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Research Article

Monitoring of Airborne Mumps and Measles Viruses in a Hospital

A new personal bioaerosol sampler, which was found suitable for continuous long-term (up to 8 h) monitoring of airborne bacteria, fungi, and viruses, was recently developed. A range of investigations under controlled laboratory conditions verified the performance of the device for both stress sensitive and robust viral particles. To decrease the detection time, the next step of the technique development was related to utilization of the sampler in combination with polymerase chain reaction (PCR) technology in the laboratory. The combined device was found to be fully feasible with the corresponding decrease of the detection time from a few days to 2.5 h. In addition, the results for targeted microorganisms were not affected by background biological particles and cross-reaction was not observed. The current study is the first trial to use the new combined device, i.e., sampler-conventional PCR device, for monitoring airborne viruses in the field. The monitoring procedures were performed in hospital infection wards with patients suffering from mumps and measles diseases to detect the corresponding disease causing viruses in the ambient air. The results for the existence of the airborne viruses were obtained for both strains. A simple procedure is also suggested for enumeration of microbial contamination in the ambient air.

Keywords: Bioaerosols; Airborne virus; Personal sampling; Bubbling; Measles; Mumps; PCR

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1 Introduction

The growing concern for human exposure to bioaerosols has created demand for the development of reliable and accurate monitoring techniques and has led to the initiation of a number of studies focused on the detection and enumeration of viable/nonviable bioaerosols in critical locations possibly contaminated by airborne pathogenic particles. Such studies have been undertaken at waste management facilities [1, 2], animal houses [3], hospitals [4, 5], clean office environments [6], and other public, industrial and agricultural facilities. However, the main focus of all the listed projects has been the identification and characterization of bacterial and fungal airborne contaminants and no viral aerosols have been taken into consideration.

A novel bioaerosol sampling technique, which utilizes the bubbling process in the collection fluid, has recently been developed and found feasible for the long-term personal sampling of airborne bacteria and fungal spores, since it maintained a high physical collection efficiency and high microbial recovery rate for robust and stress-sensitive bioaerosols [7]. Further tests have shown that the new technique also has significant potential for application in the collection of viable airborne viruses, particularly when utilized for the sampling of robust strains [8]. Various laboratory studies have been undertaken to identify the recovery rates of a number of viruses during short- and long-term sampling, detection limits of analytical techniques and other applications [9–11]. However, considering the rapid spread of some respiratory diseases caused by airborne viral particles, the standard laboratory procedures utilized for analysis of the collection liquid after air sampling are far too slow for timely detection of biological threats, e.g., bacteria should be incubated for 2-5 days and some fungi and viruses require even longer incubation periods. Clearly, there is a need for the development of rapid, and at least, qualitative analytical procedures in order to meet the expectations of potential users from anti-terrorist units, defence forces, public health and agriculture specialists. Thus, a further study by the current authors [12] was designed to explore the possibility of utilizing the conventional PCR method to rapidly obtain qualitative results on the presence of particular microorganisms in the air under controlled laboratory conditions. The advantage of such an arrangement is that if the presence of a particular pathogen in the air is detected by the PCR, the remaining collection liquid could then be analyzed further to quantify the number of live viral particles by common more time-consuming microbiological assays. The experiments were performed with a robust *Vaccinia* virus under controlled laboratory conditions. The results of the study demonstrated full capability of the combined (sampler-PCR) technique for detection of targeted airborne viral particles, even at very low concentrations. The most recent study by the current authors [13] focused on the employment of a real-time PCR in combination with a personal sampler for further shortening of the time required for identification of pathogens in ambient air.

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Considering that targeted bioaerosol monitoring is usually performed at locations with elevated concentrations of background bioaerosols, the selectivity of the combined technique was one of the main issues investigated. It is generally known that the PCR method is quite selective [14, 15]. However, some possibility of cross-reaction has been reported in the literature [16, 17]. The results of the study [13] clearly demonstrated that the combined sampling-real time PCR detection method is fully feasible for the rapid, i.e., ~2.5 h, and highly specific, i.e., no cross-reactivity, identification of the labile airborne virus in the air containing elevated concentrations of other viral particles.

The present study was the first effort to evaluate the performance of the new sampler in a ‚real world' environment for the monitoring of viruses. The entire sampling procedure was undertaken in a hospital infection ward to monitor the possible release of viruses from measles (*Paramyxovirus* of the genus *Morbillivirus*) and mumps (*Rubulavirus*) suffering patients.

2 Materials and Methods

2.1 Equipment Preparation and Location

Before being exposed to the infectious hospital wards, each personal sampler was charged with 50 mL of collecting liquid consisting of 2% Hanks solution containing bovine serum, anti-foam emulsion and antibiotics (100 U/mL of penicillin and 100 μg/mL of streptomycin). Flexible silicon tubing was used to connect each sampler to a portable sampling pump (model SP350, TSI, USA) capable of providing a flow rate of up to 4 L/min, see Fig. 1.

Two sampler-pump assemblies were placed on a nurse's protecting uniform, see Fig. 2, who then undertook the usual activities in a room (3 m · 2.5 m · 2.8 m) with one patient for a total duration of 3 h. The location of the sampler on the uniform (behind the nurse) ensured no direct exposure of the device to the droplets possibly released from the patient's throat during normal activities, e.g., breathing, coughing and sneezing. As a result, a more integrated concentration pattern could be obtained. After each 1 h interval, a sub-sample of the collection fluid (2 mL) was taken for analysis by plaque assay (live viruses) and PCR (total number of viruses live and dead). Hospital wards with mumps and measles suffering patients were used in this investigation. Both viruses are common pathogenic respiratory viral particles transmitted by air.

To obtain a representative sample of collecting liquid for the PCR negative control, another sampling device was operated for exactly the same period in a similarly sized room with no patients present.

2.2 Enumeration of Live Viruses in the Collection Liquid

After sampling, concentrations of both live viruses in the sampler collecting liquid were identified by the plaque assay of the collecting liquid. Firstly, a sample of the virus containing collecting liquid was diluted in the maintenance medium containing antibiotics. Eight ten-fold serial dilutions were then made, and 100 μL was added to confluent Vero cell monolayers in 24-well cluster plates (Costar, Pleasanton, Calif.). The virus was allowed to adsorb for 1 h at 37°C in a 5% CO_2 atmosphere of a humidified incubator. The cluster plates were shaken every 10 to 15 min, fluid was aspirated after 1 h, and 2 mL of overlay 1% agar (Difco) on RPMI-1640 [18] medium

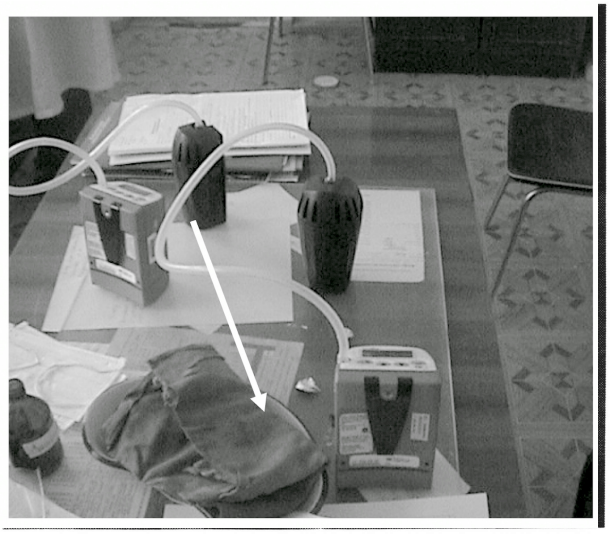

Figure 1. Personal sampling assembly prepared for sampling outside the infection ward.

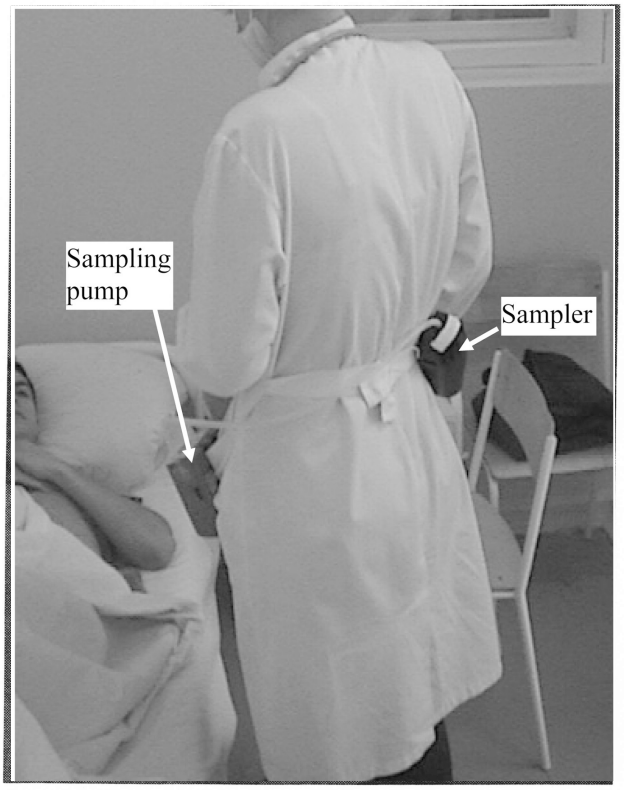

Figure 2. Arrangement of sampling devices on a nurse's uniform.

(containing 2% of fetal calf serum and antibiotics) was added. Cells were incubated for 6 days for both mumps and measles viruses at 37°C in a humidified incubator (5% CO_2 atmosphere), after which time cell monolayers were stained with neutral red and plaques were enumerated. The results were calculated in PFU per mL of suspension.

2.3 Detection of Total RNA in the Collection Liquid

2.3.1 Oligonucleotides and Primers

Oligonucleotides were synthesized in an ASM-102U DNA/RNA synthesizer (Biosset, Russia). A fragment of the measles NP (nucleoprotein) gene, 543 bp in length was amplified using primers MN1 (1196–1217, 5'ATTAGGGCAAGAGATGGTAAGG) and MN2 (1739–1722, 5'TATAACAATGATGGAGGG) [19]. MN 3 (1233-1254, 5'GTCAGTTCCACATTRGCATCTG) and MN 4 (1648–1631, 5'GTGTCCGTGTCTGAGCCT) were used for the second round primer.

A fragment of the mumps F and SH genes, 548 bp (5276–5785 using SBL strain) in length was amplified using primers N3 – (5"TTGGATCCTTGTAGCAGCCTTAGTTTTGAG) and N4 (5'CGGAATTCTGCTTTTTTCTTTATGTCTCATG) [20]. The oligonucleotides were purified by HPLC on a Lichrosorb RP-18 column (Merck).

2.3.2 Isolation of Viral RNA

RNA was extracted from specimens using the Rneasy Mini Kit (QIAGEN, Germany) according to the kit manual.

2.3.3 PCR Amplification

The virus gene regions were transcribed using the «Titan one tube RT-PCR system» (Roche, Germany), using virus specific primers according to the kit manual in a GeneAmp PCR System 6700 (Perkin-Elmer, Foster City, CA). Samples with viral RNA were amplified in 50 μL of the reaction mixture containing 10 μL 5 × buffer, 2 μL d NTP (2 mM), 2.5 μL DTT (100 mM), 1 μL RNA inhibitor, 1 μL RT-Mix, 1 μL primer 1 and 2 (25 ρM/μL) and 22.5 μl DEPC water. Before addition to the reaction mixture, samples with viral RNA (10 μL) were heated at 80°C for 5 min followed by cooling in ice (0°C) for 5 min.

In the case of the mumps virus, the reaction mixture was firstly held for at 39°C 45 min. This was followed by 40 cycles, each consisting of three steps: 30 s at 94°C, 40 s at 40°C, and 1 min at 72°C. The mixture was then incubated for 7 min at 72°C. The second round of nested PCR consisted of 40 cycles each containing three steps: 45 s at 94°C, 45 s at 56°C, and 1 min at 72°C. Finally, the mixture was incubated for 7 min at 72°C.

For the measles virus, the reaction mixture was firstly held at 39°C for 45 min. The first stage of nested PCR consisted of 20 cycles each containing three steps: 10 s at 94°C, 30 s at 37°C, and 1 min at 68°C. The mixture was then incubated for 7 min at 68°C.

The second stage of nested PCR consisted of 30 cycles each containing three steps: 1 min at 94°C, 1 min at 40°C, and 1 min at 72°C. Finally, the mixture was incubated for 7 min at 72°C.

The PCR products were analyzed by electrophoresis in 1% agarose gels in a TAE buffer (40 mM Tris-acetate and disodium ethylenediaminetetra-acetate containing ethidium bromide (0.2 μg/mL)).

The negative controls were made according to exactly the same procedure by using collecting liquid from the previously described sampling device operated in the room not occupied by a disease-suffering patient.

2.3.4 Ethical Clearance

Considering the involvement of humans in this research, all activities were undertaken under ethical clearance issued by the ethical committee IRB00001360, operated at the State Research Centre of Virology and Biotechnology "Vector" Koltsovo, Novosibirsk Region, Russian Federation (http://ohrp.cit.nih.gov/search/irbdtl.asp?IRBID=4665).

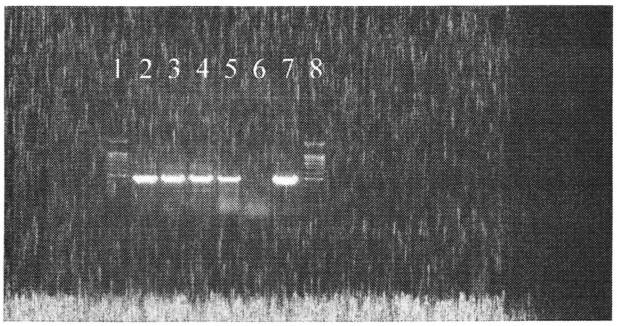

Figure 3. Identification of mumps virus by PCR after full 3 h run. 1. MW marker; 2. Patient's throat (Day 1); 3. Patient's throat (Day 2); 4. Sampler (Day 1); 5. Sampler (Day 2); 6. Negative Control; 7. Positive Control; 8. MW marker.

3 Results and Discussion

3.1 Mumps

Two repeats of the test were undertaken during two consecutive days when symptoms of the disease were very prevalent. A common print out from the PCR machine is shown in Fig. 3. As seen from Fig. 3, very sharp lines are obtained for both personal samplers and samples collected from the patient's throat. Both positive and negative control lines together with molecular weight markers clearly confirm the presence of the mumps virus in all probes. Unfortunately, the results of the plaque assay procedure demonstrated the absence of any live virus in the air. Such a result is to be expected as mumps represents a rather sensitive strain [10] with a relatively high decay rate in the air and during sampling. In addition, the main scope of this investigation was to identify the general presence of the virus in the air by the PCR method, and therefore, the time interval between sampling and analysis (should be the minimal possible time in case of identification of viable microbes) was ca. 8 to 12 h, which was long enough to allow the entire inactivation of the collected viral particles.

The following procedure was employed to identify the number of viral particles collected by the device and the corresponding virus concentration in the air. It is known that the minimal concentration of mumps [21] and measles [22] viral particles detectable by PCR machine is 10 copies per reaction on average (as reported, it can vary from 2–20 copies per reaction for both strains). Some dilutions of the collecting liquid were used to find a maximal dilution for which the viral RNA was still detectable. It was found that the maximum dilutions for which virus was still detectable are × 2, × 5 and × 8 for 1, 2 and 3 h of sampling, respectively. Such results correspond to at least 20, 50 and 80 virus particles per mL of collecting liquid for the above sampling time periods. The corresponding total number of viral particles collected by the sampler (total amount of collecting liquid is 50 mL) together with the average virus concentration in the air during related sampling period (sampling flow rate was 4 L/min) were calculated according to the main procedure and the results are shown in Tab. 1. It is clear from Tab. 1 that the discrepancy between the results is less than 30% (comparing viral concentration calculated for three time intervals), which is considered as satisfactory for such an unstable parameter.

The results represent an average of two experimental runs each involving two samplers. All devices demonstrated excellent agree-

Table 1. Monitoring of the mumps virus.

Time period, (h)	Total amount of virus particles collected, (PFU)	Average virus concentration in the air, (PFU/m^3)
1	1000	4167
2	2500	5208
3	4000	5556

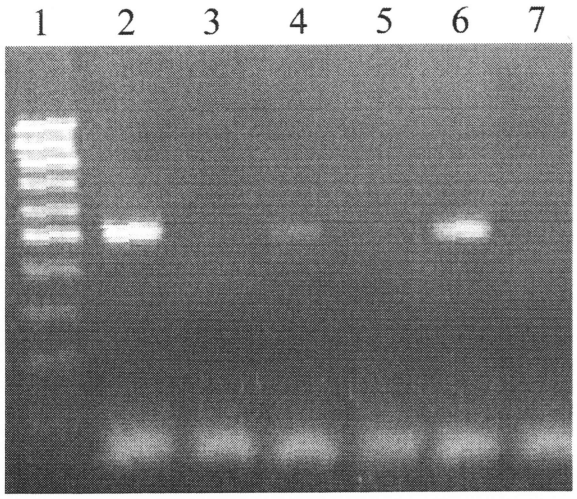

Figure 4. Identification of measles virus by PCR after full 3 h run. 1. Molecular weights; 2. Patient's throat (Day 1); 3. Patient's throat (Day 2); 4. Sampler (Day 1); 5. Sampler (Day 2); 6. Positive control; 7. Negative control.

ment with the inter-sampler variation not exceeding the experimental error and the same dilutions were detectable for all devices involved. In addition, the discrepancy between the results was found to be statistically insignificant, which was confirmed by a single-factor ANOVA test ($p > 0.05$). Such reliability looks very promising for further use of the device for various applications. It is especially important in the areas of anti-terrorism and public health where the general presence of heavy pathogens in the air is sufficient information for the requirement for immediate corresponding actions.

2.2 Measles

The second series of tests was performed in a similar size ward occupied by a patient suffering from measles disease. The procedure was similar to that described for monitoring of the mumps virus and it was also undertaken during two consecutive days. The results of the monitoring are shown in Fig. 4. Interestingly, the first day of sampling probably corresponded to the final stage of the disease and demonstrated the presence of the airborne virus in both the patient's throat and the ambient air. However, for the second day of sampling no virus was detected in the air or in the patient.

It was found that the maximal dilutions for which the measles virus was still detectable during the first day of sampling were as outlined below. No virus was detected even in the undiluted collecting liquid taken after the first hour of sampling. For the second and third hours the dilutions were $\times 2$ and $\times 3$, respectively. Such results correspond to at least 20 and 30 virus particles per mL of collecting liquid for the above sampling time periods. The corresponding total number of viral particles collected by the sampler was calculated and the results are shown in Tab. 2. As seen from Tab. 2, the results were very consistent for the second and third hours of sampling, although the lower detection limit of the device was not exceeded after the first sampling hour. In addition, it should be noted that three out of four devices used for the monitoring did show very consistent results, i.e., similar dilutions were detectable for all of them. With regards to the last sampler, the viral particles were not detected for the second hourly stage. However, the final result was in perfect agreement with the other results. This finding could possibly be explained by some error of the PCR machine used for following sample analysis. Similar to the situation for mumps, no stress sensitive live measles viruses were detected in the samples [10].

Table 2. Monitoring of the measles virus.

Time period, (h)	Total amount of virus particles collected, (PFU)	Average virus concentration in the air, (PFU/m^3)
1	Below detection limit	Below detection limit
2	1000	2083
3	1500	2083

4 Conclusions

The results obtained in the experiments demonstrated the reliable performance of the personal sample-PCR technique for the rapid monitoring of airborne viruses in the real world environment. An excellent correlation between the existence of the virus in the patient and in the ambient air was demonstrated for both viral particles used in the investigation. This result opens a very attractive opportunity for non-invasive monitoring of targeted viral particles in various public places.

It was found that the concentration of the mumps virus was higher compared to the numbers obtained for the measles virus, which can be explained by two factors. Firstly, the monitoring of the mumps virus coincided with the peck of the disease when the viral emission could be higher then the emission from the patient suffering the last stage of the measles disease. Secondly, such a result can be related to a generally smaller release of measles virus to the ambient air compared to the second microbe used. The exact answer to this question is beyond the scope of the current study and aerosol science in general, and could be addressed by corresponding research undertaken by professional virologists.

It should also be noted that in this particular investigation, no effort was made to undertake a full size study targeting total enumeration of various viruses in a hospital environment. Such studies are planned studies for the future. At this stage, a feasibility study has been performed on the capability of the new device to undertake such an investigation. In addition, the current paper does not try to identify the live viruses in the air and merely checked this factor as part of the procedure, while leaving the detailed investigation for the future. According to previous investigations, the time period between sampling and analysis should be shortest possible to identify live viruses [10] since inactivation of viruses in the collecting liquid is very rapid, i.e., on the timescale of hours. To perform the viability test in the future, a mobile laboratory is currently being constructed in a hospital to avoid any time related inactivation.

The suggested procedure of enumeration of the viral particles in the ambient air, based on some dilution of the collecting liquid, is obviously quite rough. The assumed detectable concentration at the level of 10 viral particles per mL is questionable and variable for different viral strains. However, it is not assumed that it will be recognized as the reference method for reporting airborne virus concentration results. The method is good enough for a rough approximation of half an order of magnitude and as such could be used in situations where expensive real-time quantifying PCR machines are not available. It could also be used for comparative analysis of data obtained at different locations on a sampling site in situations when the concentration of viral particles decreases with increasing distance from a source. Such a procedure could help to identify source coordinates, which in many obvious cases.

In order to obtain a comprehensive picture of the general bioaerosol presence in the ward, on completion of the sampling procedure, a certain portion of collecting liquid was spread onto selective bacteria and fungal agar plates to identify the background concentration of these particles in the ambient air (see details of the procedure by Agranovski et al., [7]). Background concentrations of airborne bacteria and fungi were within the ranges of 38 to 97 CFU/m^3 and 17 to 88 CFU/m^3 for bacteria and fungi, respectively, for the entire investigation. Special attention was given to the PCR quality control considering the presence of other biological particles in the ambient air. Finally, it should be noted that no cross-reaction was observed during the PCR procedures.

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