The Effect of Bioaerosol Sampling on the Viability of Aerosolized Influenza A

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Background

Annually, influenza virus (INF) causes 3 to 5 million cases of severe illness, and 290,000 to 650,000 respiratory deaths worldwide.[1]

Direct medical costs in the United States are estimated to be $10.4 billion annually.[2]

Influenza virus can be transmitted directly and indirectly (*i.e.,* exposure to contaminated surface or aerosolized influenza).[3,4]

Personal exposure to aerosolized INF is typically evaluated using filter-based sampling. However, few studies have directly investigated the effects of bioaerosol sampling on the viability of aerosolized INF using filter membranes.[5]

Objectives

1. Determine the average RNA copies of aerosolized influenza sampled onto a novel polystyrene (PS) filter.
2. Determine the average RNA copies of spiked influenza onto a PS filter.
3. Assess influenza virus viability (*i.e.,* percent of intact viral capsids) among aerosolized and spiked INF virus samples.

Methods

Spiked Samples:

- PS filter spiked with 200 µL of influenza A virus
- Filter placed into 37 mm 3-stage closed face cassette (CFC, SKC Inc., Eighty Four, PA), sealed, and placed in a biosafety cabinet
- INF was aerosolized in a bioaerosol chamber

Aerosolized Samples:

- Bioaerosol was collected with a CFC containing a PS filter
  - Polypropylene support pad (SKC, Inc., Eighty Four, PA)
  - Sampling pumps were operated at 4 L/min (Mesa Labs, Butler, NJ)
  - Relative humidity and temperature were monitored for all experiments

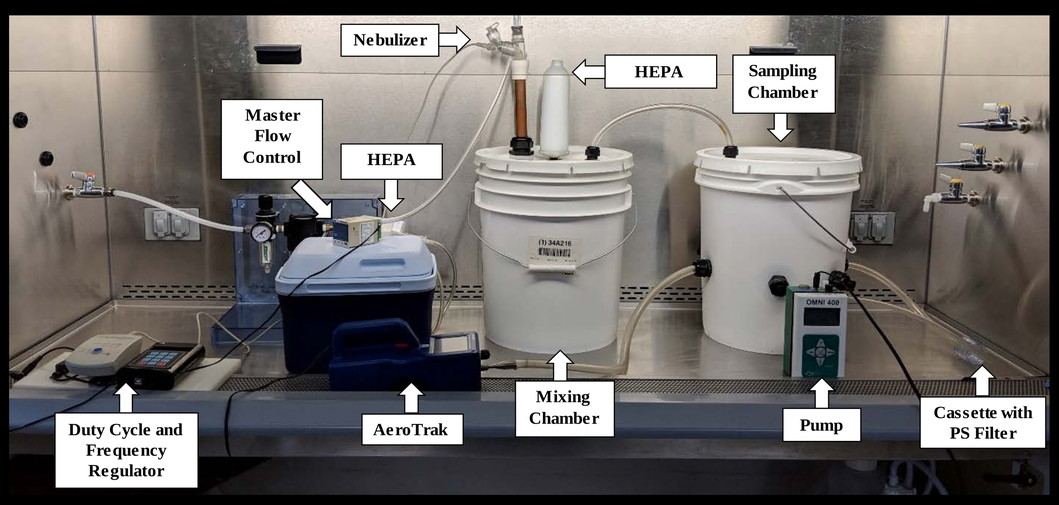
Sampled INF collection, extraction, and quantification:

- Filters were placed in 50 mL conical tubes with 1.5 mL of Hank's Balanced Salt Solution, and then vortexed for 5 minutes to remove viral particles from the filter
- Viral RNA was extracted using the QIAamp Viral RNA Mini Kit spin method (Qiagen, Hilden, Germany)
- Viral membrane integrity of INF was determined using propidium monoazide (PMA) dye (Biotium, Fremont, CA)
- PMA treated and untreated samples were quantified using reverse transcription – quantitative polymerase chain reaction
- Ten experimental trials were conducted each for 30 minutes (n=30)

The data were evaluated graphically to determine normality. Descriptive statistics were used to compare RNA copies between aerosolized and spiked samples. Relative percent of INF with intact membrane comparison was performed using two-tailed sign test with a type-1 error rate of 0.05.

Aerosolized INF samples were evaluated qualitatively using scanning electron microscopy (SEM)

Experimental Setup



Results

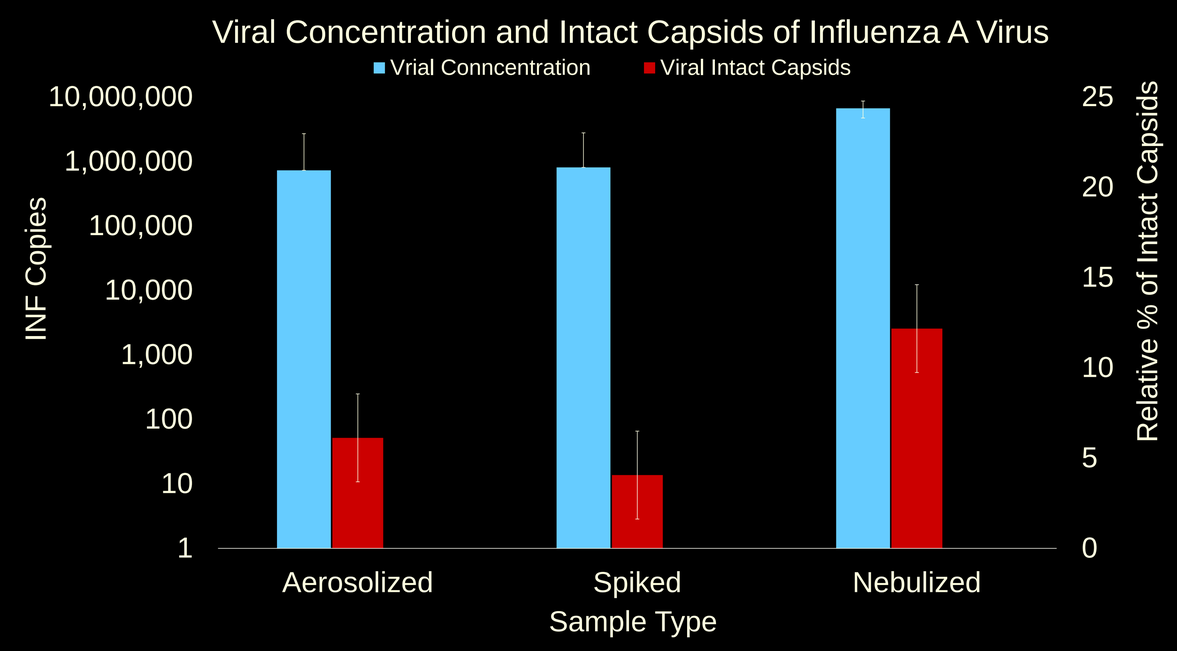


Figure 1. The average RNA copies for Influenza A samples from aerosolized, spiked, and nebulizer samples and relative % of intact capsids from all trials. Standard error bars are shown.

- Statistical comparison of the relative percent of intact capsids was p = 1.000

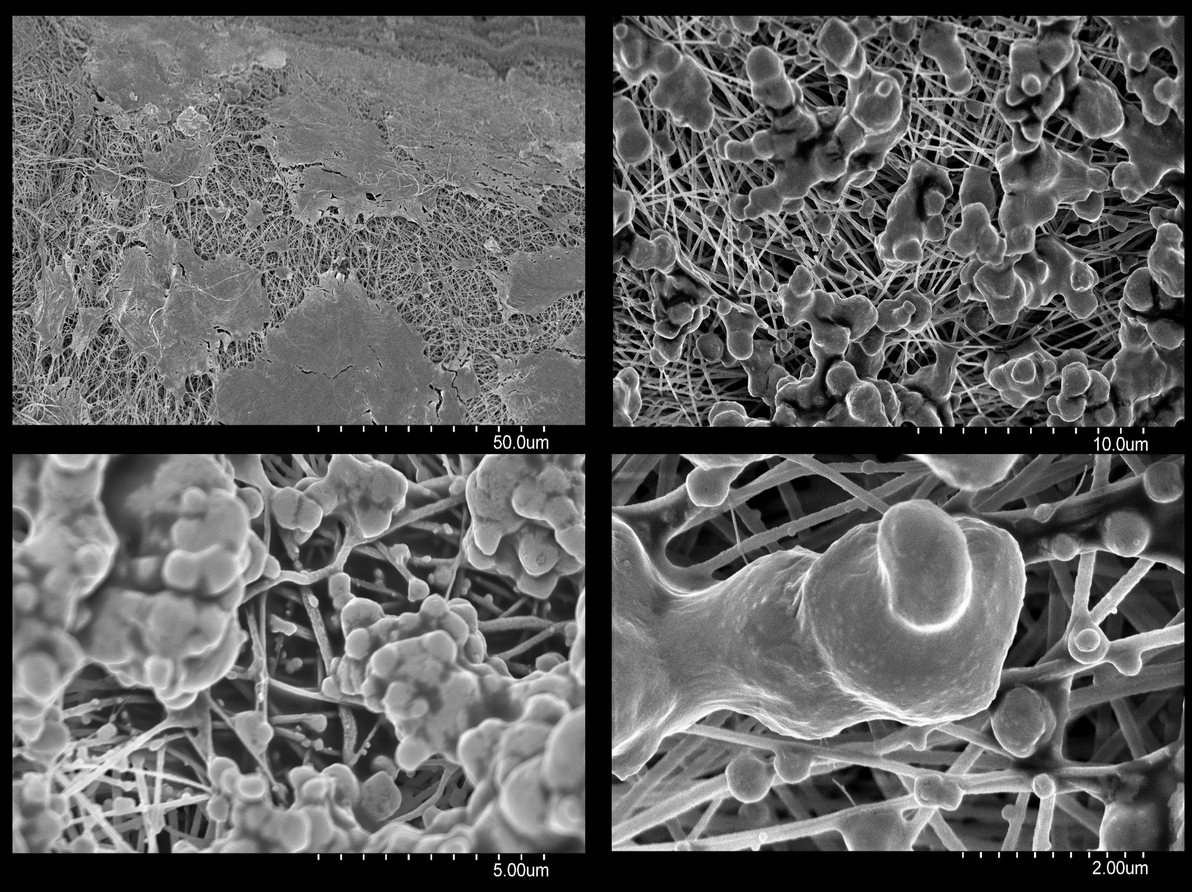


Figure 2. SEM images of aerosolized INF sampling onto a PS filter. Pictures zoom in order of Top Left, Top Right, Bottom Left, and Bottom Right.

Conclusion

Average RNA copies were less for aerosolized samples when compared to spiked filter samples

There was not enough evidence to support a difference in the relative percent of INF with intact membranes collected between aerosolized and spiked samples

This was the first study to use PMA dye to assess viability of influenza A virus aerosol samples

Future Work

Further evaluate the PMA assay for use with INF

Evaluate other filter materials for maintaining INF viability

Assess other virus and filter type interactions using TEM imaging

Acknowledgements & References

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