Testing of a new personal sampler for detection of viable viruses in aerosol state

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Received February 20, 2004

We present the results of tests of a new personal virus sampler. Viruses with different stability in the environment: influenza, measles, mumps, vaccinia, and SARS, were used for testing. It was shown that during sampling 80% of biological activity of influenza virus (labile virus) and only about 10% of activity of vaccinia virus (robust virus) was lost. Viruses in the sampler lost up to 1.5 logarithms of biological activity during sampling for 4 hours at room temperature. The potential of using the new personal sampler for detection of viable viruses in aerosol state was shown.

Introduction

Various samplers are needed to detect biological aerosol in the environment: from stationary ones with high air flow rates to easily movable personal compact devices capable of operating at low flow rates for a long time. Such personal samplers for virus-containing aerosols have not yet been described in the literature.

The aim of this work was to test the personal sampler developed at the Griffith University (Australia)[1] with viruses characterized by different stability in the environment. To obtain the information about the presence of infectious viral aerosol in the air, the sampler should ensure, first, minimum inactivation of a virus during sampling (during the transfer from the air medium into the liquid one) and, second, conservation of the infectious activity of the virus for a long time of sampler operation until extraction of the sample. In addition, it is necessary for the aerosol particles entrapped by the sampler to be kept there with high efficiency. The high efficiency during sampling and the high level of viability of various bacteria have been demonstrated earlier.[2,3] The data obtained in this work allow evaluating the potential of using this sampler for sampling virus-containing aerosols.

Materials and methods

Personal sampler

The principal layout and the appearance of the personal sampler are shown in Fig. 1. The sampler consists of two semi-tube vessels of thermostable polyurethane and has the height of 140 mm, diameter of 75 mm, and the wall thickness of 2 mm. A porous medium consisting of polypropylene fibers 12 μm in diameter with the pack density of 16% and thickness of 6 mm is placed on the lower open part of the inner vessel 45 mm in diameter (15 mm far from the sampler bottom) and glued to exclude the aerosol leaking. An extra filter (mist eliminator) catching large drops produced in the bubbling process is set near the exit. The sampler has a catch on its rear wall for its fixation.

To ensure the independence of the wind direction, air intakes are located along the sampler perimeter. The air flow from the intakes moves between the coaxial tubes downward to a sorbent liquid, turns through 180°, and passes through the porous medium submerged in the liquid. Aerosol particles are sorbed by the liquid, and the air flow leaves the sampler through the 12-mm hole in the top. The pressure drop across the sampler output is produced by a battery-supplied portable pump (224-PCXR8, SKC, Eighty Four, PA, USA) providing for the stable air flow rate of 4 l/min. The sampler geometry is chosen so to minimize the losses of aerosol particles on the sampler walls.[3]

Prior to sampling virus-containing aerosols, the sampler was filled with 50 ml of the Hanks solution containing 100 U/ml penicillin + 100 μg/ml streptomycin or the same Hanks solution with antibiotics and 2% v/v bovine serum inactivated at 56°C for 30 min. To prevent foaming during bubbling, 0.03% M-30 Dow-Corning antifoam (30% aqueous solution of dimethylpolysiloxane) was added to the bovine serum solution.

Viruses. The experiments were conducted with the following viruses: influenza, measles, mumps, vaccinia, and SARS. In the nature, these viruses are mostly spread as aerosols.

Measles and mumps viruses. The *Edmonston* culture of the measles virus was received from the ATCC Virus Collection (VR-24, 4/92). *Enders* culture of the mumps virus was also received from the ATCC Virus Collection (VR-106, 14D, 02/92). *Vero* cell culture for determination of the biological activity of these viruses was received from the collection of the Scientific Center of Virology and Biotechnology "Vektor." The biological activity of these viruses was determined by the plaque assay.[4,5]

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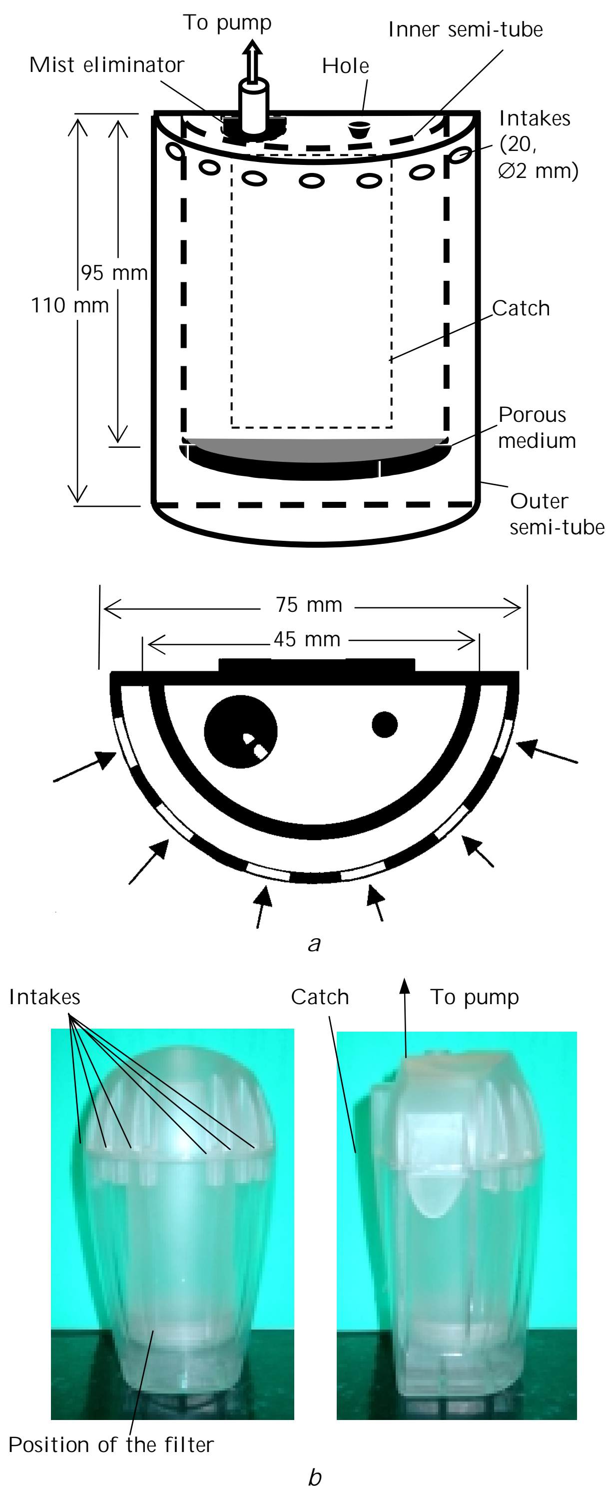

Fig. 1. Schematic layout (*a*) and photos (*b*) of personal sampler.

For this purpose, 100 μl of tenfold dilutions of the virus in the Earle's medium containing antibiotics was injected onto monolayers of the *Vero* cells in 24-well plates (Costar, Pleasanton, CÀ), and the virus was adsorbed on them for 1 h at 37°C in the ÑÎ$_2$ incubator (in the moistened atmosphere containing 5% ÑÎ$_2$). The plates were shaken every 10–15 min, and in 1 h the monolayer was rinsed from the virus and flooded with 2 ml liquid agar (1% Difco agar on RPMI-1640, containing 2% FCS + antibiotics). After 6 days of stay in the ÑÎ$_2$ incubator, the monolayers were stained by 0.001% neutral red and the plaque assay was carried out. The virus titer in plaque-forming units per milliliter (PFU/ml) was calculated by standard methods.[6] The accuracy of determination of the biological activity of the viruses by this method was about 0.3–0.4 logarithm of the determined value.

SARS virus. The SARS virus, *Frankfurt 1* culture, was received from the Institute for Medical Virology, Frankfurt University (Germany). The biological activity of this virus was determined from its cytopathic effect on the monolayers of *Vero* cell culture. For this purpose, the cells in 96-well plates (Costar, Pleasanton, CÀ) were inoculated by 10-fold dilutions of the virus, and after 1-h adsorption at 37°C the cells were rinsed from the virus and flooded by the RPMI-1640 maintaining medium with 1% fetal bovine serum. For 48 h the monolayers were partly destructed by the virus, which allowed determination of the 50% cell culture infective dose ($CCID_{50}$) by the method described on Ref. 7. The accuracy of determination of the biological activity of the SARS virus by the method described above was about 0.5 logarithm of the determined value.

Influenza virus. The *A/Aichi/2/68* (H3N2) culture of the influenza virus was received from the Ivanovskii Institute of Virology (Moscow, Russia) and has passed 12 mouse passages and 2 egg passages. Cultivation of the virus in the allantoic fluid of 9–11-day-old chicken embryos yielded the concentration of 10^8–10^9 50% embryo infective dose (EID_{50}) in 1 ml of virus-allantoic fluid (VAF), which was used in the further experiments. The biological activity of the virus in the samples was determined using 9–11-day-old chicken embryos in EID_{50} units by the method described in Ref. 4. The accuracy of determination of the viral biological activity by this method was about 0.3–0.4 logarithms of the determined value.

Vaccinia virus. The LIVP s0355 k0602 culture of the vaccinia virus was received from the Institute of Viral Medications RAMS (Moscow, Russia) in 1986; this virus has passed 10 egg passages. The virus-containing material in the concentration of 10^7 (PFU/ml) was obtained by the roll-bottle cultivation of the vaccine virus on the 4647 passages cell culture (green-monkey embryo kidney cells) with the following threefold freeze-thaw of the infected cell culture in the maintaining Eagle's medium (Ì EÌ ; Cat. # 11-100-22, ICN Biomedicals, Inc., Aurora, OH, USA). Before the beginning of the works, the virus-containing suspension was stored at −70°C. The biological activity of the virus was also determined by the plaque assay. To do this, 4647 cell monolayers in glass bottles were inoculated by 0.2 ml of tenfold dilutions of the virus-containing suspension.

After 1-h incubation at 37°C, the monolayer was flooded by the maintaining medium containing 2.4% Difco agar and 0.001% neutral red (553-24-2, Cat. #102438, ICN, USA). The cells were incubated in a dark room for 48 h at 37°C, and then plaque assay was carried out. The accuracy of determination of the viral biological activity by this method was about 0.1 logarithm of the determined value.

Bubbling experiments

To determine the viability of a virus sampled in the personal sampler for a long sampling process, the known amount of the virus-containing suspension was added to the sorbent liquid, and then the sampler was turned on in the mode of pumping of the filtered air with the volume flow rate of 4 l/min. With the interval of 1, 2, and 4 h, 0.1 ml liquid was taken from the sampler, and its biological activity was determined by the methods described above.

Aerosol experiment

The experimental setup used in aerosol experiments with the new personal sampler is schematically shown in Fig. 2.

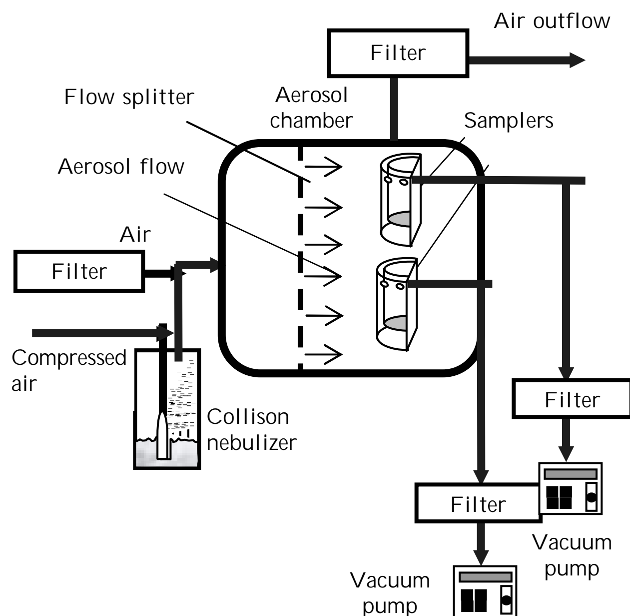

Fig. 2. Block-diagram of the aerosol experiment.

The aerosol was produced with a 3-jet Collison nebulizer (BGI Inc., Waltham, MA, USA) from the virus-containing suspension by the flow of the dry filtered air with the volume flow rate of 6 l/min. To estimate the degree of virus inactivation in the process of sampling, a fluorescent tracer (uranin $C_{20}H_{10}Na_2O_2$ solution, Fluka AG, Switzerland) of the concentration about 10^{-4} g/ml was added to the dispersant. Then the aerosol was mixed with the dry filtered air blown at the volume flow rate of 10 l/min to produce the laminar flow in the flow-through aerosol chamber. In the experiments, the flow-through horizontal aerosol chamber with the mean flow rate of 10 cm/s was used.[8] The temperature in the chamber during the experiments was 24–27°C at the relative humidity about 50%. The mean size of aerosol particles in the chamber was about 1 μm.

Two personal samplers were placed in the chamber and separated from the vacuum pump, ensuring the volume flow rate of 4 l/min through the samplers, by two filters connected in series to prevent the outflow of the infectious aerosol into the environment. Sampling was carried out with the continuously operating aerosol generator for 5 min. To reduce the level of virus inactivation in air pipes, the length of pipes used was as short as possible. The obtained samples of the virus-containing aerosol were analyzed for the biological activity by the methods described above and for the content of the fluorescent tracer using a Perkin-Elmer-1000 fluorimeter. The fluorescence intensity of uranin was measured at λ_{fl} = 520 nm (excitation at λ_{exc} = 472 nm).

Results and discussion

The conservation of the infectious activity of the virus in the personal sampler during the long sampling process was checked in two stages. At the first stage, the sampler was filled with the Hanks solution with antibiotics, but in some experiments, the virus inactivation in the process of long bubbling exceeded two orders of magnitude. Therefore, at the second stage, the solution containing antifoam and serum, described in Section "Materials and methods" was used as a sorbent liquid for labile viruses. The results obtained in all the experiments are summarized in Table 1. All the measurements were conducted at the temperature of (25 ± 2)°C and relative humidity about 50%.

It was found that bubbling for 4 h after sampling inactivates the vaccinia virus to the lowest degree (roughly, the titer decreases twice), while the other viruses lose 2 to 2.5 logarithms of activity for that time. If bovine serum and antifoam are added to the sorbent liquid, this loss can be reduced to about one order of magnitude.

The degree of virus inactivation in the process of sampling was determined for two viruses with significantly different stability, namely, influenza and vaccinia. For influenza, the biological activity of the initial suspension in the aerosol generator was $10^{7.7}$ EID_{50}/ml, and the fluorescent tracer in the suspension had the concentration of $1.63 \cdot 10^6$ ml^{-1}.

Thus, the initial suspension contained about 30.7 EID_{50} per 1 fluorescent unity. For vaccinia, the biological activity of the initial suspension in the aerosol generator was $10^{6.2}$ PFU_{50}/ml, and the concentration of the fluorescent tracer in the suspension was $0.6 \cdot 10^6$ ml^{-1}. Thus, there was about 2.64 PFU per 1 fluorescent unit in the initial suspension. The experimental results on measurement of the vital viability in the sampling process are presented in Tables 2 and 3.

Table 1. Changes in the biological activity of viruses in the personal sampler at different times of bubbling of the filtered air through the sampler

Virus	Units of biological activity	Biological activity of virus in the sampler depending on the bubbling time, in hours				
		0	1	2	3	4
Measles*	Log_{10}PFU/ml	6.4 ± 0.4	5.1 ± 0.5	4.7 ± 0.4	4.4 ± 0.5	4.2 ± 0.6
Mump*	Log_{10}PFU/ml	5.7 ± 0.4	4.5 ± 0.5	4.1 ± 0.6	3.8 ± 0.4	3.4 ± 0.4
Measles	Log_{10}PFU/ml	6.3 ± 0.5	5.8 ± 0.4	5.6 ± 0.4	N/A	4.9 ± 0.5
Mump	Log_{10}PFU/ml	5.6 ± 0.3	5.2 ± 0.5	5.0 ± 0.5	N/A	4.6 ± 0.4
Vaccinia*	Log_{10}PFU/ml	4.2 + 0.1	4.1 + 0.1	3.9 + 0.1	N/A	3.3 + 0.1
Influenza*	$Log_{10}EID_{50}$/ml	5.9 + 0.4	4.5 + 0.4	4.0 + 0.4	N/A	3.7 + 0.4
Influenza	$Log_{10}EID_{50}$/ml	6.1 + 0.4	5.9 + 0.4	5.7 + 0.4	N/A	5.5 + 0.4
SARS*	$Log_{10}CCID_{50}$/ml	4.3 + 0.7	N/A**	3.1 + 0.3	N/A	1.8 + 0.4
SARS	$Log_{10}CCID_{50}$/ml	4.2 + 0.7	N/A	3.4 + 0.5	N/A	2.4 + 0.6

* The Hanks solution with antibiotics was used as a sorbent liquid; in other experiments, serum and antifoam were added to the solution.

** Hereinafter N/A means that no measurements were conducted.

Table 2. Data on changes in the infectious activity of the influenza virus during sampling in the personal sampler

	Fluorescence intensity in the sample, ml^{-1}	Virus concentration in the sample, EID_{50}/ml	% viable virus in the sample
Sampler 1	8	31	12.7
Sampler 2	6	50	27.1
Mean	7	40.5	19.9

Table 3. Data on changes in the infectious activity of the vaccinia virus during sampling in the personal sampler

	Fluorescence intensity in the sample, ml^{-1}	Virus concentration in the sample, PFU/ml	% viable virus in the sample
Sampler 1	21	47	84.8
Sampler 2	6	15	94.0
Mean	13.5	31	89.4

From Tables 2 and 3 it can be seen that the influenza virus in the process of sampling keeps about 20% activity, while the vaccinia virus keeps up to 90%. The comparison of the results obtained with the reported data of investigations with this personal sampler shows that, in general, bacterial aerosols, especially, spore and fungi spore aerosols, are more resistant to sampling than viral aerosols.[2,3] Thus, for example, 8-hour sampling of viral and fungi spore aerosols has shown that even for the least stable gram-negative *Pseudomonas fluorescens* bacteria the viability in the sampler is (61 ± 20)%, while for the spores *Bacillus subtilis var. niger* and *Aspergillus versicolor* it is as high as (95 ± 9) and (97 ± 6)%, respectively. At the same time, it should be noted once again that this sampler is now the only one personal sampler, whose working characteristics are determined for the virus-containing aerosols. It has been shown that even simple replacement of the distilled water in the sampler with the sorbent liquid significantly improves the virus viability in the sampler. It is quite possible that application of other media and enhancement of technological solutions (for example, pre-moistening of the sampled aerosol) will also increase the virus viability in the process of sampling.

Thus, it should be concluded that this work has shown the feasibility of using the new personal sampler for detection of viable viruses in aerosol.

Acknowledgments

We would like to express our gratitude to Dr. H.W. Doerr and Dr. H.F. Rabenau from the Institute for Medical Virology, Frankfurt University, Germany for the SARS virus kindly placed at our disposal, as well as to G.A. Buryak and V.M. Generalov for the fruitful discussion of this work.

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