DEVELOPMENT OF A SAMPLER FOR THE RAPID ANALYSIS OF BIOAEROSOLS

by

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To my beloved parents and grandmother

To my beloved husband, Lei

For the endless love, support, and encouragement

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ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BCYE	Buffered charcoal yeast extract
C_c	Cunningham correction factor
$C_{e.coli}$	*E. coli* concentration
C_{est}	Estimated concentration
CFU	Colony count unit
CFU_{adj}	Adjusted and counted CFUs
CFU_{ct}	Counted CFUs
CFU_{resp}	CFU of respirable bioaerosol
d_{50}	Cut-off diameter at which 50% of particles are collected
d_{ae}	Aerodynamic diameter
DI water	Deionized (DI) water
D_j	Nozzle diameter
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
ICA	Immunochromatographic assay
LOD	Limit of detection
LpJR32	*Legionella pneumophila* JR32
n_j	Nozzle number
OD_{600}	Optical density at a wavelength of 600 nm
Q	Flow rate
Re	Reynolds number
RLU	Relative light unit
Stk_{50}	Stokes number at a 50% collection efficiency
ΔP	Pressure drop
η_c	Collection efficiency
μ	Air viscosity

ρ_0	Unit density
ρ_p	Particle density

ABSTRACT

Airborne biological aerosols (also called bioaerosols) including pathogens are found in various occupational and environmental settings. To protect human health and the environment, the first step is assessing the exposure levels of bioaerosols in the fields where the sources of bioaerosols are located. However, the conventional methods to assess the level of bioaerosols are not ideal in the field due to their delayed analysis times. To overcome these limitations, faster and convenient analysis methods are required. In this study, a size-selective bioaerosol sampler was developed and combined with two swab-based analysis methods: 1) adenosine triphosphate (ATP) bioluminescence assay for measuring the total bioaerosol concentration and 2) immunochromatographic assay (ICA) for identifying specific pathogenic bioaerosols.

The size-selective bioaerosol sampler was developed and tested in the laboratory. Specifically, the bioaerosol sampler consisted of a size-selective inlet to remove the particles larger than target size, an impactor to collect bioaerosols onto the head of a swab used for both ATP bioluminescence assay or ICA, a swab holder, and a sampling pump. An impactor nozzle was designed based on a theoretical model. The collection efficiency of the fabricated impactor was tested using aerosolized sodium chloride particles and then the particle diameter corresponding to the collection efficiency of 50% (cut-off diameter, d_{50}) was evaluated. The experimental d_{50} was 0.44 μm which means the bioaerosol sampler can theoretically collect most bioaerosols.

In the study measuring the total bioaerosol concentration, the size-selective bioaerosol sampler was used with the ATP bioluminescence assay. First, test swabs were calibrated by comparing with the colony counting methods. Specifically, correlations between ATP bioluminescence (relative light unit; RLU) from commercially available swabs having different sensitivities and colony forming unit (CFU) were examined using *Escherichia coli* (*E. coli*) suspension, and then the conversion equations from RLU to CFU were obtained. From the correlation results, the R^2 values of the two swabs were 0.53 for the normal swab and 0.81 for the sensitive swab, respectively. The conversion equations were the linear function and the slopes of the normal and sensitive swabs were 633.6 and 277.78, respectively. In the laboratory and field tests, the size-selective bioaerosol sampler with ATP bioluminescence assay and a conventional

Andersen impactor with colony counting method were compared. In the laboratory tests, concentrations of aerosolized *E. coli* collected using the size-selective bioaerosol sampler were highly correlated to those from the Anderson impactor (R^2 = 0.85). In the field tests, the concentrations measured using the bioaerosol sampler combined with ATP bioluminescence assay were higher than those from the Andersen impactor with colony counting method due to the limitations of the latter approach.

In the study identifying pathogenic bioaerosols, *Legionella pneumophila* (*L. pneumophila*) was used as a target pathogen. Prior to combining the size-selective bioaerosols sampler and ICA, the lower limit of detection of the lateral flow test kit used in the ICA was determined. In the conditions of this study, the lateral flow test kit formed a positive line when more than 1.3×10^3 CFU of *L. pneumophila* were on the sampling swab. In further experiments, *L. pneumophila* suspension was aerosolized and then collected using two identical bioaerosol samplers having different sampling times (10 and 20 min) to estimate the concentration. Andersen impactor with colony counting method was also used for measuring the concentration of *L. pneumophila* and the comparison with the developed methods. The developed method successfully collected and detected aerosolized *L. pneumophila* compared to the Andersen impactor with colony counting method.

These findings confirm that the developed bioaerosol sampler combined with swab-based analysis methods can overcome the limitations of conventional methods and allows for more rapid, inexpensive, and accurate assessments for bioaerosols in the field. These methods can be immediately used in various environmental and occupational settings.

CHAPTER 1. INTRODUCTION

1.1 Bioaerosol

Bioaerosols are aerosol particles of biological origins (e.g., bacteria, fungi, fungal spores, pollen, fragments of biofilm, etc.) (Delort and Amato, 2017; Humbal et al., 2018). Since bioaerosols can be suspended in the air for a long period, they are ubiquitous in indoor and outdoor air (Jaenicke et al., 2007; Matthias-Maser et al., 2000). Typically, a human adult inhales about 10^4-10^5 colony forming unit (CFU) through 1 m^3 of air in the outdoor environment (Li et al., 2011; Wu et al., 2018). In some contaminated places, bioaerosols could easily exceed 10^8 CFU/m^3 (Codina et al., 2008; Toivola et al., 2002). Inhaled by a human, they would be deposited in the respiratory tract, and potentially cause irritation, allergies, contagious infectious disease, acute toxic effects, cancer, and even death if the concentrations of bioaerosols are high (Aimanianda et al., 2009; Douglas et al., 2018; Kim et al., 2018; Xu et al., 2011). For example, some occupational places such as composting sites, wastewater treatment plants, food industries, livestock farms, health care centers have a high concentration of bioaerosols (Delort and Amato, 2017; Gao et al., 2018; Hsiao et al., 2019; Poh et al., 2017; Walser et al., 2015). At agricultural workplaces, high concentrations (10^5-10^7 CFU/m^3) of bacteria and fungi have caused infections and allergies, even death to farmers (Delort and Amato, 2017; Liang et al., 2013; Samake et al., 2017). People working in healthcare facilities are at higher risk of infections such as gastroenteritis and damaged skin and mucous membranes damages because of the spread of bacterias in the air (Bonifait et al., 2015; Iturriza-Gómara and Lopman, 2014; Pothier and Kaiser, 2014). Particularly, airborne infectious diseases transmitted in a form of bioaerosols pose a health threat especially for people in occupational places such as clinic centers (Tsay et al., 2020). Legionellosis, influenza, measles, and tuberculosis, are often spread by aerosols especially in poorly ventilated environments (Kundsin, 1980; Gao et al., 2016; Samake et al., 2017). The current pandemic outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can also transmit in the form of bioaerosols (Anderson et al., 2020; Kohanski et al., 2020). Other pandemics such as H1N1 influenza, and the H5N1 avian influenza have raised concerns among scientists about the importance of aerosol transmission studies (Aditama et al., 2012; Thompson et al., 2013).

1.2 Sampling Techniques

For protecting humans and the environment from biological threats, bioaerosol exposures should be evaluated even though standards for acceptable levels of bioaerosols still have not been established (Walser et al., 2015). To analyze the bioaerosols, they should be separated from the environment and then collected in the sampling media. For this purpose, aerosol behavior principles can apply to bioaerosols. So far, various sampling methods have been suggested and used to characterize bioaerosols. For exampling, general aerosol sampling techniques such as filters, impactors, and cyclones have been used to collect bioaerosols. Each sampling technique has associated benefits, requirements, and constraints, and it is important to design new sampling protocols based on specific aims. Sampling methods can be categorized based on different criteria.

1.2.1 Categories of Sampling Methods

In general, bioaerosols are collected using passive or active sampling systems in terms of mechanical forces. Passive sampling is a low-cost and simple method for collecting settleable microorganisms based on gravitational settling, generally on agar surfaces or sedimentation plates (Mainelis et al., 2020). After incubation, the collected bioaerosols are identified and counted within the area of the settling plates for the duration of a specified period. Passive sampling is convenient to use and inexpensive compared to active sampling. However, the results are treated only as qualitative as the sampled volume of air is uncertain. Otherwise, active sampling could determine the cultivable bioaerosol concentrations as well as identify microorganisms by using a pump to force air flow into the system. The volume of air from which bioaerosols are deposited can be determined. The active sampling approaches are generally composed of bioaerosol samplers, pumps, and collection sections for analysis. However, the need to sampling pumps and the related power requirements can lead to restricted sampling mobility. (Mainelis et al., 2020).

Classified by sampling zones, bioaerosol sampling techniques vary from large area samplers to small and portable personal samplers. Personal samplers are small devices that can be attached to workers' outfits to provide a representative sample of the worker's individual exposure (such as breathing zone exposure; sampling within 30 cm away from mouth and nose) while area sampling that measures the environmental (background) concentration for the working environment. Large area samplers operate with higher flow rates of about 12.5 - 800 L/min, while

personal samplers operate at about 2 - 10 L/min (Chang et al., 2012; King et al., 2012; Hall et al., 2013).

Size selection is the other category to classify the samplers. The size of bioaerosols is an important parameter to characterize bioaerosol behavior and a major determinant of their impact on human health. As shown in Figure 1.1, inhalable bioaerosols can get into the mouth and nose while thoracic bioaerosols are deposited in the upper respiratory area. The respirable bioaerosols are the smallest particles in the category and can reach the terminal bronchioles and the alveoli. Moreover, the respirable bioaerosols remain in the lungs for long durations, sometimes even for life, unless they are removed by migrating pulmonary macrophages. For this reason, the respirable bioaerosols are of particular health concern.

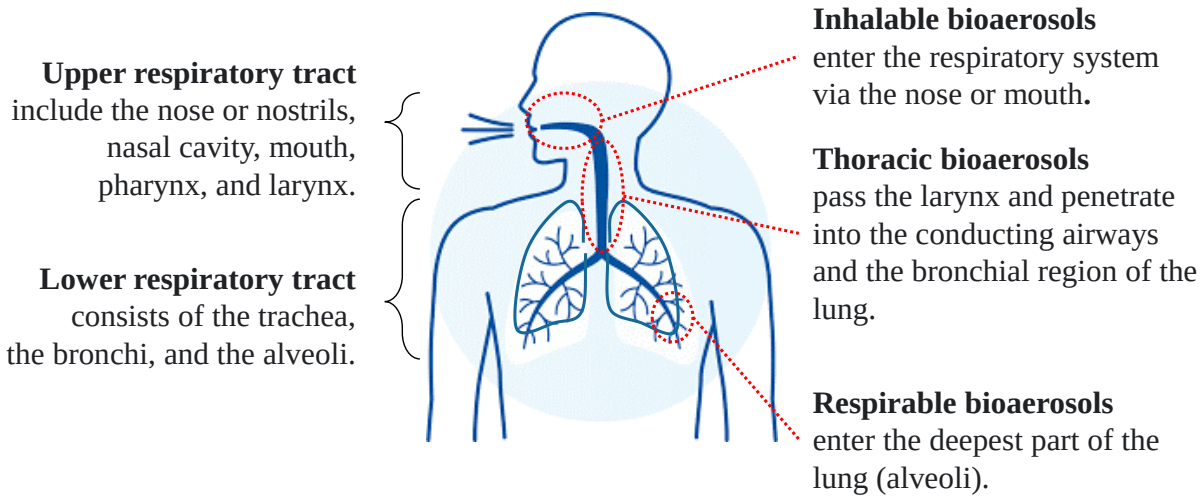

Figure 1.1 Types of bioaerosols categorized by size

Size-selective samplers are commonly used to separate and collect aerosol particles in different size fractions. For example, conventional aerosol samplers such as inhalable, thoracic, and respirable samplers have been used to collect bioaerosols by categorized size. The fraction of particles of a particular aerodynamic diameter that are collected by the sampler is defined as the collection efficiency. The aerodynamic diameter at which the collection efficiency is 50% is

defined as the cut-off diameter (d_{50}). The d_{50}s of inhalable, thoracic, and respirable samplers are 100, 10, and 4 μm, respectively (Vincent et al., 2005) (Figure 1.2).

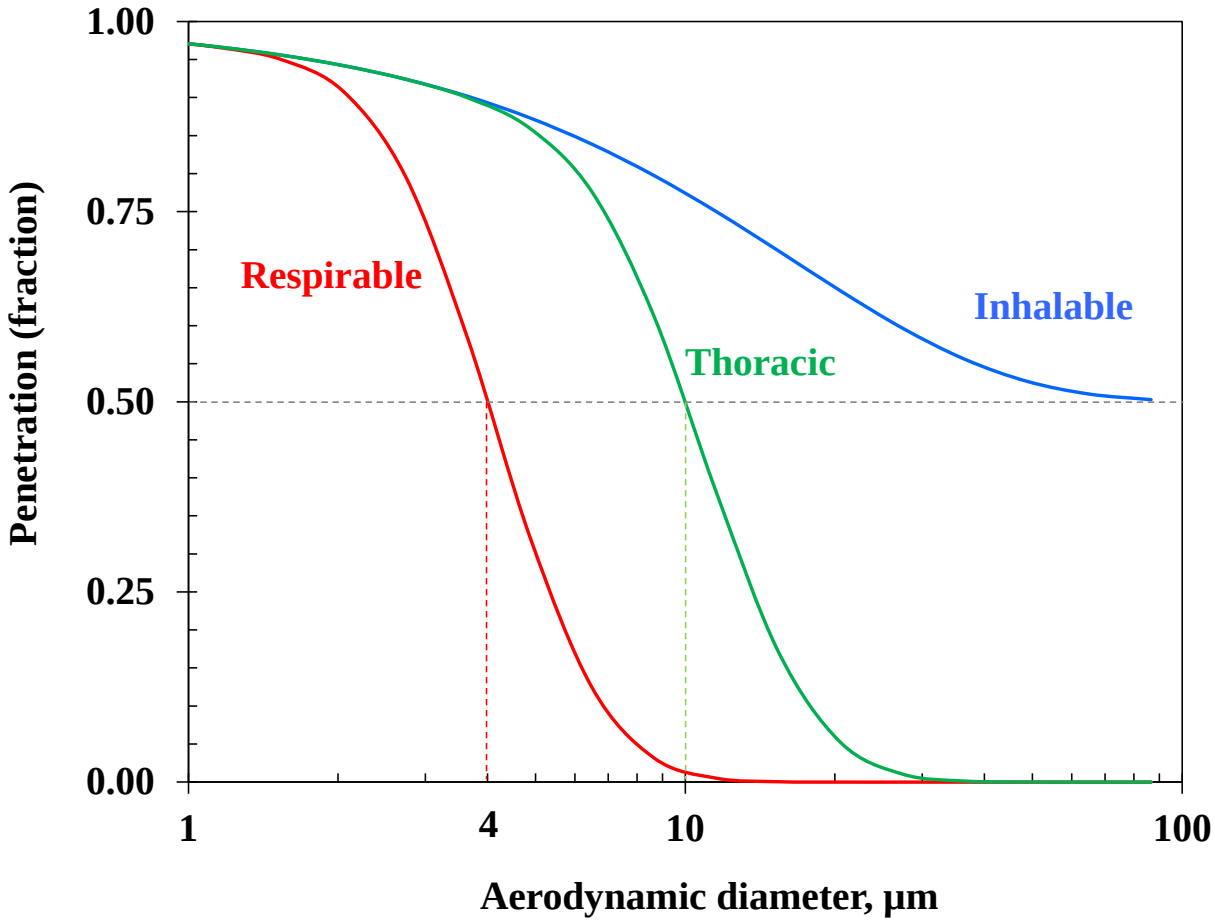

Figure 1.2 Inhalable, thoracic, and respirable sampling criteria

Currently, the most widely used bioaerosols samplers broadly fall into five categories including filters, impactors, cyclones, impingers, and electrostatic samplers.

1.2.2 Filters

Filters are widely used to capture bioaerosols. They are inexpensive, easy to use, and can be used for many types of sample analyses such as microscopy, culturing, deoxyribonucleic acid (DNA) analysis, etc. Generally, filters separate bioaerosols from the air and then collect them. The mechanisms are settling, impaction, interception, and diffusion. Most sampling filters have collection efficiencies close to 100% which means they can collect most of the bioaerosols. For

size-selective sampling, a size-selective inlet is used before the sampling filter. The collection efficiency and size selection of this combination mainly depend on the characteristics of the filter and the size-selective inlet (Burton et al., 2007; Ghosh et al., 2015). A common way of employing filters for sampling is to place the filters in a disposable two-piece or three-piece plastic cassette with a support pad to add rigidity as shown in Figure 1.3. These cassettes are generally used with close- or open-face for collecting total suspended bioaerosols. In these cases, size-selective sampling is not available. For the size selection, the filters are used in the cassettes with the respirable cyclones or special samplers having size-selective inlets as shown in Figure 1.3. The sampling filters in the Button sampler and the Institute of Occupational Medicine (IOM) sampler can collect inhalable bioaerosols.

Figure 1.3 Samplers with filters

The choice of filter materials depends on the sampling of interest and the analytical technique. For the microscopy analysis, cellulose ester or polycarbonate membranes are common choices because they have flat surfaces. For cultivation purposes, samples can be collected on water-soluble gelatin filters.

Sampling the bioaerosols on the filters would be the simplest method but they also have disadvantages to make them not used. For example, the collected bioaerosols can be dried with airflow during the sampling and the recovery fraction of collected bioaerosols can be low after washing or eluting from filters. Collected bioaerosols can not be easily washed or detached from the filters if the filter materials are hydrophobic.

1.2.3 Impactors

One of the most common collection methods for bioaerosols is inertial impaction methods (Kuo, 2015; Yoon et al., 2010). In impaction methods, the particles are separated from the airstream based on their size. Specifically, the airstream is forced to turn suddenly, and the particles that cannot follow the flow impact a surface, from which they can be collected (Figure 1.4a). Impactors can separate the bioaerosols smaller than d_{50} and collect them on the impaction stage. The number, size, and shape of the nozzle can vary. For example, a single slot nozzle is used in VersaTrap (SKC Inc., U.S.A) and Air-O-Cell (Zefon, U.S.A.), and 400-round nozzles are used in Biostage (SKC Inc., U.S.A). As an impaction plate, glass slides, membrane substrates, and the culture media (e.g. agar plate) can be used. Glass slides are generally used for the microscopy analysis, and the membrane substrates are used for various analysis methods including both non-culture and culture-based methods. Culture media is most commonly used in bioaerosols sampling to assess their concentrations and content (Lindsley et al., 2017).

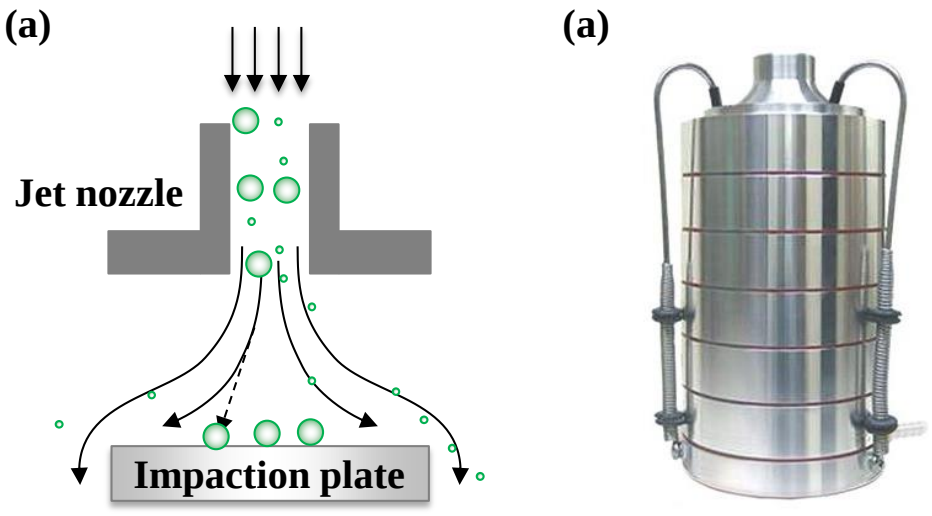

Figure 1.4 (a) Mechanism of impaction and (b) the Andersen cascade impactor

Multi-stage impactors (called cascade impactors) can provide better size information. Six-stage Andersen cascade impactors are commonly used to measure the concentration of bioaerosols by size (Figure 1.4b). This is often demonstrated to be the most efficient sampler for viable bioaerosols. Each stage consists of a multi-jet nozzle plate and a Petri dish containing agar media as the impaction plate. The main advantage of the Andersen impactor is that the instrument can divide particles into six fractions based on the aerodynamic diameter of the particles. Thus, the size distribution can be obtained. However, there are several difficulties in using the Andersen impactor. Its operational flow rate of 28.3 L/min is relatively high for high concentration environments, which can easily cause an overlap of bacteria on agar plates in several minutes (Lindsley et al., 2017). Another type of bioaerosol impactor developed by Chen and Yao (2018) had a sampling flow rate as high as 1200 L/min and also had the same overloading problems as Andersen Impactor. To achieve these high sampling flow rates, they require heavy external vacuum pumps. Other difficulties include limitations on maximum CFU value due to the number of impactor nozzles (e.g., 400 holes per stage for Andersen cascade impactor) which limits the sampling time. Moreover, jets from nozzles can dry and shear the collected bioaerosols and reduce the viability which may introduce significant measurement errors.

1.2.4 Cyclones

Cyclones are centrifugal impactors and are also frequently used for collecting bioaerosols at high airflow rates. The separation process of cyclones relies on the centrifugal accelerations that are produced when particle-laden fluid experiences a rapidly swirling motion. Lager particles are separated from the airstream due to the centrifugal force and hit the side wall while small particles exit from the cyclone with the airstream (Figure 1.5a). The cyclones collect particles smaller than d_{50} on the drywall (Figure 1.5b). Some cyclones collect the particles directly into a liquid media which are called wet-cyclones (Figure 1.5c). A commercially available wet-cyclone is the Coriolis® μ sampler (Bertin Instruments, France) which was developed by Mbareche et al. (2018). This device collects airborne particles directly into a microtube, at a rate of 300 L/min. The viability of collected samples is enhanced compared to those collected by the dry cyclone. The particle bounce which may significantly affect the collection efficiency is reduced in Coriolis® μ sampler. However, cyclone samplers were demonstrated to have lower collection efficiency in collecting bioaerosols than the Andersen samplers (Wüst et al., 2003). In addition, the collection

efficiency of the Coriolis® was lower than that of a swirl-impinger (BioSampler, SKC Inc., U.S.A) for the sampling of fungi (Chang, et al., 2019).

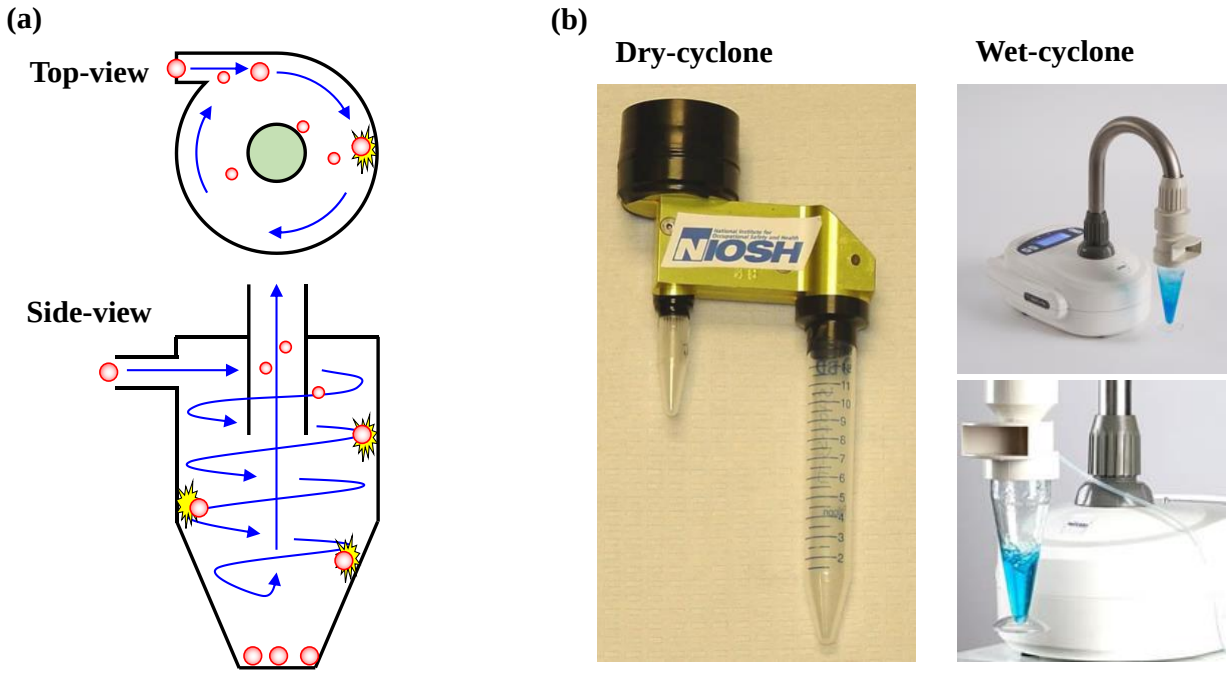

Figure 1.5 (a) Mechanism of the cyclone and (b) commercially available cyclones

1.2.5 Impingers

Impingers (known as bubblers) collect bioaerosols into designated collection liquids for analysis and utilize specially designed bubble tubes accelerating air through a nozzle into a volume of collection liquids. Similar to the impactors, larger particles are collected in the collection liquids due to their inertia but the d_{50}s of impingers are usually larger than ones of impactors. Some impingers have an angled nozzle to make a swirl increasing the collection efficiency or a multi-jet nozzle to increase the sampling flow rate. The swirling impinger, BioSampler (SKC Inc., U.S.A) is a commercially available sampler having three angled jet nozzles. The collection efficiency of this sampler is higher than one of the water-soluble gelatin filters when sampling the H1N1 influenza A virus (Li et al. 2018). Generally, impingers are gentler for living microorganisms than impactors because the liquid media enhance their viability. However, impingers still have limitations such as evaporation, re-aerosolization, and viability decrease of samples over extremely

long sampling periods (Haig et al., 2016; Han et al., 2012). Moreover, impingers require special care in handling because they are made of glass. In some cases, a water trap is required downstream of the impinger to protect a sampling pump from the water.

1.2.6 Electrostatic Samplers

Electrostatic samplers collect bioaerosols of submicron size by electrostatic forces. Before progressing through an electric field, bioaerosols are electrically charged at the inlet, then separated from the airstream and deposited on the charged plates. Compared to impactors and impingers, electrostatic samplers are operated at a slower velocity which may generate lower pressure on the bioaerosols (Mainelis et al., 1999). Recently, many researchers have been developing electrostatic samplers to collect bioaerosols. Lindsley et al. (2017) developed the electrostatic sampler collecting bioaerosols into a liquid media to help preserve the viability of microorganisms. Priyamvada et al. (2021) developed a portable, low-cost electrostatic sampler combined with a sensor for the measurement of bioaerosol concentration. However, electrostatic samplers have limitations. Ozone may be generated during sampling by electrostatics which is injurious to bioaerosols. Air ions in the system are also responsible for killing or inactivating bioaerosols. The electrical energy used by an electrostatic sampler can be substantial, resulting in a high cost for operating the device (Li et al., 2003). More investigations are needed to determine the effects of electric fields on the viability of microorganisms.

1.3 Analysis Methods

The sampling methods introduced in the previous section can be combined with various analysis methods. The analysis method for collected bioaerosols include: 1) culture-based and 2) non-culture-based methods (e.g., microscopy analysis, Nucleic Acid-Based Molecular Diagnostics, etc.).

1.3.1 Culture-Based Methods

Cultivation (Culture) of sampled microorganisms is by far the oldest means to analyze bioaerosols. Cultivation provided necessary nutrients and opportune environmental conditions to the microorganisms, allowing them to multiple to visible colonies. However, the analyzable and

effective culture sample is highly dependent on factors such as microorganism species, culture media, incubation condition, etc. Only the viable microorganisms growing on the media provided under the offered incubation conditions in the competitive microbial community are effective for analysis. Therefore, a small fraction of sampled bioaerosols that are viable and culturable can be detected. Researchers suggested that only 1-2% of all environmental bacteria are culturable (Sharma et al., 2005). Non-viable and non-culturable bioaerosols are still important from a health perspective. Moreover, organisms viable upon collection may not stay viable after long sample preparation and analysis procedure as described in the previous section (1.2.2). The collection and transfer procedure may damage the microorganisms and decrease the analyzable samples. In addition, the cultivation of viral bioaerosols is extraordinarily difficult. Viruses are obligatory intracellular parasites that require a host cell for reproduction (Tortora et al. 2015). Viruses can be extremely host-specific, even cell-line-specific. Bioaerosols of pathogenic viruses have been found in many settings to be difficult to detect (Blachere et al. 2009; Bonifait et al. 2015).

1.3.2 Non-Culture-Based Methods

At present, non-culture-based methods have become increasingly essential. They can avoid the loss of microorganisms in sample transfer and cultivation procedures and also classify nonviable and non-culturable microorganisms. Generally, non-culture-based methods include microscopy, nucleic acid-based molecular diagnostics, adenosine triphosphate (ATP) bioluminescence assay, immunochromatographic assay (ICA), etc. (Lindsley et al., 2017).

1.3.2.1 Microcopy

Microscopy includes a variety of approaches that utilize bright-field, light, fluorescence, or even electron-based approaches (Eduard et al., 2012). In bright-field or light microscopy, an ordinary microscope is used for the morphological observation of sampled bioaerosols. The electron microscopy provides images that can be used to look at aerosol particles at high magnification up to 10,000,000 times. These approaches provide a platform to visualize particle morphology and detailed structures (Flannigan et al., 2002). Particularly, it is commonly used for the identification of fungal bioaerosols based on the morphological characteristics of the spores, overcoming non-viable method limitations in viable analyses (Eduard et al. 2012). However, the

results of microscopy analysis can be confounded by observer bias, especially when it comes to differentiating bioaerosols that contain similar morphological phenotypes (Flannigan et al., 2002). This approach also cannot be conducted in short periods owing to the sample preparation and detection procedure.

1.3.2.2 Nucleic Acid-Based Molecular Diagnostics

Nucleic acid-based molecular diagnostics are rapidly becoming the preferred method for detecting and quantifying bioaerosols. Polymerase chain reaction (PCR)-based methods are commercially available and identify the pathogenic virus or bacteria without culturing (Chen et al., 2005). Three steps are generally involved in this method: extraction and purification of nucleic acids; amplification of the gene target; and detection of the amplicon. Depending on the sample and the aims of the analysis, various DNA and RNA extraction methods and kits are available. The number of copies of a target gene in a sample can be determined by quantitative PCR (qPCR) by measuring the increasing amount of PCR products in real-time using fluorescent dye or TaqMan probes. The advantage of qPCR is the ability of rapid sample quantification and species-specific identification. The qPCR method has assay sensitivity and detection specificity. However, PCR-based methods require dedicated equipment and trained personnel and are therefore difficult to use in fields (Shan et al., 2015). The practical detection limit is higher because of the losses of DNA in samples during DNA extraction. In addition, this equipment is sophisticatedly built, making it costly.

1.3.2.3 ATP Bioluminescence Assay

The ATP bioluminescence assay can quantify bioaerosol by measuring the light produced through ATP's reaction with enzyme luciferase by a luminometer (Figure 1.3) (Møretrø et al., 2019; Stewart et al., 1997). ATP is the primary carrier of energy in cells through its high-energy phosphate bonds. Dephosphorylation of ATP into adenosine diphosphate (ADP) and adenosine monophosphate (AMP) releases the energy necessary for most endergonic enzymatic reactions and transport in cells (Törnroth-Horsefield et al., 2008). The measurement of ATP quantities can be sensitive because the assay involves the enzyme luciferase from fireflies. Luciferase oxidizes d-luciferin and emits photons in the presence of ATP at a stoichiometric ratio. Since the intensity

of light produced is directly proportional to the ATP content, it is possible to quantify the microbial amount by measuring the number of photons emitted from a known volume of a sample containing luciferase and its substrate luciferin. Using conversion factors, the amount of ATP measured can be converted into the number of potentially viable cells. Thus, ATP bioluminescence assay is designed to measure the bacterial concentration on the surface without incubation.

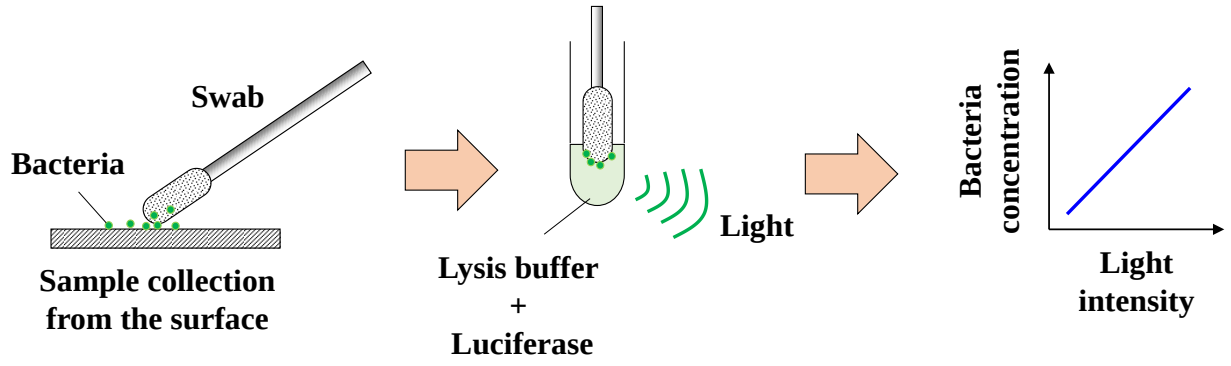

Figure 1.6 Procedure to measure the concentration of bacteria on the surface using ATP bioluminescence assay

Currently, it has been increasingly studied and utilized for monitoring microorganisms in different fields (Nante et al., 2017; Lappalainen et al., 2000; Vilar et al., 2008). The efficiency of the ATP bioluminescence assay has been evaluated in many research and some of these studies are done by comparison experiments with the colony counting method (Nante et al., 2017; Chen and Godwin, 2006; Huang et al., 2015). However, most of these tests are conducted for surface hygiene other than bioaerosol. Park et al. (2015) have demonstrated fast bioaerosol measurement by a novel sampler that captured airborne bacteria particles in a flowing liquid containing ATP bioluminescence reagents linked to a bioluminescence detector, although this device was difficult to use due to its huge size and complicated structure.

1.3.2.4 ICA

The ICA is another analytical technique for measuring a targeted antigen (Figure 1.7). The ICA has been primarily developed for rapid field testing but has also been incorporated in clinical laboratories. The method usually consists of single-use, disposable cartridges or strips (called

"lateral flow test kits") to quickly confirm the presence or absence of a compound (Wever et al., 2000; El-moamly, 2014; Ramos et al., 2017; Tominaga, 2019). In the lateral flow test kit, the antibodies mounted on a paper strip or a nitrocellulose membrane are utilized as the immobile capture antibody (test area). Capillary flow is used to move a colloidal gold or colored microparticle-labeled antibody conjugate which binds to the target antigen in the mobile phase as it moves toward the capture antibody in the immobile phase. A positive test is produced by the capture of the moving labeled antigen/antibody complex by a second immobilized anti-species antibody in the test area, and the formation of a colored line or pattern. Another control antibody to the conjugate binds the excess colloidal dye conjugate and acts as the control line (El-moamly, 2014). The control line is an indicator of the validity of the test. This analysis can be performed in approximately 25 min and requires no special equipment (Tominaga et al., 2019). To date, many commercially available lateral flow test kits have been used for identification of the infectious agent in many studies (Jørgensen et al., 2015; Sajid et al., 2015; Cruz et al., 2006). These developed test kits enable the identification of bioaerosols in the environment and provide a ready-to-use platform for the industrial hygienist. Similar to ATP bioluminescence methods, a swab is required to take a biological sample in ICA. These swab-based measurements are user-friendly, rapid, and low-cost. However, most of these tests are conducted for surface hygiene or water quality other than bioaerosol.

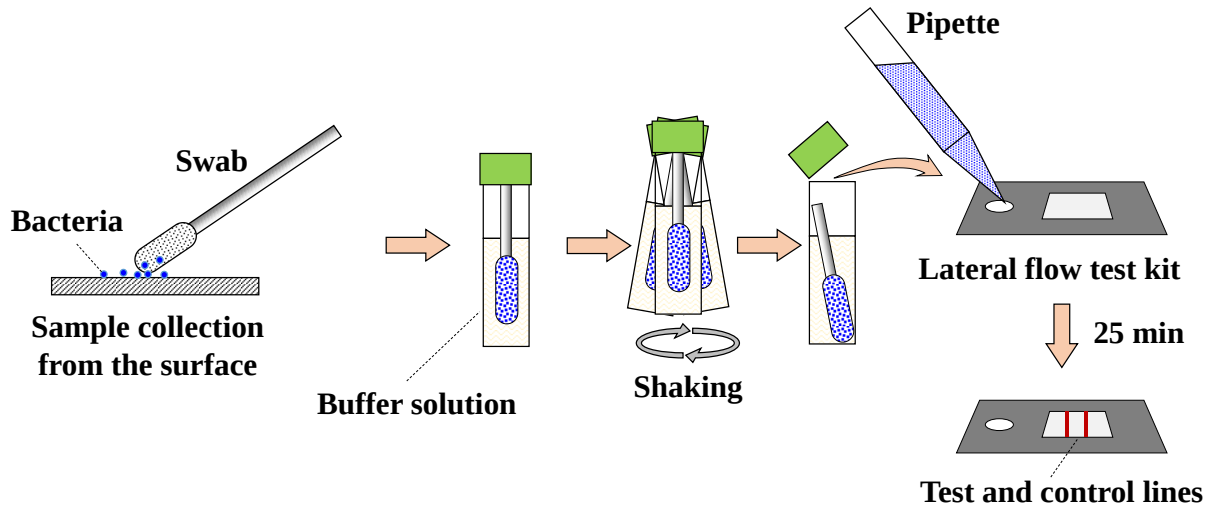

Figure 1.7 Procedure to detect the bacteria on the surface using ICA

1.4 Current Challenges

A universal bioaerosol sampler and standardized bioaerosol sampling protocols have not been established. The existing bioaerosol sampling methods relying on filtration, impaction, impingement, electrostatic precipitation, or other mechanisms to capture bioaerosols, differ in their characteristics, collection efficiency, and ability to preserve critical bioaerosol properties. At the same time, analytical tools measure for certain aspects of bioaerosol presence based on culturability and viability, ATP and deoxyribonucleic acid (DNA) amount, presence of specific antigens, or gene sequences, etc. To assess the exposure in the field, the analysis should be completed rapidly after collecting the bioaerosols. Therefore, an appropriate and practical combination of sampling and analysis methods should be selected. Currently, the disadvantages of the current sampling and analysis methods listed above create difficulties in analyzing bioaerosol in the fields. For example, the most commonly used combination, the six-stage Andersen cascade impactor with colony counting after the incubation, has several limitations. Specifically, the Andersen impactor is not portable and can not be used for personal sampling due to its bulky size and large pump. Colony counting also has limitations including time-consuming for the incubation and a limited measurable concentration range. These limitations should be overcome to better assess the exposure to bioaerosols in the field. Therefore, the ideal combination of sampling and analysis methods needs to be developed and should meet the following conditions:

a) Sampler
    - The sampler needs to be small and mobile and can be used for both personal and area sampling.
    - The sampler should collect bioaerosols falling within a specific size range (e.g., respirable bioaerosols).
    - The sampling capacity of bioaerosols should be large.
    - The sampler should have a collection efficiency higher than 90%.

b) Analysis method
    - The analysis time should be shorter than an hour.
    - The analysis should be able to be done in the field.
    - The analysis method should be economic.

Based on the conditions above, the small and wearable impactor may be considered as a sampler but should be combined with the non-culture-based method. The sampling media also has to be used in the non-culture-based method. Thus, swab-based sampling methods can be an alternative approach and easily combined with the non-culture-based method.

1.5 Objectives and Specific Aims

The main goal of this research is to develop a simple, rapid, and accurate method to analyze bioaerosols in the field which is significantly important to overcome the limitations of conventional methods. The main hypothesis is that a swab-based sampler combined with non-culture-based methods can analyze bioaerosols concentration and identify airborne pathogens more efficiently and rapidly. To achieve the research goal and the test the main hypothesis, three specific aims were established as follows:

Specific Aim 1. Develop the bioaerosol sampler to collect bioaerosols on the swab. A new size-selective sampler was designed and fabricated to collect bioaerosols onto the head of swabs used in non-culture-based methods such as the ATP bioluminescence assay or ICA. The sampler consisted of a size-selective inlet, an impactor, a swab holder, and a sampling pump. An impactor nozzle was designed based on theory. The collection efficiency and d_{50} of the fabricated impactor were evaluated using aerosolized salt particles.

Specific Aim 2. Evaluate the performance of the bioaerosol sampler combined with ATP bioluminescence assay for measuring the total bioaerosol concentrations. To measure the total bioaerosol concentration, the new sampler developed under Specific Aim 1 was combined with ATP bioluminescence assay. *Escherichia coli* (*E. coli*) was selected as test bacteria. The conversion equation was obtained to convert the relative light unit (RLU) from ATP bioluminescence assay to the CFU. The results from the developed method were compared with those from a conventional method both in lab and field tests.

Specific Aim 3. Evaluate the performance of the bioaerosol sampler combined with ICA for detecting pathogenic bioaerosols. Pathogenic bioaerosols were detected using the combination of the sampler developed under Specific Aim 1 and ICA. The commercially available lateral flow test kits were used for detecting *Legionella pneumophila* (*L. pneumophila*). The lower limit of

detection (LOD) of the test kits was evaluated. In the lab experiments, aerosolized *L. pneumophila* was sampled and analyzed using the developed and conventional methods. Results from both methods were compared. The method to estimate the concentration of pathogenic bioaerosols using the dual samplers was also suggested.

CHAPTER 2. DEVELOPMENT OF A BIOAEROSOL SAMPLER TO COLLECT BIOAEROSOLS ON THE SWAB

2.1 Introduction

Hypothesis: A size-selective sampler can collect bioaerosols directly onto the swab head for further analysis methods including the ATP bioluminescence assay and the ICA.

Rationale: For collecting airborne bacteria, the impactor should have a d_{50} below the size range of interest, which is currently assumed to be either 2.5–10 μm, as used in the U.S. Government Biological Integrated Detection System, or 1–10 μm, as used in the U.S. Government Joint Biological Point Detection System (Haglund and McFarland, 2004). A d_{50} of 0.5 μm was set for the impactor to collect even a single small bacteria cell since the size of a single bacteria cell is generally larger than 1 μm. The use of the impactor can decrease sample loss and increase the reliability and repeatability of sampling. Furthermore, a size-selective inlet, e.g., a respirable cyclone that collects fractions of aerosol that can penetrate the gas–exchange region of the respiratory tract defined as 50% for 4 μm particles and 100% for particles smaller than 1 μm, was also added. The respirable sampler can be substituted to particulate matter (PM)2.5 or PM10 impactor for environmental studies. After sampling, bioaerosols collected onto swabs can be analyzed by ATP bioluminescence assay for measuring the concentration or ICA for detecting pathogens.

2.2 Methods

2.2.1 Design and Fabrication of Bioaerosol Sampler

The schematic of the new sampler is shown in Figure 2.1. The sampler consisted of a respirable cyclone, an impactor, and a swab holder. The respirable cyclone (225-01-01, SKC, U.S.A.) was used to remove airborne particles bigger than 4 μm before the particles were collected onto the impaction section. The cyclone was made of aluminum for lightweight and conductive. The impactor nozzle plate with a diameter of 25 mm was placed in an impactor housing below the cyclone. There were nozzle holes on the impactor plate. An O-Ring (Environmental Express O-Ring, Zefon, U.S.A.) with a diameter of 25-mm was located between cyclone and impactor nozzle

plate to attain an airtight connection. Below the impactor, there was a swab holder used for installing the swab inside. The set screw was located at the end side of the swab holder used for adjusting the length of the swab holder, allowing for different swabs to be fit for it. Particle-laden air was drawn into the respirable cyclone, the impactor nozzles, and collected onto the head of a swab inserted in the holder, using a vacuum pump at the flow rate of 2.5 L/min. The impactor nozzle plate, impactor housing, O-ring, and swab holder are all made of autoclavable materials.

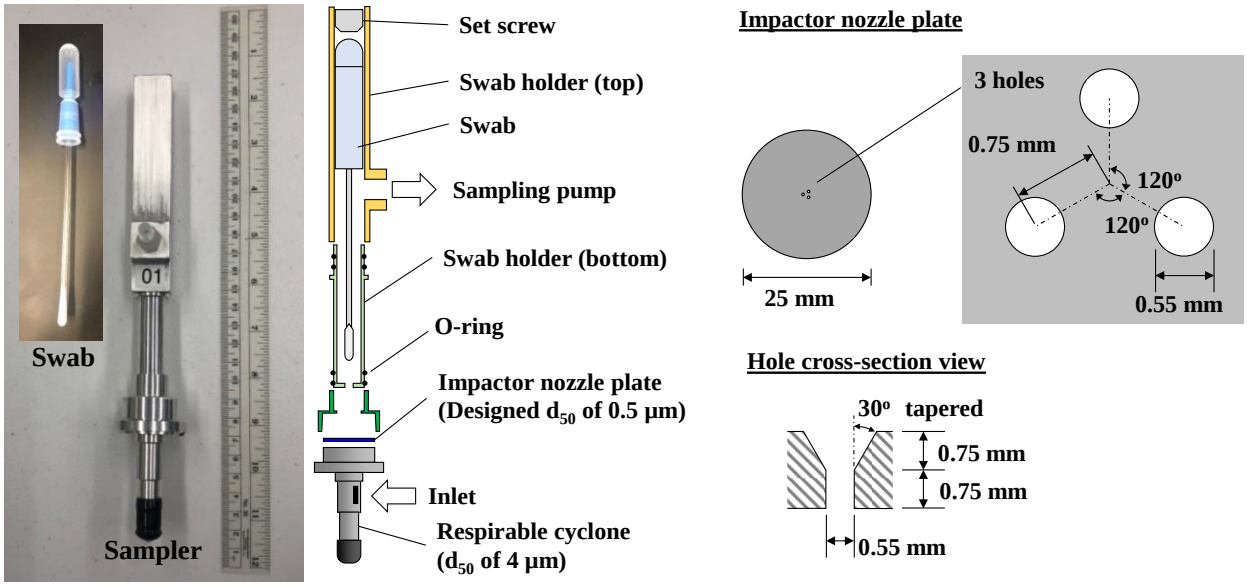

Figure 2.1 Schematic of developed bioaerosol sampler

An impactor nozzle was designed using equations from Marple and Willeke (1976) and Hinds (1999). The target d_{50} of the impactor was set to 0.5 µm to collect even a single small cell (~1 µm). The theoretical d_{50} was calculated as follows:

$$d_{50}C_c^{0.5} = \left(\frac{9\pi\mu n_j D_j (Stk_{50})}{4\rho_p Q}\right)^{0.5} \quad (1)$$

where C_c is the Cunningham correction factor, μ is the air viscosity, n_j is the nozzle number, D_j is the nozzle diameter, Stk_{50} is the Stokes number at a 50% collection efficiency, ρ_p is the particle density, Q is the flow rate. The Reynolds number (*Re*) was also calculated as follow:

$$\mathrm{Re} = \frac{4\rho_p Q C_c d_{50}^2}{\pi \mu n_j D_j}. \quad (2)$$

To control *Re* between 2000 and 3000, the values for n_j, D_j, and Q were set as 3, 550 μm, and 2.5 L/min respectively. The theoretical pressure drop (ΔP) was calculated as the following equation:

$$\Delta P = \frac{1}{2}\rho_a V_j^2 \quad (3)$$

where V_j is the jet velocity and ρ_a is the air density. The MatLab® R2017 (ver. 9.3.0, MathWorks, Inc., U.S.A.) with Simulink (ver. 9.3., MathWorks, Inc., U.S.A.) was used to calculate the equations. The theoretical *Re*, d_{50}, and ΔP were 2104, 0.527 μm, and 2 kPa, respectively. A distance between the centers of nozzles was 1.3 mm to concentrate the three-jets onto the swab head (cross-sectional diameter of 3.6 mm).

Based on these parameters, three impactor nozzles were machined on the polyether ether ketone (PEEK) plate (diameter of 25 mm) as shown in Fig. 1. Each nozzle was examined using an optical microscope.

2.2.2 Collection Efficiency of Impactor

The experimental setups for the generation of test aerosols and the evaluation of the collection efficiency of the impactor are shown in Figures 2.3 and 2.4, respectively.

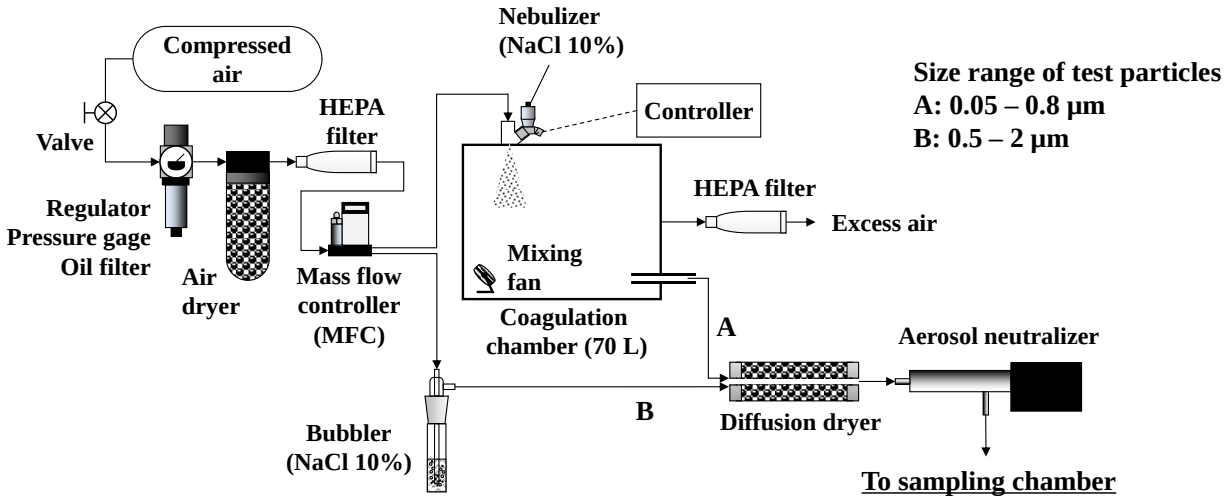

Figure 2.2 Experimental setup for aerosol generation system

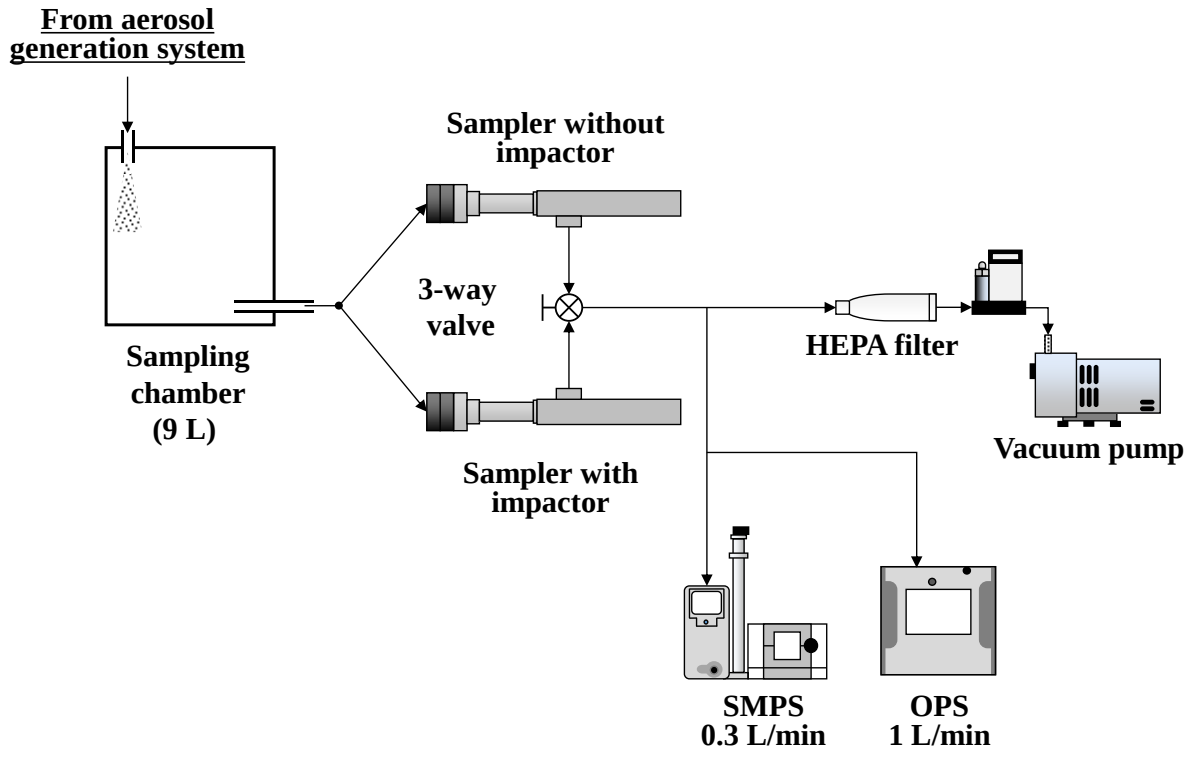

Figure 2.3 Experimental setup for collection efficiency

To determine particle collection efficiency as a function of particle size, two aerosol generation systems were used to produce particles with different size distributions. A stream of particle-free compressed air from a dry particle-free air supply system consisting of an oil filter, an air dryer, and a high-efficiency particulate air (HEPA) filter was controlled by a mass flow controller (MFC; MC-10SLPM-TFT, U.S.A.) and delivered to a vibrating mesh nebulizer (Aeroneb Solo, Aerosgen, Ireland) or a bubbler (225-36-2, SKC, U.S.A.). Sodium chloride (NaCl) 10% solution (weight/volume) was filled in both nebulizer and bubbler. The NaCl aerosols from the nebulizer or the bubbler were passed through a diffusion dryer for removing water and a neutralizer (3088, TSI Inc., U.S.A.) for neutralizing the particles. Subsequently, the NaCl aerosols were conveyed to the sampling chamber and were pulled through a three-way valve to pass through samplers. The sampler linked in the top line did not have the impactor nozzle plate and the swab while the other sampler in the bottom line had both of them. The respirable cyclone was not installed in the sampler during the collection efficiency test. Particle number concentrations downstream of samplers were measured using a scanning mobility particle sizer (SMPS; 3938NL76, TSI Inc., U.S.A.) or an optical particle sizer (OPS; 3330, TSI Inc., U.S.A.). Test particles produced from the nebulizer and the bubbler were measured using the SMPS (0.05-0.8 μm) and OPS (0.5-2 μm), respectively. The SMPS consisted of a classifier controller (3082, TSI Inc., U.S.A.), a differential mobility analyzer (DMA; 3081A, TSI Inc., U.S.A.), a condensation particle counter (CPC; 3776, TSI Inc., U.S.A.), and an aerosol neutralizer (3088; TSI Inc., U.S.A.).

The mobility diameter (d_m) measured using the SMPS was converted to volume equivalent diameter (d_{ve}) as following equations (Peters et al., 1993):

$$d_{ve} = d_m \times \frac{C_c(d_{ve})}{\chi \times C_c(d_m)} \quad (4)$$

where χ is the dynamic shape factor and assumed to be 1.08 for NaCl particles. The d_{ve} was then converted to aerodynamic diameter (d_{ae}) as follows:

$$d_{ae} = d_{ve} \times \sqrt{\frac{\rho_p \times C_c(d_{ve})}{\chi \times \rho_0 \times C_c(d_{ae})}} \quad (5)$$

where ρ_0 is the unit density (= 1000 kg/m^3) and ρ_p is the particle density. The ρ_p was assumed to be 2160 kg/m^3 for NaCl particles. The optical diameter measured using the OPS has been assumed to be equal to d_{ve} (Peters et al., 2006) and converted to d_{ae} using the equation (5). The collection efficiency by size was calculated by the following equation:

$$\eta_c(d_{ae}) = 1 - \frac{C_s(d_{ae})}{C_b(d_{ae})} \quad (6)$$

where C_s and C_b are the number concentration of particles passing through the samplers with and without impactor nozzle plate and the swab. The measurement occurred in the following sequence: C_{b1}-C_{s1}- C_{b2}-C_{s2}-C_{b3}-C_{s3}-C_{b4}. For the equation (6), values of (C_{b1} + C_{b2})/2, (C_{b2} + C_{b3})/2 and (C_{b3} + C_{b4})/2 were used and then average of three η_cs was calculated. All the tests for the collection efficiencies were performed in the same method.

A pressure drop of the impactor was measured using a differential pressure gauge (Magnehelic 2000-1000PA, Dwyer Instruments, U.S.A.).

2.3 Results and Discussion

The collection efficiency results were presented in Figure 2.4. The experimental d_{50} was 0.44 μm and in good agreement with the theoretical d_{50} (0.527 μm). The experimental d_{50} was 16.5% smaller than the theoretical d_{50}. The pressure drop of the fabricated impactor was 2.876 kPa and higher than the designed one. A smaller nozzle diameter than the designed size could create faster jet speed and result in a decrease of d_{50}. Since bioaerosols are generally larger than 1 μm in diameter and the primary concern is the respirable size (< 10 μm) (Löndahl, 2014), the fabricated impactor is capable of collecting the majority of bioaerosols.

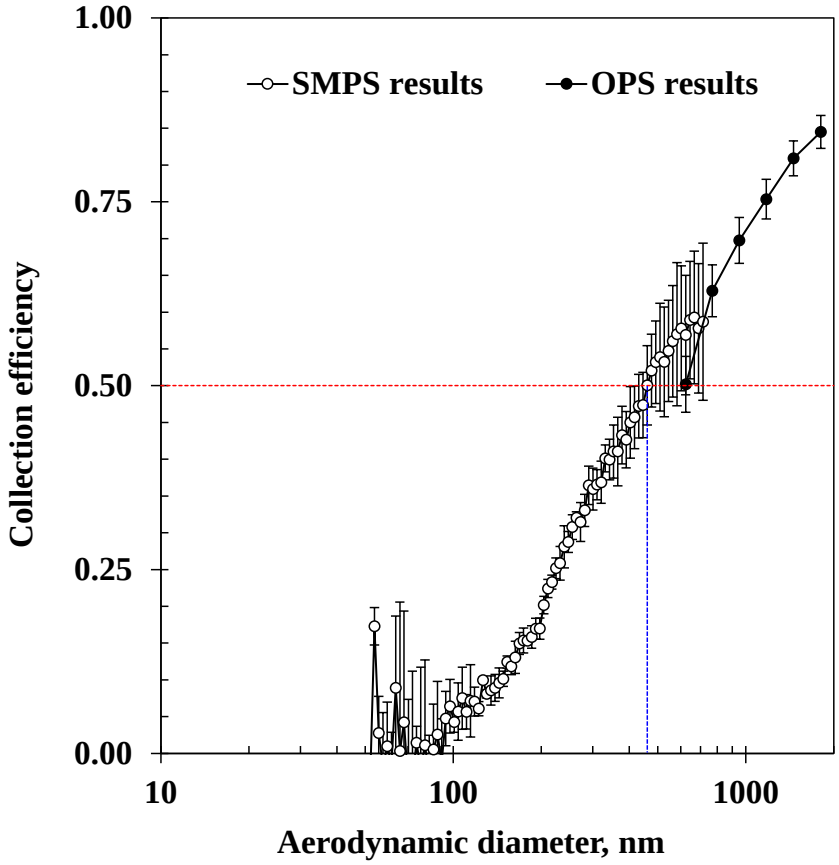

Figure 2.4 Collection efficiency of three-jet impactor

The results of this chapter were published as follows: **Liao, L.**, Byeon, J.H., Park, J.H., 2021. Development of a size-selective sampler combined with an adenosine triphosphate bioluminescence assay for the rapid measurement of bioaerosols. Environ. Res. 194, 110615. https://doi.org/10.1016/j.envres.2020.110615

CHAPTER 3. EVALUATION OF THE PERFORMANCE OF THE BIOAEROSOL SAMPLER COMBINED WITH ATP BIOLUMINESCENCE ASSAY

3.1 Introduction

Hypothesis: The developed sampler combined with the ATP bioluminescence assay can measure the bioaerosol concentrations.

Rationale: ATP bioluminescence assay is an available and affordable technique for the rapid quantification of bacteria. ATP plays a central role as an intermediate carrier of chemical energy, linking catabolism to biosynthesis within microbial cells. Firefly luciferase catalyzes a reaction between luciferin and ATP, which causes the luciferin to become excited and emit photons as it returns to its ground energy level state, with peak intensity in the 500 nm range (Karl, 1980) as per the following reaction:

ATP + luciferin–luciferase + O_2 → oxyluciferin–luciferase + adenosine monophosphate + pyrophosphate + CO_2 + luminescence

The intensity of the light produced is directly proportional to the ATP level, which in turn is proportional to the bacteria concentration (Seshadri et al., 2009). Thus, conversion equations of measured ATP amount and CFU numbers can be established through calibration of swabs and bioluminometer with bacterial suspension. Then the amount of ATP measured can be converted into the number of potentially viable cells based on the conversion equation. This method is designed for measuring bacteria on the surface and a few studies were conducted for the bioaerosols. In the study of Kim et al. (2019), the bioaerosols collected on the swab were detected by ATP bioluminescence assay. However, further research on the evaluation of ATP bioluminescence assay for measuring the concentration of bioaerosols and comparison to culture-based methods should be needed. In the current study, bacteria suspension was aerosolized and their concentrations were measured using the developed sampler with ATP bioluminescence assay and the conventional method that is a combination of Andersen cascade impactor and colony counting. The results from both methods were compared to validate the feasibility of the developed methods.

3.2 Methods

3.2.1 Calibration of Swabs and Bioluminescence with Bacterial Suspension

The swab calibration procedure is described in Figure 3.1. Two different swabs having different sensitivity were selected and examined. A sensitive swab (SuperSnap, Hygiena, LLC, U.S.A.) and a less sensitive swab (UltraSnap, Hygiena, LLC, U.S.A.) were tested and compared.

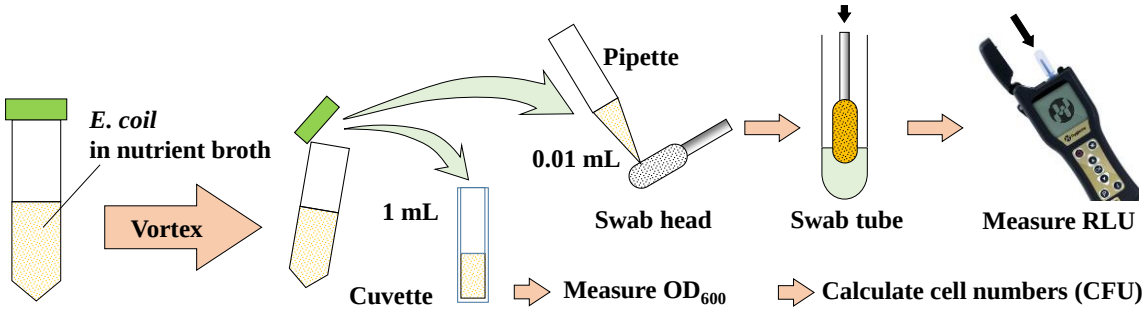

Figure 3.1 Procedure for swab calibration

E. coli (width: 0.5 μm, length: 2 μm), as a common Gram-negative bacterium, was selected to calibrate the swab used in ATP bioluminescence method. *E. coli* suspension was made from a freeze-dried *E. coli* pellet (ATCC® 11775TM). *E. coli* pellet was added to 30 mL broth (Difco™ nutrient broth 23400, BD, U.S.A.) and then grown in a shaking incubator (J-NSIL-R-110, JISICO, Korea) at 37℃ for 24 hours. The prepared *E. coli* suspension was stored at 4℃. During the experiment, temperatures of *E. coli* suspension and nutrient broth were measured by an infrared thermometer and maintained between 20-25℃.

In order to examine the correlation between ATP bioluminescence and CFU, the *E. coli* numbers in test suspensions were analyzed by the two different methods: 1) ATP bioluminometer (EnSURE, Hygiena, LLC, U.S.A.) and 2) visible spectrometer (VIS 721, Yoke Instrument Co., Ltd., China). Original *E. coli* suspension was diluted by nutrient broth to separately 0-100% and then 0.01 mL of each diluted suspension was pipetted onto the swab head. Test swab was inserted into the swab tube and the luciferase enzyme was released and reacted with diluted suspension.

After several seconds, the swab was placed in the ATP bioluminometer. The amount of light, as measured by the ATP bioluminometer, is expressed in relative light units (RLUs). Simultaneously, 1 mL of each diluted suspension was transferred to a cuvette to measure the optical density at a wavelength of 600 nm (OD_{600}) by the visible spectrophotometer. The *E. coli* concentration ($C_{e.coli}$) was calculated from OD_{600} by the following equation:

$$C_{e.coli} = OD_{600} \times 8 \times 10^8 \text{ cells/mL} \quad (7)$$

CFU of *E. coli* was calculated from $C_{e.coli}$ and the volume of suspension pipetted on the swab head. The conversion curve between RLU and CFU was obtained after calculation.

3.2.2 Evaluation of Bioaerosol Sampler combined with ATP Bioluminescence Assay by Comparison with Andersen Impactor

In the lab test, bioaerosols were collected using developed bioaerosol samplers and a conventional sampler. The experimental setup is shown in Figure 3.2.

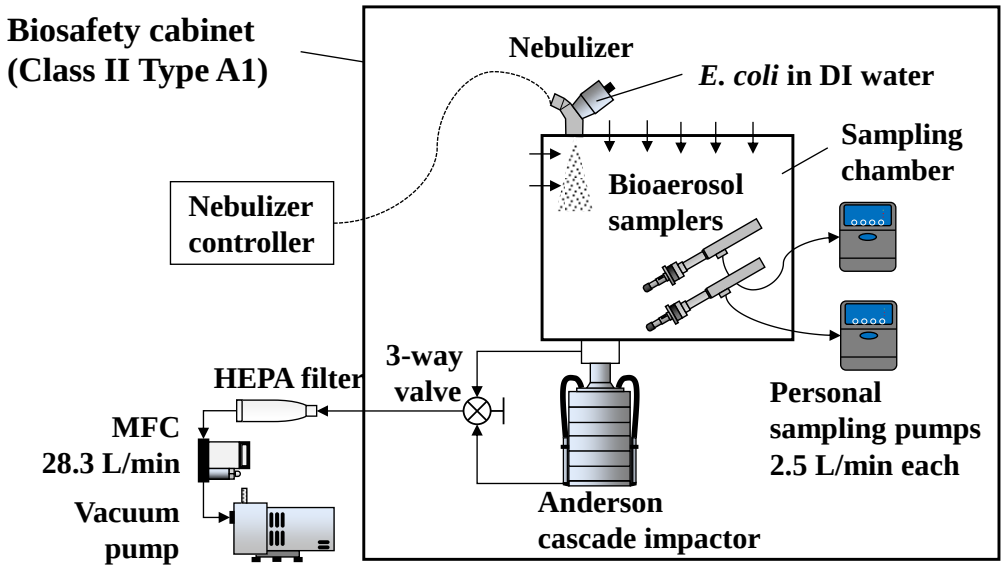

Figure 3.2 Experimental setup for bioaerosol sampling in the lab

E. coli suspension was made as described in the previous step and then washed three times with sterile deionized water using a centrifuge (CL4M, Waverly, U.S.A.) at 6000 RPM for 15 min to remove the components of the nutrient broth. A vibrating mesh nebulizer (Aeroneb Solo System, Aerogen, Ireland) was used to aerosolize *E. coli* in deionized water. For the collection of the aerosolized *E. coli*, two identical developed bioaerosol samplers were placed in the sampling chamber (9 L) and connected to the personal sampling pumps (APB-926000, Libra, U.S.A.) pulling the air at the flow rate of 2.5 L/min. Two different swabs, UltraSnap (Hygiena, LLC, U.S.A.) and SuperSnap (Hygiena, LLC, U.S.A.) were set in the samplers. After sampling the *E. coli* onto the swab head for two hours, the swab was placed into the swab tube. The swab tube was shaken for several seconds after washing the swab head and mixing the collected bacteria with an ATP releasing reagent and a luminescence reagent for the light-generating reaction. The swab tube was placed in a bioluminometer (EnSURE, Hygiena, LLC, U.S.A.) to measure the RLUs from the sampled *E. coli*. RLUs were converted to CFUs using the conversion equation obtained from the swab calibration test. Since respirable cyclone was used in the bioaerosol sampler, results from the sampler were expressed in respirable CFU (CFU_{resp}) concentrations. For calculating the concentration, CFU_{resp}s were divided by sampling volume (= sampling flow rate × sampling time). The sampling flow rates of bioaerosol samplers were calibrated using a volumetric airflow calibrator (BGI tetraCal®, Mesa Labs, Inc., U.S.A.) before and after sampling.

For the comparison, a six-stage Andersen cascade impactor (TE-10-800, Tisch Environmental, U.S.A.) was employed as a conventional sampler. This instrument divides the bioaerosols into six fractions following their aerodynamic diameters: ≥ 7.0 μm (1st stage), 7.0-4.7 μm (2nd), 4.7-3.3 μm (3rd), 3.3-2.1 μm (4th), 2.1-1.1 μm (5th), and 1.1-0.65 μm (6th). A Petri dish (diameter of 100 mm) containing agar (Difco™ nutrient agar 213000, BD, U.S.A.) was utilized as the impaction substrate for each stage of the Andersen impactor. The sampling flow rate of the Andersen impactor was set to 28.3 L/min using an MFC (MCP-50SLPM-TFT-30PSIA, Alicat, U.S.A.) and calibrated using a volumetric airflow calibrator (BGI tetraCal®, Mesa Labs, Inc., U.S.A.) before and after sampling. The sampling time, incubation temperature, and incubation time were 1 min, 37℃, and 24 hours, respectively. After incubation, the CFU of each stage was counted and adjusted using the following equation (Hinds, 1999):

$$\mathrm{CFU_{adj}} = \mathrm{CFU_{ct}} \times \left(\frac{1.075}{1.052 - \frac{\mathrm{CFU_{ct}}}{400}} \right)^{0.483} \quad \text{for } \mathrm{CFU_{ct}} < 380 \tag{8}$$

where CFU_{adj} and CFU_{ct} are adjusted and counted CFUs, respectively. For the comparison, CFU of respirable bioaerosol was calculated as follows:

$$\mathrm{CFU_{resp}} = \sum_{i=1}^{6} \left(\mathrm{CFU_{adj.i}} \times \mathrm{F_r}(\mathrm{d_i}) \right) \tag{9}$$

where i is the stage number and F_r is the respirable fraction at midpoint size of stage i (d_i). Then, CFU_{resp} was divided by sampling volume to calculate the CFU_{resp} concentration. CFU_{resp} concentrations from bioaerosol samplers and Andersen impactor were then compared.

3.2.3 Field Application of Bioaerosol Sampler combined with ATP Bioluminescence Assay

The sampling station consisting of two samplers and an Andersen impactor was used in field tests. Field tests were carried out at two different sites. The first sampling location was at Purdue West Lafayette campus (Indiana, U.S.A.; site A). The grassland approximately 30 m from the Engineer Fountain was chosen as the sampling spot. Fountains are a source of miscellaneous microorganisms because the water in a fountain can be easily contaminated with fecal bacteria such as *streptococci*, *E. coli*, and coliform from the activities of people and animals (birds, dogs, etc.) (Burkowska-But et al., 2013). Ambient air in site A was sampled by bioaerosol samplers for 60 min and Andersen impactor for 30 min. The other sampling location was stable (in Shelbyville, Indiana, U.S.A., site B) consisting of multiple stalls (3.66 m × 3.66 m each). The stall had a half-size opening with bars and a door opening with a stall guard that allowed the horse to extend head and neck into the barn aisle. An electrical fan was installed on the window and pushed the air from aisle to stall. The sampling station was placed on the aisle floor underneath the window. A high concentration of bioaerosols could be expected because of the proximity of the sources such as the animals, their feces, feed, bedding, and dirt floor (Wolny-Koładka et al., 2018). Ambient air in site B was sampled by developed bioaerosol samplers for 60 min. The sampling time of the Andersen impactor was set to 3 min to prevent the over-deposition of bioaerosols. Similar to the lab

experiments, the sampling flow rates of bioaerosols samplers (2.5 L/min) and Andersen impactor (28.3 L/min) were calibrated using a volumetric airflow calibrator (BGI tetraCal®, Mesa Labs, Inc., U.S.A.) before and after sampling.

After sampling, the swab in bioaerosol sampler was immediately inserted into the swab tube and examined using the bioluminometer by the same method used in lab tests. Each Petri dish in the Andersen impactor was sealed with parafilm tape (PARAFILM® M, Bemis, U.S.A.) and taken back to the lab to incubate at 37℃ for 24 hours. Three field blanks were prepared during sampling and the average blank value was subtracted before calculating equation (8) and RLU-CFU conversion.

3.3 Results and Discussion

3.3.1 Calibration of Swabs and Biolumometer with Bacterial Suspension

The swab calibration results were shown in Figure 3.3. RLU values from SuperSnap were proportional to CFU values when the *E. coli* numbers are smaller than 2.2×10^6 CFU. When the *E. coli* number increased from 2.2×10^6 to 5.2×10^6 CFU, RLU values from SuperSnap were not increased and kept about 8000 RLU. This means that 2.2×10^6 CFU of *E. coli* is the maximum limit measured using the SuperSnap. UltrsSnap shows similar linearity when the *E. coli* numbers are smaller than 3.3×10^6 CFU. UltraSnap shows a larger maximum measurable limit than SuperSnap. Results in linear sections of both test swabs were reorganized as a conversion plot in Figure 3.3(b). The results of linear regression analysis are summarized in Table 1. SuperSnap ($R^2 = 0.81$) shows better linear regression than UltraSnap ($R^2 = 0.53$). The slope value of SuperSnap (633.60) and larger than one of UltraSnap (277.78) which means SuperSnap is 2.3 times more sensitive than UltraSnap for *E. coli*. The slope also means a minimum resolution of the swab. For example, one RLU measured using the SuperSnap is equivalent to 277.78 CFU of *E. coli*. Since the occupational bioaerosol concentrations are often higher than environmental bioaerosol concentrations, the UltraSnap could be used for measuring bioaerosols in occupation settings and the SuperSnap could be used in both environmental and occupational settings.

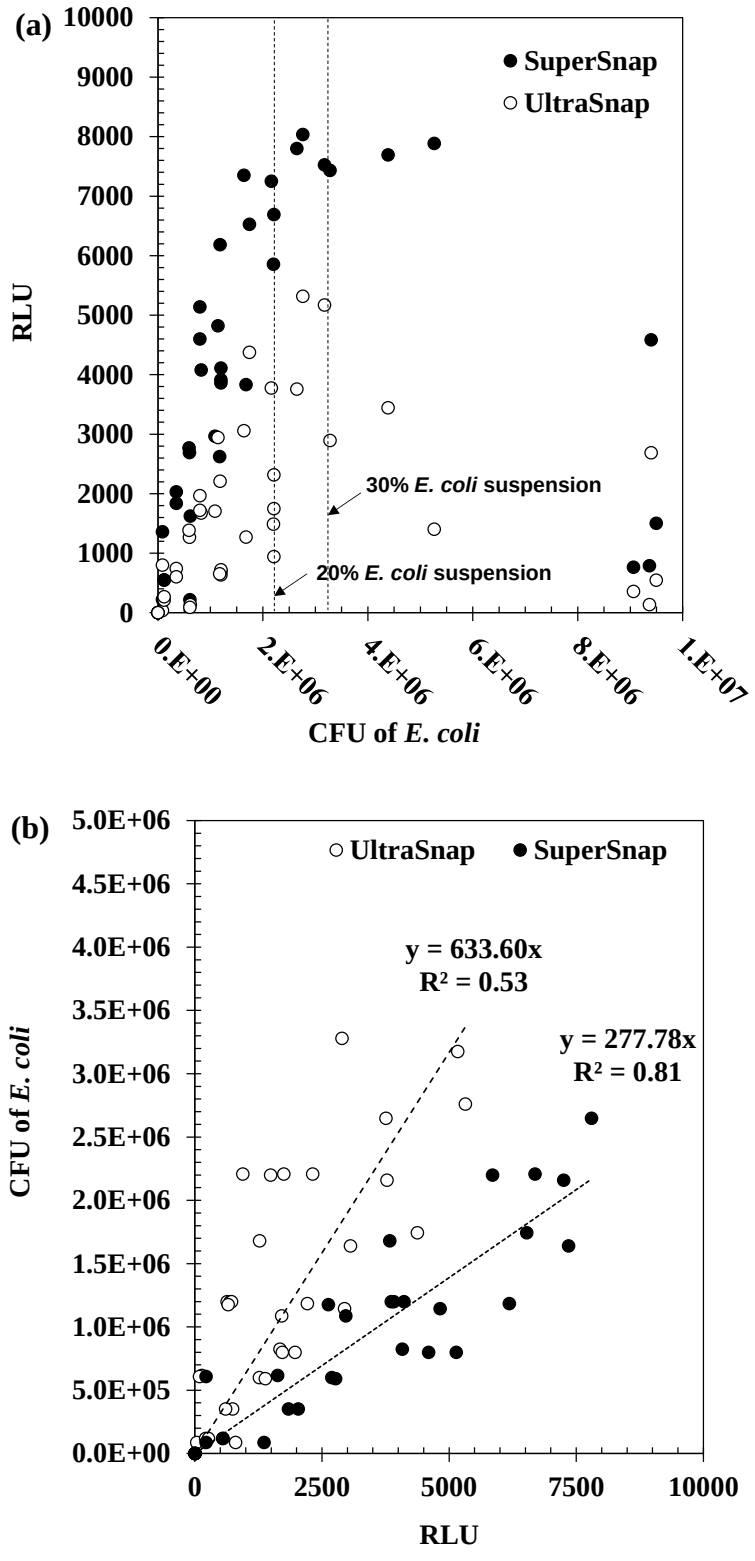

Figure 3.3 (a) Swab calibration curve, (b) RLU-CFU conversion plot (The x and y axes are swapped; RLU: relative light unit; CFU: colony forming unit)

The CFU_{resp} concentrations measured using the bioaerosol samplers and the Andersen impactor are shown in Figure 3.4. Most colonies were found in the 1st and 6th stages of the Andersen impactor in every case. Aerosolized test *E. coli* were directly settled onto the 1st stage plate through the open inlet of the Andersen impactor and other aerosolized *E. coli* were collected onto the 6th stage plate because the aerodynamic diameter of single *E. coli* is about 1 μm and larger than d_{50} of 6th stage. The CFU value from the 1st stage did not significantly affect the results since the respirable fraction at the d_i of the 1st stage is about 0.002 (Equation 9).

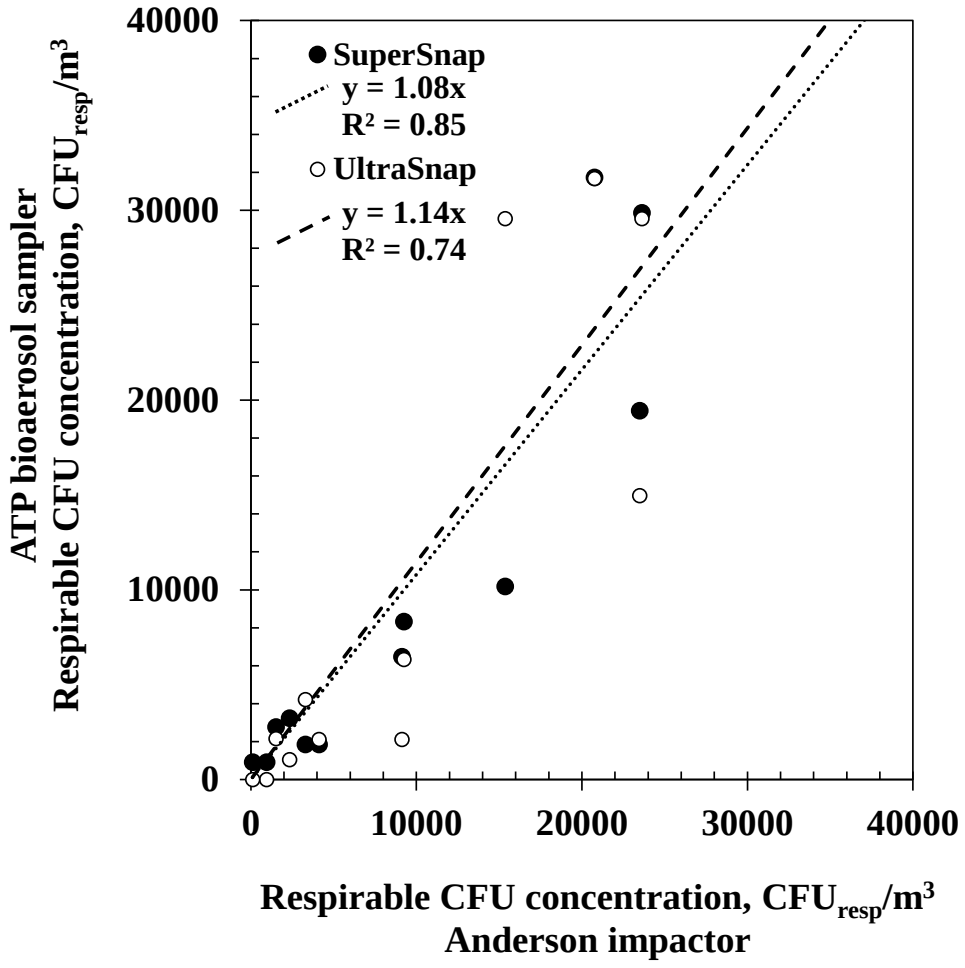

Figure 3.4 Comparison of bioaerosol sampler combined with ATP bioluminescence assay and Andersen impactor using the aerosolized E. coli

The CFU_{resp} concentrations measured using SuperSnap and UltraSnap were proportional to those measured using the Andersen impactor. The results of linear regression analysis are documented in Table 3.1. The R^2 of SuperSnap (0.85) was higher than that of UltraSnap (0.74). However, the slopes of both linear regressions were slightly larger than 1 (Figure 3.4). These deviations were presumably attributed to the sampling limitations of Andersen impactor such as desiccation stress and overloading problems (Stewar et al., 1995; Willeke et al., 1995; Xu et al., 2013). Specifically, the impaction process might decrease the viability of bacteria due to impact damage and desiccation (Haig et al., 2016). The conditions of the agar plate could also affect the culturability of collected bacteria (Therkorn and Mainelis, 2013).

Table 3.1 Results of linear regression analysis for conversion plots and comparison tests in the lab

Conversion plot (Figure 6(b))			
Variable	Coefficient	Standard errors	95% Confidence interval
UltraSnap	633.60	50.77	530.42, 736.78
SuperSnap	277.78	14.40	248.32, 307.24
Comparison of developed bioaerosol sampler and Andersen impactor in the lab (Figure 7)			
Variable	Coefficient	Standard errors	95% Confidence interval
UltraSnap	1.14	0.14	0.83, 1.46
SuperSnap	1.08	0.10	0.86, 1.30

3.3.2 Field Application of Bioaerosol Sampler

The results for field tests are documented in Table 3.2. SuperSnap shows higher respirable RLU (RLU_{resp}) concentrations than UltraSnap in both sampling locations. The RLU_{resp} is proportional to ATP concentration from the sampled bacteria. Therefore, the RLU_{resp} can be expressed in ATP concentration from the CFU_{resp} of *E. coli*. The RLU_{resp} concentrations were converted to CFU_{resp} concentrations using the conversion plot (Figure 3.3(b)). In site A, CFU_{resp} concentrations measured using UltraSnap and SuperSnap were 106,290 ± 53,075 CFU_{resp}/m^3 and

95,749 ± 31,766 CFU_{resp}/m^3, respectively, which were higher than the result measured using Andersen impactor (33 ± 27 CFU_{resp}/m^3). In site B, Andersen impactor also underestimated the concentration of bacteria compared to both UltraSnap and SuperSnap results. The main reason could be that ambient bioaerosols including fungi and plant cells contain more ATP than *E. coli* (Bajerski et al., 2018). Another reason could be the limitations of the Andersen impactor already explained in the previous section. The Andersen impactor could underestimate the concentrations because of the low viability and cultivability of sampled bacteria. The CFU_{resp} concentrations of bioaerosols sampled in the site B were similar to those in site A while the CFU_{resp} concentration in the site B measured by the Andersen impactor was 13.4 times higher than one in site A. The sampling time (30 min) for site A was longer than the sampling time (3 min) for site B. Longer sampling time can decrease the viability of sampled bacteria. The type of agar plate also can make a difference in results. Site B has more various bioaerosols including the vast majority of different bacteria, endotoxin, viruses, parasites, fungi, mycotoxin, insect parts (Millner, 2009). Stables also contain a high level of culturable microorganisms (Samadi et al., 2009). Moreover, the stable environment promotes the proliferation and growth of these microorganisms by a humid and warm microclimate.

Table 3.2 Respirable concentrations of airborne bacteria sampled in field sites

	BIOAEROSOL SAMPLER WITH ULTRASNAP		**BIOAEROSOL SAMPLER WITH SUPERSNAP**		**ANDERSEN IMPACTOR** with colony counting
SAMPLING LOCATION	RLU_{resp}/m^3	CFU_{resp}/m^{3*}	RLU_{resp}/m^3	CFU_{resp}/m^{3*}	CFU_{resp}/m^3
SITE A (CAMPUS)	168 ± 84	106,290 ± 53,075	345 ± 114	95,749 ± 31,766	33 ± 27
SITE B (STABLE)	173 ± 105	109,824 ± 66,385	660 ± 240	168,284 ± 66,660	443 ± 168

**E. coli* equivalent CFU_{resp}: RLUs were converted to CFUs using the conversion equations in Figure 3.3(b).

The CFU_{resp} concentrations of site B measured using the UltraSnap and SuperSnap were similar to those of site A which means that the ATP concentrations extracted from respirable bioaerosols sampled in both sites A and B are similar. The site B result of SuperSnap was 50%

higher than the one of UltraSnap. One possible reason could be that SuperSnap has better and more chemicals to extract ATP from the bacteria in site B.

Overall, the developed bioaerosol sampler combined with ATP bioluminescence assay can measure bacterial concentrations. RLUs values were linearly correlated with CFUs in the calibration curve. The concentrations measured using the bioaerosol sampler were higher than those from the Andersen impactor due to the limitations of the colony counting method. These findings confirm the feasibility of developing a sampler for rapid measurement of bioaerosol concentrations, offering a compact device for measuring exposure to bioaerosols, and an easy-to-use methodological concept for efficient air quality management. However, the bioaerosol sampler was not able to examine specific pathogens. A lateral flow immunochromatographic test could be an alternative method to identify the pathogenic bioaerosols.

The results of this chapter were also published as follows: **Liao, L.**, Byeon, J.H., Park, J.H., 2021. Development of a size-selective sampler combined with an adenosine triphosphate bioluminescence assay for the rapid measurement of bioaerosols. Environ. Res. 194, 110615. https://doi.org/10.1016/j.envres.2020.110615

CHAPTER 4. EVALUATE THE PERFORMANCE OF THE BIOAEROSOL SAMPLER COMBINED WITH ICA

4.1 Introduction

Hypothesis: The developed sampler combined with ICA can detect the pathogenic bioaerosols.

Rationale: The ICA relies principally on the capture of the target antigen (or sometimes antibodies) from various specimens. The assay utilizes antibodies mounted on a paper strip or a nitrocellulose membrane as the immobile capture antibody (test area). Capillary flow is used to move a colloidal gold or colored microparticle-labeled antibody conjugate which binds to the target antigen in the mobile phase as it moves toward the capture antibody in the immobile phase. A positive test is produced by the capture of the moving labeled antigen/antibody complex by a second immobilized anti-species antibody in the test area, and the formation of a colored line or pattern. Another control antibody to the conjugate binds the excess colloidal dye conjugate and acts as the control line (El-moamly, 2014). The control line is an indicator of the validity of the test. ICAs are easy-to-use, rapid non-microscopic tests that can save time and effort, and that may be more suitable under field conditions. Because the ICA is also the swab-based method, the pathogenic bioaerosols can be detected using the ICA if they are successfully collected on the swab using the developed sampler. To test the hypothesis, the developed method was tested using a pathogenic bioaerosol in the lab.

4.2 Methods

4.2.1 Test Pathogen

L. pneumophila was used as a target pathogen. *L. pneumophila* is a pathogenic group of Gram-negative bacteria and causes legionellosis including a pneumonia-type illness called Legionnaires' disease and a mild flu-like illness called Pontiac fever. The test strain was the prevalent disease-causing variant *L. pneumophila* JR32 (LpJR32) which was commonly used as a laboratory strain (Abu Khweek et al., 2013; Rao et al., 2013). LpJR32 was originally derived in the Shuman laboratory from a 1976 clinical isolate provided by Marcus Horwitz from the Centers for Disease Control (Horwitz and Silverstein, 1980).

LpJR32 was grown in buffered yeast extract (BYE) broth for 24 hours using a shaking incubator (J-NSIL-R-110, JISICO, Korea) at 37°C. The BYE broth was made of 10 g of N-(2-Acetamido)-2-aminoethanesulfonic acid (ACES; A22500, RPI, U.S.A.), 10 g of yeast extract (Y20020, RPI, U.S.A.), 5 mL of L-cysteine supplement (8 g of L-cysteine hydrochloride (C1276, EMD Millipore Corp, U.S.A.) dissolved in 100 mL of sterile deionized (DI) water (ASTM II)), and 5 mL of iron supplement (2.7 g of iron nitrate nonahydrate (F8508, EMD Millipore Corp, U.S.A.) dissolved in 100 mL of DI water) per L. The pH of the broth was measured using a benchtop pH meter (PH700, Apera Instruments, U.S.A.) and adjusted at 6.8–6.9 with potassium hydroxide (KOH) (Potassium hydroxide AC424140000, Acros Organics, U.S.A.).

LpJR32 in the DI water was used as a test suspension for the experiments. Specifically, LpJR32 in the nutrient broth was centrifuged (CL4M, Waverly, U.S.A.) at 6000 RPM for 15 min. The components of the nutrient broth were removed and then DI water was added after each centrifugation. This washing process was repeated three times.

Buffered charcoal yeast extract (BCYE) agar was used for the incubation of LpJR32 for colony counting. BCYE agar was made of 10 g of ACES, 10 g of yeast extract, 2 g of activated charcoal (Activated Carbon Darco G-60 D127-500, Fisher Scientific, U.S.A.), 18 g of agar (DifcoTM nutrient agar 213000, BD, U.S.A.), 5 mL of L-cysteine supplement, and 5 mL of iron per L. The pH of BCYE-Agar was also 6.8–6.9.

4.2.2 Determination of the Lower Limit of Detection (LOD) of the Lateral Flow Test Kits

The commercially available lateral flow test kit (Legionella Swab (Biofilm) Test Kit, 56B006401, Lovibond, U.K.) was used for detecting LpJR32. This test kit consists of a sampling swab (FLOQSwabs® 501CS01, COPAN Diagnostics, U.S.A), a test strip, and a tube containing the buffer solution (2 mL). The lower LOD of the test kit was evaluated as shown in Figure 4.1.

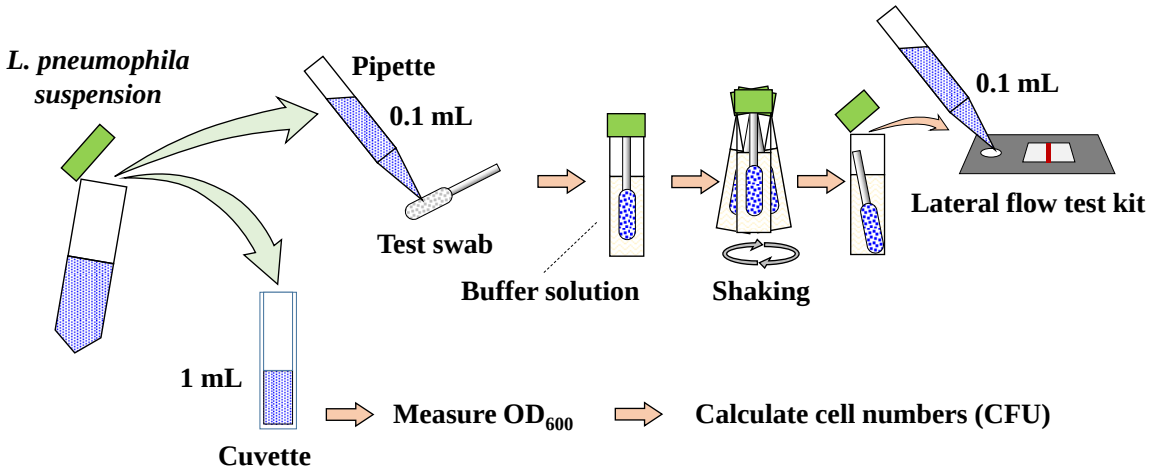

Figure 4.1 Procedure to determine the lower limit of detection of a lateral flow test kit

Test suspension (prepared in section 4.2.1.) was serially diluted with DI water and 0.1 mL of each diluted suspension was dispensed onto the head of the sampling swab. The sampling swab was broken in half and the headpiece was placed in the test tube filled with a buffer solution. LpJR32 on the swab head was washed and mixed with the buffer solution by shaking the tube for 5 s using a vortex mixer (BenchMate VM-M Mini, Oxford Lab Product, U.S.A.). 0.1 mL of the buffer solution containing LpJR32 was dispensed on the test strip. The formation of control and detection lines on the test strip was checked after 20 min. Simultaneously, an OD_{600} of each diluted suspension was measured using a visible spectrophotometer (VIS 721, Yoke Instrument Co., Ltd., China). The LpJR32 concentration (C_{jr32}) was calculated from OD_{600} by the following equation:

$$C_{jr32} = OD_{600} \times 10^9 \; in \; CFU/mL. \quad (10)$$

The total number of LpJR32 pipetted on the swab was calculated from C_{jr32} and the volume of suspension (0.1 mL) and compared with the result of the test strip.

4.2.3 Evaluation of Bioaerosol Sampler Combined with Lateral Flow Test Kits by Sampling of Aerosolized LpJR32

The bioaerosol sampler was tested and compared with a conventional sampler in the lab. The experimental setup was shown in Figure 4.2. All experiments were conducted in a biosafety

cabinet (Class II, Type A1). The sampling chamber (9 L) was sterilized by ultraviolet ray and then located in the middle of the biosafety cabinet. For the bioaerosol generation, the LpJR32 suspension was washed in the same way (described in section 2.1.), diluted with DI water, and then aerosolized using a vibrating mesh nebulizer (Aeroneb Solo System, Aerogen, Ireland) installed on the top of the sampling chamber. The concentration of test bioaerosols in the test chamber was controlled by adjusting the voltage applied to the vibrating mesh nebulizer. Three different (low, medium, and high) concentrations of bioaerosols in the chamber were tested.

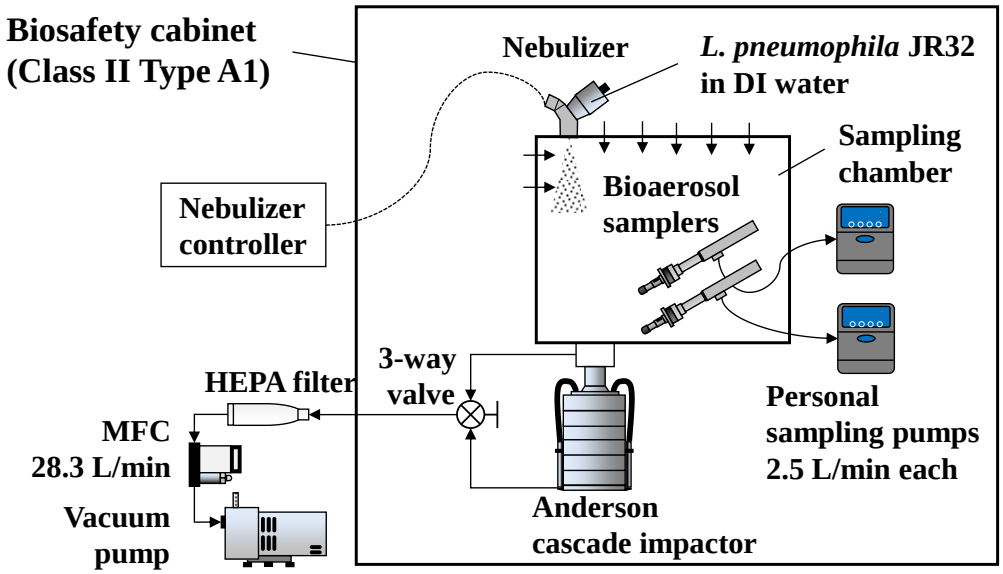

Figure 4.2 Experimental setup for sampling aerosolized *L. pneumophila* JR32

Two identical developed bioaerosol samplers were placed in the middle of the sampling chamber. The sampling times of the bioaerosol samplers were set differently to 10 min and 20 min, respectively. Samplings were conducted at various concentrations of LpJR32 and repeated three times at each condition. After sampling, each swab was broken in half and the headpiece was placed in the tube containing 2 mL of buffer solution. The tube was shaken for 5 s and then 0.1 mL of the buffer solution containing LpJR32 was dispensed on the lateral flow test strip. The color changes of control and detection lines on the test strip were checked after 20 min. The lower LOD

(determined in section 4.2.2.) was used for calculating the range of the LpJR32 concentration in the air.

For the comparison, a six-stage Andersen cascade impactor (TE-10-800, Tisch Environmental, U.S.A.) was employed as a conventional sampler. A Petri dish (diameter of 100 mm) containing BCYE agar (prepared in section 4.2.1.) was utilized as the impaction substrate for each stage. The sampling flow rate, sampling time, incubation temperature, and incubation time were 28.3 L/min, 1 min, 37°C, and 5 days, respectively. After incubation, the CFU of LpJR32 grown on BCYE agar was counted and adjusted using the equation (8). The respirable CFU (CFU_{resp}) was calculated as follows using equation (9). Then, CFU_{resp} was divided by sampling volume to calculate the CFU_{resp} concentration in the air. The results from the Andersen impactor were compared with the estimated CFU_{resp} concentration of LpJR32 from the bioaerosol samplers.

4.3 Results and Discussion

4.3.1 Determination of the Lower LOD of the Lateral Flow Test Kits

The results of the lines foamed on the lateral flow test kit are shown in Figure 4.3. Any distinct line, no matter how faint, was counted as positive based on the manual of the lateral flow test kit. The test conditions ranged from 230 to 2.6×10^5 CFU of LpJR32 on the swab. A faint positive line was formed when the CFU of LpJR32 on the swab is higher than approximately 1.3×10^3. The color of the test line formed more vivid with increasing CFU of LpJR32 on the swab. However, no test line was formed when the CFU was lower than 1.3×10^3. Thus, 1.3×10^3 CFU was assumed to be the lower LOD for further calculation. The lower LOD of 1.3×10^3 CFU is larger than the manufacturer's lower LOD of lateral test kits (200 CFU per swab). The difference of lower LODs may be caused by differences in test conditions and types of *L. pneumophila* used.

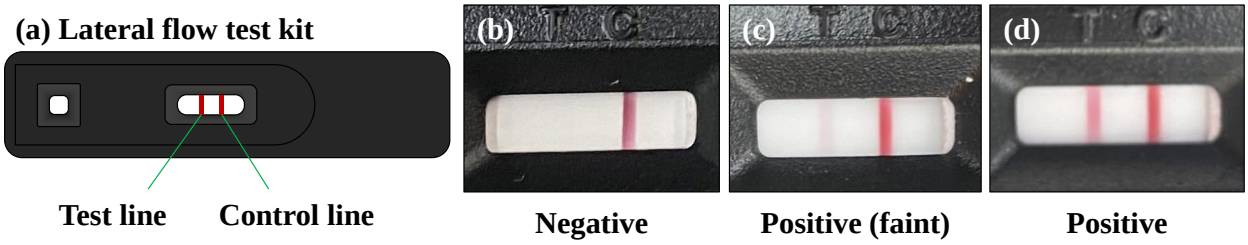

Figure 4.3 Images of (a) lateral flow test kit and results of (b) negative, (c) faint positive line, and (d) clear positive line

Based on the lower LOD of the lateral test flow kit (1.3×10^3 CFU), the conversion equation was created. When the positive result was obtained at the known sampling time, the minimum concentration of LpJR32 (C_{est}) can be estimated as the following equation:

$$C_{est} = \frac{1.3 \times 10^3}{Q \times t} \times 1000 \quad (11)$$

where Q is the sampling volume (2.5 L/min) and t is the sampling time in min. When the positive results are obtained after sampling for 10 min, the C_{est} is 5.2×10^4 CFU/m^3. Based on equation (11), the use of multiple bioaerosol samplers at different sampling times can allow estimating the range of concentrations. For example, when sampling times are 10 min and 20 min and results are negative and positive, respectively, the concentration can be assumed to be larger than 2.6×10^4 CFU/m^3 and smaller than 5.2×10^4 CFU/m^3.

4.3.2 Evaluation of bioaerosol sampler combined with lateral flow test kits by a sampling of aerosolized LpJR32

The lab sampling results are documented in Table 4.1. At the condition of low bioaerosol generation in the sampling chamber, the negative results were obtained from bioaerosol samplers at sampling times of 10 and 20 min which means respirable C_{est} in the sampling chamber was smaller than 2.6×10^4 CFU_{resp}/m^3. At medium bioaerosol generation condition, the positive lines were formed on the test strips at the sampling time of 20 min while negative results were obtained

at the sampling time of 10 min. In the same way, the respirable C_{est} ranged from 2.6×10^4 CFU_{resp}/m^3 to 5.2×10^4 CFU_{resp}/m^3. At high bioaerosol generation conditions, the positive results were obtained from bioaerosol samplers at both sampling times of 10 min and 20 min which means the respirable C_{est} is larger than 5.2×10^4 CFU_{resp}/m^3.

The results from the BBS samplers and the Andersen impactor were also compared in Table 4.1. The estimated concentrations from BBS samplers were significantly higher than the results measured by the Andersen impactor. These deviations were possibly due to the limitations of the Andersen impactor. If the bioaerosol is monodisperse and the concentration is 1×10^5 CFU/m^3, the sampling time has to be shorter than 8.06 s to avoid overload (Li et al., 2021). If the sampling time is 1 min, the Andersen impactor can measure a maximum of 13,428 CFU/m^3. In this study, bioaerosol concentrations in the chamber might be higher than the maximum concentration that the Andersen impactor can measure. In other studies, the Andersen impactor has been reported failing to detect *L. pneumophila* (Ishimatsu et al., 2001). Breiman et al. (1990) also failed to detect *L. pneumophila* using Andersen impactor with BCYE agar. The lack of growth on the plates may be caused by the dryness of the agar at the time of the sampling since the *legionella* require suitable humidity for their viability (Hambleton et al., 1983).

Table 4.1 Results of sampling aerosolized L. pneumophila JR32

Condition of bioaerosol generation in the test chamber	Low	Medium	High
Concentration range estimated using the bioaerosol sampler with ICA, CFU_{resp}/m^3	$< 2.6 \times 10^4$	$2.6 \times 10^4 – 5.2 \times 10^4$	$> 5.2 \times 10^4$
Concentration measured using the Andersen impactor, CFU_{resp}/m^3	1.4×10^3 3 overloading	$1.5 \times 10^4 \pm 9.8 \times 10^3$*	8.6×10^3, 3 overloading 1 non-grown

*Standard deviation of 3 samples

The study of this chapter will be submitted to Environmental Research for publication. The

authors and the tentative title are as follows: **Liao, L.,** Luo, Z.Q., Byeon, J.H., Park, J.H., 2021. Size-selective sampler combined with an immunochromatographic assay for the rapid detection of pathogenic bioaerosols.

CHAPTER 5. CONCLUSIONS

5.1 Problems addressed

The bioaerosol sampler was developed and evaluated. Specifically, the three-jet impactor was fabricated and tested with NaCl particles. The experimental d_{50} was 0.44 μm which indicated that the developed bioaerosol sampler can collect most of the bioaerosols since the majority of their size is bigger than d_{50}.

A commercially available respirable cyclone was also utilized as a size-selective inlet. The respirable cyclone can be replaced with PM2.5 or PM10 impactor for environmental studies. The bioaerosol sampler was designed for operating with a personal sampling pump that is also commercially available. This allows the sampler to be used for both personal samplings to assess individual exposures and area samplings to assess the background level of bioaerosols. The prototype sampler was made of aluminum and PEEK. The size and weight of the sampler can be reduced by the use of disposable plastics.

Combined with ATP bioluminescence assay, the RLU values of two different swabs were defined with CFU values of *E. coli*. In comparison with the Andersen impactor with colony counting, the bioaerosol sampler could overcome the limitations of the Andersen impactor with colony counting. Specifically, the developed method had a wider range of measurable concentrations of bioaerosols and reduced measurement time.

The detailed calibration and conversion methods were provided which allows us to define RLU values based on CFU of *E. coli* and compare RLU values among different swabs and bioluminescence assay. Currently, many researchers are developing and evaluating the ATP bioluminescence method for measuring bioaerosol concentrations. However, RLU values can not be directly compared among different swabs and bioluminometers since companies have defined their own RLU value from ATP concentration. There is no standard method to calibrate the swabs, interpret the RLU values among different luminometers and swabs, and convert RLUs to CFUs. To address this problem, a detailed method to calibrate the swabs using *E. coli* was introduced in this research. The calibration curve was also obtained. This calibration curve would be a reliable reference for researchers who want to know the contamination level from the RLUs. Using the

calibration curve, RLUs can be defined as CFU of *E. coli* having the same amount of ATP. The sensitivities of different bioluminometers and swabs can be also compared easily using the calibration method.

Two commercially available swabs, the UltraSnap and SuperSnap were verified and compared. Since the SuperSnap showed better resolution than the UltraSnap, the SuperSnap can be used for both environmental and occupational samplings. The UltraSnap showed relatively low resolution but it can be still used for occupational sampling since occupational bioaerosol concentrations are often higher than environmental bioaerosol concentrations. The maximum detection limits of UltraSnap and SuperSnap were also evaluated. The 3.3×10^6 CFU and 2.2×10^6 CFU of *E. coli* are the maximum detection limit for UltrsSnap and SuperSnap, respectively (Figure 3.3(a)). When the sampling time is assumed to be one hour, measurable maximum concentrations for UltraSnap and SuperSnap are 2.2×10^7 CFU/m^3 and 1.67×10^7 CFU/m^3, respectively. When the bioaerosol concentration is higher than the maximum limits, sampling time should be shorter than one hour. These measurable maximum concentrations are still much larger than the limits of the Andersen impactor. This deficiency of the Andersen impactor was already discussed by other studies (Lindsley et al., 2017). The Andersen impactor can collect a maximum of 400 particles per stage in ideal conditions. After applying the adjustment equation, the maximum CFU of each stage was calculated as 380 CFU (equation (8)). If the bioaerosol is monodisperse and the concentration is 1×10^5 CFU/m^3, the sampling time has to be shorter than 8.06 seconds to avoid overload. If the sampling time is one min, the Andersen impactor can measure a maximum of 13,428 CFU/m^3. Consequently, the ATP bioluminescence method can measure a wider range of bioaerosol concentrations than the Andersen impactor with colony counting.

Combined with ICA, the developed sampler could collect and detect airborne pathogens. In the lower LOD test, a faint positive line was obtained when the CFU of LpJR32 on the swab was higher than approximately 1.3×10^3 suggested as the actual lower LOD. LpJR32 was detected more easily by ICA than the Andersen impactor with colony counting. Furthermore, the lateral flow test kits are designed for disposable single-use and no contamination with the previously tested sample would occur. The limitation of the developed method would be the infeasibility of quantitative results and the sensitivity depends on the lateral flow test kit. However, using the

multiple samplers with different sampling times can allow estimating the concentration range of pathogenic bioaerosols. The developed method has great potential for the detection of pathogenic bioaerosols and allows for more rapid and accurate risk assessments because of its many advantages over conventional samplers.

Combined with ICA, the bioaerosols sampler can detect pathogenic bioaerosols rapidly and conveniently. The commercially available lateral flow test kits for diagnosis of *L. pneumophila* were used to test the presence of LpJR32 on sampled swabs. The lower LOD of 1.3×10^3 CFU for the lateral flow test kits was larger than the manufacturer's lower LOD of lateral test kits (200 CFU per swab). Although the lower LOD was larger than expected, the LpJR32 on the swabs were more easily detected by the bioaerosol sample combined with lateral flow test kits than the Andersen impactor with colony counting. Most of the samples measured by the Andersen impactor with colony counting had overloading or no-grown problems.

Overall, the results proved that the developed bioaerosol sampler combined with swab-based analysis methods can overcome the limitations of conventional methods and allows for more rapid, inexpensive, and accurate assessments for bioaerosols. Moreover, the combined methods can be immediately used in the field.

5.2 Limitations

A limitation of this study would be sampling loss due to particle bounce on the impaction substrate of both bioaerosol sampler and Andersen impactor. As shown in Figure 2.4, particle collection efficiency was approximately 0.75 at 1 μm and increased by increasing size. The collection efficiency curve is less stiff than those of conventional impactors. The impaction substrate of the bioaerosol sampler was a swab head made of cotton fibers instead of a solid plate coated with grease. Moreover, the collection efficiency was evaluated using NaCl particles which may cause the underestimation of collection efficiency since dried NaCl particles could bounce more than *E. coli* containing droplets or ambient bioaerosols. Even though the sampling loss was not significantly observed in both lab and field tests, better impaction substrates (e.g., porous plastic or metal foam) should be developed in future research.

Other limitations may include the detection threshold of ICA. In the lower LOD test, a faint positive line was obtained when the CFU of LpJR32 on the swab is higher than approximately 1.3×10^3. That means it may show negative results when the CFU of LpJR32 on the swab is lower than 1.3×10^3, resulting in a false negative outcome. Further researches on improving the sensitivity of lateral flow test kits are necessary.

5.3 Future Directions

The developed bioaerosol sampler will be optimized to be disposable for a more user-friendly application in fields. Further researches will be focused on applications of the developed bioaerosol sampler in other various environments. The effectiveness of bioaerosol sampler combined with ATP bioluminescence assay or ICA will be tested in places such as the food industry, composting site, clinic center, etc. After various applications and data collections, the methods may thus also help to establish recommendations for exposure limits of bioaerosols in open spaces as factories, hospitals, residential buildings. Further studies also include assessment of the exposure to other pathogenic bioaerosols. It can be conducted easily using the developed method if lateral flow test kits are available for the target pathogens. The sampler can also be combined with other swab-based analysis methods for diverse investigations of bioaerosols.

APPENDIX

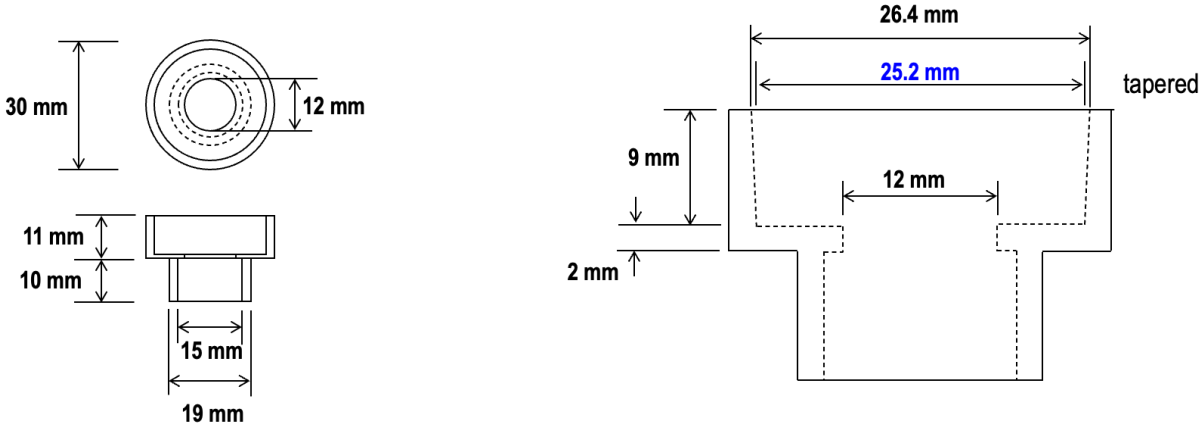

Figure A.1 Impactor housing

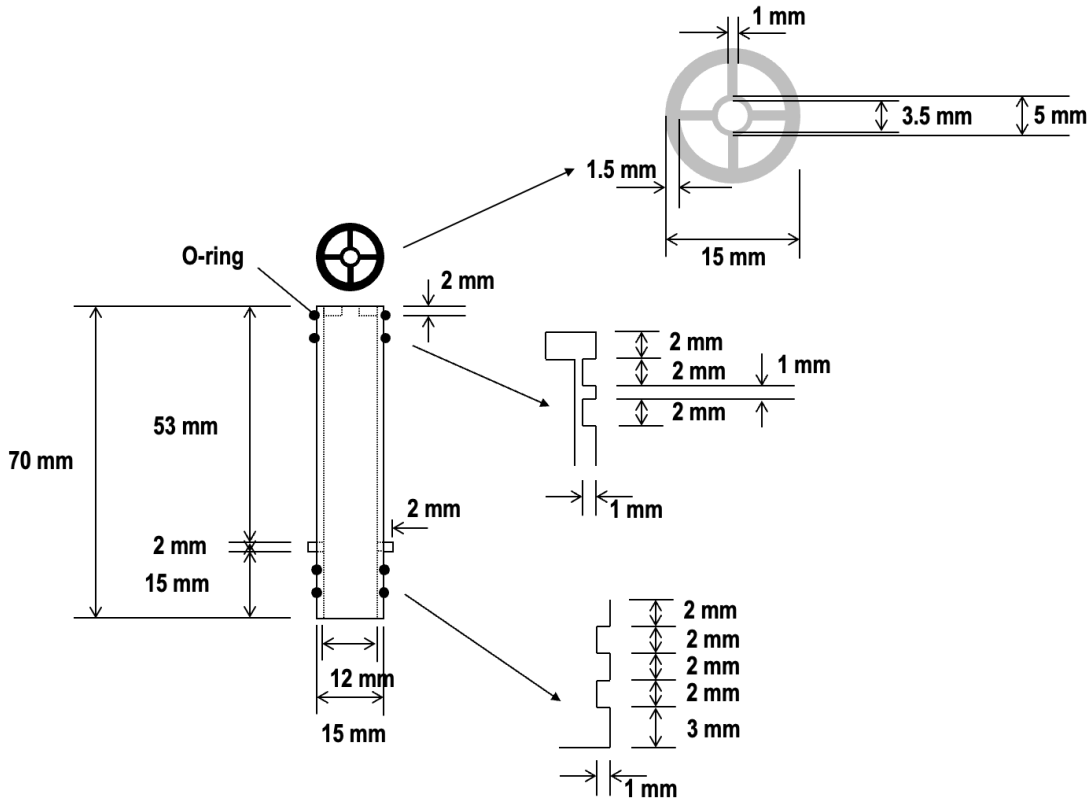

Figure A.2 Swab holder (bottom)

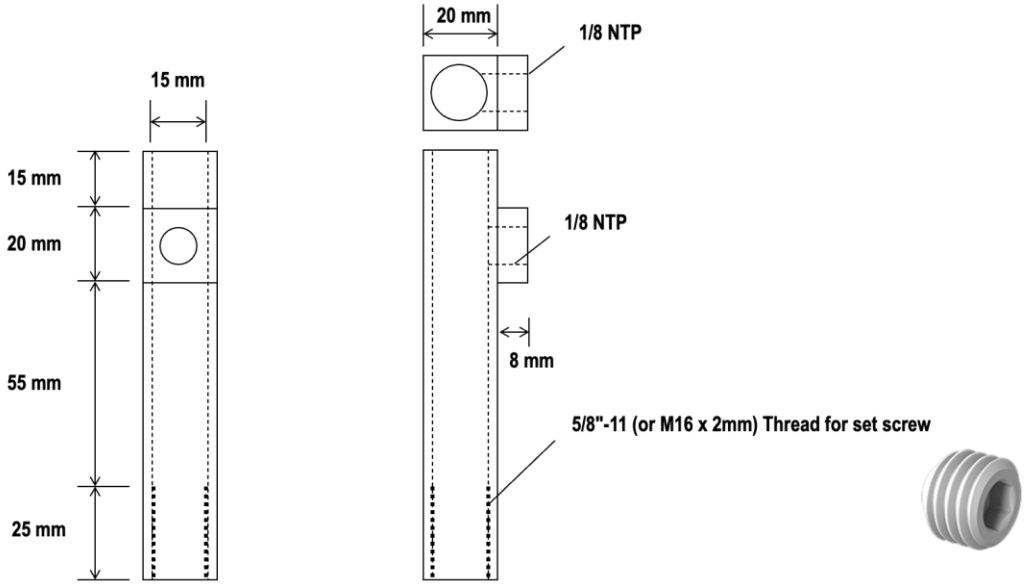

Figure A.3 Swab holder (top)

Figure A.4 Sampling station for field study

Figure A.5 Field sampling in a horse stable

Figure A.6 Field sampling on campus

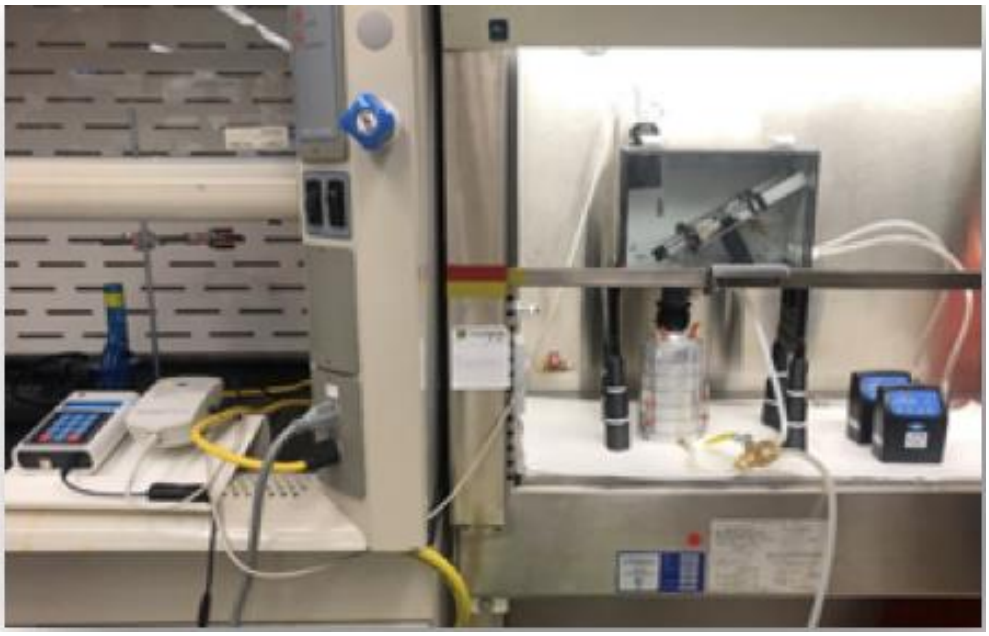

Figure A.7 Experimental setup for sampling aerosolized *E. coli*

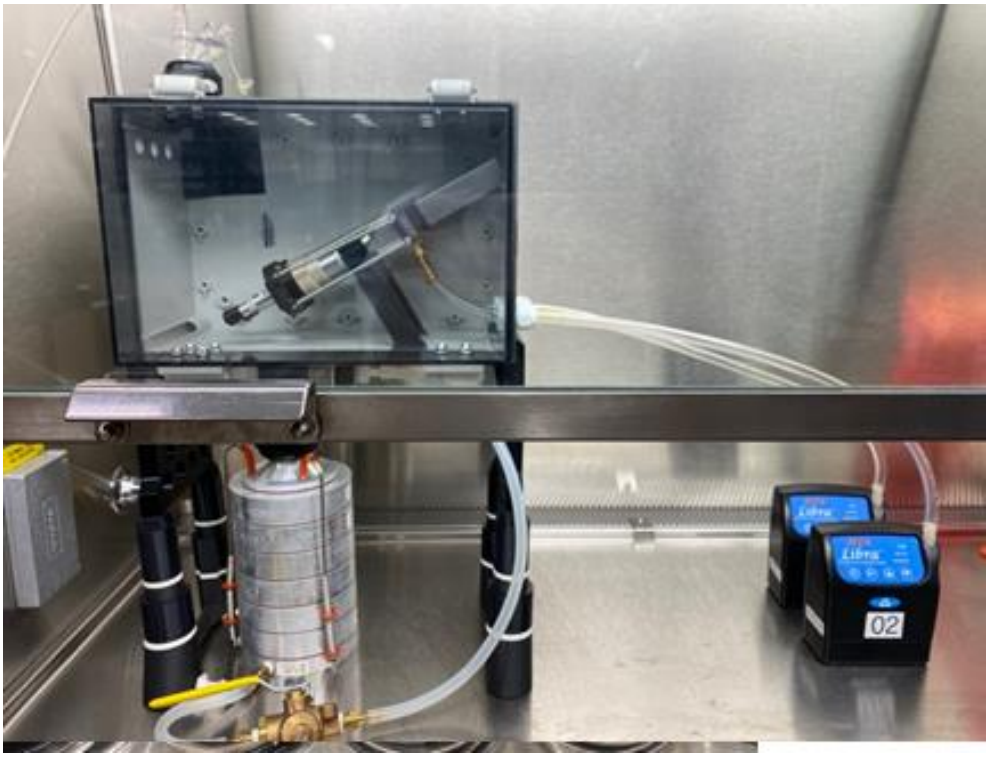

Figure A.8 Experimental setup for sampling aerosolized *L. pneumophila*

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VITA

Li Liao

EDUCATION BACKGROUND

Ph.D. in Occupational and Environmental Health Science 2021

Purdue University, West Lafayette, USA,

M.S., Food Safety and Technology 2016

Illinois Institute of Technology, Chicago, USA,

B.S., Food Quality and Safety 2013

China Agricultural University, Beijing, China

PROFESSIONAL EXPERIENCE

Ph.D. Research Assistant 01/2018 – 11/2021

Purdue University, West Lafayette, USA

- Focusing on the development of new methods to assess the occupational and environmental exposure to bioaerosols
- Developing the bioaerosol samplers innovatively combined with ATP Bioluminescence and immunochromatographic methods to field-analyze airborne virus and bacteria
- Training undergraduate and graduate students to conduct project: Development of a method for the convenient detection of *Legionella pneumophila*
  Key responsibilities: conducting the bioaerosol sampler project; evaluating the performance of the bioaerosol sampler using *E. coli*; using statistical methods to analyze and organize experiment data; conducting field study at different occupational places

Safety manager 01/2018 – 11/2021

Dr. Jae Hong Park's Laboratory at Purdue University, West Lafayette, USA

- Training undergraduate and graduate students about lab safety fundamentals
- Managing the chemical safety plan and controlling biohazard wastes
- Managing the inventory of chemical and biological materials

Master's Student Research Assistant 06/2014 – 08/2016

Food and Drug Administration, Bedford Park, USA

- Conducted studies to optimize the experimental protocol which most accurately reflects natural contamination and have negligible effects on stability and thermal resistance of *Salmonella* in low water activity food
  Achievements: thesis titled *"Efficacy of Aqueous Inoculation in Low-Water Activity Matrix on Salmonella Enterica Serotype Agona Thermal Resistance and Stability during Storage"*

Undergraduate Student Research Assistant 12/2012 - 06/2013

China Agricultural University, Beijing, China

- Evaluated the teratogenicity, carcinogenicity, and mutagenicity of antimicrobial peptide from *Pseudosciaena crocea*
- Demonstrated that antimicrobial peptide from *Pseudosciaena crocea* is a safe and economical additive that can be used in a wide range of fields including animal feeds, food storage, and clinical medications

Achievements: thesis titled "*Safety evaluation of antibiotic peptide from Pseudosciaena crocea"*

Food Analyst Intern 07/2012 – 08/2012

- National Food Quality Supervision and Inspection Center, Beijing, China
- Conducted microbiology experiments to detect *Salmonella* in foods
- Analyzing data of food quality tests for determining protein in milk powder by combustion method

Achievements: Won the honor of "Outstanding Intern"

Food Inspector Assistant 06/2011 – 08/2011

- Chongqing Municipal Technical Supervision, Chongqing, China
- Conducted food quality tests to assess the safety of foods from different production

facilities
- Determined C-4 plant sugars in honey using a stable carbon isotope ratio method
- Determined nitrite content in meat using a spectrophotometry

HONORS AND AWARDS

Graduate Teaching Award 2021
Purdue University Teaching Academy

Graduate Teaching Award 2021
School of Health Science, Purdue University

1st place gold winner of Best in Show Awards 2020
American Industrial Hygiene Conference and Exposition (AIHce)

Ken Dillon Award 2020
Biosafety and Environmental Microbiology Committee, American Industrial Hygiene Conference and Exposition (AIHce)

1st place winner of group presentation 2020
15th Annual Research Capacity Building Workshop, Cincinnati, Ohio, USA

2nd place winner 2019

Graduate Student Oral Presentation, American Industrial Hygiene Association (AIHA) Chicago Student Night, Chicago, USA

Outstanding Intern 2011
National Food Quality Supervision and Inspection Center, Beijing, China

PUBLICATIONS

Liao, L., Byeon, J.H., Park, J.H. (2021) **Development of a size-selective sampler combined with an adenosine triphosphate bioluminescence assay for the rapid measurement of bioaerosols**,

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Liao, L., Luo, Z.Q., Byeon, J.H., Park, J.H. (2021) **Size-selective sampler combined with an immunochromatographic assay for the rapid detection of pathogenic bioaerosols** (submitted to Environmental Research awaiting review scores)

CONFERENCE ACTIVITY/PARTICIPATION

Liao, L., Byeon, J.H., Park, J.H., Development of a sampler combined with adenosine triphosphate bioluminescence assay for the rapid measurement of bioaerosols, American Association for Aerosol Research (AAAR) 38th Annual Conference, Virtual, 10.05-10.09, 2020 (Oral Presentation)

Liao, L., Byeon, J.H., Park, J.H., Development of a sampler combined with adenosine triphosphate bioluminescence assay for the rapid measurement of bioaerosols, International Society for Exposure Science (ISES) 30th Annual meeting, Virtual, 09.21-09.22, 2020 (Poster Presentation)

Liao, L., Byeon, J.H., Park, J.H., Size-selective bioaerosol sampler combined with an adenosine triphosphate (ATP) bioluminescence assay, American Industrial Hygiene Conference and Exposition (AIHce), Virtual, 06.01-06.03, 2020 **[Multiple Award Winner: Best in Show Awards - 1st place gold winner; Ken Dillon Award from Biosafety and Environmental Microbiology Committee]**

Liao, L., Park, J.H., Byeon, J.H., Evaluation of a New Bioaerosol Sampler Combined with an Adenosine Triphosphate (ATP) Bioluminescence Method, Purdue Health and Disease: Science, Technology, Culture, and Policy Research Poster Session, West Lafayette, Indiana, USA, 03.05, 2020 (Poster Presentation)

Liao, L., Size-selective Bioaerosol Sampler Combined with an Adenosine Triphosphate

Bioluminescence Assay, American Industrial Hygiene Association (AIHA), Chicago Local Section Student Night, Chicago, Illinois, USA, 02.25, 2020 (Oral Presentation)

Liao, L., Park, J.H., Byeon, J.H., Evaluation of a New Bioaerosol Sampler Combined with an Adenosine Triphosphate (ATP) Bioluminescence Method**,** American Industrial Hygiene Conference and Exposition (AIHce)**,** Minnesota, Minneapolis, USA, 05.20, 2019 -05.22,2019 (Poster Presentation)

Liao, L., Liu, S., Staci, M., Kimberly, T., Impact of Bioaerosol Exposure on Inflammatory Response in Horse Farm Workers, 15th Annual Research Capacity Building Workshop, Cincinnati, Ohio, USA, 03.19,2019 (Oral Presentation) **Won 1st place group Presentation**

Liao, L., A new approach for evaluation of occupational exposure to bioaerosols, American Industrial Hygiene Association (AIHA), Chicago Local Section Student Night, Chicago, Illinois, USA, 02.27, 2019 (Oral Presentation) **Won 2nd place graduate student presentation**

Liao, L., Park, J.H., Byeon, J.H., Development of a personal sampler combined with ATP bioluminescence method for rapid quantification of bioaerosols, Purdue HHS Fall Research Day, West Lafayette, Indiana, USA, 10.17, 2018 (Poster Presentation)

Liao, L., Park, J.H., Development of a personal sampler combined with ATP bioluminescence method for rapid quantification of bioaerosols, Purdue 5th Annual C4E Environmental Community Mixer, West Lafayette, Indiana, USA, 10.12, 2018 (Poster Presentation)

Liao, L., Efficacy of Aqueous Inoculation in Low-Water Activity Matrix on Salmonella Enterica Serotype Agona Thermal Resistance and Stability during Storage, Graduate Thesis, 2016

Liao, L., Keller, S., Stam, C., Grasso, E., Anderson, N., Pickens, S., Gradl, D., Conway, C., Salmonella enterica stability and thermal resistance during extended storage of low water activity foods and inert matrices, Institute of Food Technologists (IFT), Chicago, Illinois, USA, 2015 (Poster presentation)

RESEARCH GRANTS

University of Michigan NIOSH Pilot Project Research Training program

07/01/2020 – 06/30/2021

Development of a sampler for the rapid and convenient detection of airborne pathogens

Amount: $20,000

Role: Principal Investigator

PROFESSIONAL ORGANIZATIONS

Vice president, Student Environmental Health & Safety Association 2018 – 2021