Good proof statement as to gentleness of thin film liquids to collect microorganisms

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Development of a Personal Bioaerosol Sampler Based on a Conical Cyclone with Recirculating Liquid Film

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This article describes the development of a novel, high-performance personal aerosol sampler intended to monitor occupational air pollution, specifically, microbial constituents. This prototype sampler has a horizontally positioned conical cyclone with recirculating liquid film and an ejection supply of adsorptive liquid into the inlet nozzle. Airborne pollutants were collected in the adsorptive liquid, thus improving the survivability of microbiological aerosol samples. Experimental modules of different dimensions were first evaluated. Based on the test results, a prototype sampler was fabricated and tested. Evaluation of the collection efficiency of the prototype unit indicated a higher than 90% collection efficiency for particles > 1.0 μm. The 50% cutoff diameter was between 0.70–0.75 μm. For assessment of the sampling process effect on the collected microorganisms, Bacillus thuringiensis *was tested at a concentration of about 1.0 × 10^6 cells per cm^3. The viability in the prototype sampler decreased to 78% after 60 min of operation.*

Keywords bioaerosol, cyclone, personal sampler

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INTRODUCTION

The potential for biohazard exposures exists for many occupational settings. Many of these exposures can cause adverse health effects via the respiratory route. Occupational biohazards can be defined as micro- and macroorganisms, and substances derived from these organisms that can exhibit deleterious effects on workers.[1] Known biohazards in the workplace can be broadly divided into viruses, bacteria, fungi, plant substances, and material derived and/or produced by these agents (e.g., allergens and toxins).

Biological agents (particularly bacteria and fungi) are ubiquitous in the workplace environment. Dutkiewicz et al.[1] classified 193 agents or groups of agents according to their potential risk. Exposure to these agents may lead to infections (including zoonotic infections), allergic responses, toxic reactions, and even carcinomas.[1–3] Some occupations pose greater risks than others. To date, the assessment of biological exposure in the workplace has primarily focused on agricultural (farming), health care, and laboratory environments. Individuals who work in these areas have an increased risk of deleterious health effects.[4]

Other occupational environments with the potential for biohazard exposure include but are not limited to animal handling facilities; recycling, composting, and sewage plants; food production facilities; textile, print, and paper industries; museums and libraries; forestry; horticulture; fisheries; metal and wood processing industries; and the building/construction industries. With such a broad array of potential health risks in the workplace, it is important to assess exposure accurately. Furthermore, individual assessment is necessary, as each worker will respond differently to any given biohazard.

Most present-day personal aerosol samplers (PAS) used for monitoring airborne microorganisms in occupational settings are based on air filtration (e.g., the Button Aerosol Sampler[5] and Institute of Medicine [IOM] personal sampler[6]); impaction on solid surfaces or nutrient medium (e.g., the Personal Cascade Impactor Sampler,[7] the Personal Sampler for Collecting Fungal Spores[8]); or precipitation in liquids (e.g., the Personal Rotation Cup Sampler [CIP 10-M],[9] and the Personal Sampler for Culturable Airborne Microorganisms[10]). In addition to high sampling efficiency, a bioaerosol sampler should also retain microbial viability. Existing commercial personal bioaerosol samplers using fibrous or membrane filters may affect the viability of the collected material, resulting

in inaccurate assessment of occupational exposure in critical areas, such as hospitals, agricultural settings, and research laboratory environments. Impaction on surfaces or liquid may also result in physical injuries or stress of the microorganisms.

In our opinion, the optimal sampling method for biological agents is based on the principle of creating a rotating liquid film on the inner surface of a cyclone (first suggested by Olenin et al.[11]). With this approach, the trajectory of aerosolized particles follows a path tangent to the liquid film surface, thus ensuring a gentle capture and increased microbial survivability. Detailed study and development of this method have resulted in the design and development of a bioaerosol sampler as we have described elsewhere.[12] Unfortunately, numerous characteristics inherent to that design are not appropriate for its usage as a PAS.

The main objective of this study was to develop a highly efficient personal liquid cyclone sampler to collect airborne biological materials with a lower flow rate appropriate for personal sampling. By using a gentle liquid cyclone film, we preserved microbial viability, thus allowing for a more accurate personal exposure assessment.

DESIGN AND TESTING OF EXPERIMENTAL MODULES

Sampler Design and Main Technical Characteristics

The sampler design was based on the following criteria: (1) ease of use, (2) versatility (i.e., capacity to use in different environments), (3) low aerodynamic resistance, (4) continuous operation (upward of 1 hour), (5) high collection efficiency, (6) gentle bioaerosol collection, (7) portability, (8) ease of manufacture, and (9) cost-effectiveness.

During development of the new PAS, the main focus was to design a unit with a recirculating liquid film as described previously.[12] For the device described herein, the operating principle was based on production of a rotating liquid film on the inner wall of a cylindrical cyclone as generated by airflow pulled through the system. To accomplish this, the inlet nozzle of the sampler was arranged so that its long axis was positioned tangentially to the plane surface of the cyclone. Air motion directed in this manner caused a negative pressure drop in the center of the cyclone. Due to this pressure drop, liquid (continuously pulled into the cyclone from a reservoir) traversed upward along the inner wall of the cyclone in the form of a spiral-shaped film until it reached the upper edge of the cyclone.

Because of centrifugal forces generated during sampling, aerosol particles were precipitated via inertia onto the liquid film surface. The trajectory of the aerosol stream, which is near tangential to the liquid film surface, ensured a gentler capture of bioaerosols. In addition, the unit was designed such that the liquid was provided continuously, allowing a more efficacious use of the sampling fluid, a longer sampling period (compared with known liquid samplers), and a more concentrated collection of biological agents.

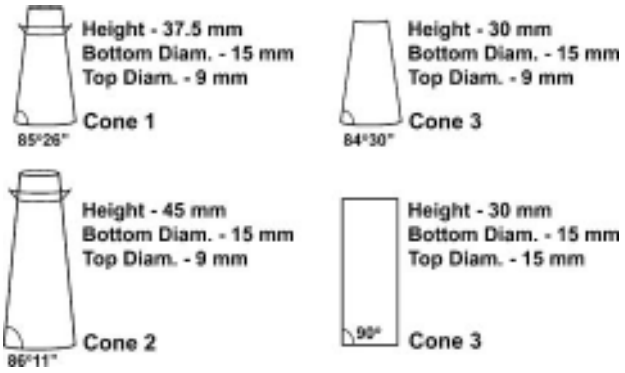

FIGURE 1. Experimental modules of the sampler

A computational fluid dynamics calculation was used to understand the flow field, the formation of the liquid film, and the particle collection process in the prototype sampler as reported previously.[12] Based on these theoretical assessments, practical design criteria for a novel PAS were formulated, and a series of experimental modules were fabricated (Figure 1).

In addition to a cylindrical module that was based on the original sampler design, several conical modules were made. Studies with the conical sampler bodies demonstrated that the airflow could make two or three revolutions versus one revolution observed in the cylinder from measurement of pressure inside the conical cylinder (Figure 2). The change

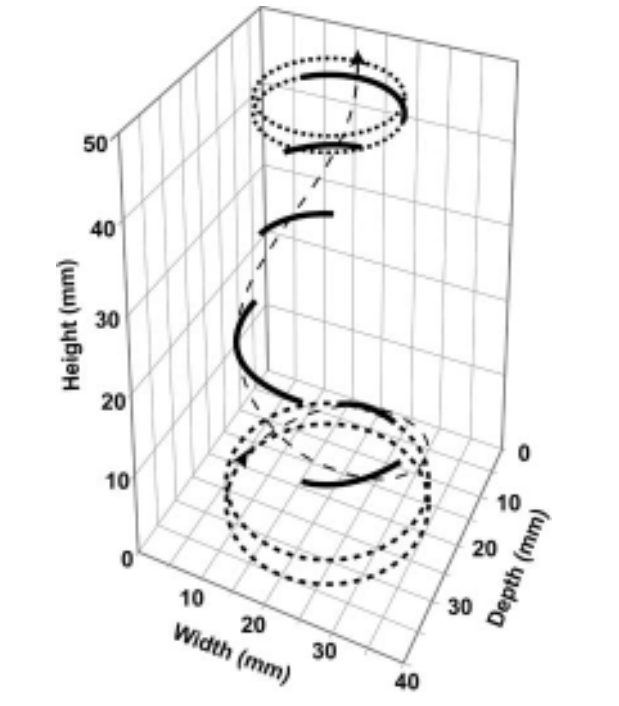

FIGURE 2. Air pressure measurements in vertical conical sampler module. The vertical axis shows the cyclone height in mm. Areas of highest negative pressure are shown in solid lines. The dashed continuous line shows a mapped rotating air stream within the cone.

in flow pattern led to increased particle capture efficiency, especially for the respirable particulate mass.

When designing the units, particular attention was paid to the top and bottom diameters, height, and angle (with the conical designs). Each module was attached to a custom-made titanium base (15 mm diameter) composed of an air inlet nozzle and liquid ejector. The inlet was designed such that the outer diameter of 2 mm narrowed to 1.8 mm at the liquid ejector interface. This allowed for vortex formation and laminar cyclonic liquid flow within the modules.

Polydisperse Aerosol Testing

The first task was to test each experimental module for particle collection efficiency and flow rate in order to determine an optimum design for the PAS. These tests were designed and performed at the Research Center for Toxicology and Hygienic Regulation of Biopreparations (RCT & HRB), Moscow, Russia.[13] Initially, each module was tested using polydisperse particles as shown in Figure 3.

Liquid polydisperse particles (physiological saline [pH 7.2], 0.02% eosin, 5% glycerol) were generated into a stainless steel, 40-L, sealed aerosol chamber (Figure 3) using a Collison-like nebulizer designed and produced at the RCT & HRB. During aerosolization, inlet flow was balanced with exhaust using a vacuum pump positioned at the top of the aerosol chamber. Particles were generated for 10 min prior to collection to establish a homogenous particle aerosol within the chamber. Particle size was determined using an Andersen six-stage viable impactor (Thermo Electron Corp., Cheswick, Pa.) equipped with metal impaction plates for each stage. Following a 5-min sample, plates were removed and washed with 10 mL of saline. The collected washes were analyzed at 490 nm using a Lumex spectrophotometer (Lumex, Ltd., St. Petersburg, Russia).

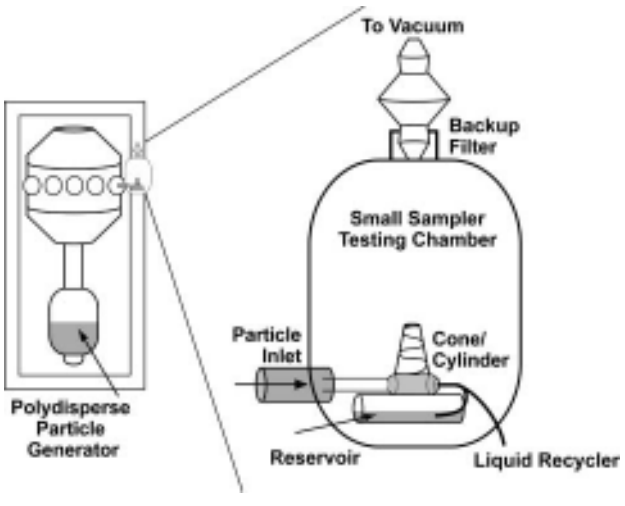

FIGURE 3. Schematic of the experimental test system at Research Center for Toxicology and Hygienic Regulation of Biopreparations

The test sampler was placed in a small enclosure connected to a sampling port of the aerosol chamber (Figure 3). Ten milliliters of phosphate buffered saline (PBS) with 0.1% Tween 20 solution (pH 7.2) was used as collection fluid in a cartridge reservoir for each trial. Tween 20 was added to allow for a better wetting surface within the modules. The liquid was circulated (vortex formation) via a vacuum source positioned at the opposite end of the makeshift chamber. Prior to particle collection, the flow rate was optimized by determining the minimal value at which a visually stable spiral liquid film would form. Particles not entrained within the collection fluid were captured at the exit of the chamber using a sterile, 47 mm diameter Gelman type E glass fiber filter (Pall Corporation, East Hills, N.Y.) secured in a stainless steel filter holder. For each module, sampling was performed for 5 min and repeated three times. The makeshift chamber and cyclone modules were thoroughly washed using ultrapure water between each test.

Following the 5-min sampling period, the vacuum was closed. The collection fluid in the cartridge was removed using a 10-mL glass syringe and transferred to a clean 15-mL glass centrifuge tube. In addition, the backup filter was removed, placed in 10 mL of sterile saline, and washed with gentle rocking for 10 min. As with the Andersen impaction plate washes, the sampler collection fluid and the filter rinse were analyzed spectrophotometrically. Absorbance readings were plotted against an eosin standard curve developed with known concentrations of eosin in 5% glycerol/saline.

The relative collection efficiency (E, %) of each sampler module was calculated using the following equation:

$$E = \frac{C_s}{C_s + C_{bf}} \times 100 \qquad \textbf{(1)}$$

where C_s was the eosin concentration in the sampler (mg/mL), and C_{bf} was the eosin concentration in the backup filter (mg/mL).

Monodisperse Aerosol Testing

From the previous test, we determined the optimum flow rates and overall collection efficiencies for each experimental module. Then we continued the development by determining the collection efficiency as a function of particle size.

Monodisperse green fluorescent polystyrene latex (PSL) particles (Duke Scientific Corp., Palo Alto, Calif.) were generated into the same chamber as described for polydisperse aerosol testing. Particles with diameters of 5.0, 3.0, 2.2, 1.0, and 0.5 μm were tested. The density of the PSL particles was 1.05. Test suspensions (10% v/v in water) were diluted from the original particle suspensions for each particle size (10% v/v in water). Particles were aerosolized using an Up-Mist medication nebulizer (Mada Medical, Carlstadt, N.J.). Each unit was filled with 15 mL of the test PSL suspension. A flow rate of 5 L/min to the nebulizer, as monitored by a rotameter (model FM-1050-VI; Matheson Tri-Gas, Montgomeryville, Pa.), was maintained for the duration of testing. Inward flow and outward flow were balanced as already described.

TABLE I. Polydisperse Particle Collection Efficiency for Different Test Modules

Experimental Module	Sampler Orientation	Flow Rates (L/min)	Collection Efficiency[A] (%)
Cone 1	Vertical	20	89.9 ± 5.3
	Horizontal	18	87.3 ± 6.0
Cone 2	Vertical	20	89.4 ± 4.1
	Horizontal	15	88.3 ± 1.1
Cone 3	Vertical	17	84.8 ± 3.0
	Horizontal	13	87.2 ± 5.1
Cylinder	Vertical	19	87.8 ± 1.2
	Horizontal	15	80.9 ± 3.4

[A]Mean and standard deviation of three duplicates.

Prior to sampling, particle concentration in the aerosol chamber was allowed to equilibrate for 8 min (5 L/min for the 40-L volume chamber). As with the previous tests, 10 mL of PBS/0.1% Tween 20 solution (pH 7.2) was used as collection fluid for each trial. Sampling was performed for 10 min. The filter was extracted in 10 mL of ethyl acetate. The collection fluid was allowed to evaporate to completion in a 90°C electric drying cabinet (Model 2B-151, manufactured in the former Soviet Union) before being resuspended in 10 mL of ethyl acetate. Samples were analyzed at an excitation wavelength of 475 nm and emission wavelength of 525 nm using a FLUORAT 02–3M flow meter (Lumex; Joint Stock Company, St. Petersburg, Russia). Horizontally positioned cyclones including Cone 1 and 3 and cylinder configurations were tested. The flow rates were 18, 13, and 15 L/min for Cones 1 and 3 and the cylinder as shown in Table I. There were three duplicates for each experimental condition.

DESIGN AND TESTING OF PROTOTYPE PERSONAL SAMPLERS

Based on mathematic modeling and lab results of experimental modules, a PAS prototype was developed[14] (Figure 4). This prototype PAS had a horizontally positioned conical cyclone with recirculating liquid film and an ejection supply of adsorptive liquid into the inlet nozzle. To maintain a low airflow rate through the unit, the Cone Model 3 configuration with a bottom cone diameter of 15 mm was selected.

A critical component of the prototype PAS was the presence of a removable cartridge used specifically to hold the collection fluid. In the cartridge, the liquid flowed through the ejection tube and formed a spirally moving liquid film on the inner surface of the cyclone. Particles that flowed onto the liquid film on the inner wall of the cyclone were then captured. Upon conclusion of a given sampling cycle, the cartridge (with captured aerosol constituents) could be easily removed and processed. The removable cartridge could be used to collect sequential samples in order to follow a temporary profile of biological aerosol concentrations in a workplace. Table II lists the dimension of the prototype sampler and its operational parameters.

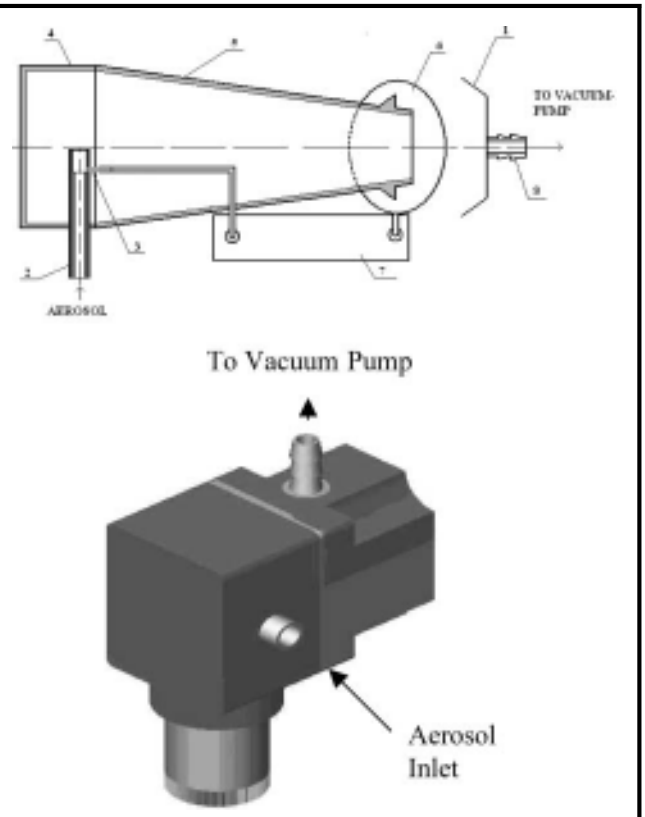

FIGURE 4. A prototype personal sampler. The aerosol enters the cyclone swirling chamber 4 via a tangential nozzle 2. Cyclone 5 consists of an open convergent cone with a horizontal long axis. Ejector 3 is placed near Intake Nozzle 2. Adsorptive liquid is supplied from Cartridge 7. While the device is operating, the liquid, which flows over the cyclone edge, is collected in the cup-shaped Collector 6, and then enters Cartridge 7.

Collection Efficiency of the Prototype Sampler

The prototype PAS was tested initially at RCT & HRB using liquid polydisperse aerosols. The purpose of this study was to

TABLE II. Dimensions and Operational Characteristics of the Prototype Sampler

Parameter	Value
Length	62 mm
Width	65 mm
Height	100 mm
Weight	180 g
Volume of adsorptive liquid	5 mL
Nominal flow rate	8–10 L/min
Operating cycle duration	Up to 60 min
Aerodynamic resistance	≤ 5.0kPa

evaluate the sampling flow rate, times of continuous operation, and consumption of fluid due to evaporation. Physiological saline was used as the adsorptive liquid in the cartridge. During the sampler operation, a continuous process of evaporation and loss of adsorptive liquid occurred.

Following the polydisperse aerosol testing, monodisperse aerosols (using fluorescent PSL particles as described previously) were used to determine the collection efficiency curve. These collection parameters were characterized at two facilities, RCT & HRB and Lovelace Respiratory Research Institute (LRRI).

Testing at RCT & HRB was conducted as described previously. The experimental apparatus was similar to that presented in Figure 3, except that the test sampler was connected directly to one sampling port of the aerosol chamber. Mass concentration and particle size distribution were monitored using an aerosol spectrometer (GRIMM model 1.108; AEROSOL Technik GmbH, Ainring, Germany). Similar testing was conducted at LRRI. Fluorescent monodisperse PSL particles were aerosolized using a Hospitak nebulizer (Hospitak, Inc., Farmingdale, N.Y.), diluted, and directed into a 15-L cylindrical aerosol chamber with a honeycomb section to provide laminar flow in the chamber.

The schematic diagram of the experimental setup at LRRI is shown in Figure 5. The outlet of the PAS was connected to a Leland Legacy personal sampling pump (SKC, Inc., Eighty Four, Pa.). Particle size and concentration were monitored using an Aerodynamic Particle Sizer (TSI, Inc., St. Paul, Minn.). After sampling, the inside walls of the sampler and backup filter were washed with 100% ethyl acetate solution. The relevant concentrations of fluorescent tracer in the solutions and collection liquid were measured with a fluorometer (model 450; Sequoia-Turner Corp., Mountain View, Calif.) and compared with a standard curve.

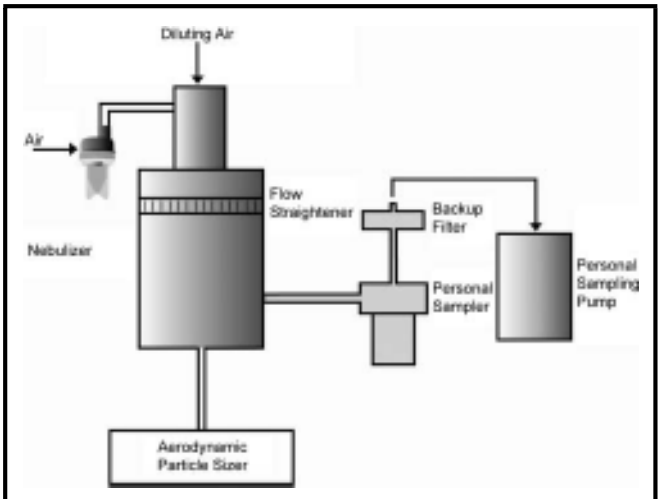

FIGURE 5. Schematic of the experimental test system at Lovelace Respiratory Research Institute

Survivability of Bioaerosols During the Sampling Process

To evaluate how the sampling process of the PAS affected survivability of microorganisms, experiments with *Bacillus thuringiensis* spores were conducted. An AGI-4 impinger (Ace Glass, Inc., Vineland, N.J.)[15,16] was tested in parallel as a reference device. Working suspensions containing spores were placed in the liquid cartridge of the sampler. The prototype device was operated by sampling filtered air at a flow rate of 10 L/min for 15, 30, 45, and 60 min. The nominal concentration of spores in the liquid was 1×10^6 cells/cm^3. On completion of the sampling period, bacterial suspensions were cultured to obtain the viability data. The suspension was serially diluted and then cultured using standard microbiological spread plating onto 90-mm tryptic soy agar plates and cultured at 37°C for 24 hr.

RESULTS AND DISCUSSION

Polydisperse Aerosol Testing of Experimental Modules

Impactor results showed that the majority of polydisperse test particles (53%) impacted onto Stages 4 and 5 (2.1–3.3 and 1.1–2.1 μm cutoff diameters, respectively). The estimated mass median aerodynamic diameter was 1.8 μm.

Results from polydisperse aerosol tests are presented in Table I and show that collection efficiencies were relatively similar (87–90%), with the exception of Cone 3 in the vertical orientation and the cylinder in the horizontal orientation, where the collection efficiencies were slightly lower. Efficiency could not be assessed accurately with these data, particularly since wall losses were not measured.

Furthermore, cutoff diameters could not be precisely calculated due to the use of polydisperse particles. These experiments did, however, demonstrate that the test sampler modules could collect a broad range of particle sizes. In addition, these data prove that in a horizontal position of the cyclone, optimal flow rate is noticeably lower than in the vertical position. This is an important aspect in designing a PAS, which has a low flow rate of $<$10 L/min.

Monodisperse Aerosol Testing of Experimental Modules

Results from monodisperse aerosol tests are presented in Table III and show that collection efficiencies for the experimental sampling modules were similar for monodisperse particles, ranging in size from 1.0–5.0 μm in diameter (94–99%). Slight decreases in efficiency became apparent with 1.0-μm particles and significant decreases when 0.5-μm particles were tested. The cutoff diameter with 50% collection efficiencies for these experimental modules was around 0.5 μm, indicating that the collection efficiency for microorganisms (particularly bacteria and fungi) would be high.

TABLE III. Monodisperse Particle Collection Efficiencies for Different Test Modules

	Collection Efficiency (%)[A]		
Particle Diameter (μm)	Cone 1	Cone 2	Cone 3
5.0[B]	100	100	100
3.0[C]	99.7 ± 0.2	99.5 ± 0.3	99.7 ± 0.2
2.2[C]	99.5 ± 0.5	99.2 ± 0.2	99.3 ± 0.6
1.0[D]	97.8 ± 0.8	93.6 ± 2.8	94.6 ± 1.5
0.5[D]	63.9 ± 5.6	49.9 ± 6.3	52.6 ± 7.5

[A] Mean and standard deviations
[B] Two replicates performed.
[C] Three replicates performed.
[D] Six replicates performed.

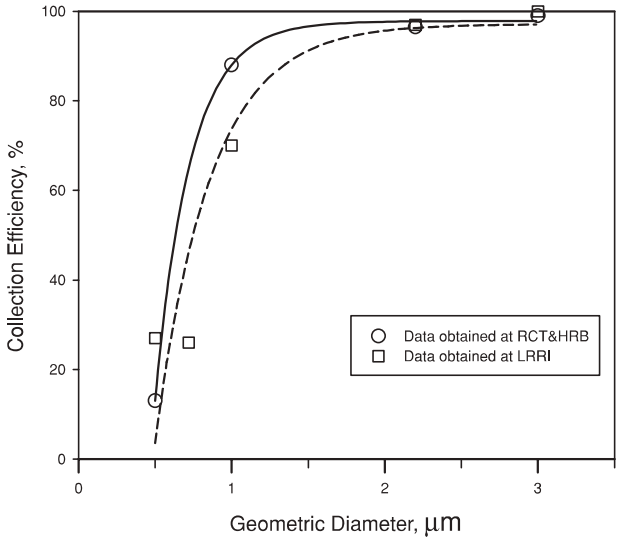

FIGURE 7. Collection efficiency of the prototype personal sampling device using the monodisperse PSL particles

Performance Evaluation of the Prototype Sampler

Figure 6 shows the amounts of liquid that remained in the cartridge as a function of time. It was apparent that the liquid slowly evaporated and remained at about 64% in the volume of original fluid in 60 min of sampling the polydisperse aerosol. This result demonstrated that the sampler could continuously operate up to 1 hr. The initial test also showed that the sampler functioned well at a flow rate of 8–12 L/min.

Figure 7 presents data on the collection efficiency of monodisperse PSL aerosols obtained in the PAS studies at LRRI and RCT & HRB. Flow rate in both cases was the same, 10 L/min. From these data it follows that the diameter for a 50% collection efficiency of monodisperse aerosol (d_{50}) was in the range of 0.7–0.75 μm. The cutoff diameter was lower than reported in the samplers developed by Chen et al.[8] and Görner et al.[9]

Figure 8 shows the viability of the *B. thuringiensis* spore as a function of time in the prototype sampler as well as an AGI-4 liquid impinger. The spore viability decreased with time in both devices. The viability in the prototype sampler decreased to 78% after 60 min of operation, which is higher than those for AGI-4 impingers. The viability of collecting airborne microorganisms in the sampler has not been tested and may have a different viability.

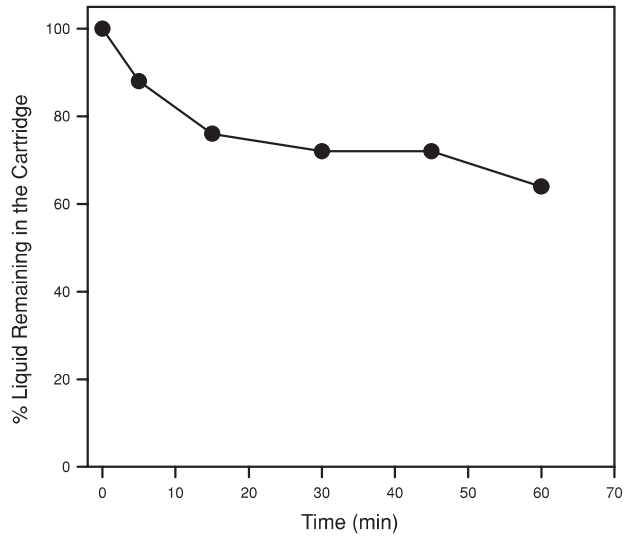

FIGURE 6. Amount of liquid remaining in the cartridge as a function of time

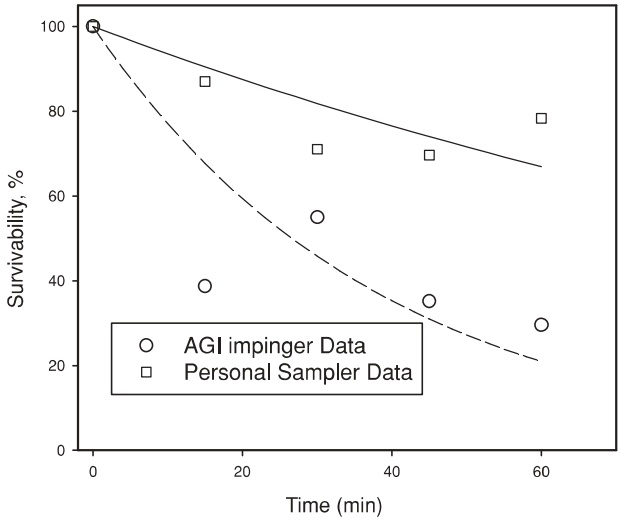

FIGURE 8. Survivability of *Bacililus thuringiensis* spores in the personal sampler and AGI impinger as a function of time for one test run

CONCLUSIONS

A prototype of a PAS was developed for sampling bioaerosols based on the recirculating liquid film cyclone. The design criteria included high collection efficiency, low flow resistance, and improved viability of the collected microorganisms. Preliminary tests of several experimental configurations showed an optimal design of lower flow resistance and high collection efficiency using a conical chamber.

A prototype PAS was designed and tested. The experiments conducted with the prototype PAS demonstrated that, on the whole, the device had appropriate characteristics to fulfill the design criteria. Conical configuration of the cyclone and ejector dispersion of adsorptive liquid in the inlet nozzle made it possible to enhance considerably the efficiency of aerosol capture. The 50% cutoff diameter (d_{50}) achieved was 0.7–0.75 μm, which exceeded the values of the known liquid cyclone-type samplers. The use of a removable cartridge for adsorptive liquid in the PAS design ensured easy operation and maintenance of the sampler. Testing of the PAS on a microbiological aerosol has proven the high survivability of *B. thuringiensis.*

ACKNOWLEDGMENTS

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Summary

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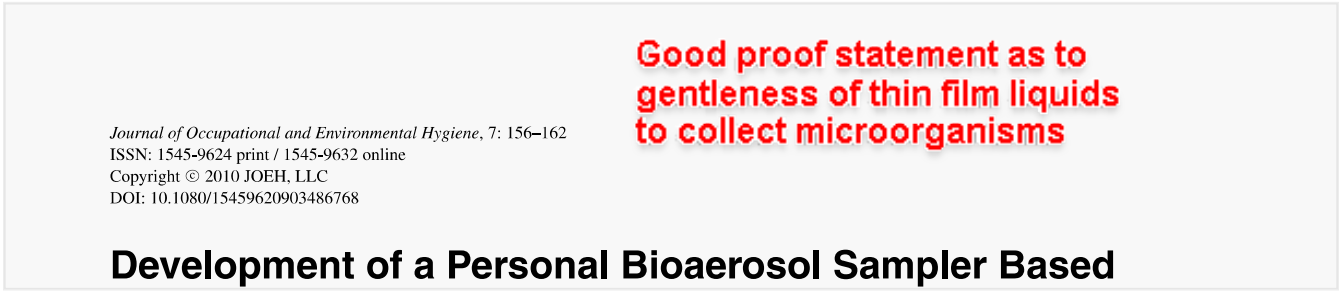

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