HIGHLY EFFICIENT COLLECTION OF VIABLE INFLUENZA VIRUS A/MEXICO/4108/2009 (pdmH1N1 AEROSOLS)

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BACKGROUND

- Local outbreaks of influenza occur most years, and they occasionally lead to epidemics or pandemics.
- Influenza A virus subtypes H1N1 and H3N2, and influenza B viruses, are the common causative agents of influenza in humans.
- Pandemics result in tens of millions of influenza cases and thousands of deaths (eg., in the USA, the 2009 pandemic resulted in 43–89 million influenza cases and 18,300 deaths)[1].

CURRENT LIMITATIONS

- There is substantial evidence that airborne transmission of infectious influenza virus poses significant risk[2]; however, the importance of airborne transmission relative to direct contact is still debated.
- Although infectious virus are generally in the nanometer size range, existing samplers for bioaerosol collection are designed for micron-sized particles such as fungal spores and bacteria.

MAIN NEED AND APPROACH

Determining the pathways whereby influenza virus (IFV) spreads is an important public health issue.

Adapt our water-based particle growth tube collector, previously tested for MS2 virus[3], for efficient collection of airborne IFV.

OBJECTIVES

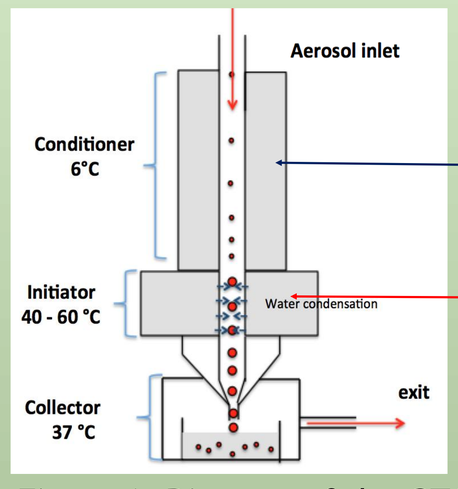
Evaluate the performance of our growth tube collector (GTC) for the collection of laboratory-generated H1N1 IFV aerosols relative to that of the industry-standard BioSampler® by:

1. Measuring the viability of IFV in the collected samples.
2. RT-PCR analyses of the IFV RNA in the collected samples.

MATERIALS AND METHODS

Test Virus: IFV A/Mexico/4108/2009 (pH1N1), a wild-type H1N1 pandemic 2009 strain.

Growth tube collector (GTC)



The **cold conditioner** serves to condition the T and RH of the aerosol.

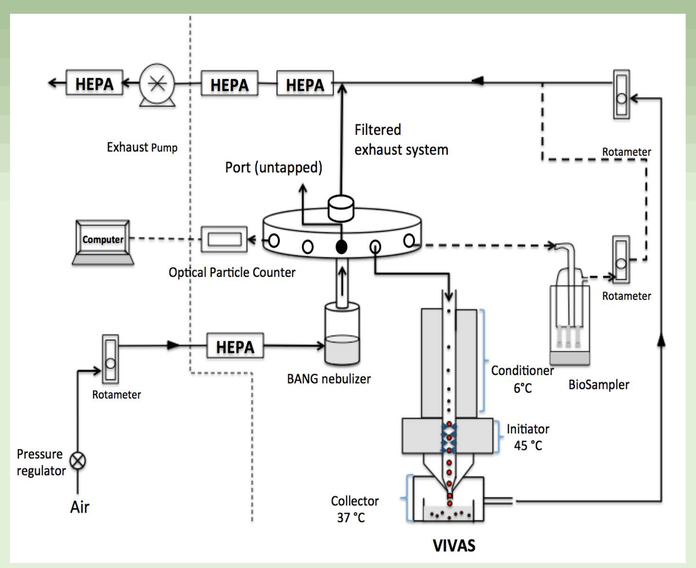
The **hot initiator** induces condensation and provides time for particle growth.

Figure 1. Diagram of the GTC

Quantification of collection efficiency

Viability: Infectious virus titers were determined in Madin Darby canine kidney cells in 96-well microtiter plates[4]. Infectious virus titers were calculated from the median (50%) tissue culture infectious dose ($TCID_{50}$) and expressed as $TCID_{50}$/mL.

EXPERIMENTAL DESIGN



BioAerosol Nebulizing Generator
Biosampler sampling at 8 lpm
Collection: 5, 10, 15 min
Collection Media: PBS + BSA

Figure 2. Schematic diagram of the experimental setup

References:

1. CDC (2011). The 2009 H1N1 pandemic: summary highlights, April 2009-April 2010. Website: http://www. cdc. gov/h1n1flu/cdcresponse. htm, Accessed on August 2.
2. Killingley, B. and Nguyen-Van-Tam, J. (2013). Routes of influenza transmission. Influenza Other Respir Viruses 7:42-51.
3. Pan, M., Fernandez, A. E., Hsieh, H., Afshar-Mohajer, N., Hering, S.V., Lednicky, J., Hugh Fan, Z. and Wu, C. Y. (2016). Efficient Collection of Viable Virus Aerosol through Laminar-Flow, Water-Based Condensational Particle Growth. J Appl Microbiol.
4. Lednicky, J. A., Hamilton, S. B., Tuttle, R. S., Sosna, W. A., Daniels, D. E. and Swayne, D. E. (2010). Ferrets develop fatal influenza after inhaling small particle aerosols of highly pathogenic avian influenza virus A/Vietnam/1203/2004 (H5N1). Virology 7.

RESULTS

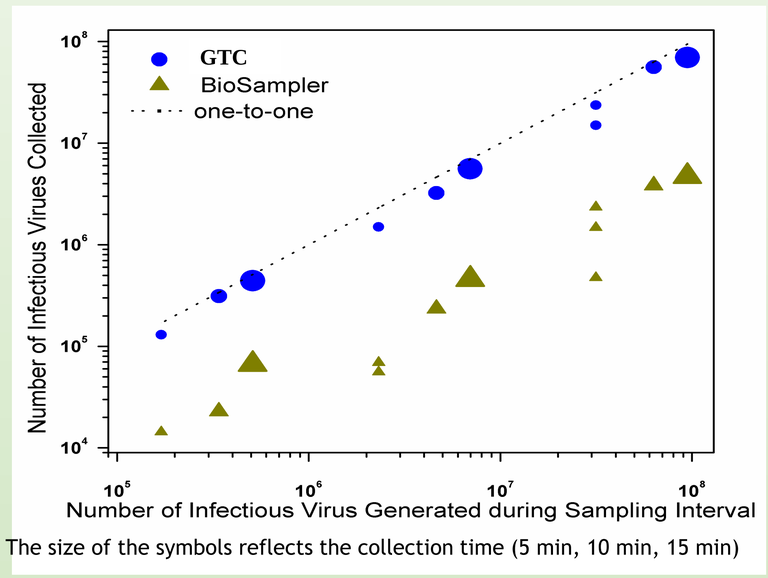
1. Collection efficiencies relative to sampling time

Table 1. Collection Efficiency (mean ± STDEV) for Infectious H1N1 Virus

		Collection Efficiency of Infectious H1N1	
Sampling time (min)	**Number of Tests**	**BioSampler**	**GTC**
5	7	4.4±2.6%	67±10%
10	3	6.0±0.8%	83±12%
15	3	8.2±4.3%	80±07%
All Runs	13	5.6±3.0%	74±12%

On average, the GTC was **12 times more efficient** than the Biosampler (average efficiency of 74%) for all sampling times.

2. Collection efficiencies relative to concentration of aerosolized IFV and collection time



The size of the symbols reflects the collection time (5 min, 10 min, 15 min)

- For both samplers, the quantity of collected IFV increases systematically with the concentration of aerosolized IFV.
- The capture efficiency of the GTC is one order of magnitude higher than the Biosampler.

3. Semi-quantification of collected IFV by RT-PCR

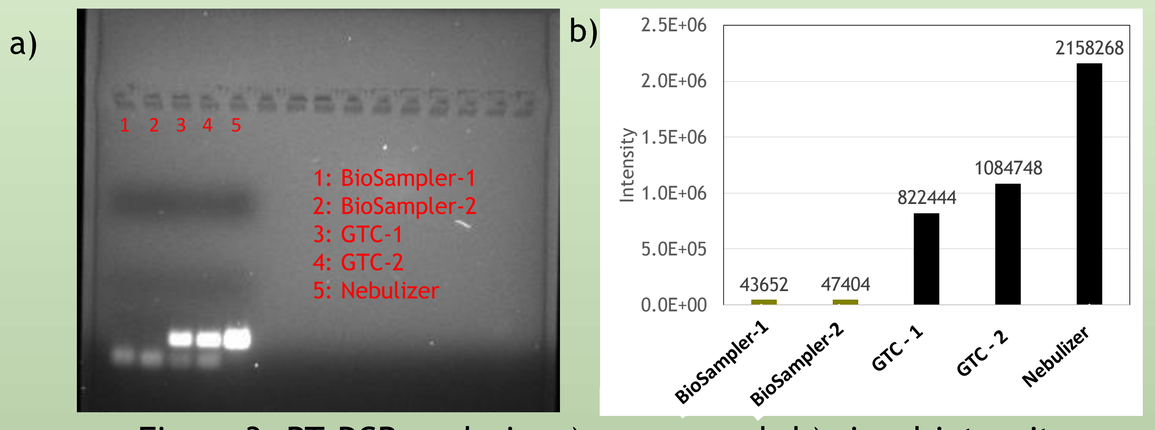


Figure 3. RT-PCR analysis; a) agarose gel; b) signal intensity

PCR amplicon signals measured by RT-PCR were at least 20 times higher for the GTC than the Biosampler, indicating much more IFV (genomic equivalents of IFV RNA) were present in the GTC samples.

Field deployment test

- The GTC was deployed at the Student Health Center at the University of Florida, Gainesville, USA.
- Viable IFVs were isolated from the GTC collection media. Preliminary tests of the IFV isolated in MDCK cells were conducted using a Quidel QuickVue Influenza A + B Kit (Figure 4)

Two types of influenza virus were detected:

1. Influenza A viruses (red line above the blue line).
2. Influenza B viruses (red line below the blue line).
3. Viable Respiratory syncytial virus A was also collected and isolated. Other viruses were also detected (information to be presented elsewhere).

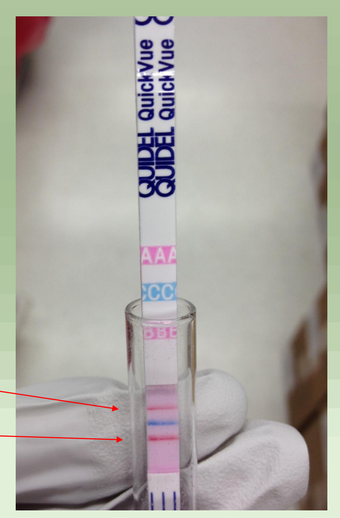


Figure 4. Quidel QuickVue assay

SUMMARY

1. With lab-generated aerosols, the GTC efficiency for infectious H1N1 capture was at least 74%, compared to 5.6% for the BioSampler
2. With lab-generated aerosols, much more IFV genomic RNA was measured in the GTC samples than those of the BioSampler.
3. The GTC successfully collected airborne IFV A and B (and other viruses) under field conditions.

Acknowledgements: National Science Foundation Grant number: IDBR-1353423, and Aerosol Dynamics Inc.