

## A. COVER PAGE

<b>Project Title:</b> Advanced Sampler for Measuring Exposure to Biological Aerosols	
<b>Grant Number:</b> 5R01OH009783-06	<b>Project/Grant Period:</b> 08/01/2016 - 07/31/2019
<b>Reporting Period:</b> 08/01/2018 - 07/31/2019	<b>Requested Budget Period:</b> 08/01/2018 - 07/31/2019
<b>Report Term Frequency:</b> Annual	<b>Date Submitted:</b> 11/30/2021
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<b>Human Subjects:</b> NA	<b>Vertebrate Animals:</b> NA
<b>hESC:</b> No	<b>Inventions/Patents:</b> No

## B. ACCOMPLISHMENTS

### B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overall goal of this competitive renewal was to improve our ability to measure exposures to airborne microorganisms, especially to their low concentrations, by completing the development of a novel electrostatics and liquid-based bioaerosol sampler featuring very high sample concentration rates. The sampler was then tested by using it to determine bioaerosol exposures in various occupational environments. This research proposal is a competing renewal of a NIOSH-funded R01 grant, where researchers developed a field-deployable prototype of an electrostatic precipitator with superhydrophobic surface (EPSS) for bioaerosol collection. Here, bioaerosol particles were drawn into the sampler, electrostatically charged and then deposited onto a narrow electrode covered by a superhydrophobic substance ("Lotus leaf" type) from which they were removed and collected by rolling liquid droplets. The developed prototype was able to achieve high sample concentration rates; however, it needed further improvement and field testing and to prepare the sampler for routine exposure assessment applications in occupational environments. The goal of project was to stabilize sample concentration rates and improve the preservation of culturability and viability of collected microorganisms. These features are important in determining exposures in occupational environments. In addition, high sample concentration rate will allow measuring exposures even to low microorganism concentrations – a feature lacking in current bioaerosol samplers – thus substantially improving our ability to identify exposure risks and protect affected populations. The main hypothesis of the proposal was that the new bioaerosol sampling system will outperform current bioaerosol samplers in their ability to determine exposure to bioaerosols. The Specific Aims were:

I. Re-design of the current sampler prototype as a two-stage collector system. The earlier developed collector prototype was a single-stage electrostatic collector of wire-to-plate design. It achieved high sample concentration rates, but it became apparent that the single-stage design could be detrimental to microorganism culturability and DNA integrity, especially in long-term sampling. Preliminary experiments showed that these issues could be solved by using a two-stage design (charging and collection sections are separated) using an innovative wire-to-wire particle charger. These concepts were to be used to redesign the sampler. All sampler components, including power supplies, air movers and controls were to be integrated into a field deployable unit.

II. Evaluation of the sampler's performance using Computational Fluid Dynamics (CFD). The sampler's performance was to be tested using Computational Fluid Dynamics (CFD). CFD simulation was needed to optimize the sampler's dimensions and operating conditions to ensure efficient particle capture.

III. Analysis of sampler's performance with non-biological particles. The system's performance (particle losses, collection efficiency and concentration rate) were to be investigated in the laboratory at different sampling flow rates with fluorescent polystyrene latex particles. The sampler will then be tested against a reference filter and using air-to-air measurements with a direct-reading instrument.

IV. Sampler's biological efficiency: performance with microorganisms and various microbial analysis methods. The complete sampling system will sample bacteria and fungi in parallel to reference samplers to determine its collection efficiency and address any potential deficiencies and shortcomings before the field testing. The collected samples will be analyzed by multiple methods to address their compatibility with electrostatic collection technique.

V. Use of the developed system to determine bioaerosol exposures in various occupational environments. The developed sampling system was to be tested in the field concurrently with two other samplers to determine bioaerosol exposures in diverse occupational environments. The samplers will be compared in terms of measured airborne concentrations of bacteria and fungi determined by various analysis methods.

#### B.1.a Have the major goals changed since the initial competing award or previous report?

No

### B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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**B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS**

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

**B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?**

NOTHING TO REPORT

**B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?**

NOTHING TO REPORT

**B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?**

Not Applicable

## Advanced Sampler for Measuring Exposure to Biological Aerosols

This study focused on developing and testing a stationary electrostatic bioaerosol collector with a high concentration rate, focusing on determining viable and culturable microorganisms. Using our earlier advances in the electrostatic collection of airborne microorganisms, we designed and optimized a Stationary Electrostatic Bioaerosol Sampler (SEBS), which incorporates our previously developed wire-to-wire charger and a newly-designed removable collector. The sample elution system was redesigned and optimized to yield a practical solution providing high elution efficiency and a high sample concentration rate. The initial development of the collection system was based on existing prototype and CFD-aided design. Multiple concepts were advanced and tested with physical PSL particles at various flowrates, charging and collection voltages. The physical and biological performance relative to filter samplers of the most promising version of SEBS was then extensively tested in the laboratory; testing was performed to optimize: 1) collector configurations and its hydrophobic coating methods, 2) collection voltages, and 3) material of sample removal tubes. The resultant SEBS is a two-stage electrostatic sampler with a wire-to-wire charger and collection section with a stainless steel electrode coated using polydimethylsiloxane (PDMS) coating technique and ultraviolet/ozone surface treatment method. The sampler's concept is shown in Fig 1. The final collection section consists of a stainless steel removable collection rod 3/16 inches in diameter and 5.1 inches in length and two grounded stainless steel plates having the shapes of the quarter cylinder (0.78×1.5×0.002 inches). The core part of SEBS (changer and collector) has a shape of a cylinder of 2.54 cm (1 inch) in diameter and ~21.2 cm (8.35 inches) in length. The omnidirectional inlet minimizes the wind effect on the aspiration efficiency. A high-voltage power supply provides the charging and collection voltages, while the airflow is provided by an air mover. Once the sampling is completed, the collection electrode is removed and transferred into a sample removal system. Several materials and designs of the removal system were investigated and analyzed. The best sampler performance was achieved with a stainless steel collection rod. Thus, a sample removal system specific for the collection rod was developed (Fig. 2). Here, post-sampling, the cylindrical collection electrode is removed from SEBS, and placed into the elution tube; either 0.2 or 1.0 mL PBS is added to the elution tube, and the particles are eluted by vortexing the tube for 1 min. The final operating parameters of SEBS were determined through a series of iterative experiments. The finalized SEBS was first tested in laboratory environment, where it was operated at 20 L/min and challenged with commonly used Gram-positive bacteria of *Bacillus atrophaeus*, Gram-negative bacteria of *Pseudomonas fluorescens*, and fungal spores of *Penicillium chrysogenum* and *Penicillium melinii*. The physical and biological performance of SEBS was compared against the Button filter sampler due to its high collection efficiency. The relative physical collection efficiency of SEBS was determined based on the concentration of airborne particles determined by SEBS relative to that determined by Button sampler; the relative viability and culturability efficiencies of SEBS were similarly determined. Overall, SEBS showed actual physical collection efficiency of ~50% when samples were eluted into 0.2 mL, and the efficiency increased to ~75% when 1 mL of elution liquid was used (Fig. 3). The average ATP-base relative viability efficiency reached close to ~80% with 1 mL elution liquid, suggesting that the sampling stress was reduced compared to the filter sampler. The relative culturability efficiency was ~60% with 1 mL elution liquid. Importantly, the use of 0.2 mL elution liquid resulted in a high sample concentration rate of  $\sim 5 \times 10^4 \text{ min}^{-1}$ .

After this extensive laboratory testing, our newly developed and optimized SEBS was used in a field study to determine bioaerosol presence in various environments and compare its performance against BioSampler (SKC, Inc., Eighty Four, PA) and an open face filter sampler (37mm PTFE, SKC Inc.). SEBS was operated at its current nominal flowrate of 20 L/min and BioSampler was operated at its nominal flowrate of 12.5 L/min with 20 ml collection liquid. The filter sampler was operated at a flowrate of 10 L/min to make it similar to the other two samplers.

For this final stage of research project, SEBS was integrated with all necessary power supplies, air movers, and our custom-built control box. Its final version weighs about 5 lbs and is a truly portable and battery-operated bioaerosol sampler (Fig. 4). The samplers were operated in occupational environments with different concentrations of airborne particles, thus representing different exposure levels. The samplers were compared for their ability to determine viable airborne microorganisms as determined by airborne ATP concentration and culturable airborne microorganisms based on determined airborne culturable microorganism concentrations. The samplers were operated for 1, 2, 5, 10, and 30 minutes to examine their detection limits and determine which sampler could serve as a fast warning system to inform about the presence of airborne biological particles in occupational environments.

Samples were collected at five different occupational sites and included athletic facilities (Site 1), office building (Site 2), laboratory environment (Site 3), agricultural facilities (horse barn) (Site 4) and, for comparison, outdoor environment (Site 5). These sites were selected because of their different occupational profiles and different levels of airborne particles.

Samplers were compared in terms of signal-to-noise ratio in their collection/elution liquid, as well as determined airborne viable and total microorganism concentration. The higher the signal-to-noise ratio,  $R_D$ , the more sensitive microorganism detection is. As an example of field investigation results,  $R_D$  for the three samplers is presented as a function of sampling time in five different sites in Fig. 5. One can see that the  $R_D$  for SEBS was always above 1 even in low concentration environments (i.e., Sites 1, 2, and 3) and, even more importantly, for short sampling periods and. For SEBS, the  $R_D$  values ranged 1.2-3.5 (Site 1), 1.5-2.6 (Site 2), 1.0-2.7 (Site 3), 4.7-31.1 (Site 4), and 2.1-48.9 (Site 5), depending on the sampling time. On the other hand, the  $R_D$  values of BioSampler and filter sampler were  $\sim 1$  or below unity in low concentration environments (i.e., Sites 1, 2, and 3) for sampling times of 10 min or shorter.  $R_D$  increased above unity for 30 min sampling time. For Site 2, Office building, these ratios by reference sampler remained close to unity even after sampling for 30 min. These data show that SEBS provides a high sampler signal-to-noise ratio allowing fast detection of airborne microorganism presence even in low concentration environments.

In summary, we used earlier advances in the electrostatic collection of airborne microorganisms and designed and optimized a Stationary Electrostatic Bioaerosol Sampler (SEBS) focusing on its ability to efficiently capture airborne microorganisms and preserve their viability and culturability. In addition, we determined that because of low pressure drop, SEBS could be operated at a relatively high flow of 20 L/min entirely by the battery power and be used as a lightweight and portable field device. The use of 0.2 mL elution liquid resulted in the concentration rate of  $4.8 \times 10^4 \text{ min}^{-1}$ . Such a high concentration rate will enable faster detection and determination of bioaerosols in low concentration environments. SEBS exhibited high viability and culturability efficiency, especially with 1 mL elution liquid. In fact, the relative viability was even higher than the relative collection efficiency, suggesting that the stress to the microorganisms is reduced compared to the filter samplers. Overall, SEBS is now one of the few electrostatic collectors, if any, that has been specifically developed and tested to measure airborne culturable microorganisms. The field testing of SEBS at five different occupational environments demonstrated the field usability and utility of the newly-developed SEBS. Namely, due to high sample concentration rate of SEBS, it was able to detect the presence of viable bioaerosols in all sites and at sampling times as short as 1 min. The difference between SEBS and other samplers was especially pronounced at low concentration occupational environments and short sampling times. SEBS also had an advantage because it was battery operated and did not require mains power to operate. In many occupational environments it is a challenge, especially if extension cords are needed to deliver the necessary power for sampler operation. Our separate measurements show that SEBS could be operated for 8 hrs or longer on a single charge. This makes the sampler uniquely suitable to be used in occupational environments, including low-concentration ones, and determine bioaerosol exposures over an entire shift.

**Figures to show examples of accomplished work**

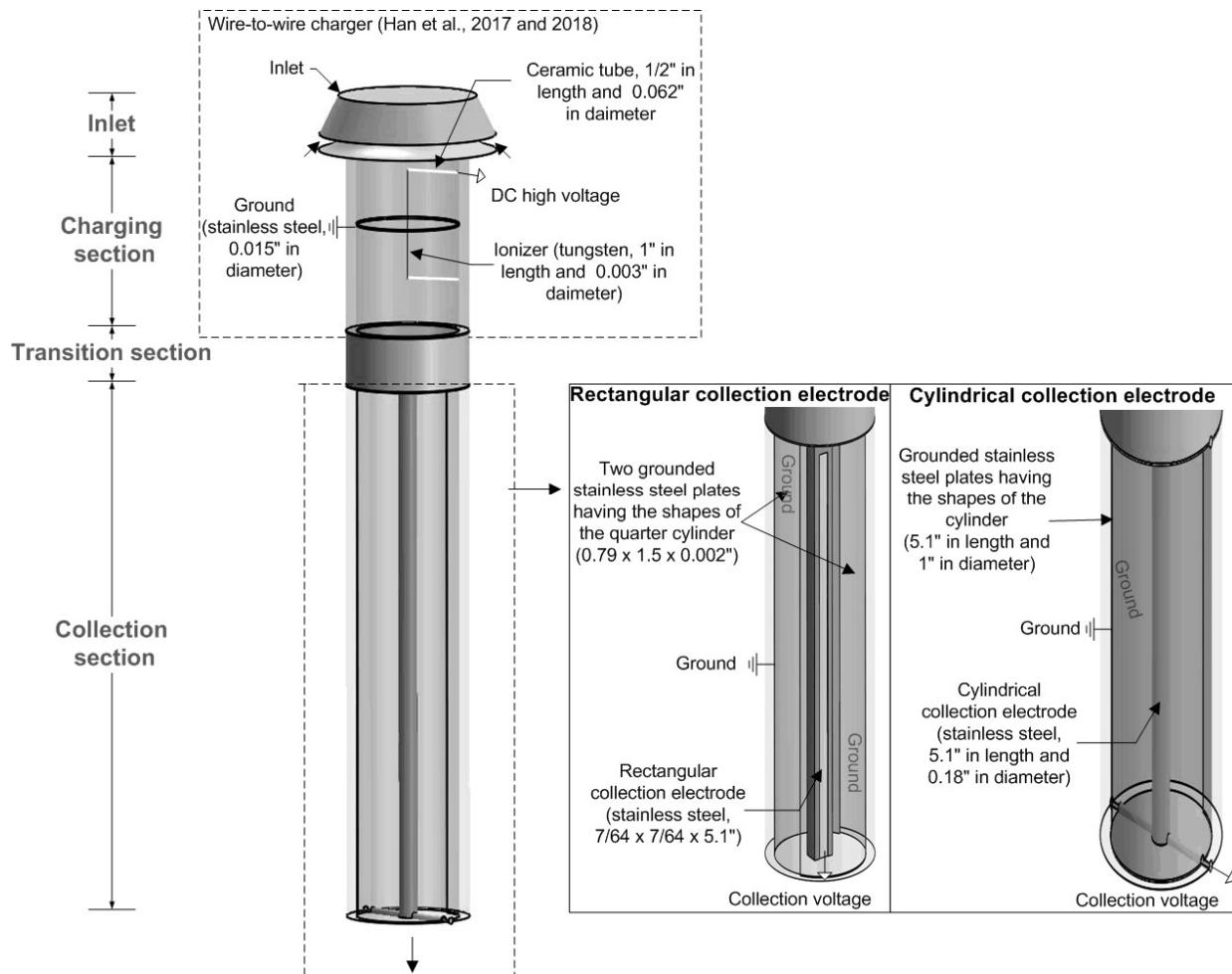


Fig. 1. Schematic diagram of Stationary Electrostatic Bioaerosol Sampler (SEBS) with a wire-to-wire charger.

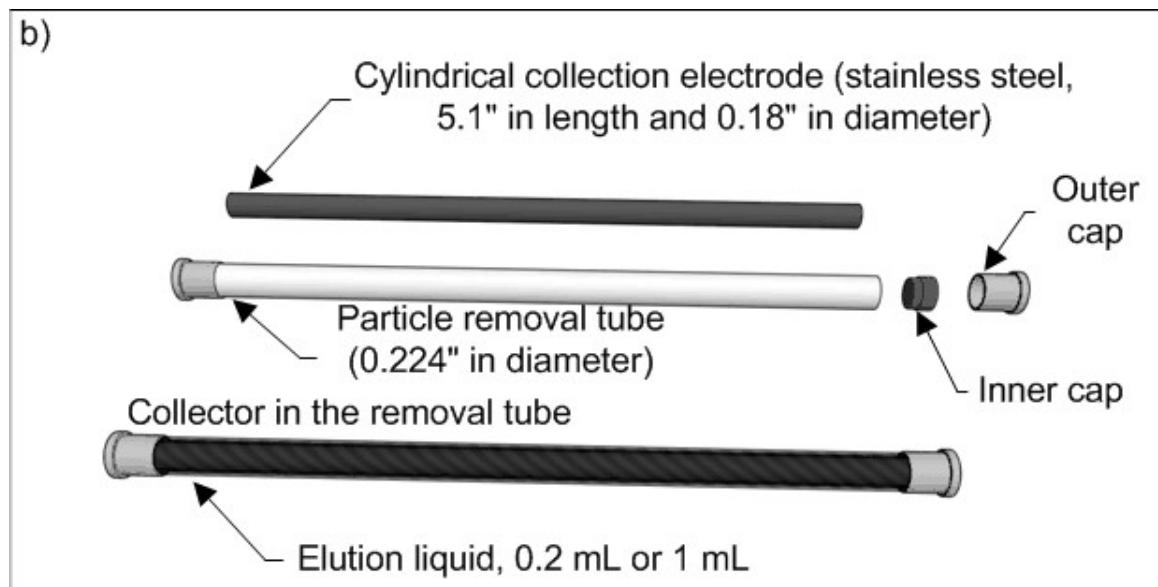


Fig. 2. A system to remove particles collected by SEBS: on a cylindrical collection electrode.

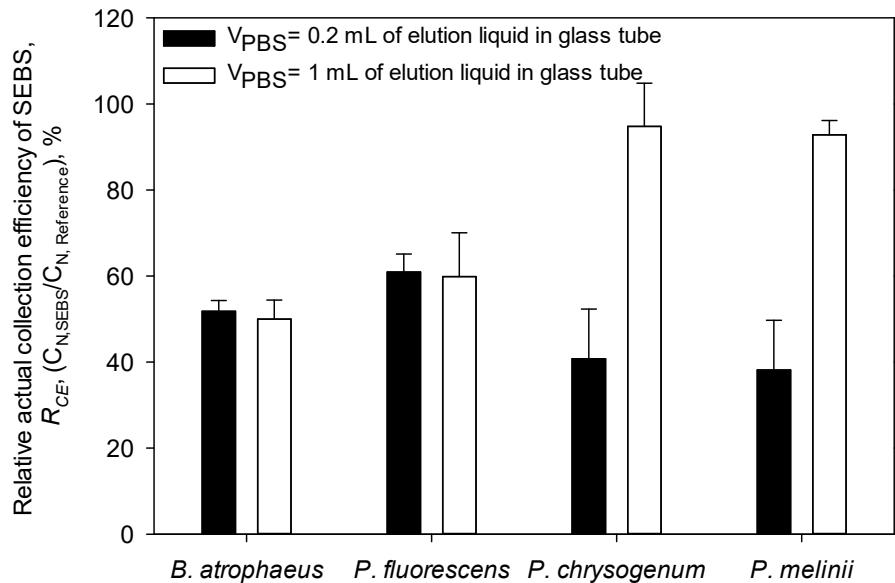


Fig. 3. Relative physical collection efficiency of finalized SEBS relative to Button sampler when sampling four microorganisms with samples eluted using 0.2 and 1 mL of PBS. The efficiency was determined by comparing the number of airborne particles ( $\text{N}/\text{m}^3$ ) determined by SEBS with that determined by the Button sampler. SEBS was operated at a 20 L/min sampling rate and +5.25 kV/-7 kV charging/collection voltages. The Button sampler was concurrently operated at 4 L/min. The test particle concentrations were  $\sim 1\text{--}2 \times 10^4/\text{L}$ . Each data point is an average of at least three repeats, and the error bars represent standard deviations.

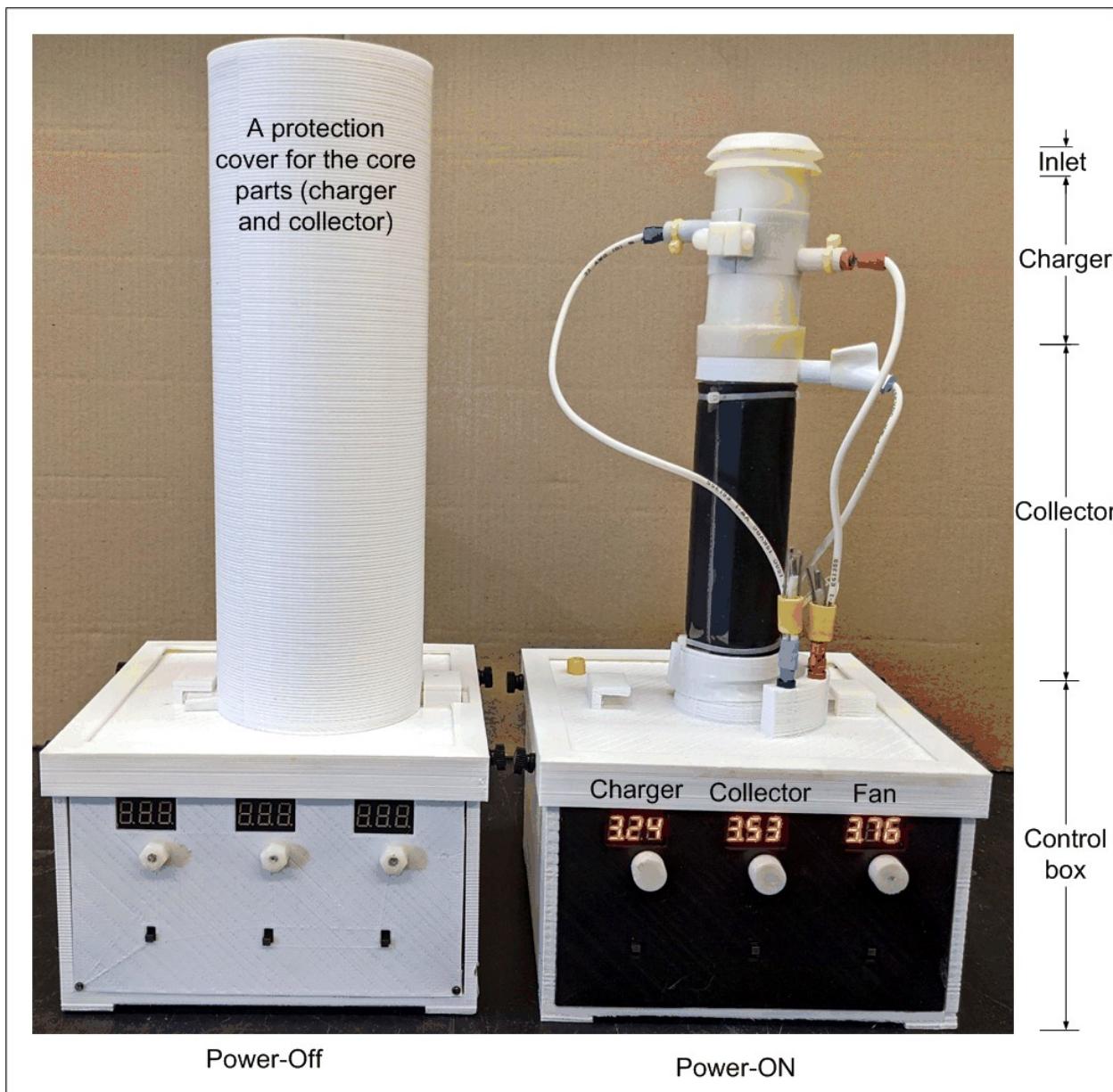


Fig. 4. A prototype of the field-deployable version of the stationary electrostatic bioaerosol sampler (SEBS) made of static dissipative material by machining and 3D printing. All electrostatic components are assembled in the control box.

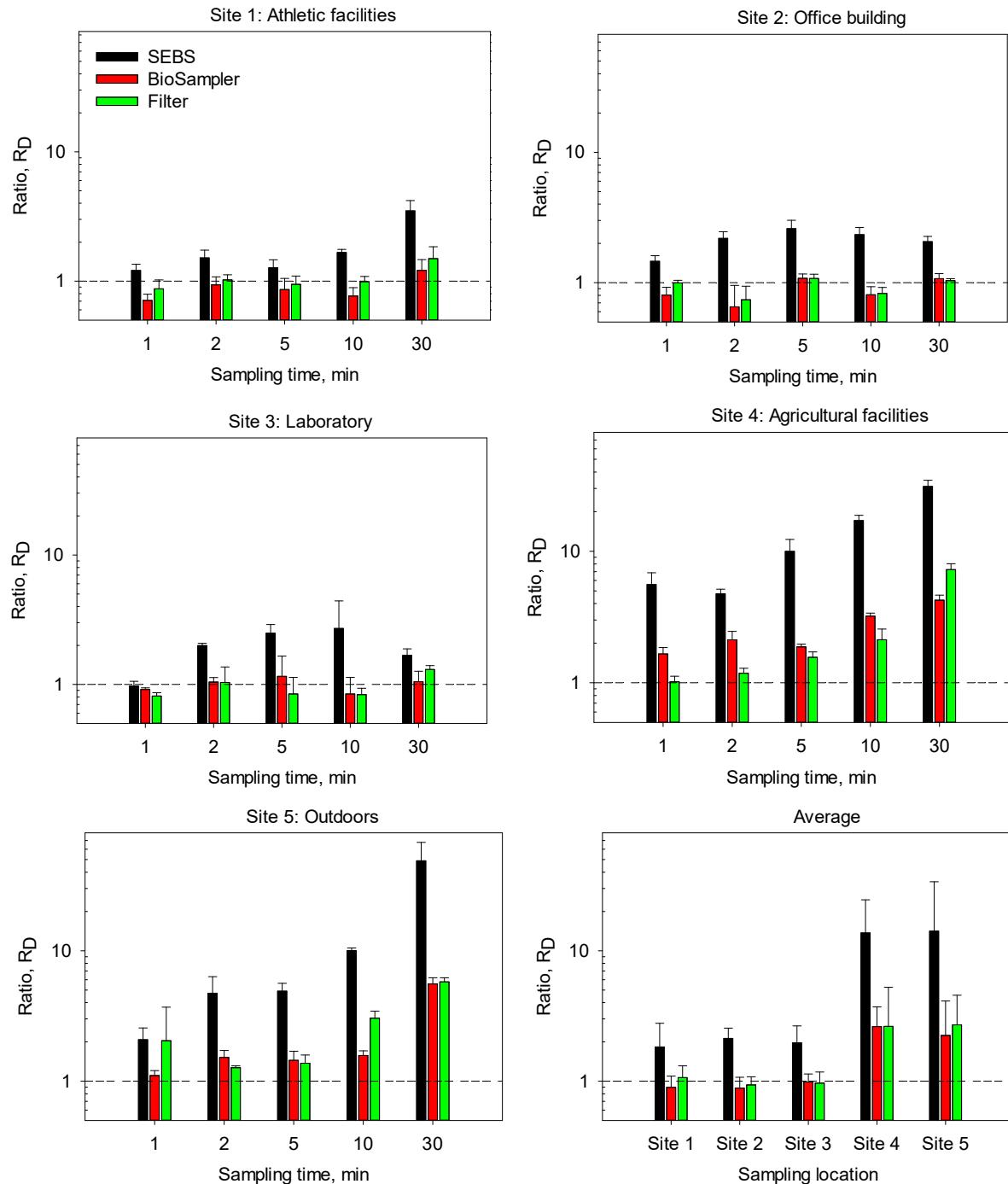


Fig. 5. Ratio of airborne ATP concentration (RLU/50  $\mu$ L) determined by samplers to that determined by background for five sampling times (1, 2, 5, 10, and 30 min). The dotted line represents  $R_D=1$ .



## C. PRODUCTS

### C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

#### Publications Reported for this Reporting Period

Public Access Compliance	Citation
N/A: Not NIH Funded	Zhen H, Krumins V, Fennell DE, Mainelis G. Development of a dual-internal-reference technique to improve accuracy when determining bacterial 16S rRNA:16S rRNA gene ratio with application to <i>Escherichia coli</i> liquid and aerosol samples. <i>Journal of microbiological methods</i> . 2015 October;117:113-21. PubMed PMID: 26241659; DOI: 10.1016/j.mimet.2015.07.023.
N/A: Not NIH Funded	Zhen H, Krumins V, Fennell DE, Mainelis G. Development of a dual-internal-reference technique to improve accuracy when determining bacterial 16S rRNA:16S rRNA gene ratio with application to <i>Escherichia coli</i> liquid and aerosol samples. <i>Journal of microbiological methods</i> . 2015 October;117:113-21. PubMed PMID: 26241659; DOI: 10.1016/j.mimet.2015.07.023.
N/A: Not NIH Funded	Han T, Wren M, DuBois K, Therkorn J, Mainelis G. Application of ATP-based bioluminescence for bioaerosol quantification: effect of sampling method. <i>Journal of aerosol science</i> . 2015 December 1;90:114-123. PubMed PMID: 26806982; PubMed Central PMCID: PMC4717491; DOI: 10.1016/j.jaerosci.2015.08.003.
N/A: Not NIH Funded	Han T, Wren M, DuBois K, Therkorn J, Mainelis G. Application of ATP-based bioluminescence for bioaerosol quantification: effect of sampling method. <i>Journal of aerosol science</i> . 2015 December 1;90:114-123. PubMed PMID: 26806982; PubMed Central PMCID: PMC4717491; DOI: 10.1016/j.jaerosci.2015.08.003.
N/A: Not NIH Funded	Mainelis G. Bioaerosol Sampling: Classical Approaches, Advances, and Perspectives. <i>Aerosol Science and Technology</i> . 2019 October 04;54:496.

### C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

### C.3 TECHNOLOGIES OR TECHNIQUES

Category	Explanation
Instruments or equipment	We used earlier advances in the electrostatic collection of airborne microorganisms and designed and optimized a Stationary Electrostatic Bioaerosol Sampler (SEBS) focusing on its ability to efficiently capture airborne microorganisms and preserve their viability and culturability. In addition, because electrostatic collectors feature low pressure drop, SEBS operates at a relatively high flow of 20 L/min entirely by the battery power for 8 hours or longer and be used as a lightweight and portable field device. The use of 0.2 mL elution liquid resulted in the concentration rate of $4.8 \times 10^4$

min<sup>-1</sup>. Such a high concentration rate enables faster detection and determination of bioaerosols, especially when sampling in low concentration environments. In addition, SEBS exhibited high viability and culturability efficiency, especially with 1 mL elution liquid. Overall, SEBS is now one of the few electrostatic collectors, if any, that has been specifically developed and tested to measure airborne culturable microorganisms. The field testing of SEBS at five different occupational environments demonstrated the field usability and utility of the newly-developed SEBS. Namely, due to high sample concentration rate of SEBS, it was the only tested sampler able to detect the presence of viable bioaerosols in all sites and at sampling times as short as 1 min.

#### C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

#### C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Research Material	<p>ADDITIONAL PUBLICATIONS</p> <p>1. Han, T.T., Myers, N. T., Manibusan, S., and Mainelis, G. (2021) Development and Optimization of Stationary Electrostatic Bioaerosol Sampler (SEBS) For Viable and Culturable Airborne Microorganisms, <i>Journal of Aerosol Science</i>, submitted.</p> <p>2. Han, T.T., Myers, N. T., and Mainelis, G. (2021) Performance of Stationary Electrostatic Bioaerosol Sampler (SEBS) in Different Field environments, <i>Aerosol Science and Technology</i>, in preparation.</p>
Research Material	<p>CONFERENCE PRESENTATIONS:</p> <p>1. Han, T. T., Thomas, N., and Mainelis, G. (2017) Development of Advanced Sampling Technologies for Measuring Exposures to Airborne Microorganisms. State of the Science Conference (Aurora, Colorado, June 21-22, 2017).</p> <p>2. Han, T. T., Thomas, N., and Mainelis, G. (2017) Advanced Electrostatic Technology for Sampling Airborne Biological</p>

Particles. The US-Korea Conference (Arlington, Virginia, August 9-12, 2017).

3. Thomas, N., Han, T. T., and Mainelis, G. (2017) Effect of Airborne Ion Emissions on Microbial Viability and Culturability. The 36th Annual Meeting of the American Association for Aerosol Research (Raleigh, North Carolina, October 16-20, 2017).

4. Han, T. T., Thomas, N., and Mainelis, G. (2018) Development and Optimization of the Electrostatic Precipitator with Superhydrophobic Surface (EPSS) Mark III for Collection of Bioaerosols. The 10th International Aerosol Conference (St. Louis, Missouri, September 2-7, 2018).

5. Han, T. T., Thomas, N., Manibusan, S., and Mainelis, G. (2019) Design, Fabrication, and Evaluation of Stationary Electrostatic Bioaerosol Sampler (SEBS) with High Concentration Rate. The 36th Annual Meeting of the American Association for Aerosol Research (Portland, Oregon, October 14-18, 2019).

## D. PARTICIPANTS

### D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
GEDI12	Y	MAINELIS, GEDIMINAS	PHD	PD/PI	1.0	0.0	0.0			NA
TAEWONHAN	Y	Han, Taewon T	PHD	PD/PI	3.0	0.0	0.0			NA

#### Glossary of acronyms:

S/K - Senior/Key  
 DOB - Date of Birth  
 Cal - Person Months (Calendar)  
 Aca - Person Months (Academic)  
 Sum - Person Months (Summer)

Foreign Org - Foreign Organization Affiliation

SS - Supplement Support

RE - Reentry Supplement

DI - Diversity Supplement

OT - Other

NA - Not Applicable

### D.2 PERSONNEL UPDATES

#### D.2.a Level of Effort

Not Applicable

#### D.2.b New Senior/Key Personnel

Not Applicable

#### D.2.c Changes in Other Support

Not Applicable

#### D.2.d New Other Significant Contributors

Not Applicable

#### D.2.e Multi-PI (MPI) Leadership Plan

Not Applicable

## E. IMPACT

**E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?**

Not Applicable

**E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?**

NOTHING TO REPORT

**E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?**

Not Applicable

**E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?**

NOTHING TO REPORT

**G. SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS****G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS**

NOTHING TO REPORT

**G.2 RESPONSIBLE CONDUCT OF RESEARCH**

Not Applicable

**G.3 MENTOR'S REPORT OR SPONSOR COMMENTS**

Not Applicable

**G.4 HUMAN SUBJECTS****G.4.a Does the project involve human subjects?**

Not Applicable

**G.4.b Inclusion Enrollment Data**

NOTHING TO REPORT

**G.4.c ClinicalTrials.gov**

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

**G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT**

NOT APPLICABLE

**G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)**

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

**G.7 VERTEBRATE ANIMALS**

Not Applicable

**G.8 PROJECT/PERFORMANCE SITES**

Not Applicable

**G.9 FOREIGN COMPONENT**

No foreign component

**G.10 ESTIMATED UNOBLIGATED BALANCE**

Not Applicable

**G.11 PROGRAM INCOME**

Not Applicable

**G.12 F&A COSTS**

Not Applicable

## I. OUTCOMES

### I.1 What were the outcomes of the award?

The reliable measurement of airborne microorganisms remains a challenge, and this research worked to address this challenge. Thus, the main output of this project is a novel bioaerosol sampling tool, where electrostatic collection technique is combined with the use of hydrophobic collection surface and novel sample elution system. The newly developed Stationary Electrostatic Bioaerosol Sampler (SEBS) features low pressure drop and could be operated at a high flow rate of 20 L/min entirely by battery power for up to 8 hours. It is a lightweight and portable field device to evaluate exposures to airborne microorganisms. When the collected samples were eluted into 0.2 mL elution liquid, SEBS featured concentration rate of  $\sim 5 \times 10^4$  min<sup>-1</sup>. The concentration rate is the ratio of microorganism concentration in the air with that in the collection liquid. The higher the concentration rate the easier it is to detect the presence of airborne microorganism. Such a high concentration rate demonstrated by SEBS will enable faster detection and determination of bioaerosols, especially when sampling in low concentration occupational environments. In addition, SEBS exhibited high viability and culturability efficiency, especially with 1 mL elution liquid. Overall, SEBS is now one of the few electrostatic collectors, if any, that has been specifically developed and tested to measure airborne culturable microorganisms. The difference in the signal-to-noise between SEBS and other samplers was especially pronounced at occupational environments with low bioaerosol concentration and short sampling times, when SEBS was able to detect microorganism presence in as little as 1 minute. Concentrations of other samplers were below detection limit for short sampling times. Overall, the new sampler enables quick detection of airborne microorganisms, especially in low concentration environments thus saving time when one needs to quickly identify exposures in occupational environment. SEBS imparts lower mechanical stress to sensitive airborne cells compared to conventional sampling methods thus minimizing bias in sample analysis and allowing to obtain a more accurate characterization of exposures in various occupational environments. Intermediate and end outcomes of this project will depend on the adaptation of this technology by health and safety professionals.