# Detection of airborne noroviruses in hospitals and lab experiments

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# Detection of airborne noroviruses in hospitals and lab experiments

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**ABSTRACT** – Norovirus is the major cause of acute gastroenteritis and it is considered to spread by food, contact, by the fecal–oral route and by droplets from splashes. However, there are studies showing disease transmission between individuals without contact, indicating airborne transmission. In our study, we have developed an experimental method for detection and infectivity assessment of aerosolized murine noroviruses. We have also sampled and detected airborne noroviruses in hospitals, close to infected patients.

## 1. INTRODUCTION

Noroviruses (NoV) cause millions of acute gastroenteritis infections every year, which are estimated to result in a loss-of-productivity of 60 billion USD per year [1]. Considering the number of infections caused by NoV worldwide, little attention has been given to improve disease prevention and control [1]. NoV is known to spread by food, by contact, by the fecal-oral route and by droplets created from splashes. There are, however case studies of NoV spread between individuals without contact, indicating transmission via air. One previous study successfully detected NoV RNA in air samples from hospitals [2]. However, important issues that remain to be understood are for instance what sources that generate airborne NoV and to what extent airborne NoV are infectious.

In order to understand what parameters that affect the infectivity of airborne NoV, we have developed an experimental setup for aerosolization and collection of murine noroviruses (MNV), a cultivable model virus for human NoV. To improve the understanding of sources that generate airborne NoV, air sampling was performed in hospital wards with infected patients.

The genomes of NoV and MNV are of positive sense strand RNA. During replication, the intermediary, complementary minus strand RNA is formed inside the infected cells. By specifically detecting the minus strand RNA in cells, infection can be determined with high sensitivity [3].

## 2. METHODOLOGY

### *2.1 Laboratory setup for aerosolization of MNV*

MNV in cell culture medium was aerosolized by an atomizer (Model 3076, TSI inc.) into a 1 m long stainless steel flow tube, and collected at the end of the flow tube into phosphate buffer saline (PBS, Life Technologies) solution with a BioSampler® impinger (SKC), see Figure 1. The particle number concentration and size distribution of the aerosol was monitored by a scanning mobility particle sizer (SMPS, built in-house, Lund University) and an aerodynamic particle sizer (APS, Model 3321,TSI inc.).

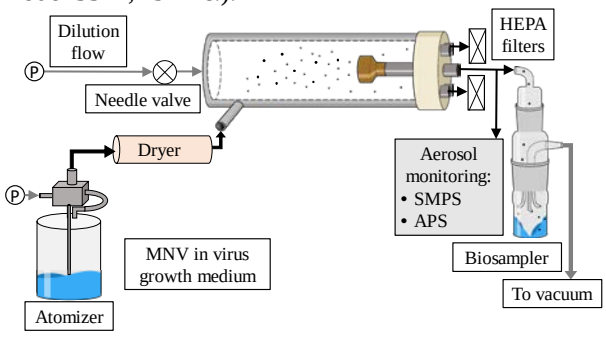


Figure 1. Schematic figure of the experimental setup for aerosolization and collection of murine noroviruses. The aerosol flow follows the black arrows. Pressurized air is marked as a circled P.

The liquid in the BioSampler® was analyzed by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) targeting the viral genome to determine the number of collected viral RNA genomes. For infectivity analysis, inoculation of RAW 264.74 cells with the collection liquid was performed during 72 h. After the incubation time, the cells and the supernatant were separated. Intra and extracellular RNA was obtained by RNeasy Mini Kit (Qiagen) and detected by strand specific plus and minus RT-qPCR as described by Vashist et al. [3].

### *2.2 Hospital air sample collection*

After confirmation of patient NoV infection, air samples were taken with a high airflow liquid cyclone, Coriolis (Bertin Instruments), operated at 200 L/min for 10 min. PBS was used as collection liquid. Samples were taken in the patient bathroom, in the patient room and in the corridor outside the patient room. Negative control samples were taken inside the hospital building but outside the wards involved. Due to patient–related issues, sampling at all locations was not possible in some cases. The collection liquid was up-concentrated with Amicon Ultra-15 centrifugal filter unit (Merck Millipore) and analyzed in the Laboratory for Clinical Microbiology, Lund University, by real-time PCR for detection of human NoV.

## 3. RESULTS AND DISCUSSION

### *3.1 Infectivity of aerosolized MNV*

The aerosolized and collected MNV that were inoculated on cell cultures showed cytopathic effect (CPE) on the cells by visual observation in a microscope. Infections were confirmed by the minus RNA RT-qPCR analysis on the cells after the incubation time. Concentrations of minus RNA in the range of $1.5 \cdot 10^3$ to $3.9 \cdot 10^6$ genomes/mL were achieved, depending on the viral concentration in the sample. Concentrations of minus RNA could be detected down to the order of $10^2$ genomes/mL.

Due to the extended inoculation time (from 8 h according to Vashist et al. [3] to 72 h), no quantitative information on the infectivity in the original sample solution could be obtained. This infectivity assay, however, serves as a sensitive qualitative determination of MNV infection. This is useful since visual CPE observations need a trained observer to be correctly analyzed. The future goal is to develop the assay to give relative quantitative information on virus infectivity.

The atomizer generated a wide size distribution of aerosol particles (Figure 2). No differences were found between the size distribution of the growth medium with and without MNV, thus the small (30 nm) MNV are likely to be incorporated together with medium solutes in the generated particles.

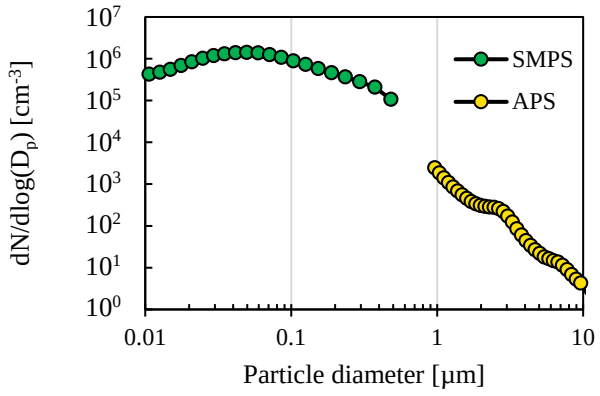


Figure 2. Number size distributions of the aerosol particles generated by the atomizer. The SMPS measured the mobility diameter (0.010-0.6 µm) and the APS measured the aerodynamic diameter (0.9-10 µm).

### *3.2 Detection of airborne human NoV in hospitals*

During three cases of patients infected with NoV in hospitals during March-May 2017, air samples were collected and analyzed for NoV by PCR. The number of NoV-positive samples are given in Table 1. In Case 1, the patient had used the toilet within one hour before the sample was taken. In Case 2, samples were taken 4 hours since last toilet visit. In Case 3, measurements were taken in two patient rooms in the same ward, ca 1 hour and 6 hours after last toilet visit, respectively.

These three cases are the first preliminary results from our hospital measurements, and although more data are needed for a proper analysis, it is notable that airborne concentrations can be detected even in the corridor outside the patient room. Flushing toilets should be deeper investigated as a potential source of airborne NoV, and more data is needed in order to conclude if all patients spread airborne NoV.

Table 1. Number of NoV positive air samples collected at three hospitals wards with infected patients.

| | Positive air samples/Total air samples | | |
|---|---|---|---|
| **Location** | **Case 1** | **Case 2** | **Case 3** |
| Patient toilet | 2/2 | - | 1/1 |
| Patient room | 1/2 | 0/1 | 0/2 |
| Corridor | 1/1 | 0/1 | 0/1 |
| Negative control | 0/1 | 0/1 | 0/1 |

## 4. CONCLUSIONS

In this study we have seen that there may be airborne NoV close to infected patients in hospitals, by detection in air samples. These measurements do, however not tell us about the potential infectivity of airborne NoV. The lab experiments, on the other hand, showed that norovirus, MNV, were infectious after aerosolization. Together, these data provide futher support for the hypothesis that NoV can be transmitted through the air.

## 5. ACKNOWLEDGEMENTS

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