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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.

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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
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 bacteria 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 ± 20%; for stress-resistant B. subtilis bacteria and A. versicolor fungal spores it was 95 ± 7% and 97 ± 6%, respectively.

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 ~10\(^4\) TCID\(_{50}\) ml\(^{-1}\) (Tissue Culture Infectious Dose) concentrated suspension of SARS...
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 41 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 milliliter 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 monolayers 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 TCID₅₀ml⁻¹ and the rate of natural decay was obtained as the ratio of concentration (TCID₅₀ml⁻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 Sterile water Hank’s solution Sterile water Hank’s solution Sterile water Hank’s solution

| Number of experiment | Initial concentration (lg TCID₅₀ml⁻¹) | Concentration after 2h, (lg TCID₅₀ml⁻¹) | Concentration after 4h, (lg TCID₅₀ml⁻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       |

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...
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:

\[ R(t) = \frac{ND(t)}{E} \]

where \( R(t) \) is the microbial recovery rate after certain
time interval since commencement of the monitoring
procedure, \( ND(t) \) 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-cm² 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 10000–60000. Examina-
tion 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 micro-
organisms 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
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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