

Bacteria Aerosol Measurements Using Ventilation Filters in Building Air Handling Units

Seung Won Kim¹, James Farnsworth²,
Sagar Goyal³, Peter Raynor¹, Thomas
Kuehn²

University of Minnesota

¹Division of Environmental Health Sciences

²Department of Mechanical Engineering

³Department of Veterinary Population Medicine

Monitoring Bioaerosol

- Increasing threat of bioterrorism
- Bioaerosol concentrations
 - ❖ Traditional evaluation
 - Impaction samplers and filtration samplers
 - Short-term sampling
 - ❖ Bioaerosol analysis
 - Culture-based (e.g. Agar plate method)
 - Nonculture-based (e.g. PCR method)

Air-Handling Units (AHUs)

- ❑ Used for a period of weeks or months
 - ❖ t
- ❑ Flow rate information
 - ❖ Q
- ❑ Denominator of the bioaerosol concentration
 - ❖ $V=tQ$



New Sampling Method

- A novel sampling method
 - ❖ Building air-handling units (AHUs) as high-volume filtration samplers
 - ❖ Heating, ventilation, and air-conditioning (HVAC) filter media in AHUs
- Advantage of this method
 - ❖ Versatile and unintrusive
 - ❖ Long-term integration
 - ❖ No sampler, no installation!

Objectives of this Study

- ❑ Evaluating the background level of airborne bacteria and their species in large public buildings
- ❑ Evaluating the HVAC filters as a high-volume bioaerosol sampling device
 - ❖ Lab tests to validate this method
 - ❖ Developing the procedure to analyze HVAC filters taken out from AHUs

Study Design

- Spiking tests
 - ❖ Direct deposit of bacteria on filter media
- Aerosolization tests
 - ❖ Small-scale filter tests through nebulization
- Full filter tests
 - ❖ According to the ASHRAE standard
- Real filter extraction

Lab Tests: Results

□ Recovery(%)

$$Recovery(\%) = \left| \frac{N_{extracted}}{N_{introduced}} \right|_{culturable} \times 100$$

□ Collection efficiency (%)

$$\eta = \left(1 - \frac{C_{downstream}}{C_{upstream}} \right) \times 100$$

Lab Tests: Test Microorganisms

- Selected based on
 - ❖ Nonpathogenicity
 - ❖ Availability
 - ❖ Relationship to known pathogens
- Surrogates for known threat agents

Pathogens and their Surrogates

Pathogen	Surrogate
<i>Bacillus anthracis</i>	<i>Bacillus subtilis</i> (<i>Bacillus atropheus</i>)
<i>Pasteurella multocida</i>	<i>Mannheimia</i> <i>haemolytica</i>
<i>Yersinia pestis</i>	<i>Yersinia ruckeri</i>

Lab Tests: Filter Media

- Fibrous filter paper
 - ❖ HE-1071, fiberglass
 - ❖ Grade 41, ashless cellulose
- HVAC filter media
 - ❖ Panel pre-filter media
 - ❖ Riga-Flo 200 PH, unused
 - ❖ Riga-Flo AP3HCP8-95, used

Spiking Tests

- ❑ Spiked 0.1 mL of bacteria suspension onto 2.26 cm² size media sample directly
- ❑ Factors tested
 - ❖ Eluents
 - DIF water, PBS, and various Tween 80 solutions
 - ❖ Agitation methods
 - Mechanical shaking (10 minutes at 70 rpm)
 - Vortex mixer (60 sec)
 - Hand shaking (500 shakes, 45 degree arc from the elbow)

Spiking Tests: Agitation Method

Agitation method	Recovery (%)
Mechanical shaking	0.4
Vortex mixer	33
Hand shaking	67

Spiking Tests: Recovery by Eluents

	DIF water	0.02% Tween 80	PBS
<i>B. subtilis</i>	102±24	114±21	N/A
<i>M. haemolytica</i>	45	33	87
<i>Y. ruckeri</i>	43	54	80

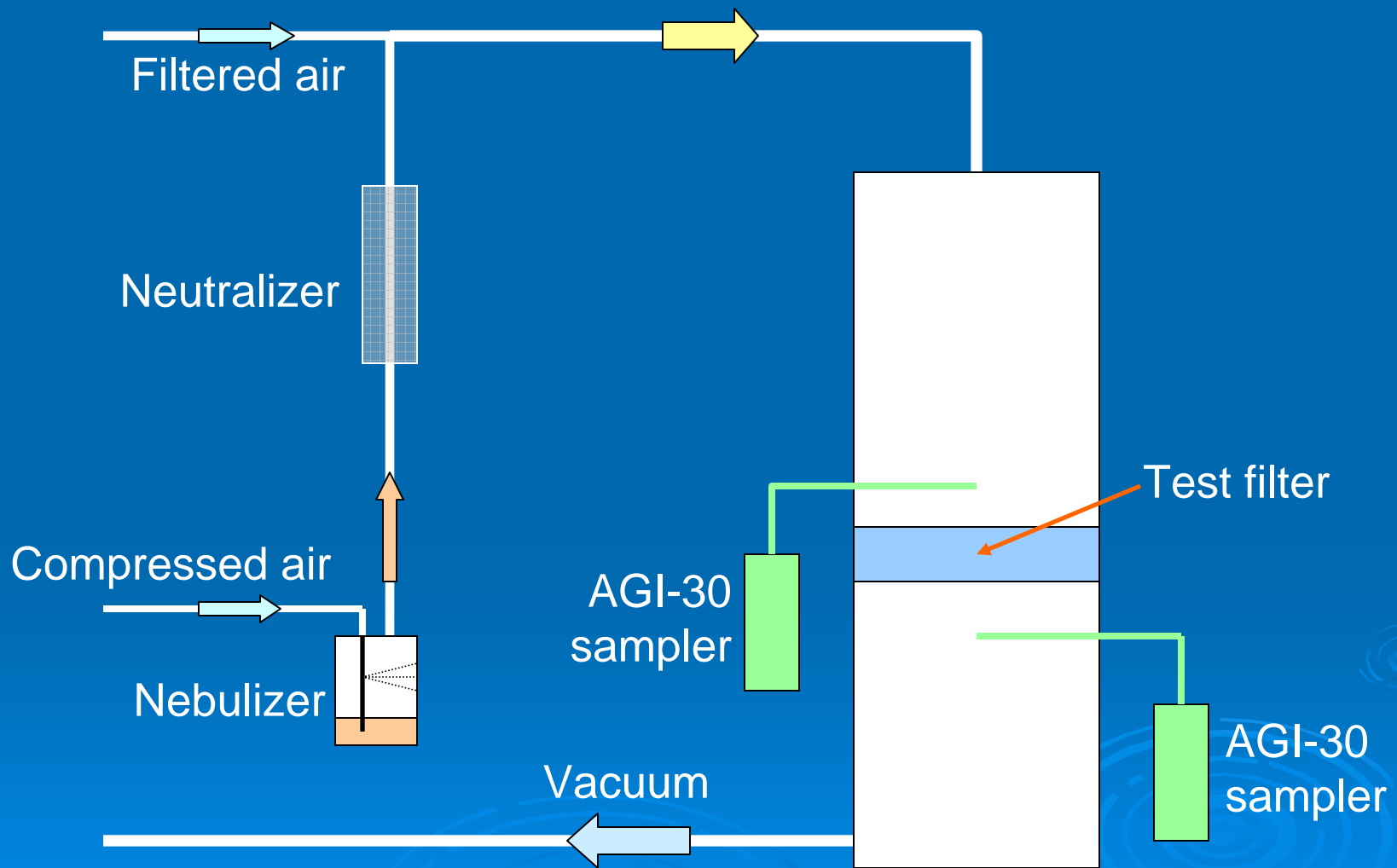
Spiking Tests: Recovery of *B. subtilis*

Filter	Recovery (%)
HE-1071 membrane filter paper	37±14
Whatman 41 ashless filter paper	31±6
AAF HVAC pre-filter media	51±17
Riga-Flo unused HVAC media	109±10
Riga-Flo used HVAC media	108±7

Aerosolization Test

- Small filter test apparatus
 - ❖ Brosseau et al. (1994); McCullough et al. (1997)
- Nebulizer
 - ❖ Retec plastic nebulizer at 3 psi
- Aerosol sampling
 - ❖ AGI-30 impingers
 - ❖ DIF water for *B. subtilis*
 - ❖ PBS for *M. haemolytica* and *Y. ruckeri*

Aerosolization Tests: Apparatus



Aerosolization Tests: *B. subtilis*

Filter	Eluent	η (%)	Recovery (%)
HE-1071 membrane filter paper	DIF water	99.6	39±5
	Tween 80	99.9	69±9
Riga-Flo unused HVAC filter media	DIF water	91.7	114±10
	Tween 80	91.7	134

Aerosolization Tests: Additional Findings

- No meaningful results from *M. haemolytica* and *Y. ruckeri*
 - ❖ Lost viability or died due to either nebulization stress or exposure to air
 - ❖ Dehydrated on the filter during air aspiration
- Temporal decay: *B. subtilis* only
 - ❖ Analyzed bacteria-loaded filter media daily
 - ❖ Recovery decreased by 50% every 8 days

Full Filter Tests

- Designed according to ASHRAE Standard 52.2-1999
 - ❖ Closed-loop wind tunnel
 - ❖ 500-2000 cfm
- Aerosol sampling
 - ❖ AGI-30 impingers and SKC Biosamplers
- Filter media
 - ❖ Viledon Mini 95-2/44 filters

Full Filter Tests: *B. subtilis*

	η (%)	Recovery (%)
AGI-30	95.6 \pm 0.5	86.7 \pm 3.4
Biosampler	96.7	98.6 \pm 8.6
OPC	97.3 \pm 0.3	n/a

Real Filter Extraction

□ Sampling

- ❖ Two batches from a public building in MN
- ❖ 3 mixed air AHUs and 1 outdoor air AHU

□ Analysis

❖ Culture-based

- Elution >> culturing >> counting >> isolation >> classification >> identification
- Identified using MicroLog System (Biolog, CA)

❖ Nonculture-based: PCR method

Real Filter Extraction: Results

□ Culture-based analysis

❖ 47 unique species

- Mostly spore-forming bacteria

❖ Total culturable bacteria concentration

- Mixed air: 0.43 – 12.1 CFUs/m³
- Outdoor air: 1.54 – 39.8 CFUs/m³

□ Nonculture-based analysis

❖ PCR to screen highly pathogenic agents

- No known pathogenic bacteria

Summary of Results

□ Spiking tests

- ❖ Handshaking for agitation method
- ❖ 0.02% Tween 80 for *B. subtilis*
- ❖ PBS for *M. haemolytica* and *Y. ruckeri*

□ Aerosolization tests

- ❖ Higher recovery from HVAC filter
- ❖ Vegetative bacteria lost viability
- ❖ Temporal decay: 8 days of half-life

Summary of Results

□ Full filter tests

- ❖ Measured collection efficiency matched well with nominal collection efficiency
- ❖ Recovery for *B. subtilis* was similar with the results from aerosolization tests.

□ Real filter extraction

- ❖ 47 unique species
- ❖ Total culturable bacteria concentration
 - Mixed air: 0.43 – 12.1 CFUs/m³
 - Outdoor air: 1.54 – 39.8 CFUs/m³

Conclusions

- ❑ The methodology of using AHUs as high-volume bioaerosol samplers was established
- ❑ Working well for spore-forming bacteria
- ❑ Not so well for vegetative bacteria
 - ❖ PCR method

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