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Rapid detection of airborne viruses by personal bioaerosol sampler combined with the PCR device

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Abstract

A new personal sampler had been previously developed and verified for monitoring of viable airborne viruses. The aims of this project were to investigate a possibility of the utilization of the polymerase chain reaction (PCR) method to speed up the time consuming analytical procedures and to evaluate a lower detection limit of the combined (sampler-PCR) device. Tenfold serial dilutions of the initial suspension of the *Vaccinia* virus were aerosolized in the chamber and airborne viruses were monitored by two simultaneously operating samplers. The results of monitoring were successfully obtained by a standard plaque assay (live microbes) and by the PCR method (total DNA). The corresponding calculations to identify the minimal detectable concentration in the ambient air were then performed. It was found that the minimal detectable concentration of airborne viruses in the ambient air depends on the sampling time. As demonstrated, such concentration should be at least 125×10^3 PFU m^{-3} for a sampling time of as short as 1 min. The detectable concentration decreases with the increase of the sampling time and reaches 25×10^3 and 10×10^3 PFU m^{-3} for 5 and 12.5 min of sampling respectively.

Keywords: Bioaerosol; Personal sampling; Airborne virus; PCR; Detection limits

1. Introduction

An investigation into the possibility of early and reliable detection of pathogenic microorganisms in the air is becoming exceptionally important. The recent spread of the SARS and Avian influenza viruses further reinforced this concept raising various scientific and public health issues related to the transmission and control of infectious agents (Lipsitch et al., 2003). The above factors, together with society's growing concern regarding 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; Macher, 1997). Based on the engineering control method (Agranovski et al., 1999; Agranovski et al., 2001), which was previously applied to the removal of particles from gas carriers, a new personal bioaerosol sampler has been developed (see a detail

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sketch in Agranovski et al., 2005a). 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 were evaluated for the monitoring of airborne *Pseudomonas fluorescens* and *Bacillus subtilis* var. *niger* bacteria, *Aspergillus versicolor* fungal spores (Agranovski et al., 2002a, b) and *Influenza* and *Vaccinia* viruses (Agranovski et al., 2004a, b, 2005a, b). As shown in the referred papers, the new sampling method was found suitable for the personal monitoring of viable airborne viruses, bacteria and fungal spores. However, standard laboratory procedures are quite time consuming (for example, bacteria should be incubated for 2–5 days and some fungi and viruses require even longer time periods) so more rapid, and at least, qualitative analytical procedures are required to meet the expectations of potential users including anti-terror units, defense forces, public health and agriculture specialists. The current study was designed to explore the possibility of utilizing of the PCR method to rapidly obtain qualitative results on the general existence of a particular microorganism in the air. The advantage of such an arrangement is that if the general existence of some pathogen in the air is detected by PCR, the remaining collecting liquid can be further analysed to find a number of live microorganisms from the total amount of DNA/RNA measured by the PCR/RT-PCR method. Special attention was given to the investigation of the lower detection limit of the sampler for enumeration of both live and total viruses collected from the air (Fig. 1).

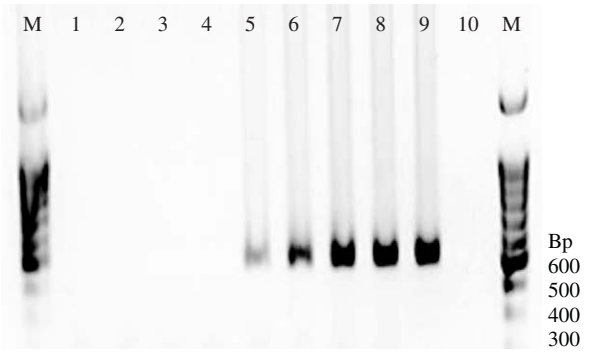

Fig. 1. PCR detection results.

2. Materials and methods

2.1. Microorganism

The *Vaccinia* virus (strain LIVP C0355 K0602) used in this research was passed 10 times in embryonated chicken eggs and the final virus containing material with concentration of 10^6 PFU ml^{-1} (Plaque Forming Unit per ml) was obtained by culturing in cells 4647 (kidney cells of *Cercopithecus aethiops* embryo) with following triple freezing/defrosting of infected cell culture in maintenance media MEM Eagle (MOD). (Cat #11-100-22, ICN Biomedicals, Inc., Aurora, OH, USA). Before use in experiments, the virus containing media was kept at the temperature of $-70\,^{\circ}$C.

2.2. Experimental procedure

The experimental setup used in this study was described in detail by Agranovski et al. (2005a). Briefly, the viral suspension was initially diluted to 2×10^4 PFU ml^{-1} and added into a 3-jet Collison nebulizer (BGI Inc., Waltham, MA), which was used to generate airborne viruses. The nebulizer operated at a flow rate of 6 L min^{-1} of dry and filtered compressed air to aerosolize 0.2 ml of suspension per min. Another portion of dry and filtered air, supplied at a flow rate of 10 L min^{-1}, was mixed with the aerosol stream from the nebulizer before the entrance to the aerosol chamber to create appropriate laminar conditions required by the chamber and to avoid particle settlement effects associated with low air velocity. A dynamic aerosol chamber with horizontal aerosol flow with the gas velocity of 10 cm s^{-1} was used in this research (Ryzhikov et al., 1995). Before each experimental run, the chamber was flushed by HEPA filtered air for, at least, 30 min. Two parallel samplers, each filled with 50 ml of collecting liquid (Hank's solution containing 2% volumetric of inactivated bovine serum, 100 U ml^{-1} of penicillin, 100 μg ml^{-1} of streptomycin and 0.03% solution of silicon anti-foam emulsion (M-30 Dow- Corning, 30% Dimethylpolysiloxane in water)), were operated for 5 min inside the chamber at the sampling flowrate of 4 l min^{-1}. To identify the lower detection limit of the technique, the concentration of viral particles in the aerosol chamber had to be varied. The only strategy to achieve such variation, considering fixed air flow requirements applied to the chamber (16 l min^{-1}) and nebulizer (6 l min^{-1}),