than the one used by Wang et al. in 2001 and is described in greater detail in Le Goff's study in 2010. Post-sampling analysis included culture-based CFU count, qPCR, and flow cytometry showing that the same filter-based samples can be analyzed in several ways.

Glass fiber filters have similarly been used in a cascade impactor (non-viable Andersen impactor) to sample for spores and analyzed via quantitative real-time PCR advanced DNA sequencing (Yamamoto et al., 2014). For sample extraction, the commercially available kit PowerMax Soil DNA Isolation Kit with modified steps was used (Yamamoto et al., 2014). These studies using cascade impactors calculated the aerodynamic diameter of each microorganism sampled based on the number of genomes detected during qPCR for each impactor stage (Yamamoto et al., 2014; Gales et al., 2015). Estimating an aerodynamic diameter for real-world microbial samples can be crucial information not only for health effects upon inhalation but also for generating bioaerosol sampling standards. While a BEE shop may not have cascade impactors on hand for collecting multiple size fractions, size-selective filter cassette sampling with cyclones could generate similar, if less precise, data.

Overall, filter-based sampling with traditional cassettes and media can still be an effective method for personal or static low-flow sampling, provided normal relative humidity conditions. Ideally filter sampling could be a component of a larger sampling study using several pieces of equipment to fully characterize a possible exposure and using more sophisticated size-selective samplers such as the Button sampler or even the IOM sampler could improve downstream data quality. Similar to general considerations for selecting a bioaerosol sampler, careful consideration must be given to the filter type as well. PC filters with pore size 0.8 μm seems to be a promising general-use sampling filter suitable for several analytical methods, but other filter types may be advantageous depending on the situation. The analytical method, sampling flowrate, and duration must be carefully chosen based on the agent of interest, sampling limitations, environment, and occupational health concern.

4.1.6 Reuter Centrifugal Sampler (RCS) High Flow Touch Microbial Air Sampler

The RCS device has been in use for about 30 years, primarily in the food and pharmaceutical industries. The RCS uses the principle of centrifugal impaction, which allows for a low impact speed, with low turbulence and airspeed, leading to better viability and reproducibility. The sampler uses a standard agar sampling substrate, for which different agar materials can be purchased depending on the pathogen of interest (Millipore Sigma, 2019). The RCS can be autoclaved and disinfected as needed and has a short warm-up time of one minute before sampling. The sampling time can be kept short due to a flow rate of 100 L/min, with a particle cutoff diameter of 1.2 μm and a detection limit of 500-600 CFU (Zhen et al., 2009). The collection efficiency increases as the particle size increases (Macher and First, 1983).

Zhen et al. conducted a comparison study with the RCS with another impaction-driven agar plate sampler, SKC's BioStage. The study was conducted in five different environments in Shanghai, China which include a train station, subway, hotel room, Honqiao Airport and a riverfront landmark known as "Wai Tan" (Zhen et al., 2009). While trends for each of the devices were similar in collection efficiency; however, the RCS had a higher bacterial and fungal culturability

with shorter sampling times, an advantage over the BioStage (Zhen, et al., 2009). Thus, this study shows that the RCS HighFlow can be effectively used in outdoor environments more typical of occupational hazard sampling than food science or pharmaceutical labs and cleanrooms. Shorter sampling times are required for the RCS than other impactors.

4.1.7 SKC BioStage

The SKC BioStage is a commercially available agar impactor bioaerosol sampler. It is a newer equivalent to the older single-stage viable Andersen N-6 sampler with improved sealing and meets the criteria given by NIOSH for bioaerosol sampling (Lindlsey et al., 2017). The autoclavable aluminum BioStage operates at 28.3 L/min, like the Andersen N-6, and is used mainly for bacterial or fungal sampling and culture. A 400-hole correction is applied to account for errors in enumeration arising from sampling biases. The BioStage has an impact velocity of 24 m/s and is compatible with any type of agar plate with a cutoff diameter of 0.65 µm. Some favorable attributes include: lightweight (~5.5 lbs when mounted to its pump), small footprint (23.6 x 21.3 x 8.9 cm), rechargeable lithium-ion battery, and samples onto 90-100 mm agar plates. The only limitations is direct-to-agar impactors; nonetheless, it is a widely used bioaerosol sampler, even considered a gold standard (Chen et al., 2018).

Xu and Yao (2011) compared the Biostage to several other widely used active samplers (Button sampler with MCE filter or gelatin filter, the BioSampler, the RCS High Flow, and an electrostatic device) to determine bacterial and fungal aerosol diversity in an indoor and outdoor environment. Their post-sampling methods included culturing, PCR, and denaturing gradient gel electrophoresis (DGGE) to determine biodiversity. The study found that the BioStage impactor was the most sensitive sampler for culturable bioaerosol diversity (determined via PCR and DGGE) for the outdoor samples. The authors concluded the BioStage was a robust sampler but also discussed the microorganisms' degree of embedding into the agar due to the high impaction velocity and the possibility of particle bounce as the agar hardens due to desiccation. Overall, this study showed that the BioStage can be an effective sampler in outdoor environments when samples are analyzed with molecular methods.

Chuang et al. (2013) used the BioStage with *B. subtilis* and *E. coli* bacterial aerosols in a controlled laboratory chamber to evaluate a bacteria inactivation method using neutral electrolyzed water for mitigating biological contamination in agricultural and food-processing facilities. Culture-based methods were used to evaluate the process, study design, and sampling to be in accordance with Taiwan Environmental analysis Laboratory guidelines (NIEA E301.11C, Taiwan Environmental Protection Agency). In contrast, Chen et al. (2016) focused on seasonal indoor air quality (IAQ) in various subway stations. While bioaerosols were not their only focus, they used the BioStage for impaction-based collection and culture-based analytical methods of bacterial and fungal contaminants. The BioStage was effective enough to determine that the overall bacterial CFU/m^3 count exceeded the indoor air quality standard of 1500 CFU/m^3, whereas the fungal standard was 1000 CFU/m^3, per Taiwan's Indoor Air Quality Management Act. These studies show that the BioStage can successfully be used to evaluate different environments against regulatory recommendations not only from NIOSH but other international regulating bodies as well. However, these studies also highlight the BioStage's portability and applicability to culture-based analyses and molecular methods, but also its limitations, namely the type of microorganism it can sample and inherent sampling stressors.

4.1.8 SKC BioSampler

The BioSampler by SKC, Inc. is an all-glass collection system that superficially resembles an impinger device. The SKC BioSampler's flow nozzles mimic the flow path through the human nose, an ideal aspect when considering personal bioaerosol collection units (SKC, 2019). The BioSampler is used in conjunction with a sonic flow pump operating at 12.5 L/min. Once the bioaerosol travels through the inlet, the particles follow an angular path and are directed through the tangential flow nozzles depositing them into the liquid medium in the collection vessel. This method is utilized to ensure trapping of particles and bacterial aggregates and increasing the collection efficiency as opposed to other standard impingers (SKC, 2019). Since the BioSampler utilizes three nozzles, the airflow allows for a gentle swirl to move the particles in the medium, minimizing the risk of re-aerosolization. The liquid of choice swirls around the walls of the collection vessel and bubbles in the entrapment chamber to ensure particles on the walls will be suspended in the liquid. A mineral oil can be used with the sampler to allow for constant collection efficiency and would likely decrease any desiccation or re-aerosolization during sampling. A disadvantage with using a higher viscosity liquid to collect samples would be that it may not be compatible with PCR analysis, unlike sterile water (SKC, 2019).

Collection efficiency for particles larger than 1.0 μm is close to 100% while decreasing to about 90% when the particle size is between 0.2-0.5 μm, compared to an older impinger the AGI-30 (SKC, 2019). It should also be noted that the SKC BioSampler had a collection efficiency of 50% for particles with an aerodynamic diameter less than 0.2 μm, which could be valuable for collecting viral or protein bioaerosol particles (West and Kimber, 2015).

While the glass BioSampler cannot be taken onboard aircraft during flight, a key operational environment needing bioaerosol sampling protocols, this instrument is still the current gold standard bioaerosol sampling device to evaluate other technologies against and would be a staple for further research (Cooper, 2010; Dybwad et al., 2014).

4.1.9 MicroBio MB2

Similar to the SKC BioStage, the MicroBio MB2 is a highly portable direct-to-agar impaction sampler and produced by Cantium Scientific. It has a higher flowrate of 100 LPM, and higher cutoff diameters. Depending on the sampling head used, the MB2 has a higher cutoff diameter (1.35 or 1.7 μm) and the particle impaction velocity is 9.65 or 10.83 m/s. The 220 hole x 1 mm stainless steel head (1.7 μm, 9.65 m/s) is standard, and the 400 hole x 0.7 mm anodized aluminum head is recommended for a higher collection sensitivity. Both sampling heads collects sample onto a 90 mm Petri dishes or for the standard head, 55/60 mm contact plates. Comparable to the BioStage, the MB2 requires positive hole correction for coincident colony counts, but the manufacturer includes Excel spreadsheets for rapid calculation. The main advantage of the MB2 is its high flowrate and light weight of 1.7 lbs. It also has a built-in tripod and is operated with 4 primary AA batteries that can be recharged in 120 minutes.

In a comparative study of various bioaerosol samplers used in the United Kingdom, Griffiths et al. also reviewed the MicroBio MB1 and MB2 (Griffiths et al., 1999). Sampler performance was evaluated with test aerosols of *Saccharomyces cerevisiae* cells and *Penicillium expansum* spores by culture colony count post-sampling. Different humidity levels were also tested, which

increased all samplers' culturability regardless of which sampler was used. Interestingly, they also found that the MB2's sampling inlet was not orientation-dependent, and post-sampling results were not significantly different. Overall, they concluded that the MicroBio MB systems were "found to be adequate as…reference sampler[s] but do not provide total counts" (Griffiths et al., 1999).

Other studies have successfully used the MB2 in commercial shell egg processing facilities (Northcutt et al., 2004), a respiratory ward (Roberts et al. 2005), and university libraries (Flores et al., 2014). In Northcutt's study, total aerobic bacteria, molds, yeasts, coliforms, and pseudomonads was calculated from 4000 L of sampled air (using four different MB2 samplers). Culture-based methods were used to enumerate plated colonies and used agar specific to the microorganisms of interest. Roberts et al. evaluated aerosol production related to nosocomial infections in a hospital respiratory ward, specifically particles 0.3-5 µm using the MB2 and an optical particle counter in tandem (Roberts et al. 2005). Based on their size-resolved particle count data and culture-derived data identifying *Staphylococcus aureus*, it was concluded that *Staphylococcus aureus* could have arisen from skin debris (sized 4-25 µm). Lastly, Flores et al. conducted studies both indoors at a university library and outdoors directly outside of the university (Flores, 2014). The study was focused specifically on fungal spore concentrations and how the concentration changed with meteorological variables (temperature, humidity, wind speed, direction, etc.). The authors found eleven fungal genera, (the main strains being *Aspergillus niger*, *Aspergillus tamarii*, and *Aspergillus oryzae*), based on colony counting, microscopy, and PCR. Fungal spore concentrations were higher for low relative humidity and increased windspeed, and a negative correlation was associated with barometric pressure, relative humidity, and dew point. Although the authors did not discuss sampler effects on their results, this study shows that the MB2 can be used in a variety of outdoor conditions.

Altogether, the literature shows the flexibility the MB2 offers for sampling in unique indoor and outdoor environments and its validity as a microbial sampler. Furthermore, it has been shown to yield not only culture-derived data but also PCR-derived data. Although specific empirical collection efficiencies were not reported, the MB2 was shown to be a valid reference sampler when used within its limits: primarily targeting larger fungal or bacterial microorganisms for culture-based methods of analysis. While its relatively high flowrate, small footprint, and high viability are significant advantages, the MicroBio MB2 is an agar impactor and suffers some drawbacks which includes: sample stressors potentially reducing culturability, not fully capturing viral, protein, or ultrafine fragmented microorganisms.

4.1.10 BioSpot™ 300P

The BioSpot^{TM} 300P by Aerosol Devices is an enhanced bioaerosol air sampler designed to efficiently collect aerosolized viruses, bacteria, spores, toxins, and exhaled proteins. Its applications have been best suited for airborne disease transmission and bio-surveillance monitoring in various locations including both heavily populated areas such as in public transportation settings, medical or laboratory facilities as well as environmental or agricultural sites (Aerosol Devices, Inc). The instrument is uniquely described as performing equivalent to a human lung with a flow rate of 8 L/min. This instrument's laminar-flow water condensation particle growth mechanism uses a condensation growth tube to capture concentrated samples then impinging droplets into either a water, buffer, or nutrient broth. This process is to assist in

the improvement of collection efficiency for particles ranging as small as 5 nm (Environmental Expert).

When compared to a wet-walled cyclone and the SKC BioSampler, the BioSpot was found to have 100% collection efficiency from 10 nm to 10 µm, whereas the wet-walled cyclone could not efficiently collect particles below 1 µm and the BioSampler was 50% efficient or less for particles under 0.3 µm (Aerosol Devices Inc.). However, limited studies are available for validating the BioSpot for bioaerosol collection and subsequent maintenance of viability for representative bacteria, fungi, or viruses. The device is rated for environmental settings; however, it is not portable as it is too heavy (65 lbs) for practical field deployment.

4.1.11 Dry Filter Unit (DFU) 1000/2000

The DFU-1000 and -2000 are air sampling devices heavily used by the military. The DFU-1000 weighs 42 lbs, making it more suitable for stationary use, lowering its overall score. The DFU-1000 is comprised of a protective casing with a 47-mm polyester felt filter on top and once power is applied a dry vacuum airflow is routed through the manifold towards the filter cartridges. Once the collection is completed, filters can be removed and suspended in liquid media for particle extraction and analysis.

The DFU-1000, designed for indoor sampling, is a primary component to the DFU-2000, which is updated with a protective enclosure and pre-separator stack attached on top. Like the XMX/2L-MIL, the pre-separator stack removes large particles (greater than 200 µm) from the airstream. The pre-separator stack is an accessory piece that only comes standard with the DFU-2000 composition and if desired with the DFU-1000 an adapter must be utilized (Army Publishing Directorate, 2016). DFUs collect and process all known biological warfare agents with a flow rate of up to 1000 L/min. It features up to 12 hours of collection time, operating temperatures in climates from -40°C to 50°C, battery-operated, and minimal training. Filter samples can be quickly recovered from the instrument and confirmatory testing can be done onsite with the necessary detection kits, making it ideal for field-deployable use (Army Publishing Directorate, 2016).

The DFU-2000 was tested in a research study against two strands of *B. thuringiensis* subsp. *Kurstaki* (BTK), barcoded (BTKb) and wild-type (BTKw), as the aerosolized agent to be collected (Emanuel et al., 2012). The goal of this study was to determine if the genetically tagged or barcoded bacterial strains were distinguishable from naturally occurring BTK (or BTKw), and if it could be reaerosolized in controlled conditions and in an outdoor environment. Multiple DFUs were deployed in a 12-day period, operating in an 8-hour time frame to collect aerosolized bacteria for offline analysis. The experimental design was to aerosolize 100 g ($1.1x10^{13}$ spores) of BTKb on day one, and on day eight, 87 g ($2.6x10^{13}$ spores) batch of BTKw. Results revealed that CFU/mL values were higher upon immediate release of the spores of both strains (Emanuel et al., 2012). This study's use of the DFU-2000 illustrates that it can be deployed into outdoor conditions and can reliably collect aerosolized agents at different concentrations over time.

A comparative study was conducted evaluating the performance of the DFU-1000 and XMX-2L/MIL in the viral aerosol sampling of a MS2 (Male-specific coliphage 2) challenge in which the virus was disseminated in a test chamber. Results showed that the DFU was ineffective in reliably collecting low concentrations of MS2, unlike the XMX-2L/MIL, which

had median sampled concentrations of PFU/(L_{air} * agent-containing particles per liter of air) 4-5x higher than the DFU (Cooper et. al, 2010).

4.1.12 XMX/2L-MIL

The XMX/2L-MIL is a device currently used by the AF and was specifically developed to service outdoor sample collection needs. It is marketed as a rapid and efficient high-volume air sampler servicing in-field use under harsh conditions with sampling times as short as five minutes. The device is designed for easy configuration and minimal required training. The internal components can be easily decontaminated thus reducing the risk of potential cross-contamination between collection missions (Life Safety Systems, Inc., n.d.).

This is a multi-component system consisting of air separation and concentrating mechanisms. This is accomplished with a combination of two collection methods: impaction and impingement. As large volumes of air gets drawn in, large particles and debris from the airstream gets separated and smaller particles of interest gets concentrated using a virtual impactor. Specifically, the primary flow path makes an abrupt turn and larger particles with high inertia to follow "fall out" of the airstream, while the secondary flow path carries the concentrated particles of interest (in this case, 1-10 μm). Subsequently, a vacuum flow pump draws in the secondary flow into a centrifuge collection vial containing liquid, impinging the particles. The collection liquid is typically phosphate buffered saline (PBS) buffer, sterile water, or a specific surfactant requested based upon collection requirements and maintained for confirmatory testing (Black, 2011). Another option for sample collection and preservation instead of a liquid medium is a dry-filter component available COTS (Dycor Technology, n.d.).

Limiting features include weight (37 lbs), power requirements in the field, and inconsistency in configurations of primary and secondary flow rate standards issued by the manufacturers. Research has presented modified primary and secondary flow rates to fit necessary preferences and/or requirements. According to the manufacturer, the primary intake flow has a standard of 800 L/min and secondary flow rates of 12 L/min. However, parameter testing studies have demonstrated that those values may not be optimal rates across all agents. More testing is required to negate the risk of false positives (Black, 2011). When utilized for the collection of aerosolized MS2, an initial operating flow rate of 600 L/min was set and results revealed a 25% sampling efficiency in comparison to glass impingers testing at low and medium aerosol concentrations (Ho, 2011). Another study was conducted to investigate the performance of the XMX/2L-MIL focusing on the secondary flow rate for capture and retention efficiency (CRE), concentration ratios (CR) as well as the limit of detection (LOD) (Black, 2011). In this case, secondary flow rates were decreased to 5 and 10 L/min which led to the further discovery of significant differences in CR and CRE that was affected by flow rates and particle sizes. It was finally concluded that intra-instrument variability was so significant that the accuracy of the lower LOD could not be indisputably determined as acceptable (Black, 2011). A follow-up study of the XMX/2L-MIL sampling device was performed to evaluate the collection efficiencies of both viral and bacterial agents, MS2 and BTK, respectively. Results for the collection of BTK indicated a median total count viability of concentrations a little over $1.0x10^7$ CFU/L and for MS2 a little under $3.0x10^6$ PFU/L when using PBS as liquid media (Enderby, 2012).

It is also important to note that even though the device has been in use for outdoor collection of agents, studies challenged the XMX/2L-MIL with aerosolized agents in test chambers rather than

in representative field deployment situations (Cooper 2010, Black, 2011, Enderby, 2012). Also, the XMX/2L-MIL underwent modifications for use in the research studies presented, specifically reducing secondary impinger flow was reduced and collecting samples into different virus-specific media (Remel M5 vs PBS) (Cooper, 2010, Black, 2011). The researchers who modified the XMX/2L-MIL suggested that the performance in MS2 collection was improved compared to unmodified XMX/2L-MIL units (Cooper, 2010).

4.2 Novel Technology

In this section, four developing technologies identified from bioaerosol literature are compared (Table 4) and described in detail below. A final score was not included for any of these systems since they are not necessarily in their final iteration. Nonetheless, these unique samplers also demonstrate emerging trends and sampling mechanisms that could supersede current COTS instruments in the future.

Table 4. Novel/Developing Bioaerosol Samplers

Device/Tech	Personal Electrostatic Bioaerosol Sampler	Rutgers Electrostatic Passive Sampler	HighBioTrap	Multi-Slit Virtual Impactor
Collection Technology	two stage electrostatic precipitation	passive electrostatics	impactor	impactor and wet-walled cyclone
Sample Recovery	suspend into liquid	suspend into liquid	Agar plate	suspend into liquid
Collection Time(s)	10 min to 4 h	infinite	desiccation occurs at > 5 h	~10 min in the paper; due to high flow rate, longer times mean higher desiccation
Flow Rate (LPM)	10	0	1200	1220
Particle Size Collection Capabilities	0.026 µm – 3.1 µm	14 nm – 5 µm	> 2.0 µm	> 2.0 µm
Operating Humidity and Temperature	not specified	not specified	not specified	not specified
Consumables	none specified	polarized, ferroelectric polymer film	agar plate	filters
Dimensions (in)	1 (diameter) x 5.5 (H)	1.1 (diameter) x 4.5 (H)	not specified	17 x 9.8 x 13
Power Source	DC voltage	passive	battery	unknown
Collection Efficiency	~80%	100% elution efficiency	10% *Pseudomonas fluorescens* ; 20% *Bacillus subtilus*	22% at 1.9 µm, 50% at 2.0 µm, 77% at 3.0 µm
Literature Reference	Han et al. (2008)	Therkorn et al. (2017)	Chen and Yao (2018)	Bergman (2004)

4.2.1 Personal Electrostatic Bioaerosol Sampler (PEBS)

The personal electrostatic bioaerosol sampler (PEBS) system is a novel device for use in determining personal exposures to bacteria and fungi. The PEBS device is a two-stage electrostatic precipitator designed with a static air blender, wire to wire charger, a collection chamber, and a transition section (Han, et al., 2018). With this design, the wire to wire charger produces a very low ozone transmission, indicated as less than 10 ppb, and the static blender is positioned to produce adequate mixing of the particles entering the device (Han, et al., 2018). The device is a self-contained and lightweight sampler allowing for increased portability.

In a study by Han et al. (2018), a *Bacillus* species and a fungal species of *Penicillium* were used to determine the performance of the PEBS compared to a BioSampler at three sampling intervals of 10, 60 and 240 min. The results of the study showed an 80% collection efficiency up to 240 min with a 10 L/min sampling rate (Han, et al., 2018). Compared to the BioSampler, the PEBS system was able to measure equal and higher concentrations of culturable bacteria, but lower concentrations of culturable fungal spores (Han et al., 2018). The system was not tested for protein or viral particles.

Since this study was published within the last year, there currently are no further details on the device. More testing is needed to determine if voltage settings could have any effect on the outcome of measurable concentrations of the bacterial and fungal species.

4.2.2 Rutgers Electrostatic Passive Sampler (REPS)

A study was conducted in which passive bioaerosol collection was performed using a polarized ferroelectric polymer film, poly(vinylidene fluoride) (PVDF) (Therkorn, et al., 2017). Using the ferroelectric films was ideal because they could be oriented in a manner that would allow for opposite polarizations to face each other, thus a continuous electric field between the films could increase the capture of bioaerosols depending on their electrostatic attractions. Furthermore, unlike electret films, ferroelectric films have a permanent electric polarization which can be strengthened by applying an electric field (as noted in Therkorn et al.'s supplementary material (2017)). Electret films have a surface charge applied to them but require an external electric field for effective particle capture; however, they are biased to oppositely charged particles and their surface charge weakens over time.

This study utilized a spiral film that was designed to maximize the surface area of the polarized ferroelectric polymer film and have the ability to fit into a 50 mL conical centrifuge tube to allow for easier storage, transport, and faster elution when processing the samples (Therkorn, et al., 2017). A study was completed to evaluate bioaerosol capture in a wind tunnel and in calm air. The results indicated that the film was able to collect particles within a range of 14 nm to 5 μm, which spans the range of viral size to fungal spore size. Also, utilizing polarized films enabled the collection of a wide variety of bacterial species. Post-processing of the samples via elution was successful, and the microorganisms were able to be removed from the films. The design of the sampler with the conical tube allowed for a small, lightweight sampling device and efficient processing with reduced container transfer after sampling.

Another factor that was determined with this study was that the PVDF film, unlike other electrostatic films used in other investigations, allowed for the electrostatic field to be

maintained, despite environmental conditions, thus possibly leading to longer sampling times in the future (Therkorn, et al., 2017).

The use of a passive collection device has benefits, for example, the microbial stress can be decreased with less desiccation. This leads to better collection, efficiency, viability, and downstream identification of the samples, particularly for long-term samples. Also, active devices utilize pumps and batteries which in turn increase their costs and can decrease overall ease of use, portability, and available sampling time.

4.2.3 HighBioTrap

The HighBioTrap is a high flow battery-operated, portable agar impactor. In the 2018 study conducted by Chen and Yao discussed the current issues surrounding the collection of a small population of aerosols, specifically in a bioterrorism event. This is important because most personal, battery-operated bioaerosol samplers do not typically go above a sampling rate of 300 L/min, which could ultimately lead to a missed collection or produce a false negative if the airborne concentration of a biological agent is low. The investigators report that with a much higher sampling rate, a larger sample could be collected in a shorter time in the event of an accidental or intentional release of a biological agent.

The large intake nozzle of 50 mm allows for a larger diversity of organisms and 1200 L/min flowrate leads to shorter sampling times. The distance between the nozzle and agar is 75 mm, and the instrument has an impaction velocity of ~10.2 m/s, resulting in a particle cutoff diameter of ~2 μm. The jet velocity was also another factor that was highly considered. The HighBioTrap was designed to have an impaction velocity comparable to or less than other portable and known samplers, such as the RCS High Flow (8 m/s) and the BioStage (24 m/s).

The study was conducted in both indoor and outdoor environments, with the HighBioTrap having a higher bacterium count but lower bacterial concentration in comparison to the BioStage in indoor and outdoor environments (Chen and Yao, 2018). In outdoor environments, most bacterial particles aggregated or adhered to other non-biological particles, allowing for a higher efficiency for the HighBioTrap despite its higher cutoff diameter than the BioStage. Longer periods of sampling will lead to desiccation but allow for microbial diversity versus other samplers. Although the high sampling rate leads to increased desiccation, the sample diversity was higher than for the BioStage impactor also used in the study. Upon investigation with aerosolized *Pseudomonas fluoresecens* and *Bacillus subtilus* bacterial particles, the HighBioTrap had collection efficiencies of 10% and 20% respectively; however, the increased microbial diversity and larger sample was considered an advantage of the HighBioTrap (Chen and Yao, 2018).

4.2.4 Multi-slit Virtual Impactor with Commercial Collector

A study was conducted to create a portable device based off the design of the USAF standard for biological agents, the XMX-2L/MIL (Bergman, 2004). The investigators sought to build a collector that is smaller than 28 L and can impinge aerosolized particles into a liquid that allows for detection analysis and can either be an independent unit or used in conjunction with a detection unit. With this unit, a multi-slit impactor with a low-pressure drop was created to concentrate aerosols while using a low power consumption blower. The collector has a sampling

rate of 1220 L/min and a flow resistance of 0.2 kPa. The device is comprised of a multi-slit virtual impactor, a wetted-wall cyclone collector (commercial product from Research International), two blowers, and associated plumbing and controls. The collection rate of the combined collector versus Research International alone produced was four times higher (Bergman, 2004). Data regarding viability after collection is currently lacking; therefore, more data is needed for a full assessment.

4.3 Direct Reading Technology

In addition to collection technology, there are also direct reading instruments that target biological aerosols. The most common example is the FLIR Integrated Bio-Active Clarifier (IBAC) 2 by FLIR Systems, Inc.

4.3.1 FLIR IBAC 2

The FLIR IBAC 2, formerly known as FIDO B2, is a fully automated indoor and outdoor bioaerosol collection technology that allows for continuous real-time air monitoring and sample collection with additional modules. Its unique alarm feature is set to trigger in 60 seconds or less at the identification of particles associated with all four classes of biothreat agents: spores, bacteria, viruses, and toxins. The device is not considered to perform high air volume collection but rather a 4 L/min with the detection limits of aerosol particle sizes ranging from 0.7-10 µm during the process of sample analysis that triggers collection. Depending on the sampling module, a flowrate of 100 or 200 L/min is used with either dry or wet collection media to collect particle sizes 1-10 µm. It can be used as a static sampler but is portable, weighing 13 lbs with battery, and up to ~20 lbs with either collection unit. The battery can operate up to 18 hours with a charge time of fewer than 4 hours. It can endure varying environmental conditions with a temperature range of -20 to 50 °C and humidity of 5 to 95% (FLIR Systems Inc.). However, known interferences such as dust, dirt, fuel products, engine exhaust, pollen, smoke, and salt may negatively influence alarm capabilities (CBRNE Tech Index).

This air sampling technology utilizes a UV Laser-Induced Fluorescence technique that distinguishes between the biological particles from unrelated organisms or dust making it possible to identify biothreat agents (CBRNE Tech Index). As air is drawn in, the method uses the absorption of light to induce an excited state of key chemical compound components of fluorescent molecules (e.g. tryptophan) within biological organisms and, when recognized, triggers an alarm within the device. Although the device includes an LED for visual indication, confirmatory testing must still be conducted to determine which bioagent was collected. Sampling can occur by dry filter method using polyester felt filters with a flow rate for collection at 100 L/min or by wet collection using the C100 collector (buffer rinse fluid provided in premeasured vials) that operates at a flow rate of up to 200 L/min (FLIR Systems Inc.).

4.3.2 WIBS-NEO

The Wideband Integrated Bioaerosol Sensor-New Electronic Option (WIBS-NEO), produced by Droplet Measurement Technologies, is another real-time bioaerosol monitor that operates upon fluorescence detection. Using two UV-filtered flashlamp sources, this device excites

fluorescence in sampled particles at 280 and 370 nm and has 3 channels to detect fluorescence emission at wavelengths 310-400 nm and 420-650 nm. These wavelengths specifically target bioaerosol components like tryptophan and nicotinamide adenine-di-nucleotide, like the FLIR IBAC 2. However, the WIBS-NEO also measures particle count, size, and asymmetry (or shape) of both fluorescent and non-fluorescent particles (Fennelly et al. 2018). The maximum overall particle concentration it can measure for sizing and counting is 9500 particles/cm^3; for fluorescent particle counting, 466 particles/cm^3. Another unique feature of the WIBE-NEO is that it is rated for use at 6562 ft.

The WIBS-NEO has an overall flowrate of 2.4 L/min (0.3 L/min sampling rate, 2.1 L/min sheath flow) and weighs 27. 5 lbs. Since the instrument's operation relies on light-scattering for single-particle measurement and characterization (e.g. asymmetry, fluorescence), its minimum detectable particle diameter is 0.3-0.5 µm and can measure particles up to 30 µm. Like the FLIR IBAC 2, certain non-biological particles such as secondary organic aerosols or polycyclic aromatic hydrocarbons can contribute to the detected fluorescence signal and must be accounted for during measurement (Fennelly et al. 2018). Nonetheless, in an occupational sampling campaign at a composting site, the WIBS-NEO was successfully used to determine the presence of fluorescing and non-fluorescing particles sized 0.5-3 µm (Fennelly et al. 2018).

5.0 CONCLUSIONS AND FUTURE DIRECTIONS

The goal of this report was to evaluate relevant and available bioaerosol sampling technologies and down-select potential candidates for sampling in compact spaces. The down-selected technologies included six COTS samplers and two novel technologies. The five commercially available technologies included the Coriolis Recon and Compact, SASS 4100, BioCapture 650, and MD8 AirPort sampler.

The Coriolis Recon is one of the most ruggedized and portable bioaerosol samplers available on the market today. For example, this specific unit is currently being utilized by the French military for their ground-based operations and has received the highest score for field use by the 2018 Global CBRN Detector Market Survey (Bertin Instruments, 2016; Global CBRN Detector Market Survey, 2018). The Recon and Micro can collect all classes of biological aerosols ranging in size from 0.5 - 10 µm, including protein and toxins that can be even smaller than viruses. Additionally, having a wet-walled cyclone collection mechanism mitigates sampling stresses for the microorganisms; conversely, the Compact's dry-wall cyclone allows for concentrating the sample to the optimal volume for offline analysis. Ultimately, the Recon is unique in its ability to perform long-term air sampling with high run-times and is in the format required to perform sample collections in a mobile environment while being an extraordinarily ruggedized system. The Micro is less ruggedized but has similar sampling capabilities and is nonetheless useful in outdoor environments. Finally, the new Compact touts similar sampling capabilities but is the smallest, lowest-flow, least expensive, and lightest Coriolis unit.

The next system recommended is the SASS 4100 Plus, a two-stage filter-based aerosol collection device that uses a proprietary electret dry filter media. The 4100 Plus boasts a unique two-stage air collection mechanism that can process over 4000 L/min of ambient air that ultimately increases the aerosol concentrations 4 - 15 times higher than the incoming air. This device is great for situations where the targeted pathogen is present at very low concentrations and therefore can be increased with the use of this air sampler. The patented filter, which is

composed of polypropylene electret micro-fiber, is capable of collecting most classes of biological aerosols with a 95% collection efficiency for particles with an aerodynamic diameter of 0.3 µm and higher. Since the aerosols are collected onto a dry filter, our group recommends that this device be paired with the SASS 3010 particle extractor that boasts extraction efficiencies typically in the range of 70-80% (Research International (c), n.d.). Unfortunately, there was no data that established how long biological particles remained viable on the electret filter without the aid of the particle extractor; however, organism viability is maximized by using low airflow velocities and a bulk electret filter media. Regarding portability, this device is relatively lightweight at 13.9 lbs and can operate in extreme environments as well as temperatures ranging from -40°C to 70°C. Currently, the SASS 4100 Plus is the highest-capacity portable aerosol dry collector in production and fulfills many of this report's criteria for a bioaerosol collection sampler.

The BioCapture 650, developed by FLIR Systems, Inc., is uniquely appealing because of its handheld capabilities and portability. Furthermore, it boasts a large single button to operate which is perfect for Airmen to quickly operate in challenging environments. This device is highly operable in sand, dust, and other inclement weather conditions and temperatures ranging from 2°C to 43°C. This unit collects and concentrate bacterial, viral, and toxin particles into a buffer solution. In addition to this, the BioCapture 650 is capable of collecting micron and submicron-sized particles in the aerodynamic diameter range of 0.5 to 10 µm. After collection, the sample is automatically deposited in an easily removed sample vial for subsequent analysis by PCR, hand-held assays, and other methods. Ultimately, BioCapture 650 provides the user with an effective and easy-to-use instrument for fast, safe, and efficient biological agent collection.

Similarly, the handheld MD8 AirPort has the high collection efficiency range air of sampling filters while also maintaining sample viability using large gelatin filters. Although gelatin filters are susceptible to desiccation, the MD8 is a high-volume sampler and therefore can collect large volumes of air per gelatin filter in a short sampling time, decreasing the chances of desiccation effects. These filters are useful for both viable and non-viable sample analysis, but the sampler can also sample directly on bacterial agar plates. However, high relative humidity will negatively affect both types of samples. Additionally, the sampler has been used in several outdoor environments and in a pressurized simulated aircraft cabin during a realistic commercial flight schedule, directly applicable for AF applications.

The two top contenders in the novel technology category are the Personal Electrostatic Bioaerosol Sampler (PEBS) and the passive sampling device utilizing polarized ferroelectric polymer film. These two were chosen based upon their sampling abilities and the recovery rates of the particles. The other two devices are noteworthy, but recovery rates declined due to higher desiccation with increased flow rates. These aspects would likely be considered downfalls in the long run.

The PEBS device was a highly ranked performer amongst the novel devices due to its 77% collection efficiency for sampling periods of up to 4 hours. The sampler was designed as a two-stage system that utilized positive corona discharge to minimize ozone production (Han, Thomas and Mainelis, 2017). During sampling it only produces about 10 ppb of ozone, minimizing any adverse effect on the viability of the particles. Ozone production also leads to adverse health effects, so minimizing this is an important factor. The PEBS device is in the early stages of

development, therefore future research would need to be conducted on continued viability and detection of the collected particles in various aerodynamic diameter ranges.

With the REPS described by Therkorn et al. (2017), charge-neutralized particles ranging in size 0.014 to 5 µm and a mixed bacterial aerosol were efficiently collected with polarized ferroelectric PVDF film relative to other sampler in both wind tunnel tests and calm air. Furthermore, the sampling film's encasement in a 50 mL conical tube enhanced the elution efficiency, and important parameter for any filter sampling. The small size and portability are a major benefit in comparison to other sampling devices, whether passive or active. Also, the study discussed the device as the first of its kind, from production of the sampler to the post sampling analysis.

While most of the recommended instrumentation is high-flow and thus effective for area sampling and/or for sampling potentially low airborne concentrations, there is still a need for low-flow and/or personal sampling options. Impingers and cyclones are generally effective at lower sampling flowrates and depositing samples directly into liquid with reduced sample loss potential in post-processing, although re-aerosolization is a concern using liquid impingers. Impinger system SKC BioSampler remains the gold standard for maintaining both high collection and biological efficiency (Dybwad et al., 2014). The SKC BioSampler is made from glass so it is easy to sterilize in the laboratory or the field; however, this makes it unsuitable for harsher environments. It may be possible to design a sleeve or enclosure for the BioSampler to mitigate concerns arising from broken glass, or to collaborate with the manufacturers to create a model made of a different material (e.g. polycarbonate). Until better COTS options for personal or low-flow static sampling are available, traditional air cassette or Button sampling with polycarbonate or polytetrafluoroethylene filters is readily available for most BEE shops.

Similarly, while the SKC BioStage and MicroBio MB2 are limited in application (e.g. the limits for which microorganisms are best collected and maintained, sampling stressors for microorganisms, and vulnerability of agar in hot or humid environments, etc.) they are attractive mid-to-high-flow options for sampling bacteria in compact spaces. The MB2 could be an alternative to the XMX-2L/MIL for some applications since it still can pull large amounts of volume but has a significantly smaller footprint and weight burden with superior portability. Its smaller and more portable battery does have a tradeoff of a shorter life (~2 hours) before recharge, but would be much simpler to handle when, for example, in flight. Additionally, although the existing BioSpot unit is not suitable for field work, the underlying sampling principle is ideal for bioaerosols and is a highly efficient technique used in direct-reading real-time aerosol instruments. A ruggedized and significantly downscaled version of this unit would be an ideal sampler.

Follow-on work to characterize these instruments is crucial, although the myriad of factors is daunting. Generally, bioaerosol samplers, both high and low volume, should be tested: a) in a representative enclosed chamber or attached to a representative hub, b) against various challenge aerosols to fully evaluate collection and retention efficiencies across aerodynamic diameters, c) against varied biological challenge aerosols to evaluate collection, retention, and biological efficiencies, and d) with general post-sampling protocols for each analytical method available, to characterize possible losses during post-processing and optimize data quality.

The recommended commercially available technologies for bioaerosol collection provide an assortment of highly efficient and capable devices to better meet criteria for high-flow area

sampling in compact spaces such as aircraft. Partnering with other research institutions to further develop promising novel samplers can also help fill future research gaps. Bioaerosol sampling and analysis is challenging, and the best sampling approach is often situation dependent. However, the recommendations in this report fulfill research criteria for sampling in a compact space without a power source.

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LIST OF ABBREVIATIONS AND ACRONYMS

AC	Alternating current
AF	Air Force
BEE	Bioenvironmental Engineering
BTK	*Bacillus thuringiensis* subsp. *Kurstaki*
CBRN	Chemical, Biological, Radiological and Nuclear
CFU	Colony-forming unit
COTS	Commercial off the shelf
DFU	Dry Filter Unit
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
GF	Glass fiber
HHA	Handheld Assay
IBAC	Integrated Bio-Active Clarifier
IOM	Institute of Medicine
LOD	Limit of detection
LPM	Liters per minute
MCE	Micro-cellulose ester
MOPP	Mission Oriented Protective Posture
MS2	Male-specific coliphage 2
NIOSH	National Institute for Occupational Safety and Health
PC	Polycarbonate
PCR	Polymerase chain reaction
PEBS	Personal Electrostatic Bioaerosol Sampler
PES	Polyethersulfone
PFU	Plaque forming unit
PPE	Personal protective equipment
PSL	Polystyrene latex
PTFE	polytetrafluoroethylene
PVDF	poly(vinylidene fluoride)
qPCR	Quantitative polymerase chain reaction
RCS	Reuter Centrifugal Sampler
REPS	Rutgers Electrostatic Passive Sampler
RNA	Ribonucleic acid
SASS	Smart Air Sampling System