Experimental assessment of aerosolized murine noroviruses

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Malin Alsved[1], Anders Widell[2], Henrik Dahlin[1], Patrik Medstrand[2], Jakob Löndahl[1]

[1]*Ergonomics and Aerosol Technology, Lund University, Sweden*
[2]*Translational Medicine, Lund University, Sweden*

Noroviruses are the cause for the majority of viral gastroenteritis cases in the world. Due to their low infectious dose and long stability in the environment, they often gives rise to outbreaks, even in hospital wards where legitimate infection control measures are practiced by the staff. Resent research has shown that noroviruses are present in air during outbreaks (Bonifait et al. 2014); however more research is needed to investigate the ability of noroviruses to transmit disease via the airborne route.

Method

In this study, a method for evaluating viral infectivity after aerosolization was developed. As there is no well-established cell-cultivation technique for human noroviruses, a murine norovirus (MNV) strain was used as model virus in this study. MNV was aerosolized from a liquid suspension into a flow tube (Figure 1) and collected into phosphate buffer saline (PBS) after 10 seconds as airborne particles. The dry size and concentration of the aerosol particles in the flow tube were monitored, and the wet droplet size was determined by model calculations. MNV infectivity after aerosolization was assessed by detection of intracellular negative sense RNA in RAW 264.7 cells after 24 h incubation. The complementary negative sense RNA strand of the MNV genome is only present during active replication and can therefore be used as an indication of infection. The collected aerosol samples were added in 10-fold end point dilution series to 24-well cell culture plates. After 24 hours, the supernatant was removed, cells lysed, RNA was extracted, and negative sense RNA detected by a tagged primer according to the method in Vashist et al. (2012).

Results

MNV preserves its infectivity after aerosolization, shown by the detection of negative sense specific RT-qPCR in the cell infectivity assay. With a dilution factor in the aerosolisation setup of about 500 times, the viral titers in the collected samples were relatively low, and the infectivity incubation time was therefore optimized for those, resulting in 24 h. Our aerosolization methodology can be further applied to other virus types, or to evaluate the effect of specific parameters in the air environment, such as temperature or humidity.

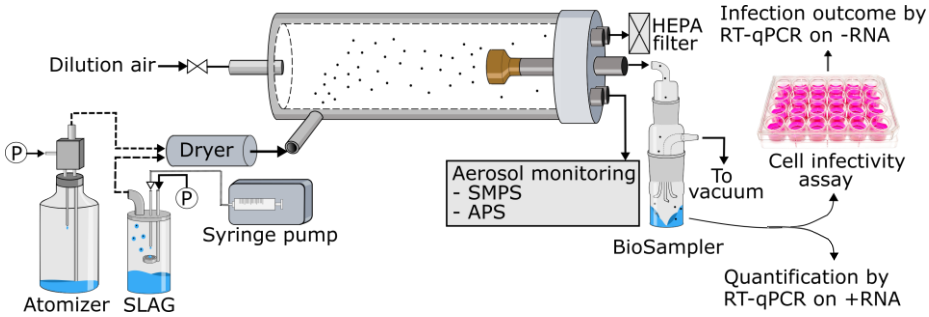

Figure 1. Experimental setup for aerosolization of murine noroviruses (by atomizer or sparging liquid aerosol generator) into a flow tube, and collection into PBS using a BioSampler. A scanning mobility particle sizer (SMPS) and an aerodynamic particle sizer (APS) was used to measure the concentration of particles in the size 0.01-0.6 µm and 0.8-20 µm respectively.

References

Bonifait L, Charlebois R, Vimont A, et al. (2015). Clin Infect Dis, 61(3), ss 299-304.
Vashist, S., Urena, L. & Goodfellow, I. (2012). J Virol Methods, 184(1-2), ss. 69-76.