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# Aerosolization of a model virus for studies of human winter vomiting disease

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## Introduction

Human Norovirus, which causes winter vomiting disease, infects millions of people every year and is estimated to cause 200 000 yearly deaths worldwide (Lopman 2015). Norovirus is known to spread via physical contact, food and through the air. Viruses can become airborne through flushing in a toilet with faeces from an infected patient, or by vomiting. Viral particles can then stay in the air for long periods and infect other humans.

An outbreak of disease at a hospital ward causes major stress on the health care: staff is reduced because of infected personnel and workload is increased because of ill patients. The knowledge of airborne transmission of viruses is limited and often overseen in disease prevention. The aim of this study is to investigate parameters that affect transmission and viability of norovirus in the air.

## Methods

The set-up for experimental analysis of aerosolized murine norovirus (MNV, a cultivable model virus for human norovirus) is schematically illustrated in Figure 1. A suspension of MNV in virus growth medium was aerosolized into a 1 m long stainless steel pipe and diluted with particle free air. The experiment was done with two aerosol generators: sparging liquid aerosol generator (SLAG, CH Technologies) at 14 lpm (30 lpm dilution) and an atomizer (model 3076, TSI) at 1.8 lpm (12.5 lpm dilution). The aerosol was collected into phosphate-buffered saline with a BioSampler impinger (SKC) at 12.5 lpm during 30 minutes and the size distribution was monitored with a scanning mobility particle sizer (SMPS, design: Lund University) and an aerodynamic particle sizer (APS, model 3321, TSI).

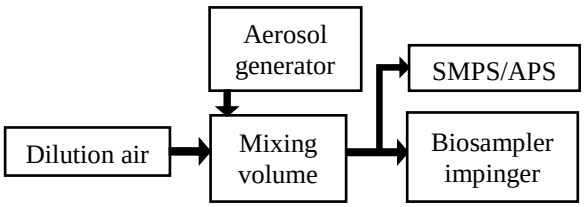


**Figure 1:** Schematic picture of the setup for aerosolizing and collecting bioaerosols.

The amount of collected MNV in the Biosampler was evaluated using quantitative polymerase chain reaction (qPCR). In addition, a technique that measures replicating minus RNA was used to assess the MNV viability after aersolization. Minus RNA qPCR specifically detects minus RNA, which is only present inside cells during replication, while mature MNV particles contain only positive RNA. We infected cells with test material and after 72 hours, when these cells should have a maximal replication of MNV, cell homogenates were prepared and analyzed for replicating MNV minus RNA.

## Results and Discussion

In this study, MNV was successfully aerosolized and the amount of collected viruses detected with qPCR. An assay for evaluation of MNV viability based on minus RNA was developed and in one experiment, positive results on the collected sample was achieved. Optimization is to be made in order to use the minus RNA assay for investigating virus viability during exposure to different types of environmental stress. Similar methodology has successfully been used by Bonifait et al. (2015), but with a different aerosol generator that produce larger droplets. We aim at producing small virus droplets (Figure 2) to reduce bias from other substances in the original suspension.

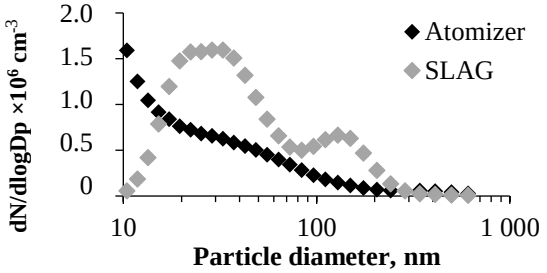


**Figure 2**: Size distributions of aerosolized virus solution measured with SMPS.

## Conclusions

Murine norovirus was successfully aerosolized and collected in a controlled laboratory environment. Positive results were obtained from the viability assay, indicating active replication of MNV. A setup for detection of aerosolized MNV is a first step to better understand the parameters and mechanisms that enable airborne spreading of human norovirus.

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Bonifait, L., et al. (2015). "Detection and quantification of airborne norovirus during outbreaks in healthcare facilities." Clin Infect Dis **61**(3): 299-304.

Lopman, B. (2015). Global burden of prospects for vaccine development, Centers for Disease Control and Prevention.

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**European Aerosol Conference, Zurich, Switzerland**
**27 August – 1 September 2017**
**Your paper T402N226**

Dear Ms Alsved,

The EAC 2017 Organising Committee is very pleased to inform you that your abstract with the title:
**Experimental set-up for studies of viability of aerosolized model organisms for infectious diseases**
has been selected for **ORAL** presentation. We have noticed that the presentation speaker will be:
**Malin Alsved**.

Your contribution has been assigned to the Session: **Airborne Pathogens and Microbes and Their Viability** and scheduled for
**Tuesday 29 August 2017, 18:00 to 18:20** hrs in **Room 15G40**.

Please note that the presentation time is 20 minutes in total, i.e. 15 minutes for the talk and 5 minutes for discussion. In view of five Sessions held in parallel and the audience possibly changing from one location to another, the 20-minutes schedule will be observed very precisely.

Please report at the registration desk one day before your talk and hand over your power point presentation to the staff. For organisational reasons no own laptops can be used for presentation. Your material will be made available on the PC installed in the respective conference room.

The overall conference programme is now being published on the EAC 2017 website and is being updated step by step. This procedure will take several days.

We are very much looking forward to seeing you in Zurich.

With best regards

Martin Gysel
Gunthard Metzig