Toward Identifying the Most Effective Samplers for Airborne Viruses

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Motivation

- Emerging zoonotic influenza viruses pose real or potential risks to swine, poultry, and veterinary workers
- Many viruses may be transmitted through air among animals or between animals and people, or have potential to develop transmissibility
- Animals in agricultural facilities generate virus-containing particles small enough to be transported substantial distances
- Little is known about typical concentrations and sizes of airborne virus-containing particles in animal agriculture, or if viruses remain infectious

Why do we care about particle size?

- We want to know how far viruscontaining particles are able to travel through air
- We want to determine where viruscontaining particles deposit in human or animal respiratory tract
- We want to identify technologies that can remove virus-containing particles from air









Research Objective

We want large samples to achieve low limits of detection

We want to do this in the real world

Identify/develop a high-volume, field-portable, size-differentiating viral aerosol sampler and use it to measure worker exposures to live airborne influenza viruses in animal agriculture facilities

Hey...we
already
talked
about this

We're working with viruses The particles that we're considering are airborne

We want to know if the viruses in the air are infectious Our focus is animal agriculture

We're collecting samples from the air

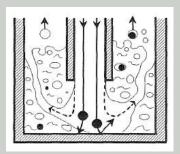


First Step: Evaluate Existing Samplers

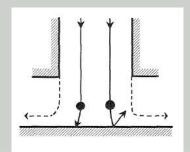
- Assemble wide range of existing samplers that collect viral aerosols by variety of principles
- Test samplers side-by-side in an isolation room using mechanically-generated influenza virus aerosols
- Determine combinations of sampling parameters and technologies that collect greatest quantity of viral RNA and live virus

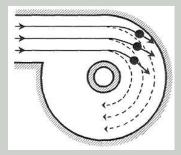
Sampling Technologies

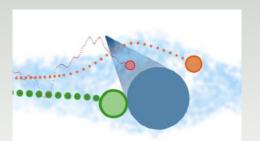
- Impingers
- Cyclones
- Impactors
- Filters
- Electrostatic collection
- Combinations

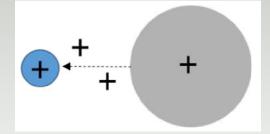


Willeke et al. (1998). *Aerosol Science and Technology*, 28:439-456











Samplers Evaluated

Group 1	Group 2	Group 3
Non-Viable Andersen	Non-Viable Andersen	Non-Viable Andersen
Cascade Impactor	Cascade Impactor	Cascade Impactor
(ThermoFisher)*	(ThermoFisher)*	(ThermoFisher)*
Cyclonic Collector	Cyclonic Collector	Cyclonic Collector
(Midwest Micro-Tek)*	(Midwest Micro-Tek)*	(Midwest Micro-Tek)*
AGI-30 impinger (Ace	47mm fiberglass filter	MOUDI (MSP Corp.)
Glass, Inc.)	47mm gelatin filter	Trichotomous Virtual
BioSampler (SKC Inc.)	PEMS PM2.5 sampler (SKC	Impactor Sampler
Cyclone Bioaerosol	Inc.)	(University of
Sampler (NIOSH)	Hi-Vol TSP sampler	Minnesota)
SpinCon II (InnovaPrep)	Electrostatic sampler	Series 230 High Volume
Bobcat (InnovaPrep)	(UNC-Chapel Hill)	Cascade Impactor (Tisch
VIVAS (UF & Aerosol		Environmental)
Dynamics)		

^{*}Sampler was used in all three groups as a control



Methods

- H3N2 swine influenza virus (SIV) grown and titrated in Madin-Darby canine kidney (MDCK) cells grown in Eagle's MEM with supplements
- Fluorescein dye added to virus suspensions to track physical collection efficiency
- SIV suspension aerosolized at pressure of 20 psi using 6-jet Collison-type nebulizer in an isolation room in the BSL-2 Veterinary Isolation Building on University of Minnesota St. Paul campus
- Simultaneous samples collected by samplers in each group for 30 minutes
- Samplers were tested in three replicate tests
- Resulting nebulizer suspensions and air samples analyzed
 - SIV titrated to determine quantities of live virus
 - Viral RNA extracted and used for qRT-PCR (quantitative real time-PCR) to determine quantities of total virus
 - Intensity of fluorescein dye measured by spectrofluorometry
- Relative recovery calculated to determine fraction of collected virus still active
- Recoveries among the samplers were compared descriptively and statistically

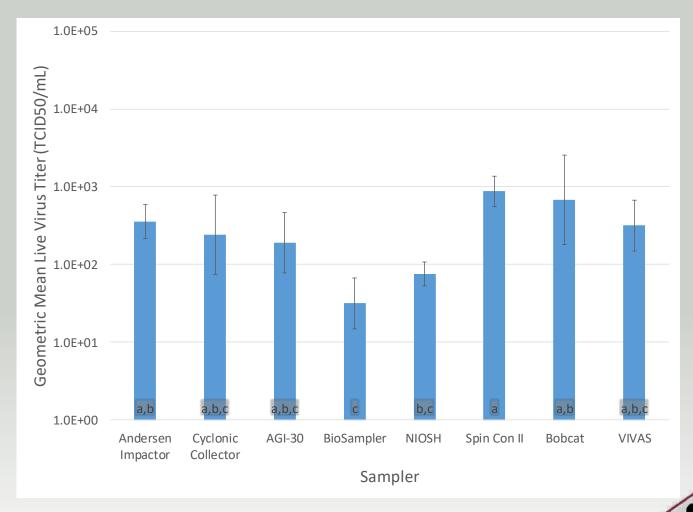
Isolation Room Setup



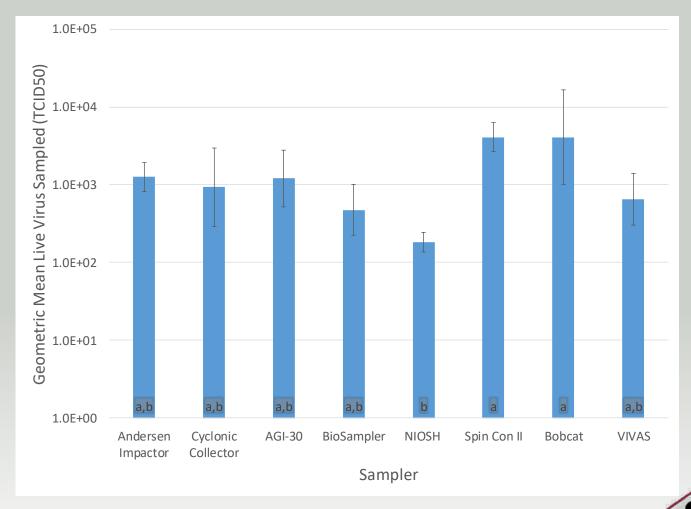




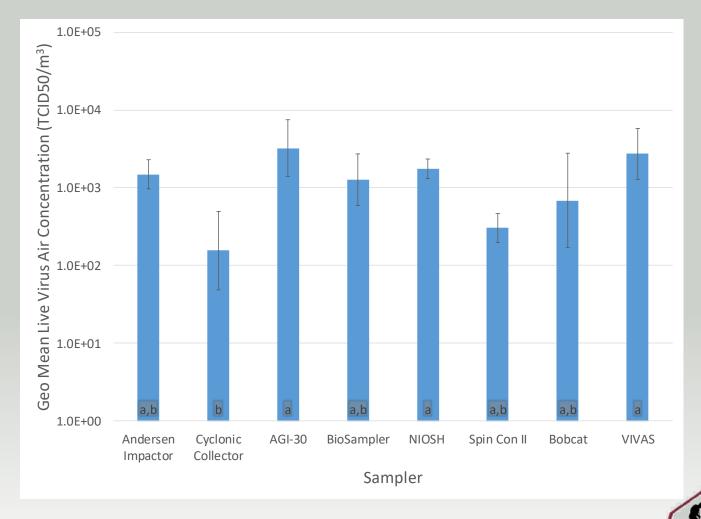
Live Virus Titer, Set #1



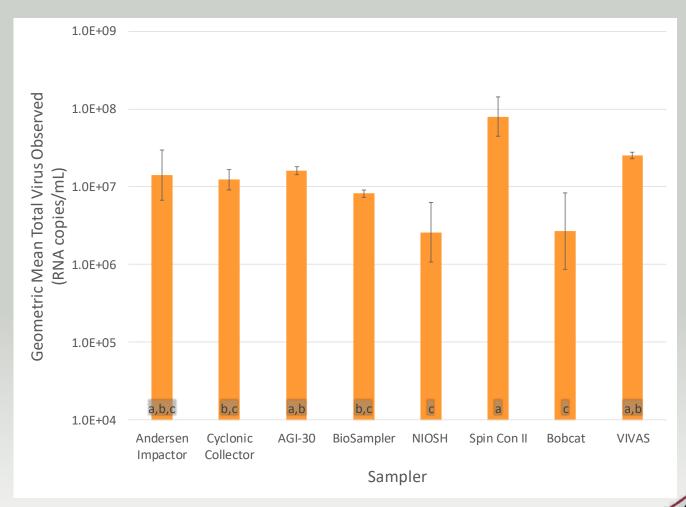
Live Virus Sampled, Set #1



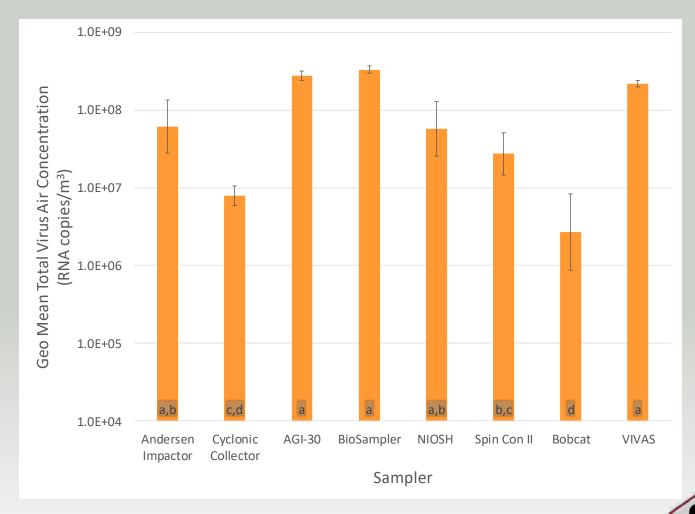
Live Virus Air Concentration, Set #1



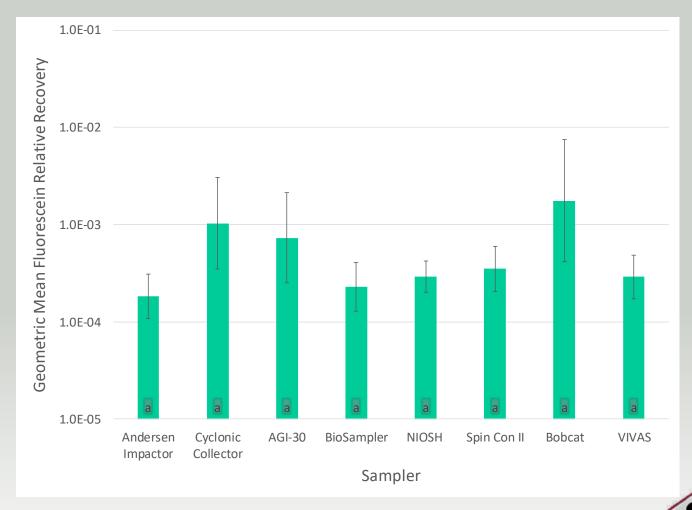
Total Virus Observed, Set #1



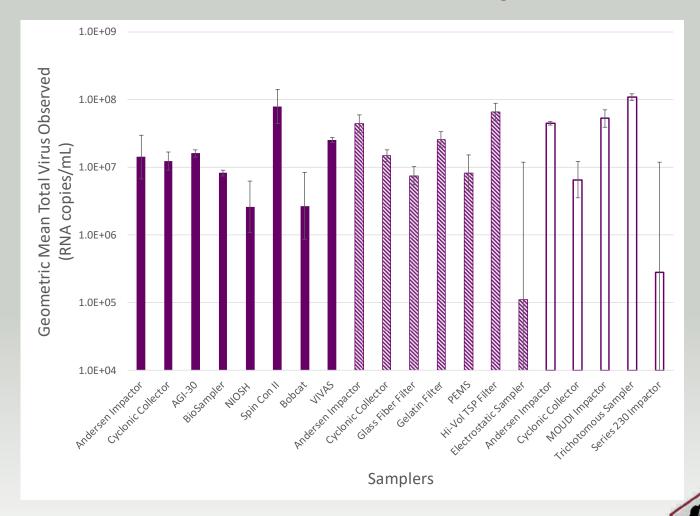
Total Virus Air Concentration, Set #1



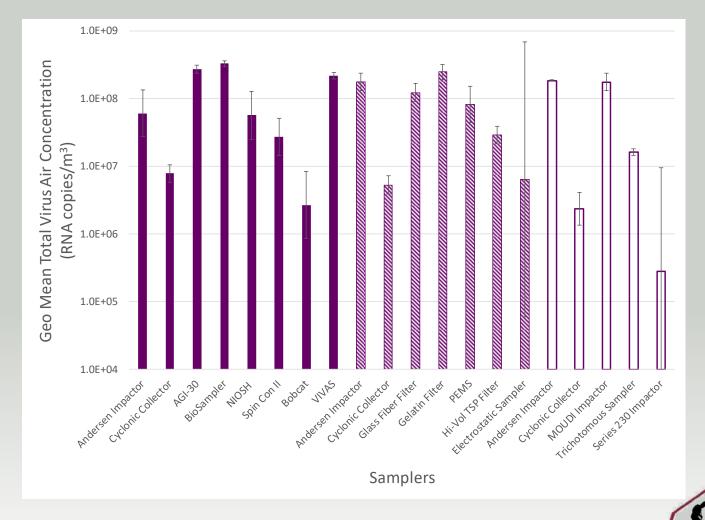
Relative Recovery, Set #1



Total Virus Observed, All Sets



Total Virus Air Concentration, All Sets



Discussion

- High flow rate samplers tend to yield higher titers/more RNA copies
 - High flow samplers consolidate sample more than lower flow samplers
 - Likely better for detection of airborne viruses at low concentrations
- Highest airborne virus concentrations observed among lower flow rate samplers



Discussion (continued)

- Impinger samplers may keep virus live more effectively than other types of samplers
- Ease of use important but should not drive decisions
- Two-sampler strategy may have benefits during outbreak investigations
 - High flow, non-sizing sampler for detection
 - Lower flow, size-separating sampler for concentration measurements



Bottom Line

No sampler that we have tested is "best" so far

Next Steps

- Compare several of best-performing samplers in field tests this flu season
- Design and build novel size-separating sampler
- Compare novel sampler to existing ones



Acknowledgements

Upper Midwest Agricultural Safety and Health (UMASH) Center funded by National Institute for Occupational Safety and Health (NIOSH) under Cooperative Agreement U54 OH010170



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