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Monitoring of viable airborne SARS virus in ambient air

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Abstract

Due to recent SARS related issues (Science 300 (5624) 1394; Nature 423 (2003) 240; Science 300 (5627) 1966), the development of reliable airborne virus monitoring procedures has become galvanized by an exceptional sense of urgency and is presently in a high demand (In: Cox, C.S., Wathers, C.M. (Eds.), Bioaerosols Handbook, Lewis Publishers, Boca Raton, FL, 1995, pp. 247–267). Based on engineering control method (Aerosol Science and Technology 31 (1999) 249; 35 (2001) 852), which was previously applied to the removal of particles from gas carriers, a new personal bioaerosol sampler has been developed. Contaminated air is bubbled through porous medium submerged into liquid and subsequently split into multitude of very small bubbles. The particulates are scavenged by these bubbles, and, thus, effectively removed. The current study explores its feasibility for monitoring of viable airborne SARS virus. It was found that the natural decay of such virus in the collection fluid was around 0.75 and 1.76 lg during 2 and 4 h of continuous operation, respectively. Theoretical microbial recovery rates of higher than 55 and 19% were calculated for 1 and 2 h of operation, respectively. Thus, the new sampling method of direct non-violent collection of viable airborne SARS virus into the appropriate liquid environment was found suitable for monitoring of such stress sensitive virus.

Keywords: SARS; Bioaerosol; Personal monitoring; Viable microorganisms; Collection efficiency

1. Introduction

Due to recent terrorist attacks in the USA and worldwide, the research of bio-terrorism related issues has become galvanized by an exceptional sense of urgency. Some more recent SARS related issues reinforced this concept even further (Lipsitch et al., 2003; Fouchier et al., 2003). The growing concern for human exposure to bioaerosols has created demand for advanced, more reliable and more efficient monitoring methods for detecting, identifying and enumerating airborne biological particles to control exposure, to evaluate controls, or to identify potentially hazardous conditions (Lacey and Dutkiewicz, 1994; Comtois and Isard, 1999). The requirements for an ideal bioaerosol sampler have been described by Macher (1997). Among others, maintaining high biological efficiency is considered to be one of the main requirements for an efficient performance of the bioaerosol samplers. Individual

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exposure to bioaerosols can best be evaluated by the use of personal aerosol monitors, as these samplers track the effects of human time-activity patterns. In regards to the personal sampling, the ideal bioaerosol monitor would be compact, would allow specific identification of particles and would maintain high physical and biological efficiencies over prolonged periods (Macher, 1999; Crook, 1995).

Based on the engineering control method, which was previously applied to the removal of non-biological particles from gas carriers (Agranovski et al., 1999, 2001), a new personal bioaerosol sampler was developed (see Fig. 1). A detail sketch of the device is also provided in our previous work (Agranovski et al., 2002b). The sampler consists of inner (45 mm internal diameter) and outer cases with a porous filter attached to the bottom of the inner case. Fifty milliliters of collecting liquid are placed in the outer case of the sampler making filter fully submerged after assembling of the device at the distance of 15 mm from the bottom of outer case. A pen-type clamp for attaching the device to the user's lapel is located at the back wall of the sampler. Portable vacuum pump is connected to the sampler to provide the required operational flow rate of $4\,l\,min^{-1}$. The shape of the air inlet ensures negligible losses of collected bioaerosols along the walls of the sampler before reaching the level of collecting fluid (Agranovski et al., 2002b). Also, the design of the sampler guarantees no leakages and spills of collecting fluid during sampling procedure even for dynamic human activities. The operational principle is based on bubbling of contaminated air through the filter submerged into a liquid layer which subsequently split into a multitude of very small bubbles. The particulates are scavenged by these bubbles, and, thus, effectively removed. Previous investigation of the performance of such process showed that the device is capable of providing efficiency higher than 95% for the range of particle sizes from 0.01 to 3.0 μm (Agranovski et al., 2001). The other very important feature of the sampler is the possibility to achieve such high efficiency at a low gas velocity of up to $0.5\,m\,s^{-1}$ which would minimize a physical stress of microbes collected by the device. The performance characteristics of the new sampler were evaluated for an 8-h continuous sampling of airborne *Pseudomonas fluorescens* and *Bacillus subtilis* var. *niger* bacterium and *Aspergillus versicolor* fungal spores (Agranovski et al., 2002a,b). It was found that the viability of sampled microorganisms remained high even after the long-term sampling: the recovery rate of stress-sensitive gram-negative *P. fluorescens* bacteria was $61\pm20\%$; for stress-resistant *B. subtilis* bacteria and *A. versicolor* fungal spores it was $95\pm9\%$ and $97\pm6\%$, respectively.

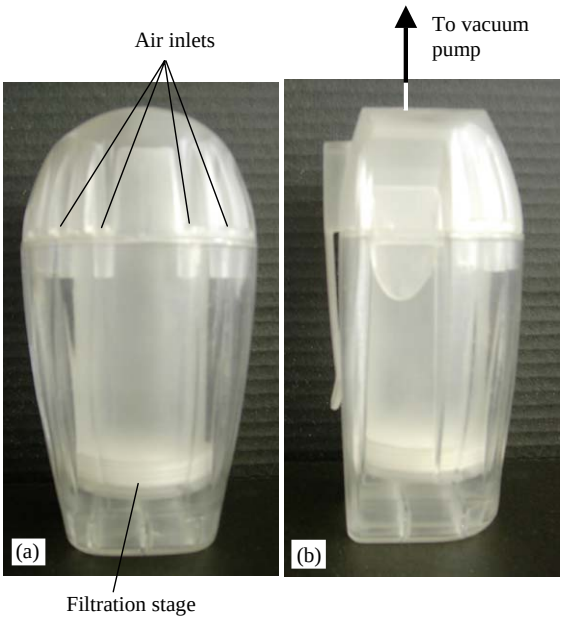

Fig. 1. New personal sampler for monitoring of viable airborne viruses.

A global concern related to SARS virus raises the necessity for development of reliable procedures of monitoring this highly pathogenic microorganism (Rota et al., 2003; Lipsitch et al., 2003). It has been found that SARS is the coronavirus (Marra et al., 2003; Guy et al., 2000), which, as most of coronaviruses, is very sensitive to physical and biological stresses. Such high sensitivity would eliminate the possibility of using most of currently available bioaerosol monitoring techniques due to their violent regimes of operation or strong desiccation of collected materials (Wang et al., 2001). As such negative effects are not relevant for the new device (the sampler is capable to operate at a low velocity which provides gentle collection of material directly into the liquid) it was decided to trial the new technique for monitoring of viable airborne SARS virus. Considering a very high physical collection efficiency of the device (higher than 96% for the SARS virus size particles) (Agranovski et al., 2001), the main investigation was focused on the issue related to the possibility of maintaining the lowest possible rate on natural decay of collected virus in the liquid for the entire collection period of at least two hours continuous operation.

2. Methods

The experiments were undertaken in the PC4 facility with HEPA filters installed in the pipeline connecting sampler and vacuum pump to prevent the equipment contamination. For the experiments, each device was filled by 50 ml of $\sim10^4$ $TCID_{50}\,ml^{-1}$ (Tissue Culture Infectious Dose) concentrated suspension of SARS

virus. The strain Frankfurt 1 of SARS virus was kindly provided by Dr. Doerr and Dr Rabenau of the Institute for Medical Virology, University Hospital Frankfurt (Frankfurt, Germany). The samplers charged with viral suspensions operated continuously aspirating clean air at 4 l min^{-1} (its standard sampling flow rate) during 4 h. The temperature of clean air was 24°C and relative humidity of around 55%. One millilitre of suspension was collected from the sampler after 0, 2 and 4 h of operation. Tenfold serial dilution were analysed by titration on Vero cells. Vero cell culture produced from the kidney of African green monkey was obtained from "Flow Laboratories" collection and was cultivated in SRC VB "Vector". The serial dilutions of absorbing fluid were inoculated onto confluent monolayers of Vero cells in wells of 96-well plates and incubated for 60 min at 37°C. Inoculated cell cultures were maintained with RPMI 1640 supplemented with 1% fetal bovine serum (Gibco BRL, Grand Island, NY). Inoculated cell cultures were examined daily for cytopathic effects of SARS virus on Vero cells. For all experiments, the first observed destruction of monolayer (distinctive rounding of Vero cells) appeared after 30 h of incubation. After 48 h up to 50% of monolayer were destroyed and after 60 h all cells were rounded and more than 80% of monolayer were destroyed (see the full procedure of titration of coronavirus in Guy et al., 2000). RT-PCR was used for control of reproduction of virus in the Vero cells. Amplification (Drosten et al., 2003) was performed with BNI in S and BNI in As primers (courtesy of Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany). The results were calculated in $TCID_{50}$ ml^{-1} and the rate of natural decay was obtained as the ratio of concentration ($TCID_{50}$ ml^{-1}) at certain time by the initial concentration of SARS in the liquid at the beginning of experiment.

Three experimental runs were undertaken with three different devices to identify the reproducibility of the results and inter sampler variation. To investigate the behaviour of the rate of natural decay in different environments, two liquids were used for preparation of viral suspensions; sterile water and microbial maintenance fluid. The maintenance fluid prepared from Hank's solution containing 2% volumetric of inactivated bovine serum, 100 U ml^{-1} of penicillin and 100 μg ml^{-1} of streptomycin was used to create conditions for lowest possible rate on natural decay. To avoid an extensive foam formation which could unfavourably interfere with the physical bubbling process, the antifoaming agent Antifoam A (Sigma Chemical Company, St. Louis) was added to the collection media. Some following observations confirmed that the virus maintenance fluid with addition of antifoaming agent did not contribute towards alternations of physical bubbling process with no excessive foam generated. Also, as was confirmed by measurements according to the previously developed procedure (Agranovski et al., 1999), physical efficiency of the sampler charged with virus maintenance fluid was the same as for the one operated with sterile water (>95%) with no increase in hydrodynamic resistance of the device detected.

3. Results and discussions

Three experimental runs for each sterile water and virus maintenance fluid were undertaken and the results of the SARS virus decay are shown in Table 1. As is seen, the natural decay of the microorganisms during first 2 h of operation was around 0.75 and 1.25 lg in average for virus maintenance fluid and water, respectively. Even after 4 h of run, the viral decay in maintenance fluid was in average of 1.76 lg. On the other hand, a much higher decay rate of the SARS virus (2.58 lg) was observed for the bubbling through viral suspension in sterile water. Taking into account a very stress sensitive nature of the SARS virus these results indicate that the device filled with virus maintenance fluid is capable of providing a relatively low level of microbial decay and can be evaluated for monitoring of such microorganisms in the air environment.

The inter sampler variation is also a very important part of the experimental procedure (Agranovski et al., 2002b). The discrepancy between all three experimental runs employing virus maintenance fluid was minimal and did not exceed 0.25 lg. This is very important

Table 1
SARS virus decay due to the bubbling process as measured during the 4-h continuous operation

Number of experiment	Initial concentration (lg $TCID_{50}$ ml^{-1})		Concentration after 2 h, (lg $TCID_{50}$ ml^{-1})		Concentration after 4 h, (lg $TCID_{50}$ ml^{-1})	
	Sterile water	Hank's solution	Sterile water	Hank's solution	Sterile water	Hank's solution
1	4.25±0.50	4.00±0.50	3.00±0.25	3.50±0.25	1.75±0.25	2.25±0.50
2	4.50±0.75	4.25±0.75	3.25±0.25	3.50±0.50	1.50±0.50	2.50±0.75
3	4.25±0.75	4.25±0.75	3.00±0.50	3.25±0.75	2.00±0.50	2.50±0.50
Average	4.33±0.67	4.17±0.67	3.08±0.33	3.42±0.50	1.75±0.42	2.41±0.58

as, taking into account the absence of any reference technique for such measurement, it was the only parameter which could be used for the evaluation of sampler's performance on monitoring viable SARS virus.

The very important characteristic of any bioaerosol monitor is the rate of microbial recovery of the device for the particular microorganism of interest. Due to impossibility of direct measurement of the recovery rate of SARS virus (no permission to aerosolize this microorganism was given at this stage) by the sampler, this parameter was theoretically evaluated by the following formula:

$$R(t) = \mathrm{ND}(t) \times E,$$

where $R(t)$ is the microbial recovery rate after certain time interval since commencement of the monitoring procedure, $\mathrm{ND}(t)$ is the corresponding rate of natural decay at the same time interval and E is the physical collection efficiency of particles with the size of the microorganism of interest. To determine the exact value of physical collection efficiency of the device for the SARS virus size particles, the microscopic research was undertaken to find out the physical dimensions of such microorganism.

The TEM photograph of SARS virus used in the experiments is shown in Fig. 2. The SARS virus was inoculated onto confluent monolayers of Vero cells in 25-cm^2 flasks and incubated for 60 min at 37°C. Inoculated cell cultures were maintained with RPMI 1640 supplemented with 1% fetal bovine serum. Cell culture supernatant fluids were examined for the presence of virus by electron microscopy. Formvar-coated grids were placed for 7 min onto drops of cell culture supernatant. Negative staining was performed with 2% phosphotungstic acid (PTA, pH 7.4) for 7 min, PTA (pH 6.0) for 1–7 min, 2% ammonium molybdate (AMo, pH 6.5) for 1 min, 2% methylamine tungstate (MAT, pH 5.8) for 1 min and 1% aqueous uranyl acetate (UAc) for 5–45 s. The samples were examined on Transmission Electron Microscope (JEM-100S, Jeol, Japan) at magnification range 10 000–60 000. Examination of cell culture supernatant revealed viral particles of rounded shapes and diameter of 80–90 nm. The particles had characteristic for coronaviruses long spikes on the surface. The physical efficiency of the device for collection of 80-90 nm NaCl particles was previously measured (Filter ♯1 in Agranovski et al., 2001) and the magnitude of 97% was obtained.

The theoretical recovery rate for the new personal sampler was then evaluated and the results are presented in Fig. 3 together with the time related results of the SARS virus natural decay during bubbling through virus maintenance fluid generally discussed before. As is seen from the figure, the theoretical recovery rate of the SARS virus was higher than 75% during first 30 min of monitoring. This figure looks very promising for utilizing of the new device for the short sampling time periods for such microorganism. It decreases only by 20% during next 30 min and remains higher than 55% for the first hour of monitoring. The recovery rate was still very high during next hour of operation and stays above 19% during this time period. Even after 3 h of sampling the recovery rate is still at the level of 5% which, in case of high initial concentration of microorganisms in the ambient air, is high enough to determine the viable airborne SARS virus in the ambient environment. Note, that above calculations are based on a fact that all virus particles reached the device at the beginning of the sampling procedure and were exposed to bubbling regime throughout entire operation of the sampler. In reality, the microorganisms are being

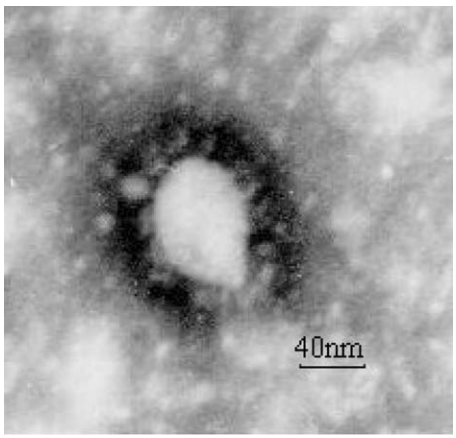

Fig. 2. TEM photograph of SARS virus used in experiments.

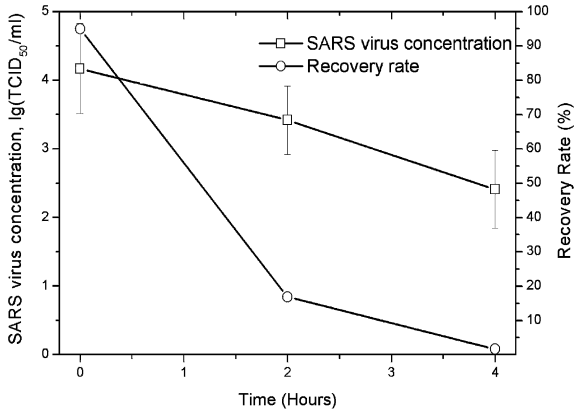

Fig. 3. Natural decay and recovery rate of SARS virus during up to 4 h monitoring procedure.

collected by the device throughout the whole sampling time which would minimize the exposure for the later coming microorganisms and correspondingly provide higher recovery rate compared to the theoretically calculated.

The other very important finding was based on a fact that the total number of virus particles (living and dead) in the virus maintenance fluid has not been changed during the entire sampling procedure. It indicates that, even in case of some decay, collecting fluid can be analysed by RT-PCR technique (Drosten et al., 2003) to qualitatively detect the existence of SARS virus in the ambient air. The early and rapid detection of this extremely pathogenic virus in public areas is extremely important, as all appropriate actions can be made before massive infection of individuals exposed by SARS.

4. Conclusion

Overall, the new sampling method of direct collection of the SARS virus into maintenance fluid was found to be feasible for the detection and enumeration of viable airborne SARS viruses. It opens the unique and revolutionary opportunity to perform such monitoring even for such stress sensitive microorganism as SARS virus. Obviously, this method would moreover be feasible for monitoring of robust and highly pathogenic viruses accidentally or purposively spread in the ambient air.

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