Long-term sampling of viable airborne viruses

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The work demonstrates a good potentiality of new personal sampler for measuring doses of long-term exposure of personnel to aerosol of robust viruses. The obtained results show that the sampler provides for a stable long-term sampling efficiency and maintains the viability of *Vaccine* virus for at least 6 hours with loss in virus recovery rate less than 10%.

Introduction

A variety of methods are currently used for monitoring of different airborne microorganisms.[1–6] The majority of bioaerosol samplers uses one of three sampling techniques: dry filtration, gravity deposition onto a nutrient (e.g., agar) or into liquid; they are principal instruments, though some other techniques, such as electrostatic precipitation, are also used for bacteria[7–9] or viruses[10] collection. Dry deposition is not recommended for long-term monitoring of viral aerosols because of fast desiccating and subsequent low level of viability; as a rule, it is used for calculation of total number (viable and non-viable) of microorganisms.[11]

Direct collection of airborne microorganisms on agar also has some limitations, connected with surface overloading,[6] impaction- and desiccation-induced stresses,[12,13] masking effect,[14,15] the need in media specific for different microorganisms,[11] etc. Collection of microorganisms into a liquid is the most preferable technique. Actually, liquid samples can be adapted by means of serial dilution or concentration to quantitative analysis of viable microorganisms in optimal conditions. Furthermore, liquid samples can be divided into parts and analyzed with various analytical procedures, including microbiological, virological, immunochemical, and molecular-biological.

Most of designed impingers, used for bioaerosol collection, produce very high sampling rates (up to 300 m/sec), which causes quite violent motion of liquid sorbent and, finally, enhances the liquid evaporation and re-aerosolization of the collected particles. This effect was quantified for the AGI-30 (Ace Glass Inc., Vineland, NJ, USA) filled with water.[16,17] Some problems of conventional impingers have been solved in the design of the Swirling Aerosol Collector,[18] which is presently manufactured as the BioSampler (SKC Inc., Aighty Four, PA, USA). The BioSampler operates at a sampling flow rate of 12.5 l/min and utilizes a viscous non-evaporative liquid sorbent. Its applicability for long-term stable sampling of airborne bacteria and fungi has been demonstrated in Refs. 18 and 19.

In general, presently available liquid impingers have been designed as stationary devices. They operate at relatively high flow rates (10–50 l/min) and require quite high pressure drop (up to 50000 Pa). Although these characteristics make them generally efficient for collecting small particles, these stationary samplers are unsuitable for the personal bioaerosol monitoring, which utilizes battery-operated pumps and requires low air flow resistance. A new approach, based on microorganism collection by air bubbling through a porous medium submerged into a liquid,[20] allows high collection efficiency to be achieved and mechanical damages of microorganisms to be reduced.

This approach has been implemented in a prototype personal sampler[21] called "the bubbler." The device consists of an inlet (45 mm internal diameter) and outlet (semi-cylinder with an internal diameter of 75 mm) faced with a porous medium fixed to the inner cylinder by a nut. The output part is filled with 50 ml of liquid sorbent so that the filter is fully submerged at a distance of 15 mm from the bottom of the outer cylinder. The operation principle is based on contaminated air bubbling through the submerged filter and subsequent splitting into a multitude of very small bubbles. The particles are scavenged by these bubbles, and, thus, effectively removed. The bubbler body is leakage-proof, hence, the liquid sorbent cannot leak during personal sampling (the latter often causes anxiety when working with impingers). A pen-type clamp is mounted on the back wall of the bubbler so that the device can be attached to the user's lapel for personal monitoring. A portable vacuum pump is connected to the bubbler's outlet to produce an air flow of 4 l/min.

The bubbler was shown to maintain the viability of bacterial and fungal microorganisms for a long-time: the recovery rate of stress-sensitive, gram-negative *P. fluorescens* bacteria was (61 ± 20)%, while stress-resistant *B. subtilis* bacteria and *A. versicolor* fungi demonstrated recovery of (95 ± 9) and (97 ± 6)%, respectively.[22] When dealing with the robust *Vaccinia* virus, the bubbler provided a recovery of 89% for 5-minute sampling.[22] Although stress-sensitive *Influenza* virus recovered only at the (19 ± 8)% level,[23] the

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"bubbling" technique showed the promise, at least, for relatively stress-resistant viruses in case of short-period sampling. As was noted, viruses often exhibit much stronger response to stress than bacteria and fungi, making their collection much more difficult. Further testing revealed a detection threshold of 125 PFU/l (PFU is the Plaque Forming Units, i.e., the number of viruses in a sample capable of forming plaque on a monolayer cell culture) of the newly developed "bubbler" when collecting airborne viruses with a volume flow of 4 l/min for the sampling time as short as 1 minute.[24] The detection threshold linearly decreases with the sampling time. However, it has not been determined whether the "bubbling" technique can ensure high virus recovery for working times exceeding 5 min, which is often required for bioaerosol monitoring. The current study was undertaken to evaluate the suitability of the prototype sampler for a long-term collection (up to 6 hours); robust *Vaccinia* virus was used as a test aerosol.

Methods

Test virus

Vaccinia virus was chosen for this study because of its resistance to environmental factors. Microscopic photograph of *Vaccinia* (a virion diameter is of about 400 nm) obtained with a Transmission Electron Microscope JEM-100S (Jeol, Tokyo, Japan) by the technique described in Ref. 25, is presented in Fig. 1.

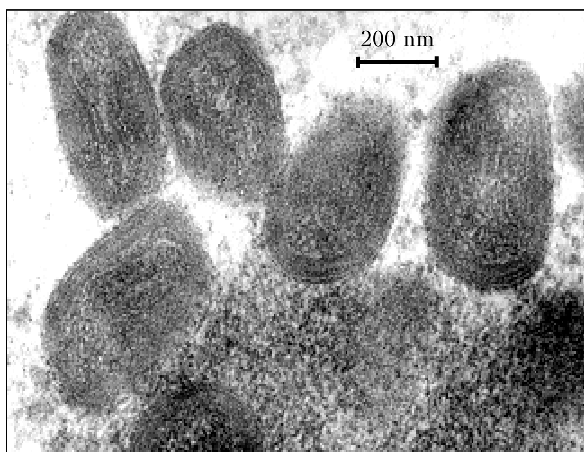

Fig. 1. Microscopic photographs of the *Vaccinia* virus.

The LIVP (C0355 K0602) viral strain, obtained from the Ivanovsky Institute of Virology (Moscow, Russia), was passed 10 times in developing chicken embryos. Virus-containing liquid with a concentration of about 10^7 PFU/ml was obtained by culturing on cells 4647 (cells of *Cercopetecus Aefiopsis 4647* embryo kidney, obtained from the "Flow Laboratories" collection and grown at the SSC "Vektor," Koltsovo, Russia) followed by a triple freezing/defrosting of the infected cell culture on the MEM maintenance media (MOM, Cat #11-100-22, ICN Biomedicals, Inc. Aurora, OH, USA). Before the use in the experiment, this virus-containing suspension was kept at −70°C.

Instruments and procedure of the experiment

A diagram of the experimental setup is shown in Fig. 2. A 3-jet Collison nebulizer (BGI Inc., Waltham, MA, USA), operating with a flow rate of 6 l/min of dry filtered air, was filled with a diluted viral suspension of $4 \cdot 10^4$ PFU/ml. The nebulizer was refilled with a fresh suspension every hour to maintain an initial liquid level and keep the viral concentration approximately the same throughout the procedure. An aerosol flow from the nebulizer mixed with dry filtered air (flow rate of 10 l/min) and then entered a 400-l dynamic test chamber, where a horizontal aerosol flow has a velocity of about 0.1 m/s.[26] A total flow through the chamber of 6 + 10 = 16 l/min allowed us to use the chamber in conditions, at which the flow was uniform over the chamber's cross section and the particle losses due to gravitational sedimentation, that may occur at low flow velocities, were minimized. The air flow uniformity was controlled at the stage of chamber activation and then after each experimental run by both an optical particle counter (aerosol concentration) and hot-wire anemometer at different points inside the chamber. The spread of values between points did not exceed 10%.

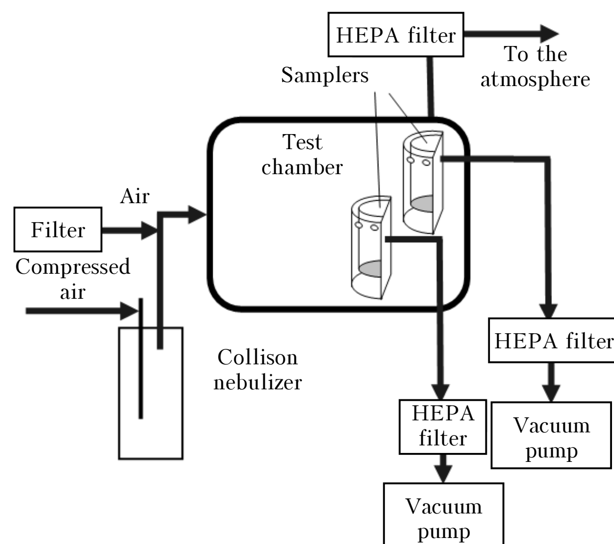

Fig. 2. Experimental setup.

As in the earlier study,[23] a fluorescence dye ($C_{20}H_{10}NA_2O_5$, Fluka AG, Buchs, Switzerland) was added to the suspension in the nebulizer. This enabled us to control the amount of viral material in the initial suspension (before aerosolization), as well as in the liquid sorbent (after sampling) by the sample fluorescence. The relationship was established between the viral concentration in the liquid and the fluorescence signal so that one fluorescence unit (FU) corresponded to one PFU. This was achieved by adding a corresponding amount of the fluorescence dye up to a concentration of $4 \cdot 10^4$ FU/ml. The amount of dye in a sample was later on determined

for the nebulizer suspension as well. The fluorescence intensity was measured by a fluorometer (FL-1, LOMO, St. Petersburg, Russia) with a sensibility of (10 ± 0.2) FU/ml.

Three identical samplers, each filled with 50 ml of Hanks solution, containing 2% of inactivated bovine serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin, were placed into the test chamber and operated in parallel at a sampling flow rate of 4 l/min for 6 hours. The above-described liquid sorbent was chosen to maximize the viral recovery rate in samplers.[21,27]

The amount of fluorescence dye in each sampler was measured after 1, 2, 4, and 6 hours, and 2-ml of the liquid sorbent was taken for biological analysis to determine the viral concentration in it. The standard virus plaque assay procedure[28] was used to determine bioactivity of viruses. Samples of initial suspension were taken from the nebulizer at the same points of time for the corresponding fluorescence measurements and PFU-titer test. To perform the procedure, the viral suspension was 10-fold diluted in Erla medium containing antibiotics and 100 μl of each dilution was added to confluent *4647* cell monolayers in 24-well cluster plates (Costar, Pleasanton, CA, USA). The cells were incubated during 1 hour at 37°C in a humidified incubator atmosphere containing 5% of CO_2. Cluster plates were shaken every 10–15 min, the supernatant was dumped in 1 hour, then the monolayers were overlaid with 2 ml of 1-% agar (Difco) in the RPMI-1640 medium[25] containing 2% of FCS and antibiotics. The plates were incubated during 48 hours at 37°C in a humidified incubator (5% of CO_2). Then the monolayers were stained and plaques were enumerated.

The relative recovery rate (RRR) of viruses was calculated as the ratio of the PFU number per one fluorescence unit, determined after sampling from the liquid sorbent, to the same variable for the initial soluble suspension:

$$\text{RRR} = \frac{(\text{PFU}/\text{FU})_{\text{sorb}}}{(\text{PFU}/\text{FU})_{\text{init}}}.$$

Since the initial suspension in our experiments was prepared with PFU/FU = 1 and its characteristics were constant at least within one-hour period of aerosolization (after which the nebulizer was refilled with a new suspension), RRR of viruses can be determined at any sampling time by direct substitution of the corresponding values in the above equation. The average RRR and its standard deviations were determined by the data of three identical samplers in three replicates.

The concentration and size distribution of particles in the air were controlled by an optical aerosol spectrometer (Model 1.108, Grimm Aerosol Technik, Ainring, Germany). The experiments were performed at a relative humidity of 50–55% and air temperature of 22–24°C. HEPA filters were installed between samplers and vacuum pumps to protect the laboratory environment from the release of viruses.

Results and discussion

The size distribution of the virus-containing particles measured by the optical aerosol spectrometer in the test chamber is shown in Fig. 3.

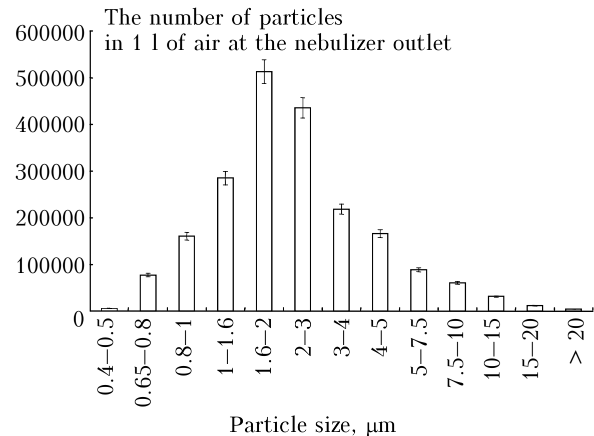

Fig. 3. Size distribution of virus-containing particles. The data represent the average values and standard deviations for five measurements.

The optical diameter of particles evidently ranges within 0.5–2 μm with a modal value of 2.2 μm; the virus was detected in one of ten aerosol particles. Since the relative humidity in the chamber was kept within a range 50–55%, the size distribution continually varied in particle traveling from the aerosolization point to a sampler. Therefore, it is important to specify that the particle size distribution was measured inside the sampler inlet, right above the liquid sorbent level.

The *Vaccinia* virus dilution in the soluble suspension, determined for one hour, did not exceed 7% on average (Fig. 4), hence, the virus aerosolization conditions did not change with time.

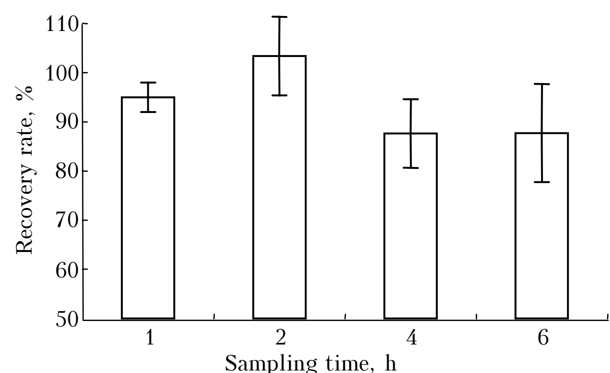

Fig. 4. Recovery rate of *Vaccinia* virus in a sampler. The data represent the average values and standard deviations for three samplers in each measurement.

Moreover, the real-time measurements of aerosol concentration in the chamber (with the optical aerosol spectrometer) showed its very high uniformity. Thus, the bioaerosol in sampling areas had similar characteristics throughout the experiment, and, therefore, any changes

in the viral concentration in the liquid sorbent are caused solely by the sampler efficiency.

Figure 5 shows the fluorescence intensity variation and viral concentration in the liquid sorbent depending on sampling time.

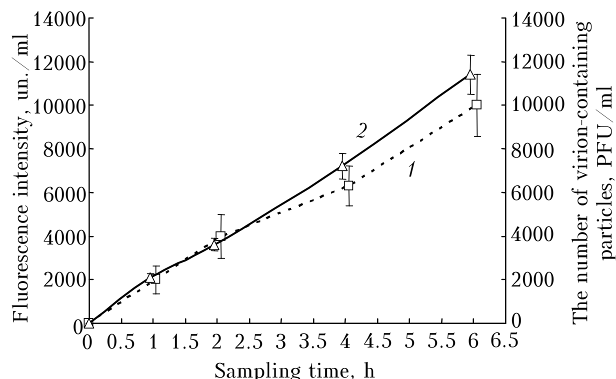

Fig. 5. Viral concentration in liquid sorbent (*1*) and fluorescence intensity (*2*) as functions of sampling time. The data represent the average values and standard deviations for three measurements with three similar samplers in each.

Both functions show close-to-linear trend. An approximately constant level of virus accumulation in the liquid sorbent over 6 hours demonstrates sustainability of sampler efficiency, which is an evidence of a good potential of the "bubbling" technique for a long-term sampling of airborne viruses. As the efficiency of aerosol particles precipitation depends on their sizes, remind that particles in our experiments were sufficiently large. The size range of virus-containing droplets generated in our tests was close to those of natural airborne viruses.

For an additional validation of the experimental technique, a material balance was calculated using the following data: a dispergating flow rate of 0.2 ml of suspension per minute; viral concentration in the nebulizer of $4 \cdot 10^4$ PFU/ml, and total air flow in the chamber of 16 l/min. Taking into account that the flow rate through each of the three samplers was 4 l/min, the maximal amount of liquid, which could be collected by each sampler per hour, is 3 ml and the corresponding amount of viral material is $12 \cdot 10^4$ PFU. This number of PFUs diluted in the 50-ml volume of the liquid in a sampler set up a concentration of $2.4 \cdot 10^3$ PFU/ml after an hour of continuous sampling. The experimentally measured viral concentration, obtained from the fluorescence-based analysis of the liquid sorbent, was $1.95 \cdot 10^3$ PFU/ml (see Fig. 5), that was only 19% lower than the theoretical maximum. This relatively small difference can be attributed to the particle losses in the system.

The data presented in Fig. 4 show that the recovery rate of *Vaccinia* virus in a sampler was as high as about (90 ± 9)% during a six-hour operation. The inter-sampler spread did not exceed 20% for all tests. In our earlier study,[23] we obtained a similar recovery rate of *Vaccinia* virus for shorter (5-min) sampling period with the bubbler. The obtained data allow essential sampling period extension. Some recovery rate decrease (represented by the regression curve at the sampling points corresponding to 4 and 6 hours) is not statistically significant ($p > 0.05$), as was shown by the one-way ANOVA test. A 10% loss in the viral recovery rate over 6 hours is very low. The level of *Vaccinia* survival in the bubbler obtained in this study is comparable to those found for stress-resistant bacteria and fungi strains, which were collected by the same samplers during a long period.[20]

It is important to note that the data presented in this work were obtained with a robust virus strains and may be inapplicable to more sensitive viruses. Nevertheless, even in case of virus inactivation under the effect of unfavorable environment or in the result of sampling and virus finding in a sampler, the presence of DNA or RNA of one or another virus (which decay much slower than virus recovery rate decreases) can be detected by chemical or molecular-biological methods. Hence, the primary problem of detecting virus aerosols in air can be solved without estimating the virus bioactivity.

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