Comparison of Bioaerosol Samplers and Media for the Collection of Aerosolized Norovirus

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Background

Norovirus is a highly contagious virus that causes enteric illness, mainly spread through person-to-person contact or the fecal-oral route.

In the U.S., norovirus causes 19-21 million cases of acute gastroenteritis, 56,000-71,000 hospitalizations, and 570-800 deaths, each year.[1]

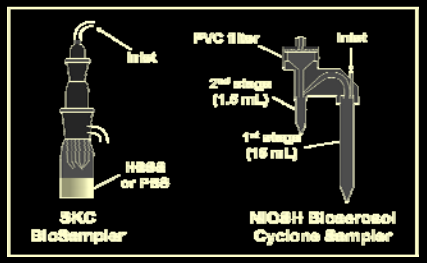
The increasing incidence of norovirus outbreaks presents a risk of exposure to employee groups across several industries.[2]

Limited evidence suggests that norovirus can become aerosolized increasing contamination and human exposure.[3,4]

Therefore, universal sampling and quantification methodologies need to be developed to characterize occupational exposure to aerosolized norovirus.

Objectives

Compare viral concentrations of aerosolized norovirus across two bioaerosol samplers:

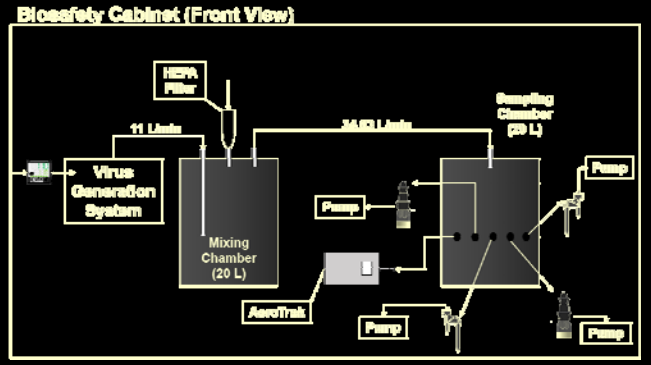


Compare viral concentrations of aerosolized norovirus across liquid sampling medias.

Compare the effect of the sampler and sampling media on membrane integrity of norovirus across samplers and sampling media.

Experimental Setup

Viral bioaerosol generation, mixing, and sampling setup for 10 trials. Airflow is shown by yellow arrows.



Methods

A norovirus surrogate, murine norovirus (MNV), was aerosolized inside of a bioaerosol chamber with a starting concentration of 10^5 PFU/mL for a total of 10 trials, each lasting 30 minutes.

After each trial, aliquots from the SKC BioSampler were transferred to a -80°C freezer. The NIOSH sampler tubes and filter were washed with either Hanks balanced salt solution (HBSS) or phosphate buffered saline (PBS). Aliquots from the washes were then transferred to a -80°C freezer.

RNA was extracted using the QIAamp Viral RNA Mini Kit spin method. RNA was quantified using RT-qPCR in triplicates for each sample. RNA concentration was calculated using qPCR output compared to a standard curve created with gBlocks.

Viral membrane integrity of MNV was determined using propidium monoazide (PMA) dye (Biotium, Fremont, CA). Integrity was quantified using PMA:RT-qPCR in triplicates for each sample. Concentration of MNV containing intact membranes were calculated using qPCR output.

Transmission electron microscopy (TEM) images were acquired by negative staining samples with uranyl acetate.

A multiple linear regression analysis, modeling (PMA/RNA) on the log scale as a function of media, sampler, temperature, relative humidity, and the log nebulizer concentration was used to analyze data.

Results

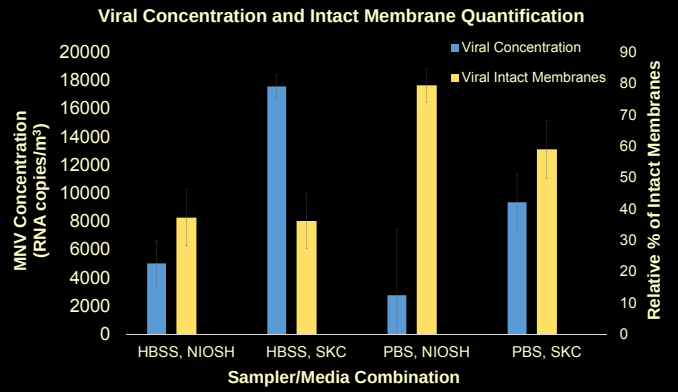


Figure 1. Arithmetic mean of MNV concentrations and relative % of intact membranes from all trials across sampler/media combination. Standard error bars are shown.

Table 1. P-value of statistical tests performed for MNV concentration and intact membrane comparisons.

Experiment	Comparison	P-value
Viral Concentration	HBSS vs. PBS	0.047*
	SKC vs. NIOSH	0.001*
Viral Intact Membranes	HBSS vs. PBS	0.002*
	SKC vs. NIOSH	0.76

* Indicates a statistically significant difference between the comparisons (α=0.05)

Results Cont.

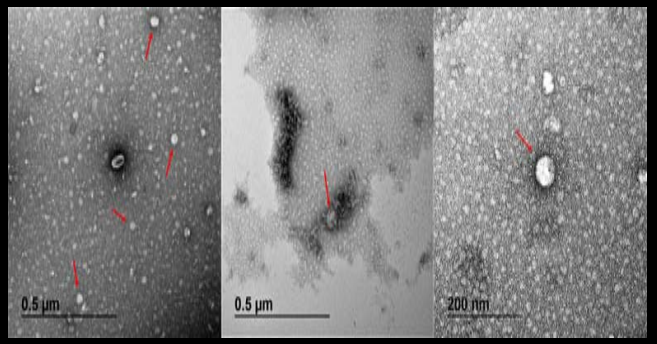


Figure 2. TEM images of murine norovirus samples. The left image is of viral stock, the center image is from a SKC BioSampler in PBS, and the right image is from a NIOSH sampler in PBS. Red arrows denote murine norovirus virions with intact membranes.

Conclusion & Discussion

The concentration of MNV was significantly higher when using HBSS media for both samplers, as well as higher in the SKC BioSampler regardless of media.

There were significantly higher percentages of intact capsids when using PBS media for both samplers. Relative percentage of intact membranes was higher in the NIOSH sampler.

Based on results, the advantages and disadvantages of using HBSS or PBS media should be considered when sampling for aerosolized norovirus.

TEM images showed minimal disruption in membrane integrity of virions sampled with PBS media.

Future Research

Develop limit of detection for the SKC BioSampler and NIOSH Bioaerosol Cyclone Sampler targeting aerosolized norovirus.

Develop quantification methods that allow for increased accuracy when characterizing norovirus aerosols.

Complete field sampling in occupational settings (*i.e.,* healthcare facilities, cruise ships, and waste water treatment facilities) for aerosolized norovirus.

Acknowledgements & References

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