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Agranovski, Igor E. and Safatov, Alexander S. and Borodulin, Alexander I. and Pyankov, Oleg V. and Petrishchenko, V. A. and Sergeev, Artemii N. and Agranovski, Victoria and Grinshpun, Sergey A. (2005) New personal sampler for viable airborne viruses: feasibility study. *Journal of Aerosol Science* 36(5-6):pp. 609-617.

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NEW PERSONAL SAMPLER FOR VIABLE AIRBORNE VIRUSES: FEASIBILITY STUDY

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Submitted to:

Journal of Aerosol Science

(Special issue on Measurement and Characterization of Bioaerosols)

MS GK/04/29
Revised October 28, 2004

Keywords: bioaerosol, personal sampling, virus, viable, bubbling, collection efficiency.

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ABSTRACT

While various sampling methods exist for collecting and enumerating airborne bacteria and fungi, no credible methodology has yet been developed for airborne viruses. A new sampling method for monitoring the personal exposure to bioaerosol particles has recently been developed and evaluated with bacteria and fungi. In this method, bacterial/fungal aerosol is aspirated and transported through a porous medium, which is submerged into a liquid layer. As the air is split into numerous bubbles, the particles are scavenged by these bubbles and effectively removed. The current feasibility study was initiated to evaluate the efficiency of the new personal sampler prototype ("bubbler") with airborne viable viruses. Two common viral strains, *Influenza* (stress-sensitive) and *Vaccinia* (robust), were aerosolized in the test chamber and collected by two identical "bubblers" that operated simultaneously for the duration of up to 5 minutes. A virus maintenance liquid, proven to be the optimum collecting environment for the test organisms, was used as a collection fluid. After sampling, the collecting fluid was analyzed and the viral recovery rate was determined. The overall recovery (affected not only by the sampling but also by the aerosolization and the aerosol transport) was 20% for *Influenza* virus and 89% for *Vaccinia* virus. The new sampling method was found feasible for the collection and enumeration of robust airborne viruses.

INTRODUCTION

The SARS outbreak has raised various scientific and public health issues related to the transmission and control of infectious agents (Lipsitch et al., 2003). The threat of bioterrorism has gained additional public attentions to these issues. The above factors, as well as a generally growing concern about human exposure to bioaerosols, have created a considerable demand for advanced, reliable and efficient monitoring methods for detecting, identifying and enumerating airborne biological particles (Lacey and Dutkiewicz, 1994; Comtois and Isard, 1999). The requirements for an ideal bioaerosol sampler have been described by Macher (1997). One of the main questions in the selection of a bioaerosol sampler is whether it can maintain high biological collection efficiency, i.e., whether the effect of the sampling on the biological status of the collected microorganisms can be minimized.

Individual exposure to bioaerosols can be adequately evaluated by the use of personal aerosol samplers. The following criteria are applied to the bioaerosol sampling: (i) the sampler should be compact and capable of maintaining high physical and biological efficiencies over prolonged time periods, and (ii) the collection media should be suitable for a variety of microbiological analyses, including microbial identification and enumeration (Crook, 1995).

Based on the engineering control method, which was previously applied to the removal of particles from gas carriers (Agranovski et al., 1999; Agranovski et al., 2001), a new personal bioaerosol sampling method was developed (Agranovski et al., 2002a; Agranovski et al., 2002b). The prototype device is shown in Figure 1. Front (a) and side (b) views photographs are presented along with the diagrams of the top (c) and vertical cut (d) of the sampler. Contaminated air, aspirated into the air inlets,

is bubbled through a porous medium, which is submerged into a liquid layer, and subsequently split into a multitude of very small bubbles. The particulates are scavenged by these bubbles, and, thus, effectively removed before the effluent air leaves the vessel. The performance characteristics of the new sampler have been evaluated in a stationary operation mode with PSL particles and found to exceed 92% for the particles of 0.318 µm and larger (Agranovski et al., 2002a). An 8-hour continuous sampling of airborne bacteria of *Pseudomonas fluorescens* and *Bacillus subtilis var. niger* and airborne fungal spores of *Aspergillus versicolor* revealed that the viability of these microorganisms remained high (Agranovski et al., 2002b). The recovery rate of the stress-sensitive gram-negative *P. fluorescens* bacteria was 61±20%. For stress-resistant *B. subtilis* bacteria and *A. versicolor* fungal spores it was 95±9% and 97±6%, respectively. The field study, in which six identical personal samplers were tested simultaneously on a simplified human manikin in an office environment during 2-, 4- and 8-hour time intervals, has shown that the viable microbial concentration was not significantly affected by the sampling time. This was attributed to a gentle collection process utilized in this novel bioaerosol sampling method. The inter-sample variation did not exceed 30%. Thus, the new sampling method was found suitable for both the stationary and personal monitoring of airborne bacteria and fungal spores.

Viruses generally exhibit much stronger response to microbial stress than bacteria and fungi. For this reason, there is a lack of methods for efficient collection of airborne viruses, although a variety of reasonably well characterized sample collection techniques are available for aerosolized bacteria and fungi. In most existing bioaerosol samplers, such as impactors and impingers, the microorganisms impact on the collection media at air velocity as high as tens and hundreds m s^{-1}

(Mainelis et al., 1999). This creates excessive microbial stress during the sampling. In contrast, the new sampler was designed to operate at an air velocity below 1 m s^{-1} to minimize the stress.

Our first (pilot) study addressed the survival of viruses in a bubbling collection fluid (Agranovski et al., 2003). The decay of viable viruses in the suspension, subjected to continuous bubbling inside the sampler's collection vessel, was determined for three stress-sensitive (*Influenza, Mumps* and *Measles*) and one robust (*Vaccinia*) viral strains. The HEPA-filtered air was continuously drawn through the virus-containing fluid at a flowrate of 4 L min^{-1} during the time of up to 4 hours. The experiments were performed with two types of fluids, including sterile water and a specially prepared virus maintenance fluid with an antifoaming agent. Fluid samples were taken at 0, 1, 2 and 4 hours and analyzed by appropriate microbiological techniques to quantify the viral response to the stress caused by the bubbling. The natural decay was also measured (for a stationary suspension with no bubbling) and used for comparison. Overall, the survival rate was relatively high given such a prolonged exposure of viruses to the stress. For stress-sensitive strains, the decay was especially pronounced when testing with sterile water: it exceeded 99% after 4 hours of the device operation. In contrast, the robust *Vaccinia* virus did not show any noticeable decay for the entire 4-hour period. The use of the virus maintenance fluid significantly decreased the decay (75%) for stress-sensitive microorganisms. The difference in the physical properties of this fluid and the sterile water did not seem to affect the physical performance characteristics of the "bubbler." No excessive foam generation was observed during its operation; the physical sampling efficiency of the device was the same with both fluids; and no increase in the hydrodynamic resistance was detected. Therefore, the pilot study data

suggested that the virus maintenance fluid is suitable for maintaining viability of airborne viruses (especially robust ones) should they be collected by the new sampling method during several hours.

In this study, the new sampler was evaluated with the viable viruses aerosolized in the test chamber and actually collected from the air into the device containing the virus maintenance fluid. The bioaerosol samples obtained during a 5-minute continuous operation of the "bubbler" were analysed to determine the recovery rate of the collected viruses.

METHODS

Test Viruses

Two common viruses with an aerosol transmission pathway, *Influenza* and *Vaccinia* were employed for the tests (see the photo in Figure 2). Both were previously tested with respect to their survival through continuous bubbling processes and found to have distinctly different stress-resistance characteristics: *Vaccinia* was much more stress-resistant than *Influenza* (Agranovski et al., 2003).

Influenza virus A/Aichi/2/68 (H3N2) was obtained from the Moscow Ivanovsky Institute of Virology. The virus was passaged 12 times in mice and twice in embryonated chicken eggs (ECE). Allantoic fluid, which was produced by cultivating the virus in ECE (9–11 day old) and had a viral concentration of $10^8 – 10^9$ EID_{50} mL^{-1}, was used in the experiments. The EID_{50} is commonly defined as the smallest amount of virus capable of initiating infection in an embryonated egg on 50% of occasions (Barrett and Inglis, 1985).

Vaccinia virus strain LIVP (C0355 K0602) was also obtained from the Moscow Ivanovsky Institute of Virology. The virus was passaged 10 times in 9 –11 day old ECE. The virus-containing material, at a concentration level of 10^7 PFU mL^{-1} (Plaque Forming Units (infectious virions capable of forming plaque) per mL), was obtained by culturing on cells 4647 (cells of *Cercopetecus Aefiopsis* embryo kidney) with the subsequent triple freezing/defrosting of the infected cell culture on the MEM Eagle maintenance media (MOD, Cat #11-100-22, ICN Biomedicals, Inc., Aurora, OH, USA). Prior to the testing, the media containing *Vaccinia* virus were kept at a temperature of -70^0C.

The viral suspension was diluted and added into a 3-jet Collison Nebulizer (BGI Inc., Waltham, MA, USA) that aerosolized viruses during experiments.

Experimental Setup

The experimental setup developed for the laboratory evaluation of the new sampler is schematically shown in Figure 3. The viral particles were aerosolized from the initially prepared suspension by the Collison nebulizer at a flow rate of 6 L min^{-1} with dry and filtered compressed air. The aerosol was then mixed with an additional flow of dry and filtered air (at 10 L min^{-1}) and entered the test chamber. The dryer was adjusted so the aerosol particles in the chamber had a prevalent size of above 1 μm. Thus, the particles aerosolized from the viral suspension contained a considerable amount of liquid. This simulated a real-life situation, as single viruses (size ~ 0.1 μm) are usually transmitted in air environments on particles-carriers of much larger size, which increases their chances to survive the desiccation stress.

A 400-L dynamic chamber with the horizontal aerosol flow velocity of 0.1 m s^{-1} was used for the tests (Ryzhikov et al., 1995). Uniform air distribution across the

chamber was achieved by using a honeycomb-based flow distribution system. The non-uniformity of the aerosol concentration did not exceed 10%.

Two identical samplers were placed in the chamber and operated in parallel at 4 L min^{-1} for 5 minutes. Each was filled with 50 mL of 2% Hanks solution containing bovine serum, a 0.03% solution of silicon anti-foam emulsion (M-30 Dow- Corning, 30% Dimethylpolysiloxane in water), and antibiotics (100 U mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin).

The concentration and size distribution of particles in the air was monitored by an optical aerosol spectrometer (Model 1.108, Grimm Aerosol Technik, Ainring, Germany), which has been previously used for real-time measurement of airborne bacteria and fungi (Gorny et al., 2001; Wang et al., 2001; Grinshpun et al., 2002). The experiments were performed at a relative humidity of 50-70 % and an air temperature of 20-24°C. The HEPA filters were installed in the sampling lines (between the sampler and the sampling pump) and in the chamber's exhaust stream to protect the laboratory environment from the release of pathogenic viruses.

Viral recovery rate

The validation tests showed that each droplet generated by the nebulizer could carry more than one virus. Such arrangement realistically represents natural environment where viral bioaerosols usually travel as clumps attached to other particles (Otten and Burge, 1999). On this basis, the droplet count provided by the real-time optical aerosol particle counter upstream of the sampler's inlet could significantly underestimate the total concentration of viruses entering the sampler. To obtain the information representing the quantity of viral material collected by the sampler, a fluorescence dye ($C_{20}H_{10}NA_2O_2$, Fluka AG, Buchs, Switzerland) was

added into the initial suspension in the nebulizer. The intensity of fluorescence was measured in Fluorescence Units (FU) and the relationship between the fluorescence intensity and number of microorganisms per mL of the initial suspension was established. The *Vaccinia* viral suspension at a concentration of 1.59×10^6 PFU mL^{-1} (after dilution) was aerosolised in the chamber. The total intensity of 0.6×10^6 fluorescence units was achieved (the corresponding balance was 2.64 PFU per 1 fluorescence unit). The concentration of *Influenza* virus in the nebulizer suspension was 5×10^7 EID_{50} mL^{-1} (after dilution). A fluorescence dye, which provided a fluorescence intensity of 1.63×10^6 FU, was added to the *Influenza* viral suspension in the nebulizer at 100 μg mL^{-1} (1 FU of initial suspension corresponds to 30.7 EID_{50}).

Once the aerosol sampling was completed, the fluid in the sampler's collection vessel was analyzed to determine its fluorescence intensity and viral concentration.

The recovery rate of the *Vaccinia* virus was calculated as:

$$RR = \frac{\left(\frac{\text{PFU}}{\text{FU}}\right)_{\text{Collection Fluid}}}{\left(\frac{\text{PFU}}{\text{FU}}\right)_{\text{Initial Suspension}}} \quad (1)$$

The recovery rate of *Influenza* virus was determined similarly, except "PFU" was substituted with "EID_{50}".

Microbiological Analytical Procedure

The fluid samples with the collected *Influenza* virus were analyzed by titration on chicken embryo to determine EID_{50} mL^{-1}, following the titration procedure described in detail by Barrett and Inglis (1985). Titration on the chicken embryo is known to be the most precise and reliable procedure to enumerate live *Influenza* viruses (Barrett and Inglis, 1985). Tenfold dilutions of the viral suspension were inoculated into the embryonated eggs' amniotic sac (10 eggs per dilution) with subsequent incubation of the eggs for 72 hours. The results of titration were expressed in EID_{50} per mL of suspension.

The fluid samples with the collected *Vaccinia* virus were analyzed using the standard plaque assay procedures – most common way to determine live viruses representing *Orthopoxviruses* genera. To perform the procedure, the viral suspension was diluted in Erla medium containing antibiotics. Tenfold serial dilutions were made, and 100 μL was added to confluent 4647 cell monolayers in 24-well cluster plates (Costar, Pleasanton, CA, USA). The cell culture produced from the kidney of African green monkey was obtained from the "Flow Laboratories" collection and cultivated in the Russian State Research Center of Virology and Biotechnology "Vector" (Koltsovo, Russia). Virus was allowed to adsorb for 1 h at 37°C in a humidified incubator in a 5% CO_2 atmosphere. Cluster plates were rocked every 10 to 15 min during 1 hour. The fluid was aspirated with an addition of 2 mL of overlay 1% agar (Difco) on the RPMI-1640 medium (containing 2% FCS and antibiotics). The cells were incubated for 48 hours at 37°C in a humidified incubator in a 5% CO_2 atmosphere. After this, the cell monolayers were stained and plaques were enumerated. The results were calculated in PFU per mL of suspension.

As described above, two different analytical techniques were employed for enumeration of live viruses in collecting liquid. They have been chosen as most accurate and reliable for particular species representing different microbial families (Barrett and Inglis, 1985). Such choice can also be justified by the fact that the raw data obtained for *Influenza* and *Vaccinia* viruses are not directly compared against each other and used separately for following calculations of dimensionless recovery rate of each microorganism.

RESULTS

The average pressure drop across the device was about 1550 Pa with the variation not exceeding 120 Pa. The evaporative loss of the collection fluid was minimal and did not exceed 1% for all experimental runs, which was anticipated given the short sampling time.

The particle size distributions of the virion-containing droplets measured in the chamber are shown in Figure 4 for the two test strains. Each data point represents the average of five experimental runs. The distributions are statistically identical: ANOVA test performed for the particle size range of each Grimm channel revealed $p>0.05$. The majority of particles range from 0.5 to 2 μm with the peak at around 1.1 μm. These size distributions suggest that the "carrier-to-virus" particle size ratio is $\sim 10^1$, which is rather common for naturally aerosolized viruses.

The results of sampling are presented in Table 1. For the stress-sensitive *Influenza* virus, the fluorescence intensity of the sample obtained with Sampler 1 was 8 FU. The corresponding viral concentration of 31 $EID_{50}\ mL^{-1}$ resulted in the

recovery rate of approximately13%. The second sampler demonstrated a higher recovery rate of 27%. The average rate of recovery was 20%.

As expected, the robust *Vaccinia* virus revealed much higher recovery with an average rate of 89%. It should be noted that many infection-causing viruses (e.g., *Small Pox*, *Ebola*, etc.) are relatively stress-resistant, especially those strains, which can be used in the event of bioterrorism or as a warfare agent. Therefore, the recovery characteristics obtained with *Vaccinia* seem to be more relevant than the data obtained with stress-sensitive microorganisms. In addition to the sampling stress, the drop in the recovery rate observed in this study can partly be attributed to the microbial stress associated with the aerosolization of viruses and their transport inside the test chamber before entering the sampling inlet. As such generation stress would not be relevant for natural sources of viral bioaerosols (infected humans and animals, plants, etc.) the recovery rate obtained in natural environments could be even higher compared to the laboratory results.

Overall, the new sampling method was found to be feasible for the detection and enumeration of airborne viruses, particularly robust ones.

ACKNOWLEDGEMENT

The project was supported by the grant 1R21 RR 17026-01A1 from the National Center for Research Resources. The authors are thankful for the technical support provided by the laboratory staff of the Russian State Research Center of Virology and Biotechnology “Vector.”

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Table 1. Recovery rate of *Influenza* and *Vaccinia* viruses after the 5-min collection.

Virus		Fluorescence Intensity (FU)	Virus concentration (EID_{50} mL^{-1})	Recovery rate (%)
Influenza	Sampler 1	8	31	13
	Sampler 2	6	50	27
	Average			**20**
Vaccinia	Sampler 1	21	47	85
	Sampler 2	6	15	94
	Average			**89**

Figure Captions.

Figure 1. Personal sampler prototype.

Figure 2. Microscopic photographs of the test viruses: *Influenza virus* (a), and *Vaccinia virus* (b). Both photos are of the same scale [shown on (a)].

Figure 3. Experimental setup.

Figure 4. Particle size distributions of the two aerosolized viral suspensions (*Influenza* and *Vaccinia*). The data represent the average values and the standard deviations of five measurements.

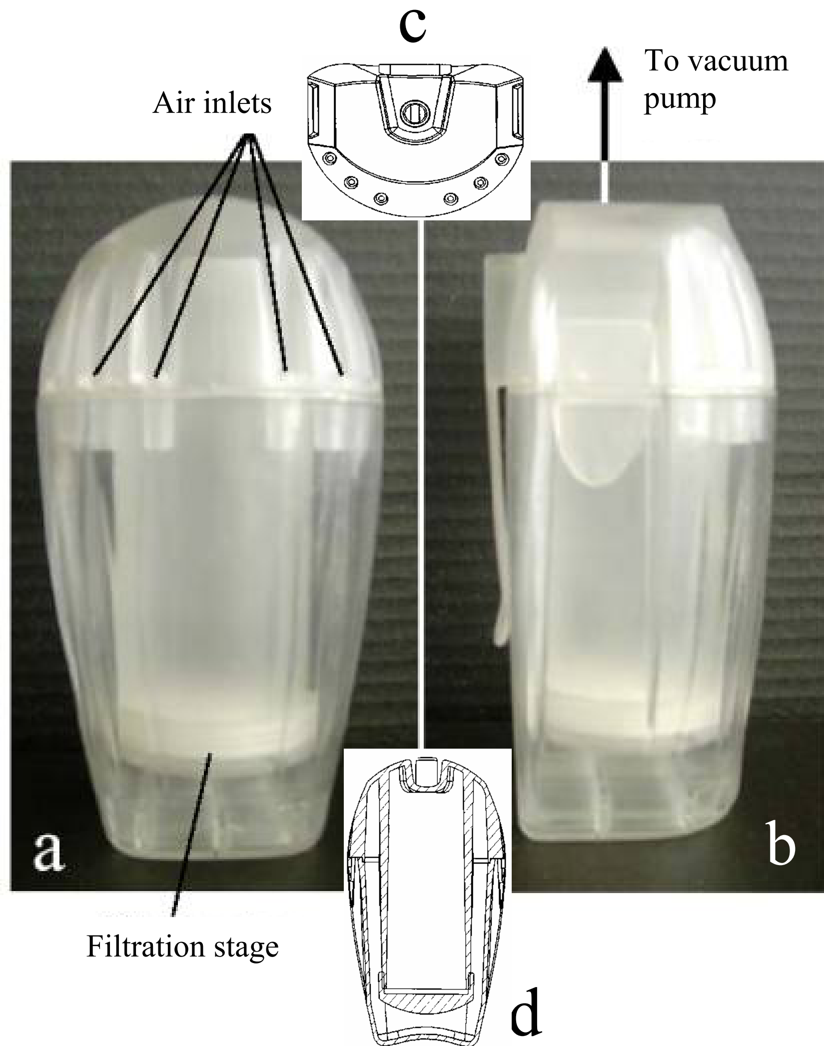

Figure 1.

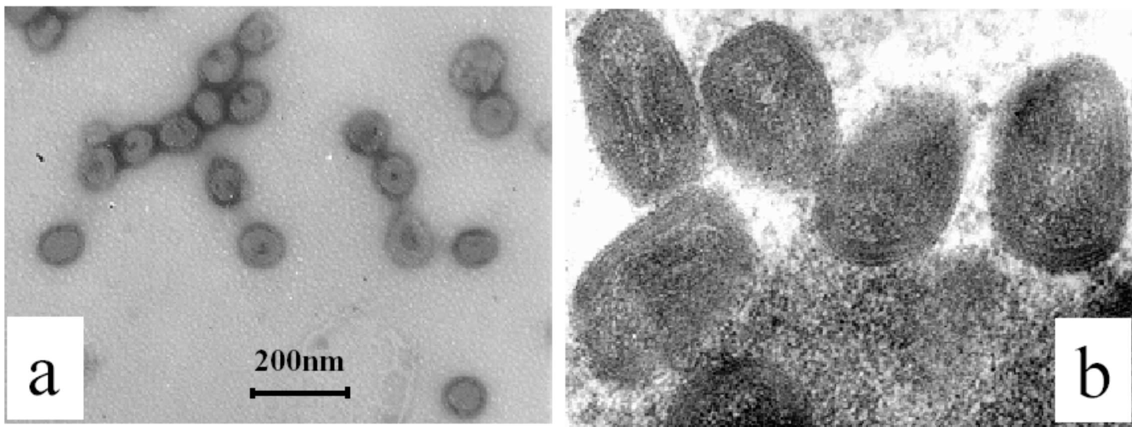

Figure 2.

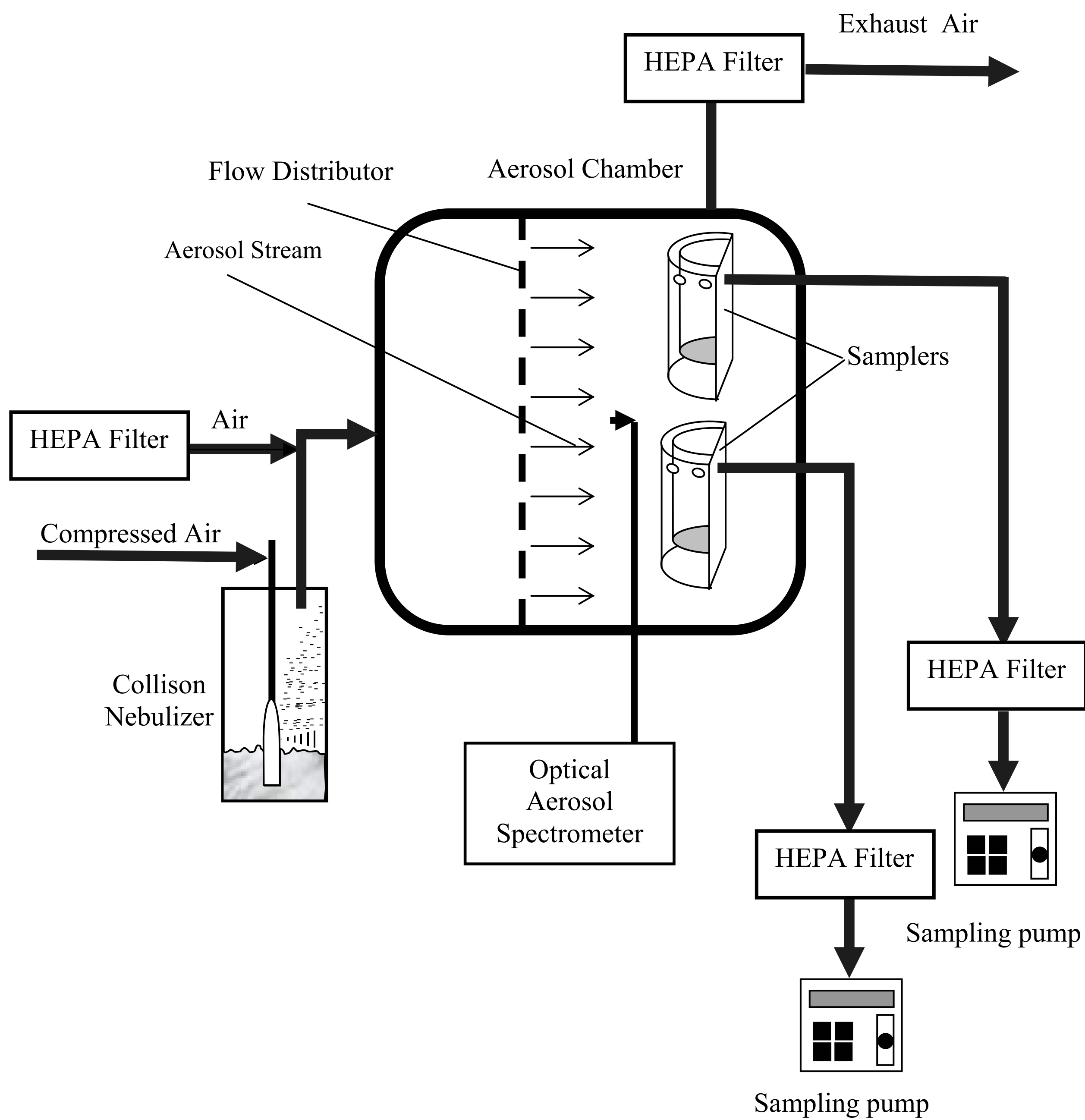

Figure 3.

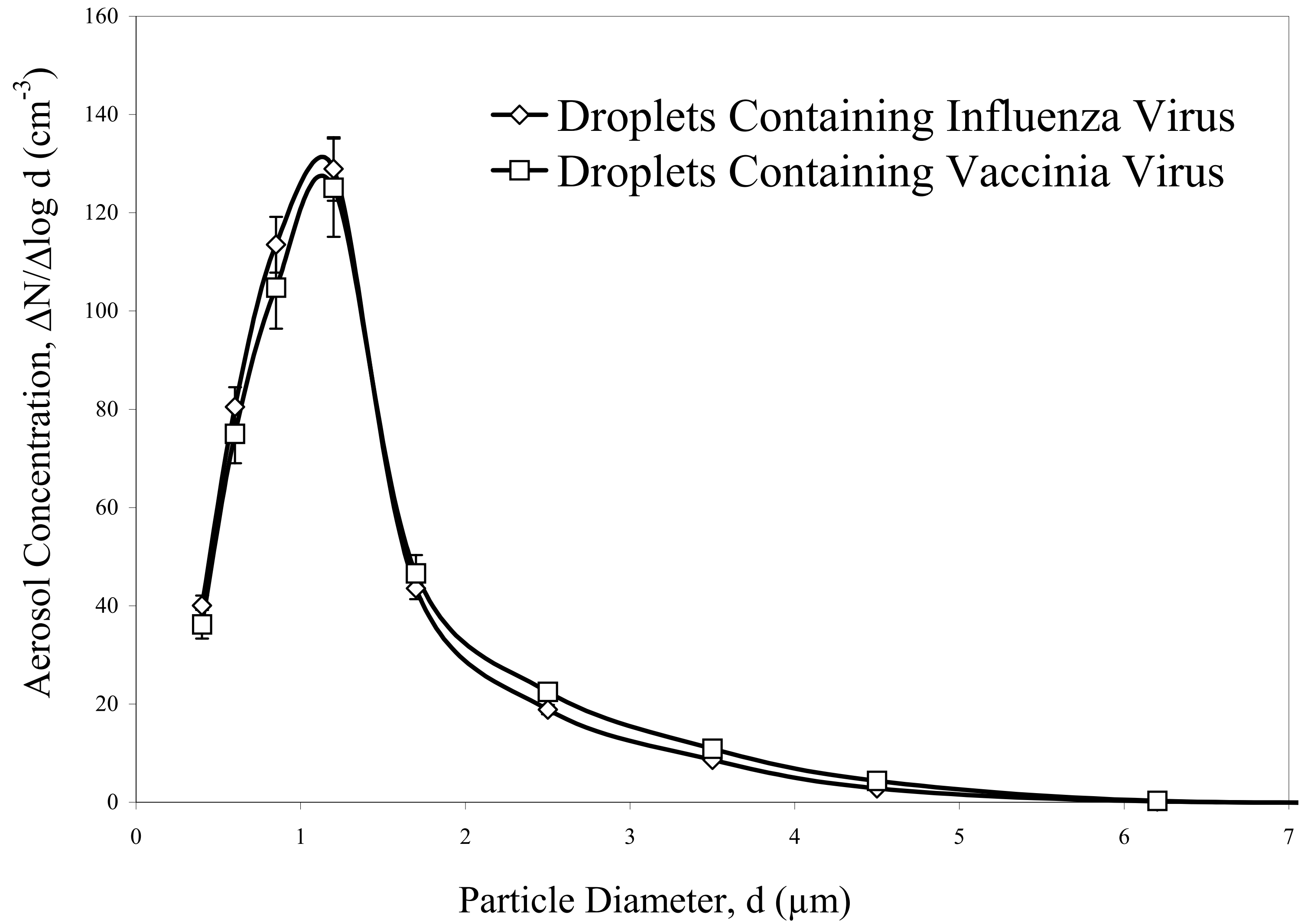

Figure 4.